Author:
Evans, Rebecca N

Title:
Impact of modifiable healthcare factors on outcome after bloodstream infection

Impact of modifiable healthcare factors on outcome after bloodstream infection

By

Rebecca Nicole Evans

Bristol Medical School

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Health Sciences.

October 2021

Word count: 58,277

ABSTRACT

There has been a range of research exploring on non-modifiable risk factors, such as patient comorbidities, for mortality in patients with a bloodstream infection (BSI). Factors of patient care which can be modified have been found to be associated with survival in other disease areas, but have not been explored in patients with a BSI to date.

In this thesis, I explore the impact of modifiable factors on mortality in patients with a BSI using data from the BSI-FOO research programme (RP-PG-0707-10043).

I begin by looking at healthcare setting related factors, before exploring the effect of duration of therapy. I then use trial emulation methods to emulate the MERINO trial, a recent randomised controlled trial comparing piperacillin-tazobactam and meropenem for the treatment of *E coli* or Klebsiella species BSI. Finally, I explore the association between the minimum inhibitory concentration (MIC) and mortality.

Healthcare setting related risk factors associated with 28-day mortality were ward speciality, ward activity, ward movements, and time to receipt of appropriate antimicrobial therapy in the first seven days. In terms of duration of therapy, the hazard of all-cause mortality for short therapy *vs* long therapy was 1.74 (95% CI 1.36, 2.24) and for intermediate *vs* long therapy was 1.09 (95 % CI 0.98, 1.22). In the emulated trial, the odds for mortality was 1.31 times higher (95% CI 0.40 to 4.26) in patients in receipt of piperacillin-tazobactam compared to meropenem, after adjustment for propensity score. This was lower than the odds ratio observed in the MERINO trial, 3.7 (95% CI 1.5 to 10.4). Finally, there was no evidence to suggest a relationship between MIC/EUCAST breakpoint ratio and 28-day mortality in patients with a Gram-negative BSI.

This thesis underlines the importance of appropriate antimicrobials within the first seven days, and the potential for ward activity, ward movements and duration of therapy to impact on survival in patients with a BSI.

DEDICATION & ACKNOWLEDGMENTS

This thesis would not have been possible without the support of many wonderful people. Firstly, I would like to thank my brilliant supervisory team, Dr. Jessica Harris, Prof. Chris Rogers and Prof. Alasdair MacGowan for their help, guidance, and feedback throughout my PhD. I am extremely privileged to have such great supervisors, and especially thankful for the time and support from Jess to keep me going during the pandemic.

There are many friends and colleagues who have supported me throughout this process. Thank you to my colleagues, friends and fellow students at Bristol Trials Centre, who were always very encouraging and supportive throughout. Special shout out to Rosie Harris and Katie Pike for the cups of teas!

I would also like to thank the participants of the BSI-FOO study, and acknowledge the contribution of the investigators, collaborators, and study teams who made this this study possible. I would like to acknowledge the funding received from the National Institute for Health Research for the BSI-FOO research programme, I also gratefully acknowledge the funding sources that made my PhD possible.

Most importantly, I would like to thank my parents, Jenny Evans and Colin Evans, my sister, Sarah Evans, and my Granny, Gloria King, for their love, support and encouragement. You have supported me in everything I do, and I couldn't wish for a better family.

And finally, thank you to my wonderful partner and best friend, Alec, for keeping me going the past 3 years and having faith I could do it.

Thank you all!

AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: Rebecca Evans DATE: 31/10/2021

PREFACE

Below is a list of publications that have arisen from research undertaken throughout this PhD along with the contributions section from each publication.

The following publication is based on Chapter 3 and also includes elements of Chapters 1, 2 and 7

Evans, R. N. , Pike, K. E., Rogers, C. A., Reynolds, R. A., Stoddart, M., Howe, R., Wilcox, M. H., Wilson, P., Gould, K., & Macgowan, A. P. Modifiable healthcare factors affecting 28-day survival in bloodstream infection: a prospective cohort study. BMC Infectious Diseases, 20, 545 (2020). https://doi.org/10.1186/s12879-020- 05262-6

Author contributions: AM & CR were involved in the study design and planning. AM, CR & RR were involved in study conduct. RE & RR cleaned the data. RE performed the analysis with KP & CR providing statistical advice. RE wrote the paper with all authors contributing to critical revisions. All authors read and approved the final manuscript.

First author: Rebecca Evans

Last author: Alasdair MacGowan

The following publication is based on Chapter 3 and also includes elements of Chapter 1 and 2.

Evans, R., Pike, K., MacGowan, A., Rogers, C. A. Analytical challenges in estimating the effect of exposures that are bounded by follow-up time: experiences from the Blood Stream Infection—Focus on Outcomes study. BMC Medical Research Methodology 21, 197 (2021). https://doi.org/10.1186/s12874-021-01393-9

Author contributions: AM & CR were involved in the study design and planning. AM, CR & RR were involved in study conduct. RE performed the analysis with KP & CR providing statistical advice. RE wrote the paper with all authors contributing to critical revisions. All authors read and approved the final manuscript.

First author: Rebecca Evans

Last author: Chris Rogers

TABLE OF CONTENTS

Chapter 1 Introduction and background 1

1.1 Research rationale 1

1.2 Background 2

1.2.1 Brief overview of bloodstream infections 2

1.2.2 Brief overview of BSI treatment 5

1.3 Literature review 7

1.3.1 Search strategy 7

1.3.2 Results 8

1.3.3 Conclusions 12

1.4 Project objective and aims 12

Chapter 2 Introduction to the data 14

2.1 Introduction 14

2.2 BSI-FOO observational study 14

2.2.1 Study Design 14

2.2.2 Data collected 15

2.2.3 Population characteristics 19

2.3 RAPIDO 28

2.3.1 Study Design 28

2.3.2 Data collected 29

2.3.3 Population characteristics 29

2.3.4 Trial conclusions 33

2.4 Summary 33

Chapter 3 Healthcare setting and treatment related risk factors 34

3.1 Introduction 34

3.2 Methods 37

3.2.1 Primary outcome 37

3.2.2 Risk factors 37

3.2.3 Analysis population 37

3.2.4 Descriptive analysis 37

3.2.5 Primary outcome 38

3.2.6 Missing data 40

3.2.7 Significance levels 40
3.2.8 Sensitivity analyses 41
3.3 Challenges and Solutions 42
3.4 Results 43
3.4.1 Descriptive analysis 43
3.4.2 Multiple imputation 61
3.4.3 Primary outcome 66
3.5 Discussion 82
3.5.1 Summary 82
3.5.2 Interpretation 82
3.5.3 Strengths and limitations 83
3.5.4 Conclusions 85
Chapter 4 Duration of therapy 86
4.1 Introduction 86
4.2 Methods 94
4.2.1 Data source 94
4.2.2 Primary outcome 94
4.2.3 Analysis population 95
4.2.4 Statistical analysis 95
4.2.5 Missing data 103
4.2.6 Sensitivity analysis 107
4.2.7 Subgroup analysis 107
4.3 Results 108
4.3.1 Population 108
4.3.2 Demographics 110
4.3.3 Treatment pathways 115
4.3.4 Missing data 117
4.3.5 Outcome 126
4.4 Discussion 131
4.4.1 Summary 131
4.4.2 Interpretation 131
4.4.3 Strengths and limitations 132

4.4.4 Conclusions 134
Chapter 5 Emulating the MERINO trial 135
5.1 Introduction 135
5.2 Methods 143
5.2.1 Data source 143
5.2.2 Eligibility criteria 143
5.2.3 Treatment strategies 145
5.2.4 Assignment of treatment 145
5.2.5 Start of follow-up 145
5.2.6 Outcome 145
5.2.7 Analysis population 146
5.2.8 Statistical analysis 146
5.3 Results 151
5.3.1 Population 151
5.3.2 Treatment 153
5.3.3 Demographics 157
5.3.4 Primary outcome 164
5.4 Discussion 169
5.4.1 Summary 169
5.4.2 Interpretation 169
5.4.3 Strengths and limitations 170
5.4.4 Conclusions 172
Chapter 6 Minimum Inhibitory Concentration (MIC) 174
6.1 Introduction 174
6.2 Methods 184
6.2.1 Data source 184
6.2.2 Primary outcome 184
6.2.3 Analysis population 184
6.2.4 Definitions 184
6.2.5 Statistical analysis 187
6.2.6 Missing data 189
6.2.7 Sensitivity analysis 189

6.3 Results 189
6.3.1 Population 189
6.3.2 Demographics 191
6.3.3 Treatment 199
6.3.4 Outcome 202
6.4 Discussion 207
6.4.1 Summary 207
6.4.2 Interpretation 207
6.4.3 Strengths and limitations 208
6.4.4 Conclusions 209
Chapter 7 Summary of findings and future work 210
7.1 Summary of main findings 210
7.1.1 Key findings 210
7.1.2 Interpretation of findings 211
7.1.3 Implication of findings 212
7.2 Strengths and limitations 212
7.3 Future research 214
7.4 Final conclusions 214
REFERENCES 215

LIST OF TABLES

Table 2.1 BSI-FOO Observational study schedule of data collection 15

Table 2.2 Participant level data collected in BSI-FOO observational study 16

Table 2.3 BSI-FOO Observational study - numbers of organisms by centre 20

Table 2.4 Further details of identity for ESBL producers and *Candida* 20

Table 2.5 BSI-FOO Observational study - patient demography/non modifiable risk factors 23

Table 2.6 RAPIDO Schedule of data collection 29

Table 2.7 RAPIDO organisms 30

Table 2.8 RAPIDO participant demography 32

Table 3.1 Lines and urinary catheter details at day/time zero 44

Table 3.2 Ward speciality on day zero 47

Table 3.3 Staffing levels and ward activity on day zero, by ward speciality 50

Table 3.4 Staffing and ward activity on day zero, by 28-day survival status 52

Table 3.5 Staffing levels and ward activity, by day since blood sample 54

Table 3.6 Staffing levels and ward activity, by day of week 57

Table 3.7 Total numbers of ward movements between day zero and day seven 58

Table 3.8 Type of ward movements between day zero and seven 58

Table 3.9 Appropriate antimicrobial therapy, by organism and survival 60

Table 3.10 Observed and imputed average number of staff, by ward speciality 61

Table 3.11 Observed and imputed ward activity, by ward speciality 61

Table 3.12 Observed and imputed number of beds, by ward speciality 61

Table 3.13 Adjusted Cox model of non-modifiable risk factors on 28-day mortality 67

Table 3.14 Model for modifiable risk factors: likelihood ratio tests for interaction terms 69

Table 3.15 Predicted and observed risk by decile of predicted risk 70

Table 4.1 Summary of studies reporting on the association between duration of therapy and outcome 87

Table 4.2 Demonstration of time-updating covariates for three example patients 96

Table 4.3 Cox regression model *vs* Pooled logistic regression - demonstration using data from the RAPIDO trial 103

Table 4.4 Temperature measurements available around missing measurement 106

Table 4.5 Summary of imputed values using two methods of imputation 106

Table 4.6 Baseline characteristics, by observed duration of therapy 111

Table 4.7 Number of active drugs prescribed per patient 115

Table 4.8 Frequency of specific active therapies 115

Table 4.9 Route of first and second administration 116

Table 4.10 Missing temperature measurement, by reason for end of follow-up 120

Table 4.11 Summary of observed and imputed temperature on last day of follow-up 120

Table 4.12 Baseline characteristics, by availability of neutrophil data 122

Table 4.13 Summary of observed and imputed neutrophil count values 126

Table 4.14 Unadjusted 28-day in-hospital mortality, by observed duration of therapy 126

Table 4.15 Exploring truncation of estimated weights 128

Table 4.16 Naïve analysis: KM estimate and hazard ratio of failure function at 28 days 129

Table 4.17 Time updated covariate: KM estimate of failure and hazard ratio at 28 days 130

Table 4.18 Hernán 3-Step Procedure: Primary analysis of 28-day mortality 131

Table 5.1 Summary of studies reporting on the comparison of carbapenems and BLBLI for the treatment of ESBL bacteraemia and mortality 141

Table 5.2 Population, intervention, comparison, outcome (PICO) table 144

Table 5.3 Treatment details for first prescription of study drug 156

Table 5.4 Duration and time to receipt of emulated intervention 157

Table 5.5 Baseline characteristics of patients in the emulated trial population, by emulated intervention 158

Table 5.6 Baseline characteristics of patients in the MERINO trial analysis population *vs* emulated trial population 162

Table 5.7 Propensity score quintiles, by treatment allocation and survival 164

Table 5.8 Primary analysis: 25-day mortality 167

Table 5.9 Post-hoc analysis: 14-day mortality 168

Table 6.1 Summary of studies reporting on the association between MIC and outcome 177

Table 6.2 Inclusion and Exclusion criteria for MIC analysis 184

Table 6.3 Antibiotics tested against centrally, by organism 185

Table 6.4 EUCAST susceptibility and resistant ranges 185

Table 6.5 Empiric therapy matching - 36 and 48 hour rule 186

Table 6.6 Cross tabulation of clinician classification and 36-hour rule classification 187

Table 6.7 Cross tabulation of clinician classification and 48-hour rule classification 187

Table 6.8 Example calculation of the cumulative average MIC/breakpoint ratio 188

Table 6.9 Baseline characteristics for Gram negative infections by inclusion in the analysis population 192

Table 6.10 Baseline characteristics, by MIC/EUCAST ratio category 195

Table 6.11 Number of drugs given during empiric and definitive epoch 199

Table 6.12 Empiric and definitive drugs given (Approach 1 – clean population) 199

Table 6.13 Treatment timelines (Approach 1 – clean population) 200

Table 6.14 MIC/breakpoint ratio on last day of follow up, by empiric/definitive therapy and survival status (Approach 1 – clean population) 202

Table 6.15 Estimates of association between MIC/breakpoint ratio category and 28-day mortality (Approach 1 – clean population) 203

Table 6.16 Cumulative average MIC/breakpoint ratio on last day of follow up, by empiric/definitive therapy and survival status (Approach 2 – full population) 204

Table 6.17 Estimates of association between MIC/breakpoint ratio category and 28-day mortality (Approach 2 – full population) 205

Table 6.18 Estimates of association between MIC/breakpoint ratio category (susceptible-borderline-resistant) and 28-day mortality (Approach 2 – full population) 205

LIST OF FIGURES

Figure 1.1 Rates of BSI per 100,000 population: 2007 to 2020 5

Figure 1.2 Literature review flowchart 10

Figure 2.1 BSI-FOO Observational study flow of participants..... 19

Figure 3.1 Ward specialty, by day since day zero 46

Figure 3.2 Box plot of average number of staff per 10 beds on day zero, by ward speciality 51

Figure 3.3 Box plot of ward activity per 10 beds on day zero, by ward..... 51

Figure 3.4 Average number of staff per 10 beds, by day since blood sample..... 53

Figure 3.5 Average ward activity per 10 beds, by day since blood sample 53

Figure 3.6 Average number of staff per 10 beds, by day of week 55

Figure 3.7 Average ward activity per 10 beds, by day of week 56

Figure 3.8 Box plot of the difference in average numbers of staff between consecutive days 63

Figure 3.9 Box plot of the difference in ward activity between consecutive days..... 64

Figure 3.10 Box plot of the difference in number of beds between consecutive days..... 65

Figure 3.11 Patient risk score by organism..... 68

Figure 3.12 Patient risk score by ward speciality on day zero..... 68

Figure 3.13 Adjusted Cox model of modifiable risk factors on 28-day mortality..... 71

Figure 3.14 SA1: Sensitivity analysis of primary outcome: stratified by centre 73

Figure 3.15 SA2: Sensitivity analysis of primary outcome: including polymicrobial infections..... 75

Figure 3.16 SA3: Sensitivity analysis of primary outcome: removal of "36-hour rule" in definition of time to appropriate therapy..... 77

Figure 3.17 SA3: Sensitivity analysis of primary outcome: 12-hour (in place of 36-hour) rule in definition of time to appropriate therapy..... 78

Figure 3.18 SA3: Sensitivity analysis of primary outcome: 24-hour (in place of 36-hour) rule in definition of time to appropriate therapy..... 79

Figure 3.19 SA4: Sensitivity analysis of primary outcome, complete case analysis 81

Figure 4.1 Visual illustration of cloning 97
Figure 4.2 Censoring rules using example timelines 99
Figure 4.3 Relationship between severity of infection and probability of censoring and mortality 100
Figure 4.4 Visualisation of time-varying confounding 100
Figure 4.5 Daily temperature, treatment duration and mortality DAG 101
Figure 4.6 Example patient temperature timelines for six patients 105
Figure 4.7 Histogram of imputed values using two methods of imputation 107
Figure 4.8 Duration of therapy flowchart 109
Figure 4.9 Histogram of duration of therapy 117
Figure 4.10 Missing temperature, by centre 118
Figure 4.11 Missing temperature by day since start of treatment 119
Figure 4.12 Missing temperature by day from end of follow-up 119
Figure 4.13 Histogram of temperature on last day of follow-up, imputed *vs* observed 121
Figure 4.14 Duration of therapy, by survival status 127
Figure 4.15 Distribution of the estimated weights 128
Figure 4.16 Kaplan-Meier curve for in-hospital 28-day mortality, by observed duration of therapy 129
Figure 5.1 Diagrams demonstrating confounder, mediator, treatment and outcome relationships 148
Figure 5.2 Emulation the MERINO trial flowchart 152
Figure 5.3 Treatment timelines for BSI-FOO patients allocated to Meropenem 153
Figure 5.4 Treatment timelines for BSI FOO patients allocated to Piperacillin-Tazobactam 154
Figure 5.5 Treatment timelines for RAPIDO patients 155
Figure 5.6 Probability density function of propensity score, by treatment allocation 165
Figure 5.7 Propensity score distribution, by treatment allocation 165
Figure 5.8 Inverse probability weighted Kaplan-Meier, by emulated intervention 166

Figure 5.9 Inverse probability weighted Kaplan-Meier of 14-day mortality, by emulated intervention 168

Figure 6.1 Visualisation of MIC testing for a strain of organism with an MIC of four 175

Figure 6.2 Flowchart for MIC analysis 190

Figure 6.3 MIC/Breakpoint ratio, by empiric/definitive therapy 201

Figure 6.4 Estimates of association between MIC/breakpoint ratio and 28-day mortality, adjusted for risk score and organism (Approach 1 – clean population) 203

Figure 6.5 Estimates of association between MIC/breakpoint ratio and 28-day mortality, adjusted for risk score and organism (Approach 2 – full population) 204

Figure 6.6 Estimates of association between MIC/breakpoint ratio category (susceptible-borderline-resistant) and 28-day mortality (Approach 2 – full population) 206

Figure 6.7 Estimates of association between MIC/breakpoint ratio category (susceptible-borderline-resistant) during first 48hrs post blood culture, and 28-day mortality (Approach 2 – full population) 206

LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
APACHE	Acute Physiologic Assessment and Chronic Health Evaluation
ARL	Antimicrobial Reference Laboratory
BLBLI	β-Lactam/β-lactamase inhibitor
BMI	Body mass index
BP	Blood pressure
BSI	Bloodstream infection
BSI-FOO	Bloodstream Infections - Focus On Outcomes
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CKD	Chronic kidney disease
CLSI	Clinical and Laboratory Standards Institute
COPD	Chronic obstructive pulmonary disease
DOT	Duration of therapy
eGFR	Estimated glomerular filtration rate: a measure of kidney function.
FU	Follow-up
EPV	Events per variable
ESBL	Extended-spectrum beta-lactamase
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
EWS	Early warning score
GLM	Generalised linear models
HCA	Healthcare assistant
HCAI	Healthcare-associated infection
HDU	High dependency unit
HIV	Human immunodeficiency virus
HR	Hazard ratio
ICU	Intensive care unit
IM	Intramuscularly
INR	International normalised ratio
IPCW	Inverse probability censoring weights
IPD	Individual patient data
IPTW	Inverse probability treatment weights
ITB	Immortal time bias

ITU	Intensive therapy unit
IQR	Interquartile range
IV	Intravenously
KM	Kaplan Meier
LOCF	Last observation carried forward
MAR	Missing at random
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant Staphylococcus aureus,
MSSA	Methicillin-susceptible Staphylococcus aureus
NHS	National Health Service
OR	Odds Ratio
PHE	Public health England
PICO	Population, intervention, comparison, outcome
PPI	Patient public involvement
RAPIDO	Randomised controlled trial testing the effect of Rapid Diagnosis on Outcome in bloodstream infection
RCT	Randomised Controlled Trial
RD	Risk difference
RR	Risk ratio
SAB	Staphylococcus aureus bacteraemia
SD	Standard deviation
SIR	Susceptible, intermediate, resistant
SMD	Standardised mean difference
UK	United Kingdom
USA	United States of America
VIF	Variation inflation factor

CHAPTER 1 INTRODUCTION AND BACKGROUND

In this chapter, I will be describing the rationale for this research (section 1.1), give an overview of the types of bloodstream infection analysed in this thesis (section 1.2.1) and a summary of treatment for bloodstream infection (section 1.2.2). I will then discuss the findings of my umbrella review on bloodstream infection and discuss directions for future research (section 1.3).

1.1 Research rationale

The focus of this thesis is to explore the relationship between modifiable risk factors such as healthcare setting and treatment related risk factors, and mortality in patients with a bloodstream infection (BSI). BSI is a major concern in British hospitals with at least 100,000 patients having an episode of BSI each year in England, Wales and Northern Ireland[1], with healthcare associated infections estimated to cost the National Health Service (NHS) just under £1,000 million per year in England[2]. The basic pathogenic causes of BSI are well known from ongoing surveillance programmes, for example Public Health England (PHE) laboratory surveillance reports[1, 3-5]. However, there has been a longstanding lack of United Kingdom (UK) and NHS specific information on factors associated with poor outcomes in patients with a BSI. Non-modifiable risk factors, such as patient comorbidities and infection severity, have been reported on in many global studies and depending on the pathogen involved, underlying patient characteristics, severity of infection and treatment provided, the death rate from these infections can reach 15-25% at 30 days and 50% at 3 years[6-8]. In contrast, the impact of healthcare setting and treatment related factors in this patient population are less well known. Such factors can be considered “modifiable” as there is the potential for human intervention or practices to change the patient exposure to these risk factors.

There have been several studies that have reported on the impact of healthcare setting related risk factors such as staffing and workload on outcome, although these have not been studied in relation to infection outcomes, and none in patients with a BSI in particular[9-11]. Timing and duration of treatment have been shown to be associated with mortality in patients with a BSI, but data are typically from single-centre studies, and information on the estimated size of these effects is limited or biased. Estimating the effect of time to initiation of treatment or duration of treatment on survival is analytically challenging since those who die shortly after the start of follow-up have not had the opportunity to be exposed to either a long time to initiation or a long duration of treatment. This introduces immortal time bias, a bias that arises when there is a period of follow-up in which the outcome, such as death, cannot occur. Studies to date have not adequately addressed this bias

in this patient population and further research is needed to inform potential intervention efforts and NHS guidelines.

This thesis uses data from a UK research programme named Bloodstream infection – Focus On Outcomes (BSI-FOO) to investigate the impact of modifaible risk factors such as healthcare setting and treatment related factors on mortalty. The BSI-FOO research programme consisted of a cohort study to assess the impact of modifiable risk factors on outcomes and a randomised controlled trial (RCT), RAPIDO, comparing two approaches to the identification of the causative microorganism(s). This chapter presents a brief introduction to BSI, treatment for BSI and the rationale for studying this topic.

1.2 Background

1.2.1 Brief overview of bloodstream infections

BSI is a large and growing burden for patients and the healthcare system. About 6% of patients in NHS hospitals develop a healthcare-associated infection (HCAI), that is, infection contracted in hospital or another healthcare setting, and public health bodies continue to reference Plowman's 1999 estimate that "300,000 patients a year in England acquire a healthcare-associated infection as a result of care within the NHS"[2, 12, 13]. The most common BSI in England, Wales and Northern Ireland are *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*[1]. Laboratory based survalence systems in England, Wales and Northern Ireland have been reporting data on BSI to PHE on a voluntary basis since the mid-1970's and the reporting of m*ethicillin-resistant Staphylococcus aureus* (MRSA) became madatory in 2001 and *Escherichia Coli (E. coli)* became madatory in 2011[14]. The mandate was introduded to allow comparison of infection rates between NHS Trusts and allow assessment of the impact of potential intervention efforts that are aimed at reducing infection rates.

The focus of this thesis will be on four specific organisms causing BSI; *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida.* These are described in turn below.

***Escherichia coli* BSI**

E. coli is a species of Gram-negative bacteria and is the most common bacterial cause of BSI in the NHS. In the 2019 financial year, 43,294 cases of *E. coli* bacteraemia were reported by NHS Trusts in England. Of these, about 18% were acquired in hospital[15]. Prior to this, the rate of *E. coli* cases per 100,000 population has risen from 60.4 in 2012 to 77.3 in 2019 (Figure 1.1)[15]. The most common sources of *E. coli* BSI are the urinary tract and gastrointestinal including hepatobiliary[15].

E.coli is often successfully treated with Beta-lactams, aminoglycosides or fluoroquinolones . Suggested duration of treatment is seven days, but for infections with no complications this can be reduced to a short course (e.g. 3 days)[16]. Upper urinary tract infections often require a longer duration of treatment i.e. 10-14 days and are usually initially treated with a broad spectrum antibiotic such as a cephalosporin (e.g. cefuroxime) or a quinolone (e.g. ciprofloxacin) if the patient is severely ill[16].

Many *E. coli* strains have become multidrug-resistant, for example those that produce Extended spectrum β-lactamases (ESBLs). These isolates are resistant to many penicillin and cephalosporin antibiotics. ESBL producing *E.coli* are associated with increased morbidity, mortality, longer hospital stay, and higher health care costs compared with infections caused by non-ESBL producing *E. coli*[17-19]. In this thesis I will include data on both ESBL producing and non-ESBL producing *E. coli.*

***Pseudomonas aeruginosa* BSI**

Pseudomonas aeruginosa (P. aeruginosa) is another Gram-negative bacteria that is often acquired in a hospital setting. The number of reported cases of *P. aeruginosa* in the UK was 4,336 in 2019/2020 of which 1,576 (36.3%) were acquired in hospital[15]. The rate of *P. aeruginosa* bacteraemia in England, Wales and Northern Ireland increased by 15.7% between 2009 and 2017, and by 26.6% (from 6.4 to 8.1 reports per 100,000 population) between 2013 and 2017 (Figure 1.1)[4]. *P. aeruginosa* is associated with infection in many body sites, including pulmonary, urinary, soft tissue and wound[20]. The most frequent source of bacteraemia for *P. aeruginosa* in 2019/2020 was the urinary tract which constituted 30.9% of cases[15].

P. aeruginosa infections are usually treated with drugs such as aminoglycosides, β-lactamase inhibitors, cephalosporins, carbapenems and fluoroquinolones[21].

***Staphylococcus aureus* BSI**

Staphylococcus aurreus (*S. aureus*) are infections caused by bacteria that normally live on people's skin and only cause an infection if they get into the skin, e.g. through a wound. *S. aureus* are usually grouped according to their resistance to methicillin, with those that are resistant to methicillin termed MRSA and those that are susceptible to methicillin termed MSSA.

In the NHS, *S. aureus* is a common cause of BSI and a total of 13,007 cases were reported to PHE in 2019/20, of which 814 (6.3%) were MRSA and 12,193 (93.7%) were MSSA[15]. Of the 814 MRSA bacteraemia reported, 260 (31.9%) were hospital-onset and of the 12,193 MSSA bacteraemia reported, 3,299 (27.1%) were hospital-onset. The rate of MRSA bacteraemia has remained steady at approximately 1.5 cases per 100,000 over the last seven years, but this is a reduction from the rate

of 8.6 per 100,000 population in 2007/08 (Figure 1.1)[15]. In contrast, rates of MSSA increased from a rate of 16.4 cases per 100,000 population in 2011/12 to 21.8 in 2019/20[15].

Skin and soft tissue was one of the most common sources of *Staphylococcus aureus* (31.1% of MRSA cases and 28.2% of MSSA cases in 2019/20). There have been large declines in the percentage of MRSA cases in which the source of bacteraemia was a catheter or line (25.6% of cases in 2007/08, to 12.8% in 2019/20) but this has remained fairly stable at 15% in MSSA bacteraemia. Pneumonia is another common source of *S. aureus* and was the primary focus of 9.3% MRSA and 12.1% MSSA in 2019/20[15].

Flucloxacillin and dicloxacillin are common treatments for the management of MSSA infections, but other treatments such as first generation cephalosporins, clindamycin, and erythromycin can be used in less serious MSSA infections or in patients with penicillin allergies. Multi-resistant MRSA strains are often treated with a combination of two antibiotics to combat the development of resistance which often develops if single agents are used[22]. Thorough guidelines for UK practice have been developed and published by a Working Party on behalf of the British Society for Antimicrobial Chemotherapy, to aid the diagnosis and management of MRSA[23].

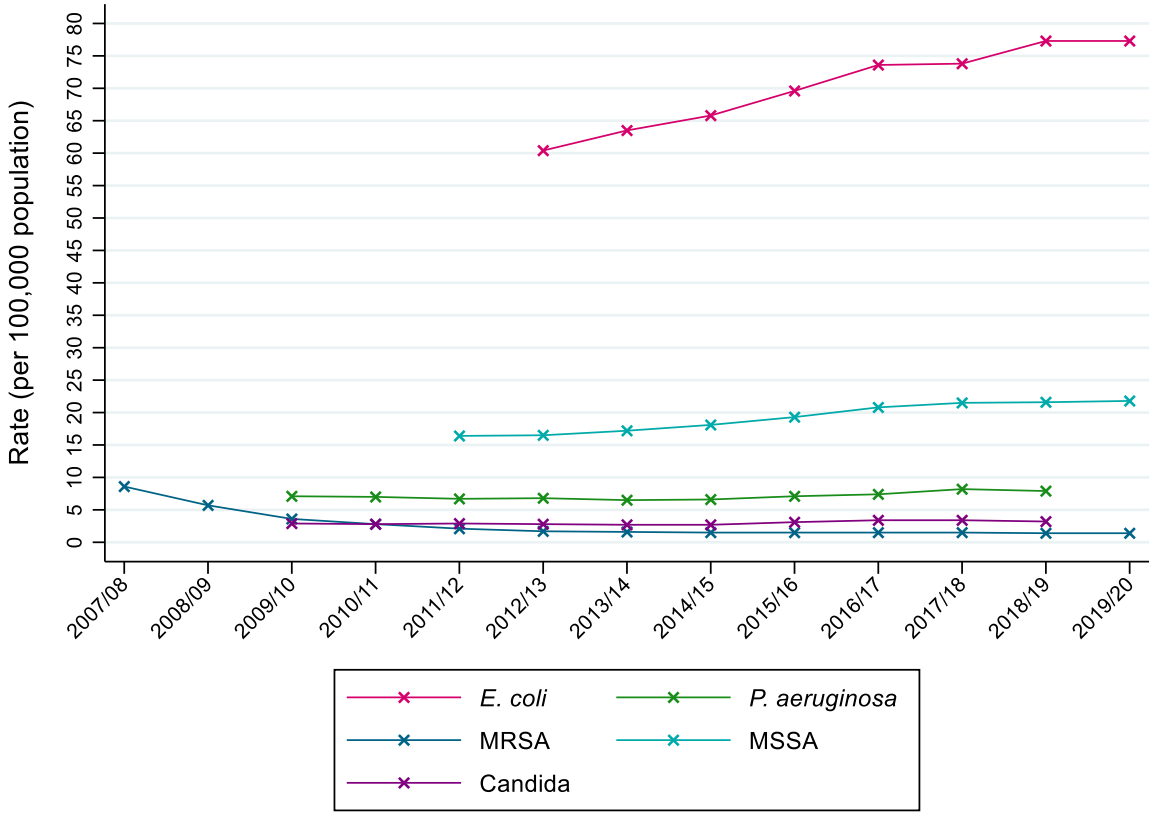
Candida BSI

Candida is a form of fungal infection, often found in the mouth and throat which is commonly known as thrush, but can also be in the bloodstream. *Candida* in the blood is known as candidaemia. *Candida* can enter the blood via various body sites. e.g. urinary tract, intravascular and gastrointestinal[24, 25]. There are a number of species of *Candida*; *C. albicans* are the most common *Candida* species to cause BSI, other less common *Candida* species are *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*[5].

The overall rate of *candidemia* in the UK was 3.3 per 100,000 population in 2018. This has remained relatively constant between 2009 and 2014 with variations between 3.0 to 3.3 per 100,000 population. The rate then increased to 3.6 per 100,000 population in 2016, at which it remained in 2017 and reduced to 3.3 in 2018 (Figure 1.1)[5].

Recommendations for the management of *candidemia* suggest sourcing control, such as removal of central venous lines, and adminstering appropriate antifungal therapy[26]. In terms of antifungal therapy, European and United States guidelines both recommend the initial use of an echinocandin i.e. anidulafungin, micafungin, caspofungin[26, 27]. Amphotericin B is a suggested alternative for cases where there limited availability or resistance to other antifungal agents[21].

Figure 1.1 Rates of BSI per 100,000 population: 2007 to 2020



Abbreviations: *BSI=Bloodstream infection, MRSA=Methicillin-resistant Staphylococcus aureus, MSSA=Methicillin-susceptible Staphylococcus aureus*

1.2.2 Brief overview of BSI treatment

Summary

BSI are mostly treated with antimicrobials which can kill or slow the growth of the bacteria. When BSI is first suspected, the exact form of the organism or its susceptibility to antimicrobials are not known. However, delaying treatment until the blood cultures have been analysed and information on the organism is known (typically two to four days later) could prolong the infection episode and the patient's condition could deteriorate. Therefore, clinicians make their "best guess" of the likely organism involved and its likely antimicrobial susceptibility based on the patient's history and local patterns of resistance for the presumed organism. Patients are then initially treated with a therapy appropriate to the clinicians "best guess" of the patient's diagnosis, this is known as *empirical* therapy. If there is uncertainty around the diagnosis, clinicians can prescribe an antimicrobial agent that is active against a wide range of organisms, these are known as a broad-spectrum agents. Broad-spectrum agents have some disadvantages such as promoting *Clostridium difficile* infection

and encouraging the development of antimicrobial resistance, consequently narrow-spectrum alternatives are used where possible. Once diagnostic results on the blood culture are available, treatment is switched to a targeted narrow-spectrum antimicrobial known to be effective against the organism involved. This is known as *definitive* therapy.

Recommendations on the reviewing and prescribing process known as the "Start Smart – then Focus" approach were published by PHE in 2011 and updated in 2015[28]. The recommendations suggest reviewing and adjusting antimicrobial prescriptions systematically 48–72 hours after the first dose, or sooner if diagnostic information is available. Adjustments include stopping treatment if there is no evidence of infection, changing the treatment to a narrower spectrum antimicrobial if possible, or changing the route of administration (e.g. from intravenous to oral).

Antimicrobial resistance

In order for antibiotics to be effective in killing the bacteria, the bacteria need to be susceptible (non-resistant) to the antibiotic being used. However, bacteria can evolve in such a way that the antibiotic which was previously effective at killing the bacteria becomes less effective or in some cases ineffective. This is known at antimicrobial resistance (AMR).

The treatment of infections with antibiotics can increase drug resistance and overuse of antibiotics could influence the development of AMR, as the antibiotic will kill off the non-resistant bacteria leaving behind the resistant bacteria and the bacteria becomes more resistant as it is passed between people. Over time the resistant bacteria reproduce which creates entire strains of resistant bacteria known as "super bugs", e.g. MRSA. Excessive or unnecessary use of antibiotics, such as for treatment of minor infections that can resolve on their own, can have an impact on the development of "super bugs" so is a major concern for the NHS. In 2019, AMR was listed as one of the top 10 world health challenges[29].

In order to retain effective treatment options against the changing range of infections the Annual Report of the Chief Medical Officer stated that "we need to do two things: First we need to preserve the effectiveness of our existing antimicrobial agents and secondly we need to encourage the development of new agents in the future"[30]. In order to preserve the effectiveness of existing antimicrobial agents, extended and inappropriate use of antibiotics should be discouraged and changes within governmental licencing need to be made to facilitate the rapid approval of antibiotics[31]. Antimicrobial stewardship (AMS) programs have been implemented across the UK with the overall aim to improve the quality and safety of healthcare whilst minimising harm to patients and society including the emergence of AMR. The aim is to do this by improving knowledge and understanding of AMR, improving rapid identification and effective treatment of BSI and minimising

unnecessary use of antibiotics by providing professionals and the public with evidence, and to stimulate the development of new antibiotics[32-34]. This has proven to be successful in reducing Clostridium difficile infection, however, evidence from well conducted and reported research studies is required to provide the program with the evidence required to update treatment guidelines and improve clinical management[35, 36].

Antimicrobial susceptibility testing

In order to identify the most suitable treatment to give to the patient, laboratory test systems are used to determine which therapies are appropriate for the bacteria being tested, in other words, which antibiotics are the bacteria susceptible to. One laboratory technique used to confirm antibiotic susceptibility is the minimum inhibitory concentration (MIC). This is the lowest concentration of drug which prevents visible growth of a bacteria. Several solutions of the antibiotic are prepared at increasing (usually doubling) concentrations and inserted into separate blood cultures. Results are then categorised as susceptible, intermediate or resistant (SIR) to a particular antibiotic by using a cut of point (break points) which are published in guidelines of a reference body such as The European Committee on Antimicrobial Susceptibility Testing (EUCAST)[37].

1.3 Literature review

1.3.1 Search strategy

I conducted a literature search to explore risk factors known to impact outcome in patients with BSI. The purpose of this review was to establish what is currently known in relation to risk factors for adverse outcomes in BSI and to provide a general insight and background to the current research being done. I conducted separate scoping reviews specific to the topic and methodology of each individual chapter and these are summarised in their respective chapters.

A literature review of papers up to 2017 was conducted as part of the BSI-FOO study itself (summarised below in section 1.3.2) so I chose to restrict my review to the period after the study ended to update the current review with more recent research. Due to the nature and requirements of this review and the high number of papers falling under the high-level search term (bacteraemia), I chose to perform an umbrella review (systematic review of reviews) of BSI, to summarise findings from other systematic reviews. Therefore, my search included all recent review papers reporting on bacteraemia.

The search was performed using Medline including articles from 01 Jan 2017 to 13 April 2021. The following search strategy was used to identify articles whose title, abstract or keywords included Bacteraemia, with the search limited to humans and review articles.

The aim of my umbrella review was to provide a comprehensive summary and description of the prevalence of BSI and establish known risk factors reported by individual reviews. Findings from this umbrella review will provide an insight into known risk factors for adverse outcomes in patients with BSI and can be used to identify areas where further research is needed.

1.3.2 Results

Literature prior to January 2017

Studies from across the world have reported on patient-related factors that have been shown to be associated with poor outcomes in patients with a BSI. In a study of over 2,000 episodes of *E. coli* BSI in Canada, significant risk factors for death were increasing age, malignancy, hospital-acquired infection, non-urinary focus and a number of comorbid illnesses[38]. Authors of a further study of 4,758 cases of *E. coli* bacteraemia conducted in Spain, reported that the two main independent risk factors for mortality were the presence of shock and the use of inappropriate antimicrobial chemotherapy[39]. Similarly in a study of community-acquired *E. coli* bacteraemia also conducted in Spain, it was found that a Pitt score (a measure of illness severity) of >1, non-urinary tract source of infection and inappropriate empirical therapy were associated with increased risk of death[40]. *P. aeruginosa* BSI shares many of the same risk factors associated with mortality as other Gram-negative cases of BSI including age, hospitalisation in the intensive care unit, coagulopathy, sepsis and the clinical condition of the patient[41]. Multiple factors influence outcome in *S. aureus* BSI. A review conducted by Sebastian J. van Hal *et al.* reported that age is the most strongly associated with all-cause and infection-related 30-day mortality[42]. The source of infection, comorbidities and the presence of shock at presentation have also been shown to be strong predictors of outcome in a study of 1,692 patients with *S. aureus* bacteraemia (SAB) in Israel[43]. The epidemiology of candidiaemia is different in intensive care units (ICU) and non-ICU patients. For those in ICU, patients are older, more likely to have more comorbidities such as renal failure, previous surgery is more likely and patients are more likely to have central venous catheters and be receiving haemodialysis or other antimicrobials. For those not in ICU, patients are more likely to have cancer, organ transplant or autoimmune disease[44]. In a multi-centre retrospective analysis of 1,392 episodes of candidemia across 22 hospitals in Brazil, older age, corticosteroid treatment and higher Acute Physiologic Assessment and Chronic Health Evaluation II (APACHE II) score were associated with an increased risk of death in ICU patients. Among non-ICU patients, mechanical ventialtion and

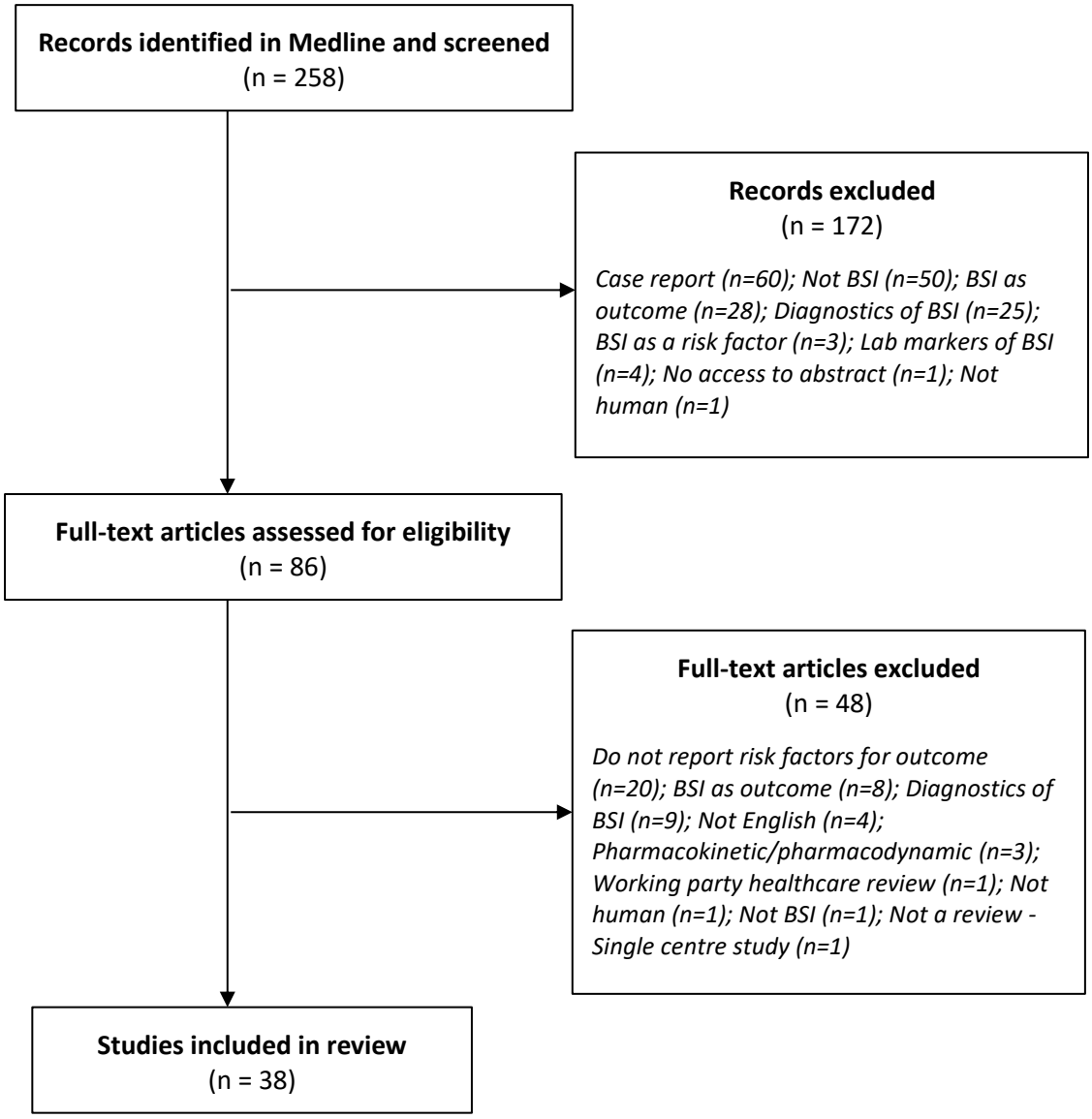
antibiotic treatment were associated with 30-day mortality[44]. In cancer patients, mortality was higher in those with a high Charlson comorbidity index, infection due to *Candida albicans*, hypoalbuminaema, high bilirubin and if no antifungal agents were given[45].

Although there are many studies exploring risk factors associated with patient outcome across the globe, data related to the UK is limited. However, a study including eight UK centres reported that older age, length of hospital stay prior to bacteraemia and unidentified infection focus were independent predictors of in-hospital death[46]. The same study also found significant variation between centres in the management of infection, specifically in the use of oral antibiotics, time to and length of therapy and use of combinations/antimicrobial chemotherapy[46].

Literature update – post January 2017

All relevant, adult, English-language, review papers published from 01 January 2017 to 13 April 2021 were evaluated. Study inclusion/exclusion was initially based on study abstracts only; full text papers of shortlisted abstracts were then reviewed (Figure 1.2). The search identified 258 reviews on BSI of which titles and abstracts were screened, and the full text of 86 articles were considered. In total, 38 reviews were included.

Figure 1.2 Literature review flowchart



Abbreviations: *BSI=Bloodstream infection*

The studies that were included in the literature review update the current research on non-modifiable risk factors, however none reported on modifiable risk factors other than the use of appropriate therapies and the use of different treatment options.

Increasing age was reported to be the "most consistent and strongest predictor" of mortality associated with *S. aureus* in a review of SAB[47]. Other factors that have been found to be associated with detrimental outcome including mortality in SAB are presence of comorbidities, sex, nosocomial acquisition, source of infection, implanted prosthetic device, inadequate choice and timing of the antibiotic treatment and time for blood culture to turn positive[47, 48]. Conversely, in patients in with Pasteurella bacteraemia, age was not found to be associated with mortality in a literature review

and case series conducted in a French hospital. The researchers of this study found that the only factor associated with mortality was a major comorbidity (odds ratio 2.78, 95% CI 1.01 to 7.70; p = 0.04), in a multivariate analysis[49].

One paper described the essentials in the management of *S. aureus* BSI in Germany and stated that patient outcomes can be improved by optimising diagnostic and therapeutic management[50]. Failure to identify a focus of infection was also found to be associated with high mortality in patients with SAB, in a review conducted in the UK. They proposed that improvements in the detection of foci would enable improved source of control and therefore contribute to improved outcomes[51]. However, another study of 247 tunnelled central venous catheters found that it is possible to successfully treat catheter related SAB without removing the tunnelled central venous catheter in haemodialysis patients[52].

Gram-negative BSI shares many of the same risk factors associated with outcome as *S. aureus* BSI including age, comorbidities, hospital acquisition, severity of infection and inadequate therapy[53]. A review investigating outcomes in patients with antibiotic resitant Gram-negative BSI in children with cancer found an increase in mortality in patients with antibiotic resistant BSI compared to non resistant BSI. Death attributed to sepsis occurred in up to 50% of children with ceftazidime resistant *K. pneumoniae*, compared to 13% of those with a susceptible BSI[54]. In addition, a large review of over 6,720 hospital records in the USA found critically ill patients with cancer are at increased risk of healthcare associated infections compared to patients without cancer and because patients with cancer are often immunocompromised, the mortality associated with these infections tends to be more severe than that in the general ICU population[55].

A systematic review including 30 studies of carbapenem-resistant BSI in adult neutropenic patients found that carbapenem-resistance was significantly associated with higher mortality among hospitalised adult patients with bacteraemia due to *K. pneumoniae*. Other variables associated with mortality were age and markers of underlying disease severity[56]. These results were in line with another systematic review looking at outcomes associated with carbapenem-resistant BSI in adult neutropenic patients which concluded that factors associated with mortality included septic shock, unresolved neutropenia, carbapenem-resistance[57]. A meta-analysis of 168 studies on bacterial infections showed a significant association between the use of inappropriate empirical therapy and all-cause mortality (OR 2.88, 95% CI 1.64 to 5.10)[58].

In terms of *Candida* BSI, in addition to severity of infection and early treatment, inflammation at insertion site and abnormal white cell counts have been found to be associated with worse prognosis in catheter related BSI[59].

The literature search also included several review papers focusing on specific treatment options and combinations[60-78]. Although there are a high number of antibiotics that are effective against MRSA BSI, the mortality associated with such infections remains high. A recent review published in 2019 highlighted the necessity of high-quality clinical trials to help identify optimal therapy for these patients[79]. If relevant, the treatment related literature will be reviewed within the chapters of my thesis.

1.3.3 Conclusions

This literature review demonstrates the amount of research that has been undertaken in investigating risk factors of adverse outcomes in patients with BSI, however the risk factors that have been explored to date have focused on non-modifiable risk factors, e.g. patient comorbidities and infection severity, and none have investigated the effect of staffing and workload in this patient population. This review highlights the need for more research in modifiable risk factors such as staffing levels, workload, and treatment related factors in this patient population to inform potential intervention efforts and NHS guidelines.

1.4 Project objective and aims

The primary aim of this thesis is to explore and quantify potential modifiable risk factors of mortality in BSI. Specifically, the following research objectives are addressed in this work:

- Develop a multivariable model to quantify the relationship between healthcare setting and treatment related risk factors associated with death within 28 days of onset of BSI. This model will include the following modifiable risk factors whilst accounting for relevant non-modifiable risk factors:
    - Ward specialty
    - Staffing levels
    - Ward activity (number of admissions/discharges)
    - Movements between and within wards
    - Antibiotic use (particularly the timing of appropriate therapy)
    - Use of intravenous lines and catheters
- Investigate whether duration of therapy is associated with 28-day mortality in patients with *S. aureus* infection, using epidemiological techniques to account for immortal time bias.
- Implement trial emulation methods to compare treatment with Piperacillin-Tazobactam compared to Meropenem on 28-day mortality in patients a BSI with *E. coli* or *Klebsiella* spp.

and to understand differences between a recently published randomised control trial (RCT) – the MERINO trial - and other published observational studies comparing these treatments.

- Explore the association between MIC and 28-day mortality in patients with a Gram-negative infection.

CHAPTER 2 INTRODUCTION TO THE DATA

In this chapter, I will give an overview of the data sources used throughout this thesis. I will be describing the study design, the data collected and population characteristics for the two studies from which data are used, namely: Bloodstream Infections – Focus on Outcomes observational study (section 2.2) and RAPIDO, a randomised controlled trial (section 2.3).

2.1 Introduction

Throughout this thesis I use data from the Bloodstream Infections – Focus on Outcomes (BSI-FOO) research programme. The programme consisted of two main studies: 1) a cohort study to assess the impact modifiable risk factors have on outcomes (BSI-FOO observational study) and 2) a multicentre open parallel group (1:1) randomised controlled trial comparing two approaches to the identification of the causative microorganism(s) of bloodstream infection (RAPIDO). The two studies used similar data collection methods allowing the two data sources to be easily combined.

This chapter presents a sumary of the study design and participant population of both studies.

2.2 BSI-FOO observational study

2.2.1 Study Design

The BSI-FOO observational study is a multicentre prospective cohort study designed to quantify modifiable risk factors for 28-day mortality in bloodstream infections[80]. The study included patients who had a BSI between November 2010 to May 2012 across five NHS acute hospital trusts in England and Wales and included BSI caused by six key pathogens: 1) MRSA; 2) MSSA; 3) non-ESBL-producing *Escherichia coli*; 4) ESBL-producing Enterobacterales; 5) *Pseudomonas aeruginosa*; 6) *Candida species*. Data were collected from routine investigations and tests and performed according to usual clinical practice i.e. the only individual patient data used was that routinely collected to support clinical care. The National Information Governance Board approved the use of such routinely collected data without individual patient consent under section 251 of the NHS Act 2006. It was important to have the approval to run the study without individual consent as seeking consent could introduce selection bias and limit generalisability and could also impose an unnecessary additional burden on patients at a time of acute illness.

Patients and eligibility

The study included adult patients (≥18 years old) receiving in-patient NHS hospital care and having a clinically significant BSI with an organism in one or more of the six key pathogen groups described

above. Non-ESBL-producing *E. coli* is one of the most prevalent pathogens of BSI in the UK, so to ensure all pathogen groups were adequately represented, a random sample of one-third of infection episodes caused by non-ESBL-producing *E. coli* and all infection episodes caused by the other five key pathogens were included. Excluded patients were those with human immunodeficiency virus infection, cystic fibrosis, on an end-of-life care pathway when the blood sample was taken, in the custody of Her Majesty's Prison Service of England or Wales, discharged on the day the sample was taken, with notes irretrievably missing or generalised refusal of consent. Multiple infection episodes per patient may be included if a patient experiences more than one infection episode during the study recruitment period, providing the blood sample of the subsequent infection episode was taken more than 14 days after their first positive sample containing the same organism and more than 28 days after the start of the preceding episode (whatever the organism). Such cases are referred to as repeat episodes and although they are included in the study, only the first infection episode per patient was included in the analysis.

2.2.2 Data collected

Timing of collection

An episode of infection began when the first positive blood sample confirming BSI i.e. diagnostic blood sample was taken. This was defined as day/time 0. Participants were followed up for 28 days from the date the diagnostic blood sample was taken. Data collection continued from day 0 until day 28, or discharge or death if earlier. Some items such as comorbidity data and temperature measurements were also collected for the 7-day period leading up to the confirmation of BSI. Different types of data were collected for different periods as summarised in Table 2.1.

Table 2.1 BSI-FOO Observational study schedule of data collection

Data type	Days -7 to -1	Day 0	Days 1 to 7	Days 8 to 28
Eligibility		✓[a]		
Positive blood culture: organism, timings, susceptibility		✓		
Hospital admission data [c]	✓	✓		
Participant characteristics, medical history, comorbidities [c]	✓	✓		
Infection severity markers, probable source of infection	✓	✓	✓	✓
Vascular lines, urinary catheters	✓[b]	✓	✓[b]	✓[b]
Early warning score	✓	✓		
Maximum and minimum daily temperature	✓	✓	✓	✓
Previous (negative) blood cultures	✓			
Antimicrobial therapy		✓	✓	✓
Ward level care: speciality, staffing, activity		✓	✓	

Notes: *[a] Eligibility data collected for all patients with a BSI at a participating centre during the study recruitment period; other data collected only for eligible participants.*

[b] Lines, catheters, infected foreign bodies and abscesses present at time 0 were recorded; their insertion and removal dates were also noted so presence/absence on other dates could be inferred.
[c] Hospital admission and records of medical history/comorbidity could be before day -7.

Medical history and outcome

Data on participants' health and care up to the start of the infection episode included participant demography and characteristics (age, gender, weight and height), hospital admission data (date admitted and prior residence in nursing or care home), recent medical history, long-term comorbidities, measures of illness severity at or shortly before time 0 (temperature and other clinical signs, blood test results and medical interventions), and speciality of consultant at time 0. Maximum temperature and early warning score were also noted for up to seven days leading up to day 0.

Probable sources of the bloodstream infections were recorded using The Centres for Disease Control and Prevention (CDC) criteria for infection at specific sites[81]. Dates of insertion and dates and times of removal were noted for any vascular lines, urinary catheters or infected foreign bodies present at time 0; dates and times of drainage were noted similarly for abscesses.

Dates of death, discharge and *C. difficile* infection were recorded from day 0 up to day 28. Maximum temperatures were recorded twice daily (a.m. and p.m.) while participants remained in hospital. Participant and organism data collected are given in Table 2.2.

Table 2.2 Participant level data collected in BSI-FOO observational study

Type	Factors
Organisational	Centre
	Admission from nursing or care home
	Length of in-patient stay, prior to day 0 (days)
	Speciality of consultant at time 0
Organism / infection	Organism identity (target organism group)
	Source of infection (CDC criteria)
Patient measures	Age
	Gender
	Height (cm)
	Weight (kg)
Medical history (up to day 0)	Leukaemia within 5 years before day 0
	Lymphoma within 5 years before day 0
	Solid tumour within 5 years before day 0
	Any other (second) tumour within 5 years before day 0
	Chemotherapy in month before day 0
	Surgery requiring overnight stay within 7 days before day 0
	Burn requiring hospital admission within 7 days before day 0

Type	Factors
	Cardiac arrest within 7 days before day 0
	Myocardial infarction, symptomatic within 7 days before day 0
	Renal support within 7 days before day 0
Comorbidities ongoing at day 0	Ascites
	Diabetes without organ damage
	Diabetes with organ damage
	Chronic obstructive pulmonary disease
	Congestive heart failure
	Connective tissue disease
	Cerebrovascular disease
	Dementia
	Hemiplegia
	Peptic ulcer disease
	Peripheral vascular disease
	Abscess at time 0
	Infected foreign body (non-surgical) at time 0
	Infected prosthesis or similar surgical item at time 0
Infection severity measures at or nearest before time 0	Mental Disorientation (scale 0-4) at time 0
	Temperature (°C) at time 0
	Systolic blood pressure (mmHg) at time 0
	Early warning score at time 0
	INR[a] at time 0, or nearest within 7 days before
	eGFR[b] (mL/min/1.73 m^2) at time 0, or nearest within 7 days before
	Serum albumin (g/L) at time 0, or nearest within 7 days before
	Bilirubin (total, micromol/L) at time 0, or nearest within 7 days before
	Neutrophil count ($\times 10^9$/L) at time 0, or nearest within 7 days before
	Receiving intravenous fluids on day 0, at or before time 0
	Receiving artificial ventilation on day 0, at or before time 0
	Receiving vasopressor drugs on day 0, at or before time 0
	Received systemic corticosteroids in 24 hours before time 0

Abbreviations: *CDC= Centers for Disease Control and Prevention, eGFR=Estimated glomerular filtration rate, INR=International normalised ratio*

Antimicrobial therapy

All potentially relevant antimicrobial prescriptions were recorded from day 0 until day 28 or discharge or death if earlier. Data included: antimicrobial name, prescribed dose, route and frequency of administration, the number of doses actually taken each day, and the date and time of the first and last dose taken.

Care environment data

Information on ward speciality, staffing and activity was collected at ward level from day 0 to 7, for the ward where the participant spent most of their day. The number of occupied beds was counted

at the end of the day (23:59), and the number of admissions, discharges, and day case (non-admitted) participants was recorded for the 24 hours leading up to that time (i.e. midnight to midnight). The number of healthcare assistants, Trust-employed nurses and agency nurses on duty was recorded for each of the three shifts (early, late and night-shift); the night-shift was that at the end of the day concerned, running into the following morning. Ward speciality and total number of beds were also recorded.

Microbiological test data

The identities of up to four organisms were recorded together with the date and time that samples had been put on the blood culture machine. All available local antimicrobial susceptibility results for the organisms were extracted from local laboratory systems.

Additional centralised testing

Microbial isolates were stored frozen at each centre and later supplied to the Antimicrobial Reference Laboratory (ARL) at North Bristol NHS Trust for more specialised testing. The additional tests were completed at the end of the study period and had no influence on the participants' clinical care. Target bacteria (*S. aureus*, *P. aeruginosa* and Enterobacteriaceae, as identified by the study centres) were tested at the ARL by measurement of minimum inhibitory concentrations (MIC) of a range of antimicrobial agents the Clinical and Laboratory Standards Institute (CLSI) M7-A8 agar dilution method with Mueller Hinton agar[82].

S. aureus isolates were tested against cefoxitin, ciprofloxacin, daptomycin, erythromycin, fusidic acid, gentamicin, linezolid, rifampicin, teicoplanin and vancomycin. Enterobacteriaceae isolates were tested against amoxicillin/clavulanate, ampicillin, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, meropenem, and piperacillin/tazobactam. *P. aeruginosa* isolates were tested against ceftazidime, ciprofloxacin, colistin, gentamicin, meropenem, and piperacillin/tazobactam. A doubling dilution series was used for all antimicrobials except daptomycin, vancomycin and teicoplanin, which were tested using a 0.25 mg/L step dilution series.

Candida isolates were tested at the Mycology Reference Laboratory of the Statens Serum Institut (Copenhagen, Denmark). MICs for micafungin, anidulafungin, fluconazole, voriconazole and isavuconazole were measured by the EUCAST Edef 7.2 broth dilution method [83] and MICs for amphotericin and caspofungin were measured by Etest.

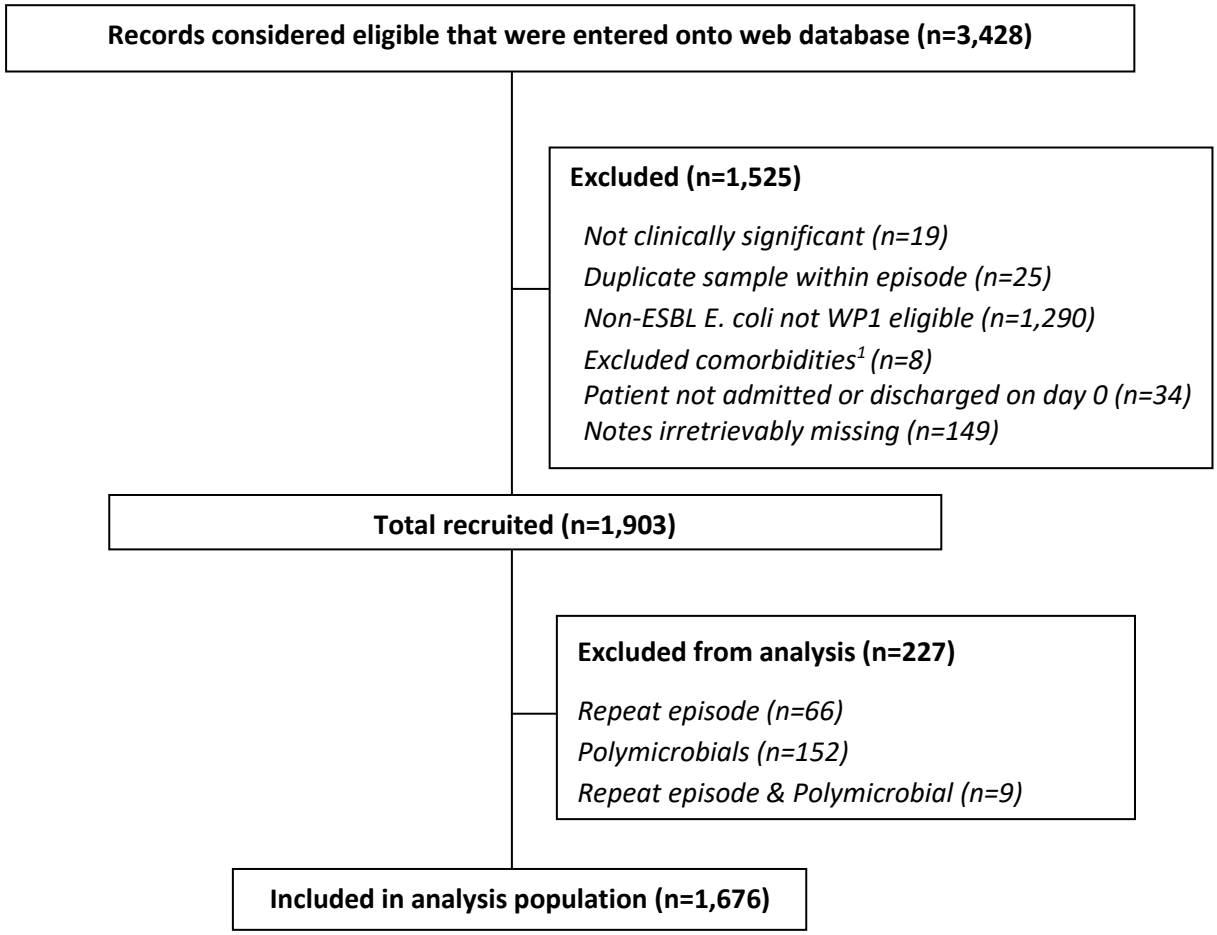
2.2.3 Population characteristics

Participant population

Data was provided for 3,428 episodes of infection from patients over 18 years old who had a bloodstream infection with one of the key organisms and did not meet any of the exclusion criteria (Figure 2.1).

A total of 1,525 episodes were excluded after being entered onto the database due to ineligibility. Therefore 1,903 eligible infection episodes (1,828 patients) were recruited. A total of 227 repeat and/or polymicrobial episodes were excluded from the analysis population (66 repeat, 152 polymicrobial, nine that were both repeat and polymicrobial) and therefore the analysis population consisted of 1,676 participants.

Figure 2.1 BSI-FOO Observational study flow of participants



***Notes**:[1] Excluded comorbidities: Having Cystic Fibrosis, Human Immunodeficiency Virus (HIV) positive, Patients on the end of life pathway*
***Abbreviations**: ESBL= Extended Spectrum Beta-Lactamase*

Organism details

Of the 1,676 participants included in the analysis population, the most common organism was non-ESBL-producing *E. coli* (32.3%) and least common was MRSA (6.0%), see Table 2.3. There were slight differences in the distribution of organisms across the centres. The most common organism in Bristol and Newcastle was MSSA (41.7% and 31.1%, respectively), whereas Cardiff, Leeds and London all had a higher proportion of non-ESBL-producing *E. coli* compared to other organisms (32.7%, 37.1% and 31.2%, respectively).

Table 2.3 BSI-FOO Observational study - numbers of organisms by centre

Centre	Non-ESBL *E. coli*	ESBL producer	Candida	MRSA	MSSA	P. aeruginosa	Total
Bristol	61 (30.7%)	9 (4.5%)	10 (5.0%)	17 (8.5%)	83 (41.7%)	19 (9.5%)	199
Cardiff	91 (32.7%)	37 (13.3%)	13 (4.7%)	24 (8.6%)	87 (31.3%)	26 (9.4%)	278
Leeds	218 (37.1%)	55 (9.4%)	33 (5.6%)	39 (6.6%)	173 (29.4%)	70 (11.9%)	588
London	72 (31.2%)	28 (12.1 %)	20 (8.7%)	9 (3.9%)	52 (22.5%)	50 (21.6%)	231
Newcastle	100 (26.3 %)	39 (10.3%)	40 (10.5%)	11 (2.9%)	118 (31.1%)	72 (18.9%)	380
Total	**542**	**168**	**116**	**100**	**513**	**237**	**1676**

Notes: *Data are presented as n (%)*

Abbreviations: *ESBL= Extended Spectrum Beta-Lactamase, MRSA=Methicillin-resistant Staphylococcus aureus, MSSA=Methicillin-susceptible Staphylococcus aureus*

Further details of the identity of the organisms for ESBL producers and *Candida* are given in Table 2.4 below. The majority of ESBL producers were *Escherichia coli* (88.7%) and the most common species of *Candida* were *C. albicans* and *C. glabrata* (46.6% and 33.6%, respectively).

Table 2.4 Further details of identity for ESBL producers and *Candida*

Identity of target organism	n (%)
ESBL producer:	
Escherichia coli	149 (88.7%)
Enterobacter spp.	3 (1.8%)
Klebsiella spp.	15 (8.9%)
Other ESBL producer	1 (0.6%)
Candida:	
Candida albicans	54 (46.6%)
Candida dubliniensis	2 (1.7%)
Candida glabrata	39 (33.6%)
Candida guilliermondii	2 (1.7%)
Candida krusei	1 (0.9%)
Candida lusitaniae	3 (2.6%)
Candida parapsilosis	9 (7.8%)
Candida tropicalis	5 (4.3%)
Candida spp.	1 (0.9%)

Abbreviations: *ESBL= Extended Spectrum Beta-Lactamase*

Participant demographics

Participant measures, medical history, infection severity measures, participant comorbidities at date 0, source of infection and organisational factors are given in Table 2.5*,* by survival status.

The median age on day 0 was 68.5 years (IQR 53.0 to 80.0), and this was higher in participants who died (74.0 years; IQR 62.0 to 83.5 *vs* 67.0 years; IQR 51.0 to 70.0). Approximately 55% of the participants were male and this was well balanced between surviving participants and those who died. The mean body mass index (BMI) was 26.0 kg/m^2 (SD 6.6) and this was slightly higher in participants who survived (26.1 kg/m^2; SD 6.7 *vs* 25.3 kg/m^2; SD 5.9).

Just over a third of participants had a tumour within the last five years and tumours were more prevalent in participants who died compared to those who survived (44.8% *vs* 31.6%).

Renal support in the past week was higher (12.6% *vs* 6.1%) and the prevalence of abscesses at time 0 lower (3.5% *vs* 7.2%) in participants who died. All other medical history variables were broadly similar between participants who died and those who survived. In terms of the indicators of infection severity, the mean temperature at day/time 0 (time of blood culture) was 38.1 °C (SD 1.1) and this was higher in surviving participants compared to those who died (38.2 °C; SD 1.1 *vs* 37.7 °C; SD 1.2), where a normal temperature is generally considered to be 37°C. The median estimated glomerular filtration rate (eGFR) was 62.0 ml/min/1.73 m^2 (IQR 1.1 to 1.5) and the mean serum albumin was 31.5 g/L (SD 7.9). eGFR is a measure of kidney function, where a higher number indicates better kidney function, with values over 60 consider normal kidney function. Serum albumin is a measure of albumin in the blood which can be used to measure liver function, the concentration of albumin in the blood reduces when the liver is severely damaged. Both measures were higher in participants who survived compared to those who died, which suggests survivors had better kidney and liver function. Neutrophil count, INR and bilirubin were similar in participants who died and who survived. The mean systolic blood pressure was 121.9 mmHg (SD 26.8) and this was higher in participants who survived compared to those who died. 37% of participants were on intravenous fluids, 9% on ventilation and 6% on vasopressor drugs at day 0; all were more prevalent for participants who died compared to those who survived. Systemic corticosteroids (anti-inflammatory treatment) were taken by 14% of participants in the previous 24 hours, with a higher proportion of participants who died taking them compared to those who survived (23.3% *vs* 11.3%).

All comorbidities were more prevalent in the participants who died with the exception of connective tissue disease, the rate of which was similar in those who survived and those who died (8.8% *vs* 8.6%). Diabetes had the highest overall prevalence (21.3%) compared to all other comorbidities; 16.3% had diabetes without organ damage and 5% with organ damage. The median Child-Pugh[84]

(measure of severity of liver disease) score was 7.0 (IQR 6.0 to 8.0), where scores of 5-6 indicate least severe liver disease, 7-9 moderate liver disease and 10-15 most severe liver diseases. The Charlson score is comorbidity score based on the adjusted risk of mortality for a range of comorbidities where a score of zero indicates that no comorbidities were found and higher scores indicate a larger presence of comorbidities associated with predicted 10-year mortality [85]. The median Charlson score was 3.0 (IQR 2.0 to 4.0), and this was similar in participants who survived and those who died. Early warning score is a score based on six measurements that are routinely measured (respiratory rate; oxygen levels; temperature; SBP; heart rate and level of consciousness), to help identify acutely ill patients, including those with sepsis[86]. Higher scores indicate a higher clinical risk and need for medical review and possible intervention. The proportion of participants with an early warning score >3 was 34.1% and this was slightly lower in participants who survived (31.9% *vs* 42.8%). The source of infection varied between participants who survived and those who died, with lower respiratory tract infections more common in participants who died (16.4% *vs* 4.6%) and urinary tract infections more common in survivors (29.1% *vs* 17.5%). The median length of prior hospital stay was 1.0 days (IQR 0.0 to 11.0) and was greater in participants who died (5.0 days; IQR 0.0 to 14.0 *vs* 1.0 days; IQR 0.0 to 10.0). Just under half of participants had a hospital-acquired infection (44.7%) and this was higher in participants who died (57.8% *vs* 41.3%). Finally, the proportion of participants with an assigned consultant in major surgery on day 0 was higher in those who survived compared to those who died (29.2% *vs* 18.0%). Conversely, the proportions of participants with an assigned consultant in medicine or high-dependency medicine were slightly lower in participants who survived compared to those who died (45.9% *vs* 51.4% and 16.6% *vs* 24.0% respectively).

Table 2.5 BSI-FOO Observational study - patient demography/non modifiable risk factors

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Patient measures			
Age	67.0 (51.0, 79.0)	74.0 (62.0, 83.5)	68.5 (53.0, 80.0)
Male	728/1328 (54.8%)	191/348 (54.9%)	919/1676 (54.8%)
Height (cm) [a]	168.4 (10.3)	167.5 (10.0)	168.2 (10.3)
Weight (kg) [b]	74.6 (20.5)	69.9 (18.9)	73.7 (20.3)
Body Mass Index [c]	26.1 (6.7)	25.3 (5.9)	26.0 (6.6)
Medical history			
Chemotherapy in month before date 0	201/1328 (15.1%)	57/348 (16.4%)	258/1676 (15.4%)
Any tumour within last 5 years	419/1328 (31.6%)	156/348 (44.8%)	575/1676 (34.3%)
Leukaemia within last 5 years	90/1328 (6.8%)	34/348 (9.8%)	124/1676 (7.4%)
Lymphoma within last 5 years	75/1328 (5.6%)	20/348 (5.7%)	95/1676 (5.7%)
Solid tumour within last 5 years	258/1328 (19.4%)	101/348 (29.0%)	359/1676 (21.4%)
Any other tumour within last 5 years	60/1327 (4.5%)	22/348 (6.3%)	82/1675 (4.9%)
Surgery requiring overnight stay within 7 days before date 0	118/1327 (8.9%)	34/348 (9.8%)	152/1675 (9.1%)
If yes:			
Elective surgery	77/118 (65.3%)	14/34 (41.2%)	91/152 (59.9%)
Surgical Speciality			
Cardiothoracic surgery	13/118 (11.0%)	4/34 (11.8%)	17/152 (11.2%)
General surgery	59/118 (50.0%)	19/34 (55.9%)	78/152 (51.3%)
Neurosurgery	14/118 (11.9%)	6/34 (17.6%)	20/152 (13.2%)
Plastic surgery	2/118 (1.7%)	2/34 (5.9%)	4/152 (2.6%)
Trauma & orthopaedics	8/118 (6.8%)	0/34 (0.0%)	8/152 (5.3%)
Urology	16/118 (13.6%)	3/34 (8.8%)	19/152 (12.5%)
Ear nose & throat	2/118 (1.7%)	0/34 (0.0%)	2/152 (1.3%)
Oral & maxillo facial surgery	1/118 (0.8%)	0/34 (0.0%)	1/152 (0.7%)
Obstetrics and gynaecology	3/118 (2.5%)	0/34 (0.0%)	3/152 (2.0%)
Body area of surgery			

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Superficial	4/117 (3.4%)	3/34 (8.8%)	7/151 (4.6%)
Head & neck	17/117 (14.5%)	6/34 (17.6%)	23/151 (15.2%)
Upper limbs	4/117 (3.4%)	0/34 (0.0%)	4/151 (2.6%)
Lower limbs	7/117 (6.0%)	2/34 (5.9%)	9/151 (6.0%)
Thoracic cavity	16/117 (13.7%)	4/34 (11.8%)	20/151 (13.2%)
Abdominal cavity	69/117 (59.0%)	19/34 (55.9%)	88/151 (58.3%)
Burn requiring admission within 7 days before date 0	3/1326 (0.2%)	1/347 (0.3%)	4/1673 (0.2%)
Cardiac arrest within 7 days before date 0	5/1328 (0.4%)	5/348 (1.4%)	10/1676 (0.6%)
Renal support within 7 days before date 0	81/1328 (6.1%)	44/348 (12.6%)	125/1676 (7.5%)
Myocardial infarction within 7 days before date 0	128/1328 (9.6%)	44/348 (12.6%)	172/1676 (10.3%)
Infection severity measures			
Mental Disorientation:			
None	1113/1327 (83.9%)	257/348 (73.9%)	1370/1675 (81.8%)
Grade I	66/1327 (5.0%)	20/348 (5.7%)	86/1675 (5.1%)
Grade II	86/1327 (6.5%)	42/348 (12.1%)	128/1675 (7.6%)
Grade III	54/1327 (4.1%)	20/348 (5.7%)	74/1675 (4.4%)
Grade IV	8/1327 (0.6%)	9/348 (2.6%)	17/1675 (1.0%)
Temperature (°C) at time 0 [d]	38.2 (1.0)	37.7 (1.2)	38.1 (1.1)
INR [e]	1.2 (1.1, 1.4)	1.2 (1.1, 1.6)	1.2 (1.1, 1.5)
eGFR (mL/min/1.73m^2) [f]	65.0 (37.0, 90.0)	52.5 (26.5, 84.0)	62.0 (35.0, 90.0)
Serum Albumin (g/L) [g]	32.6 (7.5)	27.2 (7.9)	31.5 (7.9)
Bilirubin total (micromol /L) [h]	12.0 (7.0, 20.5)	13.0 (8.0, 23.0)	12.0 (8.0, 21.0)
Neutrophil count at day 0 or closest (x10^9/L) [i]	9.3 (5.4, 13.8)	10.2 (4.8, 15.3)	9.5 (5.3, 14.1)
Systolic BP at day 0 or closest (mmHg) [j]	122.9 (26.2)	117.9 (28.7)	121.9 (26.8)
On IV fluids at day 0	450/1324 (34.0%)	165/348 (47.4%)	615/1672 (36.8%)
On ventilation at day 0	90/1323 (6.8%)	66/348 (19.0%)	156/1671 (9.3%)
On vasopressor drugs at day 0	60/1327 (4.5%)	48/348 (13.8%)	108/1675 (6.4%)
Systemic corticosteroids in last 24 hours	149/1324 (11.3%)	81/347 (23.3%)	230/1671 (13.8%)

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
EWS score nearest to day 0			
≤ 3	468/687 (68.1%)	99/173 (57.2%)	567/860 (65.9%)
>3	219/687 (31.9%)	74/173 (42.8%)	293/860 (34.1%)
Comorbidities at date 0			
Congestive heart failure	151/1328 (11.4%)	61/348 (17.5%)	212/1676 (12.6%)
Peripheral vascular disease	103/1328 (7.8%)	43/348 (12.4%)	146/1676 (8.7%)
Cerebrovascular disease	198/1328 (14.9%)	74/348 (21.3%)	272/1676 (16.2%)
Hemiplegia	50/1328 (3.8%)	18/348 (5.2%)	68/1676 (4.1%)
Dementia	99/1327 (7.5%)	39/348 (11.2%)	138/1675 (8.2%)
COPD	160/1327 (12.1%)	57/348 (16.4%)	217/1675 (13.0%)
Connective tissue disease	117/1328 (8.8%)	30/348 (8.6%)	147/1676 (8.8%)
Peptic ulcer disease	86/1328 (6.5%)	31/348 (8.9%)	117/1676 (7.0%)
Ascites	48/1328 (3.6%)	32/348 (9.2%)	80/1676 (4.8%)
Diabetes:			
None	1052/1328 (79.2%)	267/348 (76.7%)	1319/1676 (78.7%)
Without organ damage	212/1328 (16.0%)	61/348 (17.5%)	273/1676 (16.3%)
With organ damage	64/1328 (4.8%)	20/348 (5.7%)	84/1676 (5.0%)
Child-Pugh score [k]	6.0 (6.0, 7.0)	7.0 (6.0, 8.0)	7.0 (6.0, 8.0)
Charlson score [l]	3.0 (2.0, 4.0)	4.0 (2.0, 5.0)	3.0 (2.0, 4.0)
Abscess at time 0	96/1327 (7.2%)	12/347 (3.5%)	108/1674 (6.5%)
Infected foreign body at time 0	16/1327 (1.2%)	3/347 (0.9%)	19/1674 (1.1%)
Surgical prosthesis time 0	19/1327 (1.4%)	3/347 (0.9%)	22/1674 (1.3%)
Source of infection			
Bone and joint	59/1327 (4.4%)	6/348 (1.7%)	65/1675 (3.9%)
Cardiovascular system	25/1327 (1.9%)	5/348 (1.4%)	30/1675 (1.8%)
Central nervous system	9/1327 (0.7%)	0/348 (0.0%)	9/1675 (0.5%)
Eye, ear, nose, throat or mouth	3/1327 (0.2%)	1/348 (0.3%)	4/1675 (0.2%)
Gastrointestinal system	134/1327 (10.1%)	16/348 (4.6%)	150/1675 (9.0%)

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Line infection – central venous line	123/1327 (9.3%)	15/348 (4.3%)	138/1675 (8.2%)
Line infection – peripheral venous line	20/1327 (1.5%)	7/348 (2.0%)	27/1675 (1.6%)
Lower respiratory tract	61/1327 (4.6%)	57/348 (16.4%)	118/1675 (7.0%)
Reproductive tract	9/1327 (0.7%)	2/348 (0.6%)	11/1675 (0.7%)
Skin and soft tissue	98/1327 (7.4%)	20/348 (5.7%)	118/1675 (7.0%)
Surgical site infection	37/1327 (2.8%)	4/348 (1.1%)	41/1675 (2.4%)
Systemic Infection	10/1327 (0.8%)	8/348 (2.3%)	18/1675 (1.1%)
Urinary tract infection	386/1327 (29.1%)	61/348 (17.5%)	447/1675 (26.7%)
Site uncertain	353/1327 (26.6%)	146/348 (42.0%)	499/1675 (29.8%)
Organisational factors			
Admission from nursing home (Y)	97/1327 (7.3%)	40/348 (11.5%)	137/1675 (8.2%)
Length of prior hospital stay (days)	1.0 (0.0, 10.0)	5.0 (0.0, 14.0)	1.0 (0.0, 11.0)
Hospital or community acquired infection			
Hospital	548/1328 (41.3%)	201/348 (57.8%)	749/1676 (44.7%)
Community	780/1328 (58.7%)	147/348 (42.2%)	927/1676 (55.3%)
Speciality of consultant on day 0:			
Medicine	559/1217 (45.9%)	171/333 (51.4%)	730/1550 (47.1%)
High dependency medicine	202/1217 (16.6%)	80/333 (24.0%)	282/1550 (18.2%)
Major surgery	355/1217 (29.2%)	60/333 (18.0%)	415/1550 (26.8%)
Minor surgery	9/1217 (0.7%)	3/333 (0.9%)	12/1550 (0.8%)
Other	92/1217 (7.6%)	19/333 (5.7%)	111/1550 (7.2%)

Notes: *Data are presented as median (IQR), mean (SD) or n (%)*

[a] *Data missing for 761 participants (578 survived, 183 died)*

[b] *Data missing for 490 participants (357 survived, 133 died)*

[c] *Data missing for 799 participants (604 survived, 195 died)*

[d] *Data missing for 30 participants (20 survived, 10 died)*

[e] *Data missing for 1011 participants (815 survived, 196 died)*

[f] *Data missing for 118 participants (98 survived, 20 died)*

[g] *Data missing for 200 participants (161 survived, 39 died)*

[h] *Data missing for 267 participants (216 survived, 51 died)*

[i] *Data missing for 139 participants (110 survived, 29 died)*

[j] *Data missing for 246 participants (196 survived, 50 died)*

[k] *Data missing for 1075 participants (867 survived, 208 died)*

[l] *Data missing for 377 participants (299 survived, 78 died)*

Abbreviations: *BP=Blood pressure, COPD=Chronic obstructive pulmonary disease, eGFR=Estimated glomerular filtration rate, EWS=Early warning score, INR=International normalised ratio, IV= Intravenous*

2.3 RAPIDO

2.3.1 Study Design

Increasing the speed and improving the accuracy of infection diagnostics can help improve the management of infection as well as reducing the adverse effects of inappropriate antimicrobial use[87]. Conventional diagnostics may take 24–60 hours to confirm the diagnosis of BSI and provide antimicrobial susceptibility results, hence the use of a rapid diagnostic in the laboratory may provide information to clinical staff more quickly impacting on clinical care and improving outcomes. RAPIDO was designed with the objective to compare conventional diagnostic methods with microbial identification by MALDI-TOF (a rapid diagnosis technique) with the primary outcome of 28-day survival. RAPIDO was a multicentre open parallel-group (1:1) randomised controlled trial in hospitalised adult patients with an episode of BSI at seven centres in England and Wales[87]. It aimed to compare two approaches to the identification of the causative microorganism(s) of bloodstream: i) MALDI-TOF spectrometry in addition to conventional microbiological culture ("RAPIDO" arm) and ii) conventional culture only ("Conventional" arm). Patients were recruited and randomised between 30th July 2012 and 31st August 2014. The primary outcome was 28-day survival.

In addition to the centres taking part in BSI-FOO observational study, RAPIDO included two additional centres Whittington Health NHS Trust and Plymouth.

It was essential that patients were randomised promptly when the machine flagged positive so that rapid diagnosis could begin immediately. Therefore, randomisation preceded consent and enrolment. Patients were approached for consent when they and clinical staff judged that they had recovered enough and had capacity; if patients did not have capacity and were thought unlikely to regain it, a relative or close friend as consultee was approached, if available. Prior ethical approval was given to collect full study data for patients who died before being approached for consent. Approval was also given to retain minimal data, sufficient to analyse mortality outcomes only, from patients who survived to at least 28 days but who either lacked capacity to consent and had no suitable consultee available, or who were discharged to supported living before being approached, these are referred to as unapproached survivors.

Patients and eligibility

Adult patients under the care of NHS having a positive blood sample culture for bacteria or fungi were eligible for inclusion. Only a patient's first infection episode in the study period was eligible, and only if both conventional and intervention (MALDI-TOF) tests were available when the first

blood sample entered the diagnostic pathway, and if presence of bacteria or fungi in blood was later confirmed by microbiological culture. Patients were excluded if they were in in custody; on an end-of-life care pathway; or, judged unsuitable by the attending physician.

2.3.2 Data collected

Data collection was similar to BSI-FOO observational study, with the exception that data on antimicrobial therapy was only collected up to day 7 and vascular lines, urinary catheters and early warning score were not collected. Care environment data such as staffing and ward activity were also not collected as these were the primary focus of the BSI-FOO observational study. Different types of data were collected for different periods as summarised in Table 2.6.

Table 2.6 RAPIDO Schedule of data collection

Data type	Days -7 to -1	Day 0	Days 1 to 7	Days 8 to 28
Eligibility, age and gender [d]		✓[a]		
Hospital admission data [d]	✓[b]	✓		
Patient characteristics, medical history, comorbidities [d]	✓[b]	✓		
Infection severity markers, probable source of infection [d]		✓		
Organism identity, clinical significance, susceptibility [d]		✓[c]	✓[c]	✓[c]
Laboratory process data		✓	✓	✓
Maximum and minimum daily temperature [d]		✓	✓	
Antimicrobial therapy [d]		✓	✓	
Outcomes: death, discharge, *C. difficile* infection, entry to end-of-life pathway	N/A	✓	✓	✓

[a] *Eligibility data collected for all patients; other data collected only for enrolled participants.*

[b] *Hospital admission and records of medical history/comorbidity could be before day -7.*

[c] *Organism data relates to the organism(s) at day 0, but the information became available over following days.*

[d] *Data also collected in BSI-FOO Observational study*

2.3.3 Population characteristics

Patient population

In total 3,127 patients consented to the study, 1,341 died (and therefore consent was not required) and 1,082 were unapproached survivors (and therefore included in the analysis population for mortality outcomes but not for other outcomes). The analysis population therefore consisted of 5,550 participants for the mortality outcomes (2,740 in the RAPIDO group and 2,810 in the Conventional group), and 4,468 participants for the other outcomes (2,197 in the RAPIDO group and 2,271 in the Conventional group).

Organism details

Of the 4,468 participants included in the analysis population for non-mortality outcomes, the most common organisms were Coagulase-negative staphylococcus (27.5%) and *E. coli* (20.6%), see Table 2.7.

Table 2.7 RAPIDO organisms

	Randomised to RAPIDO (n=2197)	Randomised to Conventional (n=2271)	Overall (n=4468)
Coagulase-negative staphylococcus	616 (28.0%)	611 (26.9%)	1227 (27.5%)
Escherichia coli	439 (20.0%)	481 (21.2%)	920 (20.6%)
MSSA	165 (7.5%)	165 (7.3%)	330 (7.4%)
Klebsiella pneumoniae	76 (3.5%)	83 (3.7%)	159 (3.6%)
Streptococcus pneumoniae	56 (2.5%)	82 (3.6%)	138 (3.1%)
Pseudomonas aeruginosa	55 (2.5%)	59 (2.6%)	114 (2.6%)
Corynebacterium spp.	37 (1.7%)	34 (1.5%)	71 (1.6%)
Proteus mirabilis	27 (1.2%)	35 (1.5%)	62 (1.4%)
Enterobacter cloacae	22 (1.0%)	35 (1.5%)	57 (1.3%)
MRSA	28 (1.3%)	25 (1.1%)	53 (1.2%)
Other single organism	508 (23.1%)	510 (22.5%)	1018 (22.8%)
Polymicrobial (≤1 clinically significant organism)	76 (3.5%)	61 (2.7%)	137 (3.1%)
Polymicrobial (>1 clinically significant organism)	92 (4.2%)	90 (4.0%)	182 (4.1%)

Notes: *Data are presented as n (%)*

Abbreviations: *MRSA=Methicillin-resistant Staphylococcus aureus, MRSA=Methicillin-susceptible Staphylococcus aureus*

Patient demographics

Demography, medical history, infection severity measures, comorbidities at date 0, source of infection and organisational factors are given in Table 2.8, by randomised allocation.

Approximately 55% of participants were male, with a median age of 69 years (IQR 55 to 80). The average Charlson score was 3 (IQR 2 to 4), 0.5% had cystic fibrosis, and 5.2% had had a prior transplant. Very few participants (1.6%) had a recent (in last 7 days) cardiac arrest recorded, 11.1% had chemotherapy in the last month and 8.3% had undergone recent surgery. As measures of illness severity at entry to the study, the median neutrophil count was 9.2 10^9/l (IQR 5.4 to 13.7), the median systolic blood pressure was 120 mmHg (IQR 105 to 139), the median temperature was 38.1°C (IQR 37.2 to 38.7°C, with 67.7% defined as having a fever), and 8.5% of participants were ventilated. Small proportions of participants were on vasopressor drugs, systemic corticosteroids or immunosuppressive drugs (8.1%, 5.3% and 11.4% respectively), but 53.6% had mental disorientation and 43.0% were receiving intravenous fluids. The BSI was classified as hospital-acquired in 37.1% of cases.

Characteristics were mainly well balanced between the groups, although there were slightly more males (56.4% versus 54.6%) and slightly more participants on intravenous fluids at day 0 (44.9% versus 41.0%) in the conventional group, compared to the RAPIDO group.

Table 2.8 RAPIDO participant demography

	Randomised to RAPIDO (n=2197)	Randomised to Conventional (n=2271)	Overall (n=4468)
Demography			
Males	1200/2197 (54.6%)	1281/2271 (56.4%)	2481/4468 (55.5%)
Age	69.0 (55.0, 80.0)	69.0 (55.0, 80.0)	69.0 (55.0, 80.0)
Recent medical history			
Cardiac arrest in last 7 days	31/2102 (1.5%)	36/2172 (1.7%)	67/4274 (1.6%)
Chemotherapy in last month	223/2103 (10.6%)	250/2172 (11.5%)	473/4275 (11.1%)
Surgery requiring overnight stay in last 7 days	177/2104 (8.4%)	179/2171 (8.2%)	356/4275 (8.3%)
Clinical data (days -7 to 0)			
Neutrophil count at day 0 or closest (10^9/L) [a]	9.2 (5.5, 13.6)	9.2 (5.3, 13.8)	9.2 (5.4, 13.7)
On ventilation at day 0	177/2078 (8.5%)	184/2157 (8.5%)	361/4235 (8.5%)
Temperature nearest to time 0 (°C) [b]	38.1 (37.3, 38.7)	38.1 (37.2, 38.7)	38.1 (37.2, 38.7)
Fever present nearest to time 0	1363/2013 (67.7%)	1416/2094 (67.6%)	2779/4107 (67.7%)
Systolic blood pressure at day 0 or closest (mmHg) [c]	120.5 (105.0, 140.0)	120.0 (104.0, 138.0)	120.0 (105.0, 139.0)
On intravenous fluids at day 0	838/2043 (41.0%)	946/2105 (44.9%)	1784/4148 (43.0%)
On vasopressor drugs at day 0	177/2058 (8.6%)	162/2140 (7.6%)	339/4198 (8.1%)
Systemic corticosteroids (shock) day 0, 1 or 2	99/2049 (4.8%)	122/2133 (5.7%)	221/4182 (5.3%)
Suspected hospital-acquired infection [d]	797/2101 (37.9%)	794/2182 (36.4%)	1591/4283 (37.1%)
Comorbidities at day 0			
Charlson score [e]	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)
Cystic fibrosis	9/2101 (0.4%)	12/2172 (0.6%)	21/4273 (0.5%)
Mental disorientation [f]	1145/2102 (54.5%)	1147/2171 (52.8%)	2292/4273 (53.6%)
Any prior transplant	115/2101 (5.5%)	109/2170 (5.0%)	224/4271 (5.2%)
On immunosuppressive drugs at time0	242/2064 (11.7%)	236/2141 (11.0%)	478/4205 (11.4%)

Notes: *Data are presented as median (IQR) or n (%)*

[a] *Data missing for 338 participants (156 RAPIDO, 182 Conventional)*

[b] *Data missing for 361 participants (184 RAPIDO, 177 Conventional)*

[c] *Data missing for 535 participants (273 RAPIDO, 262 Conventional)*

[d] *Defined as more than 2 days between admission and blood sampling.*

[e] *Data missing for 1063 participants (510 RAPIDO, 553 Conventional). 484 participants (243 RAPIDO, 241 Conventional) had a Charlson score of zero.*

[f] *Data also included in liver disease component of Charlson score*

2.3.4 Trial conclusions

The trial concluded that 28-day survival was similar between the groups (81.5% RAPIDO *vs* 82.3% conventional, p=0.42). In subgroup analyses, there was no evidence to suggest that this effect differed by clinical significance or by organism. Microbial identity was supplied more quickly in the RAPIDO group (median 38.5 *vs* 50.3 hours after sampling, IQR 26.7 to 50.3 *vs* 47.1 to 72.9, p<0.0001) but this did not translate into shorter times to receiving effective antimicrobial therapy[87].

2.4 Summary

In summary, I will use data from both the BSI-FOO observational study (n=1,676) and the RAPIDO trial (n=4,468) throughout this thesis. I will define the analysis populations derived from these studies within each chapter along with a descriptive analysis of the data items specific to each chapter.

CHAPTER 3 HEALTHCARE SETTING AND TREATMENT RELATED RISK FACTORS

In this chapter, I will discuss the methods and results of a multivariable model which I developed to explore the relationship between healthcare setting related risk factors such as staffing levels, ward activity (number of admissions/discharges), movements between wards, antibiotic use (particularly the timing of appropriate therapy), and use of intravenous lines and catheters, and the outcome of 28-day mortality. I will start by discussing the current literature on these risk factors (section 3.1) and then move on to present the methods (section 3.2) and results of the multivariable risk factor model (section 3.3). At the end of the chapter, I reflect on the findings as well as the strengths and limitations of the methodology used (section 3.4).

3.1 Introduction

Staffing levels, which can be considered as a modifiable factor, have been shown to impact on a range of care quality outcomes including patient mortality[10, 11, 88]. In England, a cross-sectional analysis conducted in over 100,000 patients across 30 acute trusts reported that mortality was 26% higher in hospitals with the highest patient to nurse ratio[89]. Another retrospective longitudinal study using routinely collected data reported the hazard of death was increased by 3% for every day a patient experienced below the average registered nurse staffing levels[9]. Similarly, Needleman *et al.* estimated that the risk of death increased by 2% for each shift that was below the staffing level target[90]. This has been shown to differ in patients who stay in ICU to those who do not. A retrospective observational study of approximately 130,000 patients found an association between nurse staffing and mortality for patients without an ICU stay, but not for patients who experienced an ICU stay[91]. In addition, the level of nursing skill and the use of non-permanent staff was associated with increased rates of hospital-acquired infection[92]. A cross-sectional analysis conducted in Pennsylvania found that for each 10% increase in the proportion of degree-level qualified nurses was associated with a 5% decrease in the risk of mortality within 30 days of admission[93]. Workload has also been found to be associated with survival. Needleman *et al.* estimated that the risk of death increased by 4% for each high-turnover shift to which a patient was exposed, where turnover was equal to the sum of admissions, transfers and discharges[90]. Griffiths *et al*. conducted a scoping review investigating the impact of organisation and management factors on infection control performance in acute hospitals[94]. Most of the studies included were observational and they focused on different staffing issues and outcome measures, however they all found an inverse relationship between nurse staffing and risk of infection. A meta-analysis reported by Kane *et al* showed that for

each additional full-time-equivalent registered nurse per patient day the odds of death was reduced; by 9% for patients in ICU, 16% for surgical patients and 6% for medical patients[95]. Although there have been several studies and reviews on the impact of staffing and workload on outcome, these have not been studied in relation to infection outcomes, and none have been in patients with a BSI in particular.

Timely appropriate antimicrobial chemotherapy has also been found to be beneficial, but data are typically from single-centre studies, and information on the estimated size of these effects is limited. Inappropriate initial antimicrobial therapy has been found to be associated with higher in-hospital mortality[96] and a meta-analysis of 70 studies reported that inappropriate empirical antibiotic treatment was significantly associated with all-cause mortality in patients with sepsis (over half of the studies included were of BSI)[97]. A delay in starting effective antimicrobial therapy in *P. aeruginosa* BSI has been shown to be associated with higher mortality[98]. A study of 37 patients with carbapenem-resistant *P. aeruginosa* BSI found that delayed therapy of >48h had an impact on microbiological but not clinical outcome however this was a single centre study limited to a small number of patients[99].

Estimating the effect of time to initiation of treatment on survival is analytically challenging since it requires the person to survive until the day they receive treatment. This means that only those who survive a long time can wait a long time to start treatment and those that die shortly after start of follow-up have not had the opportunity to be exposed to a long time to initiation. This introduces immortal time bias, a bias that arises when there is a period of follow-up in which the outcome, such as death, cannot occur. For example, a person who starts treatment on day 7 is considered "immortal" for the first seven days. Similarly, the number of ward movements in this study is bounded by the survival time. Patients who die shortly after the blood culture was taken have not had an opportunity to become exposed to a large number of movements between or within wards and will have no or few movements by definition.

Different approaches to control for the bias that arises when estimating the effect of treatment initiation on outcome have been studied in the statistical literature by several authors. Zheng Zou *et al* compared methods to control for survival bias associated with treatment initiation[100]. One method classified patients into users (those who started treatment) and non-users (those who did not start treatment) at the end of follow-up, however this resulted in an overestimate of the treatment effect as it used patients' future exposure to define the groups and therefore the event-free time in the user group was inflated. To control for this, another method proposed was to change time 0 to be the start of treatment for users, and randomly assign a time according to a

uniform distribution for those who do not start treatment (non-users). However, this method still introduced survival bias if the distribution of start of follow-up for users was different to the uniform distribution assigned for the non-users. To overcome this, a method described as prescription time-distribution matching which assigns time 0 to be start of treatment for users and assigns a time 0 selected at random from the set of times for users was described. This ensures that the overall distribution of time 0 of the users and nonusers are the similar. However, this method excludes nonusers who experienced the event before the given time 0. Another approach proposed was to start follow-up after a given exposure time (e.g., 90 days) allowing all patients 90 days exposure to start treatment. Patients who experience the event within the 90 days exposure are excluded, and those who do not experience the event within the 90 days are classified into users and non-users at this time and followed up from the end of this exposure time. However, this method loses a lot of study information (the first 90 days follow-up is excluded). The final approach described used a time-dependent variable for treatment which assigned the value of the treatment variable as 0 before the time of first treatment and changes to 1 when the treatment starts. For the non-user, the value remains as 0 throughout the whole follow-up. This method accurately represents the exposure status without the need to exclude participants and has shown to reduce bias in other studies[101-104]. The two methods which Zou *et al* concluded best controlled for survival bias were prescription time-distribution matching and using a time-dependent variable for treatment. However, a simulation study by Karim *et al* comparing the prescription time-distribution matching approach to time-dependent Cox-model found that prescription time matching was not adequate in addressing immortal time bias[105]. Gleiss *et al* introduced the "Landmark" method to deal with immortal time bias in which follow-up time is split at prespecified time point in which exposure is defined[106]. However, a simulation study concluded that time-dependent Cox regression outperformed the Landmark method in terms of bias[107].

In this chapter I explore the effect of modifiable risk factors on 28-day mortality, whilst considering the analytical challenges associated with exposures that are bounded by survival time such as time to initiation of therapy. Modifiable risk factors that are considered in this chapter include: staffing levels; ward activity (number of admissions/discharges); movements between wards; antibiotic use (in particular the timing of appropriate therapy); and use of intravenous lines and catheters.

3.2 Methods

3.2.1 Primary outcome

The primary outcome was time to death up to 28 days of taking the diagnostic positive blood sample. Patients were followed up for 28 days following blood culture, including mortality tracing after hospital discharge.

3.2.2 Risk factors

Any factors present before time 0 were considered non-modifiable. Modifiable risk factors considered were aspects of hospital care received from time 0 onwards, which included staffing levels, ward activity (number of admissions/discharges), movements between wards, timing of appropriate therapy and continuing presence of intravenous lines and catheters (see section 2.2.2). Overall staffing levels, including healthcare assistants (HCA), trust-employed nurses and agency nurses, was averaged across three shifts (early, late and night) and defined as staff:bed ratio (number of staff per bed). Ward activity per ped was defined similarly. The presence or absence of central lines, peripheral lines and urinary catheters was observed on day 0, and their presence on days 1 to 28 determined using the date of removal.

Antimicrobial therapy was defined as 'appropriate' if the organism was susceptible to the antimicrobial prescribed, and therapy continued for at least 36 hours to allow therapeutic effect. If treatment was changed from one appropriate antimicrobial to another, this was treated as a single period of appropriate therapy providing that the next therapy began within 24 hours of the last dose of the previous therapy.

3.2.3 Analysis population

In this analysis, I included all BSI-FOO observational study participants, excluding repeat episodes and patients with polymicrobial episodes (as described in Chapter 2). Ward level data, such as staffing levels and ward activity, were not collected in RAPIDO and therefore it was not possible to include RAPIDO participants in this analysis.

3.2.4 Descriptive analysis

I summarised continuous variables via the mean and standard deviation (SD) or median and interquartile range (IQR) if distributions were skewed and categorical variables as numbers and percentages.

I described the presence and number of lines and catheters on day 0, timing of insertion and timing of removal of lines and catheters by 28-day survival status. I described ward speciality by day since day 0; and I also described ward speciality on day 0 by 28-day survival status. Similarly, I described staffing levels (nurses, HCA and agency staff) and ward activity (admissions + discharges) by: 28-day survival status; ward speciality on day 0; the number of days from day 0; and day of week. I described the number and type of ward movements and details of timing to receipt and duration of appropriate antimicrobial therapy by 28-day survival status.

3.2.5 Primary outcome

The primary aim of this analysis was to investigate modifiable factors in the care of patients with BSI. However, I needed to account for relevant non-modifiable factors as they could be an important source of confounding and/or variability in mortality rates. There were many variables collected which were potential confounders, however adjusting for all important confounders in a multivariable model would result in the number of events per variable (EPV) exceeding 10 and this could impact the stability of estimates and validity of the statistical models[108] . Therefore, to avoid over fitting and to maximise the degrees of freedom available, I built the statistical model in two stages. Firstly, I modelled the non-modifiable risk factors and calculated a "risk score" for individual patients. I then modelled the modifiable risk factors with the risk score from the first stage included as a covariate. The stages are described in more detail below:

Stage one

Firstly, I performed univariate Cox regression models on each of the non-modifiable risk factors and identified factors associated with mortality at the 20% significance level. I then took all the variables identified on univariate analysis and used backwards elimination methods with p<20% threshold to select variables that were predictive of mortality in a multivariable model. To select the best-fitting functional form for continuous variables, I used multivariable fractional polynomials[109]. I then derived a risk score for each patient using the model estimates from the final multivariable model.

Stage two

I then modelled the modifiable risk factors using Cox regression, with the risk score for the non-modifiable risk factors calculated in stage one included as a covariate. To allow for the longitudinal nature of the data and time varying covariates, I split the episodes (using the -stsplit- Stata command[110]) at daily intervals from day 0 to 28 with ward speciality, presence of central line, peripheral line, urinary catheter, ward movements, staffing levels, ward activity, and antimicrobial therapy variable values updated at each interval. Ward data was not recorded post day 7 so I

assumed for patients who survived and were not discharged prior to day 7, ward variables and ward movements for days 7 to day 28 or death/discharge were constant for this period.

I included all modifiable risk factors under consideration in one adjusted model, along with risk score and organism. I did not include duration of appropriate antimicrobial therapy in the model as it was bounded by survival time and highly correlated with time to receipt of appropriate antimicrobial therapy, therefore including both could lead to biased and/or uninterpretable results. I decided to only include time to receipt of appropriate antimicrobial therapy as it was of highest clinical interest at that time and I explored duration of therapy within a separate analysis (Chapter 4). I included interaction terms between ward speciality and: a) ward activity and b) staffing levels in the model (regardless of statistical significance) to ensure that interpretation of staffing and ward activity estimates could be restricted to patients who were still in hospital at that time. I then considered the following further interaction terms for potential inclusion in the model: organism by risk score, organism by central line, organism by peripheral line, organism by urinary catheter, organism by time to appropriate antimicrobial therapy, and ward speciality by within-ward speciality movements. These were pre-specified after discussions with Clinicians about potential interactions. Due to the low numbers of patients with a line or catheter within each organism, I categorised organisms into two groups (*Candida*, MSSA and MRSA versus ESBL-producing Enterobacteriaceae, ESBL-non-producing *E. coli* and *P. aeruginosa*) when considering the interaction between organism and line/catheter. These groups were chosen as *Candida*, MSSA and MRSA were considered to be of most importance in terms of line presence. I applied a forward stepwise approach to select which interactions were to be included in the final model, using likelihood ratio tests to compare nested models using a 10% significance level (see section 3.2.7 for further details on significance levels). I do not present interactions which were not statistically significant in the likelihood ratio tests. Therefore, the final model included the risk score, organism, modifiable covariates, and any identified interaction terms.

I assessed the proportional hazards assumption based on Schoenfeld residuals and log-log plots of survival. In the stage one modelling process, if non-proportional hazards were indicated for a particular variable, I then stratified the model by that variable (or a categorised version for continuous variables) as interpretation of coefficients was not required in this model. In stage two, if non-proportional hazards were indicated, I then categorised time into periods where proportional hazards appeared valid and added an interaction between this categorised time and the variable causing non-proportional hazards to the model – that is, fitting a piecewise Cox model (estimating different effects of the variable for each of the categorised time periods).

I assessed the fit of the final model using standard methods (e.g. Brier Score, plots of deviance and martingale residuals to identify any influential observations/outliers and assess functional form i.e. non-linearity of continuous variables). It was not possible to calculate Harrell's concordance (c) statistic due to the time varying nature of the data. I assessed the calibration by comparing the observed event rate for patients in each decile of predicted event rates. I assessed collinearity during the model fitting process using the variance inflation factor (VIF) and variables with a VIF>5 were investigated further.

3.2.6 Missing data

I imputed missing values using multiple imputation methods (fully conditional specification) under the assumption that data were missing at random. The imputation was applied to stage one and stage two models. I transposed the data to be in wide format so that variables that were measured at multiple time points e.g., ward variables from day 0 to day 7 were considered as distinct variables. I included all variables that were in the primary analysis model, auxiliary variables (based on clinical expertise or predictors of the missing status based on statistical tests), indicator for death and the log of survival time in the imputation procedure.

I imputed ward variables for each day conditional on patients being alive and in hospital on that day.

I transformed non-normally distributed variables prior to imputation, with the most suitable transformation being selected using Stata's -gladder- command[111]. If a suitable transformation could not be found or the imputation procedure imputed values outside valid ranges, then I used predictive mean matching for the imputation of that variable. I set the number of imputations (m) to be equal to the value of the percentage of missing data for the variable with the highest proportion of missing data (m = 45)[112]. I checked the validity of the imputations of continuous variables by comparing the distributions of the imputed data to the distributions of the observed data. I assessed longitudinal data by looking at the change in values from the previous day and assessing differences in this change between imputed and observed data. I checked categorical variables by comparing the frequencies and percentages of observed and imputed values within each level of the categorical variable. Within all analysis models, I used Rubin's rule to summarise data across the m datasets[113].

3.2.7 Significance levels

For the non-modifiable risk factor model (stage one), I considered two-tailed p-values <0.2 statistically significant. A p-value of 0.2 was chosen rather than the conventional 0.05 as the purpose of this model was purely to derive a risk score and not to explore strength of associations. To ensure

the risk score encompassed all important non-modifiable risk factors for mortality I did not wish to impose a stringent criterion for inclusion in the model.

The threshold of 0.2 was not used in determining the inclusion of variables in the multivariable model of modifiable risk factors (stage two), it was simply used to highlight factors that appeared to show some evidence of association with outcome and therefore were of main interest. This value was prespecified (prior to data collection and analysis) and was chosen as the focus of the study was not on conventional statistical significance, but rather quantifying the effects of covariates on outcome. However, I used a 10% level when determining which interaction terms to retain in the stage two model in order to limit the number of parameters estimated, following the guidelines of having at least 10 events per parameter to be fitted[108]. I wanted the threshold for interactions to be greater than the conventional 0.05 as the power of a statistical test for an interaction is typically lower compared to tests of main effects. A more stringent p-value than 0.2 but higher than conventional 0.05 was therefore chosen to limit the number of interactions and therefore parameters estimated to those considered the most important.

I did not make any formal adjustment for multiple testing, however I gave consideration to the number of tests performed when interpreting the results.

3.2.8 Sensitivity analyses

I performed four sensitivity analyses which are each described below. I did not repeat the model selection process for these analyses: instead, I fitted the model from the main analysis to the sensitivity analysis datasets and compared the resulting estimates.

Centre

To allow the baseline hazard to vary by centre, I re-fitted the primary outcome model including stratification by centre.

Exclusion of polymicrobial episodes

I assessed the effect of excluding polymicrobial episodes from the main analysis by including them in this sensitivity analysis. I classified polymicrobial infection episodes with two target organisms according to the organism with the higher overall mortality risk e.g., an infection with both MRSA and non-ESBL-producing *E. coli,* would be analysed as an MRSA episode, as MRSA has a higher mortality rate than non-ESBL-producing *E. coli.* I repeated the multiple imputation procedure with polymicrobial episodes included.

Appropriate antimicrobial definition

I assessed the potential impact of defining antimicrobial therapy as 'appropriate' if the organism was susceptible to the antimicrobial prescribed and the therapy continued for at least 36 hours. In the main analysis, an antimicrobial treatment would not be considered appropriate if the patient died within 36 hours of starting it. However, this may lead to inflated estimate effects as the death could be viewed as a consequence of not receiving the therapy. I performed a sensitivity analysis with the "36-hour rule" removed to assess this possibility.

Complete case analysis

I assessed the impact of the multiple imputation by fitting a complete-case model for the primary outcome, i.e., refitting the primary outcome model (both stage one and stage two models) only for patients with complete data for all variables included in the model.

3.3 Challenges and Solutions

I intended to allow for any interaction terms that were candidates for inclusion in the primary analysis model in the imputation procedure by imputing separately for each category of one of the variables involved in any interactions, e.g. if there was an interaction between ward speciality and one of the other variables, the imputation would be done separately for the different types of ward speciality. All the interactions in the final primary analysis model involved either organism type or ward speciality. Models including the six categories of organism type did not converge, while the time-varying nature of ward speciality made it difficult to choose a time point at which to split the dataset in order to perform the imputation on a "one row per patient" model; data was also missing in ward speciality itself. Unfortunately, these computational difficulties meant I was unable to include interactions in the imputation procedure.

The imputation of repeated measures resulted in convergence problems which may have been caused by over-fitting and/or collinearity. Therefore, I decided to limit the variables in the individual imputation models for ward variables to variables considered most important and predictive without causing convergence problems. I therefore imputed ward variables using data from the other ward variables on that day only and using data from the variable itself on all other days. For example, ward activity on day 3 was imputed (conditional on the patient being alive and in hospital on day 3) using ward activity on all other days, staffing on day 3, ward speciality on day 3 and ward movements on day 3.

Due to the small number of patients with more than one ward movement, ward movement was included as a binary variable (one or more *vs* none) in the model, treated as a time-varying covariate (0 until the patient moves wards, then updated to 1 on day of their first ward movement).

3.4 Results

3.4.1 Descriptive analysis

Lines and urinary catheters

Approximately two-thirds of the sample had a line present at time 0 (Table 3.1), and the maximum number of lines present was five (0.2%). Both central and peripheral lines were more common in patients who died (30.5% *vs* 22.6%, and 62.5% *vs* 46.8%, respectively). A higher proportion of patients who died had a urinary catheter present at time 0 compared to those who survived (48.1% *vs* 26.9%).

The median time (prior to day 0) that central lines were present was eight days (IQR 3.0 to 20.0) and was lower in patients who died (4.0 days; IQR 1.0 to 8.0 *vs* 11.0 days; IQR 4.0 to 28.0). Peripheral lines were present for a median of one day (IQR 0.0 to 2.0) and this was slightly higher in patients who died (1.0 day; IQR 0.0 to 2.0 *vs* 0.0 days; IQR 0.0 to 1.0). Urinary catheters were present for a median of four days (IQR 1.0 to 10.0) and the length of time was similar for those who survived and those who died.

The presence of a central and/or peripheral line and length of time they were present for prior to day 0 varied across organisms. Lines were most common in patients with *Candida* (59.5% for central line and 62.1% for peripheral line, 89.7% had at least one line of either type). The length of time prior to day 0 that lines were present was highest in patients with *P. aeruginosa* for central lines (12.0 days; IQR 4.0 to 33.0) and in patients with *Candida* for peripheral lines (2.0 days; IQR 1.0 to 4.5). The proportion of patients with a urinary catheter at time 0 and the time present prior to day 0 also varied between organisms; catheters were most common in patients with *Candida* (57.8%) and were in place for the longest time in patients with *P. aeruginosa* (6.0 days; IQR 1.0 to 15.0).

Table 3.1 Lines and urinary catheter details at day/time zero

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Total number of lines (central or peripheral) present at time 0			
0	505/1326 (38.1%)	85/347 (24.5%)	590/1673 (35.3%)
1	633/1326 (47.7%)	182/347 (52.4%)	815/1673 (48.7%)
2	144/1326 (10.9%)	45/347 (13.0%)	189/1673 (11.3%)
3	33/1326 (2.5%)	23/347 (6.6%)	56/1673 (3.3%)
4	9/1326 (0.7%)	11/347 (3.2%)	20/1673 (1.2%)
5	2/1326 (0.2%)	1/347 (0.3%)	3/1673 (0.2%)
Central lines:			
Number of central lines present at time 0			
0	1026/1326 (77.4%)	241/347 (69.5%)	1267/1673 (75.7%)
1	248/1326 (18.7%)	70/347 (20.2%)	318/1673 (19.0%)
2	52/1326 (3.9%)	36/347 (10.4%)	88/1673 (5.3%)
Number of days central line present prior to day 0 [a]	11.0 (4.0, 28.0)	4.0 (1.0, 8.0)	8.0 (3.0, 20.0)
Number of days from day 0 to central line removal [b]	3.0 (1.0, 6.0)	2.0 (1.0, 4.0)	2.0 (1.0, 6.0)
Central line implicated infection	123/1327 (9.3%)	15/348 (4.3%)	138/1675 (8.2%)
Number of days from day 0 to central line removal for those with a central line implicated infection [c]	2.0 (1.0, 4.0)	2.0 (1.0, 3.0)	2.0 (1.0, 4.0)
Peripheral lines:			
Number of peripheral lines present at time 0			
0	705/1326 (53.2%)	130/347 (37.5%)	835/1673 (49.9%)
1	531/1326 (40.0%)	188/347 (54.2%)	719/1673 (43.0%)
2	87/1326 (6.6%)	27/347 (7.8%)	114/1673 (6.8%)
3	3/1326 (0.2%)	2/347 (0.6%)	5/1673 (0.3%)
Number of days peripheral line present prior to day 0 [d]	0.0 (0.0, 1.0)	1.0 (0.0, 2.0)	1.0 (0.0, 2.0)
Number of days to peripheral line removal from day 0 [e]	1.0 (1.0, 3.0)	1.0 (1.0, 2.0)	1.0 (1.0, 3.0)
Peripheral line implicated infection	20/1327 (1.5%)	7/348 (2.0%)	27/1675 (1.6%)
Number of days to peripheral line removal for those with a peripheral line implicated infection [f]	0.0 (0.0, 2.0)	1.5 (0.0, 2.0)	0.0 (0.0, 2.0)
Urinary catheters:			

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Urinary catheter present at time 0	357/1326 (26.9%)	167/347 (48.1%)	524/1673 (31.3%)
Number of days urinary catheter present for prior to day 0 [g]	4.0 (1.0, 12.0)	4.0 (1.0, 9.5)	4.0 (1.0, 10.0)
Number of days to urinary catheter removal from day 0 [h]	4.0 (1.0, 10.0)	4.0 (1.0, 8.0)	4.0 (1.0, 8.0)

Notes: *Data are presented as median (IQR), mean (SD) or n (%)*

Missing data:

[a] *Data missing for 38 patients (32 survived, 6 died)*

[b] *Data missing for 82 patients (59 survived, 23 died)*

[c] *Data missing for 13 patients (13 survived, 0 died)*

[d] *Data missing for 88 patients (70 survived, 18 died)*

[e] *Data missing for 190 patients (141 survived, 149 died)*

[f] *Data missing for 2 patients (1 survived, 1 died)*

[g] *Data missing for 103 patients (76 survived, 27 died)*

[h] *Data missing for 178 patients (132 survived, 46 died)*

Abbreviations: *IQR=Interquartile range, SD=Standard deviation*

Ward speciality

On day 0, approximately 56% of patients were in a medical ward, 14% in a critical care ward, 24% in major surgery, 2% in minor surgery & 4% in wards classified as "Other", which includes A&E, emergency assessment, fracture clinics and related units, obstetrics & gynaecology, imaging, diagnostics and telemetry and Services - not medical, surgical or Intensive therapy unit/high dependency unit (ITU/HDU), and not listed elsewhere. These proportions remained constant for the remainder of the days (Figure 3.1)

With respect to survival, the proportion of patients in a medical ward on day 0 was broadly similar for patients who survived and those who died (Table 3.2). However, a higher proportion of patients who died were in critical care (27.8%) compared to those who survived (10.5%). Approximately 24% of all patients were in a major surgery ward on day 0 and this proportion was higher in patients who survived compared to those who died (27.0% *vs* 14.5%).

Figure 3.1 Ward specialty, by day since day zero

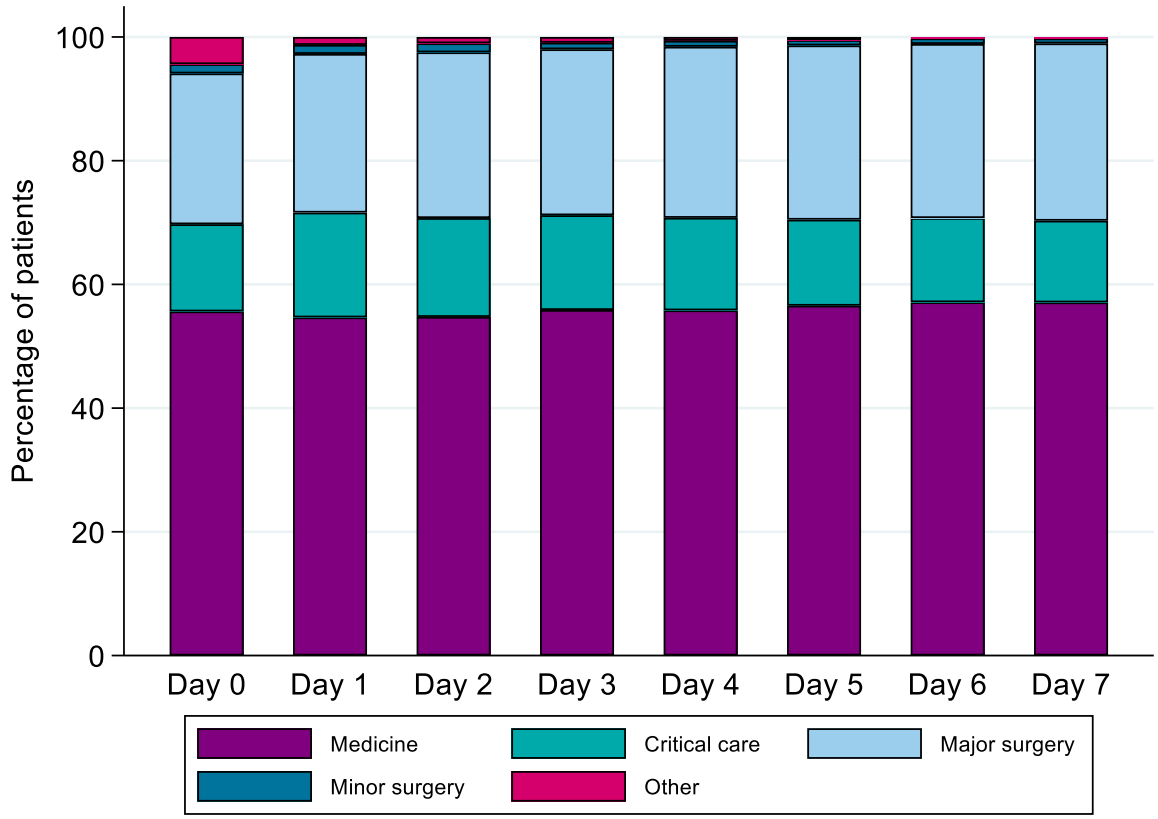


Table 3.2 Ward speciality on day zero

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Medicine	**738/1316 (56.1%)**	**186/345 (53.9%)**	**924/1661 (55.6%)**
Medical: General medical (no declared speciality)	37/1316 (2.8%)	10/345 (2.9%)	47/1661 (2.8%)
Medical: Acute medical admissions and pre-admissions	269/1316 (20.4%)	53/345 (15.4%)	322/1661 (19.4%)
Medical: Cardiology/cardiovascular/coronary	38/1316 (2.9%)	13/345 (3.8%)	51/1661 (3.1%)
Medical: Care of the Elderly	59/1316 (4.5%)	39/345 (11.3%)	98/1661 (5.9%)
Medical: Dermatology/rheumatology	2/1316 (0.2%)	0/345 (0.0%)	2/1661 (0.1%)
Medical: Diabetes/endocrinology	4/1316 (0.3%)	0/345 (0.0%)	4/1661 (0.2%)
Medical: Gastroenterology/gastrology/liver	29/1316 (2.2%)	4/345 (1.2%	33/1661 (2.0%)
Medical: Haematology/oncology	184/1316 (14.0%)	44/345 (12.8%)	228/1661 (13.7%)
Medical: Infectious disease/travel medicine	23/1316 (1.7%)	2/345 (0.6%)	25/1661 (1.5%)
Medical: Nephrology/renal/dialysis	48/1316 (3.6%)	7/345 (2.0%)	55/1661 (3.3%)
Medical: Neurology/neurosciences/neuromedical	6/1316 (0.5%)	0/345 (0.0%)	6/1661 (0.4%)
Medical: Respiratory	23/1316 (1.7%)	9/345 (2.6%)	32/1661 (1.9%)
Medical: Stroke	16/1316 (1.2%)	5/345 (1.4%)	21/1661 (1.3%)
Critical care	**138/1316 (10.5%)**	**96/345 (27.8%)**	**234/1661 (14.1%)**
ITU/HDU : General (not specified as surgical, medical or specialist)	86/1316 (6.5%)	79/345 (22.9%)	165/1661 (9.9%)
ITU/HDU: General medical	12/1316 (0.9%)	1/345 (0.3%)	13/1661 (0.8%)
ITU/HDU: General surgical	2/1316 (0.2%)	1/345 (0.3%)	3/1661 (0.2%)
ITU/HDU: Cardiac	15/1316 (1.1%)	5/345 (1.4%)	20/1661 (1.2%)
ITU/HDU: Neurology/neurosurgery	22/1316 (1.7%)	10/345 (2.9%)	32/1661 (1.9%)
ITU/HDU: Theatre recovery areas	1/1316 (0.1%)	0/345 (0.0%)	1/1661 (0.1%)
Major surgery	**355/1316 (27.0%)**	**50/345 (14.5%)**	**405/1661 (24.4%)**
Surgery: Admissions/pre-admissions units	15/1316 (1.1%)	1/345 (0.3%)	16/1661 (1.0%)
Surgery: Cardiothoracic/thoracic	12/1316 (0.9%)	3/345 (0.9%)	15/1661 (0.9%)
Surgery: General including gastrointestinal, breast, vascular	177/1316 (13.4%)	18/345 (5.2%)	195/1661 (11.7%)
Surgery: Neurosurgery	24/1316 (1.8%)	5/345 (1.4%)	29/1661 (1.7%)
Surgery: Orthopaedic/trauma	48/1316 (3.6%)	5/345 (1.4%)	53/1661 (3.2%)
Surgery: Plastics/burns	10/1316 (0.8%)	1/345 (0.3%)	11/1661 (0.7%)
Surgery: Urology/renal	69/1316 (5.2%)	17/345 (4.9%)	86/1661 (5.2%)

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Minor surgery	**22/1316 (1.7%)**	**3/345 (0.9%)**	**25/1661 (1.5%)**
Surgery: Ear, nose, throat, oral & maxillo-facial, and opthalmic units	13/1316 (1.0%)	3/345 (0.9%)	16/1661 (1.0%)
Surgery: Short stay and daycase units	9/1316 (0.7%)	0/345 (0.0%)	9/1661 (0.5%)
Other	**63/1316 (4.8%)**	**10/345 (2.9%)**	**73/1661 (4.4%)**
Other: A&E, emergency assessment, fracture clinics and related units	45/1316 (3.4%)	9/345 (2.6%)	54/1661 (3.3%)
Other: Imaging, diagnostics and telemetry	1/1316 (0.1%)	0/345 (0.0%)	1/1661 (0.1%)
Other: Obstetrics & gynaecology	16/1316 (1.2%)	1/345 (0.3%)	17/1661 (1.0%)
Other: Services - not medical, surgical or ITU/HDU, and not listed elsewhere	1/1316 (0.1%)	0/345 (0.0%)	1/1661 (0.1%)

***Notes**: Data are presented as n (%)*

***Abbreviations:** A&E= Accident and emergency department, ITU/HDU= Intensive therapy unit/high dependency unit*

Staffing and ward activity

The average number of staff (split by staff type) and ward activity (number of admissions + number of discharges per ward) by ward speciality are given in Table 3.3 and shown in Figure 3.2.

Unsurprisingly, the median number of nurses per 10 beds was highest in critical care (8.0 nurses; IQR 7.1 to 9.0), and similar across all other ward specialities (1.8 nurses, IQR 1.5 to 2.0 in minor surgery; 1.3 nurses, IQR 1.0 to 2.1 in "other"; 1.4 nurses, IQR 1.1 to 1.8 in medicine and 1.3 nurses, IQR 1.1 to 1.5 in major surgery). The median number of HCA per 10 beds was very similar across all specialities (approx. 0.6); however, there was wide variation within specialities (see Table 3.3 and Figure 3.2*).* The median number of agency staff per 10 beds was also similar across the specialities, with less variation within specialities compared to the other staffing types. Unsurprisingly, ward activity was highest in "other" ward specialities with a median of 8.1 patients admitted or discharged per 10 beds (IQR 2.3 to 16.4) and lowest in medicine, critical care and major surgery with a median of 3.9 (IQR 1.8 to 9.7) in medicine, 3.3 (IQR 2.2 to 5.0) in critical care and 3.4 (IQR 2.0 to 5.1) in major surgery (see Figure 3.3*).*

Staffing levels and ward activity on day 0 by 28-day survival status are given in Table 3.4. The average number of nurses per 10 beds was slightly higher for patients who died compared to those who survived (median 1.7 nurses; IQR 1.2 to 7.0 *vs* 1.4; IQR 1.1 to 2.1), perhaps reflecting the fact that patients who died were more likely to be in critical care wards. The number of HCA and agency staff per 10 beds on day 0 was similar for patients who survived and those who died with an overall median 0.6 HCA (IQR 0.4 to 0.8) and 0.0 agency staff (IQR 0.0 to 0.3). Ward activity was also similar for patients who survived and those who died with a median of 3.7 admissions and discharges per 10 beds (IQR 1.9 to 7.3) for patients who survived and 3.5 admissions and discharges per 10 beds (IQR 1.8 to 5.5) for patients who died.

Table 3.3 Staffing levels and ward activity on day zero, by ward speciality

	Staffing: number per 10 beds, averaged over the 3 shifts			Ward activity per 10 beds
	NHS-employed nurses	HCA	Agency	
Medicine (n=924) [a]	1.4 (1.1, 1.8)	0.7 (0.5, 0.9)	0.1 (0.0, 0.3)	3.9 (1.8, 9.7)
Critical care (n=234) [b]	8.0 (7.1, 9.0)	0.6 (0.2, 0.9)	0.0 (0.0, 0.4)	3.3 (2.2, 5.0)
Major surgery (n=405) [c]	1.3 (1.1, 1.5)	0.6 (0.4, 0.8)	0.1 (0.0, 0.3)	3.4 (2.0, 5.1)
Minor surgery (n=25) [e]	1.8 (1.5, 2.0)	0.5 (0.0, 0.7)	0.0 (0.0, 0.2)	6.3 (3.9, 8.3)
Other (n=73) [e]	1.3 (1.0, 2.1)	0.6 (0.5, 0.9)	0.3 (0.0, 0.6)	8.1 (2.3, 16.4)

***Notes**: Data are presented as median (IQR)*

Other includes: A&E, emergency assessment, fracture clinics and related units; Imaging, diagnostics and telemetry; Obstetrics & gynaecology; Services - not medical, surgical or HDU/ITU, and not listed elsewhere

Ward activity is defined as the number of patients admitted + number of patients discharged

Missing data:

[a] NHS-employed nurses (n=154); HCA (n=155); Agency (n=158); Ward activity (n=72)

[b] NHS-employed nurses (n=8); HCA (n=8); Agency (n=10); Ward activity (n=1)

[c] NHS-employed nurses (n=33); HCA (n=33); Agency (n=39); Ward activity (n=8)

[d] NHS-employed nurses (n=8); HCA (n=8); Agency (n=8); Ward activity (n=3)

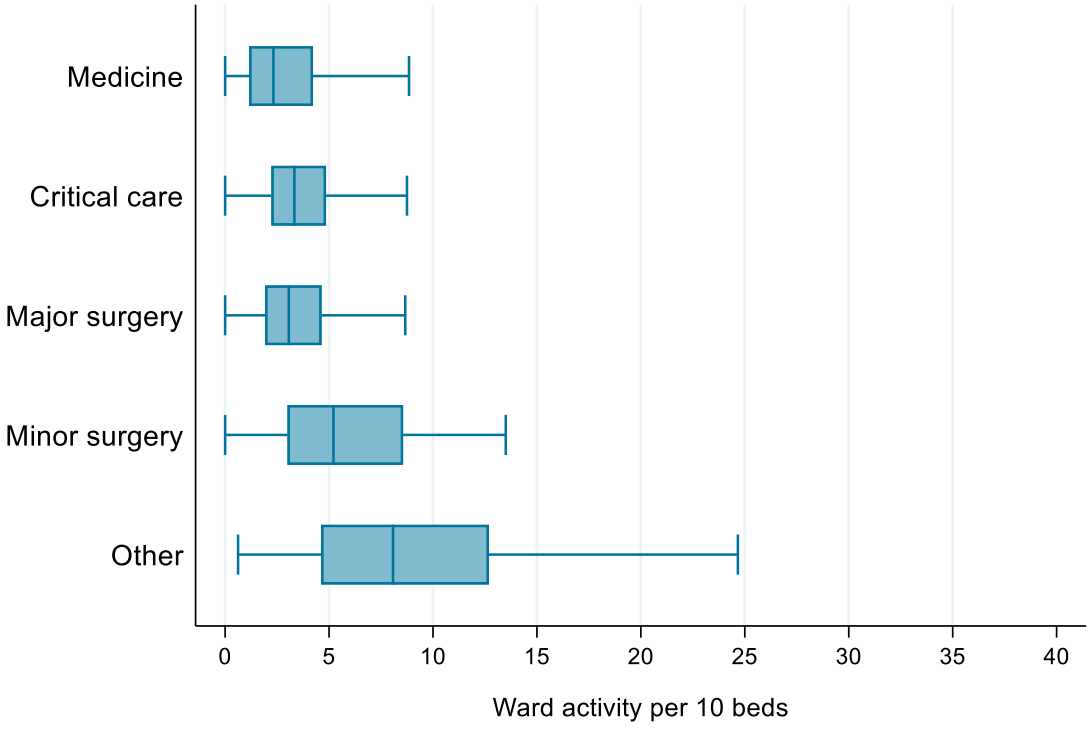
[e] NHS-employed nurses (n=58); HCA (n=58); Agency (n=58); Ward activity (n=54)

***Abbreviations**: HCA=Health care assistant, IQR=Inter-quartile range, NHS=National Health Service*

Figure 3.2 Box plot of average number of staff per 10 beds on day zero, by ward speciality

Notes: *Box represents median and IQR, tails represent the range within 1.5*IQR*
Abbreviations*: HCA=Health care assistant, IQR=Inter-quartile range*

Figure 3.3 Box plot of ward activity per 10 beds on day zero, by ward



Notes: *Box represents median and IQR, tails represent the range within 1.5*IQR*
Abbreviations*: IQR=Interquartile range*

Table 3.4 Staffing and ward activity on day zero, by 28-day survival status

	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Average number of nurses per 10 beds [a]	1.4 (1.1, 2.1)	1.7 (1.2, 7.0)	1.4 (1.1, 2.4)
Average number of HCA per 10 beds [b]	0.6 (0.4, 0.8)	0.7 (0.4, 0.9)	0.6 (0.4, 0.8)
Average number of agency per 10 beds [c]	0.0 (0.0, 0.3)	0.1 (0.0, 0.3)	0.0 (0.0, 0.3)
Average number of total staff per 10 beds [d]	2.3 (1.9, 2.8)	2.6 (2.0, 7.4)	2.3 (1.9, 3.1)
Ward activity per 10 beds [e]	3.7 (1.9, 7.3)	3.5 (1.8, 5.5)	3.7 (1.9, 6.9)

Notes: *Data are presented as median (IQR)*

Ward activity is defined as the number of patients admitted + number of patients discharged

Missing Data:

[a] *Data missing for 276 patients (234 survived, 42 died)*

[b] *Data missing for 277 patients (235 survived, 42 died)*

[c] *Data missing for 288 patients (245 survived, 43 died)*

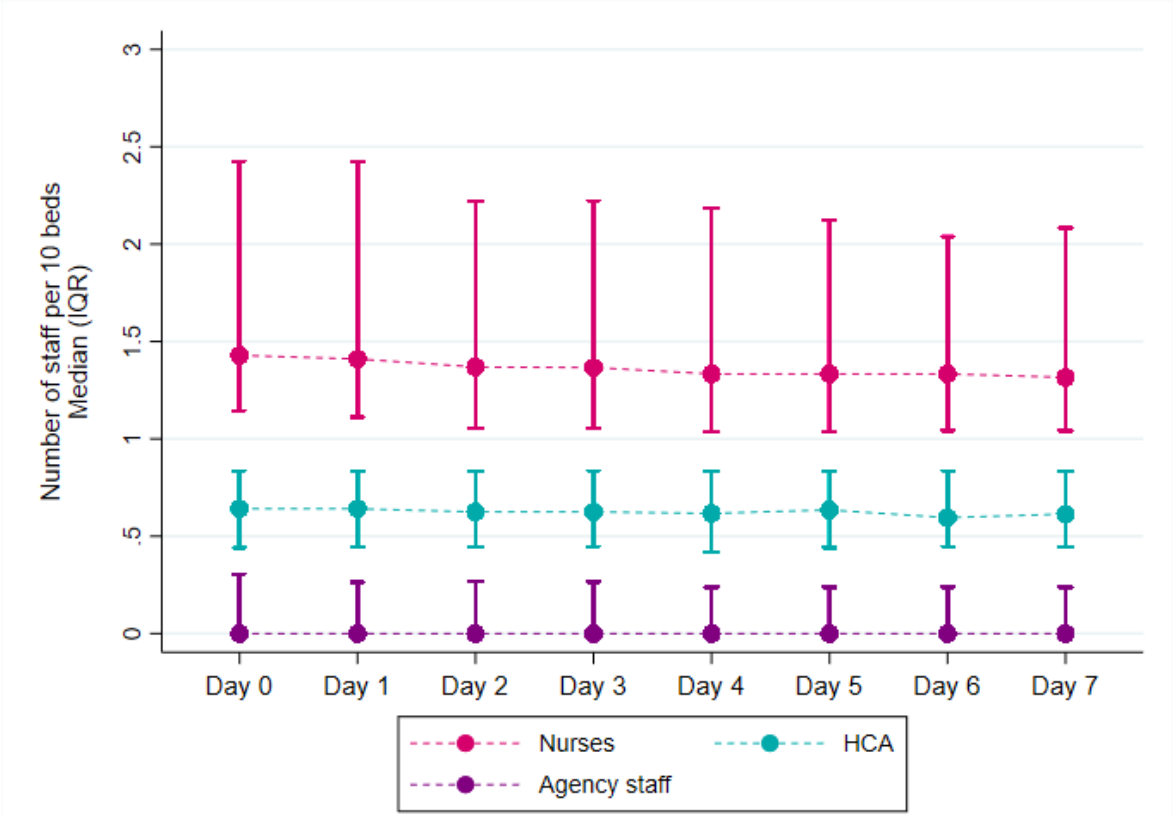
[d] *Data missing for 300 patients (255 survived, 45 died)*

[e] *Data missing for 153 patients (133 survived, 20 died)*

Abbreviations: *IQR=Interquartile range, HCA=Health care assistant*

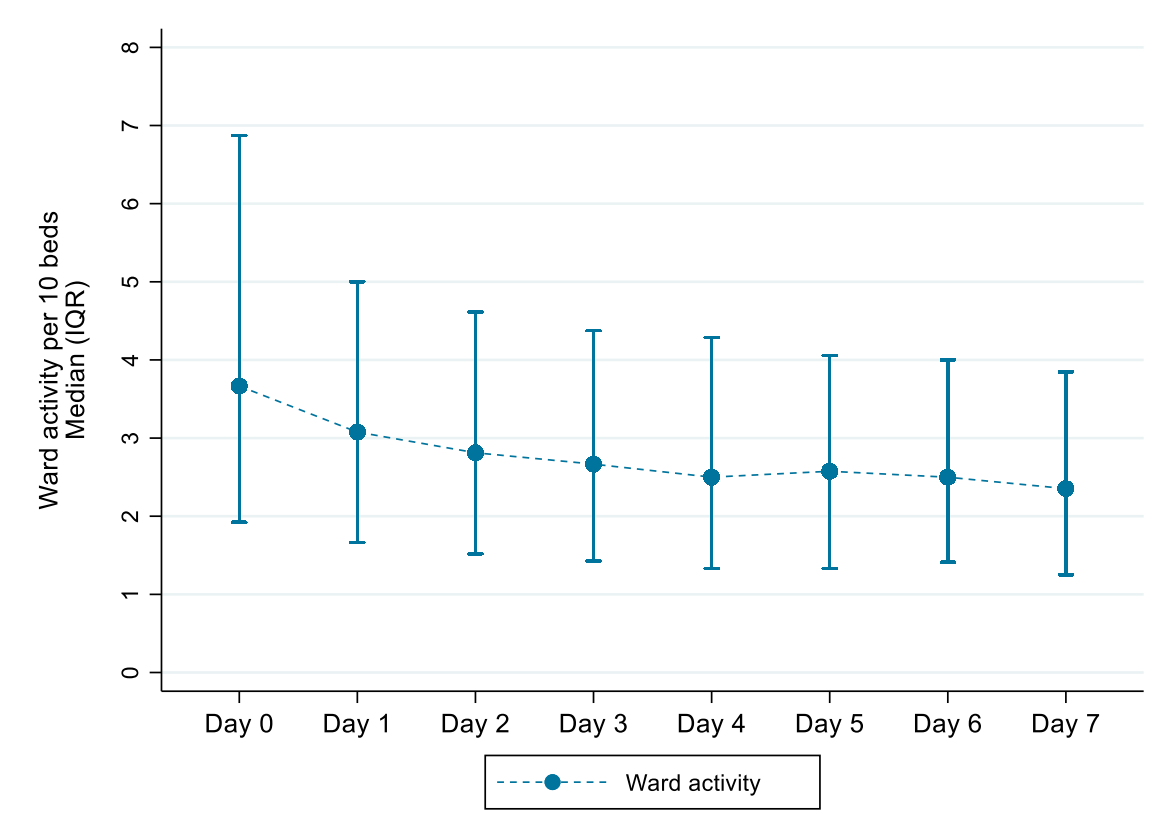
Over time the number of nurses per 10 beds pooled across all ward specialities decreased very slightly with a median of 1.4 nurses (IQR 1.1 to 2.4) on days 0 to 3 and a median of 1.3 nurses (IQR 1.0 to 2.2) on days 4 to 7 (Table 3.5 and Figure 3.4). HCA and agency staff remained stable. There was a large amount of variation in the number of nurses, HCA and agency staff on each day, although the variability on each day was similar across the 7 days. Ward activity was highest on day 0 with a median of 3.7 admissions or discharges per 10 beds (IQR 1.9 to 6.9) and steadily decreased across the 7 days to 2.4 (IQR 1.3 to 3.8) by day 7 (Table 3.5 and Figure 3.5).

Figure 3.4 Average number of staff per 10 beds, by day since blood sample



Abbreviations: *HCA=Health care assistant, IQR=Interquartile range*

Figure 3.5 Average ward activity per 10 beds, by day since blood sample



Abbreviations: *IQR=Interquartile range*

Table 3.5 Staffing levels and ward activity, by day since blood sample

		Average number per 10 beds over the 3 shifts			Ward activity per 10 beds
Day	N patients	NHS-employed nurses	HCA	Agency	
0 [a]	1676	1.4 (1.1, 2.4)	0.6 (0.4, 0.8)	0.0 (0.0, 0.3)	3.7 (1.9, 6.9)
1 [b]	1654	1.4 (1.1, 2.4)	0.6 (0.4, 0.8)	0.0 (0.0, 0.3)	3.1 (1.7, 5.0)
2 [c]	1581	1.4 (1.1, 2.2)	0.6 (0.4, 0.8)	0.0 (0.0, 0.3)	2.8 (1.5, 4.6)
3 [d]	1518	1.4 (1.1, 2.2)	0.6 (0.4, 0.8)	0.0 (0.0, 0.3)	2.7 (1.4, 4.4)
4 [e]	1470	1.3 (1.0, 2.2)	0.6 (0.4, 0.8)	0.0 (0.0, 0.2)	2.5 (1.3, 4.3)
5 [f]	1401	1.3 (1.0, 2.1)	0.6 (0.4, 0.8)	0.0 (0.0, 0.2)	2.6 (1.3, 4.1)
6 [g]	1335	1.3 (1.0, 2.0)	0.6 (0.4, 0.8)	0.0 (0.0, 0.2)	2.5 (1.4, 4.0)
7 [h]	1266	1.3 (1.0, 2.1)	0.6 (0.4, 0.8)	0.0 (0.0, 0.2)	2.4 (1.3, 3.8)

Notes: *Data are presented as median (IQR)*

Ward activity is defined as the number of patients admitted + number of patients discharged

Missing data:

[a] NHS-employed nurses (n=276); HCA (n=277); Agency (n=288); Ward activity (n=153)

[b] NHS-employed nurses (n=174); HCA (n=174); Agency (n=188); Ward activity (n=69)

[c] NHS-employed nurses (n=150); HCA (n=150); Agency (n=158); Ward activity (n=41)

[d] NHS-employed nurses (n=128); HCA (n=128); Agency (n=142); Ward activity (n=30)

[e] NHS-employed nurses (n=132); HCA (n=132); Agency (n=144); Ward activity (n=39)

[f] NHS-employed nurses (n=120); HCA (n=121); Agency (n=133); Ward activity (n=37)

[g] NHS-employed nurses (n=112); HCA (n=113); Agency (n=125); Ward activity (n=31)

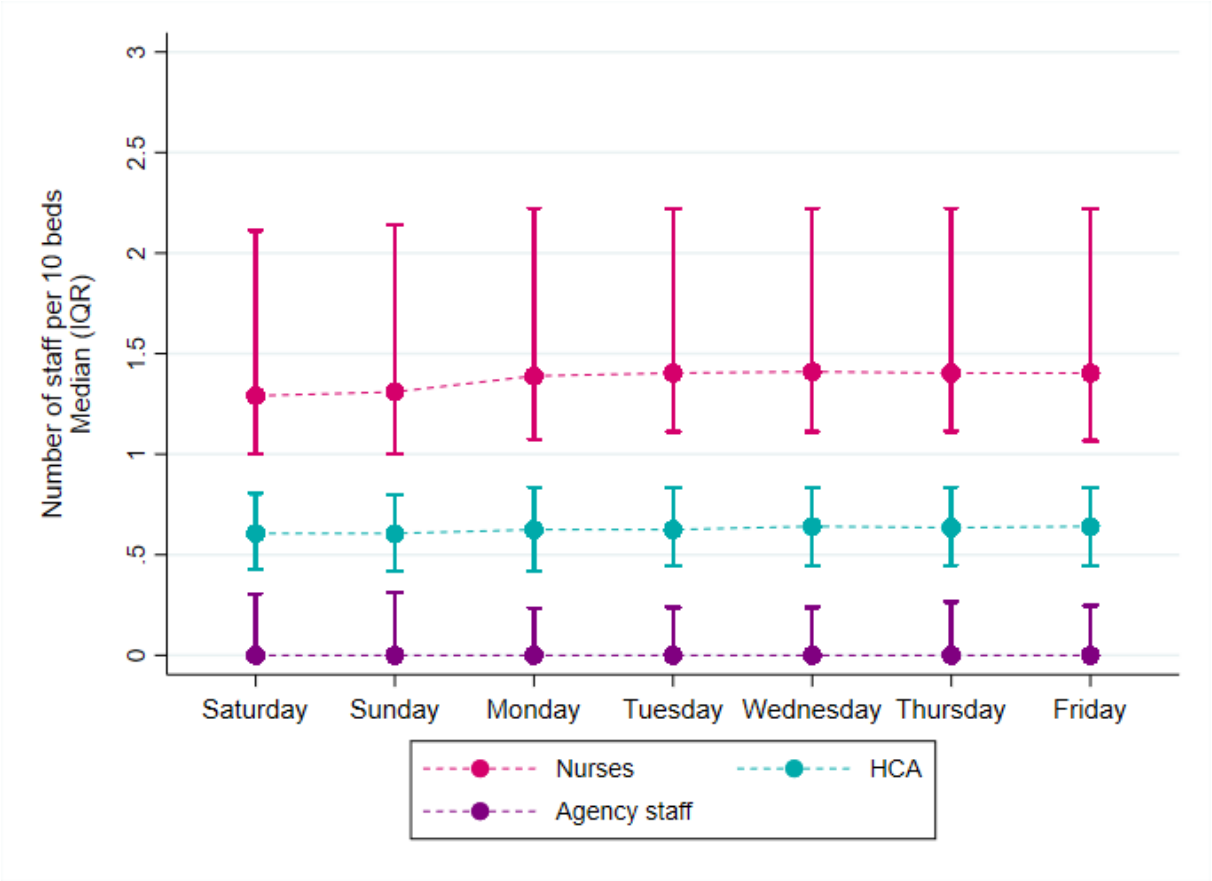
[h] NHS-employed nurses (n=115); HCA (n=116); Agency (n=127); Ward activity (n=34)

Abbreviations*: HCA=Health care assistant, IQR=Interquartile range, NHS=National Health Service*

There was some suggestion of slightly reduced numbers of nurses at weekends compared to weekdays but the average number of HCA and agency staff did not appear to change throughout the course of a week (see Table 3.6 and Figure 3.6).

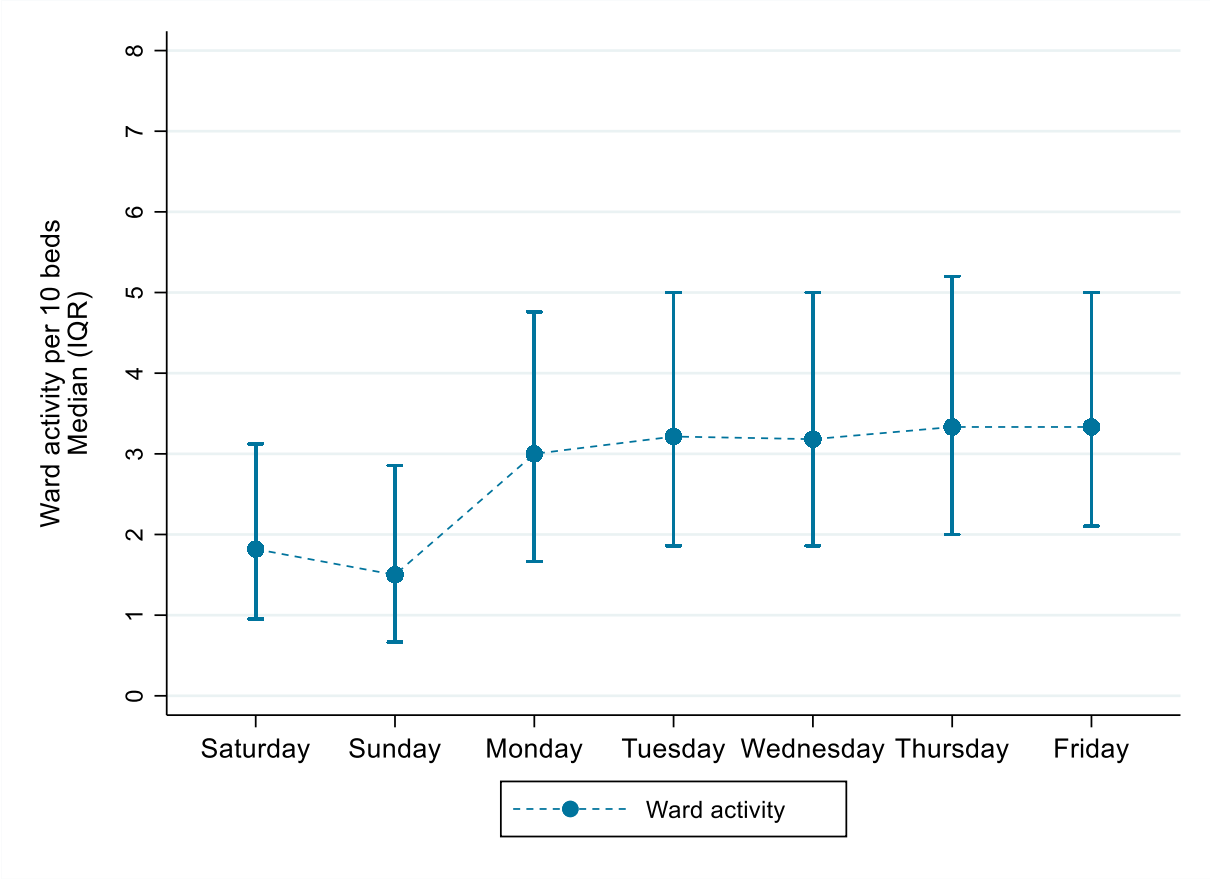
Ward activity followed a similar trend to that of nursing levels, the quietest days being Saturday and Sunday with a median of 1.5 (IQR 0.7 to 2.9) and 1.8 (IQR 1.5 to 4.5) admissions or discharges per 10 beds, respectively, compared to a median above 3 on all other days (Table 3.6 and Figure 3.7).

Figure 3.6 Average number of staff per 10 beds, by day of week



Abbreviations: *HCA=Health care assistant, IQR=Interquartile range*

Figure 3.7 Average ward activity per 10 beds, by day of week



Abbreviations: *IQR=Interquartile range*

Table 3.6 Staffing levels and ward activity, by day of week

		Average number per 10 beds over the 3 shifts			Ward activity per 10 beds
Day of week	**n**	**NHS-employed nurses**	**HCA**	**Agency**	
Sunday [a]	1648	1.3 (1.0, 2.1)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	1.5 (0.7, 2.9)
Monday [b]	1705	1.4 (1.1, 2.2)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	3.0 (1.7, 4.8)
Tuesday [c]	1718	1.4 (1.1, 2.2)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	3.2 (1.9, 5.0)
Wednesday [d]	1717	1.4 (1.1, 2.2)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	3.2 (1.9, 5.0)
Thursday [e]	1706	1.4 (1.1, 2.2)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	3.3 (2.0, 5.2)
Friday [f]	1690	1.4 (1.1, 2.2)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	3.3 (2.1, 5.0)
Saturday [g]	1626	1.3 (1.1, 2.2)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	1.8 (1.5, 4.5)

Notes: *Data are presented as median (IQR)*

Ward activity is defined as the number of patients admitted + number of patients discharged

Missing data:

[a] NHS-employed nurses (n=149); HCA (n=151); Agency (n=162); Ward activity (n=40)

[b] NHS-employed nurses (n=169); HCA (n=169); Agency (n=184); Ward activity (n=54)

[c] NHS-employed nurses (n=160); HCA (n=160); Agency (n=173); Ward activity (n=50)

[d] NHS-employed nurses (n=162); HCA (n=162); Agency (n=178); Ward activity (n=56)

[e] NHS-employed nurses (n=165); HCA (n=165); Agency (n=179); Ward activity (n=51)

[f] NHS-employed nurses (n=156); HCA (n=157); Agency (n=170); Ward activity (n=49)

[g] NHS-employed nurses (n=155); HCA (n=156); Agency (n=168); Ward activity (n=43)

Abbreviations: *HCA=Health care assistant, IQR=Interquartile range*

Ward movements

The number of ward movements per patient between days 0 and 7 for patients who were alive and in hospital on day 7 (as the number of movements is bounded by number of days alive for participants who died or were discharged before day 7) are given in Table 3.7. Overall, 58.5% did not move wards at all and 29.7% moved just once. The maximum number of ward movements was four which was experienced by three patients (0.2%), all of whom survived. The proportion of patients who moved wards in the first 7 days was higher in patients who survived to day 28 compared to those who died (42.7% *vs* 34.1%).

Table 3.7 Total numbers of ward movements between day zero and day seven

	Survived (n=1,096)	Died after day 7 (n=170)	Overall (n=1,266)
Number of ward movements			
0	617/1076 (57.3%)	110/167 (65.9%)	727/1243 (58.5%)
1	329/1076 (30.6%)	40/167 (24.0%)	369/1243 (29.7%)
2	105/1076 (9.8%)	15/167 (9.0%)	120/1243 (9.7%)
3	22/1076 (2.0%)	2/167 (1.2%)	24/1243 (1.9%)
4	3/1076 (0.3%)	0/167 (0.0%)	3/1243 (0.2%)

Note: *Data are presented as n (%)*
This table is restricted to patients still in hospital and alive on day 7

The most common movement was within a speciality (e.g. medicine to medicine), experienced by 24.1% of patients (Table 3.8) and was more common in patients who survived compared to those who died (26.7% *vs* 14.2%). Similarly, movements from critical care to any other speciality, and from medicine to surgery, were more common in patients who survived than for those who died (7.5% *vs* 2.4% and 6.3% *vs* 2.1%). However, rates of movements to a critical care unit and from surgery to medicine were similar in survivors and patients who died (6.7% *vs* 8.9% and 2.3% *vs* 1.5%).

Table 3.8 Type of ward movements between day zero and seven

Type of movement	Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Movement to critical care	87/1301 (6.7%)	30/337 (8.9%)	117/1638 (7.1%)
Movement from critical care	98/1301 (7.5%)	8/337 (2.4%)	106/1638 (6.5%)
Movement within a ward speciality	347/1301 (26.7%)	48/337 (14.2%)	395/1638 (24.1%)
Movement from medicine to surgery	82/1301 (6.3%)	7/337 (2.1%)	89/1638 (5.4%)
Movement from surgery to medicine	30/1301 (2.3%)	5/337 (1.5%)	35/1638 (2.1%)

Note: *Data are presented as n (%)*

Antimicrobial therapy

Details of appropriate antimicrobial therapy, including the number of patients who were classified as having appropriate therapy for 36 hours or longer, and the median time to receipt and duration are given in Table 3.9 by survival status and organism. A higher proportion of patients who survived received an appropriate antimicrobial therapy compared to patients who died (91.1% *vs* 59.5%). Patients with

MSSA were most likely to receive appropriate antimicrobial therapy (89.5%) and patients with *Candida* were the least likely (61.2%).

Candida had the longest median time to receipt of appropriate antimicrobial therapy (72.0 hours, IQR 46.0 to 114.0) and those with non-ESBL-producing *E. coli*, MSSA and *P. aeruginosa* had the shortest (5.0 hours, IQR 1.0 to 26.0; 5.0 hours, IQR 1.0 to 30.0; and 5.0 hours, IQR 1.0 to 33.0, respectively). Overall time to receipt of appropriate antimicrobial therapy was slightly longer in patients who died; a median of 8.0 hours (IQR 1.0 to 38.0) compared to 7.0 hours (IQR 1.0 to 40.0) in patients who survived. However, this differed between organisms. For patients with *Candida* and MRSA, the time to receipt of appropriate therapy was longer, on average, for those who died compared to those who survived. The opposite was seen for ESBL producers and *P. aeruginosa*, with a shorter average time to receipt to appropriate antimicrobial therapy for patients who survived. For non-ESBL-producing *E. coli* and MSSA there were similar average times to receipt of appropriate therapies in patients who survived and those who died.

The overall duration of appropriate antimicrobial therapy was shorter for patients who died compared to those who survived; a median of 6.3 days (IQR 3.7 to 9.3) compared to 8.0 days (IQR 5.4 to 13.3), which will, at least in part, be due the fact that the duration of appropriate antimicrobial treatment is bounded by survival time. This trend was seen in all organisms except for non-ESBL-producing *E. coli* where there was a shorter median duration of appropriate antimicrobial therapy for patients who survived compared to those who died (6.6 days; IQR 4.5 to 9.0 *vs* 6.9 days; IQR 4.6 to 9.4). However, non-ESBL-producing *E. coli* had the highest survival rate of the six organisms. The longest median duration of appropriate antimicrobial therapy was for patients with MSSA (11.9 days; IQR 6.8 to 18.3) and the shortest for patients with non-ESBL-producing *E. coli* and *P. aeruginosa* (6.6 days, IQR 4.5 to 9.0; 6.6 days, IQR 4.8 to 9.2 respectively).

Table 3.9 Appropriate antimicrobial therapy, by organism and survival

		Survived (n=1,328)	Died (n=348)	Overall (n=1,676)
Non-ESBL *E. coli*	Received appropriate therapy	422/470 (89.8%)	52/72 (72.2%)	474/542 (87.5%)
	If Yes, median (IQR) time to receipt (hours)	5.0 (1.0, 27.0)	3.5 (0.0, 16.5)	5.0 (1.0, 26.0)
	If Yes, median (IQR) duration (hours)	158.5 (108.0, 216.0)	166.5 (111.5, 225.0)	159.5 (108.0, 217.0)
ESBL producer	Received appropriate therapy	123/134 (91.8%)	19/34 (55.9%)	142/168 (84.5%)
	If Yes, median (IQR) time to receipt (hours)	15.0 (1.0, 48.0)	28.0 (1.0, 51.0)	19.0 (1.0, 48.0)
	If Yes, median (IQR) duration (hours)	173.0 (138.0, 222.0)	134.0 (73.0, 184.0)	168.0 (133.0, 218.0)
Candida	Received appropriate therapy	60/82 (73.2%)	11/34 (32.4%)	71/116 (61.2%)
	If Yes, median (IQR) time to receipt (hours)	74.0 (45.5, 118.0)	60.0 (48.0, 96.0)	72.0 (46.0, 114.0)
	If Yes, median (IQR) duration (hours)	301.5 (120.0, 366.0)	158.0 (69.0, 234.0)	240.0 (119.0, 349.0)
MRSA	Received appropriate therapy	67/71 (94.4%)	13/29 (44.8%)	80/100 (80.0%)
	If Yes, median (IQR) time to receipt (hours)	37.0 (7.0, 69.0)	29.0 (3.0, 50.0)	35.5 (5.5, 66.0)
	If Yes, median (IQR) duration (hours)	280.0 (162.0, 590.0)	126.0 (74.0, 170.0)	209.0 (123.0, 424.0)
MSSA	Received appropriate therapy	387/406 (95.3%)	72/107 (67.3%)	459/513 (89.5%)
	If Yes, median (IQR) time to receipt (hours)	5.0 (1.0, 29.0)	5.5 (1.0, 31.5)	5.0 (1.0, 30.0)
	If Yes, median (IQR) duration (hours)	312.0 (183.0, 496.0)	169.5 (90.5, 273.5)	286.0 (164.0, 440.0)
P. aeruginosa	Received appropriate therapy	151/165 (91.5%)	40/72 (55.6%)	191/237 (80.6%)
	If Yes, median (IQR) time to receipt (hours)	4.0 (0.0, 35.0)	9.5 (1.5, 32.0)	5.0 (1.0, 33.0)
	If Yes, median (IQR) duration (hours)	165.0 (122.0, 232.0)	116.5 (88.0, 178.0)	159.0 (114.0, 222.0)
Overall	Received appropriate therapy	1210/1328 (91.1%)	207/348 (59.5%)	1416/1676 (84.5%)
	If Yes, median (IQR) time to receipt (hours)	7.0 (1.0, 40.0)	8.0 (1.0, 38.0)	7.0 (1.0, 40.0)
	If Yes, median (IQR) duration (hours)	192.0 (130.0, 320.0)	152.0 (89.0, 224.0)	184.0 (120.0, 306.0)

Note: *Data are presented as median (IQR) or n (%)*

Abbreviations: *ESBL= Extended Spectrum Beta-Lactamase, IQR=Interquartile range, MRSA=Methicillin-resistant Staphylococcus aureus, MSSA=Methicillin-susceptible Staphylococcus aureus*

3.4.2 Multiple imputation

I describe the comparisons of observed and imputed data for continuous variables below. The average number of staff (total NHS-employed nurses, HCAs and agency staff, averaged across the 3 daily shifts) by ward speciality can be seen in Table 3.10. Average staffing numbers in the observed data are broadly similar to the imputed data.

Table 3.10 Observed and imputed average number of staff, by ward speciality

Ward speciality	Observed data					Imputed data				
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
Medicine	5,890	5.8	2.5	1.3	29.3	36,875	6.0	2.8	0.0	33.0
Critical care	1,684	17.8	8.3	2.0	37.0	3,532	15.4	8.1	0.0	37.0
Surgery	3,010	6.3	2.9	0.0	19.3	18,858	6.6	3.1	0.0	31.7

***Note**: For each patient there are up to eight days of data (days 0 to 7) and 45 records for each day in the imputed data (one for each of the 45 imputed datasets).*
***Abbreviations:** SD=Standard deviation*

In terms of ward activity (Table 3.11), mean observed and imputed values are similar for surgical and critical care wards but somewhat higher in the imputed data for medicine ward specialities. With similar estimates in the imputed and observed data for the other ward specialities I considered that the imputation procedure was adequate. After the main analysis model was fitted the influence of this finding was explored if unexpected or extreme results for the medicine ward specialities were found. Finally, the mean numbers of beds were similar in the observed and imputed data for all three ward specialities (Table 3.12).

Table 3.11 Observed and imputed ward activity, by ward speciality

Ward speciality	Observed data					Imputed data				
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
Medicine	6,453	9.8	12.9	0.0	124.0	11,536	16.8	17.8	0.0	151.0
Critical care	1,742	7.0	4.9	0.0	70.0	886	7.8	8.1	0.0	83.0
Surgery	3,323	11.3	11.8	0.0	162.0	4,768	10.9	11.1	0.0	104.0

***Note**: For each patient there are up to eight days of data (days 0 to 7) and 45 records for each day in the imputed data (one for each of the 45 imputed datasets).*
***Abbreviations:** SD=Standard deviation*

Table 3.12 Observed and imputed number of beds, by ward speciality

Ward speciality	Observed data					Imputed data				
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
Medicine	6,445	26.3	8.7	3.0	56.0	11,896	28.2	9.0	3.0	62.0
Critical care	1,747	20.3	9.4	3.0	35.0	661	20.7	10.6	3.0	56.0
Surgery	3,315	29.9	10.5	5.0	62.0	5,128	28.8	7.6	3.0	62.0

***Note**: For each patient there are up to eight days of data (days 0 to 7) and 45 records for each day in the imputed data (one for each of the 45 imputed datasets).*
***Abbreviations:** SD=Standard deviation*

A box plot of the difference in average numbers of staff between consecutive days is given in Figure 3.8. The differences are similar in observed and imputed data, with more overall variability and narrower IQR in the observed data due to the larger amounts of data available. This was done for all other days and yielded similar results. Similar patterns are seen in the corresponding plots for ward activity and numbers of beds (Figure 3.9 and Figure 3.10).

Figure 3.8 Box plot of the difference in average numbers of staff between consecutive days

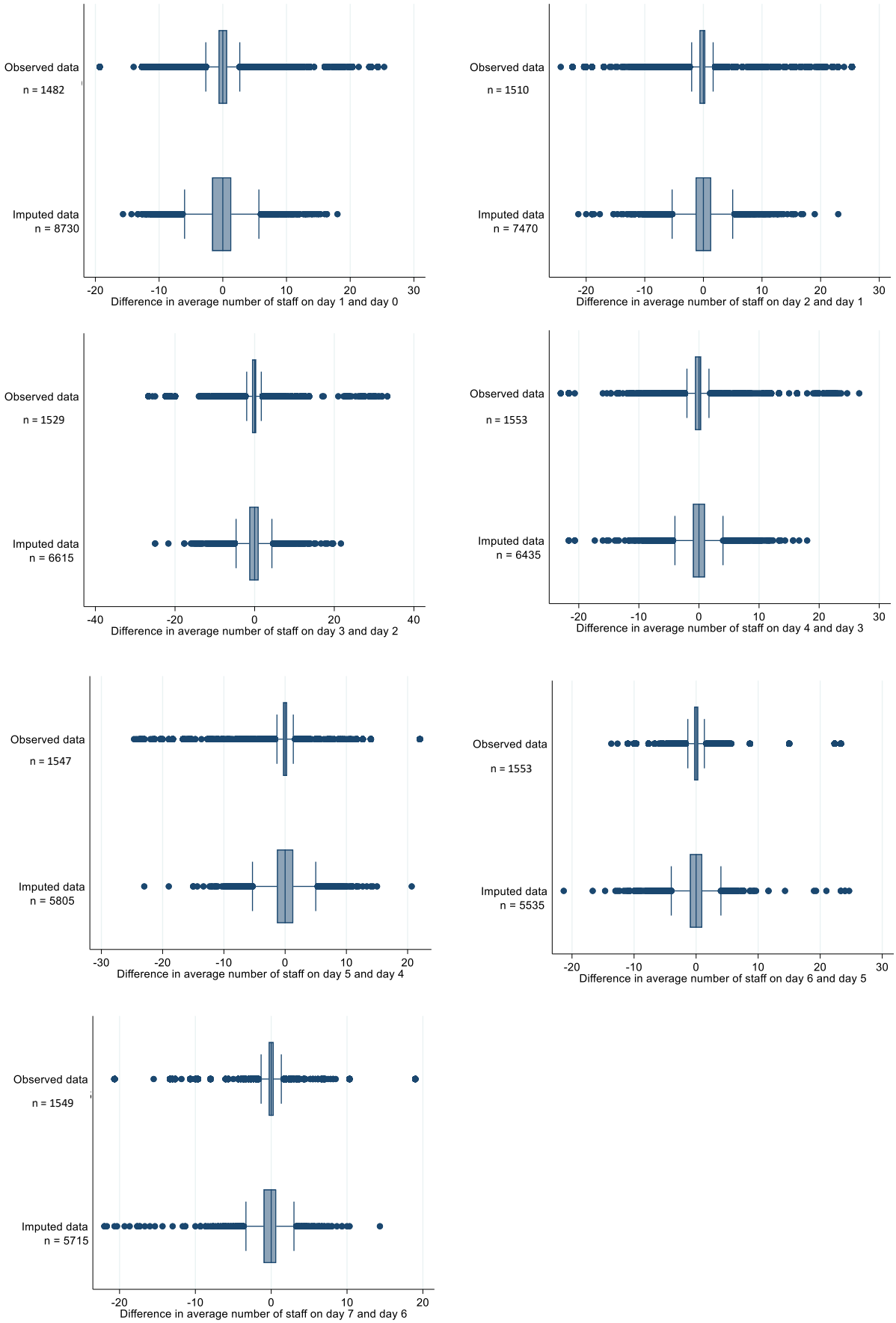


Figure 3.9 Box plot of the difference in ward activity between consecutive days

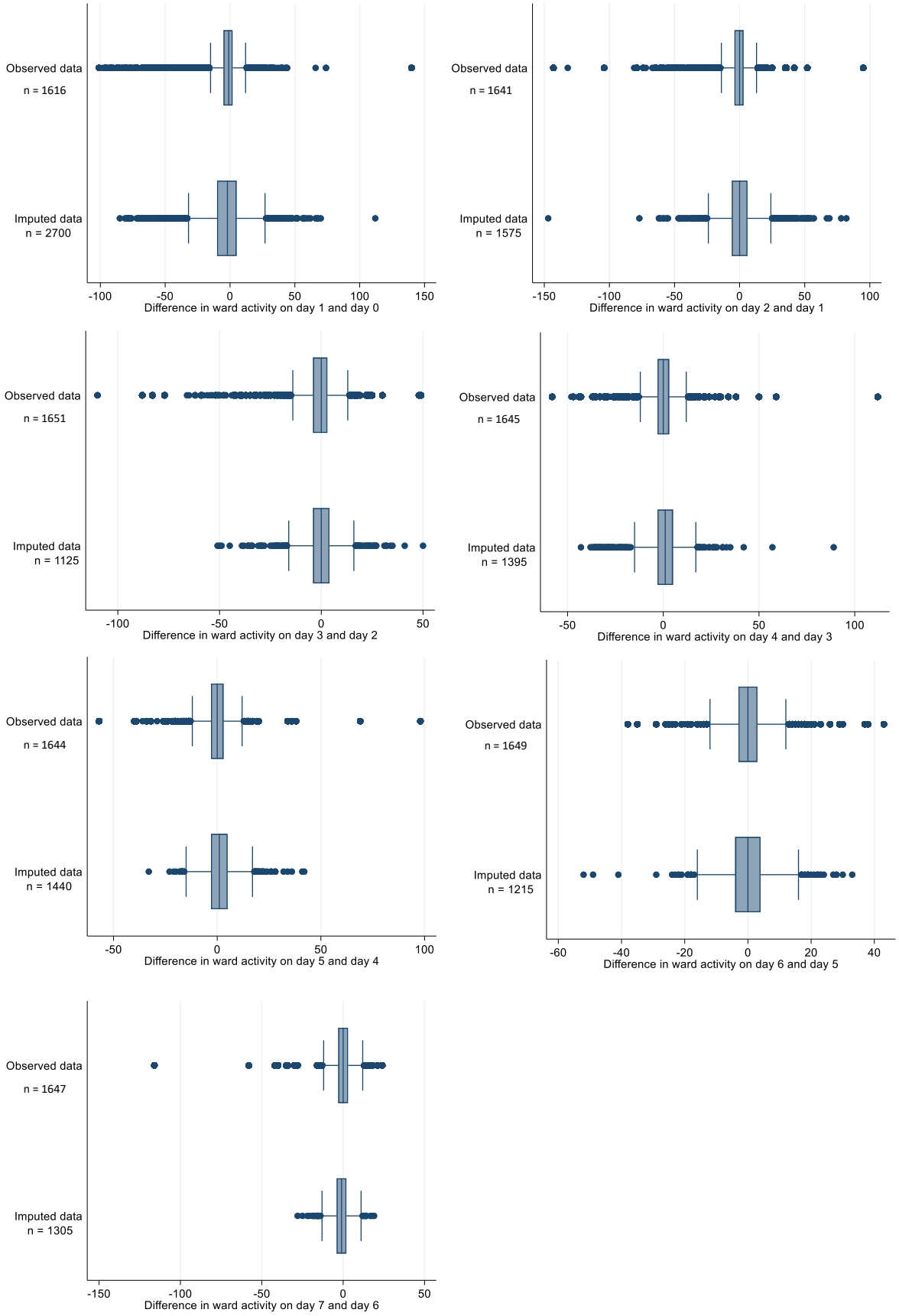
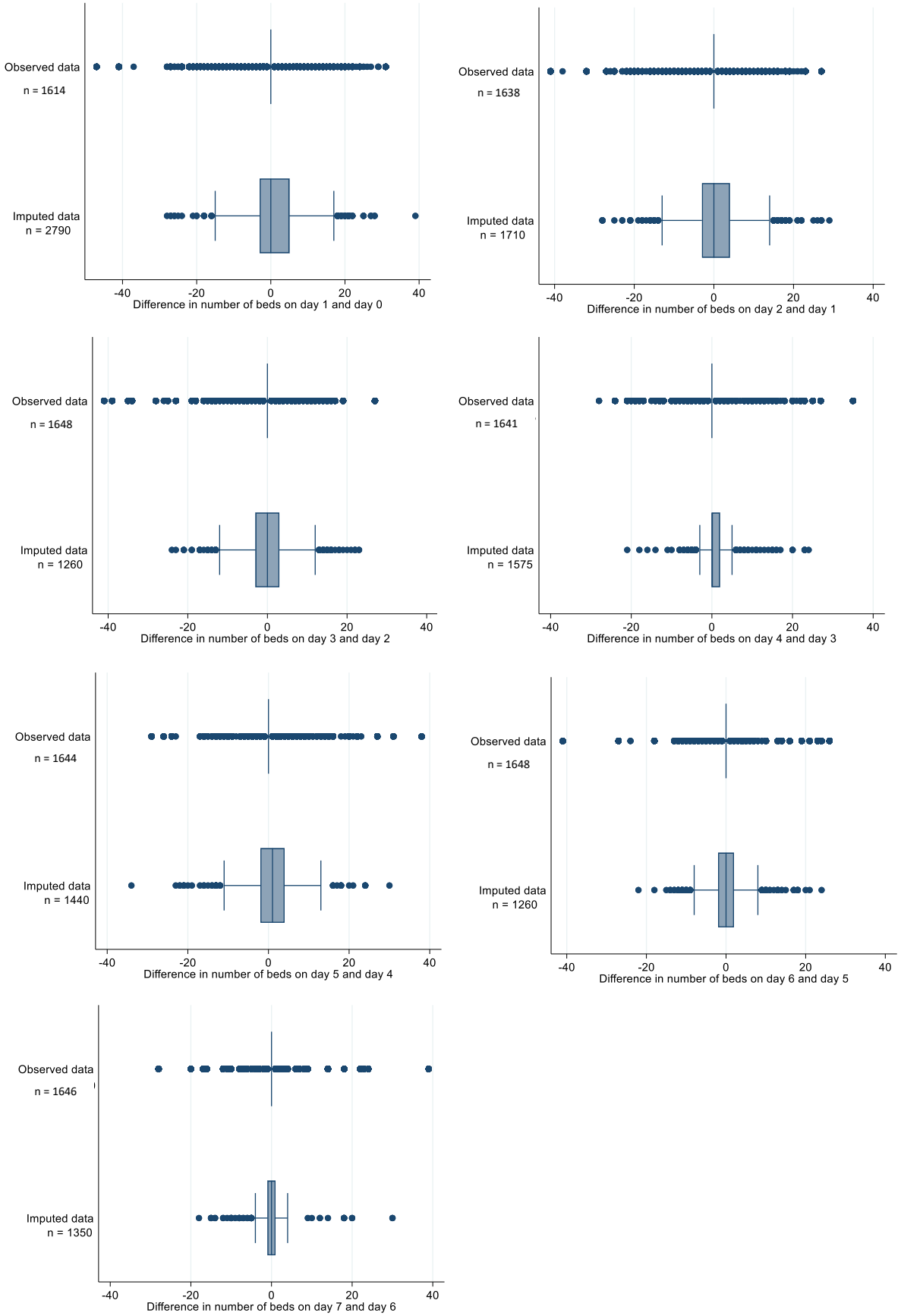


Figure 3.10 Box plot of the difference in number of beds between consecutive days



3.4.3 Primary outcome

Stage 1: Non-modifiable risk factors

In univariate analyses the non-modifiable characteristics associated with mortality (at the 20% level) were: age, weight, admission from nursing home, source of infection, myocardial infarction, cardiac arrest in previous seven days, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, mental disorientation, chronic obstructive pulmonary disease, peptic ulcer disease, ascites, renal support in past week, leukaemia within last 5 years, solid tumour within last 5 years, any other tumour within last 5 years, eGFR at day 0 (or nearest within previous seven days), serum albumin, bilirubin (total), neutrophil count at day 0 or closest, temperature at time 0, systolic blood pressure, on intravenous fluids at day 0, on ventilation at day 0, on vasopressor drugs at day 0, systemic corticosteroids in last 24 hours, abscess at time 0 and length of prior hospital stay (days). These variables were taken forward into the multivariable model selection process, and the final model can be seen in Table 3.13.

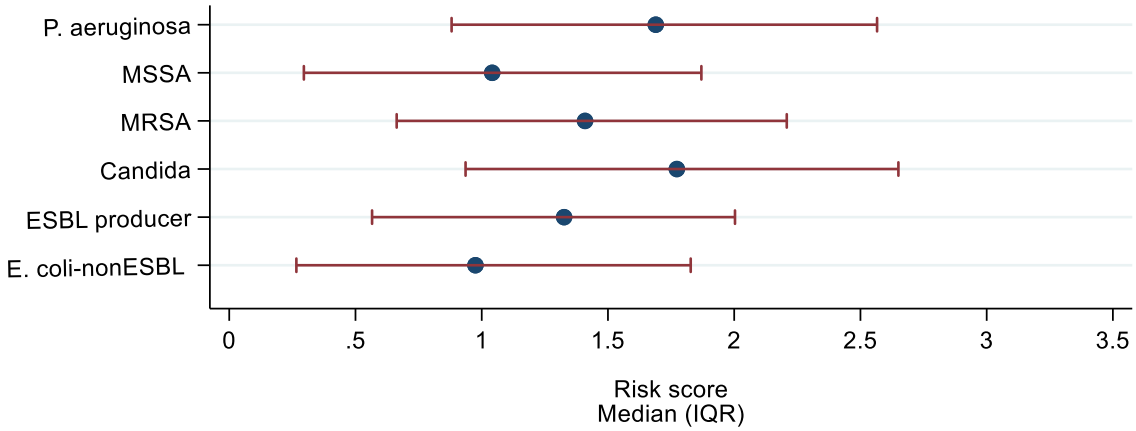
Table 3.13 Adjusted Cox model of non-modifiable risk factors on 28-day mortality

		Hazard ratio	95% CI	p-value
Age (years)		1.02	(1.01, 1.03)	<0.001
Temperature at time 0 (°C)		0.83	(0.75, 0.92)	<0.001
Weight (kg)		0.99	(0.98, 1.00)	0.14
Systolic BP at day 0 or closest (mmHg)		1.43	(1.17, 1.75)	<0.001
Admission from nursing home		1.36	(0.94, 1.98)	0.10
Serum albumin (g/L)		0.94	(0.92, 0.96)	<0.001
Bilirubin total (µmol/L)		1.00	(1.00, 1.00)	0.19
Renal support within 7 days before date 0		1.53	(1.01, 2.31)	0.044
On ventilation at day 0		1.33	(0.94, 1.90)	0.11
On intravenous fluids at day 0		1.21	(0.96, 1.53)	0.11
Systemic corticosteroids in last 24 hours		1.75	(1.35, 2.28)	<0.001
Abscess at time 0		0.63	(0.34, 1.15)	0.13
Congestive heart failure		1.35	(1.00, 1.84)	0.053
Peripheral vascular disease		1.50	(1.06, 2.12)	0.021
Cerebrovascular disease		1.32	(1.00, 1.74)	0.050
Peptic ulcer disease		1.38	(0.93, 2.05)	0.11
Ascites		2.04	(1.33 ,3.12)	0.001
Leukaemia within last 5 years		2.01	(1.36, 2.96)	<0.001
Solid tumour within last 5 years		1.38	(1.07, 1.79)	0.015
Any other tumour within last 5 years		1.54	(0.97, 2.45)	0.070
Centre	A	1	-	
	B	0.53	(0.35, 0.81)	
	C	1.25	(0.86, 1.84)	<0.001
	D	1.04	(0.67, 1.62)	
	E	0.93	(0.61, 1.41)	
Mental disorientation:	None	1	-	
	Grade I	0.89	(0.35, 1.44)	
	Grade II	1.54	(1.08, 2.19)	0.14
	Grade III	1.01	(0.61, 1.67)	
	Grade IV	1.40	(0.64, 3.06)	
eGFR	Normal/Stage 1	1	-	
	Stage 2 CKD	0.94	(0.66, 1.33)	
	Stage 3 CKD	0.88	(0.61, 1.27)	0.007
	Stage 4 CKD	1.55	(1.06, 2.26)	
	Stage 5 CKD	0.82	(0.48, 1.42)	
Source of infection	Gastrointestinal system	1	-	
	Line	1.62	(0.83, 3.15)	
	Lower respiratory tract	4.71	(2.58, 8.58)	
	Skin and surgical site	1.73	(0.89, 3.37)	<0.001
	Systemic & site uncertain	3.37	(1.96, 5.78)	
	Urinary tract	1.29	(0.73, 2.30)	
	Other	1.56	(0.74, 3.30)	

Abbreviations: *CI=Confidence interval, CKD=chronic kidney disease*

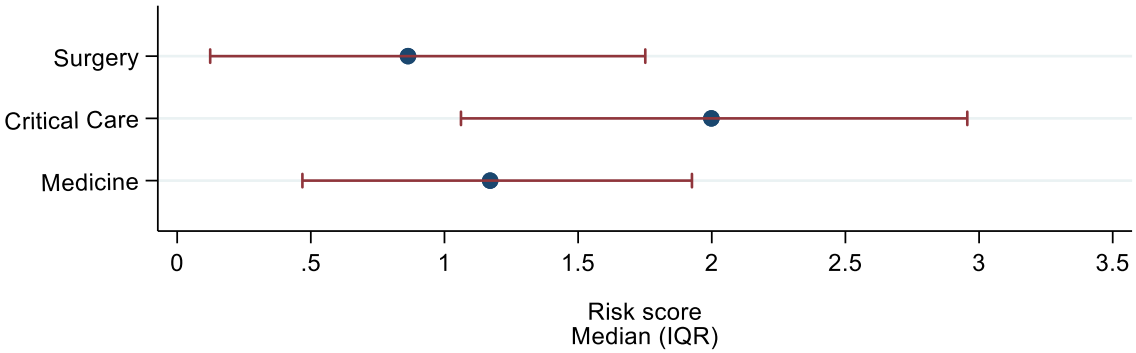
The risk score summarised by organism can be seen in Figure 3.11 and by ward speciality on day 0 in Figure 3.12. Patients with *Candida* had on average the highest risk score, median 1.77 (IQR 0.94 to 2.65) and those with non-ESBL-producing *E. coli* the lowest, median 0.98 (IQR 0.27 to 1.83). As expected, patients in a critical care ward had on average a higher risk score than patients in surgery or medicine, median 2.00 (IQR 1.06 to 2.96) compared to 0.86 (IQR 0.12 to 1.75) in surgery and 1.17 (IQR 0.47 to 1.93) in medicine.

Figure 3.11 Patient risk score by organism



Abbreviations: *ESBL= Extended Spectrum Beta-Lactamase, MRSA=Methicillin-resistant Staphylococcus aureus, MSSA=Methicillin-susceptible Staphylococcus aureus, IQR=Interquartile range*

Figure 3.12 Patient risk score by ward speciality on day zero



Abbreviations: *IQR=Interquartile range*

Stage 2: Modifiable risk factors

Interaction terms that were statistically significant and included in the model were i) risk score by ward speciality, ii) time to receipt of appropriate antimicrobial therapy by organism. Interactions of ward speciality with average staff and ward activity were forced in, *a priori*. Likelihood ratio test results for all tests of interactions are given in Table 3.14.

Table 3.14 Model for modifiable risk factors: likelihood ratio tests for interaction terms

	Risk factor	P-value
Interaction with organism:	Risk score	0.83
	Central line	0.51
	Peripheral line	0.63
	Urinary catheter	0.44
	Time to appropriate antimicrobial therapy	0.0145
Interaction with ward speciality:	Risk score	<0.001
	Average staff (forced into model)	0.98
	Ward activity (forced into model)	0.061
	Within ward movement	0.57

Note: *p <0.1 suggests that the effect of the risk factor on 28-day mortality differs between organisms or ward specialities, respectively. For example, the effect of time to appropriate antimicrobial therapy on 28-day mortality is not the same for different types of organism*

Model checks suggested that the hazards for time to receipt of appropriate antimicrobial therapy were not proportional. Further examination of the data suggested that the hazards were proportional within each of three intervals: days 0–6, days 7–13, and day 14 onwards, but not across the three intervals. Therefore, follow-up time was categorised into these intervals and the effect of time to appropriate antimicrobial therapy was estimated separately for each interval. After fitting the model with this interaction, the proportional hazards assumption was met on the basis of Schoenfeld residuals with an overall p-value of 0.51.

The final model indicated that ward speciality, ward activity, movement within ward speciality, movement from a critical care ward and time to receipt of appropriate antimicrobial therapy in the first 7 days were associated with mortality within 28 days, after adjustment for other factors (Figure 3.13).

The effect of risk score on mortality was greatest for patients in surgical (HR 2.89; 95% CI 2.13 to 3.90) and medical wards (HR 2.77; 95% CI 2.35 to 3.27). For patients in critical care, the effect was still highly statistically significant, but with a smaller effect size (HR 1.84; 95% CI 1.54 to 2.19). The presence of central lines, peripheral lines and urinary catheters were not significantly associated with 28-day mortality.

The average staff per 10 beds was not significantly associated with 28-day mortality, although the estimated effect was greater for surgical wards (HR 0.95; 95% CI 0.63 to 1.44) compared to medicine (HR 1.00; 95% CI 0.86 to 1.16) and critical care (HR 0.99; 95% CI 0.96 to 1.02). In terms of ward activity, in a medical ward for each increase in 1 admission or discharge per 10 beds there was a 4% (95% CI +1% to +6%) increased hazard of death within 28 days. This increase in hazard was slightly

higher in a critical care ward (12%; 95% CI +6% to +19%) and negligible in a surgical ward (3%; 95% CI -9% to +16%).

Patients who had moved wards within a speciality had a 33% reduced (95% CI: -7% to -52%) hazard of death within 28 days. There was also a 48% reduction (95% CI -76% to +11%) for patients who had moved out of a critical care ward. There was no evidence to suggest movement to a critical care ward, movement from surgery to medicine or from medicine to surgery impacted on 28-day mortality.

The effect of time to receipt of appropriate antimicrobial therapy varied depending on organism and time. During the first week, there was a highly significant effect for all organisms. The effect was greatest for MSSA with a 102% increase (95% CI +71% to +138%) in hazard of mortality associated with each day delay until the receipt of first appropriate antimicrobial therapy, and lowest for MRSA, with a corresponding 39% increase (95% CI +3% to +88%) in hazard of mortality. For patients who survived to day 7, the effect of time to receipt of first appropriate therapy on 28-day mortality was generally not associated with mortality.

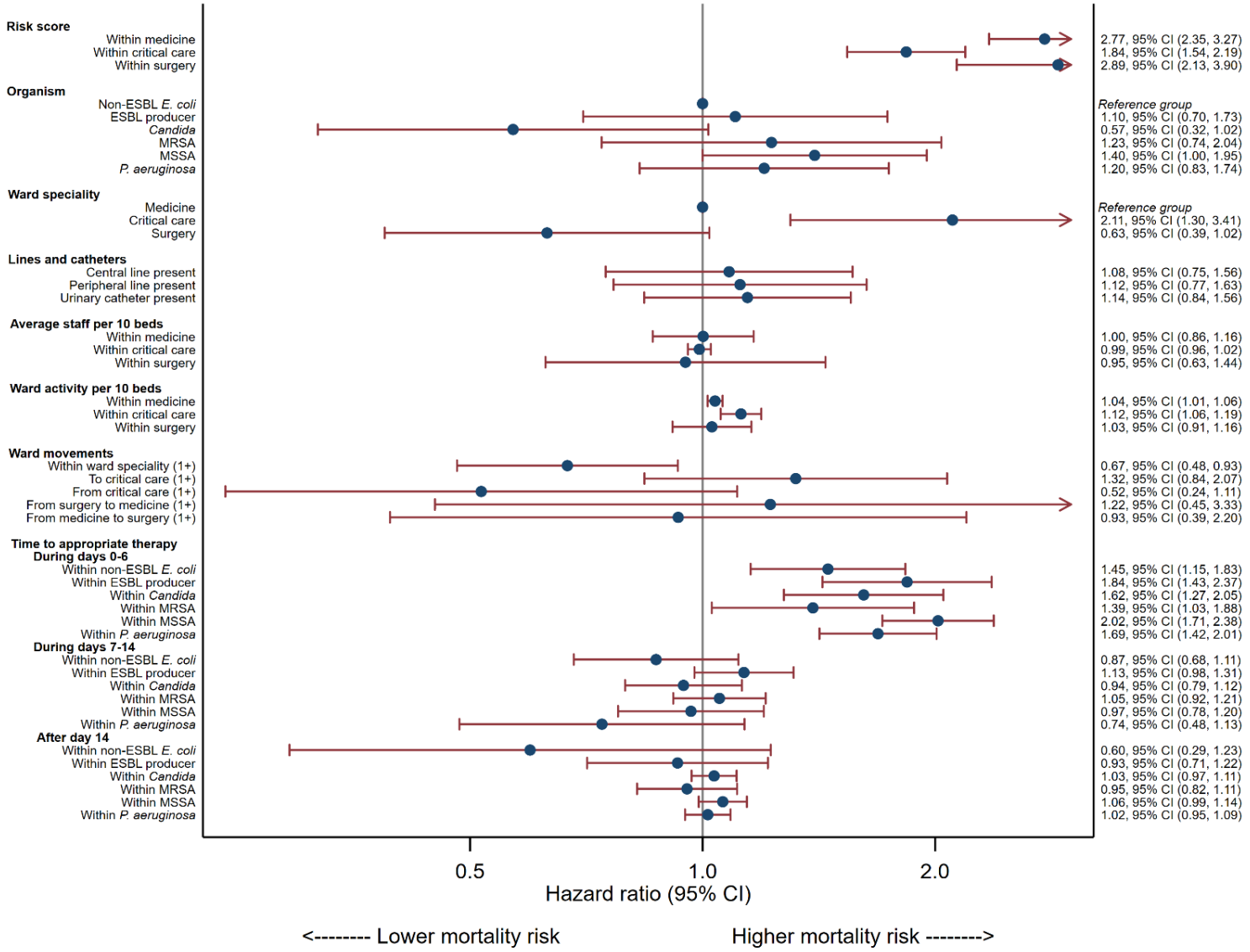
Deviance residuals were symmetrically distributed about zero and did not identify any outlying observations (all were within 2.5 standard deviations of zero). For the continuous variables, Martingale residual plots did not show any pattern and therefore I considered the linear assumption reasonable. The VIF was less than five for all variables and therefore I did not consider collinearity to be present. Predicted and observed risks are given by deciles of predicted risk for 28-day mortality in Table 3.15. The Brier Score was 0.003 which indicates a good measure of accuracy in prognostic classification.

Table 3.15 Predicted and observed risk by decile of predicted risk

Decile of predicted risk	Observed risk	Predicted risk*
1	0.01	0.01
2	0.07	0.02
3	0.08	0.03
4	0.09	0.05
5	0.15	0.07
6	0.18	0.11
7	0.21	0.15
8	0.28	0.22
9	0.32	0.34
10	0.29	0.65

** Mean predicted risk within each risk decile*

Figure 3.13 Adjusted Cox model of modifiable risk factors on 28-day mortality



[a] *Effect of organism is given for the time period 0 to 6 days and when time to appropriate therapy is 1 day*

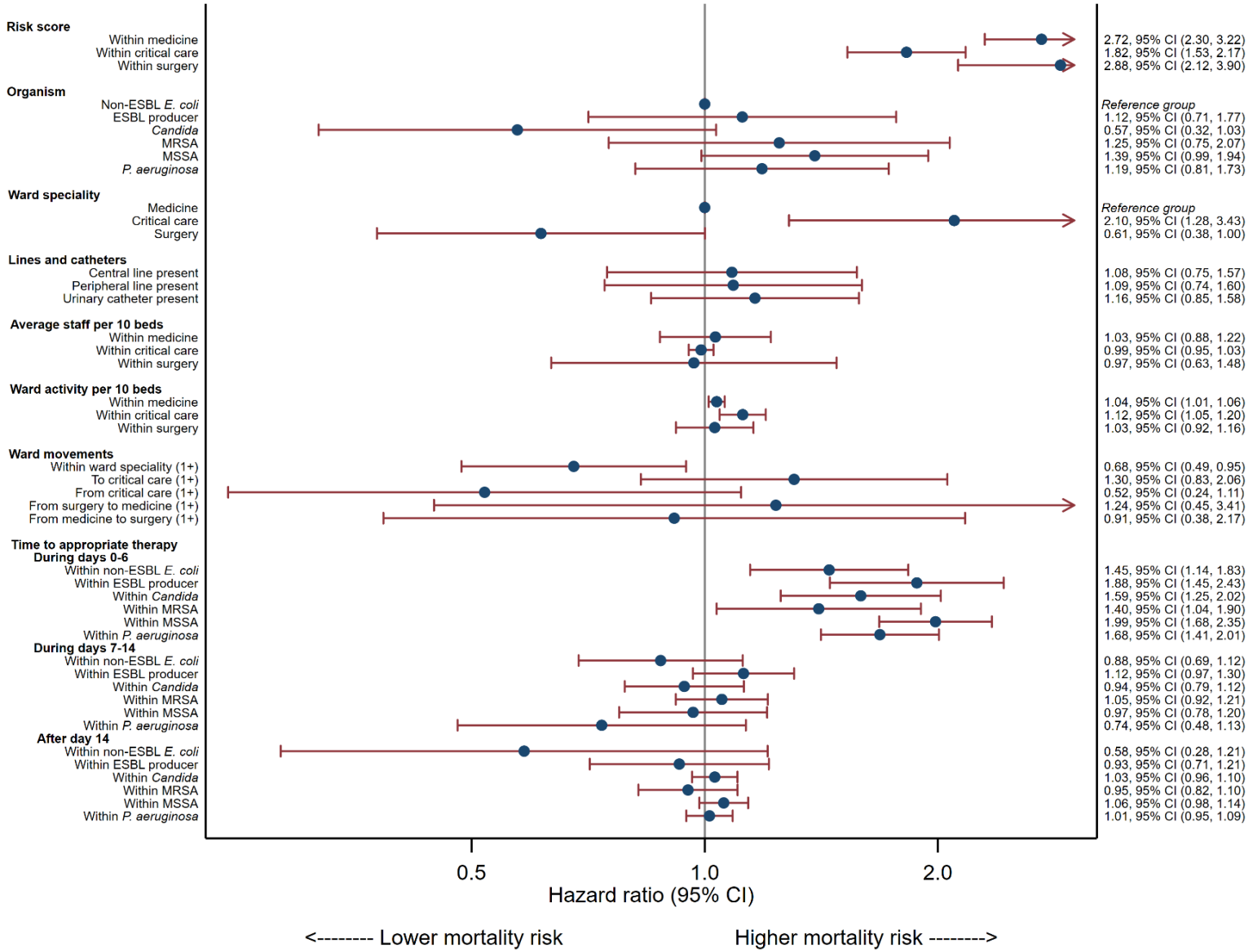
[b] *Effect of ward speciality is given for the median number of staff per 10 beds, median ward activity and median risk score*

Sensitivity analyses

SA1: Stratification by centre

Sensitivity analyses allowing the baseline hazard to vary across centres showed very little effect on primary outcome estimates (Figure 3.14).

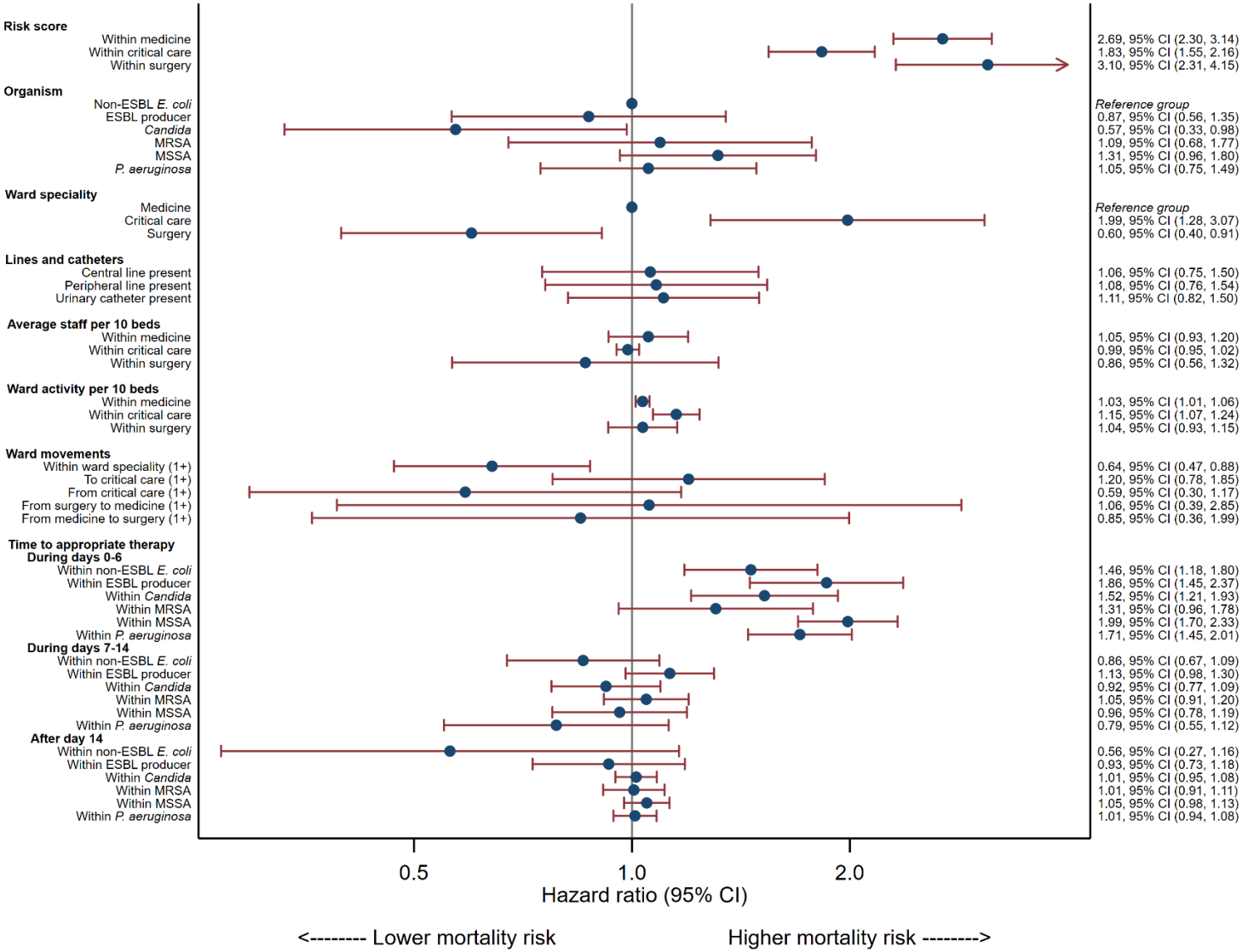
Figure 3.14 SA1: Sensitivity analysis of primary outcome: stratified by centre



SA2: Inclusion of polymicrobial infections

The sensitivity analysis assessing the potential impact of removing polymicrobial infections is summarised in Figure 3.15. There were a total of 1,837 patients in this analysis, with a total of 386 deaths. The impact of removing polymicrobial infections was judged to be minimal, with very little change to estimated hazard ratios.

Figure 3.15 SA2: Sensitivity analysis of primary outcome: including polymicrobial infections



[a] Effect of organism is given for the time period 0 to 7 days, when time to appropriate therapy is 1 day and when central lines are not present

[b] Effect of ward speciality is given for the median number of staff per 10 beds, median ward activity and median risk score

SA3: Removing "36-hour rule" for appropriate therapy

The sensitivity analysis assessing the impact of the removing the "36-hour rule" in defining time to appropriate antimicrobial therapy is given in Figure 3.16. Compared to the primary analysis: the effect of organism was increased, with all hazard ratios greater than 1 compared to the reference group of non-ESBL-producing *E. coli*, and the effect of time to appropriate antimicrobial therapy within the first week (days 0–6) was considerably reduced.

One factor that may contribute to this is that patients who die very soon after starting therapy may not have received the treatment long enough for it to take effect but, with no minimum duration required, the therapy is still defined as 'appropriate'. This would dilute the effect of time to receipt of truly appropriate therapy. This hypothesis is supported by the finding that the sensitivity analysis had the least impact for patients with *Candida*; the time to appropriate therapy for such patients is on average longer than for other organisms and so proportionately more deaths would have happened before receiving an appropriate treatment, irrespective of how long it was received for.

This sensitivity analysis was also performed increasing the minimum duration to 12 hours ("12-hour rule") and 24 hours ("24-hour rule") which showed similar effects to when using a minimum duration of 36 hours (the 36-hour rule), suggesting that the use of a minimum time on appropriate therapy in the definition is sensible: see Figure 3.17 and Figure 3.18.

Therefore, although removing the 36-hour rule completely did have an impact, it was pre-defined and therefore not subject to post hoc bias and I believe that the definition with no minimum duration at all is not a true reflection of appropriate therapy in clinical practice.

Figure 3.16 SA3: Sensitivity analysis of primary outcome: removal of "36-hour rule" in definition of time to appropriate therapy

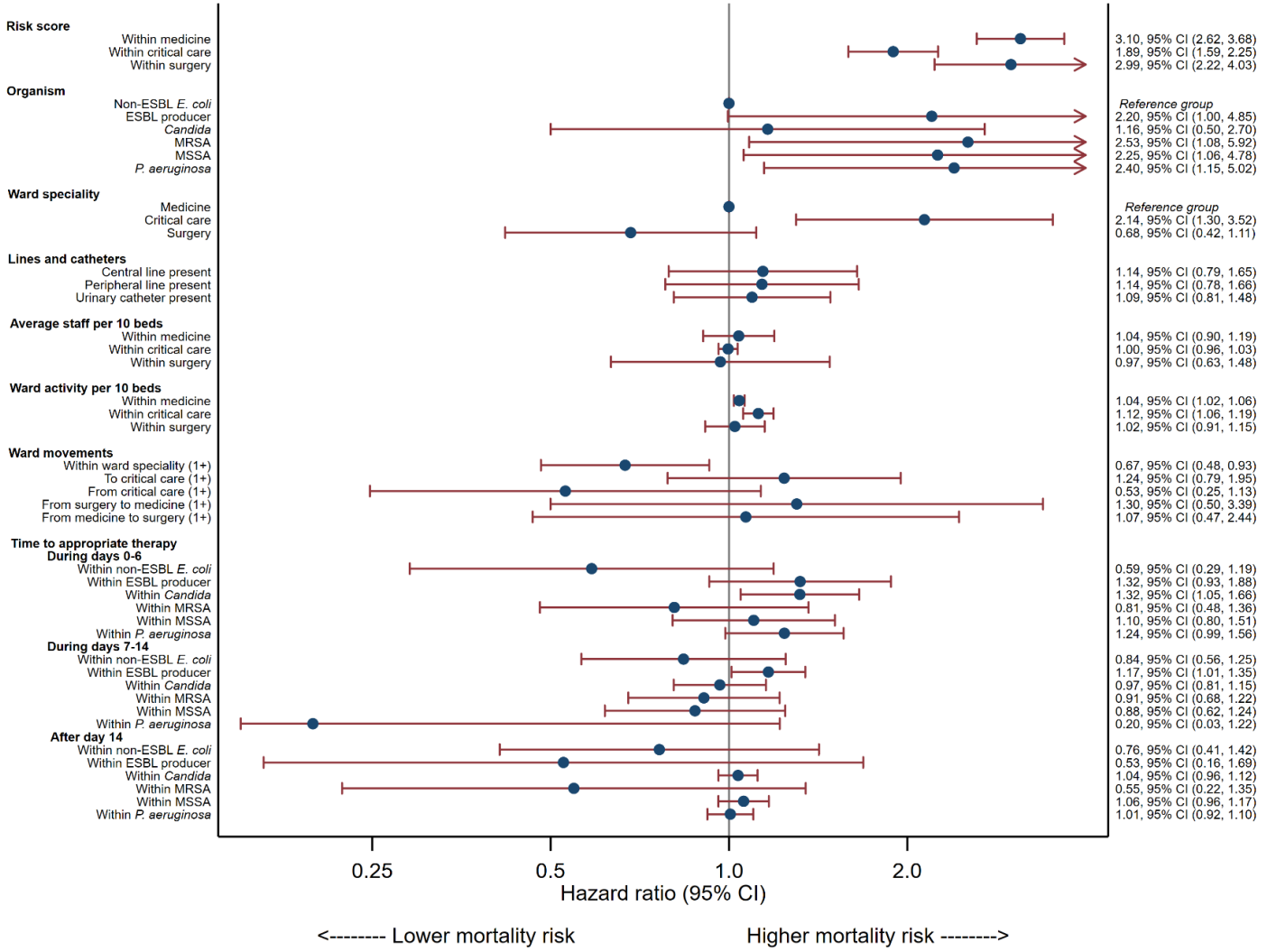


Figure 3.17 SA3: Sensitivity analysis of primary outcome: 12-hour (in place of 36-hour) rule in definition of time to appropriate therapy

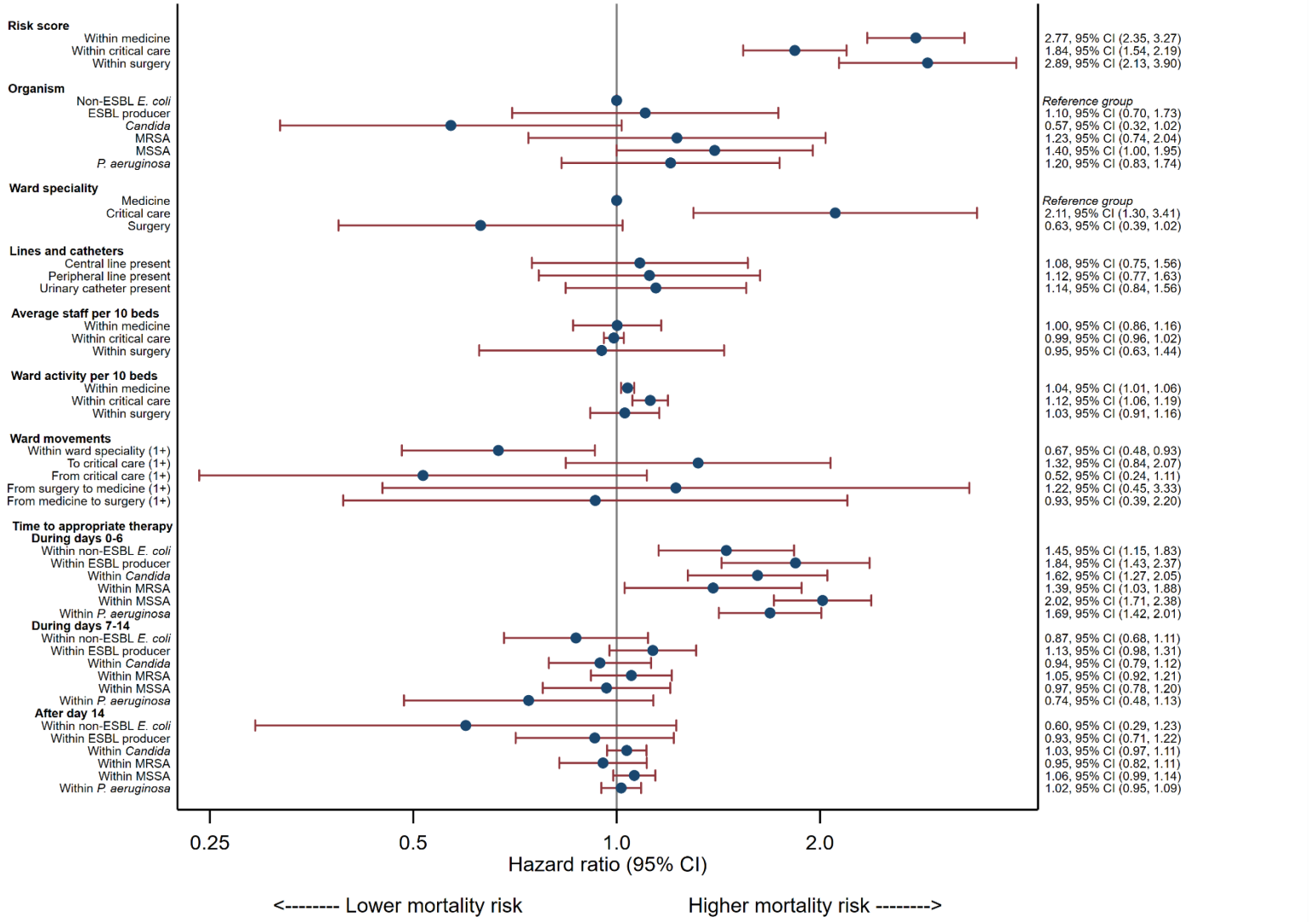
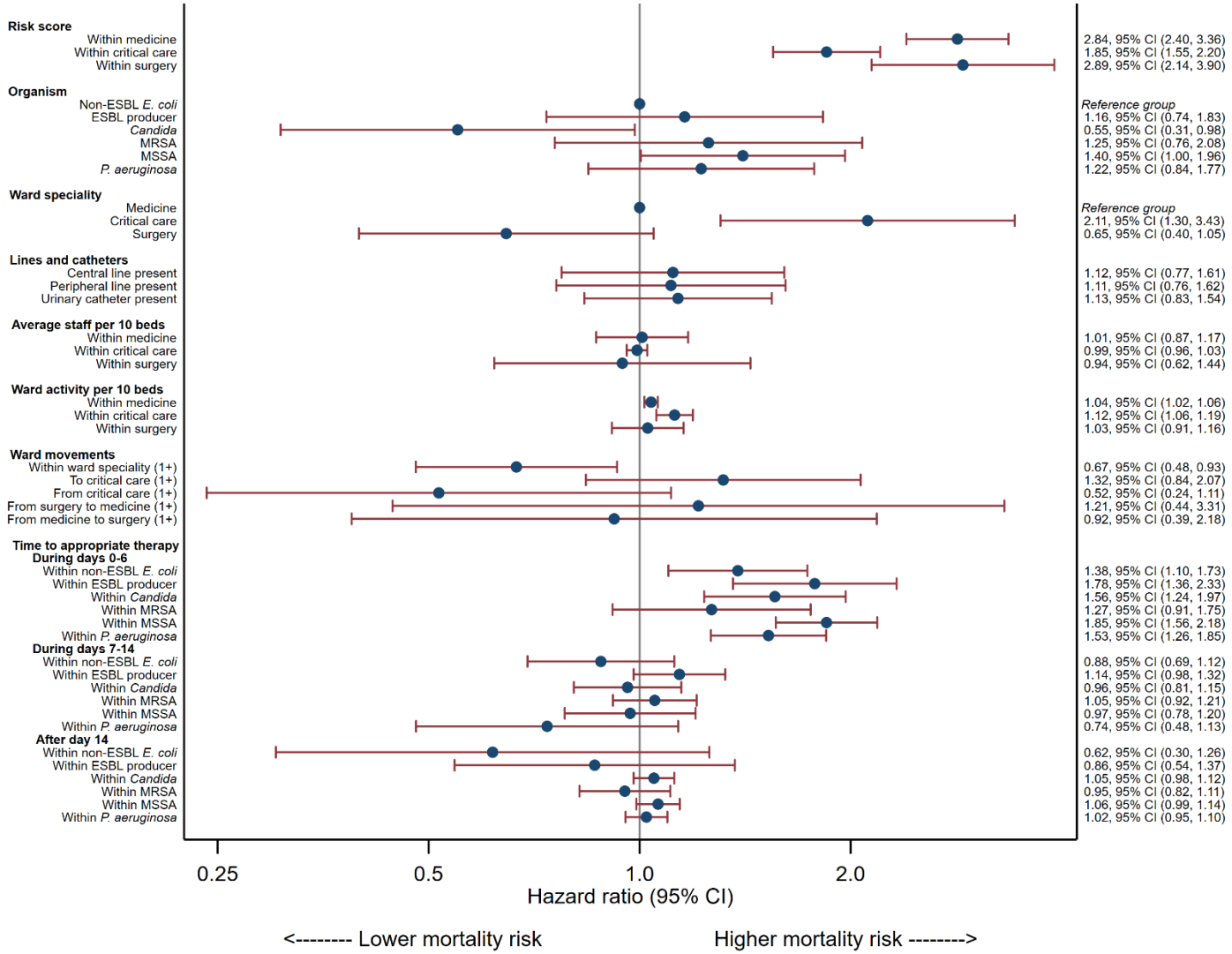


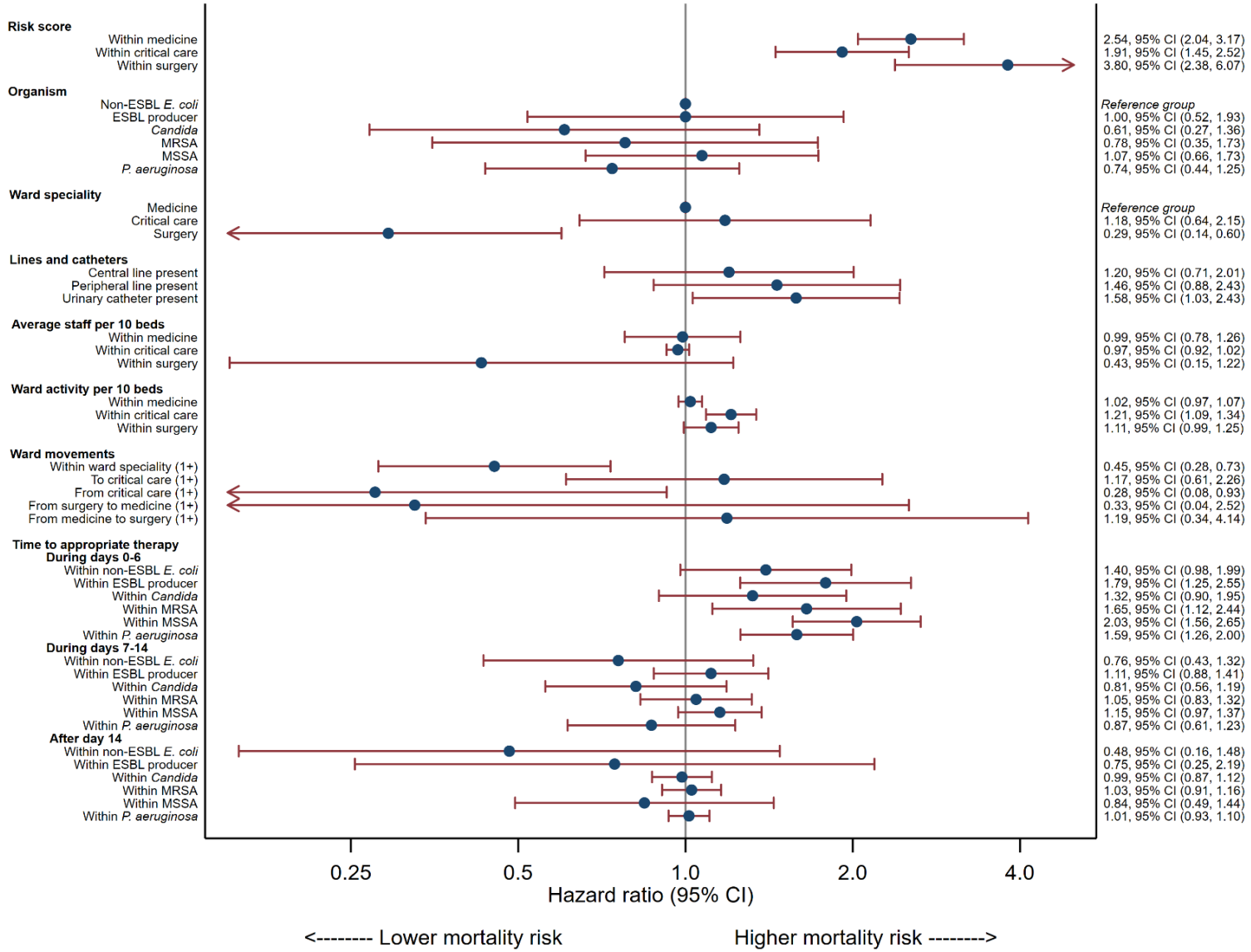
Figure 3.18 SA3: Sensitivity analysis of primary outcome: 24-hour (in place of 36-hour) rule in definition of time to appropriate therapy



SA4: Complete case analysis

Finally, the complete case analysis for the primary outcome is given in Figure 3.19 below. There are some changes to the estimated effects compared to the main primary outcome model, most noticeably the effect of risk score within surgery (HR 3.80; 95% CI 2.38 to 6.07) and the effects of ward speciality (HRs for critical care *vs* medicine 1.18; 95% CI 0.65 to 2.16, for surgery *vs* medicine 0.29; 95% CI 0.14 to 0.60). However, confidence intervals overlap and there is no impact on the interpretation of the results.

Figure 3.19 SA4: Sensitivity analysis of primary outcome, complete case analysis



3.5 Discussion

3.5.1 Summary

In this observational analysis, for the pathogen groups studied, ward speciality, ward activity, ward movement within speciality, movement from critical care and time to receipt of appropriate antibiotics were all independently associated with mortality.

3.5.2 Interpretation

There are a large number of publications relating organisational and management factors to infection control performance in acute hospitals including workforce and workload[94]. However, there are no previous studies relating ward staffing and activity to BSI outcomes. In our descriptive analysis of modifiable factors, patients who died were on wards where the average number of nurses per ten beds on day 0 was slightly higher than for those who survived, but this may reflect the higher mortality in intensive care units where nursing staffing was much higher. After adjustment for other factors there was no evidence of an effect of staffing levels on 28-day mortality, although the estimated effect was lowest in critical care, which has also been reported in other studies[91]. Interestingly, the number of NHS-employed nurses, healthcare assistants or agency staff working did not vary greatly by day of week, although nurse numbers were slightly lower at the weekend. However, ward activity was markedly lower at weekends – which is perhaps not surprising despite the current drive in the NHS towards a seven day working week[114]. Ward activity was highest on the day blood samples were taken and diminished over the following week, possibly as patients are moved from high activity settings such as emergency departments or admissions units to wards having more stable populations of patients undergoing recovery. It has been shown that increasing exposure to shifts with high turnover of patients is associated with an increase in the risk of death, however there is less information on the impact of workload on infection outcomes in particular[90]. In an adjusted model where ward activity was updated daily to reflect the ward activity where the patient spent most of the day, increased ward activity was associated with an increased hazard of death within 28 days. We also found that ward movements are associated with reduced hazard of death within 28 days. It is likely that movements within ward speciality and movements from critical care are related to improving patient condition as patients are moved from high intensity ward areas to those offering lower levels of immediate care.

Appropriate antimicrobial therapy has been shown to reduce mortality based on a large number of publications over the last 20 years[115]. There are several more recent systematic reviews and meta-analyses indicating that appropriate antimicrobial therapy has survival benefit in both BSI[116] and

severe sepsis[97, 117]. Our data shows that delays in administration of appropriate antimicrobials impact on outcome in BSI over days 0–6. Patients who received prompt appropriate antimicrobial therapy were less likely to die in the first week, and those patients who did survive the first week had apparently similar survival prospects over the next three weeks with or without the benefit of previous early appropriate therapy.

There was concern about the possibility of reverse causation in the conclusions of appropriate therapy, as it was predefined as treatment for at least 36 hours with an antimicrobial to which the organism was susceptible. This meant that deaths within 36 hours of a first dose of suitable antimicrobial could be associated with a lack of appropriate therapy and therefore strengthen the apparent effect of receiving appropriate therapy on survival. I therefore repeated the analysis of 28-day mortality with 24-hour, 12-hour and 0-hour rules in place of the 36-hour rule. Compared to the 36-hour rule, the apparent impact of time to appropriate therapy was reduced slightly with the 24-hour rule and slightly more so with the 12-hour rule. These results are consistent with reverse causation inflating the estimated effect, and shorter defined minimum periods reduced the extent of this. The 0-hour rule, however, gave quite different estimates with the estimated impact of time to appropriate therapy being greatly reduced, though I deemed this not to be a true reflection of appropriate therapy. Patients who die very soon after starting therapy are likely to have not received the treatment long enough for it to take effect and therefore it is unsurprising that the effect of time to receipt of truly appropriate therapy is highly diluted.

3.5.3 Strengths and limitations

A large number of data items were collected which enabled us to adequality control for potential confounding in the analysis and also allowed us to include variables that are predictive of missing data in a covariate of interest in the multiple imputation procedure making the missing at random assumption plausible.

There are some limitations of the study. Firstly, this study focussed on NHS-employed nurses, health-care assistants and agency nurses, but did not explore the impact of other medical staffing levels such as junior doctors and consultants which may merit analysis in future research. Number of consultant reviews was included in the original research plan however the data quality was deemed insufficient and was missing for >40% cases and therefore removed from the research plan.

The analysis of risk factors for mortality was split into those classified as non-modifiable factors and those that could be modified by changes in organisation or patient management. The complexity of addressing the analysis issues simultaneously resulted in several analytical challenges. The use of multivariable fractional polynomials within survival analysis models using imputed data can be useful

to find a best fitting model for a dataset with missing data, however it may not be appropriate to apply this method where straightforward interpretability of coefficients is required. The code to achieve this is available in standard statistical packages, such as Stata.

I used multiple imputation using chained equations to impute missing data. Many data items were collected in the study which enabled me to include variables that were predictive of missing data in a covariate of interest in the multiple imputation procedure. I therefore deem the missing at random assumption plausible. Conditional imputation can be used to impute longitudinal data, e.g. ward variables for each day were imputed conditional on patients being alive and in hospital on that day, using data from other ward variables on the other days. I considered two-fold imputation as an alternative approach to impute longitudinal data[118] in order to overcome the convergence issues however after exploring this approach I felt that it was less flexible in its specification (e.g. unable to use predictive matching). In addition, it requires numerous iterations in each time period which makes it computationally intensive. It has also been shown that the two-fold method produced slightly more biased and less precise estimates than the standard approach[119, 120]. As repeated measures were only collected over seven days, I felt that the standard fully conditional specification imputation approach would be most suitable with specifications on individual imputation models in place to avoid convergence problems.

Within the imputation procedure, I intended to allow for any interaction terms that were in the main analysis model by imputing separately for each category of one of the variables involved in any interactions. For example, if there was an interaction between ward speciality and one of the other variables, the imputation would be done separately for the different types of ward speciality. Unfortunately, computational problems prevented this. All the interactions involved either organism type or ward speciality. Models including the six categories of organism type did not converge, while the time-varying nature of ward speciality made it difficult to choose a time point at which to split the dataset in order to perform the imputation on a "one row per patient" model; data was also missing in ward speciality itself. I was also unable to include ward movements in the imputation model for ward speciality, again due to convergence problems. After running the imputation model, some variables had imputed values outside valid ranges and therefore the imputation was adapted to use predictive mean matching for these variables.

To account for risk factors which can vary across the study period, episodes can be split at intervals with risk factors being updated at each interval. Where risk factors are bounded by the outcome, a cumulative count can be used within the Stata command "stsplit" framework to help overcome this. This ensures that, for each day of risk, the maximum number of days exposed/unexposed does not

exceed the time at risk. This enabled me to reduce the risk of immortal time bias and can also be used when estimating the effect of duration of treatment on survival. Time dependent Cox models with time-varying indicators to classify exposure have been shown to reduce bias in other studies[101-104]. I applied a similar approach but extended the methodology by including an updated count variable rather than an indicator of exposure to examine the impact of time-to-receipt of therapy and number of ward movements.

Ward data was not recorded post day seven, so I assumed for patients who survived and were not discharged prior to day seven, ward variables and ward movements for days 7 to day 28 or death/discharge were constant for this period. I believe this is a reasonable assumption as it is not anticipated that many patients would move wards after day seven so staffing/activity would be expected to be constant.

The hazards for time to receipt of appropriate antimicrobial therapy were not proportional and therefore follow-up time was categorised into three intervals: days 0–6, days 7–13, and day 14 onwards and the effect of time to appropriate antimicrobial therapy was estimated separately for each interval. However, caution needs to be taken when interpreting time varying period specific hazard ratios due the potential 'built in' selection bias[121] i.e. the calculation of the hazard ratio for period 'x' to 'y' is restricted to people who survive to time 'x' and they may be a select cohort of the population at time 0. Effect estimates should therefore be interpreted as associations and not causal effects.

3.5.4 Conclusions

Applying these methods enabled me to determine that ward speciality, ward activity, ward movement within speciality, movements from critical care, and time to receipt of appropriate antibiotics, were all risk factors associated with mortality within 28 days. Using cumulative counts within a one row per day framework in a survival analysis can reduce the risk of bias. The approach that I followed uses already established methodology, so it is easily implemented in standard statistical packages, including Stata.

CHAPTER 4 DURATION OF THERAPY

In this chapter, I will investigate the relationship between duration of treatment and mortality. I will discuss methods to deal with immortal time bias in assessing the effect of duration of treatment on survival and then apply these methods to compare duration of treatment on 28-day mortality in patients with *Staphylococcus aureus* BSI (MRSA or MSSA). I will start by discussing the current literature on duration of therapy for the treatment of MRSA and MSSA BSI (section 4.1) and then move on to present methods for dealing with immortal time bias (section 4.2) and results of these analyses (section 4.3). At the end of the chapter, I reflect on the findings as well as the strengths and limitations of the methodologies used (section 4.4).

4.1 Introduction

Published guidelines suggest long course duration of 4-6 weeks of therapy for treatment of complicated BSI, but this can be reduced to two weeks for uncomplicated infections[122-124]. Long course duration is advised to reduce the risk of relapse of infection, however, there remains uncertainty around the optimum length of duration of therapy for the treatment of *S. aureus* bacteraemia (SAB)[47]. Long course therapy has the obvious benefit of maximising the chance of infection resolution and reducing the risk of relapse of infection but can lead to increased costs and unnecessary antibiotic exposure leading to increased adverse events and also increasing the risk of antibiotic resistance[125]. Reducing the exposure of antibiotics by shortening the duration of treatment could lower the risk of adverse effects of treatment and reduce the risk of antibiotic resistance development which is a growing problem worldwide[29, 126, 127].

Evidence on the use of shorter therapy is limited and has been based on observational data which are subject to confounding and bias. Confounding is often appropriately described and addressed in these studies, however the presence of immortal time bias is often ignored. This bias arises in a survival comparison between individuals with longer and shorter treatment duration, as only patients who survive a long time can receive treatment for a long time i.e. there is a period of follow-up in which the outcome cannot occur[128]. This can artificially inflate the effect of duration of treatment and is known as immortal time bias, as discussed in Chapter 3 section 3.1.

Table 4.1 provides a summary of studies reporting on the association between duration of therapy and outcome in patients with SAB. Key studies are discussed further below.

Table 4.1 Summary of studies reporting on the association between duration of therapy and outcome

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings	Approach used to address ITB
Thorlacius-Ussing (2021)[129]	Retrospective multi centre cohort/Denmark (6 hospitals)	1005 patients	Low risk MS-SAB	Primary: 90-day all-cause mortality. Secondary: 90-day relapse.	DOT 6-10 days *vs* 11-16 days	No significant differences in 90-day mortality: OR 1.05 (95% CI, 0.71–1.51).	Index date defined as the date of completed antimicrobial therapy
Abbas (2020)[130]	Retrospective single-centre cohort study/Switzerland	530 patients	SAB	Primary: 90-day all-cause mortality. Secondary: 90-day relapse.	DOT ≤14 days *vs* >14 days	Uncomplicated SAB: aHR 0.85 (95% CI 0.41 to 1.78), p= 0.67. Complicated SAB: aHR 0.32 (95% CI 0.16 to 0.64), p=0.001.	Excluded patients who died before day 14.
Eichenberger (2020)[131]	Review/Multi-country	Review: Variable sample sizes	SAB	Review: Variable outcomes	Review: Variable exposures	Insufficient evidence to justify widespread adoption of shorter DOT.	Review: Variable adjustment
Berrevoets (2019)[132]	Retrospective multi centre cohort/The Netherlands (2 hospitals)	76 patients	SAB	3-month SAB-specific mortality, recurrent infection, overall mortality	Identifying patients at risk of complicated SAB without metastatic infection using F-FDG PET/CT and echocardiography who may be successfully treated with a short course	No difference in outcome was observed between patients identified as high risk without signs of metastatic infection on 18F-FDG PET/CT, and patients with uncomplicated SAB when treated with a 14-d course of IV antibiotic therapy.	Patients treated <7days excluded.

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings	Approach used to address ITB
					treatment (14 days).		
Kim (2019)[133]	Prospective multi-centre cohort/Korea (11 hospitals)	1866 patients	SAB	Composite outcome of 90-day mortality or 30-day recurrence	Short therapy sufficient group (SS): <14 days *vs* ≥14 days. Warrant longer therapy group (LW): <28 *vs* ≥28 days.	SS group: <14 days *vs* ≥14 days OR 1.24 (95% CI 0.69 to 2.25), p=0.471. LW group: <28 *vs* ≥28 days OR 1.68 (95% CI 1.00 to 2.83), p=0.050	Deaths before completing antibiotic treatment excluded.
Thorlacius-Ussing (2019)[134]	Protocol for multi-centre RCT/ Denmark (up to 10 centres)	Target sample size 284 patients	Uncomplicated SAB	90-day survival without failure to treatment or infection relapse	DOT 7 days *vs* 14 days	Trial not yet completed	Analysed on an ITT basis.
Kempley (2015)[135]	Retrospective cohort/UK (2 hospitals)	90 patients	Neonatal SAB	Adverse event, recurrence of SAB, mortality	DOT <14 days *vs* 14- 27 days *vs* >27 days.	No difference between adverse events 22% *vs* 41% *vs* 47% (p= 0.46) or recurrence of SAB 0% *vs* 6% *vs* 5% (p=1.00). No deaths in any group after excluding deaths whilst on antibiotics.	Deaths before completing antibiotic treatment excluded.
Chong (2013)[136]	Prospective single-centre cohort/Korea	111 patients	Uncomplicated SAB	12 week relapse/recurrent of SAB, crude mortality, and treatment failure	Short-course therapy (<14 days) *vs* intermediate course therapy (≥14 days).	Recurrence: 7.9% *vs* 1.4%, p=0.12. Treatment failure: 26.3% *vs* 21.9%, p=0.64. Mortality 18.4% *vs* 21.9%, p=0.67	No adjustment.

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings	Approach used to address ITB
Havey (2013)[137]	Retrospective single centre cohort/Canada	100 patients	Critically ill patients with BSI	Relapse of bacteraemia within 30 days, relapse of infectious syndrome within 30 days, secondary infection with other pathogen(s) or infectious syndrome(s), incident infection with C difficile during hospital stay, in-hospital mortality.	DOT ≤10 days *vs* > 10 days	Relapse of bacteraemia: 5% *vs* 8%, p=1.00, Relapse of infectious syndrome: 5% *vs* 6%, p=1.00, Secondary BSI: 26% *vs* 17%, p=0.50, *C.difficile*: 21% *vs* 9%, p=0.23. Mortality: 26% *vs* 25%, p=1.00	Excluded patients who died whilst on therapy within 10 days.
Asgeirsson (2011)[138]	Retrospective multicentre cohort/Iceland (15 hospitals)	300 patients	SAB	30-day mortality, relapse of infection,	Uncomplicated: Optimal ≥14 days *vs* acceptable 10-13 days *vs* inadequate < 10 days. Complicated: Optimal ≥28 days *vs* acceptable 24-27 days *vs* inadequate < 24 days.	No statistically significant association between adequacy of antibiotic therapy and mortality or relapse of infection	Patients who died before 14 days were excluded.
Kreisel (2006)[139]	Retrospective single centre cohort/US	397 patients	SAB	Recurrence of SAB	DOT ≤14 days *vs* >14 days	No association between DOT ≤14 days and risk of relapse (RR 0.68, 95% CI 0.44 to 1.04), p=0.10	Excluded deaths occurring before having completed antibiotics.

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings	Approach used to address ITB
Fatkenheuer (2004)[140]	Retrospective single centre cohort/Germany	229 patients	SAB	One-year mortality	DOT ≤14 days *vs* >14 days	No difference in survival compared to between patients treated with ≤14 days therapy and patients treated with >14 days	No adjustment.
Pigrau (2003)[141]	Retrospective single centre cohort/Spain	87 patients	SAB	Survival and infection recurrence within 3 to 12 months.	Short-course therapy (10–14 days) – no comparator	Uncomplicated catheter-related SAB was treated effectively with short-course therapy with high doses of antibiotics	No adjustment.
Chang (2003)[142]	Prospective multi-centre cohort/Country not specified (6 hospitals)	505 patients	SAB	Relapse of infection	Optimal *vs* acceptable *vs* suboptimal.	Duration of antibiotic treatment was not associated with relapse (14.0% *vs* 7.7% *vs* 9.9%)	Patients who died before 14 days were excluded.
Jensen (2002)[143]	Prospective multi-centre cohort/Denmark (4 hospitals)	278 patients	SAB	3-month survival and recurrence of infection.	DOT <14 days *vs* ≥14 days	Overall mortality 34%. DOT <14 days was associated with mortality: OR 0.84 (95% CI 0.76 to 0.94), p=0.001.	Only included patients whose observation time was longer than DOT.
Zeylemaker (2001)[144]	Retrospective single centre cohort/Netherlands	49 patients	Catheter associated SAB	Favourable outcome, mortality, attributable mortality, and complications.	DOT 0 days *vs* 1–7 days *vs* 7–14 days *vs* >14 days.	Favourable outcome was higher in the shorter therapy group (1-14 days) compared to longer therapy (>14 days) 41% *vs* 33%), complications were lower (48% *vs* 53%), and attributable death higher	No adjustment

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings	Approach used to address ITB
						(31% *vs* 20%), and death due to underlying disease higher (41% *vs* 33%).	
Malanoski (1995)[145]	Retrospective single centre cohort/US	102 patients	Catheter related SAB	Relapse and complications	DOT <10 days *vs* 10-15 days *vs* >15 days	Relapse rate in patients treated for 10-15 days was 0% *vs* 4.7% in those treated >15 days; risk -4.7% (95% CI -13.9% to 4.3%).	No adjustment
Jernigan (1993)[146]	Meta-analysis of 11 studies	132 patients	Uncomplicated catheter related SAB	Late complication rate	Short-course therapy (≤2 weeks)	Overall late complication rate 6.1% (95% CI 2.0% - 10.2%)	N/A – descriptive with no comparator group
Raad (1992)[147]	Retrospective multi-centre cohort/Florida (2 hospitals)	55 patients	Catheter related SAB	Relapse of infection within 3 months	DOT <10 days *vs* ≥10 days	Relapse 16% *vs* 0% <10 days *vs* 10 days (p=0.05)	No adjustment
Ehni (1989)[148]	*Prospective multi-centre cohort/US (3 hospitals)*	*13 patients*	*Catheter associated SAB*	*Relapse of infection*	*Efficacy of short-course therapy (<17 days) – no comparator*	*The relapse rate in patients treated with short course therapy (<17 days) of therapy was 7.7%*	*N/A – descriptive with no comparator group*

Abbreviations: *aHR= Adjusted hazard, BSI=Bloodstream infection, CI=Confidence Interval, DOT=Duration of therapy, HR= Hazard ratio, ITB= Immortal time bias, MS=Methicillin susceptible, OR= Odds ratio, RCT=Randomised control trial, RR=Risk ratio, SAB= S. aureus bacteraemia ratio*

Of the 20 studies included, 17 were observational studies (8 single centre cohort/9 multi centre cohort, 12 retrospective/5 prospective). The remaining three were: a protocol for a randomised trial (trial currently in recruitment); a review of the literature; and a meta-analysis of 11 studies[131, 134, 146].

The most recent and largest study to date is a retrospective cohort study of 1,005 patients by Thorlacius-Ussing *et al*[129]. They compared short course therapy defined as 6-10 days of treatment to pro-longed course defined as 11-16 days in patients with low-risk methicillin-susceptible SAB. They excluded complicated infections defined as >16 days of treatment, the presence of endocarditis, meningitis, osteomyelitis, arthritis, spondylodiscitis, "other" secondary manifestation, infection involving a foreign body, pneumonia, or a positive follow-up blood culture for *S. aureus* obtained >48 hours after treatment initiation. They concluded that outcomes, including 90-day all-cause mortality, were similar in patients treated with short course therapy to those with prolong course therapy (90-day all-cause mortality OR= 1.05, 95% CI, 0.71 to 1.51). They addressed confounding by indication using inverse probability of treatment weights and immortal time bias by setting the index date to be the date of completed antimicrobial therapy in both treatment groups and excluding patients who did not complete their antimicrobial treatment of SAB. Although this minimises immortal time bias, it can introduce selection bias as failure to complete treatment may be related to severity of illness.

Another recent study, a retrospective cohort study of 530 patients by Abbas *et al*. and commentary by E.M. Eichenberger *et al*., investigated the impact of duration of therapy (≤14 days *vs* >14 days) on 90-day mortality and relapse of infection in patients with SAB[130, 131]. They defined infections as complicated if any of endocarditis, presence of implanted prosthesis, duration of SAB >2 days, fever >3 days were present. They found that treatment duration for greater than 14 days was associated with higher survival rates for patients with complicated SAB (aHR 0.32, 95% CI 0.16 to 0.64, p=0.001), however there was no strong evidence to suggest duration of therapy was associated with mortality in patients with uncomplicated SAB (aHR 0.85, 95% CI 0.41 to 1.78, p= 0.67). Within their analyses, they adjusted for confounding by indication using propensity scores and accounted for immortal time bias by excluding patients who died before day 14. However, this exclusion may introduce selection bias as those who die early on are likely to be older, sicker patients. These results were consistent with a prospective study by Jensen *et al*. that found duration of treatment >14 days was associated with lower mortality (OR 0.84, 95% CI 0.76 to 0.94, p=0.001), but again these conclusions are subject to bias as they only included patients whose observation time was longer than their duration of therapy[143].

Kim *et al.* looked at duration of therapy separately for patients who were classified as warranting longer or shorter antibiotic therapy where factors necessitating longer treatment included persistent bacteraemia, metastatic infections and any prosthetic devices[133]. Within those warranting shorter antibiotic therapy, patients were classified as <14 days or ≥14 days and in the longer antibiotic therapy group patients were classified as <28 days or ≥28 days. They observed that shorter duration of therapy than recommended was related to worst outcome (composite outcome of 90-day all-cause mortality and 30-day relapse) in the longer duration groups but not in the short duration group. However, deaths that occurred before the patient completed antibiotic treatment were excluded. Chong *et al*. performed a prospective cohort study of patients with uncomplicated SAB at a Korean hospital between August 2008 and September 2010[136]. They categorised patients into short-course therapy defined as <14 days and intermediate-course therapy defined as ≥14 days according to the patient's observed duration of treatment. They did not find evidence to suggest a difference in mortality between the groups, however, less than 14 days was significantly associated with an increase in relapse of infection. Again, they excluded patients who died whilst they were still in receipt of antibiotic therapy from the analysis which may have introduced bias as the minimum time to death would be 15 days in the long therapy group compared to 7 days in the short therapy group.

In fact, of the 20 studies summarised in Table 4.1, 10 excluded patients who died within a pre-specified period or whilst still receiving antibiotics and six studies do not describe how immortal time bias was addressed so it is likely that these results are also subject to bias. All studies published to date are observational and do not adequately address immortal time bias which may have an impact on the conclusions that can be confidently drawn. In addition, a review and meta-analysis of 11 studies reporting on the effectiveness of short duration of treatment (two weeks or less) in patients with a catheter related SAB concluded that the available data regarding the safety of short-course therapy for catheter related SAB are flawed due to the bias from the observational nature of all studies[146].

In order to overcome the immortal time bias patients need to be assigned to an intervention group without looking forward in time, either using appropriate statistical methodology within observational studies or by conducting an RCT where patients can be analysed according to an intention to treat approach. To date, there has not been an RCT evaluating optimal duration of therapy for the treatment of SAB, however there is currently a trial in recruitment comparing the efficacy of seven and fourteen days of antibiotic treatment in uncomplicated *S. aureus*[134]. However, until the study concludes evidence must be based on observational studies alone and immortal time bias must be appropriately addressed to obtain unbiased estimates. Hernán proposed a three-step

procedure to compare the treatment strategies whilst eliminating the induced immortal time bias[149]. Other methods that can account for immortal time bias include time-updating covariates where the intervention assigned to each individual is compatible with their duration of treatment up to that time and is updated once duration of therapy received exceeds the threshold in question, discussed in further detail in Chapter 3 section 3.2[100].To date, there has not been a study that has appropriately addressed immortal time bias when assessing the effect of duration of therapy on mortality in patients with *Staphylococcus aureus* bacteraemia, in particular the three-step procedure as described by Hernán has not been implemented in this setting.

In this analysis I aimed to investigate the effect of duration of therapy on 28-day mortality in patients with *Staphylococcus aureus* bacteraemia (MRSA or MSSA) using statistical methodology to eliminate the potential immortal time bias. I will be applying the methodology proposed by Hernán whilst also presenting a naïve approach and an approach using time-updated covariates.

4.2 Methods

4.2.1 Data source

For this analysis, I used patient-level data from the BSI-FOO observational study. It was not possible to include data from RAPIDO as data on treatment was only collected up to day 7 post blood culture.

4.2.2 Primary outcome

The primary outcome is 28-day all-cause mortality. Patients were followed up for 28 days following blood culture in BSI-FOO, however time 0 in this analysis is start of active therapy so some patients will have shorter follow-up if they start active treatment after their initial blood culture. I will therefore censor patients 28 days after blood culture.

Appropriate antibiotics were defined as antibiotics to which the pathogen showed susceptibility. The duration of appropriate antibiotic treatment (active therapy) was defined as the time interval between the first appropriate antibiotic administration and the last appropriate antibiotic administration in the 28 days post blood culture or death/end of follow-up if earlier. Duration of therapy was categorised into three groups for comparison. The three groups are:

1. Short therapy: Duration of active therapy <10 days
2. Intermediate therapy: Duration of active therapy 10 to 18 days
3. Long therapy: Duration of active therapy ≥19 days

These were based on the distribution of the data ensure enough patients and events in each of the groups whilst maintaining clinical relevance.

4.2.3 Analysis population

Patients were included if they had a BSI with MRSA or MSSA and were in receipt of antibiotic treatment with known antimicrobial activity to MRSA/MSSA. Patients who did not receive treatment were excluded as the aim of this analysis was to explore the effect of duration of treatment from the point in which the clinician decides to treat the patient, not the effect of treating the patient or not. I also excluded polymicrobial infections and repeat infection episodes.

4.2.4 Statistical analysis

I will be applying the methodology proposed by Hernán whilst also presenting a naïve approach and an approach using time-updating covariates. I describe the methods for each of the three approaches below.

Approach 1: Naïve approach

A naïve approach to assess the effect of duration of therapy on mortality where duration of therapy group is assigned according to their observed duration of therapy could lead to biased results[150]. As discussed earlier in this chapter, these results are subject to immortal time bias as only patients who survive a long time can be assigned to the longer duration groups e.g. in order to be assigned to the long therapy group, there is a period of immortal time as patients would have to survive at least 19 days. I will implement this approach to demonstrate the inflation of estimates that can arise when this bias is ignored.

Approach 2: Time-updating covariates

To overcome the presence of immortal time bias, I need to assign patients to an intervention group without looking forward in time. One method that can be used to help minimise this bias is time-updating covariates[100]. In this approach, patients can only be assigned to a treatment duration group that is consistent with their observed duration up to that time. For example, on day seven patients could only be assigned to duration group one (<10 days), after day 10 this could be updated to duration group two for those who continue to receive treatment. The process of updating covariates is demonstrated for three example patients in Table 4.2.

Table 4.2 Demonstration of time-updating covariates for three example patients

	Patient 1: Duration of therapy = 12 days, survive	**Patient 2: Duration of therapy = 7 days, died on day 20**	**Patient 3: Duration of therapy = 26 days, died on day 26**
Day	**Duration group: Updated covariate**	**Duration group: Updated covariate**	**Duration group: Updated covariate**
1	Group 1	Group 1	Group 1
2	Group 1	Group 1	Group 1
3	Group 1	Group 1	Group 1
4	Group 1	Group 1	Group 1
5	Group 1	Group 1	Group 1
6	Group 1	Group 1	Group 1
7	Group 1	Group 1	Group 1
8	Group 1	Group 1	Group 1
9	Group 1	Group 1	Group 1
10	Group 2	Group 1	Group 2
11	Group 2	Group 1	Group 2
12	Group 2	Group 1	Group 2
13	Group 2	Group 1	Group 2
14	Group 2	Group 1	Group 2
15	Group 2	Group 1	Group 2
16	Group 2	Group 1	Group 2
17	Group 2	Group 1	Group 2
18	Group 2	Group 1	Group 2
19	Group 2	Group 1	Group 3
20	Group 2	Group 1	Group 3
21	Group 2	-	Group 3
22	Group 2	-	Group 3
23	Group 2	-	Group 3
24	Group 2	-	Group 3
25	Group 2	-	Group 3
26	Group 2	-	Group 3
27	Group 2	-	-
28	Group 2	-	-

Note: *Red lines represent the thresholds for short, intermediate, and long therapy. Group 1 (short therapy): Duration of active therapy <10 days; Group 2 (intermediate therapy): Duration of active therapy 10 – 18 days; Group 3 (long therapy): Duration of active therapy ≥19 days*

Approach 3: Three step procedure

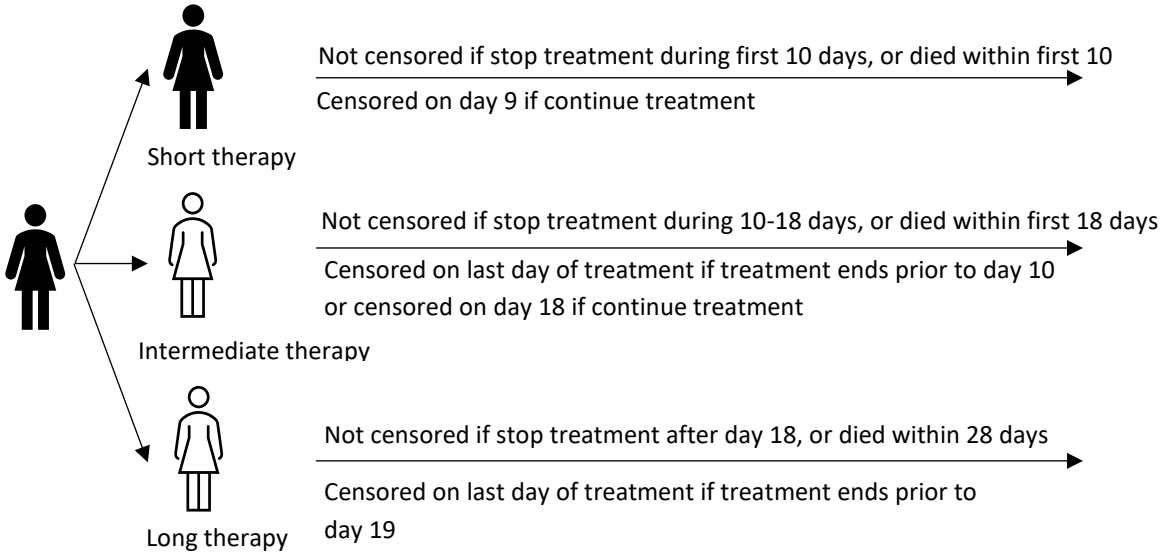
Hernán proposed a 3-step procedure to compare the treatment strategies whilst eliminating the induced immortal time bias[149]. The first stage is to clone all participants, so that each patient is assigned to each treatment strategy once. The second stage is to censor observations when an individual's data becomes inconsistent with their assigned strategy. The third stage is to apply

inverse probability weights to adjust for the potential selection bias introduced by censoring. These steps are described in more detail below:

STEP 1: CLONING

Each patient is triplicated within the dataset so that each patient is represented by three observations: one assigned to their observed duration of therapy group, and the other two assigned to the remaining two groups respectively (Figure 4.1).

Figure 4.1 Visual illustration of cloning



STEP 2: CENSORING

Each observation is followed-up and censored at the point they deviate from their assigned strategy. The events and person time that occur after the patient deviates from their assigned protocol will be discarded. Example patients and censoring rules are described below and given in Figure 4.2.

Individual 1: Started treatment on day of blood culture and received active therapy for full 28 days of follow-up. Their complete follow-up contributes to Group 3 (≥19 days) as they do not deviate from that protocol. However, only their follow-up before day 10 contributes to the Group 1 clone (<10 days), resulting in "artificial" censoring at day 9 as this is the point they deviate from that protocol. Their follow-up time before day 18 contributes to Group 2 (10-18 days) clone and they are artificially censored at day 18.

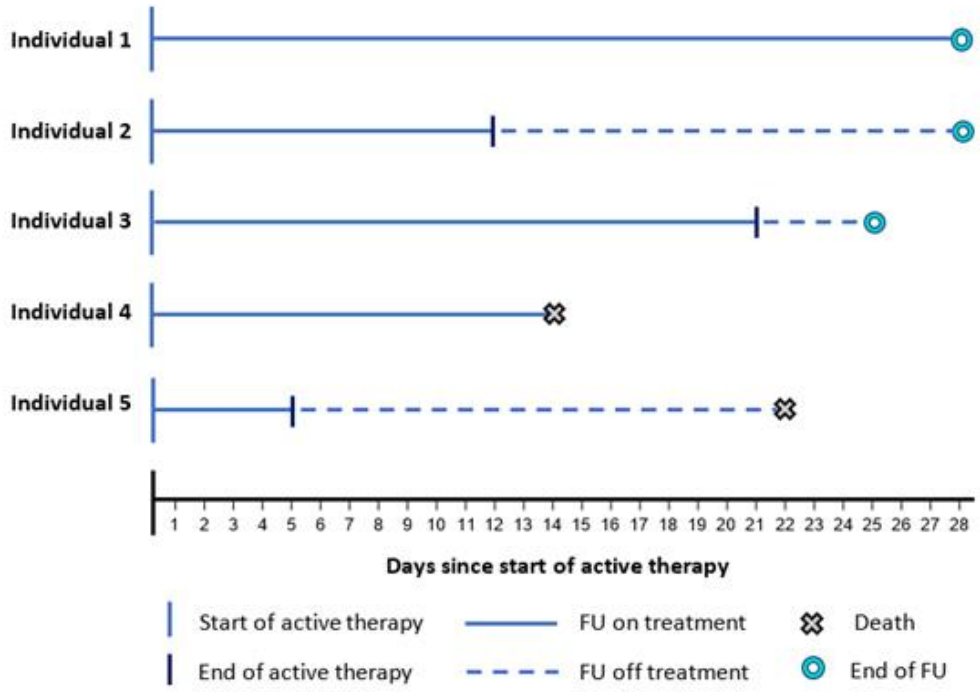
Individual 2: Started treatment on day of blood culture, received active therapy for 12 days. Their complete follow-up contributes to Group 2, and they are artificially censored at day 9 in Group 1 clone and day 12 in Group 3 clone.

Individual 3: Started treatment 3 days after blood culture (so has 25-day follow-up available), received active therapy for 21 days. Their complete follow-up contributes to Group 3 and they are artificially censored at day 9 in Group 1 clone and day 18 in Group 2 clone.

Individual 4: Started treatment on day of blood culture, died on day 14. Their complete follow-up contributes to Group 2, and they are artificially censored at day 9 in Group 1 clone. Their follow-up does not contribute to Group 3 clone as they do not reach day 18.

Individual 5: Started treatment on day of blood culture, received active therapy for five days and died on day 18. Their complete follow-up contributes to Group 1 clone, and they are artificially censored at day 5 in Group 2 and 3 clones.

Figure 4.2 Censoring rules using example timelines



	Group 1 (clone) Treated < 10 days	Group 2 (clone) Treated 10-18 days	Group 3 (clone) Treated ≥19 days
Individual 1	Censored at day 9	Censored at day 18	Uncensored
Individual 2	Censored at day 9	Uncensored	Censored at day 12
Individual 3	Censored at day 9	Censored at day 18	Uncensored
Individual 4	Censored at day 9	Uncensored	-
Individual 5	Uncensored	Censored at day 5	Censored at day 5

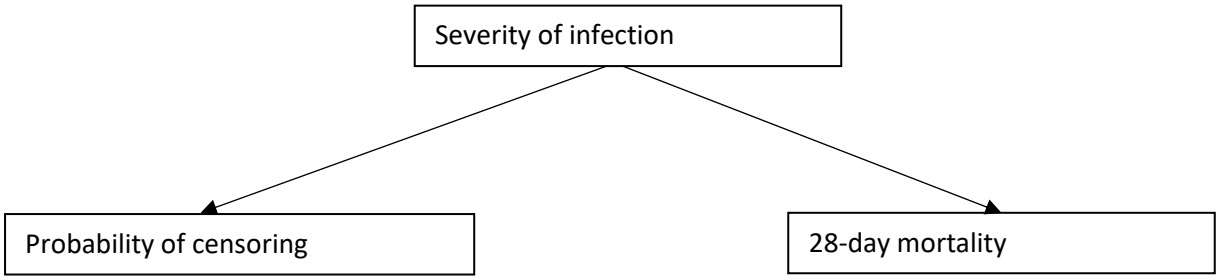
Abbreviations: *FU= Follow-up*

STEP 3: WEIGHTING

Time varying confounding

Censoring patients when they deviate from their assigned strategy is a form of informative censoring, as sicker patients/patients with more severe infections are more likely to be artificially censored as they are more likely to continue treatment for a longer duration and therefore "cross-over". In addition, sicker patients are also more likely to die (Figure 4.3). This induces selection bias.

Figure 4.3 Relationship between severity of infection and probability of censoring and mortality



William and Ravani state that "The mechanism that causes this informative censoring can be thought of as another form of a time-dependent confounder because the probability of censoring depends on the outcome the subject would have had in the absence of censoring."[151]

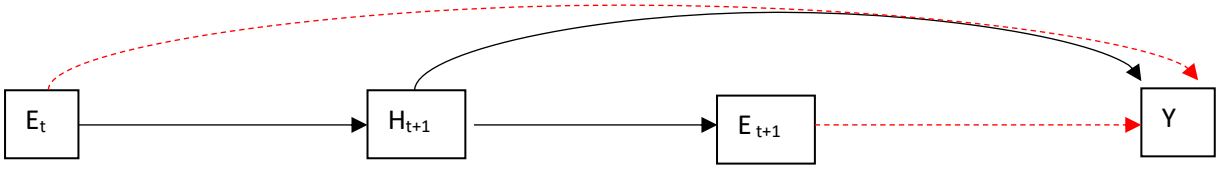
A covariate is a time-varying confounder for the effect of treatment on outcome if:

1. past covariate values predict current treatment

2. current covariate value predicts outcome

For example, an indication of poor health status/severity of infection (*H*), which is also associated with the outcome (*Y*), is affected by previous exposure (E_t) and, in turn, increases the probability of subsequent exposure (E_{t+1}) would be considered a time-varying confounder (Figure 4.4).

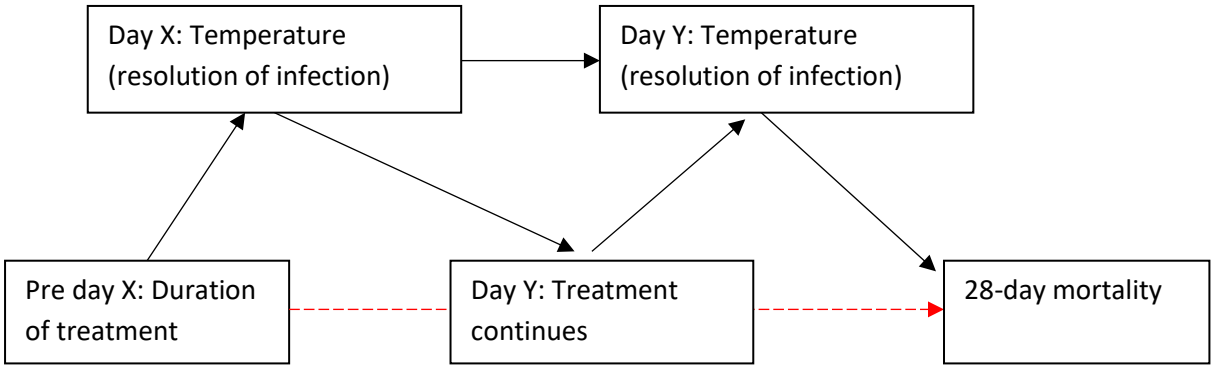
Figure 4.4 Visualisation of time-varying confounding



An example of this relationship within the current analysis is the relationship between duration of treatment, temperature and mortality. Previous temperature measurements may predict whether

treatment will continue and current temperature predicts outcome (28-day mortality), see Figure 4.5.

Figure 4.5 Daily temperature, treatment duration and mortality DAG



Note: *Day 'Y' is any day after day 'X'*

In this case, temperature is a time-varying confounder that is affected by the previous exposure, and therefore over time temperature plays both the role of confounder and mediator of the effect of treatment on outcome. If I ignore temperature then there will no longer be a causal interpretation of duration of therapy on survival because temperature is a confounder (affects treatment duration and survival), but I cannot simply adjust for temperature as it lies on the causal pathway so this would induce bias and again there would no longer be a causal interpretation of duration of therapy on survival.

This time-varying confounding and informative censoring can be corrected for by using marginal structural models as introduced by Hernán *et al.* which use inverse probability-of-censoring weights to up-weight uncensored observations to represent censored observations with similar characteristics which will an allow unbiased estimation of the effect[152]. This involves assigning time-varying inverse probability weights that are calculated by estimating the cumulative probabilities of remaining uncensored, dependent on the baseline and time varying variables that are predictive of the censoring mechanism, i.e. variables that predict adherence to treatment duration strategy.

In the analysis of duration of therapy, variables that predict adherence to the assigned treatment duration strategy were specified a *priori.* At baseline, I included neutrophil count. Neutrophils are a type of white blood cell that fight against infection. High levels (neutrophilia) are a sign that an infection is present and low levels (neutropenia) mean your body cannot fight infection and is often caused by chemotherapy. Patients with neutropenia are often given a longer duration of therapy. Blood cultures after day 0 would identify cases of infection resolution, which would be a predictor of adherence to duration of therapy strategy. Unfortunately, data from blood cultures after day 0 were

not recorded in BSI-FOO so I will use daily temperature records as a surrogate measure of infection severity/infection resolution. The calculation of weights is described in more detail below.

Calculation of weights

The probability of remaining un-censored, C_{ij}, is calculated for each time point using the patient's past covariate history, H_{ij}, and treatment received, T_{ij}, where i denotes person and j denotes time. The covariate vector H_{ij} which is available on each day, j, includes the time-varying covariate, temperature, as well as the baseline covariates. C_{ij} =1 if participant is censored by day j, and zero otherwise. The weight is then calculated as follows:

$$W_{ij} = \prod_{k=0}^{t} \frac{P(C_{ik} = 0 \mid \bar{C}_{ik-1} = \bar{0}, T_{ik}, B_{i0})}{P(C_{ik} = 0 \mid \bar{C}_{ik-1} = \bar{0}, T_{ik}, \bar{H}_{ik-1})}$$

The denominator of the weight is calculated as the probably of remaining uncensored at time k, given that the patient has remained uncensored up to time k, their treatment on day k, and their covariate history up to day k. This adjusts for the bias induced by informative censoring. Standard weights tend to be highly variable (i.e., very small or very large) therefore subsequent estimation based on them tends to become unstable. The numerator of the weight is added to help stabilised the weights in order to reduce the variance and yield narrower 95% confidence intervals[153]. The aim is to minimise the difference between the numerator and denominator of the weights so that the only difference that remains reflects the confounding due to time-varying covariates[154].

Pooled logistic regression vs Cox regression

Finally, I fitted a weighted pooled logistic regression model (see below) regressing mortality on cloned duration of treatment group weighted using the weights calculated as above. I included cubic splines of follow-up (knots at 10%, 25%, 50%, 75% and 90% percentiles), within the pooled logistic regression to mirror the underlying mortality risk.

In a Cox model, it is not possible to allow for within-individual time-varying weights and ignoring this will produce inaccurate standard errors. I therefore used a discrete time model (pooled logistic regression) to estimate the hazard ratio. Pooled logistic regression will give results that approximates the Cox model, but I need to model the baseline hazard, as the functional form of the underlying hazard is not specified in a Cox model. I will do this using splines (flexible curves where the shape is estimated from the data) to model the underlying hazard function of Cox regression, i.e. allowing the underlying risk of death to vary from day to day without computing a separate intercept term for each time period[155, 156].

Implementation of pooled-logistic regression vs Cox regression using RAPIDO data

In order to check the implementation of the pooled-logistic regression model did approximate the Cox proportional hazards model, I used the RAPIDO data with randomised allocation as the exposure to compare estimates from the two models. Estimates were comparable, and identical up to 1dp, and confidence intervals slightly more conservative in the pooled-logistic regression model but the same up to 2dp (Table 4.3). I am therefore confident in implementing this approach in the main analysis.

Table 4.3 Cox regression model *vs* Pooled logistic regression - demonstration using data from the RAPIDO trial

	Hazard ratio	95% CI
Cox regression (unweighted)	1.068	(0.901, 1.266)
Pooled logistic (unweighted)	1.069	(0.899, 1.271)
Cox regression (weighted [a] [b])	1.084	(0.875, 1.343)
Pooled logistic (weighted [b])	1.084	(0.873, 1.346)

[a] *Standard error not adjusted for within-patient correlation*

[b] *Weights estimated from a logistic regression model estimating each patient's probability of remaining uncensored conditional on their age and sex (for demonstration purposes).*

Abbreviations: *CI=Confidence interval*

Obtaining valid confidence intervals

In calculating confidence intervals, robust standard errors can be used to account for the cloning that leads to an "artificial" increase in number of events. However, I also need to take into account the uncertainty in the estimation of inverse probability weights. It has been shown that confidence intervals based on the robust variance estimator are valid but they are conservative[157]. An alternative approach that provides less conservative confidence intervals and has been used in recent studies that have implemented the cloning approach is bootstrapping[150, 158-161]. I therefore decided to use the non-parametric bootstrap with 500 bootstraps to obtain 95% confidence intervals as this is the least conservative whilst maintaining validity.

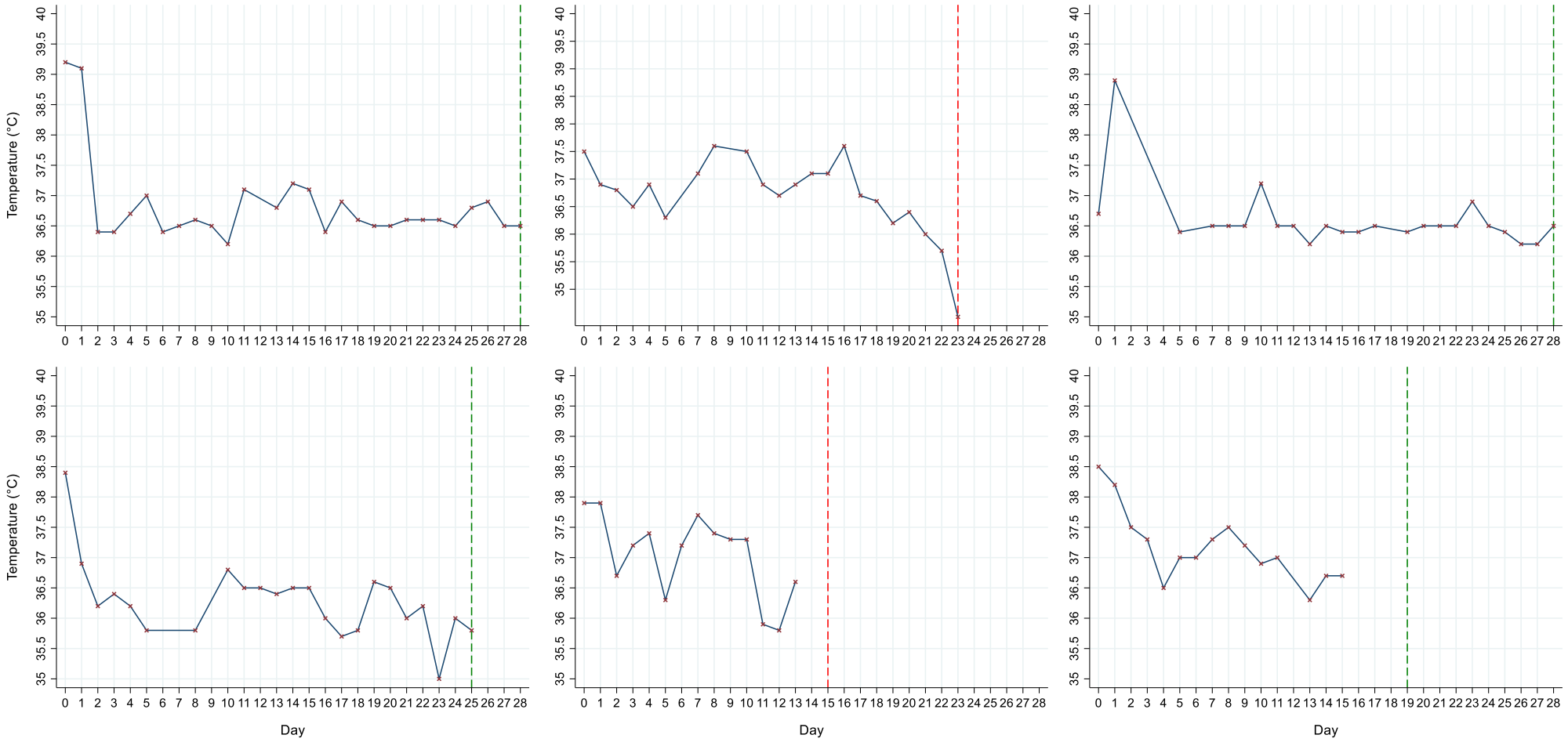
4.2.5 Missing data

Daily temperature measurements are likely to be missing at random (MAR), since it is routinely measured during patient's hospital stay. However, temperature measurements were not recorded post-discharge and patients who are discharged are likely to have their fever resolved. I will therefore not impute missing data post-discharge, but I will censor patients at hospital discharge.

Data were missing for 8.2% (849/10,378) patient in-hospital days. After consideration I decided that given the nature of the analysis approach (cloning & bootstrapping) that multiple imputation would

be computationally intensive to implement. I therefore decided to use a less computationally intensive method to impute missing temperature during patients in-hospital stay. I first looked at some example patients' temperature profiles over time to get a feel for potential temperature patterns during hospital stay to ensure my choice of imputation method was appropriate (Figure 4.6).

Figure 4.6 Example patient temperature timelines for six patients



***Note**: Green dash line = Discharge/End of follow-up. Red dash line = death*

Given the longitudinal nature of the data, temperature measurements nearest to the day of the missing data are likely to be similar to the missing temperature measurement itself. Previous temperature measurements are known for 810/849 of the missing temperatures and future temperature measurements known for 302/849 of the missing measurements (Table 4.4).

Table 4.4 Temperature measurements available around missing measurement

Temperature measurements available	N (days)	%
Only future values	16	1.9%
Only past values	508	59.8%
Future and past values	302	35.6%
No values	23	2.7%
Total	849	100.0%

I therefore considered two approaches:

Method 1: Use last observation carried forward (LOCF), replacing all missing values with the last value that was recorded for that particular participant[162]

Method 2: Use mean of previous and future value if possible (n=302), otherwise use LOCF (n=508).

To aid my decision, I have summarised the imputed data using the two approaches below (Table 4.5 and Figure 4.7).

Table 4.5 Summary of imputed values using two methods of imputation

	Temperature (°C)			
	Mean (SD)	**Median (IQR)**	**Min**	**Max**
Method 1* (n=810)	37.1 (1.05)	36.8 (36.5, 37.6)	33.4	40.1
Method 2 **(n=810)	37.0 (0.95)	36.8 (36.5, 37.5)	33.4	40.0

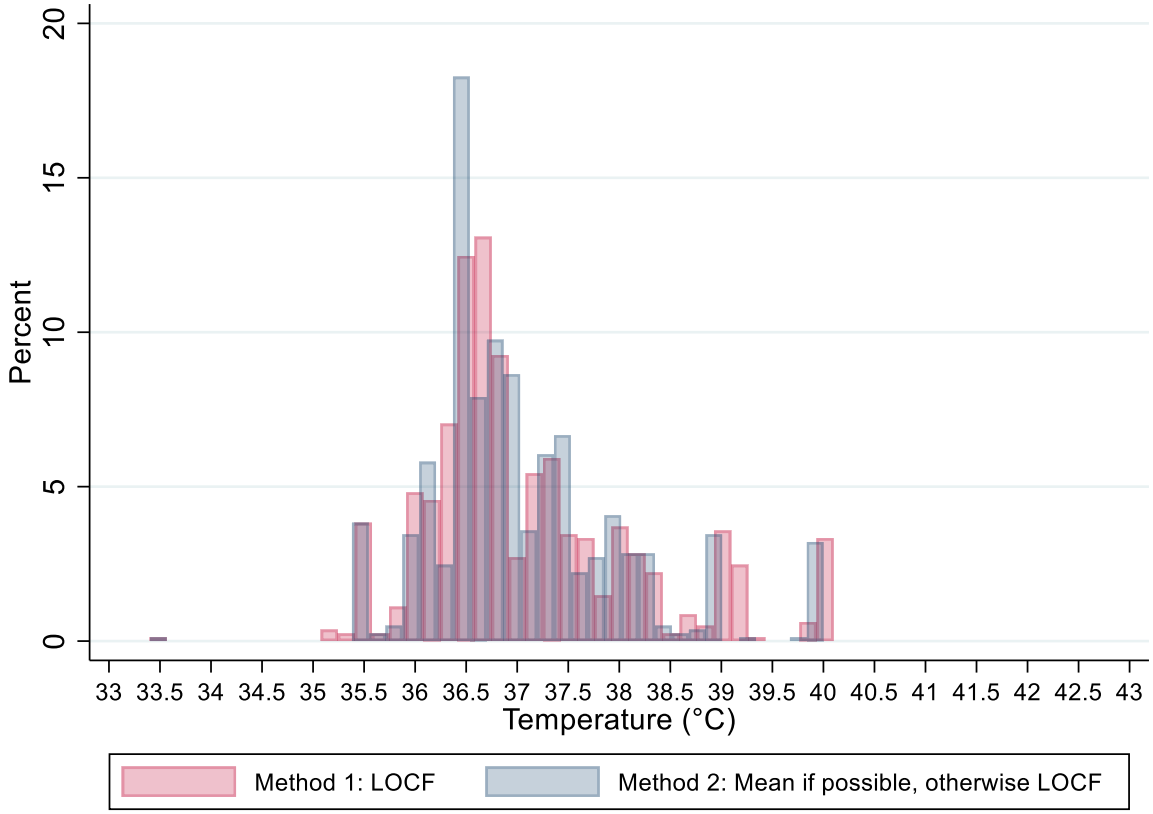
Note: *8 patients (39 days) do not have previous temperature measurements and therefore LOCF cannot be implemented. Hence n=810 (849-39)*

** Method 1 = LOCF*

*** Method 2 = mean of previous and future value if possible, otherwise LOCF*

Abbreviations: *IQR=Interquartile range, LOCF= Last observation carried forward*

Figure 4.7 Histogram of imputed values using two methods of imputation



Abbreviations: *LOCF= Last observation carried forward*

As both approaches provided similar imputed values and I am imputing values within a small range (35-40^{o}C) the impact of choice of approach used should be minimal. I decided to use the LOCF approach so that a consistent approach was used for all missing values.

The only other variable used within this analysis with missing data was neutrophil count. As this was measured at baseline only it was not possible to use a LOCF approach. I therefore decided to impute neutrophil count with age- and sex-adjusted averages.

4.2.6 Sensitivity analysis

I performed a sensitivity analysis to explore the impact of censoring at hospital discharge by including follow-up and events after hospital discharge. Daily temperature measurements were not recorded post-discharge, so I assumed normal temperature (37^{o}C) for this period.

4.2.7 Subgroup analysis

I performed a sub-group analysis by complicated SAB, where infections were defined as complicated when any of the following were present: persistent fever at 72 hours, presence of prosthesis,

cardiovascular system source of infection. This definition was based on the definitions used in other studies and that were applicable to data collected in BSI-FOO[123, 125, 130, 134]. Persistent fever at 72 hours was only known for patients who survived to 72 hours, therefore patients who died before 72 hours were classified based on their data temperature measurements recorded up until that point. Some criteria in the literature could not be applied as the data were not collected in BSI-FOO, e.g. duration of bacteraemia (do not have blood sample results after day 0), skin examination findings suggesting acute systemic infection and evidence of metastatic sites of infection.

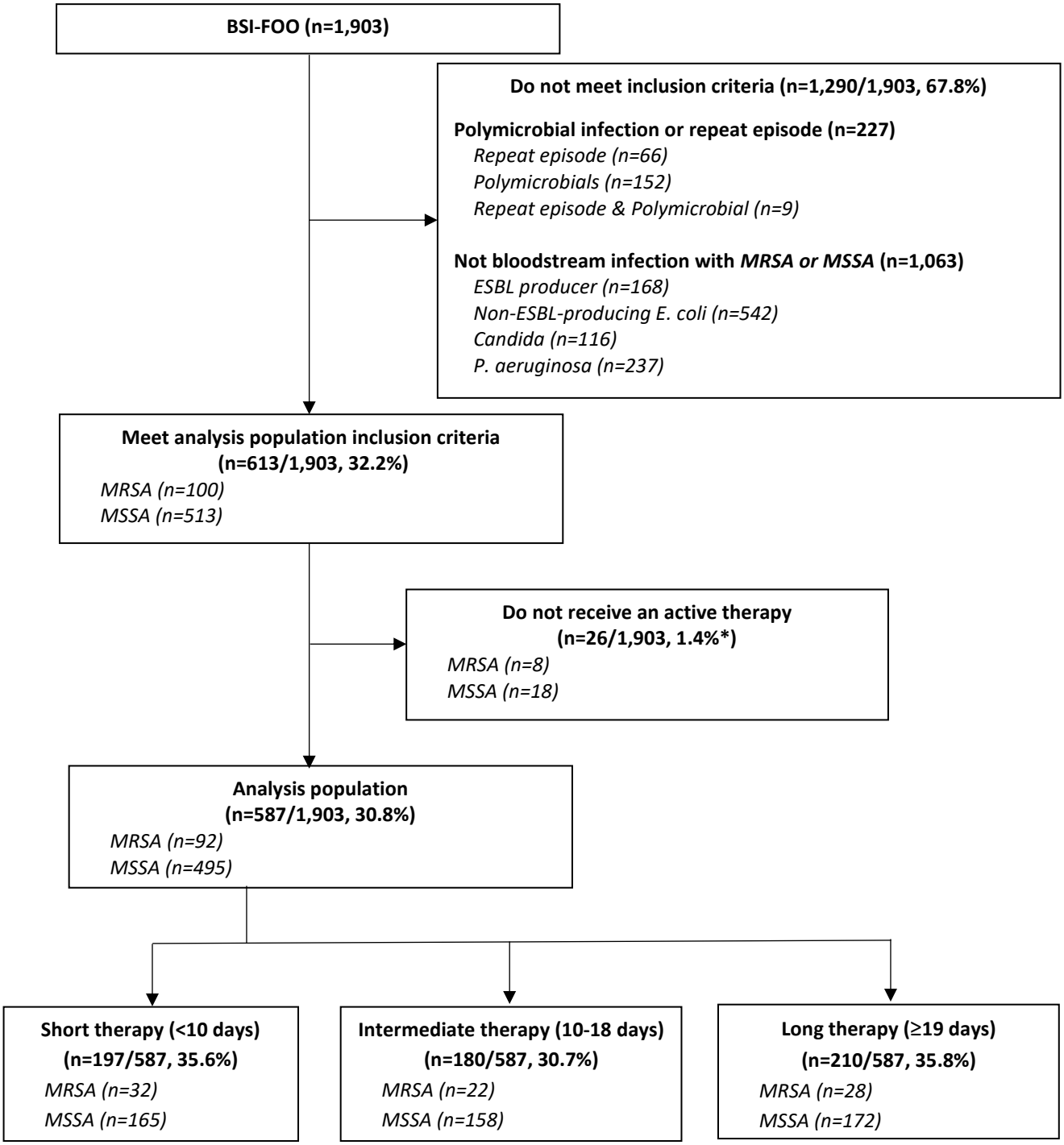
I also performed a sub-group analysis by MRSA and MSSA to investigate whether the association of duration of therapy and mortality differed by type of *S. aureus* BSI.

4.3 Results

4.3.1 Population

Of the 1,903 participants in the BSI-FOO study, 1,063 did not have MRSA or MSSA and 227 were polymicrobial or repeat episodes and were therefore excluded. There were 26 patients that were not in receipt of an active therapy of which 13 died within two days of blood culture. I considered including these patients in the analysis with a duration of zero days, however I am interested in the effect of duration of therapy once the decision to treat the patient is made. I therefore decided to exclude these patients, and after the exclusion was applied 587 (92 MRSA and 495 MSSA) met the eligibility criteria and were included in the analysis (Figure 4.8). Based on observed duration of therapy i.e. before cloning, 33.6% (197/587) received active treatment for <10 days, 30.7% (180/687) received active treatment for 10-18 days and 35.8% (210/587) received active treatment for ≥19 days.

Figure 4.8 Duration of therapy flowchart



** n=13 died within 2 days of blood culture, n=11 survived but did not receive any treatment, n=2 received treatment but with therapy inactive against pathogen (1 died on day 20, one survived)*

Abbreviations: *ESBL= Extended Spectrum Beta-Lactamase, MRSA=Methicillin-resistant Staphylococcus aureus, MSSA=Methicillin-susceptible Staphylococcus aureus*

4.3.2 Demographics

Table 4.6 summarises the distribution of baseline characteristics, by group based on observed duration of therapy. The median age was 66.0 years (IQR 49.0, 77.0) and 64.4% (378/387) were male. Baseline characteristics were broadly similar across the groups, however there were some differences in some of the clinical measures. eGFR was on average higher indicating better kidney function in patients in the short therapy group compared to the intermediate and long therapy groups (median 78.0 *vs* 69.0 *vs* 69.5 respectively). Source of infection was more commonly skin and soft tissue (19.3% *vs* 14.4% *vs* 11.0%) and site uncertain (42.1% *vs* 28.3% *vs* 22.4%) and less commonly bone and joint (1.5% *vs*, 10.6% *vs* 18.6%) in patients in the short therapy group compared to the intermediate and long therapy. A larger proportion were on vasopressor drugs on day 0 (9.1% *vs* 3.3% *vs* 3.8%) and systemic corticosteroids (15.2% *vs* 10.6% *vs* 7.6%). However, this difference is maybe a reflection of fact that sicker patients are more likely to die early and therefore more likely to be in the short therapy group as they did not survive long enough to receive longer duration of therapy. Therefore, these descriptive summaries are subject to immortal time bias as described previously. Once cloned, all participants were represented by a clone in the short, intermediate, and long therapy group, therefore baseline characteristics of the three groups were perfectly balanced.

Table 4.6 Baseline characteristics, by observed duration of therapy

	Short therapy (<10 days) (n=197)	Intermediate therapy (10-18 days) (n=180)	Long therapy (≥19 days) (n=210)	SMD (I – S)	SMD (L – S)	SMD (L – I)	Overall (n=587)
Patient measures							
Age	67.0 (45.0, 77.0)	66.0 (51.0, 76.0)	66.0 (48.0, 78.0)	0.07	0.03	0.03	66.0 (49.0, 77.0)
Male	120/197 (60.9%)	123/180 (68.3%)	135/210 (64.3%)	0.16	0.07	0.09	378/587 (64.4%)
Body Mass Index [a]	26.1 (5.9)	26.1 (6.0)	26.2 (6.5)	0.01	0.01	0.02	26.1 (6.2)
Patient medical history							
Chemotherapy in month before date 0	29/197 (14.7%)	18/180 (10.0%)	22/210 (10.5%)	0.14	0.13	0.02	69/587 (11.8%)
Any tumour within last 5 years	58/197 (29.4%)	52/180 (28.9%)	50/210 (23.8%)	0.01	0.13	0.12	160/587 (27.3%)
Surgery requiring overnight stay within 7 days before date 0	17/197 (8.6%)	22/180 (12.2%)	12/210 (5.7%)	0.12	0.11	0.23	51/587 (8.7%)
Burn requiring admission within 7 days before date 0	1/196 (0.5%)	1/179 (0.6%)	2/210 (1.0%)	0.01	0.05	0.05	4/585 (0.7%)
Cardiac arrest within 7 days before date 0	0/197 (0.0%)	2/180 (1.1%)	0/210 (0.0%)	0.15	-	0.15	2/587 (0.3%)
Renal support within 7 days before date 0	19/197 (9.6%)	21/180 (11.7%)	16/210 (7.6%)	0.07	0.07	0.14	56/587 (9.5%)
Myocardial infarction within 7 days before date 0	25/197 (12.7%)	24/180 (13.3%)	17/210 (8.1%)	0.02	0.15	0.17	66/587 (11.2%)
Infection severity measures							
Temperature (°C) at time 0 [b]	38.2 (37.5, 38.9)	38.0 (37.2, 38.6)	38.2 (37.3, 38.8)	0.02	0.05	0.08	38.1 (37.3, 38.8)
INR [c]	1.2 (1.1, 1.6)	1.1 (1.1, 1.5)	1.2 (1.1, 1.5)	0.05	0.10	0.08	1.2 (1.1, 1.5)
eGFR (mL/min/1.73m^2) [d]	78.0 (38.0, 91.0)	69.0 (36.0, 90.0)	69.5 (34.0, 90.0)	0.11	0.09	0.02	72.0 (36.0, 90.0)
Serum Albumin (g/L) [e]	31.5 (8.4)	32.8 (6.9)	31.2 (7.2)	0.16	0.04	0.23	31.8 (7.6)
Bilirubin total (umol/L) [f]	11.0 (7.0, 18.0)	11.0 (7.0, 18.0)	11.5 (8.0, 20.0)	0.10	0.01	0.11	11.0 (7.0, 19.0)
Neutrophil count at day 0 or closest (x10^9/L) [g]	8.6 (4.9, 12.8)	8.8 (5.5, 12.8)	10.0 (6.9, 14.0)	0.07	0.17	0.10	9.2 (5.8, 13.3)
Systolic BP at day 0 or closest (mmHg) [h]	124.4 (27.9)	126.6 (26.9)	125.1 (25.0)	0.08	0.03	0.06	125.3 (26.5)
On IV fluids at day 0	66/197 (33.5%)	56/180 (31.1%)	59/210 (28.1%)	0.05	0.12	0.07	181/587 (30.8%)
On ventilation at day 0	20/197 (10.2%)	7/178 (3.9%)	17/209 (8.1%)	0.24	0.07	0.18	44/584 (7.5%)
On vasopressor drugs at day 0	18/197 (9.1%)	6/180 (3.3%)	8/210 (3.8%)	0.24	0.22	0.03	32/587 (5.5%)
Systemic corticosteroids in last 24 hours	30/197 (15.2%)	19/180 (10.6%)	16/210 (7.6%)	0.14	0.24	0.10	65/587 (11.1%)
EWS score nearest to day 0 [i]	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)	2.5 (1.0, 5.0)	0.16	0.00	0.17	2.0 (1.0, 4.0)

	Short therapy (<10 days) (n=197)	Intermediate therapy (10-18 days) (n=180)	Long therapy (≥19 days) (n=210)	SMD (I – S)	SMD (L – S)	SMD (L – I)	Overall (n=587)
Patient comorbidities at date 0							
Congestive heart failure	31/197 (15.7%)	26/180 (14.4%)	32/210 (15.2%)	0.04	0.01	0.02	89/587 (15.2%)
Peripheral vascular disease	19/197 (9.6%)	20/180 (11.1%)	17/210 (8.1%)	0.05	0.05	0.10	56/587 (9.5%)
Cerebrovascular disease	42/197 (21.3%)	30/180 (16.7%)	27/210 (12.9%)	0.12	0.23	0.11	99/587 (16.9%)
Hemiplegia	10/197 (5.1%)	13/180 (7.2%)	6/210 (2.9%)	0.09	0.11	0.20	29/587 (4.9%)
Dementia	17/197 (8.6%)	8/180 (4.4%)	12/210 (5.7%)	0.17	0.11	0.06	37/587 (6.3%)
COPD	23/197 (11.7%)	17/180 (9.4%)	24/210 (11.4%)	0.07	0.01	0.06	64/587 (10.9%)
Connective tissue disease	13/197 (6.6%)	19/180 (10.6%)	20/210 (9.5%)	0.14	0.11	0.03	52/587 (8.9%)
Peptic ulcer disease	13/197 (6.6%)	15/180 (8.3%)	10/210 (4.8%)	0.07	0.08	0.14	38/587 (6.5%)
Ascites	16/197 (8.1%)	9/180 (5.0%)	10/210 (4.8%)	0.13	0.14	0.01	35/587 (6.0%)
Diabetes:							
None	147/197 (74.6%)	141/180 (78.3%)	169/210 (80.5%)	0.13	0.19	0.06	457/587 (77.9%)
Without organ damage	40/197 (20.3%)	28/180 (15.6%)	28/210 (13.3%)				96/587 (16.4%)
With organ damage	10/197 (5.1%)	11/180 (6.1%)	13/210 (6.2%)				34/587 (5.8%)
Child-Pugh score [j]	7.0 (6.0, 8.0)	6.0 (6.0, 7.0)	7.0 (6.0, 7.0)	0.38	0.28	0.15	7.0 (6.0, 8.0)
Charlson score [k]	3.0 (1.0, 4.0)	3.0 (1.0, 5.0)	2.0 (1.0, 4.0)	0.02	0.15	0.12	3.0 (1.0, 4.0)
Abscess at time 0	12/197 (6.1%)	21/180 (11.7%)	33/210 (15.7%)	0.20	0.31	0.12	66/587 (11.2%)
Infected foreign body at time 0	2/197 (1.0%)	1/180 (0.6%)	8/210 (3.8%)	0.05	0.18	0.22	11/587 (1.9%)
Surgical prosthesis time 0	0/197 (0.0%)	2/180 (1.1%)	14/210 (6.7%)	0.15	0.38	0.29	16/587 (2.7%)
Source of infection							
Bone and joint	3/197 (1.5%)	19/180 (10.6%)	39/210 (18.6%)	0.53	0.88	0.53	61/587 (10.4%)
Cardiovascular system	6/197 (3.0%)	5/180 (2.8%)	18/210 (8.6%)				29/587 (4.9%)
Central nervous system	2/197 (1.0%)	1/180 (0.6%)	6/210 (2.9%)				9/587 (1.5%)
Eye, ear, nose, throat or mouth	0/197 (0.0%)	1/180 (0.6%)	2/210 (1.0%)				3/587 (0.5%)
Gastrointestinal system	4/197 (2.0%)	4/180 (2.2%)	7/210 (3.3%)				15/587 (2.6%)
Line infection – central venous line	24/197 (12.2%)	25/180 (13.9%)	28/210 (13.3%)				77/587 (13.1%)

	Short therapy (<10 days) (n=197)	**Intermediate therapy (10-18 days) (n=180)**	**Long therapy (≥19 days) (n=210)**	**SMD (I – S)**	**SMD (L – S)**	**SMD (L – I)**	**Overall (n=587)**
Line infection - peripheral venous line	8/197 (4.1%)	9/180 (5.0%)	6/210 (2.9%)				23/587 (3.9%)
Lower respiratory tract	14/197 (7.1%)	15/180 (8.3%)	10/210 (4.8%)				39/587 (6.6%)
Reproductive tract	1/197 (0.5%)	2/180 (1.1%)	0/210 (0.0%)				3/587 (0.5%)
Skin and soft tissue	38/197 (19.3%)	26/180 (14.4%)	23/210 (11.0%)				87/587 (14.8%)
Surgical site infection	3/197 (1.5%)	7/180 (3.9%)	14/210 (6.7%)				24/587 (4.1%)
Systemic Infection	2/197 (1.0%)	4/180 (2.2%)	1/210 (0.5%)				7/587 (1.2%)
Urinary tract infection	9/197 (4.6%)	11/180 (6.1%)	9/210 (4.3%)				29/587 (4.9%)
Site uncertain	83/197 (42.1%)	51/180 (28.3%)	47/210 (22.4%)				181/587 (30.8%)
Lines and catheters							
Central line present at time 0	57/197 (28.9%)	45/180 (25.0%)	50/210 (23.8%)	0.09	0.12	0.03	152/587 (25.9%)
Peripheral line present at time 0	104/197 (52.8%)	95/180 (52.8%)	96/210 (45.7%)	0.00	0.14	0.14	295/587 (50.3%)
Urinary catheter present at time 0	67/197 (34.0%)	45/180 (25.0%)	57/210 (27.1%)	0.20	0.15	0.05	169/587 (28.8%)
Organisational factors							
Centre:							
A	26/197 (13.2%)	30/180 (16.7%)	41/210 (19.5%)	0.25	0.18	0.21	97/587 (16.5%)
B	41/197 (20.8%)	27/180 (15.0%)	39/210 (18.6%)				107/587 (18.2%)
C	70/197 (35.5%)	58/180 (32.2%)	67/210 (31.9%)				195/587 (33.2%)
D	17/197 (8.6%)	26/180 (14.4%)	18/210 (8.6%)				61/587 (10.4%)
E	43/197 (21.8%)	39/180 (21.7%)	45/210 (21.4%)				127/587 (21.6%)
Ward specialty on day 0:							
Medicine	109/193 (56.5%)	100/177 (56.5%)	102/209 (48.8%)	0.28	0.38	0.17	311/579 (53.7%)
Critical care	28/193 (14.5%)	21/177 (11.9%)	26/209 (12.4%)				75/579 (13.0%)
Major surgery	42/193 (21.8%)	49/177 (27.7%)	70/209 (33.5%)				161/579 (27.8%)
Minor surgery	1/193 (0.5%)	3/177 (1.7%)	6/209 (2.9%)				10/579 (1.7%)
Other	13/193 (6.7%)	4/177 (2.3%)	5/209 (2.4%)				22/579 (3.8%)

Notes: *Data are presented as median (IQR), mean (SD) or n (%)*

Abbreviations: *BP=Blood pressure, eGFR=Estimated glomerular filtration rate, EWS= Early warning score, I=Intermediate therapy, INR= international normalised ratio, IQR =Interquartile range, IV= Intravenous, L= Long therapy, S=Short therapy, SD=Standard deviation, SMD= Standardised mean difference*

4.3.3 Treatment pathways

Over three quarters of the patients received up to three different drugs during their hospital stay (24.7% received just one, 30.3% received two and 22.7% received three). The maximum number of treatments received was eight and this was in one patient (Table 4.7).

Table 4.7 Number of active drugs prescribed per patient

Number of drugs prescribed	MRSA (n=92)		MSSA (n=495)		Overall (n=587)	
1	43	46.7%	102	20.6%	145	24.7%
2	22	23.9%	156	31.5%	178	30.3%
3	26	28.3%	107	21.6%	133	22.7%
4	1	1.1%	68	13.7%	69	11.8%
5	0	0.0%	44	8.9%	44	7.5%
6	0	0.0%	16	3.2%	16	2.7%
7	0	0.0%	1	0.2%	1	0.2%
8	0	0.0%	1	0.2%	1	0.2%

Notes: *Data are presented as n (%)*
Abbreviations: *MRSA=Methicillin-resistant Staphylococcus aureus, MSSA= Methicillin-susceptible Staphylococcus aureus*

The frequencies of each active therapy administered are given in Table 4.8. The most common therapies prescribed for MRSA were Vancomycin (77.2%) followed by Rifampicin (28.3%). In patients with MSSA, the most common prescribed treatment was Flucloxacillin (78.0%) followed by Piperacillin-tazobactam (39.0%).

Table 4.8 Frequency of specific active therapies

	MRSA (n=92)		MSSA (n=495)		Overall (n=587)	
Penicillin						
Benzylpenicillin sodium (Penicillin G)	0/92	0.0%	7/495	1.4%	7/587	1.2%
Phenoxymethylpenicillin (Penicillin V)	0/92	0.0%	1/495	0.2%	1/587	0.2%
Flucloxacillin	0/92	0.0%	386/495	78.0%	386/587	65.8%
Amoxicillin	0/92	0.0%	5/495	1.0%	5/587	0.9%
Co-amoxiclav	0/92	0.0%	93/495	18.8%	93/587	15.8%
Piperacillin-tazobactam	0/92	0.0%	193/495	39.0%	193/587	32.9%
Cephalosporins						
Cefalexin	0/92	0.0%	6/495	1.2%	6/587	1.0%
Cefuroxime	0/92	0.0%	35/495	7.1%	35/587	6.0%
Cefotaxime	0/92	0.0%	6/495	1.2%	6/587	1.0%
Ceftriaxone	0/92	0.0%	17/495	3.4%	17/587	2.9%
Carbapenems						
Ertapenem	0/92	0.0%	4/495	0.8%	4/587	0.7%
Meropenem	0/92	0.0%	52/495	10.5%	52/587	8.9%
Tetracyclines						
Doxycycline	2/92	2.2%	3/495	0.6%	5/587	0.9%

	MRSA (n=92)		MSSA (n=495)		Overall (n=587)	
Minocycline	0/92	0.0%	1/495	0.2%	1/587	0.2%
Tigecycline	0/92	0.0%	1/495	0.2%	1/587	0.2%
Aminoglycosides						
Gentamicin	12/92	13.0%	66/495	13.3%	78/587	13.3%
Macrolides, lincosamides and streptogramins						
Clarithromycin	1/92	1.1%	30/495	6.1%	31/587	5.3%
Erythromycin	0/92	0.0%	7/495	1.4%	7/587	1.2%
Clindamycin	3/92	3.3%	50/495	10.1%	53/587	9.0%
Glycopeptides and lipoglycopeptides						
Teicoplanin	14/92	15.2%	43/495	8.7%	57/587	9.7%
Vancomycin	71/92	77.2%	123/495	24.8%	194/587	33.0%
Oxazolidonones						
Linezolid	21/92	22.8%	31/495	6.3%	52/587	8.9%
Fluoroquinolones						
Ciprofloxacin	1/92	1.1%	26/495	5.3%	27/587	4.6%
Levofloxacin	0/92	0.0%	1/495	0.2%	1/587	0.2%
Moxifloxacin	0/92	0.0%	1/495	0.2%	1/587	0.2%
Misc						
Chloramphenicol	3/92	3.3%	6/495	1.2%	9/587	1.5%
Daptomycin	10/92	10.9%	25/495	5.1%	35/587	6.0%
Trimethoprim	3/92	3.3%	10/495	2.0%	13/587	2.2%
Co-Trimoxazole	2/92	2.2%	14/495	2.8%	26/587	4.4%
Rifampicin	26/92	28.3%	95/495	19.2%	121/587	20.6%

Notes: *Data are presented as n (%)*

Abbreviations: *MRSA=Methicillin-resistant Staphylococcus aureus, MSSA= Methicillin-susceptible Staphylococcus aureus*

The majority of patients (89.8%) were started on an intravenous (IV) therapy of which 52.7% subsequently received oral antibiotics, and 47.2% received IV only (Table 4.9). This was similar across patients with MRSA and MSSA.

Table 4.9 Route of first and second administration

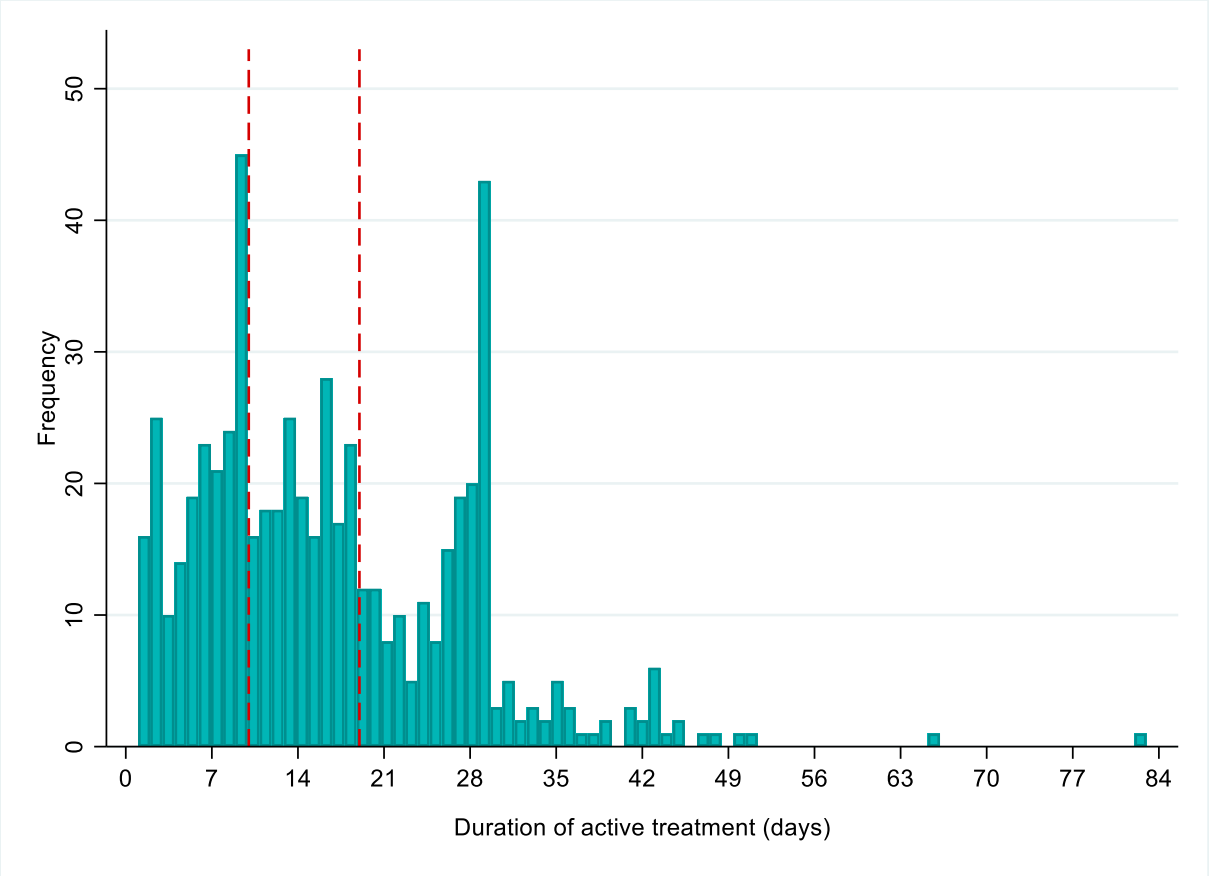
Route of administration	MRSA (n=92)		MSSA (n=495)		Overall (n=587)	
Route 1: IV	86/92	93.5%	441/495	89.1%	527/587	89.8%
Route 2: IV only	50/86	58.1%	199/441	45.1%	249/527	47.2%
Route 2: IV→ IM	0/86	0.0%	1/441	0.2%	1/527	0.2%
Route 2: IV → Oral	36/86	41.9%	241/441	54.6%	277/527	52.6%
Route 1: Oral	5/92	5.4%	54/495	10.9%	59/587	10.1%
Route 2: Oral only	0/5	0.0%	10/54	18.5%	10/59	16.9%
Route 2: Oral → IV	5/5	100.0%	44/54	81.5%	49/59	83.1%
Route 1: IM	1/92	1.1%	0/495	0.0%	1/587	0.2%
Route 2: IM only	1/1	100.0%	-	-	1/1	100.0%

Notes: *Data are presented as n (%)*

Abbreviations: *IM= Intramuscularly, IV= Intravenously*

The median duration of active therapy was 15 days (IQR 8, 25). The distribution of duration of active therapy is shown in Figure 4.9.

Figure 4.9 Histogram of duration of therapy

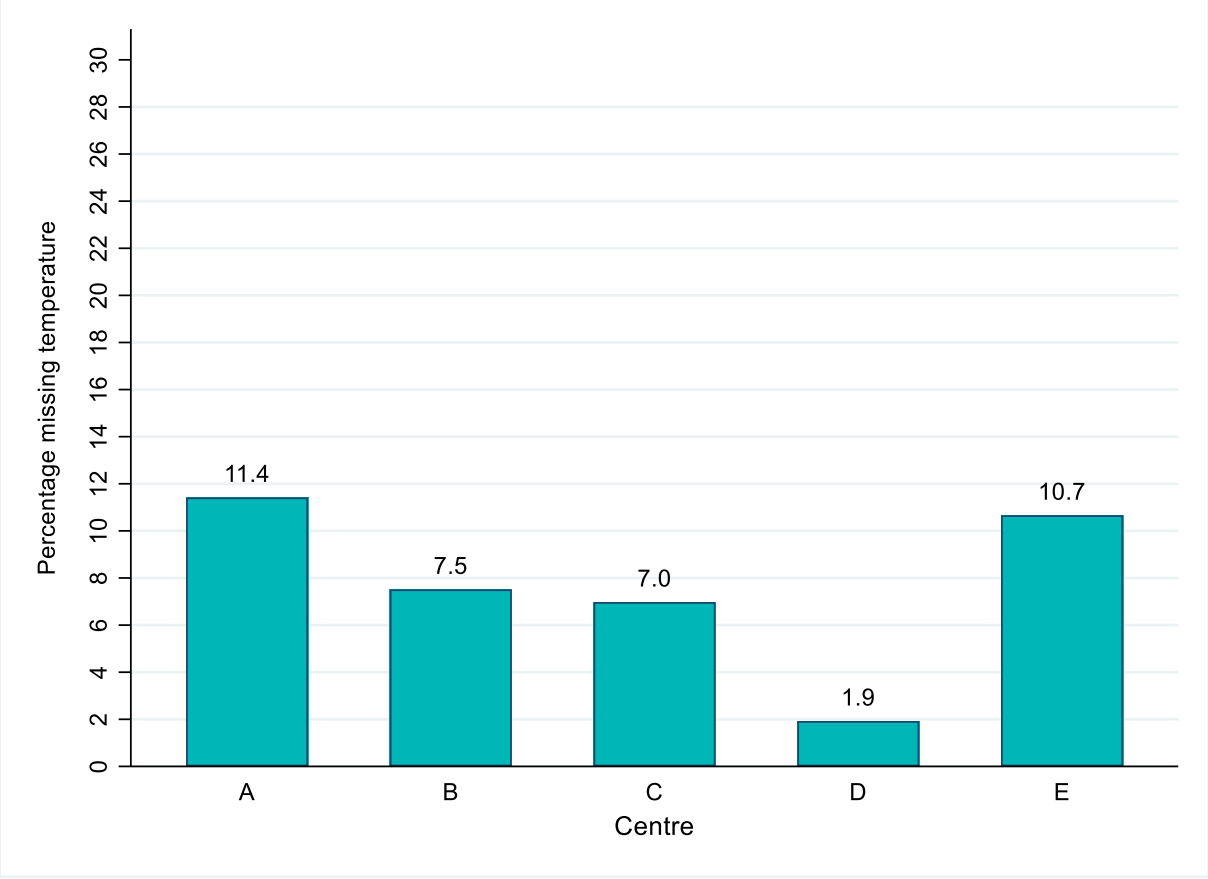


Note: *Red dashed lines represent the thresholds for the three comparative groups (<10 days, 10-18 days, ≥19 days)*

4.3.4 Missing data

Temperature was missing for 8.2% of patient hospital days. I summarised missing temperature measurements by centre to check that it was not one centre contributing most to the missing data rates. Temperature completeness was highest at centre D with only 1.9% missing, and lowest at centre A and E with 11.4% and 10.7% measurements missing respectively (Figure 4.10). However, data were missing across all centres and I did not consider there to be a pattern of missingness across centres. This is in line with the assumption that measurements are likely to be missing at random.

Figure 4.10 Missing temperature, by centre



I then summarised missingness by day since start of treatment (Figure 4.11) and day prior to end of follow-up (Figure 4.12) to check for any patterns e.g. higher proportion missing data later in hospital stay. Data were missing for between 2.6% of patients (day 0) and 13.4% of patients (day 28). Missingness appeared to increase with day since start of treatment, but this fluctuated over time. A similar pattern was seen when assessed by day prior to end of follow-up and temperature was missing for over a quarter of patients on their last day of follow-up. This may have arisen if the patient died or was discharged early in the day, they would not be in hospital for the measurement to be taken.

Figure 4.11 Missing temperature by day since start of treatment

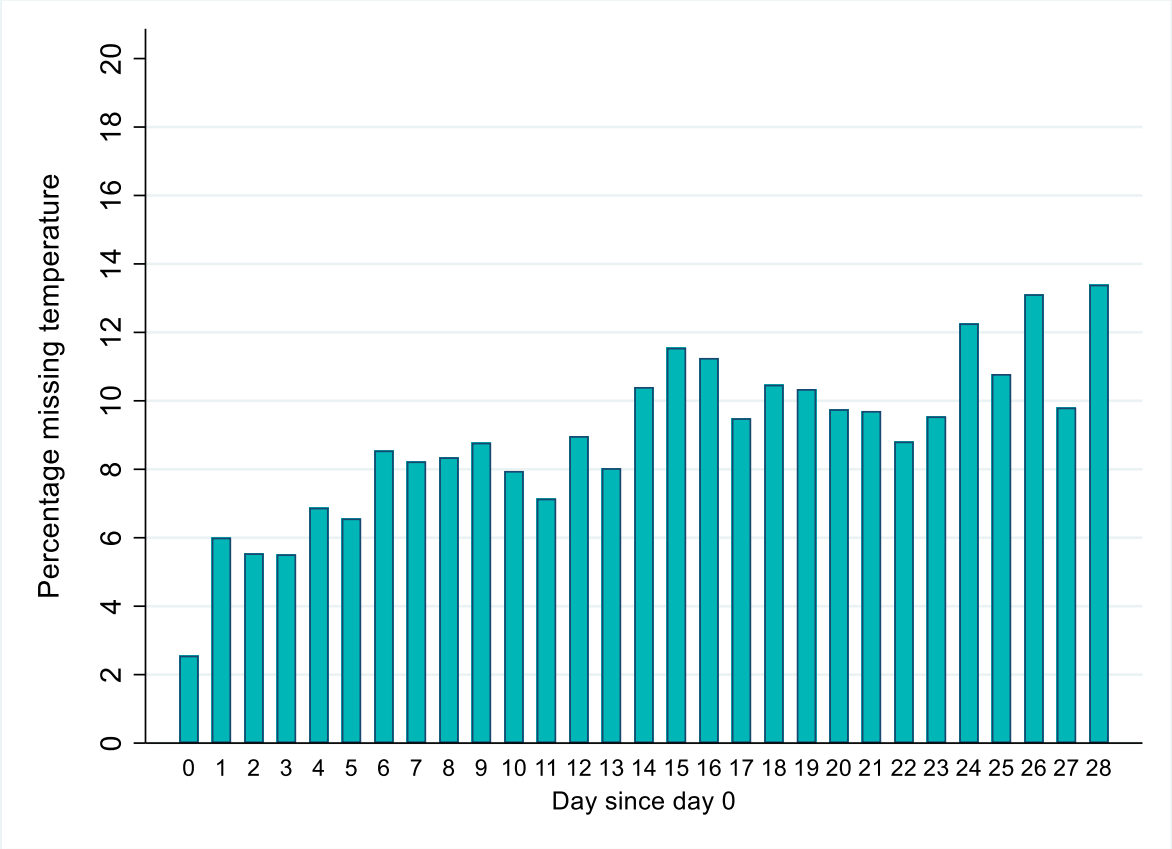
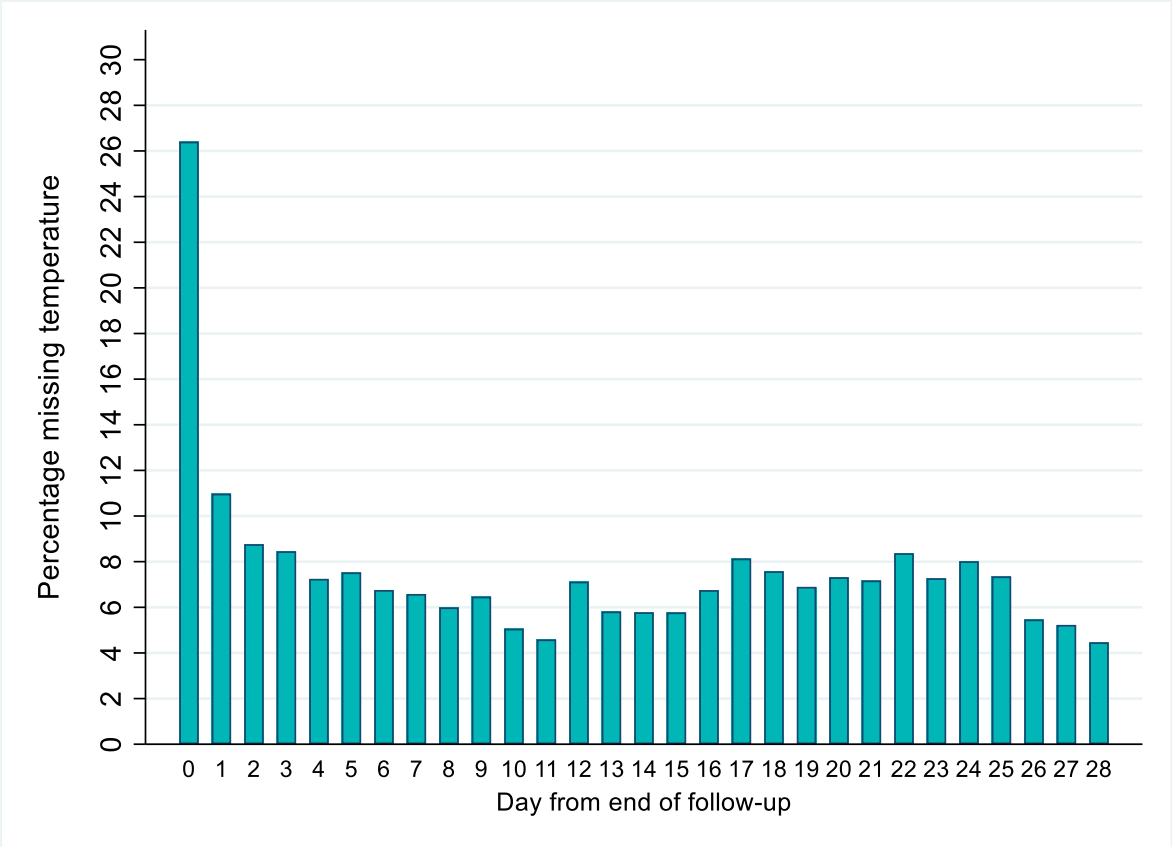


Figure 4.12 Missing temperature by day from end of follow-up



I assessed whether this missingness was different on last day of follow-up for the reasons why follow-up was ended (Table 4.10). It was missing for a higher percentage of those who died (56.6%) compared to those who were discharged (24.5%) or reached end of study follow-up (11.2%). This was expected as those who were discharged are likely to have their temperature measurement taken to assess fitness for discharge. Similarly temperature on the last day of study follow-up is likely to be the least missing as patients would have been in hospital for the full day.

Table 4.10 Missing temperature measurement, by reason for end of follow-up

Reason for end of follow-up	Total (n)	Missing (n)	%
Died	113	64	56.6%
Discharged	286	70	24.5%
End of study follow-up	188	21	11.2%

I used last observation carried forward to impute the missing temperature measurements. Given that a larger proportion of patients were missing temperature on their last day of follow-up (26.4%), I looked at the distribution of imputed values *vs* observed values on this day to check the imputation was reasonable (Table 4.11 and Figure 4.13). Imputed values were similar, and I therefore deemed the imputation acceptable.

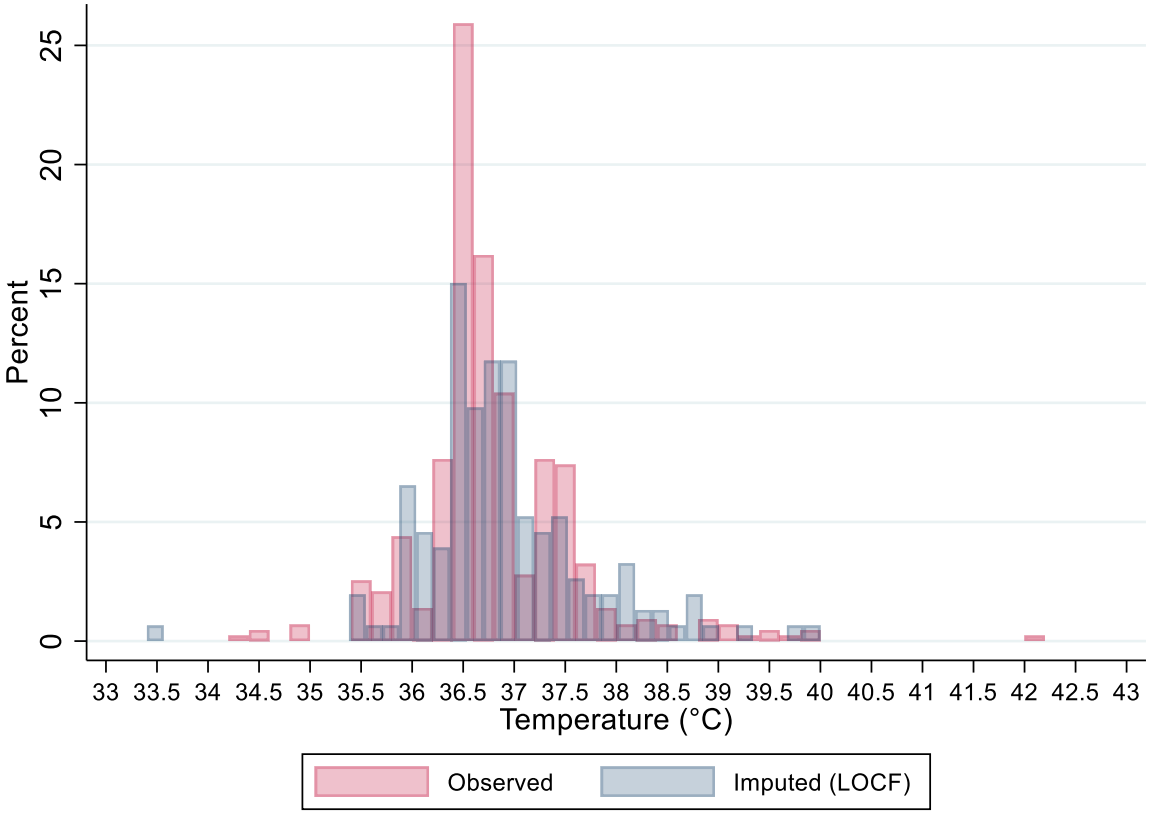
Table 4.11 Summary of observed and imputed temperature on last day of follow-up

	Temperature (°C)			
	Mean (SD)	**Median (IQR)**	**Min**	**Max**
Observed (n=432)	36.8 (0.79)	36.7 (36.4, 37.2)	34.2	42.2
Imputed - LOCF (n=153)	36.9 (0.85)	36.7 (36.4, 37.2)	33.4	40.0

Note: *Data missing for 155 patients on last day of follow-up. Two patients have no temperature measurements so LOCF is not possible.*

Abbreviations: *IQR=Interquartile range, LOCF=Last observation carried forward, SD=Standard deviation*

Figure 4.13 Histogram of temperature on last day of follow-up, imputed *vs* observed



Abbreviations: *LOCF=Last observation carried forward*

Neutrophil count on day 0 was missing for 54/587 (9.2%) of patients. There were some differences between those with neutrophil data available and those with data missing (Table 4.12). Patients with missing data had on average lower eGFR (median 57.0 *vs* 72.0, SMD=0.21), a lower proportion on IV fluids on day 0 (16.7% *vs* 32.3%, SMD=0.37), lower proportion had congestive heart failure (7.4% *vs* 15.9%, SMD=0.27), and a lower proportion had a central line 7.4% *vs* 27.8%, SMD=0.55) or peripheral line (37.0% *vs* 51.6%, SMD 0.30). It also varied by source of infection and centre, SMD 0.84 and 0.94 respectively. Although missingness varied by these factors, they were not considered to be predictive of neutrophil count itself.

Table 4.12 Baseline characteristics, by availability of neutrophil data

	Neutrophil data available (n=533)	Neutrophil data missing (n=54)	SMD (A-M)	Overall (n=587)
Patient measures				
Age	66.0 (49.0, 77.0)	64.0 (49.0, 79.0)	0.06	66.0 (49.0, 77.0)
Male	345/533 (64.7%)	33/54 (61.1%)	0.07	378/587 (64.4%)
Body Mass Index [a]	26.0 (6.1)	27.0 (6.8)	0.15	26.1 (6.2)
Patient medical history				
Chemotherapy in month before date 0	67/533 (12.6%)	2/54 (3.7%)	0.33	69/587 (11.8%)
Any tumour within last 5 years	149/533 (28.0%)	11/54 (20.4%)	0.18	160/587 (27.3%)
Surgery requiring overnight stay within 7 days before date 0	49/533 (9.2%)	2/54 (3.7%)	0.22	51/587 (8.7%)
Burn requiring admission within 7 days before date 0	3/531 (0.6%)	1/54 (1.9%)	0.12	4/585 (0.7%)
Cardiac arrest within 7 days before date 0	2/533 (0.4%)	0/54 (0.0%)	0.09	2/587 (0.3%)
Renal support within 7 days before date 0	54/533 (10.1%)	2/54 (3.7%)	0.26	56/587 (9.5%)
Myocardial infarction within 7 days before date 0	60/533 (11.3%)	6/54 (11.1%)	0.00	66/587 (11.2%)
Infection severity measures				
Temperature (°C) at time 0 [b]	38.2 (37.3, 38.8)	38.1 (37.7, 38.9)	0.10	38.1 (37.3, 38.8)
INR [c]	1.2 (1.1, 1.5)	1.4 (1.1, 2.1)	0.00	1.2 (1.1, 1.5)
eGFR (mL/min/1.73m^2) [d]	72.0 (36.0, 90.0)	57.0 (29.0, 90.0)	0.21	72.0 (36.0, 90.0)
Serum Albumin (g/L) [e]	31.8 (7.4)	32.3 (10.5)	0.06	31.8 (7.6)
Bilirubin total (umol/L) [f]	11.0 (7.0, 19.0)	17.0 (8.0, 35.0)	0.33	11.0 (7.0, 19.0)
Neutrophil count at day 0 or closest (x10^9/L) [g]	9.2 (5.8, 13.3)		-	9.2 (5.8, 13.3)
Systolic BP at day 0 or closest (mmHg) [h]	125.5 (26.7)	123.2 (24.8)	0.09	125.3 (26.5)
On IV fluids at day 0	172/533 (32.3%)	9/54 (16.7%)	0.37	181/587 (30.8%)
On ventilation at day 0	42/531 (7.9%)	2/53 (3.8%)	0.18	44/584 (7.5%)
On vasopressor drugs at day 0	32/533 (6.0%)	0/54 (0.0%)	0.36	32/587 (5.5%)
Systemic corticosteroids in last 24 hours	59/533 (11.1%)	6/54 (11.1%)	0.00	65/587 (11.1%)
EWS score nearest to day 0 [i]	2.0 (1.0, 4.0)	3.0 (0.0, 5.0)	0.08	2.0 (1.0, 4.0)

	Neutrophil data available (n=533)	Neutrophil data missing (n=54)	SMD (A-M)	Overall (n=587)
Patient comorbidities at date 0				
Congestive heart failure	85/533 (15.9%)	4/54 (7.4%)	0.27	89/587 (15.2%)
Peripheral vascular disease	47/533 (8.8%)	9/54 (16.7%)	0.24	56/587 (9.5%)
Cerebrovascular disease	93/533 (17.4%)	6/54 (11.1%)	0.18	99/587 (16.9%)
Hemiplegia	27/533 (5.1%)	2/54 (3.7%)	0.07	29/587 (4.9%)
Dementia	34/533 (6.4%)	3/54 (5.6%)	0.03	37/587 (6.3%)
COPD	61/533 (11.4%)	3/54 (5.6%)	0.21	64/587 (10.9%)
Connective tissue disease	50/533 (9.4%)	2/54 (3.7%)	0.23	52/587 (8.9%)
Peptic ulcer disease	35/533 (6.6%)	3/54 (5.6%)	0.04	38/587 (6.5%)
Ascites	29/533 (5.4%)	6/54 (11.1%)	0.21	35/587 (6.0%)
Diabetes:				
None	415/533 (77.9%)	42/54 (77.8%)	0.12	457/587 (77.9%)
Without organ damage	86/533 (16.1%)	10/54 (18.5%)		96/587 (16.4%)
With organ damage	32/533 (6.0%)	2/54 (3.7%)		34/587 (5.8%)
Child-Pugh score [j]	7.0 (6.0, 8.0)	7.5 (6.0, 8.0)	0.15	7.0 (6.0, 8.0)
Charlson score [k]	3.0 (1.0, 4.0)	3.0 (0.0, 5.0)	0.08	3.0 (1.0, 4.0)
Abscess at time 0	60/533 (11.3%)	6/54 (11.1%)	0.00	66/587 (11.2%)
Infected foreign body at time 0	9/533 (1.7%)	2/54 (3.7%)	0.12	11/587 (1.9%)
Surgical prosthesis time 0	16/533 (3.0%)	0/54 (0.0%)	0.25	16/587 (2.7%)
Source of infection				
Bone and joint	50/533 (9.4%)	11/54 (20.4%)	0.84	61/587 (10.4%)
Cardiovascular system	22/533 (4.1%)	7/54 (13.0%)		29/587 (4.9%)
Central nervous system	8/533 (1.5%)	1/54 (1.9%)		9/587 (1.5%)
Eye, ear, nose, throat or mouth	3/533 (0.6%)	0/54 (0.0%)		3/587 (0.5%)
Gastrointestinal system	15/533 (2.8%)	0/54 (0.0%)		15/587 (2.6%)
Line infection – central venous line	76/533 (14.3%)	1/54 (1.9%)		77/587 (13.1%)
Line infection - peripheral venous line	23/533 (4.3%)	0/54 (0.0%)		23/587 (3.9%)

	Neutrophil data available (n=533)	Neutrophil data missing (n=54)	SMD (A-M)	Overall (n=587)
Lower respiratory tract	37/533 (6.9%)	2/54 (3.7%)		39/587 (6.6%)
Reproductive tract	3/533 (0.6%)	0/54 (0.0%)		3/587 (0.5%)
Skin and soft tissue	76/533 (14.3%)	11/54 (20.4%)		87/587 (14.8%)
Surgical site infection	23/533 (4.3%)	1/54 (1.9%)		24/587 (4.1%)
Systemic Infection	6/533 (1.1%)	1/54 (1.9%)		7/587 (1.2%)
Urinary tract infection	27/533 (5.1%)	2/54 (3.7%)		29/587 (4.9%)
Site uncertain	164/533 (30.8%)	17/54 (31.5%)		181/587 (30.8%)
Lines and catheters				
Central line present at time 0	148/533 (27.8%)	4/54 (7.4%)	0.55	152/587 (25.9%)
Peripheral line present at time 0	275/533 (51.6%)	20/54 (37.0%)	0.30	295/587 (50.3%)
Urinary catheter present at time 0	158/533 (29.6%)	11/54 (20.4%)	0.22	169/587 (28.8%)
Organisational factors				
Centre:				
A	90/533 (16.9%)	7/54 (13.0%)	0.94	97/587 (16.5%)
B	100/533 (18.8%)	7/54 (13.0%)		107/587 (18.2%)
C	159/533 (29.8%)	36/54 (66.7%)		195/587 (33.2%)
D	58/533 (10.9%)	3/54 (5.6%)		61/587 (10.4%)
E	126/533 (23.6%)	1/54 (1.9%)		127/587 (21.6%)
Ward specialty on day 0:				
Medicine	286/528 (54.2%)	25/51 (49.0%)	0.33	311/579 (53.7%)
Critical care	70/528 (13.3%)	5/51 (9.8%)		75/579 (13.0%)
Major surgery	147/528 (27.8%)	14/51 (27.5%)		161/579 (27.8%)
Minor surgery	8/528 (1.5%)	2/51 (3.9%)		10/579 (1.7%)
Other	17/528 (3.2%)	5/51 (9.8%)		22/579 (3.8%)

Notes: *Date and time 0 = date/time of sampling for blood culture*

Data are presented as median (IQR), mean (SD) or n (%)

Missing data (Neutrophil not missing, neutrophil missing):[a] Data missing for 301 patients (269, 32);[b] Data missing for 9 patients (6, 3);[c] Data missing for 353 patients (306, 47);[d] Data missing for 44 patients (13, 31);[e] Data missing for 72 patients (40, 32);[f] Data missing for 101 patients (66, 35);[g] Data missing for 54 patients (0, 54);[h] Data missing for 93 patients (75, 18);[i] Data missing for 278 patients (257, 21);[j] Data missing for 379 patients (329, 50);[k] Data missing for 128 patients (89, 39)
Abbreviations: *BP=Blood pressure, COPD=Chronic obstructive pulmonary disease, eGFR=Estimated glomerular filtration rate, EWS=Early warning score, INR=International normalised ratio, IV=Intravenous, IQR=Interquartile range, SD=Standard deviation, SMD=Standardised mean difference,*

Neutrophil count was imputed using age- and sex- adjusted averages. Imputed and observed values are summarised in Table 4.13. The averages are similar across the imputed and observed values but by nature of the imputation the variability of imputed values is considerably lower than the variability of the observed values.

Table 4.13 Summary of observed and imputed neutrophil count values

	Neutrophil count on day 0			
	Mean (SD)	**Median (IQR)**	**Min**	**Max**
Observed (n=533)	10.3 (7.14)	9.2 (5.8, 13.3)	0	68
Imputed (n=54)	10.3 (0.91)	10.6 (9.8, 10.8)	8.5	11.4

Abbreviations: *IQR=Interquartile range, SD=Standard deviation*

4.3.5 Outcome

In the overall cohort, a total of 113/587 (19.3%) patients died in hospital within 28-days. This was lowest in the patients in the long therapy group (10/210, 4.8%) and highest in the short therapy group (82/197, 41.6%), see Table 4.14. However, it is important to note that these are unadjusted and subject to bias. Overall, 140 patients had an infection defined as complicated. In-hospital mortality was higher in patients with a complicated infection across all three duration of therapy groups (Table 4.14).

Table 4.14 Unadjusted 28-day in-hospital mortality, by observed duration of therapy

	Short therapy (<10 days) (n=197)	**Intermediate therapy (10-18 days) (n=180)**	**Long therapy (≥19 days) (n=210)**	**Overall (n=587)**
Overall				
28-day mortality	82/197 (41.6%)	21/180 (11.7%)	10/210 (4.8%)	113/587 (19.3%)
Died whilst on treatment	28/82 (34.2%)	8/21 (38.1%)	3/10 (30.0%)	39/113 (34.5%)
Complicated *				
N	31	40	69	140
28-day mortality	14/31 (45.2%)	5/40 (12.5%)	4/69 (5.8%)	23/140 (16.4%)
Non-complicated *				
N	154	137	134	425
28-day mortality	61/154 (39.6%)	15/137 (11.0%)	6/134 (4.5%)	82/425 (19.3%)

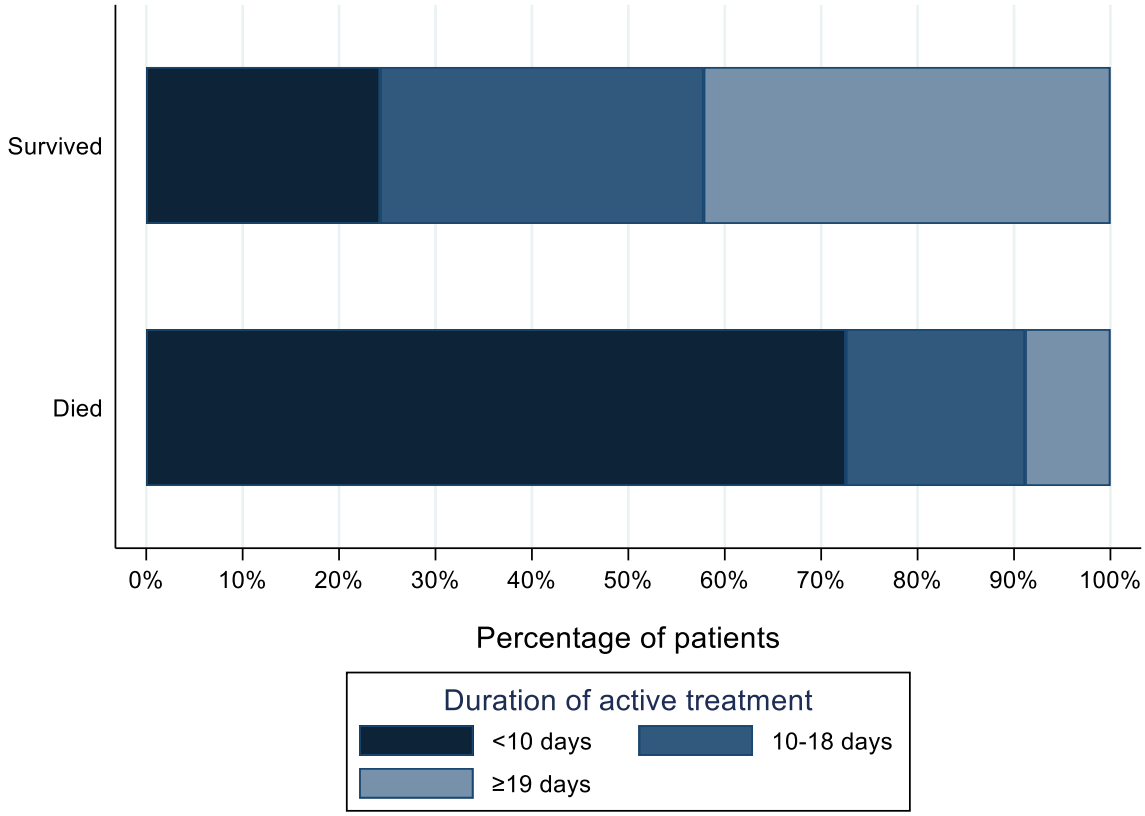
** Complicated if any of the following apply:*

- *Persistent fever at 72 hours*
- *Presence of prosthesis*
- *Cardiovascular system source of infection (endocarditis surrogate)*

Uncomplicated if none of these apply.

Of the 113 patients who died, 72.6% were in the short therapy group, 18.6% were in the intermediate therapy group and 8.9% in the long therapy group (Figure 4.14). The proportion of patients in each duration of therapy group were more similar in patients that survived, with 24.3% in the short therapy group, 33.5% in the intermediate group and 42.2% in the long therapy group. Again, these figures should be interpreted with caution as they are unadjusted and subject to immortal time bias.

Figure 4.14 Duration of therapy, by survival status

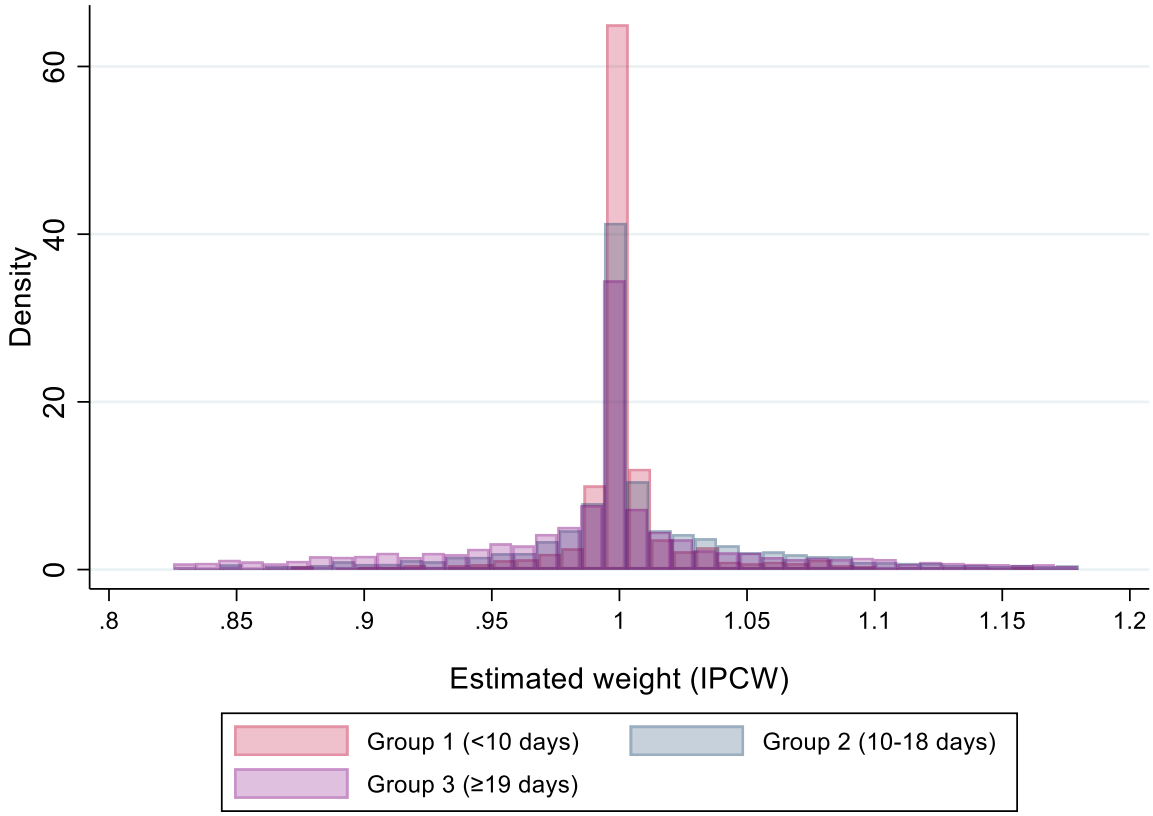


Estimation of weights

The estimated cumulative probability of remaining uncensored (un-stabilised), dependent on the baseline and time varying variables, ranged from 0.004 to 0.999. If the weights were not stabilised, this would equate to inverse-probability-of-censoring weights ranging from 1.00 (1/0.999) to 250 (1/0.004). This would mean that some observations would be represented by one copy of themselves, whereas others would be represented by 250 copies. To avoid just a few people contributing most of the observations to the analysis, the weights were stabilised as per the methods (see section 4.2.4). The range of the stabilised weights were narrower, 0.19–3.59, and are normally distributed around a mean of one (Figure 4.15). Extreme weights may contribute to instability in estimates, I therefore examined the change in precision in weights by truncating the

weights at various percentiles, namely 1st and 99th, 5th and 95th and 10th and 90th percentiles[154]. As the percentiles in which the weights are truncated increases, the precision also increases (Table 4.15). However, the untruncated weights do not have such extreme values that are likely to lead to instability of estimates. Therefore, to maintain sufficient number and variability of participants in the model I decided truncation was not necessary.

Figure 4.15 Distribution of the estimated weights



Note: *Weights truncated at 5th and 95th percentiles*
Abbreviations: *IPCW=Inverse probability of censoring weights,*

Table 4.15 Exploring truncation of estimated weights

Percentiles truncated	Estimated weight (IPCW)		
	Mean (SD)	Minimum	Maximum
0, 100 (no truncation)	1.00 (0.13)	0.19	3.59
1, 99	1.00 (0.09)	0.63	1.46
5, 95	1.00 (0.05)	0.83	1.18
10, 90	1.00 (0.03)	0.90	1.09

Abbreviations: *IPCW=Inverse probability of censoring weights, SD=Standard deviation*

Approach 1: Naive approach

In an unadjusted analysis where treatment strategy was based on the observed exposure status, before cloning; the Kaplan-Meier estimate of 28-day mortality was highest in the short therapy group (72.3%, 95% CI 59.9 to 83.6), followed by intermediate therapy (20.1%, 95% CI 12.9 to 30.6) and lowest in those in the long therapy group (5.7%, 95% CI 3.1 to 10.3), see Table 4.16 and Figure 4.16. In this naïve approach, those who received 18 days of treatment, must have lived at least 18 days to receive 18 days of treatment. They were by definition "immortal" during that time, and therefore results are subject to bias.

Figure 4.16 Kaplan-Meier curve for in-hospital 28-day mortality, by observed duration of therapy

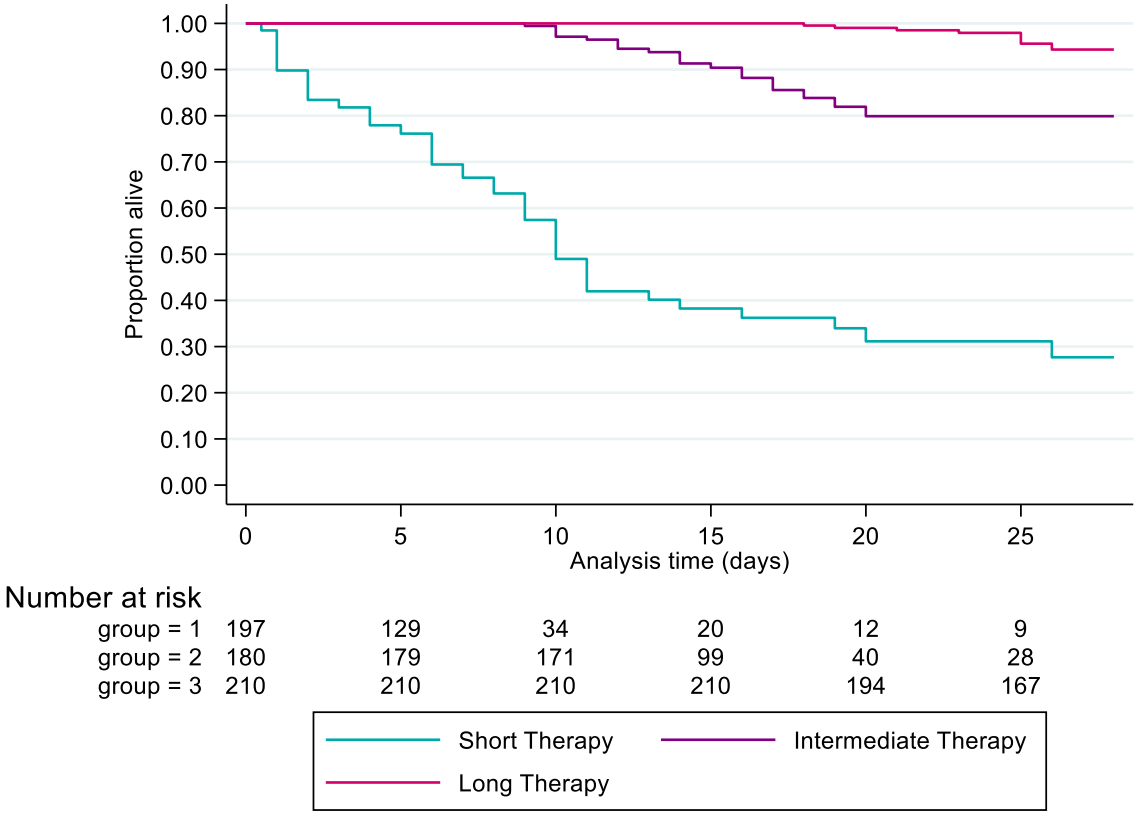


Table 4.16 Naïve analysis: KM estimate and hazard ratio of failure function at 28 days

	Short Therapy	Intermediate Therapy	Long Therapy
KM estimate: Proportion died (95% CI)	72.3% (59.9, 83.6)	20.1% (12.9, 30.6)	5.7% (3.1, 10.3)
Hazard Ratio (95% CI)	37.4 (18.9, 74.4)	4.1 (1.9, 8.9)	Ref.

Abbreviations: *CI=Confidence interval, KM=Kaplan Meier*

Approach 2: Time updating covariates

When applying time-updating covariates, the un-weighted Kaplan-Meier estimate of 28-day in hospital mortality decreased to 57.7% (95% CI 41.3 to 75.0) in the short therapy group, 10.2% (95% CI 5.3 to 19.0) in the intermediate group and 5.2% (95% CI 2.7 to 9.8) in the long therapy group. After weighting, with reference to the long therapy group, the estimated hazard ratios are 7.82 (95% CI 2.10, 29.17) in the short therapy group and 1.37 (95% CI 0.38, 4.97) in the intermediate therapy group, showing evidence of a benefit of longer therapy *vs* short therapy (Table 4.17).

Table 4.17 Time updated covariate: KM estimate of failure and hazard ratio at 28 days

	Short Therapy	Intermediate Therapy	Long Therapy
KM estimate: Proportion died (95% CI)	57.7% (41.3, 75.0)	10.2% (5.3, 19.0)	5.2% (2.7, 9.8)
Hazard Ratio (95% CI)	7.82 (2.10, 29.17)	1.37 (0.38, 4.97)	Ref.

Note: *KM estimates are unweighted. Hazard ratios estimated from a weighted pooled logistic regression model, using inverse probability of censoring weights calculated using daily temperature measurements.*
Abbreviations: *CI=Confidence interval, KM=Kaplan Meier*

In this approach, the bias is reduced because at each time point patients are assigned to the treatment duration group consistent with their data up to that time point (not looking forward in time) i.e. all patients are assigned to <10 days up to day 10, at which point those who continue treatment will be updated and assigned 10-18 days and those who do not will continue to be assigned <10 days.

Approach 3: Three-step procedure

After cloning, the weighted estimates of hazard ratios of all-cause mortality were 1.74 (95% CI 1.36, 2.24) for short therapy *vs* long therapy and 1.09 (95% CI 0.98, 1.22) for intermediate *vs* long (Table 4.18). The sensitivity analysis including time and events after hospital discharge showed similar but weaker associations, possibly explained by deaths after discharge being unlikely to be attributed to infection/treatment therefore biasing the results towards the null. The effect size is smaller than those found using time updated covariates. This may be explained by early deaths contributing to all three groups when using cloning approach but would only contribute to the short therapy group using the updated covariate approach[150].

The effect was strongest in the infections defined as complicated with an estimated hazard ratio of 3.04 (95% CI 1.32, 7.00) and 1.25 (95% CI 0.87, 1.79) compared to 1.70 (95% CI 1.26, 2.29) and 1.08 (95% CI 0.95, 1.22) in infections defined as non-complicated, but the difference between sub-groups was not statistically significant (p-value for interaction=0.43, Table 4.18). Similarly, the effect of short *vs* long therapy was stronger in MRSA infections with an estimated hazard ratio 2.92 (95% CI 1.45, 5.88) compared to 1.54 (95% CI 1.19, 2.00) in MSSA, but estimates of intermediate *vs* long therapy

were similar in MRSA and MSSA infections with estimated hazard ratios of 1.07 (95% CI 0.92, 1.25) and 1.10 (95% CI 0.97, 1.24) respectively, p-value for interaction=0.12 (Table 4.18).

Table 4.18 Hernán 3-Step Procedure: Primary analysis of 28-day mortality

	Short Therapy	Intermediate Therapy	Long Therapy
KM estimate: Proportion died (95% CI)	57.6% (41.1, 75.0)	18.6% (13.2, 25.8)	18.5% (14.7, 23.1)
Hazard Ratio (95% CI)	1.74 (1.36, 2.24)	1.09 (0.98, 1.22)	Ref.
Sens 1: Including time after discharge*	1.36 (1.14, 1.64)	0.97 (0.88, 1.08)	Ref.
Subgroup analysis 1 **			
Complicated (n = 140)	3.04 (1.32, 7.00)	1.25 (0.87, 1.79)	Ref.
Non-complicated (n=425)	1.70 (1.26, 2.29)	1.08 (0.95, 1.22)	Ref.
Subgroup analysis 2 ***			
MRSA (n=92)	2.92 (1.45, 5.88)	1.07 (0.92, 1.25)	Ref.
MSSA (n=495)	1.54 (1.19, 2.00)	1.10 (0.97, 1.24)	Ref.

Note: *KM estimates are unweighted. Hazard ratios estimated from a weighted pooled logistic regression model, using inverse probability of censoring weights calculated using daily temperature measurements.*

** Assuming normal temperature (37^{o}C) after hospital discharge*

*** p-value for interaction=0.43*

**** p-value for interaction =0.12*

Abbreviations: *CI=Confidence interval, KM=Kaplan Meier, MRSA=Methicillin-resistant Staphylococcus aureus, MSSA= Methicillin-susceptible Staphylococcus aureus*

4.4 Discussion

4.4.1 Summary

There is limited trial evidence to guide optimal duration of therapy for the treatment BSI. Results of a clinical trial in *E. coli* were recently published concluding that treatment for seven days is noninferior to 14 days of treatment, however, trial evidence is lacking in SAB[163]. Previous observational studies examining duration of therapy have been criticised for the presence of immortal time bias[130, 133, 136, 144]. I implemented a novel approach to address the bias introduced by confounding and immortal time and our estimates suggest longer treatment (≥18 days) is beneficial compared to <10 days of treatment however the effect of reducing duration to 10-18 days is less certain with our estimates compatible with a range of 2% benefit to 22% harm in survival.

4.4.2 Interpretation

We observed a 74% increase in hazard of all-cause in hospital mortality (HR 1.74, 95% CI 1.36, 2.24) for short therapy (<10 days) compared to long therapy (≥19 days), showing evidence of a benefit of longer therapy compared to short therapy. An increase in hazard of mortality was also found in the intermediate therapy group (10-18 days) compared to long therapy, with a 9% increase in hazard observed, however there was more uncertainty in the effect with a 95% CI ranging from 2% decrease in hazard to 22% increase. A recent single centre cohort study reported a 68% reduction in 90-day

all-cause mortality in patients treated for >14 days compared to ≤14 days (adjusted HR (aHR) 0.32, 95% CI 0.16 to 0.64) in patients with complicated SAB and reported no association in patients with uncomplicated SAB (aHR 0.85, 95% CI 0.41 to 1.78), but these estimates are subject to bias as described above[130]. Similarly, I found a reduction in effect of duration of therapy on mortality in the subgroup of non-complicated infections, however the number of events within our subgroups was small so the power to detect a clinically important difference may be limited in the subgroup of patients. Similar results were observed in a prospective study of 155 patients with SAB that found duration of treatment >14 days was associated with lower mortality (OR 0.84, 95% CI 0.76 to 0.94), but again these conclusions are subject to bias as they only included patients whose observation time was longer than their duration of therapy. I attempted to eliminate immortal time bias by implementing a novel approach involving 'cloning' and 'censoring' that does not require exclusions based on survival time. I contrasted the estimates from this approach with estimates from a Cox regression model where confounding and immortal time bias are ignored (naïve approach) and also from a Cox regression model with time-updated treatment covariates. The effect size was largest in the naïve approach, however the estimates are likely to be extremely biased. The effect sizes were reduced when using time updated covariates, but these remained higher than the estimates from the cloning approach. This may be explained by early deaths contributing to all three arms when using cloning approach but these would only contribute to the short therapy group using the updated covariate approach.

4.4.3 Strengths and limitations

A key strength of this analysis is that the application of the 3-step procedure described by Hernán enabled us to adequately control for immortal bias, which has been a frequent criticism of other studies. BSI-FOO was a large multi-centre study which meant I was able to include a large number of patients in the analysis (over 500) increasing the precision of the estimates.

There are several limitations to our study. Firstly, there are many reasons why patients may cease treatment at a particular time, it may be according to a prespecified treatment strategy, or they may cease or change treatment due to side effects or if their condition has improved so that no further treatment is necessary. The retrospective nature of the study meant that information on reason for continuing/discontinuing treatment was not captured. I accounted for time-varying confounding using inverse-probability weights which included neutrophil count at baseline and daily temperature measurements, however it is not possible to rule out unmeasured confounding factors such as C-Reactive protein which may be associated with clinician's decision in discontinuing treatment. I also did not consider dosing in the estimates which could be another important factor. However, dosing

would have been prescribed as per recommended clinical guidelines and would vary depending on drug prescribed and adjustment for renal impairment. Therefore, there are a very large number of potential dosing and duration strategies that could be compared most likely resulting in an insufficient number of patients to evaluate each strategy separately. Therefore, treatment strategies were defined to be broad and did not include dosing regimen.

Data collection was designed for a different protocol and therefore not tailored for this analysis. This meant that important data items for this analysis were not collected and the definition of start of follow-up differed. Start of follow-up was defined as date of diagnostic blood culture in BSI-FOO, however in this analysis date 0 was defined as start of active therapy. Therefore, the full 28-day follow-up was not available for all participants. However, this is unlikely to have a significant impact as later deaths are unlikely to be a result of treatment. I also restricted the all-cause mortality outcome to in-hospital deaths only in this analysis as temperature measurements post-discharge were not collected therefore weights after hospital discharge could not be reliably calculated. Deaths after hospital discharge are unlikely to be related to the episode of infection during their hospital stay and therefore, I did not consider this to bias the results. Infection-related mortality may be considered a more appropriate outcome, however it was not possible to define this clearly enough to distinguish robustly from other causes of death as cause of death was not collected in BSI-FOO.

The use of an observational dataset defined for a different study protocol also limited the definition of complicated infection that could be applied for the sub-group analysis. Follow-up blood cultures were not recorded in the dataset, so it was not possible to identify positive blood cultures after the initial blood culture, and I was therefore not able to identify persistent infections. Endocarditis was also not recorded so I used cardiovascular system source of infection as a surrogate. Other criteria which have been used in the literature that I was not able to apply as the data were not collected were skin examination findings suggesting acute systemic infection and evidence of metastatic sites of infection[123, 125].

To account for missing data, I used a last observation carried forward approach to impute missing temperature measurements. This meant that other covariates which may be predictive of temperature were not included in the imputation. An alternative approach to imputing the missing data, given that the data is assumed to be missing at random, is multiple imputation. However, this would be challenging and very computationally intensive to combine with cloning and bootstrapping with the given sample size. I also did not perform a complete case analysis as this would reduce the sample size and in turn the power of the study and would likely be bias limiting the generalisability

of the results. Temperature measurements do not fluctuate by large amounts (~35-40^{o}C) and I therefore consider LOCF to be the most efficient and appropriate in this analysis to maintain the sample size and reduce the bias caused by the missing data. However, it was not possible to use the same approach to impute missing neutrophil count as this was only measured on day 0. I therefore imputed neutrophil count using age-sex- adjusted averages. Chemotherapy or radiotherapy can lower the levels of neutrophil in the blood so I considered including this in the imputation, however, only two participants who received chemotherapy in month prior to day 0 had missing neutrophil so this was not included in the imputation for ease of interpretation and consistency.

Finally, I only looked at one outcome in this analysis – all-cause in hospital mortality within 28 days. Other outcomes such as relapse of infection and complication rate may also be of importance in determining efficacy of reducing treatment duration, however such data were not collected in BSI-FOO so it was not possible to investigate these in this study. Clinically it would be relevant to look at the time after completion of treatment to longer term outcomes e.g. deaths at 90-days. However, this is not possible in the dataset, so future work may consider additional linkage of mortality data to address this.

4.4.4 Conclusions

To date, there has not been an RCT evaluating duration of treatment for SAB, however there is currently a trial in recruitment comparing the efficacy of seven and fourteen days of antibiotic therapy in uncomplicated *S. aureus*[134]. Until the results of this trial are published, on the basis of these findings presented here, we do not recommend duration of therapy to be <10 days for SAB. Duration 10-18 days may be adequate for uncomplication infections, however reducing duration of therapy in clinical practice should be adopted with caution until sufficiently powered studies are published allowing more accurate and precise estimation of the effect.

CHAPTER 5 EMULATING THE MERINO TRIAL

In this chapter, I will discuss the methods and results of a trial emulation that I performed to compare the results of a recently published RCT (the MERINO Trial) to published results from observational studies comparing treatments for ESBL-producing bacteraemia. I will start by discussing the current literature on treatment with carbapenems and β-Lactam/β-lactamase inhibitor (BLBLI) (section 5.1) and then move on to present the trial emulation methods (section 5.2) and results of the emulated trial (section 5.3). At the end of the chapter, I reflect on the findings as well as the strengths and limitations (section 5.4).

5.1 Introduction

ESBL producing bacteria are a frequent cause of bloodstream infection, in particular ESBL producing *E. coli* and Klebsiella species. Carbapenems are commonly regarded as the antibiotic of choice for treatment of infections caused by ESBL producers, especially for the treatment of severe infections[164, 165]. However, it has been shown that increased use of carbapenems is associated with increased incidence of carbapenem resistant Enterobacteriaceae[166]. Alternative treatments are therefore needed to reduce the use of carbapenems to help contain the spread and frequency of carbapenem resistance.

BLBLI combination antibiotics, such as Piperacillin-Tazobactam, have been used as an alternative treatment for treatment of ESBL producers[167, 168]. There have been a number of observational studies that have shown that BLBLIs are an effective treatment for infections caused by ESBL producers[169-173], and recent reviews have shown the effect to differ depending on the infection severity[174, 175]. A summary of studies comparing carbapenems and BLBLIs for the treatment of ESBL bacteraemia is given in Table 5.1.

Despite the number of published studies, the majority have been observational which are subject to bias and confounding. However, an RCT has been recently published, "Randomized Controlled Trial of Meropenem Versus Piperacillin-Tazobactam for Definitive Treatment of Bloodstream Infections Due to Ceftriaxone Non-susceptible *E. Coli* and Klebsiella Species" (the MERINO trial), and results were not consistent with results published in these observational studies[176]. The MERINO trial is an international RCT to determine whether definitive therapy with a carbapenem-sparing treatment (Piperacillin-Tazobactam) is noninferior to a carbapenem (Meropenem) in patients with BSI caused by ceftriaxone-non-susceptible *E. coli* or *K. pneumoniae* with respect to 30-day mortality[176]. A noninferiority margin of 5% was used. The trial analysis included 378 participants; 23/187 participants (12.3%) in the Piperacillin-Tazobactam died within 30-days compared to 7/191 (3.7%) in

the Meropenem group with an unadjusted risk difference of 8.6% (95% CI 3.0 to 14.5). They concluded that definitive treatment with Piperacillin-Tazobactam was inferior in terms of 30-day mortality compared to definitive treatment with Meropenem, which was conflicting to some of the observational studies published prior to the trial (described below). In a further analysis excluding strains with piperacillin/tazobactam MIC values > 16, the 30-day mortality difference was reduced with a risk difference of 5% (95% CI -1 to 10)[177].

Gutierrez *et al*. investigated the efficacy of definitive therapy with BLBLIs compared to carbapenems in an international cohort study of 601 patients[170]. After adjustment for propensity score, they concluded no difference in 30-day mortality between those who received definitive therapy with carbapenems and those who received BLBLI (13.9% *vs* 9.8% respectively, adjusted OR 0.65 (95% CI 0.23 to 1.65; p=0.86). Gudiol *et al*. performed a multicentre retrospective study of patients with haematological neutropenia who had ESBL producing Gram-negative BSI by comparing 30-day all-cause mortality after onset of BSI in patients who received carbapenems to patients who received BLBLIs as their empiric or definitive therapy (n=174 patients in empiric therapy comparisons and 251 in definitive therapy comparisons)[169]. They found no statistically significant difference in mortality between the groups in either the empiric therapy comparison or definitive therapy comparison although the direction of the effect favoured carbapenem in the empiric comparisons (13.4% carbapenem versus 20.8% BLBLI empiric treatment, p=0.33). The 30-day mortality rate was higher in patients receiving carbapenems in the definitive therapy comparisons (15.8% carbapenem versus 5.8% BLBLI) however, this did not reach statistical significance (p=0.99) and may be explained by the larger number of patients treated with carbapenem compared to BLBLI (234 versus 17 respectively). After using a propensity score matching approach for the definitive therapy comparison, they found no statistically significant difference in 30-day mortality between 15 matched pairs between the patients treated with carbapenems and those who received BLBLIs (6.5% versus 12.5%), although again this may be explained by the small number of matched pairs. Another multicentre cohort study evaluating the use of empirical treatment with non-carbapenem treatment to empirical treatment with carbapenems was conducted in 232 patients with ESBL bacteraemia in Korea[171]. They used the inverse probability of treatment weights (IPTW) to account for imbalance between treatments and found that 30-day mortality was not statistically significantly different in patients treated with non-carbapenems (6.3%) to patients treated with carbapenems (11.4%), p=0.42. They also conducted a subgroup analysis comparing only Piperacillin Tazobactam to carbapenem as this was the most used non-carbapenem treatment and similar results were observed. They concluded that appropriate non-carbapenems were not inferior to carbapenems as empirical therapy for ESBL producing bacteraemia. Ming *et al*. conducted a retrospective cohort study across two hospitals in

Singapore to compare 30-day mortality of patients with empirical treatment of Piperacillin-Tazobactam *vs* a carbapenem[172]. This study included 394 patients with ESBL producing *E. coli* or Klebsiella pneumoniae and did not find a statically significant difference in 30-day mortality between those who were treated with Piperacillin-Tazobactam and those who were treated with a carbapenem (30.9% *vs* 29.8% respectively) with a corresponding odds ratio of 1.00 (95% CI 0.45 to 2.17, p=0.99) after adjustment for propensity score.

Rodriguez *et al.* performed a post hoc analysis comparing 30-day mortality in patients treated with an active BLBLI or carbapenem in patients with ESBL producing *E. coli* BSI from six prospective cohorts[173]. Confounding was controlled by multivariate analysis or propensity score adjustment. In the cohort comparing definitive treatments, the 30-day mortality rates were 9.3% *vs* 16.7% for those treated with BLBLI versus carbapenems respectively. After adjustment for confounders, they concluded 30-day mortality was equivalent in those treated with BLBLI or carbapenem as definitive therapy (adjusted HR: 0.76, 95% CI 0.28 to 2.07). However, it is worth noting the confidence intervals were wide and the majority of BSIs were urinary tract which are known to be more responsive to treatment. In addition, Piperacillin-Tazobactam was given in relatively higher doses compared with the usual practice in hospitals and countries, which might have contributed to overall effect (4,500mg every 6 hours in this study compared to the usual dose of 3,375mg every 6 to 8 hours or 4,500mg every 8 hours). The higher doses administered during the study is likely to be due to β-lactams having high concentrations excreted in urine therefore higher concentrations are administered.

Ofer-Friedman *et al.* performed an analysis focussing on non-urinary source ESBL producing *E. coli* which included 79 patients treated with either a carbapenem or Piperacillin-Tazobactam[178]. After adjustment for confounders, they found that 90-day mortality was higher in patients treated with Piperacillin-Tazobactam (adjusted odds ratio 7.9, 95% CI 1.2 to 53.0, p=0.03) and therefore concluded that in ESBL BSIs of a non-urinary course, carbapenems can be viewed as a superior treatment to BLBLIs. However, this study only included 10 patients treated with Piperacillin-Tazobactam which resulted in a very wide confidence interval.

Tsai *et al.* performed a multicentre retrospective study of 47 patients with ESBL-producing P. mirabilis BSI in Taiwan comparing the outcomes of patients treated with Piperacillin-Tazobactam or a carbapenem[179]. The 30-day mortality rate of patients in receipt of carbapenem was lower than that of those in receipt of Piperacillin-Tazobactam (14.3% *vs* 23.1%, p = 0.65) and concluded that carbapenem therapy could be considered as the treatment of choice for ESBL-producing P. mirabilis BSI. However, only patients who received antimicrobial therapy for more than 48 hours were

included, so patients who died within 48 hours of treatment would have been excluded which could have resulted in patients who were more ill or had more severe infections being excluded.

Tamma *et al.* compared 14-day mortality of Piperacillin-Tazobactam *vs* carbapenems as empiric therapy in a cohort of 331 patients with ESBL bacteraemia who all received definitive therapy with a carbapenem[180]. Fourteen-day mortality, after applying inverse probability weights of propensity of receiving Piperacillin-Tazobactam, was 1.92 times higher for patients receiving empiric Piperacillin-Tazobactam compared with empiric carbapenem therapy (95% CI, 1.07 to 3.45, p=0.03) and therefore they concluded that Piperacillin-Tazobactam is inferior to carbapenems for the empiric treatment of ESBL bacteraemia.

In 2012, Vardakas *et al.* performed a meta-analysis of 21 studies (1,584 patients) reporting comparisons of carbapenems *vs* alternative treatments which included BLBLIs for the treatment of ESBL-producing Enterobacteriaceae BSI[164]. No statistically significant differences in mortality were observed between patients treated with carbapenems and patients treated with BLBLIs as definitive therapy (risk ratio (RR) 0.52, 95% 0.23 to 1.13) or empiric therapy (RR 0.91, 95% CI 0.66 to 1.25), but direction favoured carbapenems. However, as mentioned in their discussion, none of the studies included in the meta-analysis were RCTs and were therefore likely to be subject to confounding and data were not available to adjust for confounding factors. Treating clinicians may select a broader spectrum antibiotic or combination therapy for more serious infections which may impact on outcome. In addition, the source of bacteriemia varied significantly between the included studies.

An RCT is considered the gold standard design in clinical research, however, they can be time-consuming and are not always financially or ethically feasible to conduct. Therefore, an observational approach is often used but these studies can be subject to bias and confounding. Such biases include selection bias and immortal time bias. These limitations can be avoided by designing the observational analysis in a way that it can viewed as an emulation of a 'target' trial, that is a hypothetical trial that would answer the research question, but with no blinding[181]. Emulating a target trial is an approach designed to "mimic" trial practice using observational data and if successful, should yield similar results with the exception of sampling variation[182].

Trial emulation methods can be used to analyse data to answer research questions where a randomised trial is not feasible, too time consuming or too costly[182]. The methods can also be applied to compare results of observational analysis to randomised trials where they are conflicting. An example of the latter was performed to assess the effect of oestrogen/progestin therapy which has been shown to reduce risk of coronary heart disease in observational analysis, but an increased risk was found in a RCT[183-185]. To explore the discrepancies, an analysis of the observational data

using an emulated trial design was performed. The emulated trial yielded similar estimates to the RCT, suggesting that the discrepancies were primarily due to different analytic approaches[186].

There are a number of components that need to be considered in the trial emulation: eligibility criteria, start of follow-up, treatment strategies, assignment of procedures, outcomes, causal contrast, and analysis plan[182]. Firstly, the eligibility needs to be defined, such that the eligibility criteria that would be used in a trial is applied in the observational setting. It is important to note that the eligibility criteria cannot use data that are identified from events occurring post baseline e.g. including only patients who received three days of treatment, as in a true RCT it would not be possible to apply these at the time of randomisation and could introduce bias. Secondly, assignment of treatment strategy needs to be done such that strategies are consistent with patients' baseline data.

We then need to emulate the random assignment of strategies by adjusting for all confounding factors. The adjustment for baseline confounders can be done using various methods e.g. propensity score matching, multivariable regression, inverse probability weighting, g-estimation[157].

The outcome for each patient is determined using the observed data. Choice of outcome is important in trial emulation, as treatment blinding will generally not be implemented so it is worth considering whether the outcome can be impacted by the outcome assessor's knowledge of the treatment. The choice of effect i.e. intention to treat or per protocol effect, also needs to be decided prior to writing an analysis plan. The choice would be based on what would be of interest in the design of a RCT if one was performed. If the intention-to-treat and per-protocol effects would be compared in a trial, then an attempt to estimate both effects should be done in the emulated trial. An analysis plan would then be written prior to analysis. In an RCT, to estimate the intention-to-treat effect, outcomes would be compared according to randomised allocation regardless of adherence. However, in an observational setting, this is not possible as randomisation was not performed. Instead, a similar comparison would be a comparison by treatment initiation i.e. according to which treatment the patient started on, but this requires ensuring that baseline confounding factors are adjusted for. A per-protocol or as-treated effect would analyse patients by adherence and would need to account for post-baseline confounding factors that are associated with adherence to the treatment strategies and may be affected by prior adherence.

Defining time zero, the start of follow-up, is important in trial emulation. Eligibility criteria need to be met at this point and outcomes counted from this point forward. In trials, start of follow-up is often defined as the time of randomisation. In an emulated trial, patients are not randomised and therefore a comparable time would be at the time of initiation of a treatment strategy. Starting

follow up after this could introduce selection bias as outcome occurrence between starting treatment or randomisation in a trial and start of follow-up would be excluded.

As the MERINO trial results were conflicting to some of the results published using observational data, I decided to use trial emulation methods to emulate the MERINO trial to see if I could obtain similar results to the trial and to explore whether the observed differences between the observational studies and the MERINO trial are due to the lack of randomisation and potential bias in the observational studies. In addition, no UK patients were recruited to the MERINO trial, so applying trial emulation methods to UK data could provide information on how a trial such as the MERINO trial would have performed in the NHS. The aim of this chapter was to use data from BSI-FOO observational study and the RAPIDO trial to emulate the MERINO trial.

Table 5.1 Summary of studies reporting on the comparison of carbapenems and BLBLI for the treatment of ESBL bacteraemia and mortality

Study	Design/Country	Numbers analysed	Organism	Primary outcome	Comparator groups	Mortality rate		
						Carbapenem	BLBLI	p-value
Harris (2018)[187]	Randomised clinical trial, noninferiority/multi-country (9 countries, 26 centres)	n=391	E.coli or Klebsiella spp	30-day mortality	P&T *vs* Meropenem	3.7%	12.3%	p=0.9 (noninferior)
Ko (2018)[171]	Retrospective cohort study/Korea (4 hospitals)	n=232	ESBL-E.coli & Klebsiella pneumoniae	30-day mortality	Non-carbapenem *vs* Carbapenem	11.4%	6.3%	p=0.42
Gudiol (2017)[169]	Retrospective cohort study/multi-country (9 countries, 22 centres)	Empirical therapy: n= 174 Definitive therapy: n=251	ESBL gram negative bacteraemia	30-day mortality	BLBLI *vs* Carbapenems	ETC: 13.4% DTC: 15.8%	ETC: 20.8%. DTC: 5.8%	ETC: p=0.33 DTC: p=0.99
Gutierrez-Gutierrez (2016)[170]	Retrospective cohort study/multi-country (12 countries, 37 centres)	Empirical therapy: n= 365 Definitive therapy: n=601 Global cohort: n=627	ESBL-Enterobacteriaceae	Clinical response at 14 days and 30-dy mortality	BLBLI *vs* Carbapenems	ETC: 20.0% DTC: 13.9%	ETC: 17.6% DTC: 9.8%	ETC: p=0.6 DTC: p=0.28
Ming (2016)[172]	Retrospective cohort study/Singapore (2 hospitals)	n=151	ESBL-E.coli & Klebsiella pneumoniae	30-day mortality	P&T *vs* Carbapenem	29.8%	30.9%	p=0.89
Ofer-Friedman (2015)[178]	Retrospective cohort study/Multi-country (Israel - 1 centre, USA - 1 centre)	n=79	ESBL-E.coli & Klebsiella pneumoniae & P.mirabilis	In hospital mortality, 30-day mortality and 90-day mortality	P&T *vs* Carbapenem	34.0%	60.0%	p=0.10
Tamma (2015)[180]	Retrospective cohort study/US (Single centre)	n=231	ESBL bacteraemia	14-day mortality	P&T *vs* Carbapenem	8.0%	17.0%	p=0.03
Tsai (2014)[179]	Retrospective cohort study/Taiwan (5 centres)	n=40	ESBL- Proteus mirabilis	30-day mortality	P&T *vs* Carbapenem	14.3%	23.1%	p=0.653

Study	Design/Country	Numbers analysed	Organism	Primary outcome	Comparator groups	Mortality rate		
						Carbapenem	BLBLI	p-value
					vs Other agents			
Vardakas (2012)[164]	Meta analysis/21 articles	n=1,584	ESBL bacteraemia	All-cause mortality	Various, including Carbapenems *vs* BL/BLIs	ETC: 20%. DTC: 19%	ETC: 21%. DTC: 20%	
Rodriguez-Bano (2012)[173]	Six prospective cohort studies/Spain	Empirical therapy: n= 103 Definitive therapy: n=174	ESBL-E.coli	30-day mortality	Amoxicillin-clavulanic acid or P&T *vs* carbapenem	ETC: 19.4%. DTC: 16.7%	ETC: 9.7%. DTC: 9.3%	p=0.1

Abbreviations: *BLBLI= β-Lactam/β-lactamase inhibitor, DTC=Definitive therapy cohort, ESBL= Extended-spectrum β-lactamase, ETC=Empirical therapy cohort, P&T= Piperacillin-Tazobactam*

5.2 Methods

5.2.1 Data source

I used patient-level data from both BSI-FOO and RAPIDO to emulate the MERINO trial eligibility criteria, treatment strategy, and statistical analysis. The two studies had similar data collection, therefore to maximise the potential sample size of the emulated trial, I included data from both studies.

5.2.2 Eligibility criteria

The first step in trial emulation is to define the study population. I applied the MERINO trial inclusion/exclusion criteria to BSI-FOO and RAPIDO patients (Table 5.2). Patients were included if they had a BSI with *E. coli* or *Klebsiella* spp. that was resistant to Ceftriaxone and/or Cefotaxime and susceptible to both Meropenem and Piperacillin-Tazobactam and treatment was started within 72 hours of blood culture. I excluded patients that would have otherwise been eligible but did not start either of the study drugs (Meropenem or Piperacillin-Tazobactam) within the 72 hours window. All patients were aged 18 years and over. Informed consent was not required for the BSI-FOO observational study or for patients who died before being approached for consent in RAPIDO, so it was not possible to replicate consent for this population. It was also not possible to determine survival expectation and therefore to avoid using observed survival data which could introduce bias, I did not apply these criteria to the emulated trial. Data on allergies were not collected in the BSI-FOO observational study or RAPIDO but it was assumed that if the patient was given a penicillin or a carbapenem that they had no known allergies to these drugs. Polymicrobial infections and repeat episodes were excluded. End of care pathway was also an exclusion criterion in BSI-FOO and RAPIDO, so no extra restriction was needed for the emulated population. Data on pregnancy and breast-feeding were not collected in the BSI-FOO observational study and therefore I could not apply these criteria, however the average age in BSI-FOO is approximately 70 years and since the exclusion was likely to be a safety measure I did not anticipate it to affect the comparison of results.

Table 5.2 Population, intervention, comparison, outcome (PICO) table

PICO component		MERINO trial[187]	Emulated trial (BSI-FOO & RAPIDO)
Patient/population	Inclusion	"Bloodstream infection with *E. coli* or Klebsiella spp. with proven non-susceptibility to third generation cephalosporins and susceptibility to Meropenem and Piperacillin-Tazobactam"	• ESBL producing *E.coli* AND Klebsiella – ESBL • Resistant to Ceftriaxone or Cefotaxime • Susceptible to Meropenem AND Piperacillin-Tazobactam
		"No more than 72 hours has elapsed since the first positive blood culture collection"	• Start treatment within 72 hours of blood culture • Received either Meropenem or Piperacillin-Tazobactam in that window
		Patient is aged 18 years and over	All BSI-FOO patients
		The patient or approved proxy is able to provide informed consent	Not applied
	Exclusion	Patient not expected to survive more than 4 days	Not applied
		Patient allergic to a penicillin or a carbapenem	Assume if in receipt of drug then no known allergy
		Patient with significant polymicrobial bacteraemia	Polymicrobial infections
		Previously enrolled	Repeat episodes
		Treatment is not with the intent to cure the infection (that is, palliative care is an exclusion).	End of care pathway excluded in BSI-FOO
		Pregnancy or breast-feeding	Not applied
		"Use of concomitant antimicrobials in the first 4 days after enrolment with known activity against Gram-negative bacilli (except trimethoprim/sulfamethoxazole may be continued as Pneumocystis prophylaxis)."	Not applied
Intervention	Piperacillin-Tazobactam	"4.5g administered every 6 hours intravenously. Dose adjustment for renal impairment was made."	As prescribed, determined by treating clinician* Doses may be lower due to renal impairment.
Comparison	Meropenem	"1g will be administered every 8 hours intravenously."	As prescribed, determined by treating clinician** Doses may be lower due to renal impairment.
		"Each dose will be given over 30 minutes. The study drug was to be administered for a minimum of 4 days and given for as long as 14 days. The total duration of therapy	

PICO component		MERINO trial[187]	Emulated trial (BSI-FOO & RAPIDO)
		was be determined by the treating clinician." Dose adjustment for renal impairment was made.	
Outcome	Follow up	Starts at assignment to intervention and ends at death or 30 days.	Start on date/time of first prescribed study drug
	Primary outcome	30-day mortality	25-day mortality

** Dose: 4.5g (99%), 2.25g (1%). Frequency: 3/day (68%), 2/day (13%), 1/day (1%), stat (17%).*
*** Dose: 1g (74%), 2g (3%), 0.5g (23%). Frequency: 3/day (54%), 2/day (23%), 1/day (5%), stat (18%).*
Abbreviations: *ESBL= Extended-spectrum β-lactamase; PICO=Population, intervention, comparison, outcome*

5.2.3 Treatment strategies

The trial interventions in the MERINO trial were treatment with Piperacillin-Tazobactam or Meropenem. Both drugs were administered intravenously. Meropenem was given at a dose of 1g and was administered every 8 hours and Piperacillin-Tazobactam was given at a dose of 4.5g administered every 6 hours. These were to be administered for a minimum of 4 days and maximum of 14 days, with duration determined by the treating clinician and dose adjusted for renal impairment i.e. if creatinine clearance ≤ 50 mL/min or on renal replacement therapy. In the emulated trial, treatments were given as prescribed (Table 5.2).

5.2.4 Assignment of treatment

As per the inclusion/exclusion criteria defined above, all patients received either Meropenem or Piperacillin-Tazobactam within 72 hours of blood culture. In the emulated trial population patients were assigned to a "emulated intervention" based on their treatment timeline and allocated to the first study drug received.

5.2.5 Start of follow-up

The start follow-up was defined as the time in which individuals became eligible for a regimen. In practice, this would be when susceptibility results on the two study drugs become available. These data were not available for the emulated trial, so I assumed that when either study drug was given that susceptibility was known at this time. Therefore, the start of follow-up was defined as the date in which the patient started their first dose of their assigned intervention.

5.2.6 Outcome

The primary outcome of the MERINO trial was all-cause mortality at 30 days after randomisation. It was not possible to analyse 30-day mortality for the emulated trial as follow-up was limited to 28

days in the BSI-FOO observational study and RAPDIO, where start of follow-up for these studies was defined as the date the blood sample was taken. All patients started their emulated intervention within three days of blood sample; therefore, I chose to analyse 25-day mortality to ensure full follow-up was available for all patients. Only one patient died (randomised to Piperacillin-Tazobactam) after day 25 in the MERINO trial so I decided analysing 25-day mortality would not impact the comparison of results. In addition, I also decided to investigate all-cause 14-day mortality as a post-hoc analysis, as deaths after day 14 are unlikely to be attributable to treatment. The MERINO trial did not report 14-day mortality, but I calculated the number of events up to day 14 for comparison using the Kaplan-Meier graph.

5.2.7 Analysis population

The MERINO trial primary analysis population was defined as any randomised participant receiving at least one dose of the allocated study drug, regardless of their adherence with the study protocol, for example administered for a minimum of 4 days. This was supported by a per-protocol analysis. By definition, in the emulated trial population, all patients received at least one dose of allocated drug and were therefore included in the primary analysis population. The per-protocol analysis required patients to receive their allocated treatment for four days and not receive a second Gram-negative active agent in days 1-5 post randomisation. However, few patients received the allocated treatment with no other active therapy for the required four days in the emulated trial population, therefore I did not emulate the per-protocol analysis.

5.2.8 Statistical analysis

Descriptive analysis

Continuous data were summarised using mean and standard deviation (or median and IQR if distributions were skewed) and categorical data as numbers and percentages. Demographics, comorbidities and medical history were summarised by emulated intervention. Standardised mean differences (SMD) were calculated to quantify imbalances in baseline characteristics by the emulated intervention group[188]. Mortality over 25-days was summarised by emulated intervention using inverse probability weighted survival curves[189]. Weights are assigned to each subject where the weight is equal to the inverse of the probability of receiving the treatment that they actually received conditional on the observed covariates. The probabilities were estimated from a logistic regression of treatment received regressed on the covariates (the same regression used to estimate the propensity score, see below) and estimates of the predicted probabilities were obtained from the fitted model. The weighted data is then used to produce a weighted survival curve to show the

covariate adjusted survival graphically, to try and provide the best comparison to the MERINO trial where randomisation should have ensured balance in patients characteristics.

Primary outcome

To emulate the trial analyses, I calculated absolute risk differences using generalised linear models (GLM), with 95% CI calculated using the Miettinen-Nurminen method (to be consistent with the approach in the MERINO trial). I used the Meropenem arm as the reference group for all analyses, as in the MERINO trial.

For the adjusted analyses (adjustments described below), GLM models would not converge. I therefore decided to fit a logistic regression model as these are more robust and unlikely to have the same convergence issues. I was able to calculate odds ratios for the MERINO trial for comparison.

To test non-inferiority, it was necessary to convert the 5% risk difference (non-inferiority margin) to the odds scale which I did as follows. Using the values (a, b, c, d) in the below table, the odds ratio of a death is calculated by $\frac{c/d}{a/b}$.

	Meropenem	**Pip + Taz**	**Overall**
Died	a	c	a+c
Survived	b	d	b+d
Overall	a+b	c+d	a+b+c+d

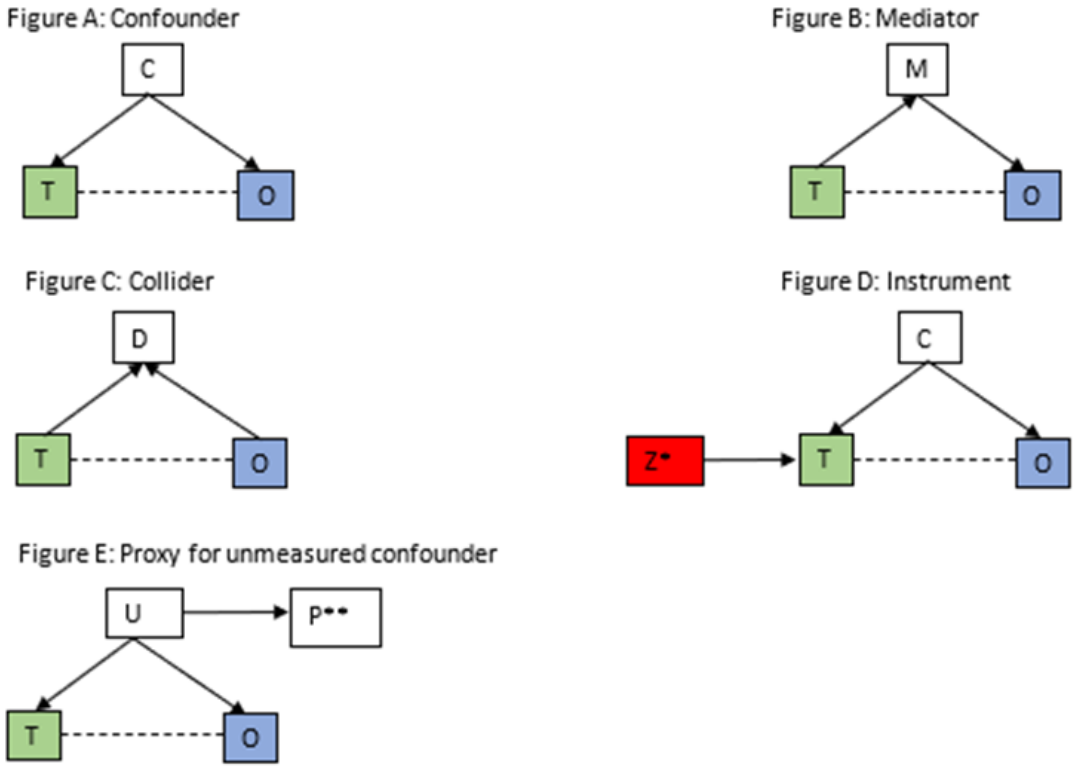
Based on the MERINO trial protocol, the mortality rate was estimated to be 14% in control group (Meropenem) and therefore 19% in the Piperacillin-Tazobactam group equates to the upper limit of the 95% confidence interval for non-inferiority given the 5% risk difference non-inferiority margin. The non-inferiority limit on the odds scale is therefore $\frac{0.19/0.81}{0.14/0.86} = 1.4$.

Identification of confounders

As the emulated trial was not randomised, I needed to account for potential confounding factors in the analysis. I needed to include confounders in my adjustment to account for any factors that might be responsible for the relationship between the receipt of Meropenem or Piperacillin-Tazobactam and survival (Figure 5.1, A). To do this, I considered the causal pathways. To avoid adjusting for variables which are on the causal pathway, known as mediators (Figure 5.1, B), which could introduce bias and "block" some of the effect, I only considered pre-exposure variables. Controlling for all pre-exposure variables could introduce bias if adjustment is made for a "collider" variable (Figure 5.1, C), that is a variable that is a common effect of exposure and outcome, however I did not consider this a problem in this analysis as the outcome is death and death cannot be the cause.

Controlling for all pre-exposure variables that are common causes of exposure **and** outcome can sometimes be too conservative, therefore I decided to use the disjunctive cause criterion, that is to control for variables that are associated with exposure and/or survival[190]. I decided to discard any variables that are instruments (cause of exposure, but otherwise totally unrelated to outcome except through the exposure, Figure 5.1, D) and include any proxy confounders that is a variable that could act as a proxy for an unmeasured variable that is known to be common cause of both the exposure and the outcome (Figure 5.1, E). I decided to only adjust for variables collected in both studies and exclude variables with less than five events or greater than 50% missing.

Figure 5.1 Diagrams demonstrating confounder, mediator, treatment and outcome relationships



C: Pre-exposure variable (confounder)
M: Post-exposure variable (mediator)
D: Pre-exposure variable (collider)
*Z: Pre-exposure variable (instrument)**
*P: Proxy***
U: Unmeasured confounder
T: Treatment (M or PT)
O: Outcome (survival)
** Z = Cause of exposure, but otherwise totally unrelated to outcome except through the exposure*
*** P = A variable that could act as a proxy for an unmeasured variable that is known to be common cause of both the exposure and the outcome*

I compared baseline characteristics between the two emulated intervention groups and calculated standardised mean differences for all patient demography and history factors. However, potential confounders included in the adjustment were based on clinician expertise specified *a priori* and not based on statistical tests as statistical tests cannot distinguish between confounders that ought to be controlled for and mediators.

Propensity score

As there were few events (n=20/121), I wanted to ensure that the most important confounders were adjusted for whilst minimising the number of degrees of freedom used to ensure stability of estimates. As there were many potential confounders, using the conventional adjustment method (multivariable model) would use many degrees of freedom. I therefore considered using an instrumental variable analysis approach to control for the confounding, as it can adjust for measured and unmeasured confounding[191]. However, instrumental variable methods require large sample sizes, as they have lower statistical power than standard regression models since the instrument only explains some of the variance in treatment. There are also specific requirements that a variable needs to meet to be considered an instrumental variable; (i) it has a casual effect on exposure, (ii) it affects the outcome variable only through exposure (does not have a direct influence on outcome), and (iii) there is no confounding for the effect of instrumental variable on outcome[192]. After careful consideration I decided that there were no suitable instruments that met the requirements of an instrumental variable, and therefore I decided to account for the confounding using propensity score adjustment.

I chose propensity score adjustment as there are few events (n=20/121) so the number of confounders that could be included in a multivariable adjusted model was limited whilst maintaining statistical power and stability of estimates. An advantage of propensity score is if the outcome is rare (but there are more numbers in treatment group) then you can gain statistical power by using fewer variables in the model for the outcome but include more variables in the regression model for the treatment (the model for the propensity score)[193, 194]. In some cases, individual confounders are included in the model for the outcome as well as adjustment for propensity score (doubly robust) however, as one of the reasons I chose to use propensity was due to the small number of events in this analysis, I decided to adjust for propensity score only[195]. I used propensity score adjustment rather than propensity score matching as matching excludes patients without a match which "discards" data and with a small sample this method may lead to few matched pairs in the analysis[196]. As the analysis sample size was already small (n=121), I wanted to maximise the sample size as much as possible and therefore opted for propensity score adjustment.

I developed a propensity score model using logistic regression with emulated intervention as the outcome and the confounders identified as explanatory variables. For causal effect to be valid all patients have to be eligible to receive both interventions. Confoundment by indication, such as more severe infections only likely to receive one of the treatments, can be addressed by dropping people at extreme end of propensity score. Tails should be excluded if there is a group of patients who were only ever going to receive one of the interventions meaning there is no comparator equivalent for this group of patients e.g. patients that were sick that they would never be given Drug A for example. I examined the number of patients and deaths in each emulated intervention group within strata defined by propensity score quintiles. If there were strata for which there were no patients or deaths in either group then I excluded patients in that strata to ensure that the analyses were restricted to patients likely to be eligible to receive either treatment strategy. I did not anticipate this to be many patients as by design of the study all are eligible for the target trial and therefore should be eligible to receive either intervention.

I modelled propensity scores using restricted cubic splines with three knots at 10^{th}, 50^{th} and 90^{th} percentiles to capture potential non-linear associations with the outcome.

Confounders included in the propensity score model were: centre, age, sex, temperature at time 0, neutrophil count on day 0 or closest before day 0, systolic blood pressure on day 0 or closest, on IV fluids at day 0, on ventilation at day 0, cerebrovascular disease, Charlson score and source of infection.

Missing data

Treatment allocation will not be missing, by design of the study. Missing values of variables included in the propensity score were imputed with age- and sex-adjusted averages. Elements of Charlson comorbidity index were imputed separately, again by age and sex.

Sensitivity analysis

I carried out two sensitivity analyses: (a) imputing missing categorical values with worst case values i.e. disease present before calculating propensity score; (b) propensity score model using restricted cubic splines at 25^{th}, 50^{th} and 75^{th} percentiles to assess the robustness of the results to the location of knots.

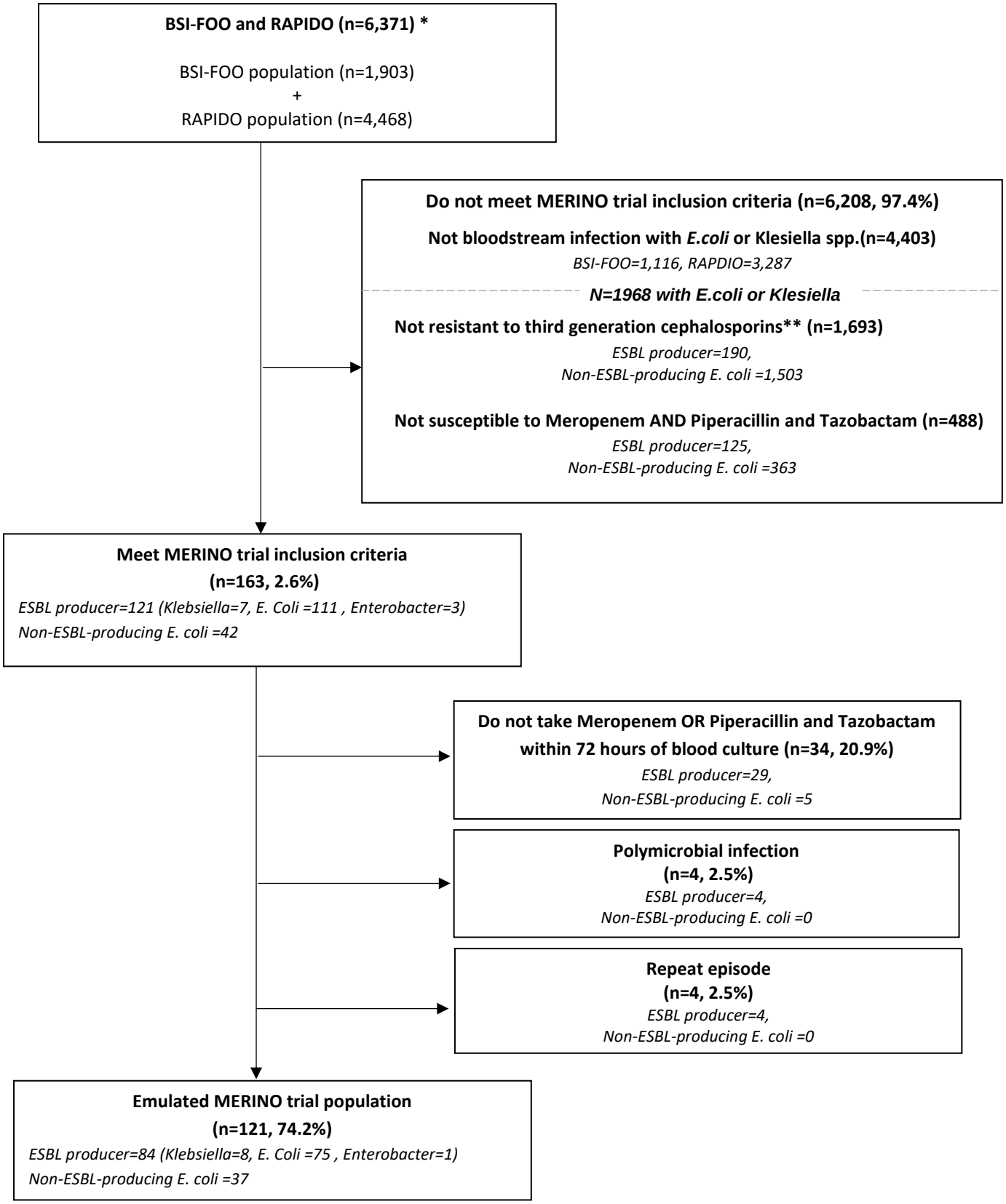
I planned to perform a subgroup analysis in just BSI-FOO patients, but there were too few events in the subgroup population to make this feasible.

5.3 Results

5.3.1 Population

Of the 6,371 BSI-FOO/RAPIDO patients, 1,968 had a BSI with *E. coli* or *Klebsiella* spp. of which 163 had proven non-susceptibility to third generation cephalosporins and proven susceptibility to Meropenem and Piperacillin-Tazobactam. Of these, 34 were not in receipt of either Meropenem or Piperacillin-Tazobactam within 72 hours of blood culture. Of the remaining 129 patients, four repeat episodes and four polymicrobial infections were excluded, thus 121 met the eligibility criteria (Figure 5.2) and were included in the analysis population. Of these, 91 were from BSI-FOO observational study and 30 from RAPIDO (16 conventional arm, 14 MALDI arm). No observations were excluded based on propensity scores.

Figure 5.2 Emulation the MERINO trial flowchart



** All BSI-FOO/RAPIDO participants were ages 18 years and over and RAPIDO participants provided informed consent*

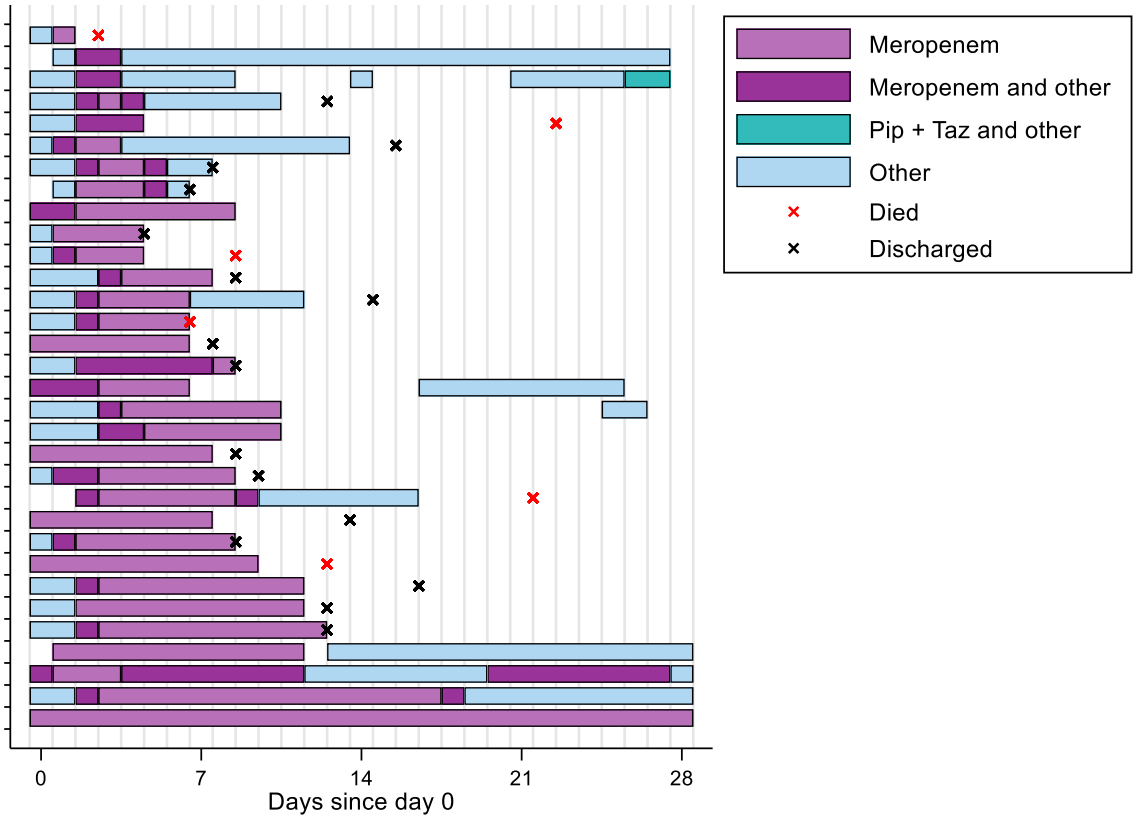
*** Ceftriaxone OR Cefotaxime*

Abbreviations: *ESBL= Extended-spectrum β-lactamase*

5.3.2 Treatment

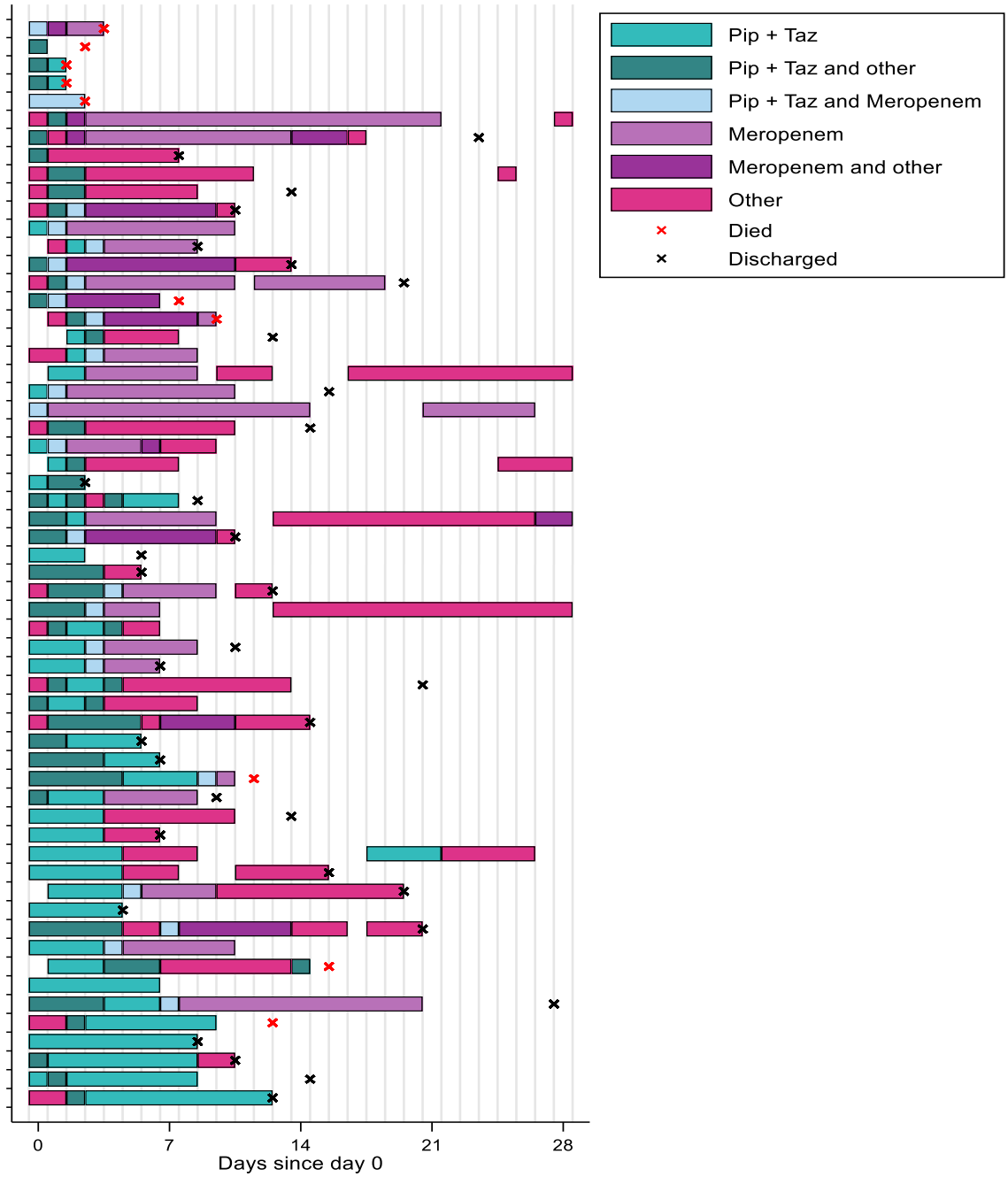
Of the 121 patients who met the emulated trial eligibility criteria, 82 were assigned to Piperacillin-Tazobactam and 39 to Meropenem, according to their first study drug received. The treatment timelines from blood culture to day 28 are shown for each patient in Figure 5.3 and Figure 5.4 for BSI-FOO observational study patients and Figure 5.5 for RAPIDO patients.

Figure 5.3 Treatment timelines for BSI-FOO patients allocated to Meropenem



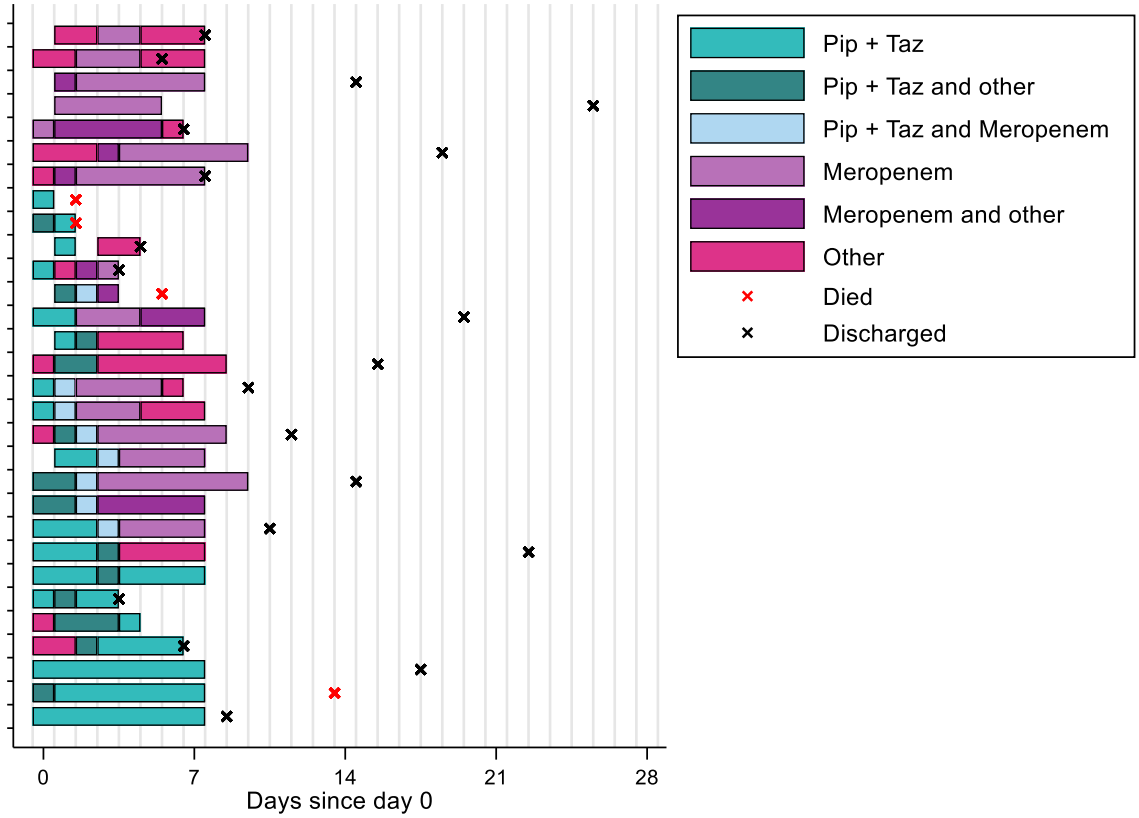
Abbreviations: *Pip + Taz = Piperacillin-Tazobactam*

Figure 5.4 Treatment timelines for BSI FOO patients allocated to Piperacillin-Tazobactam



Abbreviations: *Pip + Taz = Piperacillin-Tazobactam*

Figure 5.5 Treatment timelines for RAPIDO patients



Abbreviations: *Pip + Taz = Piperacillin-Tazobactam*

Meropenem was given at a dose of 500mg for approximately 23% (9/39) of patients and 1000mg (MERINO trial protocol) in 75% (29/39) and 3 times a day in 53.8% (21/39) of patients. Piperacillin-Tazobactam was given at a dose of 4500mg (MERINO trial protocol) for all but one patient (98.8%) assigned to Piperacillin-Tazobactam (Table 5.3). The lower doses administered are likely to be due to renal impairment, with an average eGFR of 43.9 mL/min/1.73m^2 (SD 20.8) on day 0 in patients given 500mg of Meropenem compared to 67.3 mL/min/1.73m^2 (SD 62.9) in patients given 1000mg of meropenem. Similarly, the lower frequencies are likely to be attributed to renal impairment, with an average eGFR of 12.5 (SD 3.5), 33.3 (SD 26.0), 63.8 (SD 32.0) mL/min/1.73m^2 for patients given treatment 1/day, 2/day and 3/day respectively.

Table 5.3 Treatment details for first prescription of study drug

	Meropenem (n=39) n (%)	Piperacillin-Tazobactam (n=82) n (%)
Dose (mg)		
500	9/39 (23.1%)	0/82 (0.0%)
1000	29/39 (74.4%)	0/82 (0.0%)
2000	1/39 (2.6%)	0/82 (0.0%)
2250	0/39 (0.0%)	1/82 (1.2%)
4500	0/39 (0.0%)	81/82 (98.8%)
Frequency		
1/day	2/39 (5.1%)	1/82 (1.2%)
2/day	9/39 (23.1%)	11/82 (13.4%)
3/day	21/39 (53.8%)	56/82 (68.3%)
Stat	7/39 (18.0%)	14/82 (17.1%)
Route		
IV	39/39 (100.0%)	82/82 (100.0%)

Abbreviations: *IV=Intravenous*

The median time to receipt of study drug was longer in the Meropenem group at 38 hours (IQR 8, 54) compared to the Piperacillin-Tazobactam group at 6 hours (IQR 0, 19) and duration of allocated drug treatment was also longer in the Meropenem group (7 days (IQR 4, 8) *vs* 3 days (IQR 2, 5)). The MERINO trial protocol required the study drug to be administered for a duration of four to fourteen days, with the duration determined by the treating clinician. In the emulated trial population, 31/39 (79.5%) of those allocated to Meropenem were in receipt of their study drug for the four days, of which four patients received an additional active treatment during this time. Similarly, of those allocated to Piperacillin-Tazobactam, 38/82 (46.3%) were in receipt of their study drug for a minimum of four days, but 15 of these received another active treatment in addition to Piperacillin-Tazobactam during this time. After the first dose of emulated intervention, 1/39 (2.6%) patients allocated to Meropenem switched to Piperacillin-Tazobactam, conversely, 39/82 (47.6%) switched from Piperacillin-Tazobactam to Meropenem (Table 5.4). In the MERINO trial, the treating clinician had the option of changing treatment on day 5 (either ceasing treatment, continuing on allocated treatment or changing treatment). Of those randomised to Piperacillin-Tazobactam, 20.2% were changed to a carbapenem, and of those randomised to Meropenem 2.6% were changed to Piperacillin-Tazobactam.

Table 5.4 Duration and time to receipt of emulated intervention

	Meropenem (n=39) n (%)	Piperacillin-Tazobactam (n=82) n (%)	Overall (n=121) n (%)
Time to receipt of allocated drug			
Median hours (IQR)	38 (8, 54)	5 (0, 19)	7 (1, 32)
Total duration of allocated drug *			
Median days (IQR)	7 (4, 8)	3 (2, 5)	4 (2, 7)
Duration category			
Died within 4 days	1/39 (2.6%)	7/82 (8.5%)	8/121 (6.6%)
Intervention received <4 days	7/39 (17.9%)	37/82 (45.1%)	44/121 (36.4%)
Intervention received ≥4 days	31/39 (79.5%)	38/82 (46.3%)	69/121 (57.0%)
In combination with other active drug	4/39 (10.3%)	15/82 (18.3%)	19/121 (15.7%)
Allocated drug only	27/39 (69.2%)	23/82 (28.0%)	50/121 (41.3%)
Crossover			
Switch to other intervention during follow-up	1/39 (2.6%)	39/82 (47.6%)	40/121 (33.1%)

** Total duration of study drug in MERINO (Median, IQR): Meropenem = 6 days (5, 9); Piperacillin-Tazobactam = 6 days (5, 10)*

Abbreviations: *IQR=Interquartile range*

5.3.3 Demographics

Demographic characteristics and medical history are shown by emulated intervention in Table 5.5 and *vs* MERINO trial population in Table 5.6. Overall, the patients' characteristics were similar to the MERINO trial population with the exception of the Charlson comorbidity index which was slightly higher (median 3.0 *vs* 2.0) and moderate-severe renal dysfunction which was present in a higher proportion of patients (61% *vs* 16%) in the emulated trial population. Patients in receipt of Piperacillin-Tazobactam in the emulated trial population were on average older (median 74.5 years *vs* 70.0 years, SMD -0.26), had a higher proportion of males (56.1% *vs* 38.5%, SMD 0.36), a lower proportion on ventilation (4.9% *vs* 15.4%, SMD 0.35) and a lower early warning score (2.0 *vs* 4.0, SMD 0.62).

Table 5.5 Baseline characteristics of patients in the emulated trial population, by emulated intervention

	Meropenem (n=39)	Piperacillin-Tazobactam (n=82)	SMD (M-PT)	Overall (n=121)
Patient measures				
Age	70.0 (54.0, 82.0)	74.5 (63.0, 84.0)	0.29	73.0 (61.0, 83.0)
Male	15/39 (38.5%)	46/82 (56.1%)	0.36	61/121 (50.4%)
Body Mass Index [a]	25.3 (9.2)	24.7 (5.0)	0.07	24.9 (6.6)
Patient medical history				
Chemotherapy in month before date 0	1/39 (2.6%)	15/82 (18.3%)	0.53	16/121 (13.2%)
Any tumour within last 5 years	12/39 (30.8%)	29/82 (35.4%)	0.10	41/121 (33.9%)
Surgery requiring overnight stay within 7 days before date 0	2/39 (5.1%)	3/82 (3.7%)	0.07	5/121 (4.1%)
Burn requiring admission within 7 days before date 0	0/32 (0.0%)	0/59 (0.0%)	-	0/91 (0.0%)
Cardiac arrest within 7 days before date 0	0/39 (0.0%)	0/82 (0.0%)	-	0/121 (0.0%)
Renal support within 7 days before date 0	2/39 (5.1%)	2/82 (2.4%)	0.14	4/121 (3.3%)
Myocardial infarction within 7 days before date 0	3/39 (7.7%)	9/82 (11.0%)	0.11	12/121 (9.9%)
Infection severity measures				
Temperature (°C) at time 0 [b]	38.4 (38.0, 39.0)	38.0 (37.1, 38.5)	0.48	38.2 (37.4, 38.7)
INR [c]	1.3 (1.2, 2.8)	1.1 (1.1, .)	0.04	1.2 (1.1, 1.5)
eGFR (mL/min/1.73m^2) [d]	53.0 (31.7, 81.0)	49.0 (29.0, 77.4)	0.12	49.5 (29.0, 79.0)
Neutrophil count at day 0 or closest (x10^9/L) [e]	10.3 (6.9, 13.3)	11.2 (4.9, 16.2)	0.03	10.8 (5.1, 15.3)
Systolic BP at day 0 or closest (mmHg) [f]	129.6 (28.8)	116.3 (29.2)	0.46	120.7 (29.6)
On IV fluids at day 0	16/39 (41.0%)	37/82 (45.1%)	0.08	53/121 (43.8%)
On ventilation at day 0	6/39 (15.4%)	4/82 (4.9%)	0.35	10/121 (8.3%)
On vasopressor drugs at day 0	3/39 (7.7%)	1/82 (1.2%)	0.32	4/121 (3.3%)
Systemic corticosteroids in last 24 hours	5/39 (12.8%)	9/82 (11.0%)	0.06	14/121 (11.6%)
EWS score nearest to day 0 [g]	4.0 (2.0, 6.0)	2.0 (1.0, 3.5)	0.62	2.0 (1.0, 4.0)
Patient comorbidities at date 0				
Congestive heart failure	4/39 (10.3%)	10/82 (12.2%)	0.06	14/121 (11.6%)
Peripheral vascular disease	4/39 (10.3%)	9/82 (11.0%)	0.02	13/121 (10.7%)

	Meropenem (n=39)	**Piperacillin-Tazobactam (n=82)**	**SMD (M-PT)**	**Overall (n=121)**
Cerebrovascular disease	10/39 (25.6%)	20/82 (24.4%)	0.03	30/121 (24.8%)
Hemiplegia	0/39 (0.0%)	5/82 (6.1%)	0.36	5/121 (4.1%)
Dementia	5/39 (12.8%)	10/82 (12.2%)	0.02	15/121 (12.4%)
COPD	6/39 (15.4%)	11/82 (13.4%)	0.06	17/121 (14.0%)
Connective tissue disease	2/39 (5.1%)	6/82 (7.3%)	0.09	8/121 (6.6%)
Peptic ulcer disease	4/39 (10.3%)	6/82 (7.3%)	0.10	10/121 (8.3%)
Ascites	1/39 (2.6%)	3/82 (3.7%)	0.06	4/121 (3.3%)
Diabetes:				
None	29/39 (74.4%)	57/82 (69.5%)	0.11	86/121 (71.1%)
Without organ damage	8/39 (20.5%)	16/82 (19.5%)	0.03	24/121 (19.8%)
With organ damage	2/39 (5.1%)	9/82 (11.0%)	0.22	11/121 (9.1%)
Child-Pugh score [h]	6.0 (5.0, 8.0)	6.0 (6.0, 9.0)	0.36	6.0 (6.0, 8.0)
Charlson score [i]	4.0 (2.0, 5.0)	3.0 (2.0, 4.0)	0.01	3.0 (2.0, 4.5)
Abscess at time 0	0/32 (0.0%)	2/59 (3.4%)	0.26	2/91 (2.2%)
Infected foreign body at time 0	1/32 (3.1%)	0/59 (0.0%)	-	1/91 (1.1%)
Surgical prosthesis time 0	0/32 (0.0%)	1/59 (1.7%)	0.19	1/91 (1.1%)
Source of infection				
Bone and joint	1/39 (2.6%)	0/82 (0.0%)		1/121 (0.8%)
Gastrointestinal system	6/39 (15.4%)	10/82 (12.2%)		16/121 (13.2%)
Line infection – central venous line	1/39 (2.6%)	1/82 (1.2%)		2/121 (1.7%)
Lower respiratory tract	1/39 (2.6%)	1/82 (1.2%)		2/121 (1.7%)
Reproductive tract	1/39 (2.6%)	0/82 (0.0%)	0.57	1/121 (0.8%)
Skin and soft tissue	1/39 (2.6%)	0/82 (0.0%)		1/121 (0.8%)
Surgical site infection	0/39 (0.0%)	1/82 (1.2%)		1/121 (0.8%)
Systemic Infection	1/39 (2.6%)	0/82 (0.0%)		1/121 (0.8%)
Urinary tract infection	20/39 (51.3%)	46/82 (56.1%)		66/121 (54.5%)
Site uncertain	7/39 (17.9%)	23/82 (28.0%)		30/121 (24.8%)

	Meropenem (n=39)	Piperacillin-Tazobactam (n=82)	SMD (M-PT)	Overall (n=121)
Lines and catheters				
Central line present at time 0	4/32 (12.5%)	11/59 (18.6%)	0.17	15/91 (16.5%)
Peripheral line present at time 0	15/32 (46.9%)	34/59 (57.6%)	0.22	49/91 (53.8%)
Urinary catheter present at time 0	7/32 (21.9%)	21/59 (35.6%)	0.31	28/91 (30.8%)
Organisational factors				
Centre:				
A	1/39 (2.6%)	8/82 (9.8%)		9/121 (7.4%)
B	8/39 (20.5%)	14/82 (17.1%)		22/121 (18.2%)
C	13/39 (33.3%)	31/82 (37.8%)		44/121 (36.4%)
D	9/39 (23.1%)	10/82 (12.2%)	0.54	19/121 (15.7%)
E	5/39 (12.8%)	17/82 (20.7%)		22/121 (18.2%)
F	1/39 (2.6%)	0/82 (0.0%)		1/121 (0.8%)
G	2/39 (5.1%)	2/82 (2.4%)		4/121 (3.3%)
Ward specialty on day 0:				
Medicine	20/39 (51.3%)	52/82 (63.4%)		72/121 (59.5%)
Critical care	4/39 (10.3%)	6/82 (7.3%)		10/121 (8.3%)
Major surgery	12/39 (30.8%)	16/82 (19.5%)	0.37	28/121 (23.1%)
Minor surgery	0/39 (0.0%)	2/82 (2.4%)		2/121 (1.7%)
Other	3/39 (7.7%)	6/82 (7.3%)		9/121 (7.4%)

Notes: *Data are presented as n (%). Date and time 0 = date/time of sampling for blood culture*

[a] *Data missing for 78 patients (24 Meropenem, 54 Piperacillin-Tazobactam)*

[b] *Data missing for 3 patients (3 Meropenem, 0 Piperacillin-Tazobactam)*

[c] *Data missing for 56 patients (19 Meropenem, 37 Piperacillin-Tazobactam)*

[d] *Data missing for 3 patients (2 Meropenem, 1 Piperacillin-Tazobactam)*

[e] *Data missing for 3 patients (1 Meropenem, 2 Piperacillin-Tazobactam)*

[f] *Data missing for 12 patients (3 Meropenem, 9 Piperacillin-Tazobactam)*

[g] *Data missing for 76 patients (26 Meropenem, 50 Piperacillin-Tazobactam)*

[h] *Data missing for 80 patients (26 Meropenem, 54 Piperacillin-Tazobactam)*

[i] *Data missing for 29 patients (10 Meropenem, 19 Piperacillin-Tazobactam)*

Abbreviations: *BP=Blood pressure, COPD=Chronic obstructive pulmonary disease, eGFR=Estimated glomerular filtration rate, EWS=Early warning score, INR=International normalised ratio, IQR=Interquartile range, IV=Intravenous, SD= Standard deviation, SMD=Standardised mean difference*

Table 5.6 Baseline characteristics of patients in the MERINO trial analysis population *vs* emulated trial population

		MERINO trial analysis population				Emulated trial population			
		Piperacillin-Tazobactam (n=188)		Meropenem (n=191)		Piperacillin-Tazobactam (n=82)		Meropenem (n=39)	
		n	%	n	%	n	%	n	%
Organism									
E. coli		162/188	86.2%	166/191	86.9%	77/82	93.9%	36/39	92.3%
Klebsiella spp.		26/188	13.8%	25/191	13.1%	5/82	6.1%	3/39	7.7%
Stratification *									
E1: *E. coli,* less severe		159/188	84.6%	162/191	84.8%	74/79	93.7%	32/35	91.4%
E2: *E. coli,* more severe		3/188	1.6%	3/191	1.6%	1/79	1.3%	0/35	0.0%
K1: Klebsiella, less severe		23/188	12.2%	25/191	13.1%	4/79	5.1%	3/35	8.6%
K2: Klebsiella, more severe		3/188	1.6%	1/191	0.5%	0/79	0.0%	0/35	0.0%
Patient measures									
Age (years)	Median (IQR)	70.0	(55.0, 78.0)	69.0	(59.0, 78.0)	74.5	(63.0, 84.0)	70.0	(54.0, 82.0)
Male		101/188	53.7%	97/191	50.8%	46/82	56.1%	15/39	38.5%
Weight (kg) [a]	Mean (SD)	67.2	18.1	69.3	19.3	70.0	15.9	72.1	24.6
Acquisition **									
Hospital-acquired		52/188	27.7%	46/191	24.1%	35/82	42.7%	16/39	41.0%
Health-care associated		55/188	29.3%	61/191	31.9%				
Community associated		81/188	43.1%	84/191	44.0%	47/82	57.3%	23/39	59.0%
Source of infection									
Bone and joint		0/188	0.0%	0/191	0.0%	0/82	0.0%	1/39	2.6%
Gastrointestinal system		0/188	0.0%	0/191	0.0%	10/82	12.2%	6/39	15.4%
Intra-abdominal infection		34/188	18.1%	28/191	14.7%	0/82	0.0%	0/39	0.0%
Line infection – central venous line		0/188	0.0%	0/191	0.0%	1/82	1.2%	1/39	2.6%
Vascular catheter-related		3/188	1.6%	3/191	1.6%	0/82	0.0%	0/39	0.0%
Lower respiratory tract		0/188	0.0%	0/191	0.0%	1/82	1.2%	1/39	2.6%
Reproductive tract		0/188	0.0%	0/191	0.0%	0/82	0.0%	1/39	2.6%

		MERINO trial analysis population				Emulated trial population			
		Piperacillin-Tazobactam (n=188)		Meropenem (n=191)		Piperacillin-Tazobactam (n=82)		Meropenem (n=39)	
Skin and soft tissue		4/188	2.1%	1/191	0.5%	0/82	0.0%	1/39	2.6%
Surgical site infection		8/188	4.3%	4/191	2.1%	1/82	1.2%	0/39	0.0%
Systemic Infection		0/188	0.0%	0/191	0.0%	0/82	0.0%	1/39	2.6%
Urinary tract infection		103/188	54.8%	128/191	67.0%	46/82	56.1%	20/39	51.3%
Pneumonia		9/188	4.8%	3/191	1.6%	0/82	0.0%	0/39	0.0%
Mucositis/neutropenia		12/188	6.4%	7/191	3.7%	0/82	0.0%	0/39	0.0%
Musculoskeletal		1/188	0.5%	0/191	0.0%	0/82	0.0%	0/39	0.0%
Other		2/188	1.1%	1/191	0.5%	0/82	0.0%	0/39	0.0%
Site uncertain		12/188	6.4%	16/191	8.4%	23/82	28.0%	7/39	17.9%
Other patient measures									
Surgery requiring overnight stay within past 14 (MERINO) or 7 (BSI-FOO) days		19/188	10.1%	14/191	7.3%	3/82	3.7%	2/39	5.1%
ICU admission		13/188	7.0%	14/191	7.3%	6/82	7.3%	4/39	10.3%
Charlson score [b]	Median (IQR)	2.0	(1.0, 4.0)	2.0	(1.0, 4.0)	3.0	(2.0, 4.0)	4.0	(2.0, 5.0)
Pitt score [c]	Median (IQR)	1.0	(0.0, 2.0)	1.0	(0.0, 2.0)	1.0	(0.0, 2.0)	0.0	(0.0, 2.0)
Neutropenia		16/188	8.5%	9/191	4.7%	11/79	13.9%	2/38	5.3%
Urinary catheter/nephrostomy ***		51/188	27.1%	37/191	19.4%	21/59	35.6%	7/32	21.9%
Moderate-sever renal dysfunction		31/188	16.5%	30/191	15.7%	51/81	63.0%	21/37	56.8%
Diabetes		59/188	31.4%	79/191	41.4%	25/82	30.5%	10/39	25.6%
Liver disease		12/188	6.4%	18/191	9.4%	13/69	18.8%	5/31	16.1%

Notes: *Data are presented as n (%).*

** Severity definition: More severe= nonurinary source and Pitt score >4. Less Severe= Urinary source, or nonurinary source and Pitt score ≤4.*

*** Hospital acquired if date of blood culture is >48 hours after date of admission in BSI-FOO. Data on healthcare associated infections was not collected in BSI-FOO.*

**** Urinary catheter only in BSI-FOO*

[a] *Data missing for 57 BSI-FOO patients (14 Meropenem, 43 Piperacillin-Tazobactam)*

[b] *Data missing for 29 BSI-FOO patients (10 Meropenem, 19 Piperacillin-Tazobactam)*

[c] *Data missing for 12 BSI-FOO patients (4 Meropenem, 8 Piperacillin-Tazobactam)*

Abbreviations: *ICU=Intensive care unit, IQR=Interquartile range, SD= Standard deviation*

5.3.4 Primary outcome

Propensity score

Confounders included in the propensity score model are given in section 5.2.8. I did not include cardiac arrest, previous surgery, renal support, or vasopressor drugs in the propensity score model as there were less than five observations in a category. I also did not include ward specialty on day 0 as the model would not converge due to perfect prediction. Additionally, many of the source of infections were experienced by only one patient, therefore I categorised source of infection into three groups (gastrointestinal, urinary tract infection and other) in the calculation of the propensity score.

I examined the number of patients in each emulated intervention group and the number of deaths within strata defined by propensity score quintiles, although due to the small number of deaths I did not exclude any patients based on this.

Table 5.7 Propensity score quintiles, by treatment allocation and survival

Propensity score quintile	Meropenem (n=38)		Piperacillin-Tazobactam (n=82)	
	Survived	Died	Survived	Died
<20 (n=24)	12 (80.0%)	3 (20.0%)	8 (88.9%)	1 (11.1%)
20-40 (n=24)	10 (83.3%)	2 (16.7%)	11 (91.7%)	1 (8.3%)
40-60 (n=24)	3 (75.0%)	1 (25.0%)	12 (60.0%)	8 (40.0%)
60-80 (n=24)	5 (100.0%)	0 (0.0%)	18 (94.7%)	1 (5.3%)
≥80 (n=24)	2 (100.0%)	0 (0.0%)	19 (86.4%)	3 (13.6%)
Overall (n=120)	**32 (84.2%)**	**6 (15.8%)**	**68 (82.9%)**	**14 (17.1%)**

Note: *n=120/121 due to centre F being omitted from the propensity score model as it only had one patient. Percentages are row percentages. Propensity score quintiles defined as <0.46, 0.46 – 0.69, 0.70 – 0.78, 0.79-0.88, ≥0.89.*

To ensure the populations are comparable and not two distinct populations, I assessed the probability density function to ensure they overlap. Based on the probability density function (Figure 5.6) and the distribution of propensity score (Figure 5.7) I considered overlap to be approximately satisfied given the sample size.

Figure 5.6 **Probability density function of propensity score, by treatment allocation**

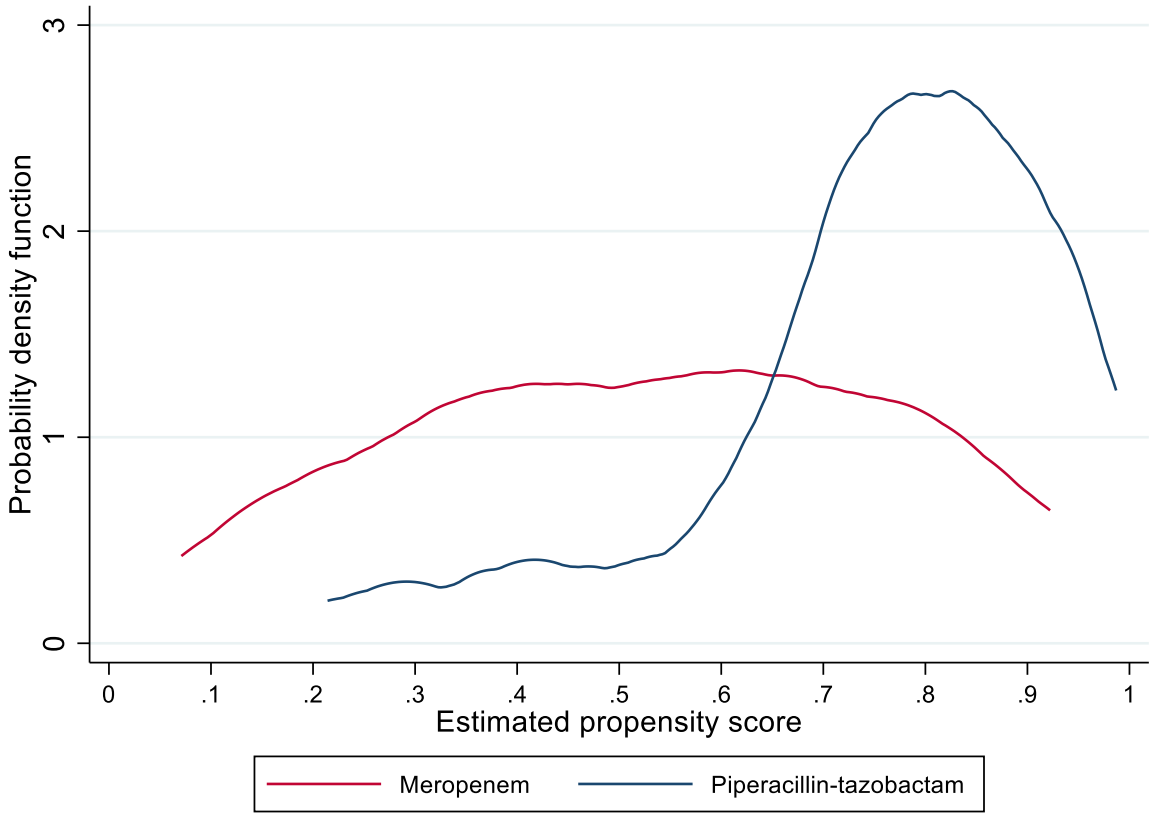
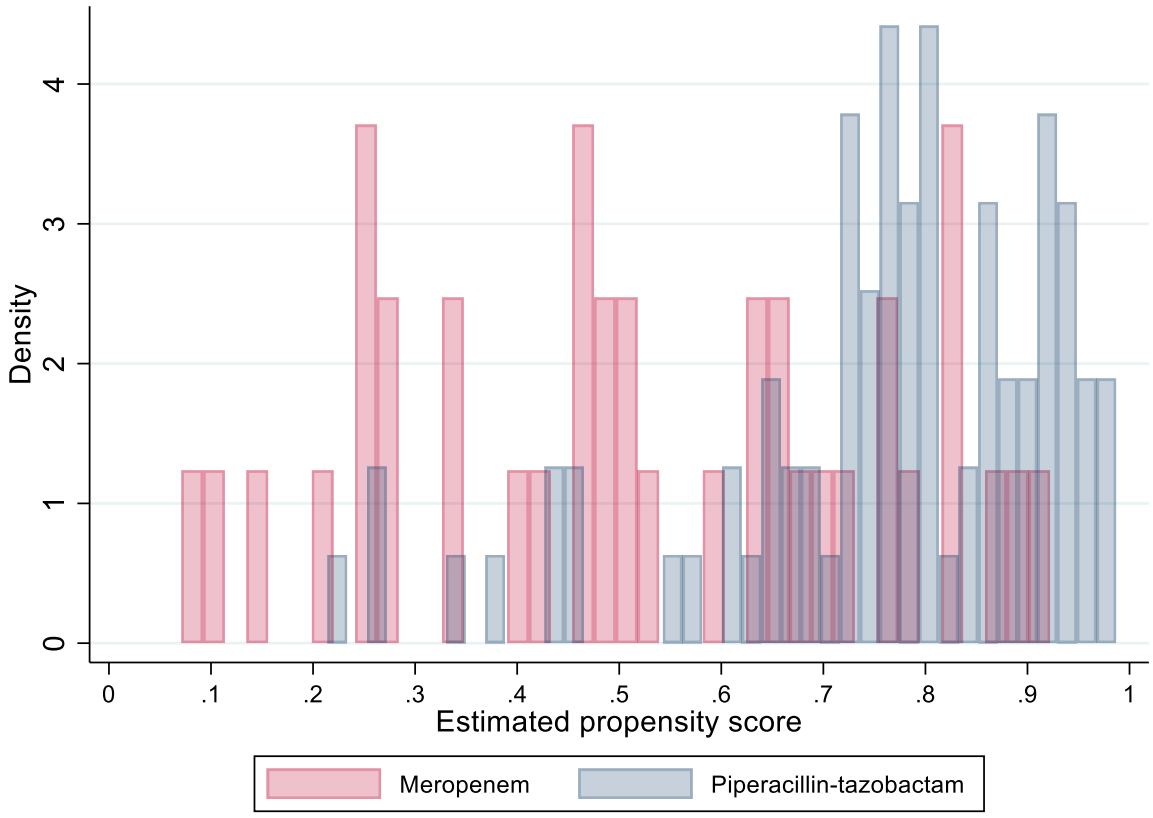


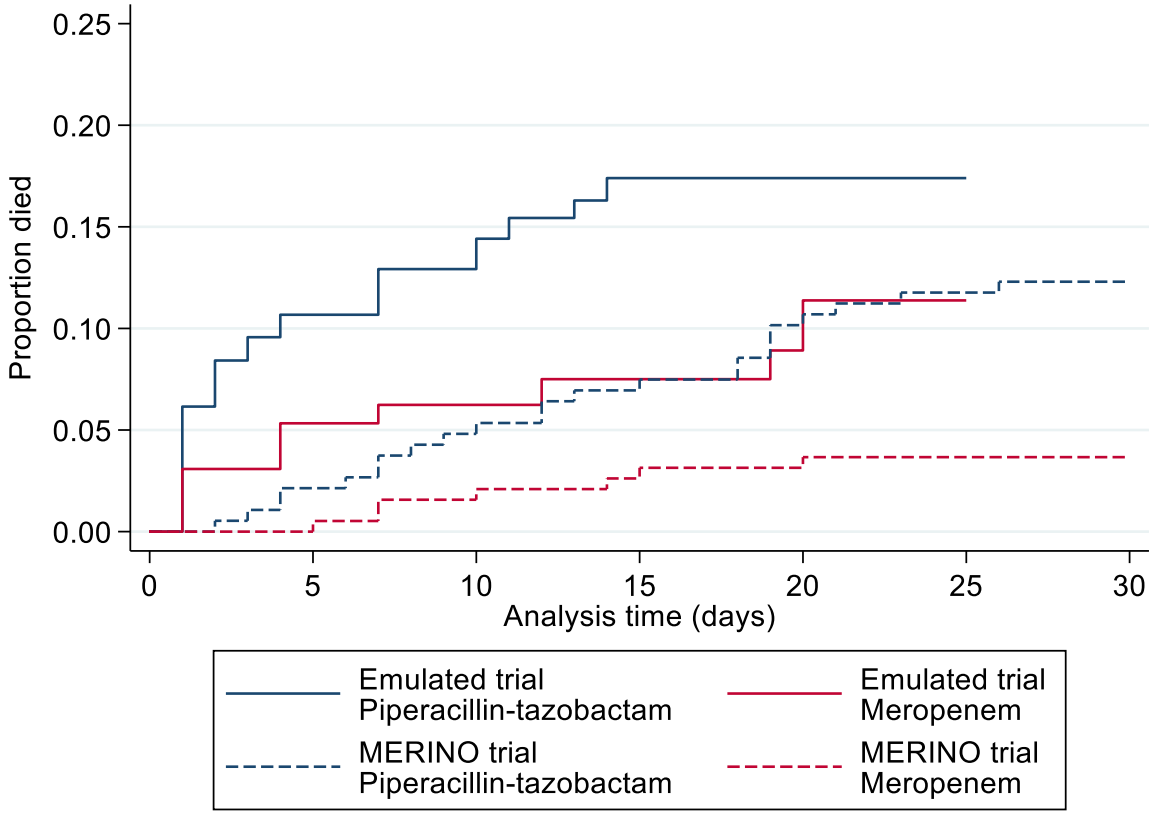
Figure 5.7 **Propensity score distribution, by treatment allocation**



25-day mortality

The overall 25-day mortality rate was 20/121 (16.5%) compared to 30/378 (7.9%) 30-day mortality in the MERINO trial population[176]. Inverse probability weighted Kaplan-Meier curves displaying time to death according to emulated intervention group are shown in Figure 5.8.

Figure 5.8 Inverse probability weighted Kaplan-Meier, by emulated intervention



In this emulated trial, a total of 14/82 patients (17.1%) allocated to Piperacillin-Tazobactam met the primary outcome of all-cause mortality at 25 days compared with 6/39 (15.4%) in the Meropenem group (risk difference 1.7%, 95% CI -12.26 to 15.64). The corresponding unadjusted odds ratio is 1.13 (95% CI 0.40 to 3.21). After adjustment for propensity score, the odds ratio increased to 1.31 (95% CI 0.40 to 4.26). The non-inferiority margin on the odds scale (1.4) falls within the 95% confidence interval so there is no evidence to suggest Piperacillin-Tazobactam is non-inferior to Meropenem, meaning non-inferiority is not demonstrated. Sensitivity analysis gave similar results (Table 5.8). These differences are lower than observed in the MERINO trial, where 30-day mortality was 23/187 patients (12.3%) in the Piperacillin-Tazobactam and 7/191 (3.7%) in the Meropenem group with an unadjusted risk difference 8.6 (95% CI 3.0 to 14.5) and odds ratio of 3.69 (95% CI 1.48 to 10.41).

Table 5.8 Primary analysis: 25-day mortality

Population	Meropenem n (%)	Piperacillin-Tazobactam n (%)	N	Estimate RD/OR (95% CI)
MERINO TRIAL (30-day mortality)	7/191 (3.7%)	23/187 (12.3%)		
Unadjusted risk difference			378	RD = 8.6 (95% CI 3.4 to 14.5)
Unadjusted odds ratio			378	OR = 3.7 (95% CI 1.5 to 10.4)
EMULATED TRIAL (25-day mortality)	6/39 (15.4%)	14/82 (17.1%)		
Unadjusted risk difference			121	RD = 1.69 (95% CI -12.26 to 15.64)
Unadjusted odds ratio			121	OR = 1.13 (95% CI 0.40 to 3.21)
Propensity score adjusted *			120	OR = 1.31 (95% CI 0.40 to 4.26)
Sensitivity analysis 1 **			120	OR = 1.38 (95% CI 0.43 to 4.45)
Sensitivity analysis 2 ***			120	OR = 1.29 (95% CI 0.40 to 4.17)

** n=120/121 due to centre F being omitted from the propensity score model as it only had one patient. Propensity score adjustment. Propensity score calculated using centre, age, sex, chemotherapy in month before date 0, temperature at time 0, neutrophil count at day 0, SBP, on IV fluids, on ventilation, cerebrovascular disease, Charlson score, and source of infection. Charlson comorbidity index, temperature at time 0, neutrophil count and SBP imputed using conditional mean imputation. Propensity score modelled using restricted cubic splines with 3 knots at 10th, 50th and 90th percentiles.*

*** SA1: Adjusted for propensity score: Charlson comorbidity index imputed using worst case scenario (liver disease present and moderate/severe kidney disease)*

**** SA2: Adjusted for propensity score: Propensity score modelled using restricted cubic splines with 3 knots at 25th, 50th and 75th percentiles.*

Abbreviations: *IV=Intravenous, OR=Odds ratio, RD=Risk difference, SBP=Systolic blood pressure*

14-day mortality

Upon examining the Kaplan-Meier curve, it was apparent that most of the deaths occurred within the first 15 days. The first week could be considered as the most critical in terms of treatment related deaths, therefore it is possible that deaths during days 7-14 could be attributable to treatment or unrelated, e.g. due to comorbidity, and post day 14 deaths are unlikely to be attributable to suboptimal treatment. I therefore decided to perform a post-hoc analysis of 14-day mortality. Inverse probability weighted Kaplan-Meier curves and results of the post-hoc analyses are given in Figure 5.9 and Table 5.9.

In this emulated trial, a total of 14/82 patients (17.1%) allocated to Piperacillin-Tazobactam met the outcome of all-cause mortality at 14 days compared with 4/39 (10.3%) in the Meropenem group (unadjusted risk difference 6.8%, 95% CI -8.1 to 18.7). After adjustment for propensity score, the odds ratio is 2.14 (95% CI 0.56 to 8.13) which is similar to the odds ratio of 2.78 (95% CI 0.90 to 10.14) observed in the MERINO trial.

Figure 5.9 Inverse probability weighted Kaplan-Meier of 14-day mortality, by emulated intervention

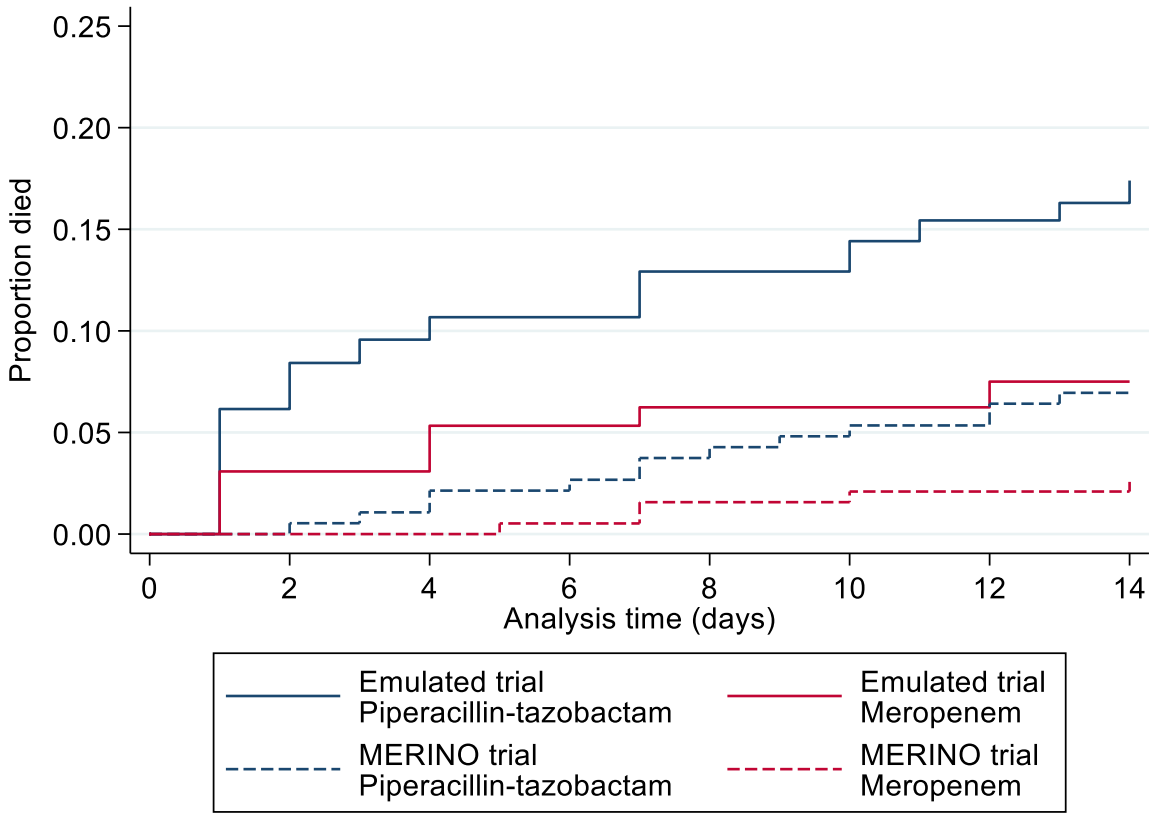


Table 5.9 Post-hoc analysis: 14-day mortality

Population	Meropenem		Piperacillin-Tazobactam		N	Estimate
	n	%	n	%		RD/OR (95% CI)
MERINO TRIAL (14-day mortality)	5/191	2.7%	13/187	7.0%		
Unadjusted risk difference					378	RD = 4.3 (95% CI 0.0 to 9.2)
Unadjusted odds ratio					378	OR = 2.78 (95% CI 0.90 to 10.14)
EMULATED TRIAL (14-day mortality)	4/39	10.3%	14/82	17.1%		
Unadjusted risk difference					121	RD = 6.8 (95% CI -8.1 to 18.7)
Unadjusted odds ratio					121	OR = 1.80 (95% CI 0.55 to 5.88)
Propensity score adjusted *					120	OR = 2.14 (95% CI 0.56 to 8.13)
Sensitivity analysis 1 **					120	OR = 2.25 (95% CI 0.60 to 8.44)
Sensitivity analysis 2 ***					120	OR = 2.01 (95% CI 0.47 to 8.49)

** n=120/121 due to centre F being omitted from the propensity score model as it only had one patient.*
Propensity score adjustment. Propensity score calculated using centre, age, sex, chemotherapy in month before date 0, temperature at time 0, neutrophil count at day 0, SBP, on IV fluids, on ventilation, cerebrovascular disease, Charlson comorbidity index, and source of infection. Charlson comorbidity index, temperature at time

0, neutrophil count and SBP imputed using conditional mean imputation. Propensity score modelled using restricted cubic splines with 3 knots at 10th, 50th and 90th percentiles.
*** SA1: Adjusted for propensity score: Charlson score imputed using worst case scenario (liver disease present and moderate/severe kidney disease)*
*** SA2: Adjusted for propensity score: Propensity score modelled using restricted cubic splines with 3 knots at 25th, 50th and 75th percentiles.*
Abbreviations: *IV=Intravenous, OR=Odds ratio, RD=Risk difference, SBP=Systolic blood pressure*

5.4 Discussion

5.4.1 Summary

In an emulated trial population, the overall mortality rate was more than double the mortality rate in the MERINO trial, but similar to mortality rates reported in other observational studies[169, 170]. The difference between mortality rates in Piperacillin-Tazobactam and Meropenem was weaker than that observed in the MERINO trial, however a similar treatment effect for 14-day mortality was observed.

5.4.2 Interpretation

There have been a number of observational studies that have shown that BLBLIs are an effective treatment for infections caused by ESBL producers[169-173], however, observational analyses are subject to bias and a recent RCT provided results that were conflicting to some of these studies. A meta-analysis of 21 studies by Vardakas et al. which reported no statistically significant differences in mortality between patients treated with carbapenems and patients treated with BLBLIs as empiric therapy (RR 0.91, 95% CI 0.66 to 1.25), but direction favoured carbapenems[164]. This is in contrast to observational analysis that have shown BLBLIs to be a safe alternative treatment for ESBL producing bacteraemia, with the direction favouring carbapenem sparing options[170, 171, 173]. In the analyses presented here, I aimed to minimise the bias that may arise in observational studies by implementing trial emulation methods to existing observational data to emulate the MERINO trial in an attempt to understand the mechanisms behind the differences observed[176]. Applying trial emulation methods to existing observational data produced different mortality rates to the published MERINO trial and a smaller treatment effect to the trial's results. Despite the higher mortality rates observed in the emulated trial compared to the MERINO trial, the observed treatment effect was in the same direction and 95% CI of the primary model includes the observed odds ratio reported in the MERINO trial. This suggests that the data reported in this study are potentially consistent with the MERINO trial given the amount of uncertainty in our estimates.

There are several differences in the study design and population characteristics that could explain the lower mortality rates observed in the MERINO trial compared to the emulated trial. Firstly, there

are differences in the demographics, comorbidities and severity of illness between the emulated trial population and the MERINO trial population. We aimed to minimise these differences by applying the same eligibility criteria as the trial, however the distribution of comorbidities and severity of illness may still differ depending on the sampling population. In the MERINO trial, there was a higher proportion of patients in the meropenem group with a urinary tract source (67.0% *vs* 54.8%) which are known to be more responsive to treatment. This was more balanced in the emulated trial (51.3% *vs* 56.1%). The treatment effect may differ across different levels of infection severity or presence of comorbidities e.g. Piperacillin-Tazobactam may be inferior to carbapenems in patients with severe infections but non-inferior in less severe infections such as urinary tract infections[174, 175], however we did not have a large enough sample size to explore this. Further research is required to investigate this, but this may in part explain the conflicting results published in the MERINO trial to other observational studies where the populations and severity of illness may differ.

5.4.3 Strengths and limitations

Strengths and limitations

One of the strengths of this study is the trial emulation approach used in the design and analysis. Trial emulation using observational data ensures that eligibility criteria and assumptions are explicit before analysis and minimises common biases that can arise in observational data analyses. In addition, a number of the published observational studies exclude patients who are not in receipt of either intervention for more than 48 hours[171-173], meaning patients who die within 48 hours of receipt treatment are excluded from the population which gives potential for introducing survival bias. Applying trial emulation methods enabled us to include all patients who would be eligible for a trial, without using data after start of follow-up in the inclusion/exclusion criteria, therefore minimising survival bias.

There are several limitations to this study and a number of compromises were made regarding eligibility criteria and treatment strategy comparisons. Firstly, it was not possible to emulate all elements of the MERINO trial. In terms of eligibility, the MERINO trial did not include patients who were not expected to survive more than 4 days. This could have also resulted in the sicker patients who would otherwise be eligible for the trial being excluded, which could lead to an underestimation of the true mortality rate. This was acknowledged by the authors as a limitation of the MERINO study. We did not impose the 96-hour restriction in the trial emulation as it was not possible to determine survival expectation and using observed survival could introduce bias. In addition, the process of obtaining patient consent in MERINO could result in the sickest patients not being captured. Consent was not required for the BSI-FOO observational study and therefore all eligible

patients were included. Both these points could have resulted in sicker patients that would have been ineligible for the trial being included in the emulated trial.

Data on allergies were not collected in the BSI-FOO observational study or RAPIDO so it was assumed that if the patient was given a penicillin or a carbapenem that they had no known allergies and I did not consider this likely to have impacted the results. Data on pregnancy and breast-feeding were not collected in the BSI-FOO observational study and therefore I could not apply these criteria, however the average age in BSI-FOO is approximately 70 years and since the exclusion was likely to be a safety measure I did not anticipate it to affect the comparison of results. The MERINO trial had 30-day follow-up, we did not have 30-day follow-up for all BSI-FOO and RAPIDO patients and therefore we analysed 25-day mortality. However, this is unlikely to have a significant impact as later deaths can be considered unlikely to be a result of treatment. Upon examination of the Kaplan-Meier curves, there was a larger difference in mortality rate up to 14 days. When 14-day mortality was formally compared in a post-hoc analysis, the treatment estimates in the emulated trial were similar to the MERINO trial although mortality rates were still lower in the MERINO trial across both interventions. There were 10 deaths after day 14 in the Piperacillin-Tazobactam arm of the MERINO trial compared to two in the Meropenem arm however it can be argued that 14-day mortality may be a more clinically meaningful outcome for studies investigating mortality in blood-stream infection as it is the time period most reflective of death attributable to suboptimal therapy.

Secondly, it was not possible to emulate the per-protocol analysis as few patients received the allocated treatment for four days and restricting analyses to those who are in receipt for four or more days would introduce immortal time bias (bias induced by a period of follow-up during which, by design, the outcome cannot occur). Our approach made it hard to attribute differences in the intention-to-treat analysis because some patients received both treatments with many of the patients assigned to Piperacillin-Tazobactam swapping to Meropenem, leading to contamination of drug exposure. This “cross-over” would make the groups more similar potentially diluting any treatment effect. This makes it difficult to draw any firm conclusions and compromises our ability to make many inferences from the results. It was not feasible to perform a “per-protocol” analysis restricting the comparison to those patients who did not switch as the sample size and number of events in the per-protocol population was low (n=6 in Meropenem group, n=8 in Piperacillin-Tazobactam group). This approach is also prone to severe selection bias because switching is usually related to prognosis. I considered the use of inverse probability weights and g-formula to account for treatment switching but did not pursue them for the following reasons: i) inverse probability weights are appropriate for time-to-event models but are not appropriate for logistic regression, which is the model used in the emulated trial analysis and ii) both approaches require an adjustment for post

baseline prognostic factors that affect treatment status and are also affected by past treatment. This requires data to be available on all prognostic factors for mortality that independently predict the probability of switching. Unfortunately, such information (factors that affect the clinicians' decision to change treatment from Piperacillin-Tazobactam to meropenem) such as raised CRP or WBC, failure to improve clinically or a clinical deterioration, repeat diagnostic imaging showing progression of infection, were not collected in BSI-FOO or RAPIDO. Therefore, the assumption of no residual confounding was not satisfied, and it was not appropriate to implement these approaches.

In addition to cross-overs, patients' empirical treatment and treatment pathways other than the "allocated" intervention were not controlled for, so any observed differences could be attributed to the effects of empirical therapy or treatments received after classification of "trial drug". There were also differences in the time to receipt of "allocated" treatment between the two groups.

Another aspect of treatment strategy that it was not possible to emulate was the dosing regimen. The MERINO trial protocol specified Meropenem to be administered at a dose of 1g every 8 hours and Piperacillin-Tazobactam at a dose of 4.5g every 6 hours. In the emulated trial, doses were as prescribed and were consistent with the MERINO trial protocol in 75% of patients in the Meropenem arm and 99% in the Piperacillin-Tazobactam arm. In the Meropenem arm of the emulated trial, 23% received Meropenem at a lower dose (500mg) however dose adjustment for renal impairment was made in the MERINO trial so actual doses given in the trial may have differed to those specified in the protocol.

Finally, it was not possible to emulate blinding, so the validity of our estimates depends on the assumption that all confounding factors were correctly adjusted for. We allowed for differences in baseline characteristics by adjusting for propensity score. However, due to the data collection being designed for a different study protocol we were only able to control for variables that had been collected specific to that study and there is the risk that unmeasured confounders may impact the results. I could also only adjust for variables that were collected in both BSI-FOO observational study and RAPIDO. I considered an IV analysis with centre practice as the instrument which could account for both measured and unmeasured confounding, however this technique has lower power and after exploring centre prescription practices, I did not consider it to meet the assumptions of a valid instrument.

5.4.4 Conclusions

In summary, the mortality rate in an emulated trial population was more than double the mortality rate in the MERINO trial and the difference between mortality rates in Piperacillin-Tazobactam and Meropenem was weaker, but in the same direction. Our findings suggest that the discrepancies

between the MERINO trial and observational studies estimates could be partly explained by differences in the sample population and also due to biases that arise from observational studies, e.g. survival bias and bias from unmeasured or uncontrolled confounding and in treatments received. This methodology attempts to address the concern that previous results could be explained by such biases and compliments the literature with data from the UK. However, our estimates are still subject to some of the biases that arise in observational studies, so further clinical trials with adequate power and refined eligibility criteria are required to determine efficacy. A new trial (PeterPen) designed to answer the same question as the MERINO trial is due to complete in April 2024, although no UK sites are planned in this trial[197].

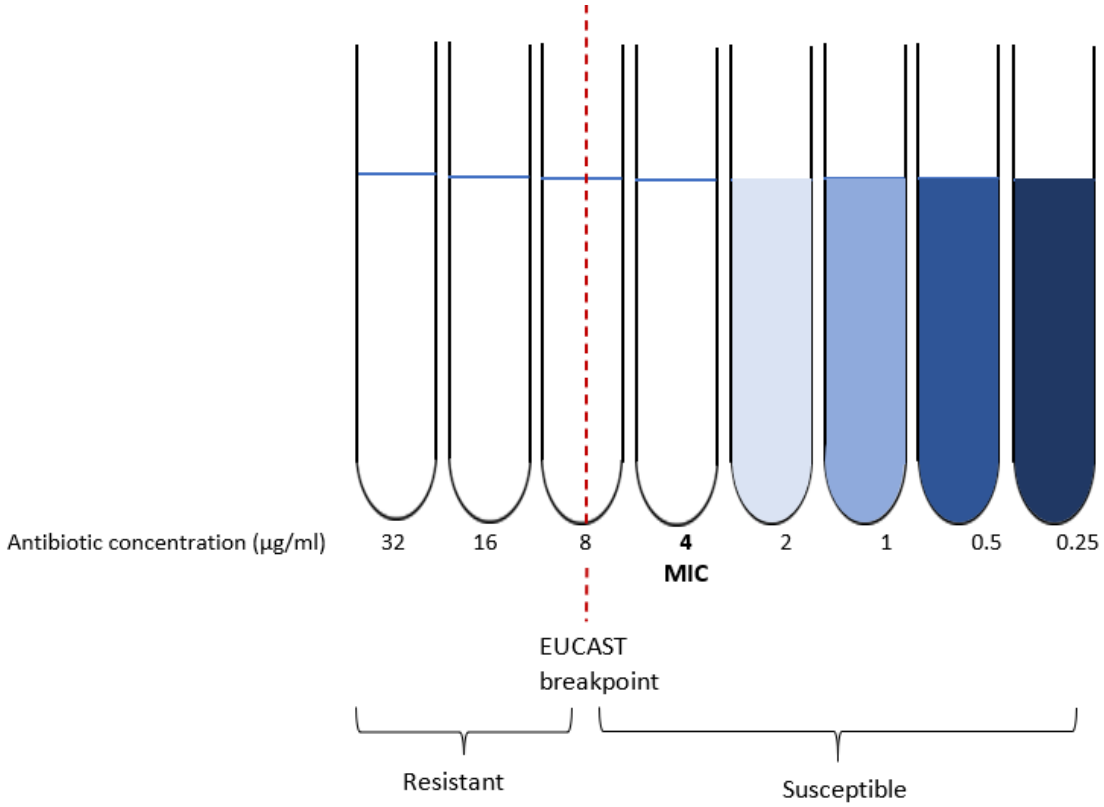
CHAPTER 6 MINIMUM INHIBITORY CONCENTRATION (MIC)

In this chapter, I will discuss the methods and results of an analysis investigating the relationship between MIC and mortality in patients with a Gram-negative bloodstream infection. I will start by discussing what MIC is and what it is used for, and I will then discuss the current literature on MIC and mortality (section 6.1). I will then move on to present methods (section 6.2) and results of these analyses (section 6.3). At the end of the chapter, I reflect on the findings as well as the strengths and limitations (section 6.4).

6.1 Introduction

As briefly mentioned in Chapter 1, the MIC is a more detailed measure of antimicrobial susceptibility and resistance than the S-I-R classification and is defined as the lowest concentration of a particular antimicrobial required to inhibit growth of the organism. It is used to classify organisms as susceptible, intermediate or resistant using 'breakpoint' MIC values, published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) at http://www.eucast.org. The breakpoints published by EUCAST are set primarily based on the pharmacokinetics, pharmacodynamics simulation modelling processes, microbiological resistance data and information from clinical trials. The MIC varies between patients and organism strains, and it is customarily tested at fixed concentrations in a doubling dilution series above and below 1 µg/L, e.g. 0.25, 0.5, 1, 2, 4 µg/L. Strains with an MIC below the breakpoint are classified as susceptible and are considered more likely to respond to treatment and strains with an MIC above the breakpoint are classified as resistant and considered less likely to respond to treatment. Figure 6.1 is visualisation of a blood culture being tested at doubling solutions of a particular antibiotic, where the different shades of blue represent the different levels of growth of organism and white represents no growth of organism. In this visualisation, the MIC for this particular strain of organism for the drug being tested is 4µg/ml as this is the lowest concentration which prevents visible growth of the organism, i.e. the lowest concentration with no blue visible. The EUCAST breakpoint is 8 µg/ml for the corresponding drug and organism, and as four falls below the breakpoint, this strain of organism would be classified as susceptible to the drug and the drug would be considered an appropriate treatment.

Figure 6.1 Visualisation of MIC testing for a strain of organism with an MIC of four



Susceptibility results from MIC tests play a key role in treatment decision making and guide the clinician as to which treatments patients are likely to respond or not respond to. However, there is a grey area around the breakpoint where strains may not respond as well even though the MIC is within the susceptible range. It has been shown in some studies that high MIC in the susceptibility range have worse outcomes, and this may help clinicians explain a slower response to treatment for some patients or suggest benefits of including an index of the degree of susceptibility to the treatments, so a binary classification as susceptible/resistant is not used[198, 199]. However, studies to date have focussed on the MIC of a particular drug e.g. Vancomycin MIC for the treatment of MRSA, and there is a lack of research exploring the overall relationship between MIC and patient outcomes.

I performed a literature review including studies that report on the association between MIC and mortality in patients with a bloodstream infection. There were 37 papers that were included in this review. The details of these are listed in Table 6.1, along with the key information about the type of study, the organism, the sample size, the MIC exposure and the study findings. Of the 37 studies included, 36 (97.3%) reported solely on the MIC of one drug of interest, of which 22 (59.5%) focussed on vancomycin MIC. MICs of antibiotics explored in the remainder of the studies included Teicoplanin (n=1), Carbapenem (n=2), Cefepime (n=2), Cefepime or ceftazidime (n=1), Fluconazole (n=1), Levofloxacin (n=1), Imipenem (n=1), Piperacillin/tazobactam (n=3), Piperacillin (n=1),

Tigecycline (n=1). The one remaining study was a meta-analysis by Falagas *et al* which compared strains of Gram-negative BSI with MICs equal to the breakpoint or one dilution lower (high MIC) to strains with MICs more than one dilution lower than the breakpoint (low MIC) for various drugs[200]. They found a higher all-cause mortality in patients with high MICs among non-Salmonella Enterobacteriaceae (RR, 2.03, 95% CI, 1.05 to 3.92), and Gram-negative non-fermentative bacilli (RR, 2.39, 95% CI, 1.19 to 4.81), however this study included infections other than bacteraemia.

The organisms studied varied, but the majority focussed on SAB (MRSA and/or MSSA) and the sample sizes ranged from 19 to 8,291. In terms of Gram-negative bloodstream infections, Rhodes *et al* found an increase in mortality was associated with cefepime MIC of 4 mg/L and 64 mg/L compared to 1mgL after adjusting for modified APACHE II score and days to positive culture[201]. O'Donnell *et al* performed a meta-analysis of four studies including 115 patients with Enterobacteriaceae BSI and found an increase in each meropenem MIC dilution was significantly associated with an increase in 30-day mortality (OR 1.51; 95% CI 1.06 to 2.15)[202]. Conversely, a retrospective cohort of 275 patients with Enterobacteriaceae BSI found no difference in mortality between patients with low Piperacillin-tazobactam MIC: 10.5% in the low MIC group (≤4 mg/L) and 11.1% in the borderline MIC group (8-16 mg/L, relative risk=1.06, 95% CI 0.34–3.27).

The studies included in this review all focussed on the MIC of a particular drug and only one explored a general effect of MIC. We aimed to explore whether MIC values closer to the EUCAST breakpoints, are associated with worse outcomes than lower MIC values in infections caused by Gram-negative bloodstream infection (*E. coli* or *P. aeruginosa*).

Table 6.1 Summary of studies reporting on the association between MIC and outcome

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings
Shi C 2021[203]	Meta-analysis/International	15 studies/ 2,487 patients	MSSA	Mortality	Vancomycin MIC	Mortality was significantly higher in isolates with a high vancomycin MIC than isolates with a low MIC (OR 1.44, 95% CI 1.12 to 1.84, p=0.004)
Papadimitriou-Olivgeris M 2020[204]	Retrospective cohort/ Greece	302 patients	Carbapenemase-producing Klebsiella pneumoniae	30-day mortality	Tigecycline MIC	Mortality was higher in patients with Tigecycline MIC 0.75–2 mg/L compared to MIC ≤ 0.5 mg/L (50.9% *vs* 20.0%, p = 0.042).
O'Donnell JN 2020[202]	Meta-analysis/International	4 studies/ 115 patients	Enterobacteriaceae BSI	30-day mortality	Carbapenem MIC	A significant increase in mortality was observed with increasing meropenem MIC dilution (OR 1.51; 95% CI 1.06 to 2.15)
Kagami K 2018[205]	Retrospective cohort/Japan	19 patients	MRSA	Treatment failure and 60-day mortality	Teicoplanin MIC	Treatment failure was higher in patients with Teicoplanin MIC >2 µg/mL compared to MIC ≤2 µg/mL (100.0% *vs* 26.7%, p=0.018). Mortality was also higher in patients with MIC >2 µg/mL compared to MIC ≤2 µg/mL (100.0% *vs* 13.3%, p=0.004)
Abelenda Alonso GA 2018[206]	Retrospective cohort /Spain	98 patients	SAB	30-day mortality	Vancomycin MIC	30-day mortality was similar in patients with Vancomycin MIC ≥ 2 mg/L compared to Vancomycin MIC < 2 mg/L (23.25% *vs* 27.7%).
Ko JH 2018[207]	Retrospective cohort /Korea	197 patients	*Candida* glabrata BSI	30-day mortality	Fluconazole MIC	Infections with fluconazole MIC ≤ 16 showed a better survival compared to those with fluconazole MIC = 32µg/mL (p<0.001)

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings
Adani S 2018[208]	Retrospective cohort /USA	166 patients	MRSA BSI	30-day in-hospital mortality	Vancomycin MIC	Vancomycin MIC of 2 µg/ml was not significantly associated with 30-day in hospital mortality compared to a MIC of <2 µg/ml (24.0% *vs* 13.2%, p=0.072).
Bouiller K 2017[209]	Prospective cohort/ France	250 patients	MSSA BSI	30-day mortality	Vancomycin MIC	No significant difference in 30-day mortality in patients with vancomycin MIC <1.5mg/L versus patients with vancomycin MIC ≥1. 5mg/L (24.7% *vs* 28.1%, p= 0.592)
Su TY 2017[210]	Retrospective cohort /Taiwan	90 patients	P. aeruginosa BSI	30-day mortality	Cefepime MIC	Cefepime MIC <4 mg/L was associated with lower mortality compared to MIC ≥4 mg/L (27.4% *vs* 76.5%, p < 0.0001).
Yang YS 2017[211]	Retrospective cohort/ Taiwan	224 patients	Acinetobacter BSI	30-day mortality	Carbapenem MIC	Mortality was higher in infections with MIC ≥8 mg/L than in those with isolates with MICs of ≤4 mg/L (53.1% *vs* 25.5%, p<0.001)
Hentzien 2017[212]	Retrospective cohort/ France	269 patients	CoNS bacteraemia	30-day in-hospital mortality	Vancomycin MIC	Vancomycin MIC ≥2 mg/l was not associated with 30-day in-hospital mortality (adjusted HR 0.8; 95% CI 0.30 to 2.19, p=0.67).
Gentry 2017[213]	Retrospective cohort/ USA	354 patients	P. aeruginosa bacteraemia	30-day all-cause mortality	Piperacillin/tazobactam MIC	No difference in 30-day all-cause mortality was found between elevated MIC (32–64 mg/L) and low MIC (≤16 mg/L) (24.5% *vs* 22.6% respectively, p = 0.79).
Ratliff 2017[214]	Retrospective cohort/ USA	103 patients	P. aeruginosa bacteraemia	30-day all-cause mortality	Cefepime or ceftazidime MIC	All-cause 30-day mortality was not statistically significant between the low MIC (≤2 µg/mL) group and the high MIC group (4–8 µg/mL)

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings
						(17.2% *vs* 27.6%, p = 0.34)
Song 2017[215]	Retrospective cohort/ Korea	1,027 isolates	Invasive *S. aureus* infections	30-day all-cause mortality	Vancomycin MIC	Vancomycin ≥1.5 mg/L was not associated with all-cause 30-day mortality (30.0% *vs* 26.7%, p=0.351).
Baxi 2016[216]	Prospective cohort/ USA	418 patients	SAB	30- or 90-day mortality	Vancomycin MIC	Vancomycin MIC of <2 µg/ml compared to 2 µg/ml was not associated with 30 day or 90 day mortality (HR 0.86; 95 CI 0.41 to 1.80, p=0.70 and HR 0.91; 95% CI 0.49 to 1.69, p=0.77 respectively)
Delgado-Valverde 2016[217]	Prospective cohort/ Spain	275 patients	Enterobacteriaceae BSI	Treatment failure and 30-day mortality	Piperacillin/tazobactam MIC	No difference in mortality: 10.5% in the low MIC group and 11.1% in the borderline MIC group (relative risk=1.06, 95% CI 0.34 to 3.27, p=1.00).
Rhodes 2015[201]	Retrospective cohort/ USA	91 patients	Gram-negative BSI	In-hospital mortality.	Cefepime MIC	Increased odds of mortality when MIC = 4 mg/L (aOR 6.47, 95% CI 1.25 to 33.4) and MIC= 64 mg/L (aOR 6.54, 95% CI 1.03 to 41.4).
Kalil 2014[218]	Meta-analysis/International	38 studies/ 8,291 patients	SAB	All-cause mortality	Vancomycin MIC	Mortality was 26.8% in patients with MIC ≥1.5mg/L compared with 25.8% in patients MIC <1.5mg/L (adjusted RD= 1.6%; 95% CI −2.3% to 5.6%, p=0.43).
Caston 2014[219]	Retrospective cohort/ Spain	53 patients	MSSA	30-day all-cause mortality	Vancomycin MIC	High MIC (>2 µg/ml) was associated with mortality compared to MIC=2 µg/ml (OR= 9.3, 95% CI 1.31 to 63.20, p=0.027)
Park 2013[220]	Prospective cohort/ South Korea	94 patients	MRSA	Attributable mortality or 30-day mortality	Vancomycin MIC	No difference in 30-day mortality between high-vancomycin-

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings
						MIC group (2 µg/ml) and a low-vancomycin-MIC group (<1.0 µg/ml): 19% *vs* 24 % respectively, p=0.79
Hope 2013[221]	Prospective cohort/ UK	228 patients	MRSA	resolution of bacteraemia or mortality	Vancomycin MIC	Mortality was higher in patients with isolates with MICs of 0.5–0.7 mg/L compared with isolates with vancomycin MICs of ≥1 mg/L (OR=2.55, 95% 1.08 to 6.01, p=0.054)
Retamar 2013[222]	IPD from 6 prospective cohort studies/ Spain	39 patients	ESBL-producing *Escherichia* coli	All-cause 30-day mortality	Piperacillin/tazobactam MIC	Mortality was higher for patients with MIC >8 mg/L compared to <8 mg/L (RR 0.21; 95% CI 0.06 to 0.75, p=0.01) and for patients with MICs >2 mg/L compared to MIC ≤2mg/L (41.1% versus 0%; RR 0.13; 95% CI 0.01 to 0.98, p= 0.002).
Holmes 2013[223]	Retrospective cohort/ Australia and New Zealand	410 patients	SAB	All-cause 30-day mortality	Vancomycin MIC	Mortality higher in patients with vancomycin MIC >1.5mg/L (28.9% *vs* 12.7%, p<0.001)
Jacob 2013[224]	Meta-analysis/International	20 studies/ 2439 patients	MRSA	Treatment failure and mortality	Vancomycin MIC	Mortality risk was greater in patients with MIC ≥1mg/L than in patients with MIC <1mg/L (RR 1.42, 95% CI 1.08–1.87).
Woods 2012[225]	Retrospective cohort/ USA	99 patients	MRSA	In-hospital mortality	Vancomycin MIC	MIC of 2mg/L was associated with higher mortality compared to MIC of <2mg/L (adjusted OR = 13.9, 95% CI 1.1 to 171.2)
Mavros 2012[226]	Meta-analysis/International	33 studies/ 6,210 patients	SAB	All-cause mortality and treatment failure	Vancomycin MIC	Group with MIC >1 mg/L but ≤2 mg/L) had higher mortality (RR= 1.21, 95% CI 1.03 to 1.43) and more treatment

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings
						failures (RR = 1.67, 95% CI 1.26 to 2.21) compared to group with MIC ≤ 1 mg/L
Esterly 2012[199]	Retrospective cohort/ USA	71 patients	Pseudomonas *aeruginosa*, Acinetobacter baumannii, and ESBL-producing Gram-negative bacteria	All-cause in hospital mortality	Imipenem MIC	76.9% of patients with MIC of ≥4 mg/L died *vs* 16.1% who died with a MIC of ≤2 mg/L (p <0.01).
Tamma 2012[227]	Retrospective cohort/ USA	170 patients	P. aeruginosa BSI	30-day all-cause mortality	Piperacillin MIC	30-day mortality was lower in children with a piperacillin MIC of ≤16 µg/mL compared to 32–64 µg/mL, respectively (OR, 3.23; 95% CI, 1.30–8.08).
Wi 2012[228]	Retrospective cohort/ South Korea	137 patients	MRSA	30-day mortality	Vancomycin MIC	Vancomycin MIC ≥1 µg /mL was associated with higher mortality (aHR = 7.0, 95% CI 2.2 to 22.1, p = 0.001)
Falagas 2012[200]	Meta-analysis/International	13 studies/ 1,469 patients	Gram-negative bacteria.	All-cause (30-day or in-hospital) mortality and treatment failure	High MIC (equal to breakpoint or 1 dilution lower) *vs* Low MIC (more than 1 dilution lower than breakpoint) – various drugs	Higher all-cause mortality in patients with high MICs (RR, 2.03, 95% CI, 1.05 to 3.92), among non-Salmonella Enterobacteriaceae. Mortality rate for patients with infections with Gram-negative nonfermentive bacilli with high MICs was also higher than for those with low MICs (RR, 2.39, 95% CI, 1.19 to 4.81).
Yeh 2012[229]	Retrospective cohort/ Taiwan	140 patients	MRSA	In-hospital mortality	Vancomycin MIC	No significant difference in in-hospital mortality rate between patients with MRSA isolates with

Study	Design/Country	Sample size	Organism	Outcome(s)	Exposure	Findings
						MICs ≥1.5 mg/L or < 1.5 mg/L (p=0.54)
Van Hal 2012[230]	Meta-analysis/International	22 studies/ 3,332 patients	MRSA	30-Day Mortality	Vancomycin MIC	Vancomycin MIC was significantly associated with mortality high vancomycin MIC (≥1.5 lg/mL) compared to low MIC (>1.5 lg/mL) (OR= 1.64; 95% CI 1.14 to 2.37, p = 0.01).
Brown 2011[231]	Retrospective review/ USA	50 patients	Complicated MRSA	Attributable mortality	Vancomycin MIC	Vancomycin AUC/MIC ratio of <211 was associated with attributable mortality (OR= 10.4, 95% CI 3.89 to 16.77. p=0.01)
Defife 2009[198]	Retrospective cohort/ USA	312 patients	Gram-negative BSI	All-cause in-hospital mortality	Levofloxacin MIC	No significant difference in mortality between patients with MIC ≤0.25 mg/L (12.5%), MIC = 0.5 mg/L (11.5%) and MIC = 1 or 2 mg/L (14.3%), p=0.91.
Lodise 2008[232]	Retrospective cohort/ USA	92 patients	MRSA	Treatment failure, including 30-day mortality	Vancomycin MIC	No significant difference in mortality between patients with MIC ≥1.5 mg/L (18.2%) and patients with MIC low (<1.5 mg/L (11.5%), p=0.5.
Soriano 2008[233]	Prospective cohort/ Spain	414 patients	MRSA	30-day mortality	Vancomycin MIC	Receipt of empirical vancomycin and an isolate with a vancomycin MIC of 2 mg/Ml was associated with an increase in mortality (OR = 6.39; 95% CI 1.68 to 24.3)
Maclayton 2006[234]	Case-control study/ USA	50 patients	MRSA	30-day mortality	Vancomycin MIC	Mortality was significantly higher in patients with MIC 2 mg/L compared with patients with MIC <0.5 mg/L and control groups (35% *vs* 24% and 15%, respectively, p = 0.022).

Abbreviations: *aHR= Adjusted hazard ratio, BSI=Bloodstream infection, CI=Confidence Interval, HR= Hazard ratio, IPD=Individual participant data, MIC=Minimum inhibitory concentration, MRSA=Methicillin-resistant Staphylococcus aureus, MSSA= Methicillin-susceptible Staphylococcus aureus, OR= Odds ratio, RCT=Randomised control trial, RD=Risk difference, RR=Relative risk, SAB= S. aureus bacteraemia*

6.2 Methods

6.2.1 Data source

For this analysis, I used patient-level data from the BSI-FOO observational study. It was not possible to include data from RAPIDO as MIC data was not collected.

6.2.2 Primary outcome

The primary outcome is 28-day all-cause mortality from date of blood culture.

6.2.3 Analysis population

Organism samples were sent by the five contributing centres to Bristol, where they were tested centrally against a selected range of antimicrobials and MICs recorded. A large number of isolates were not sent, generally because the labs had failed to retain them (administrative error). Therefore, MIC information was not available for all isolates. The inclusion and exclusion criteria for this analysis are given in Table 6.2. I focussed this analysis on Gram-negative infections as these isolates have a wider range of MIC values and there has been less research in this group of infections (Table 6.1). I excluded infection episodes that were identified as a different organism between the main BSI-FOO dataset and the central MIC dataset as it was not possible to determine which was the correct classification using the data available. MIC was also only tested for a select number of drugs, I therefore excluded patients who were not in receipt of a drug where MIC data was available or if they were in receipt of any additional drugs where MIC was not tested, therefore I required MIC data to be available for all drugs administered.

Table 6.2 Inclusion and Exclusion criteria for MIC analysis

Inclusion criteria	Exclusion criteria
• Gram negative BSI: *E. coli* or *P. aeruginosa* • Isolate sent to Bristol for central testing	• MIC organism does not match main dataset • Do not take any of the drugs in which MIC was tested or inferred* • MIC data not available for all drugs administered

** see section 6.2.4 for details on inferring MIC*

Abbreviations: *BSI=Bloodstream infection, MIC=Minimum inhibitory concentration*

6.2.4 Definitions

MIC classification

MICs were measured using the CLSI M7-A8 agar dilution method with Mueller Hinton agar[82]. A doubling dilution series was used for all antimicrobials. Enterobacteriaceae isolates were tested against co-amoxiclav, ampicillin, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, meropenem,

and piperacillin/tazobactam. *P. aeruginosa* isolates were tested against ceftazidime, ciprofloxacin, colistin, gentamicin, meropenem, and piperacillin tazobactam (Table 6.3). MIC for some antimicrobials that were not tested were inferred from those tested (see footnotes 1 to 4 of Table 6.3).

Table 6.3 Antibiotics tested against centrally, by organism

E. coli	P. aeruginosa
Ampicillin	Ceftazidime
Ceftriaxone	Ciprofloxacin
Ciprofloxacin	Colistin
Co amoxiclav	Gentamicin
Ertapenem	Meropenem
Gentamicin	Piperacillin tazobactam
Meropenem	Tobramycin[2]
Piperacillin tazobactam	Levofloxacin[3]
Amoxicillin[1]	
Tobramycin[2]	
Levofloxacin[3]	
Cefotaxime[4]	

[1] *Amoxicillin MIC inferred from Ampicillin MIC*
[2] *Tobramycin MIC inferred from Gentamicin MIC*
[3] *Levofloxacin MIC inferred from Ciprofloxacin MIC*
[4] *Cefotaxime MIC inferred from Ceftriaxone MIC*

I used EUCAST v9.0[37]. breakpoints for susceptibility classification, with susceptibility and resistant ranges given in Table 6.4

Table 6.4 EUCAST susceptibility and resistant ranges

	E. coli		P. aeruginosa	
Antibiotic	**Susceptible (≤)**	**Resistant (>)**	**Susceptible (≤)**	**Resistant (>)**
Ampicillin	8	8	-	-
Ceftriaxone	1	2	-	-
Ciprofloxacin	0.25	0.5	0.5	0.5
Co amoxiclav	8	8	-	-
Ertapenem	0.5	0.5	-	-
Gentamicin	2	4	4	4
Meropenem	2	8	2	8
Piperacillin tazobactam	8	16	16	16
Amoxicillin	8	8	-	-
Tobramycin	2	4	4	4
Levofloxacin	0.5	1	1	1
Cefotaxime	1	2	-	-
Ceftazidime	-	-	8	8
Colistin	-	-	2	2

Note: *Breakpoints for antibiotics that are not relevant for the bug are shown as "-" in this table*

The MICs cannot be directly compared between drugs because the breakpoints for each drug are different[235]. For that reason, I defined a ratio of MIC to EUCAST breakpoint (MIC/breakpoint). When susceptible to an antibiotic, the strain has a ratio of <1, and when resistant, the strain has a ratio of ≥1.

Empiric and definitive therapy

I wanted to estimate the association between MIC/breakpoint ratio and mortality for empiric and definitive treatments separately. However, the clinical choice of empiric and definitive therapy was not collected in BSI-FOO, therefore I needed to replicate the decision-making process using an algorithm. Timing of availability of susceptibly results would provide a good indication of which drugs were empiric, i.e. drugs given before susceptibility results were available, and definitive i.e. drugs given after availability of susceptibility results. However, the timing of availability of susceptibility results was not collected. Susceptibility results are usually available within 36-48 hours of blood culture. I therefore defined empiric and definitive treatment with a 36-hour and 48-hour threshold and compared the derived results to a clinician's best guess (Alasdair MacGowan) which was based on the individual treatment timelines for a sample of 75 patients (selected based on complexity of timeline to ensure the sample included patients with both simple and complex treatment timelines). The 75 patients were prescribed a total of 421 drugs (min=2 per patient, max=19 per patient). The algorithm was defined as:

- Empiric therapy: Treatments administered <96 hours prior to and within 36/48 hours post blood culture AND duration of treatment<72 hours
- Definitive therapy: Treatments administered (> 36/48 hours of blood culture AND within seven days of blood culture) OR (administered within 36/48 hours of blood culture and duration of treatment ≥72 hours)

Table 6.5 Empiric therapy matching - 36 and 48 hour rule

	n	%
36-hour rule		
Algorithm and clinical assessment disagreed	132	31.4%
Algorithm and clinical assessment agreed	289	68.6%
48-hour rule		
Algorithm and clinical assessment disagreed	108	25.7%
Algorithm and clinical assessment agreed	313	74.3%
Total	**421**	

Table 6.6 Cross tabulation of clinician classification and 36-hour rule classification

	36-hour rule classification			
Clinician classification	**Empiric**	**Definitive**	**Not empiric or definitive**	**Total**
Empiric	101	54	1	156
Definitive	12	153	8	173
Not empiric or definitive	11	46	35	92
Total	**124**	**253**	**44**	**421**

Note: *Numbers are presented at drug level. Kappa= 0.4952*

Table 6.7 Cross tabulation of clinician classification and 48-hour rule classification

	48-hour rule classification			
Clinician classification	**Empiric**	**Definitive**	**Not empiric or definitive**	**Total**
Empiric	119	18	19	156
Definitive	38	126	9	173
Not empiric or definitive	5	19	68	92
Total	**162**	**163**	**96**	**421**

Note: *Numbers are presented at drug level. Kappa= 0.6044*

The agreement when applying a 48-hour rule improved when compared to the agreement after applying the 36-hour rule (74.3% match vs 68.6% match), see Table 6.5, Table 6.6 and Table 6.7. Therefore, I decided to use a 48-hour rule. This enabled each patient's follow-up time to be easily split into two epochs, with the first epoch defined as the period of empiric treatment (days 0 and 1) and the second epoch defined as the period of definitive treatment (day 2 onwards).

6.2.5 Statistical analysis

Data management

The most critical period for choice and timing of therapy in relationship to 28-day mortality can be considered as the first seven days post blood culture (see Chapter 3). I therefore chose to explore the MIC of drugs administered in the first seven days only. The drugs that are administered can vary daily meaning the MIC can vary day to day; I therefore performed the analysis using two approaches:

Approach 1 – "Clean" population: In this first approach, I restricted the analysis to patients whose therapy remained unchanged in the first epoch of time (first 48 hours), and unchanged in the second epoch of time (days 2 to 7). This was the simplest approach but has the potential to introduce selection bias as the patients whose therapy remains unchanged are likely to be patients who are responding well to treatment. It also reduced the sample size to 236 patients (approximately 50% of the target analysis population) resulting in less statistical power.

Approach 2 – "full" population: In this approach I included the full analysis population including patients who change treatments during follow-up. To allow for changes in treatment, I split the

infection episodes (using the -*stsplit*- Stata command) at daily intervals from day 0 to 7 with MIC/breakpoint ratios updated at each daily interval. As the therapeutic effect can last longer than a day, I wanted to include the MICs of previous drug exposures in the estimation of effect, e.g. on day 5, I wanted to include MIC of drug exposure up to day 5 rather than the MIC of the drug taken on day 5 only. I therefore calculated a cumulative average MIC/breakpoint ratio which was updated daily from days 0 to 7. I excluded drugs that were administered with an MIC/breakpoint ratio ≥4 from the calculation of the cumulative average as the therapeutic effect of such drugs is likely to be minimal/similar to receiving no therapy, and I wanted the average to be a reflection of drugs administered with some potential therapeutic effect. An example calculation of the cumulative average MIC/breakpoint ratio is given in Table 6.8 for a patient who was in receipt of Gentamicin on days 0 and 1, Piperacillin tazobactam on day 2 and Ceftriaxone on days 2, 3 & 4. The MIC/breakpoint ratio for gentamicin was excluded from the calculation of cumulative average as it was ≥4.

Table 6.8 Example calculation of the cumulative average MIC/breakpoint ratio

Day	MIC/breakpoint ratio			Cumulative average (median)
	Gentamicin (In receipt on day 0 and 1)	Piperacillin tazobactam (In receipt on day 2 only)	Ceftriaxone (In receipt on days 2, 3 &4)	
0	16			-
1	16			-
2		0.0625	0.03	0.04625
3			0.03	0.03
4			0.03	0.03
5				0.03
6				0.03
7 to 28				0.03

Analysis

In both approaches, I performed a Cox regression analysis to estimate the association between MIC/breakpoint ratio and mortality, where MIC/breakpoint ratio was categorised into the following groups, and updated daily based on the cumulative average MIC/breakpoint as described above:

- <0.125 (Susceptible (S))
- 0.125 – <0.25 (S)
- 0.25 – <0.5 (S)
- 0.5 - <1 (S)
- = 1 (S)
- >1- <4 (Resistant (R))
- ≥4 (R)

I adjusted for the risk score calculated in Chapter 3 and organism. I planned to include an interaction term between empiric and definitive therapy epochs of time to estimate the effect of empiric and definitive therapy separately, but there were too few events in the empiric epoch to provide reliable estimates. I therefore summarise the number events by MIC/breakpoint ratio category for empiric and definitive epochs separately, but only provide model estimates for the whole period. I planned to use MIC/breakpoint ratio=1 as the reference category for the analysis as this group are of most clinical interest, however due to such few events in this group I decided to use the least susceptible group as the reference category to improve the precision of estimates.

For all models, I assessed the proportional hazards assumption based on Schoenfeld residuals and log-log plots of survival.

6.2.6 Missing data

MIC data was not missing for any drugs administered in the analysis population as this was an inclusion criterion of the analysis population. Missing values of variables included in the risk score were imputed using multiple imputation as described in Chapter 3. I planned to modify the imputation procedure to include the exposure variable of this analysis (MIC/breakpoint ratio), however the model would not converge due to the smaller population size which resulted in small numbers in many of the categorical variables and perfect prediction issues. I attempted to remove the problematic variables, however this resulted in a model with different specifications for each variable and I felt the original specification without MIC would provide a better estimate of the risk score. I therefore used the same imputation as Chapter 3 and performed a complete case analysis as a sensitivity analysis (see section 6.2.7).

6.2.7 Sensitivity analysis

I assessed the impact of using multiple imputation for missing risk score components by fitting a complete-case model, i.e., refitting the primary outcome model only for patients with complete data for all variables included in the model used to derive the risk score.

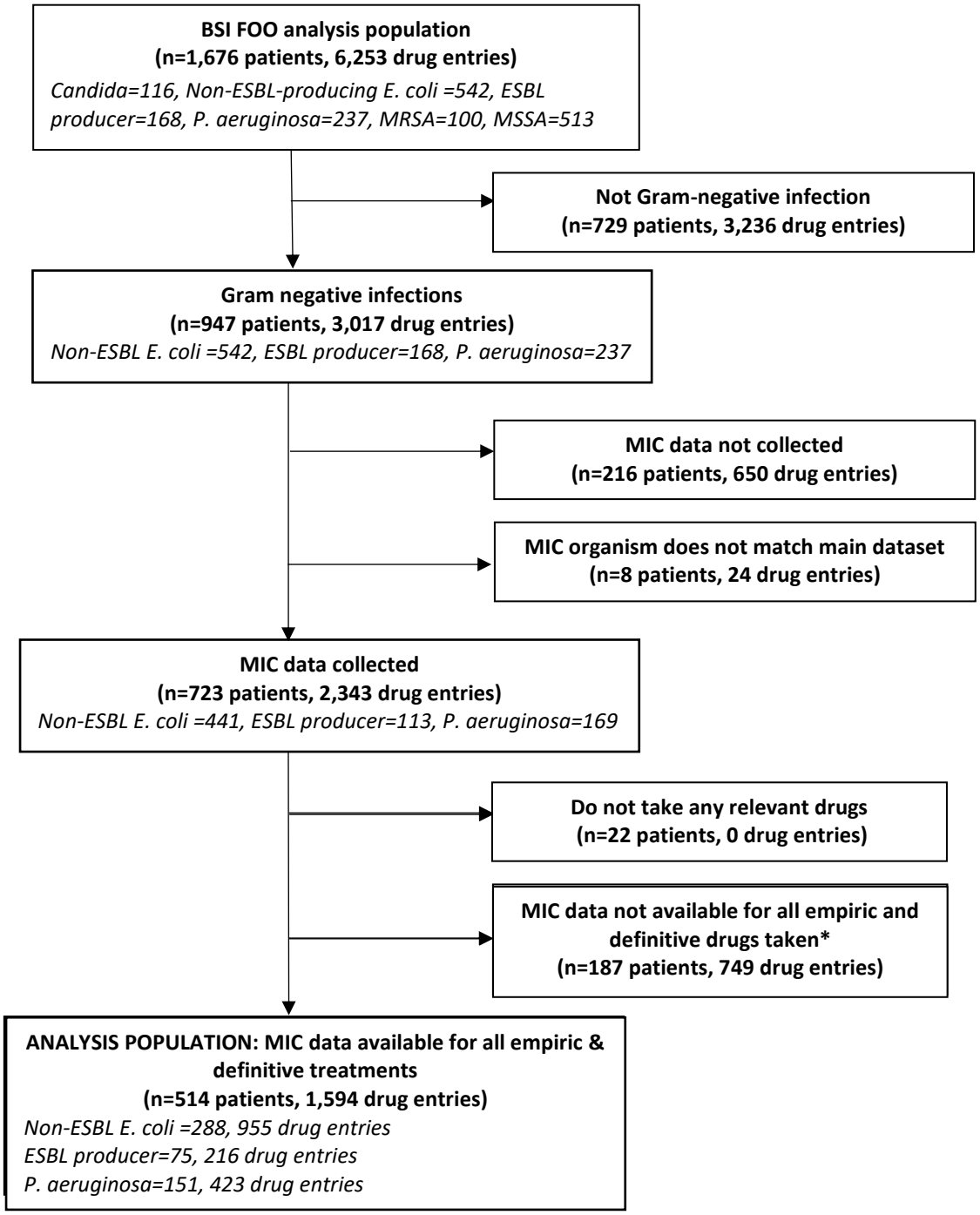
6.3 Results

6.3.1 Population

Of the 1,676 participants in the BSI-FOO analysis population (after exclusion of polymicrobial and repeat episodes), 729 did not have a Gram-negative infection and were therefore excluded. There were 216 patients whose isolates were not sent to Bristol for central testing and eight patients were excluded as their organism recorded in the MIC dataset did not match the organism recorded in the

main BSI-FOO dataset. After excluding patients that received an empiric or definitive therapy which MIC data was not collected for, 514 patients met the eligibility criteria and were included in the analysis (Figure 6.2).

Figure 6.2 Flowchart for MIC analysis



* See Table 6.3 for list of drugs with MIC data available

6.3.2 Demographics

Demographic characteristics and medical history are shown by inclusion status in Table 6.9 and MIC/EUCAST breakpoint category in Table 6.10. Overall, the patients' characteristics are generally similar in the analysis population to the excluded patients. The included patients are slightly older (median 74 years *vs* 68 years) and source of infection was less commonly a urinary tract (39.7% *vs* 45.5%) with a higher proportion site uncertain (30.7% *vs* 25.4%). Despite these, other characteristics are similar, and I did not consider the exclusion criteria to introduce any selection bias that would impact the generalisability of the results. Baseline characteristics were also broadly similar across the MIC/EUCAST breakpoint categories (Table 6.10). The average age was youngest in the >1 MIC category with a median of 66 years (IQR 54.0, 79.0) and oldest in the 0.25-<0.5 category with a median of 75.5 years (62.0, 82.0).

Table 6.9 Baseline characteristics for Gram negative infections by inclusion in the analysis population

	Excluded (n=433)	Included (n=514)	SMD	Overall (n=947)
Patient measures				
Age	68.0 (53.0, 80.0)	74.0 (60.0, 82.0)	0.23	71.0 (57.0, 82.0)
Male	212/433 (49.0%)	253/514 (49.2%)	0.00	465/947 (49.1%)
Body Mass Index [a]	26.3 (6.9)	25.8 (6.7)	0.07	26.0 (6.8)
Patient medical history				
Chemotherapy in month before date 0	76/433 (17.6%)	93/514 (18.1%)	0.01	169/947 (17.8%)
Any tumour within last 5 years	155/433 (35.8%)	214/514 (41.6%)	0.12	369/947 (39.0%)
Surgery requiring overnight stay within 7 days before date 0	35/433 (8.1%)	34/513 (6.6%)	0.06	69/946 (7.3%)
Burn requiring admission within 7 days before date 0	0/433 (0.0%)	0/513 (0.0%)	-	0/946 (0.0%)
Cardiac arrest within 7 days before date 0	1/433 (0.2%)	4/514 (0.8%)	0.08	5/947 (0.5%)
Renal support within 7 days before date 0	18/433 (4.2%)	27/514 (5.3%)	0.05	45/947 (4.8%)
Myocardial infarction within 7 days before date 0	45/433 (10.4%)	50/514 (9.7%)	0.02	95/947 (10.0%)
Infection severity measures				
Temperature (°C) at time 0 [b]	38.2 (37.6, 38.8)	38.2 (37.6, 38.8)	0.01	38.2 (37.6, 38.8)
INR [c]	1.2 (1.1, 1.4)	1.2 (1.1, 1.4)	0.01	1.2 (1.1, 1.4)
eGFR (mL/min/1.73m^2) [d]	61.0 (37.5, 90.0)	54.0 (33.0, 88.0)	0.11	58.0 (35.0, 90.0)
Serum Albumin (g/L) [e]	32.3 (8.0)	31.7 (7.7)	0.08	32.0 (7.9)
Bilirubin total (umol/L) [f]	12.0 (8.0, 22.0)	13.0 (8.0, 22.0)	0.03	12.0 (8.0, 22.0)
Neutrophil count at day 0 or closest (x10^9/L) [g]	9.4 (5.0, 13.5)	10.1 (4.9, 14.8)	0.15	9.7 (4.9, 14.3)
Systolic BP at day 0 or closest (mmHg) [h]	121.4 (27.4)	119.3 (26.9)	0.07	120.3 (27.2)
On IV fluids at day 0	169/430 (39.3%)	187/514 (36.4%)	0.06	356/944 (37.7%)
On ventilation at day 0	26/433 (6.0%)	40/513 (7.8%)	0.07	66/946 (7.0%)
On vasopressor drugs at day 0	20/432 (4.6%)	28/514 (5.4%)	0.04	48/946 (5.1%)
Systemic corticosteroids in last 24 hours	72/429 (16.8%)	67/514 (13.0%)	0.11	139/943 (14.7%)
EWS score nearest to day 0 [i]	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)	0.13	2.0 (1.0, 4.0)
Patient comorbidities at date 0				

	Excluded (n=433)	Included (n=514)	SMD	Overall (n=947)
Congestive heart failure	47/433 (10.9%)	58/514 (11.3%)	0.01	105/947 (11.1%)
Peripheral vascular disease	34/433 (7.9%)	45/514 (8.8%)	0.03	79/947 (8.3%)
Cerebrovascular disease	68/433 (15.7%)	88/514 (17.1%)	0.04	156/947 (16.5%)
Hemiplegia	16/433 (3.7%)	20/514 (3.9%)	0.01	36/947 (3.8%)
Dementia	42/432 (9.7%)	55/514 (10.7%)	0.03	97/946 (10.3%)
COPD	64/433 (14.8%)	65/514 (12.6%)	0.06	129/947 (13.6%)
Connective tissue disease	41/433 (9.5%)	43/514 (8.4%)	0.04	84/947 (8.9%)
Peptic ulcer disease	27/433 (6.2%)	42/514 (8.2%)	0.07	69/947 (7.3%)
Ascites	19/433 (4.4%)	16/514 (3.1%)	0.07	35/947 (3.7%)
Diabetes:				
None	337/433 (77.8%)	411/514 (80.0%)	0.07	748/947 (79.0%)
Without organ damage	73/433 (16.9%)	82/514 (16.0%)		155/947 (16.4%)
With organ damage	23/433 (5.3%)	21/514 (4.1%)		44/947 (4.6%)
Child-Pugh score [j]	7.0 (6.0, 8.0)	6.0 (6.0, 7.0)	0.02	7.0 (6.0, 7.0)
Charlson score [k]	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	0.08	3.0 (2.0, 4.0)
Abscess at time 0	22/433 (5.1%)	12/514 (2.3%)	0.15	34/947 (3.6%)
Infected foreign body at time 0	3/433 (0.7%)	3/514 (0.6%)	0.01	6/947 (0.6%)
Surgical prosthesis time 0	2/433 (0.5%)	2/514 (0.4%)	0.01	4/947 (0.4%)
Source of infection				
Bone and joint	0/433 (0.0%)	4/514 (0.8%)	0.27	4/947 (0.4%)
Eye, ear, nose, throat or mouth	0/433 (0.0%)	1/514 (0.2%)		1/947 (0.1%)
Gastrointestinal system	56/433 (12.9%)	68/514 (13.2%)		124/947 (13.1%)
Line infection - central venous line	16/433 (3.7%)	19/514 (3.7%)		35/947 (3.7%)
Line infection - peripheral venous line	1/433 (0.2%)	0/514 (0.0%)		1/947 (0.1%)
Lower respiratory tract	31/433 (7.2%)	30/514 (5.8%)		61/947 (6.4%)
Reproductive tract	2/433 (0.5%)	3/514 (0.6%)		5/947 (0.5%)
Skin and soft tissue	14/433 (3.2%)	13/514 (2.5%)		27/947 (2.9%)

	Excluded (n=433)	Included (n=514)	SMD	Overall (n=947)
Surgical site infection	2/433 (0.5%)	11/514 (2.1%)		13/947 (1.4%)
Systemic Infection	4/433 (0.9%)	3/514 (0.6%)		7/947 (0.7%)
Urinary tract infection	197/433 (45.5%)	204/514 (39.7%)		401/947 (42.3%)
Site uncertain	110/433 (25.4%)	158/514 (30.7%)		268/947 (28.3%)
Lines and catheters				
Central line present at time 0	78/432 (18.1%)	104/514 (20.2%)	0.06	182/946 (19.2%)
Peripheral line present at time 0	220/432 (50.9%)	237/514 (46.1%)	0.10	457/946 (48.3%)
Urinary catheter present at time 0	106/432 (24.5%)	176/514 (34.2%)	0.21	282/946 (29.8%)
Organisational factors				
Centre:				
A	48/433 (11.1%)	41/514 (8.0%)	0.56	89/947 (9.4%)
B	52/433 (12.0%)	102/514 (19.8%)		154/947 (16.3%)
C	117/433 (27.0%)	226/514 (44.0%)		343/947 (36.2%)
D	74/433 (17.1%)	76/514 (14.8%)		150/947 (15.8%)
E	142/433 (32.8%)	69/514 (13.4%)		211/947 (22.3%)

Notes: *Date and time 0 = date/time of sampling for blood culture*

Data are presented as median (IQR), mean (SD) or n (%)

Missing data (Excluded, Included):

[a] *Data missing for 439 patients (187, 252)*

[b] *Data missing for 12 patients (7, 5).*

[c] *Data missing for 574 patients (275, 299).*

[d] *Data missing for 64 patients (21, 43).*

[e] *Data missing for 115 patients (48, 67).*

[f] *Data missing for 150 patients (63, 87).*

[g] *Data missing for 76 patients (37, 39).*

[h] *Data missing for 130 patients (49, 81).*

[i] *Data missing for 449 patients (244, 205).*

[j] *Data missing for 610 patients (289, 321).*

[k] *Data missing for 219 patients (95, 124).*

Abbreviations: *BP=Blood pressure, COPD=Chronic obstructive pulmonary disease, eGFR=Estimated glomerular filtration rate, EWS=Early warning score, INR=International normalised ratio, IQR=Interquartile range, IV=Intravenous, SMD=Standardised mean difference, SD= Standard deviation*

Table 6.10 Baseline characteristics, by MIC/EUCAST ratio category

	MIC/EUCAST breakpoint ratio on last day of follow-up					
	<0.125 (n=121)	**0.125 – <0.25 (n=98)**	**0.25 – <0.5 (n=140)**	**0.5 - <1 (n=90)**	**= 1 (n=36)**	**>1 (n=29)**
Patient measures						
Age	72.0 (58.0, 82.0)	71.5 (62.0, 82.0)	75.5 (62.0, 82.0)	75.5 (60.0, 83.0)	75.5 (64.5, 83.5)	66.0 (53.0, 79.0)
Male	63/121 (52.1%)	52/98 (53.1%)	72/140 (51.4%)	37/90 (41.1%)	14/36 (38.9%)	15/29 (51.7%)
Body Mass Index [a]	26.4 (7.7)	24.8 (5.4)	25.6 (7.3)	25.9 (6.9)	26.0 (4.2)	27.1 (5.4)
Patient medical history						
Chemotherapy in month before date 0	18/121 (14.9%)	22/98 (22.4%)	34/140 (24.3%)	10/90 (11.1%)	5/36 (13.9%)	4/29 (13.8%)
Any tumour within last 5 years	41/121 (33.9%)	44/98 (44.9%)	67/140 (47.9%)	34/90 (37.8%)	17/36 (47.2%)	11/29 (37.9%)
Surgery requiring overnight stay within 7 days before date 0	7/121 (5.8%)	7/98 (7.1%)	6/140 (4.3%)	5/89 (5.6%)	5/36 (13.9%)	4/29 (13.8%)
Burn requiring admission within 7 days before date 0	0/121 (0.0%)	0/98 (0.0%)	0/140 (0.0%)	0/89 (0.0%)	0/36 (0.0%)	0/29 (0.0%)
Cardiac arrest within 7 days before date 0	1/121 (0.8%)	0/98 (0.0%)	2/140 (1.4%)	1/90 (1.1%)	0/36 (0.0%)	0/29 (0.0%)
Renal support within 7 days before date 0	9/121 (7.4%)	2/98 (2.0%)	8/140 (5.7%)	2/90 (2.2%)	1/36 (2.8%)	5/29 (17.2%)
Myocardial infarction within 7 days before date 0	13/121 (10.7%)	11/98 (11.2%)	12/140 (8.6%)	5/90 (5.6%)	3/36 (8.3%)	6/29 (20.7%)
Infection severity measures						
Temperature (°C) at time 0 [b]	38.1 (37.5, 38.7)	38.5 (38.0, 39.1)	38.1 (37.6, 38.6)	38.2 (37.4, 38.8)	38.3 (37.5, 38.6)	38.5 (37.6, 39.0)
INR [c]	1.2 (1.1, 1.3)	1.1 (1.1, 1.5)	1.1 (1.0, 1.3)	1.2 (1.1, 1.3)	1.3 (1.0, 1.5)	1.3 (1.1, 2.3)
eGFR (mL/min/1.73m^2) [d]	49.0 (25.0, 90.0)	62.0 (45.0, 90.0)	60.0 (33.0, 90.0)	53.0 (35.0, 90.0)	41.0 (31.0, 65.0)	51.0 (30.0, 75.0)
Serum Albumin (g/L) [e]	30.9 (8.2)	33.9 (7.2)	32.5 (7.6)	30.8 (7.8)	30.3 (6.3)	29.2 (8.4)
Bilirubin total (umol/L) [f]	12.0 (8.0, 21.5)	14.0 (8.0, 25.5)	12.0 (9.0, 22.0)	16.0 (9.0, 24.0)	11.5 (9.0, 21.5)	12.5 (7.5, 18.5)
Neutrophil count at day 0 or closest (x10^9/L) [g]	10.2 (5.0, 14.0)	8.4 (1.7, 14.1)	9.4 (4.5, 15.6)	11.9 (7.5, 16.6)	10.1 (7.5, 15.6)	11.8 (6.3, 14.4)

	MIC/EUCAST breakpoint ratio on last day of follow-up					
	<0.125 (n=121)	**0.125 – <0.25 (n=98)**	**0.25 – <0.5 (n=140)**	**0.5 - <1 (n=90)**	**= 1 (n=36)**	**>1 (n=29)**
Systolic BP at day 0 or closest (mmHg) [h]	121.9 (23.4)	122.1 (29.9)	117.7 (27.3)	118.4 (28.3)	112.4 (23.0)	117.9 (29.5)
On IV fluids at day 0	53/121 (43.8%)	33/98 (33.7%)	46/140 (32.9%)	31/90 (34.4%)	11/36 (30.6%)	13/29 (44.8%)
On ventilation at day 0	9/121 (7.4%)	6/98 (6.1%)	10/140 (7.1%)	9/89 (10.1%)	1/36 (2.8%)	5/29 (17.2%)
On vasopressor drugs at day 0	9/121 (7.4%)	4/98 (4.1%)	6/140 (4.3%)	5/90 (5.6%)	0/36 (0.0%)	4/29 (13.8%)
Systemic corticosteroids in last 24 hours	14/121 (11.6%)	15/98 (15.3%)	22/140 (15.7%)	9/90 (10.0%)	6/36 (16.7%)	1/29 (3.4%)
EWS score nearest to day 0 [i]	3.0 (1.0, 5.0)	2.0 (1.0, 4.0)	3.0 (1.0, 5.0)	2.0 (1.0, 3.0)	2.0 (1.0, 4.0)	3.0 (1.5, 3.0)
Patient comorbidities at date 0						
Congestive heart failure	17/121 (14.0%)	11/98 (11.2%)	18/140 (12.9%)	8/90 (8.9%)	3/36 (8.3%)	1/29 (3.4%)
Peripheral vascular disease	12/121 (9.9%)	9/98 (9.2%)	12/140 (8.6%)	6/90 (6.7%)	4/36 (11.1%)	2/29 (6.9%)
Cerebrovascular disease	24/121 (19.8%)	13/98 (13.3%)	26/140 (18.6%)	16/90 (17.8%)	5/36 (13.9%)	4/29 (13.8%)
Hemiplegia	4/121 (3.3%)	4/98 (4.1%)	7/140 (5.0%)	2/90 (2.2%)	2/36 (5.6%)	1/29 (3.4%)
Dementia	13/121 (10.7%)	12/98 (12.2%)	12/140 (8.6%)	12/90 (13.3%)	2/36 (5.6%)	4/29 (13.8%)
COPD	18/121 (14.9%)	11/98 (11.2%)	19/140 (13.6%)	6/90 (6.7%)	7/36 (19.4%)	4/29 (13.8%)
Connective tissue disease	17/121 (14.0%)	4/98 (4.1%)	11/140 (7.9%)	3/90 (3.3%)	6/36 (16.7%)	2/29 (6.9%)
Peptic ulcer disease	9/121 (7.4%)	8/98 (8.2%)	11/140 (7.9%)	8/90 (8.9%)	2/36 (5.6%)	4/29 (13.8%)
Ascites	5/121 (4.1%)	4/98 (4.1%)	3/140 (2.1%)	0/90 (0.0%)	3/36 (8.3%)	1/29 (3.4%)
Diabetes:						
None	85/121 (70.2%)	81/98 (82.7%)	116/140 (82.9%)	78/90 (86.7%)	28/36 (77.8%)	23/29 (79.3%)
Without organ damage	24/121 (19.8%)	16/98 (16.3%)	19/140 (13.6%)	10/90 (11.1%)	7/36 (19.4%)	6/29 (20.7%)
With organ damage	12/121 (9.9%)	1/98 (1.0%)	5/140 (3.6%)	2/90 (2.2%)	1/36 (2.8%)	0/29 (0.0%)
Child-Pugh score [j]	7.0 (6.0, 8.0)	7.0 (6.0, 7.0)	6.0 (6.0, 7.0)	6.0 (5.0, 7.0)	7.0 (6.0, 8.0)	7.0 (7.0, 8.0)
Charlson score [k]	3.0 (2.0, 5.0)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	4.0 (3.0, 5.0)	3.0 (2.0, 4.0)
Abscess at time 0	5/121 (4.1%)	3/98 (3.1%)	1/140 (0.7%)	1/90 (1.1%)	0/36 (0.0%)	2/29 (6.9%)
Infected foreign body at time 0	0/121 (0.0%)	0/98 (0.0%)	1/140 (0.7%)	2/90 (2.2%)	0/36 (0.0%)	0/29 (0.0%)
Surgical prosthesis time 0	1/121 (0.8%)	0/98 (0.0%)	1/140 (0.7%)	0/90 (0.0%)	0/36 (0.0%)	0/29 (0.0%)
Source of infection						

	MIC/EUCAST breakpoint ratio on last day of follow-up					
	<0.125 (n=121)	**0.125 – <0.25 (n=98)**	**0.25 – <0.5 (n=140)**	**0.5 - <1 (n=90)**	**= 1 (n=36)**	**>1 (n=29)**
Bone and joint	1/121 (0.8%)	0/98 (0.0%)	2/140 (1.4%)	1/90 (1.1%)	0/36 (0.0%)	0/29 (0.0%)
Eye, ear, nose, throat or mouth	1/121 (0.8%)	0/98 (0.0%)	0/140 (0.0%)	0/90 (0.0%)	0/36 (0.0%)	0/29 (0.0%)
Gastrointestinal system	11/121 (9.1%)	15/98 (15.3%)	16/140 (11.4%)	13/90 (14.4%)	5/36 (13.9%)	8/29 (27.6%)
Line infection - central venous line	0/121 (0.0%)	2/98 (2.0%)	13/140 (9.3%)	1/90 (1.1%)	2/36 (5.6%)	1/29 (3.4%)
Lower respiratory tract	3/121 (2.5%)	4/98 (4.1%)	7/140 (5.0%)	11/90 (12.2%)	3/36 (8.3%)	2/29 (6.9%)
Reproductive tract	1/121 (0.8%)	0/98 (0.0%)	0/140 (0.0%)	2/90 (2.2%)	0/36 (0.0%)	0/29 (0.0%)
Skin and soft tissue	5/121 (4.1%)	3/98 (3.1%)	5/140 (3.6%)	0/90 (0.0%)	0/36 (0.0%)	0/29 (0.0%)
Surgical site infection	2/121 (1.7%)	1/98 (1.0%)	4/140 (2.9%)	4/90 (4.4%)	0/36 (0.0%)	0/29 (0.0%)
Systemic Infection	0/121 (0.0%)	0/98 (0.0%)	1/140 (0.7%)	1/90 (1.1%)	0/36 (0.0%)	1/29 (3.4%)
Urinary tract infection	54/121 (44.6%)	34/98 (34.7%)	54/140 (38.6%)	33/90 (36.7%)	18/36 (50.0%)	11/29 (37.9%)
Site uncertain	43/121 (35.5%)	39/98 (39.8%)	38/140 (27.1%)	24/90 (26.7%)	8/36 (22.2%)	6/29 (20.7%)
Lines and catheters						
Central line present at time 0	25/121 (20.7%)	21/98 (21.4%)	33/140 (23.6%)	13/90 (14.4%)	4/36 (11.1%)	8/29 (27.6%)
Peripheral line present at time 0	60/121 (49.6%)	41/98 (41.8%)	65/140 (46.4%)	45/90 (50.0%)	12/36 (33.3%)	14/29 (48.3%)
Urinary catheter present at time 0	48/121 (39.7%)	27/98 (27.6%)	43/140 (30.7%)	38/90 (42.2%)	8/36 (22.2%)	12/29 (41.4%)
Organisational factors						
Centre:						
A	15/121 (12.4%)	10/98 (10.2%)	10/140 (7.1%)	3/90 (3.3%)	2/36 (5.6%)	1/29 (3.4%)
B	31/121 (25.6%)	12/98 (12.2%)	22/140 (15.7%)	18/90 (20.0%)	11/36 (30.6%)	8/29 (27.6%)
C	36/121 (29.8%)	54/98 (55.1%)	62/140 (44.3%)	47/90 (52.2%)	14/36 (38.9%)	13/29 (44.8%)
D	26/121 (21.5%)	11/98 (11.2%)	20/140 (14.3%)	12/90 (13.3%)	4/36 (11.1%)	3/29 (10.3%)
E	13/121 (10.7%)	11/98 (11.2%)	26/140 (18.6%)	10/90 (11.1%)	5/36 (13.9%)	4/29 (13.8%)

Notes: *Date and time 0 = date/time of sampling for blood culture*

Data are presented as median (IQR), mean (SD) or n (%)

Missing data (<0.125, 0.125 – <0.25, 0.25 – <0.5, 0.5 - <1, = 1, >1)

[a] *Data missing for 252 patients (63, 46, 61, 44, 21, 17)*

[b] *Data missing for 5 patients (4, 0, 0, 0, 0, 1)*

[c] *Data missing for 299 patients (63, 56, 85, 54, 20, 21)*

[d] *Data missing for 43 patients (8, 12, 9, 9, 3, 2)*

[e] *Data missing for 67 patients (14, 21, 14, 11, 4, 3)*

[f] *Data missing for 87 patients (21, 22, 16, 19, 4, 5)*

[g] *Data missing for 39 patients (4, 11, 10, 7, 4, 3)*

[h] *Data missing for 81 patients (16, 14, 21, 19, 5, 6)*

[i] *Data missing for 205 patients (59, 32, 59, 29, 13, 13)*

[j] *Data missing for 321 patients (70, 61, 90, 56, 21, 23)*

[k] *Data missing for 124 patients (30, 29, 25, 25, 6, 9)*

Abbreviations: *BP=Blood pressure, COPD=Chronic obstructive pulmonary disease, eGFR=Estimated glomerular filtration rate, EWS=Early warning score, INR=International normalised ratio, IQR=Interquartile range, IV=Intravenous, SMD=Standardised mean difference, SD= Standard deviation*

6.3.3 Treatment

Overall, 514 patients were in receipt of 1,312 drugs varying from one drug per patient (33.7% of patients) to five drugs per patients (0.4% of patients). Of the 1,312 treatments administered, 675 (51.4%) were giving during the empiric epoch (days 0-2) and 637 (48.6%) were given during the definitive epoch (days 2+)

Table 6.11 Number of drugs given during empiric and definitive epoch

	Empiric (n=472) n (%)	**Definitive (n=453) n (%)**	**Overall (n=514) n (%)**
Total number of drug entries*	832	752	1,584
Total number of unique drug entries	675	637	1,312
Number of unique drugs per patient			
1	300/472 (63.6%)	289/453 (63.8%)	173/514 (33.7%)
2	142/472 (30.1%)	145/453 (32.0%)	218/514 (42.4%)
3	29/472 (6.1%)	18/453 (4.0%)	102/514 (19.8%)
4	1/472 (0.2%)	1/454 (0.2%)	19/514 (3.7%)
5	0/472 (0.0%)	0/454 (0.0%)	2/514 (0.4%)
Same drug during empiric/definitive epoch	300/472 (63.6%)	289/453 (63.8%)	236/514 (45.9%) **

** Patients may have multiple entries per drug if frequency, route or dose changes*

*** Treatment during empiric epoch may not necessarily be the same as the treatment during definitive epoch, but only one treatment given during each epoch of time*

Of the 514 patients, 236 received the same drug during the empiric epoch and same drug during definitive epoch and are included in the analysis of approach one. Of the 236 patients, 209 were in receipt of therapy during the empiric epoch of time and 196 were in receipt of therapy during the definitive epoch of time (169 in receipt of both). The most frequent treatment administered during the empiric epoch of time was Piperacillin-tazobactam which was administered in 64.6% of patients (n=135). Piperacillin-tazobactam remained the most frequent during the definitive epoch of time, however the proportion in receipt was lower (n=86, 43.9%), with 20.4% (n=40) in receipt of Meropenem and 17.4% (n=34) in receipt of Co-amoxiclav (Table 6.12).

Table 6.12 Empiric and definitive drugs given (Approach 1 – clean population)

Drug	**Empiric (n=209)**	**Definitive (n=196)**
Amoxicillin	2/209 (1%)	12/196 (6.1%)
Co-amoxiclav	28/209 (13.4%)	34/196 (17.4%)
Piperacillin tazobactam	135/209 (64.6%)	86/196 (43.9%)
Cefotaxime	1/209 (0.5%)	0/196 (0%)
Ceftazidime	0/209 (0%)	3/196 (1.5%)
Ertapenem	2/209 (1%)	3/196 (1.5%)
Meropenem	24/209 (11.5%)	40/196 (20.4%)
Gentamicin	5/209 (2.4%)	1/196 (0.5%)
Ciprofloxacin	10/209 (4.8%)	15/196 (7.7%)
Levofloxacin	2/209 (1%)	2/196 (1%)

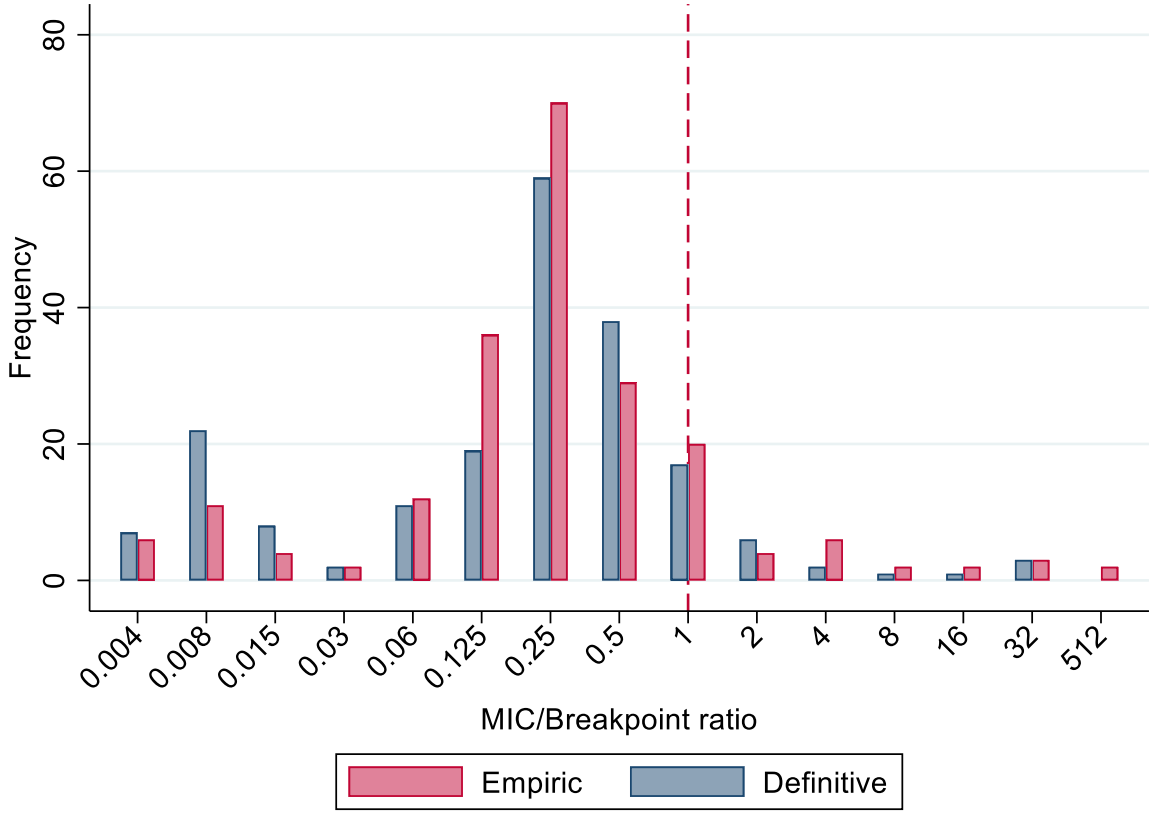
Over a quarter of the 236 patients were in receipt of Piperacillin tazobactam during the empiric and definitive epoch of time, 7.2% changed from Piperacillin tazobactam to Co-amoxiclav and 5.9% changed from Piperacillin tazobactam to Meropenem (Table 6.13).

Table 6.13 Treatment timelines (Approach 1 – clean population)

Empiric therapy		Definitive therapy	n (%)
Amoxicillin (n=2)	→	Amoxicillin	2/236 (0.8%)
Co-amoxiclav (n=28)	→	Co-amoxiclav	17/236 (7.2%)
	→	Piperacillin tazobactam	4/236 (1.7%)
	→	Meropenem	3/236 (1.3%)
	→	No definitive therapy	4/236 (1.7%)
Piperacillin tazobactam (n=135)	→	Amoxicillin	10/236 (4.2%)
	→	Co-amoxiclav	17/236 (7.2%)
	→	Piperacillin tazobactam	65/236 (27.5%)
	→	Ceftazidime	1/236 (0.4%)
	→	Ertapenem	2/236 (0.8%)
	→	Meropenem	14/236 (5.9%)
	→	Ciprofloxacin	4/236 (1.7%)
	→	No definitive therapy	22/236 (9.3%)
Cefotaxime (n=1)	→	Meropenem	1/236 (0.4%)
Ertapenem (n=2)	→	Piperacillin tazobactam	1/236 (0.4%)
	→	Ertapenem	1/236 (0.4%)
Meropenem (n=24)	→	Piperacillin tazobactam	1/236 (0.4%)
	→	Meropenem	15/236 (6.4%)
	→	Levofloxacin	1/236 (0.4%)
	→	No definitive therapy	7/236 (3%)
Gentamicin (n=5)	→	Piperacillin tazobactam	1/236 (0.4%)
	→	Meropenem	1/236 (0.4%)
	→	Gentamicin	1/236 (0.4%)
	→	Ciprofloxacin	1/236 (0.4%)
	→	No definitive therapy	1/236 (0.4%)
Ciprofloxacin (n=10)	→	Meropenem	1/236 (0.4%)
	→	Ciprofloxacin	4/236 (1.7%)
	→	No definitive therapy	5/236 (2.1%)
Levofloxacin (n=2)	→	Levofloxacin	1/236 (0.4%)
	→	No definitive therapy	1/236 (0.4%)
No empiric therapy (n=27)	→	Piperacillin tazobactam	14/236 (5.9%)
	→	Ceftazidime	2/236 (0.8%)
	→	Meropenem	5/236 (2.1%)
	→	Ciprofloxacin	6/236 (2.5%)

The distribution of MIC/EUCAST breakpoint ratio is given by empiric/definitive treatment in Figure 6.3. The distributions are fairly similar for the two epochs of time (empiric/definitive), with a very slightly larger number of patients with lower ratios in the definitive epoch of time.

Figure 6.3 MIC/Breakpoint ratio, by empiric/definitive therapy



Note: *Red dashed line represents MIC = EUCAST breakpoint*

Abbreviations: *MIC=Minimum inhibitory concentration*

6.3.4 Outcome

Approach 1 – "Clean" population

Within the 236 patients who were in receipt of one therapy during each of the empiric and definitive epochs of time, the highest mortality rate was observed in patients treated with an antibiotic with an MIC/breakpoint ratio of ≥4 (45.4%) and the lowest mortality was observed in patients treated with an antibiotic with an MIC/breakpoint ratio of one (5.0%), although it is worth noting the small number of patients in some groups (Table 6.14).

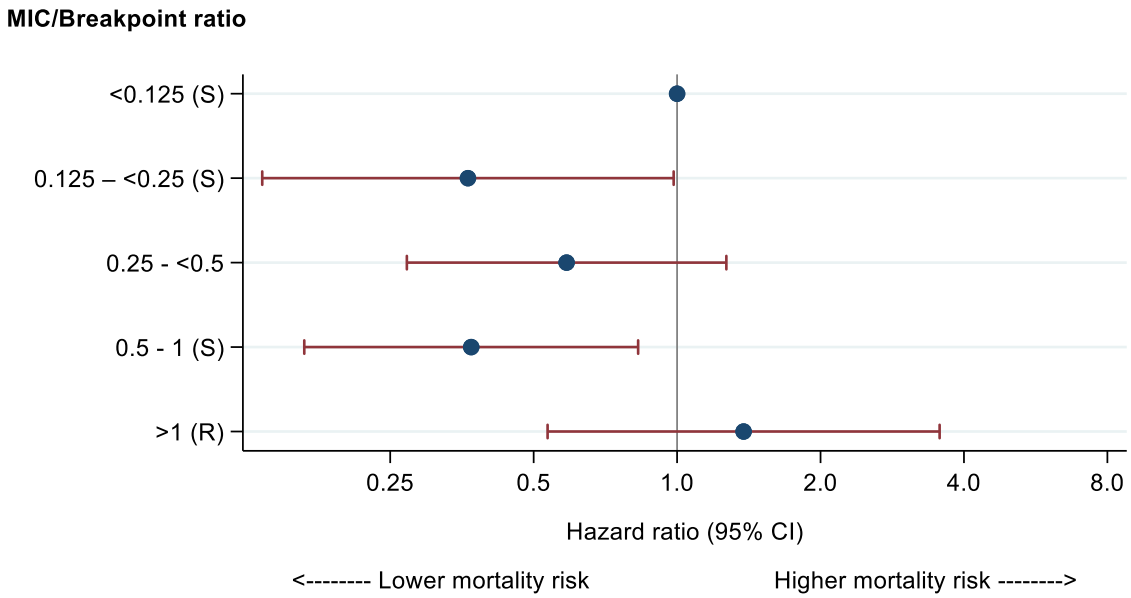
Table 6.14 MIC/breakpoint ratio on last day of follow up, by empiric/definitive therapy and survival status (Approach 1 – clean population)

MIC/breakpoint ratio group	N	Survived n (%)	Died n (%)		
			Overall	During empiric epoch	During definitive epoch
<0.125 (S)	57	40/57 (70.2%)	17/57 (29.8%)	4/57 (7%)	13/57 (22.8%)
0.125 – <0.25 (S)	24	18/24 (75.0%)	6/24 (25.0%)	2/24 (8.3%)	4/24 (16.7%)
0.25 – <0.5 (S)	75	56/75 (74.7%)	19/75 (25.3%)	3/75 (4%)	16/75 (21.3%)
0.5 - <1 (S)	42	30/42 (71.4%)	12/42 (28.6%)	1/42 (2.4%)	11/42 (26.2%)
= 1 (S)	20	19/20 (95.0%)	1/20 (5.0%)	1/20 (5%)	0/20 (0%)
>1- <4 (R)	7	6/7 (85.7%)	1/7 (14.3%)	0/7 (0%)	1/7 (14.3%)
≥4 (R)	11	6/11 (54.6%)	5/11 (45.4%)	2/11 (18.2%)	3/11 (27.3%)
Overall	**236**	**175/236 (74.2%)**	**61/236 (25.8%)**	**13/236 (5.5%)**	**48/236 (20.3%)**

Abbreviations: *MIC=Minimum inhibitory concentration, S= Susceptible, R=Resistant*

Due to small numbers of events (<5) in some MIC/breakpoint groups, the following categories were combined for the analysis model: >1 to <4 and ≥4; 0.5 - <1 and =1. After adjusting for risk score and organism, the final model indicated that MIC/breakpoint ratio was not associated with 28-day mortality, although there was a suggestion of reduced hazard of mortality in patients in receipt of drugs with a higher MIC/breakpoint ratio within the susceptible range, but this did not reach statistical significance (p=0.09). The complete case analysis provided similar but less precise results due to the smaller sample size (Table 6.15).

Figure 6.4 Estimates of association between MIC/breakpoint ratio and 28-day mortality, adjusted for risk score and organism (Approach 1 – clean population)



Note: *The hazard ratios and confidence intervals are given in Table 6.15.*
Abbreviations: *CI=Confidence interval, MIC=Minimum inhibitory concentration*

Table 6.15 Estimates of association between MIC/breakpoint ratio category and 28-day mortality (Approach 1 – clean population)

		Hazard ratio (95% CI)		
MIC/breakpoint ratio group	**n**	**Unadjusted**	**Adjusted***	**Complete case****
<0.125 (S)	57	Ref	Ref	
0.125 – <0.25 (S)	24	0.67 (0.26, 1.71)	0.36 (0.13, 0.98)	0.19 (0.41, 0.90)
0.25 – <0.5 (S)	75	0.77 (0.40, 1.49)	0.59 (0.27, 1.27)	0.65 (0.27, 1.55)
0.5 - 1 (S)	62	0.64 (0.31, 1.31)	0.37 (0.17, 0.83)	0.42 (0.16, 1.07)
>1 (R)	18	1.07 (0.42, 2.73)	1.38 (0.53, 3.56)	2.33 (0.69, 7.82)

** Adjusted for risk score and organism*

*** Complete case analysis, n=164 patients: <0.125 (n=39), 0.125 – <0.25 (n=18), 0.25 – <0.5 (n=53), 0.5 - 1 (n=43), >1 (n=11). Adjusted for risk score and organism.*

Abbreviations: *CI=Confidence interval, MIC=Minimum inhibitory concentration*

Approach 2 – Full population

Similarly, when the MIC/breakpoint ratio was updated daily using a cumulative average, the highest mortality rate was observed in patients treated with antibiotics with an average MIC/breakpoint ratio of ≥4 (50.0%) and the lowest mortality was observed in patients treated with antibiotics with an average MIC/breakpoint ratio of one (11.1%), see Table 6.16.

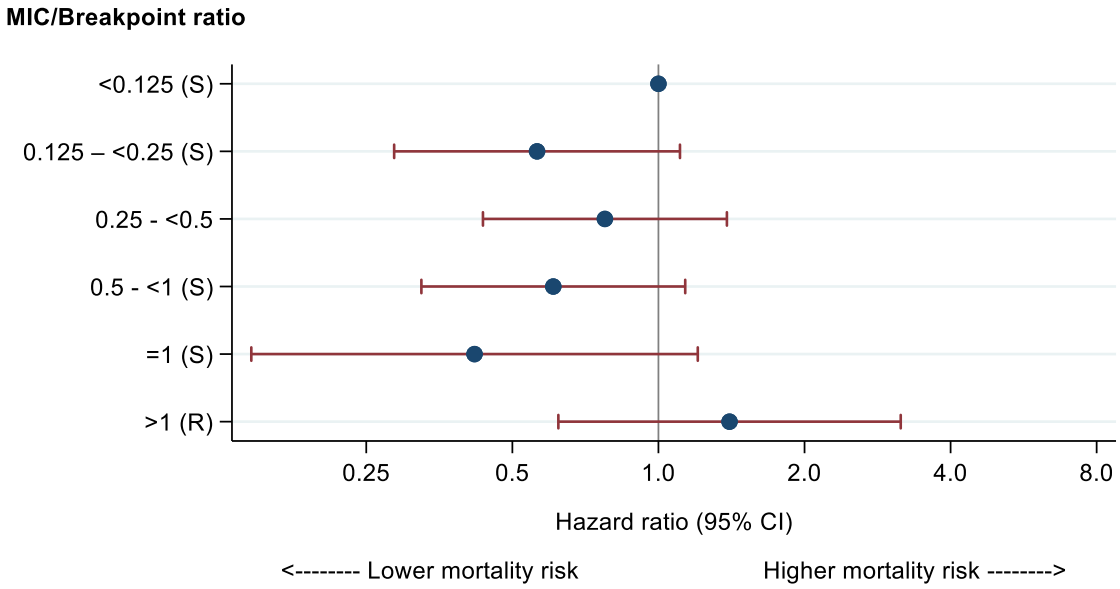
Table 6.16 Cumulative average MIC/breakpoint ratio on last day of follow up, by empiric/definitive therapy and survival status (Approach 2 – full population)

MIC/breakpoint ratio group	N	Survived n (%)	Died n (%)		
			Overall	During empiric epoch	During definitive epoch
<0.125 (S)	121	95/121 (78.5%)	26/121 (21.5%)	5/121 (4.1%)	21/121 (17.4%)
0.125 – <0.25 (S)	98	84/98 (85.7%)	14/98 (14.3%)	3/98 (3.1%)	11/98 (11.2%)
0.25 – <0.5 (S)	140	111/140 (79.3%)	29/140 (20.7%)	4/140 (2.9%)	25/140 (17.9%)
0.5 - <1 (S)	90	71 /90 (78.9%)	19/90 (21.1%)	2/90 (2.2%)	17/90 (18.9%)
= 1 (S)	36	32/36 (88.9%)	4/36 (11.1%)	1/36 (2.8%)	3/36 (8.3%)
>1- <4 (R)	21	17/21 (80.9%)	4/21 (19.1%)	0/21 (0%)	4/21 (19%)
≥4 (R)	8	4/8 (50.0%)	4/8 (50.0%)	3/8 (37.5%)	1/8 (12.5%)
Overall	**514**	**414/514 (80.5%)**	**100/514 (19.5%)**	**18/514 (3.5%)**	**82/514 (16%)**

Abbreviations: *MIC=Minimum inhibitory concentration, S= Susceptible, R=Resistant*

Due to a larger number of patients in this population, only the two resistant categories were combined for the analysis (>1 - <4 and ≥4). Again, after adjusting for risk score and organism, MIC/breakpoint ratio was not associated with 28-day mortality with consistent results in the complete case analysis (Figure 6.5 and Table 6.17).

Figure 6.5 Estimates of association between MIC/breakpoint ratio and 28-day mortality, adjusted for risk score and organism (Approach 2 – full population)



Note: *The hazard ratios and confidence intervals are given in Table 6.17*
Abbreviations: *CI=Confidence interval, MIC=Minimum inhibitory concentration*

Table 6.17 Estimates of association between MIC/breakpoint ratio category and 28-day mortality (Approach 2 – full population)

		Hazard ratio (95% CI)		
MIC/breakpoint ratio group	**n**	**Unadjusted**	**Adjusted***	**Complete case****
<0.125 (S)	121	Ref	Ref	Ref
0.125 – <0.25 (S)	98	0.58 (0.30, 1.11)	0.56 (0.29, 1.11)	0.42 (0.17, 1.04)
0.25 – <0.5 (S)	140	0.85 (0.50, 1.45)	0.78 (0.43, 1.38)	0.77 (0.40, 1.49)
0.5 - <1 (S)	90	0.87 (0.48, 1.57)	0.61 (0.32, 1.14)	0.65 (0.32, 1.34)
=1 (S)	36	0.43 (0.15, 1.22)	0.42 (0.14, 1.21)	0.57 (0.19, 1.69)
>1 (R)	29	1.23 (0.58, 2.83)	1.40 (0.62, 3.16)	1.62 (0.54, 4.88)

** Adjusted for risk score and organism*

*** Complete case analysis, n=375 patients: <0.125 (n=94), 0.125 – <0.25 (n=68), 0.25 – <0.5 (n=106), 0.5 - <1 (n=61), =1 (n=28), >1 (n=18). Adjusted for risk score and organism.*

***Abbreviations:** CI=Confidence interval, MIC=Minimum inhibitory concentration*

These results were not in line with our hypothesis that patients in receipt of treatments with a lower MIC/breakpoint ratio have better outcome than patients with a larger MIC/breakpoint ratio (nearer to the breakpoint). This may be because the breakpoint is set correctly (mortality is higher only in the group with an MIC above the breakpoint), or it may be explained by a "ceiling" effect, that is antibiotics that are active against the organism are sufficient to kill the organism regardless of the level of susceptibility. To explore this further, I performed the analysis with broader categories to compare those that are very susceptible to the grey area around the breakpoint and those that are resistant. These were defined as Susceptible: MIC/breakpoint ratio <0.5; Borderline: MIC/breakpoint ratio 0.5-1; and Resistant: MIC/breakpoint ratio >1. The results were consistent with the primary analysis, with similar mortality in the borderline and susceptible MIC/breakpoint ratio groups (18.3% and 19.2% respectively) and highest mortality in the resistant group (27.6%). The results are presented in Table 6.18 and Figure 6.6.

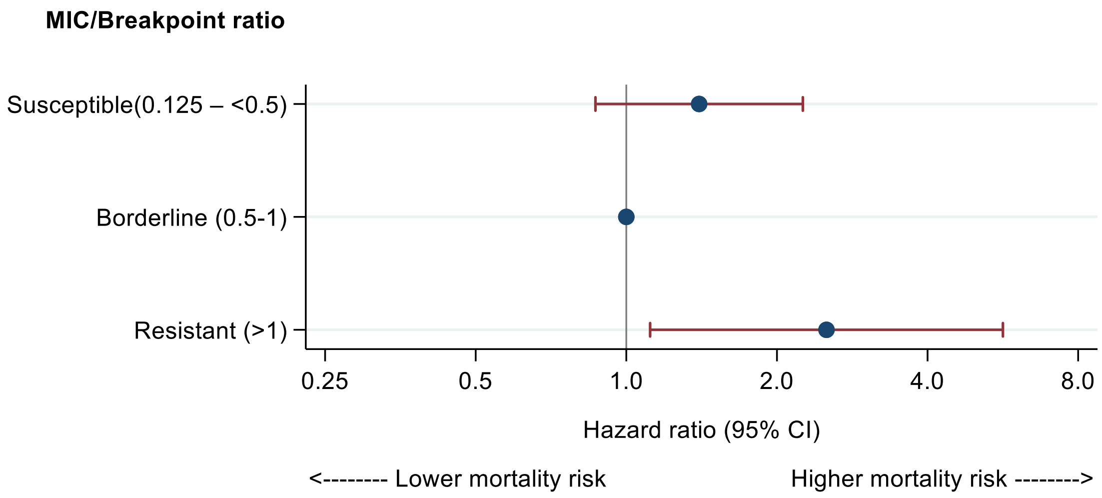
Table 6.18 Estimates of association between MIC/breakpoint ratio category (susceptible-borderline-resistant) and 28-day mortality (Approach 2 – full population)

MIC/breakpoint ratio group	**Died n (%)**	**Hazard ratio (95% CI)***
0.125 – <1 (Susceptible)	69/359 (19.2%)	1.40 (0.87, 2.25)
0.5 - 1 (Borderline)	23/126 (18.3%)	Ref
>1 (Resistant)	8/29 (27.6%)	2.51 (1.12, 5.67)

** Adjusted for risk score and organism*

***Abbreviations:** CI=Confidence interval, MIC=Minimum inhibitory concentration*

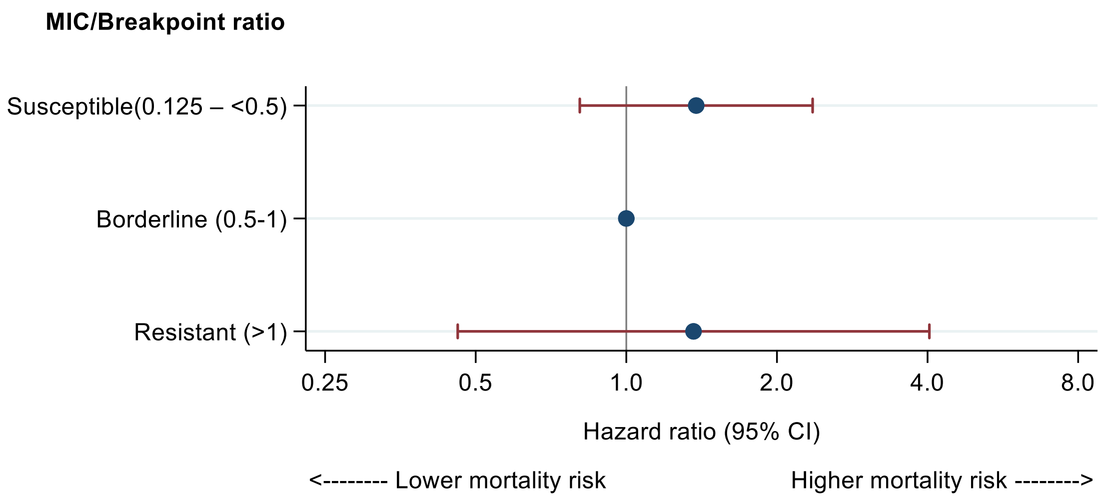
Figure 6.6 Estimates of association between MIC/breakpoint ratio category (susceptible-borderline-resistant) and 28-day mortality (Approach 2 – full population)



Abbreviations: *CI=Confidence interval, MIC=Minimum inhibitory concentration*

This analysis was repeated for treatments administered during the empiric epoch only (first 48 hours post blood culture) ignoring any effect of definitive treatment, i.e. the updated covariate stops updating at day 2 and remains constant, with similar results (Figure 6.7).

Figure 6.7 Estimates of association between MIC/breakpoint ratio category (susceptible-borderline-resistant) during first 48hrs post blood culture, and 28-day mortality (Approach 2 – full population)



Abbreviations: *CI=Confidence interval, MIC=Minimum inhibitory concentration*

The proportional hazards assumption was assessed and met in all models.

6.4 Discussion

6.4.1 Summary

In this analysis, there was no evidence to suggest a relationship between MIC/EUCAST breakpoint ratio and 28-day mortality in patients with a Gram-negative BSI. The lack of relationship was maintained in an adjusted model controlling for a risk score that was calculated using potential confounding variables, with missing data of elements of the risk score imputed. This was complimented with a complete case analysis.

6.4.2 Interpretation

Although there have been a number of studies exploring the association between MIC and outcome, to date these have focussed on the MIC of a particular drug in question. In this analysis I attempted to investigate whether there was a general effect of MIC by exploring the MIC/EUCAST breakpoint ratio. I did not find an association between MIC/breakpoint ratio and 28-day mortality in patients with a Gram-negative BSI, although I did observe an increase in hazard of mortality in patients with a strain with an MIC/breakpoint ratio >1 which is consistent with data showing that receipt of appropriate therapy improves outcome. There are a number of reasons that may explain the lack of relationship; 1) the study was underpowered, 2) if an organism is susceptible to a drug, the drug works in terms of reducing mortality regardless of the level of susceptibility, 3) the population is too heterogenous with multiple drugs administered during follow-up, distorting any relationship. We attempted to overcome this by using a cumulative average MIC/breakpoint ratio which was updated daily.

A study investigating the impact of piperacillin-tazobactam MIC on 30-day mortality in patients with bacteraemia caused by Enterobacteriaceae found no associations, with a reported 30-day mortality of 10.5% in the low MIC group and 11.1% in the borderline MIC group[217]. Similarly, in a propensity score matched cohort of patients with *P. aeruginosa*, 30-day mortality was not statistically significant different between patients with low and high cefepime or ceftazidime MIC[214]. However, a recent individual patient data meta-analysis including 115 patients with Enterobacteriaceae BSI treated with a carbapenem found for each increase in meropenem MIC was associated with an increase 30-day mortality[202]. The conflicting results in the literature may be down to the heterogeneity of the populations being studied, such as different bugs and also due to the drug in question, e.g. the MIC of some drugs may be related to mortality but not others. Most patients in this analysis were treated with Piperacillin-tazobactam where the MIC has been shown to be unrelated to mortality in other studies[213, 217]. Due to the observational nature of the data source

used in this analysis, most patients were in receipt of more than one treatment during follow-up so it was not feasible to include an interaction term in the analysis to estimate the effect of specific MIC per drug, meaning a direct comparison to these studies was not possible.

6.4.3 Strengths and limitations

A strength of this analysis is the use of a MIC/breakpoint ratio enabling the MIC of a broad range of drugs to be included. This meant the analysis population did not have to be restricted to patients in receipt of a particular drug allowing inclusion of a larger sample size and improving generalisability of results. Start and end dates were also collected for each prescribed drug which enabled me to estimate a cumulative exposure of MIC. Another strength of this analysis is that the MIC testing was performed centrally using the same methodology for all isolates rather than depending on each hospitals recording which could vary between sites. There are a number of limitations to this analysis. First, as it was observational there is a risk of unmeasured confounding effects. I attempted to adjust for confounding by adjusting for a risk score calculated using baseline variables associated with mortality, full details in Chapter 3. Within the imputation procedure for the elements of the risk score, I had intended to include variables that are in the main analysis model. This involved adding MIC to the imputation procedure for this analysis, however convergence problems prevented this. The imputation procedure was therefore not modified to include MIC and the risk score remained the same as the risk score used in Chapter 3. Even after adjustment for risk score, there is still chance that residual confounding remains. For example, clinicians may prioritise/perform more regular reviews of patients who are treated with a drug with a high MIC due to the perception that they may be less likely to respond, and more regular reviews may lead to a lower risk of mortality. This was not measured and could bias the results, however I considered this to be unlikely in this dataset as MIC values themselves are not widely used to aid choice of antibiotic within the NHS, rather the broader classification of susceptibility i.e. S-I-R are used. In addition, potential confounding variables attributable to differential antibiotic exposure can be difficult to measure as they can be based on clinician's personal care preferences.

As well as residual confounding, there is also the possibility of measurement error within the measurement of MIC. Methods of measuring MIC can sometimes be inaccurate which could have led to misclassification of the exposure. However, as mentioned above, the MIC testing was performed centrally using the same methodology for all isolates in this study, maximising within study consistency.

Another limitation is that MIC was only collected for a subset of participants. The subset was shown to be similar in terms of baseline demography and comorbidities so I feel the results are

generalisable to the wider population, however, the resultant smaller sample size limited the comparisons that could be made, and the low number of events in some of the MIC/breakpoint categories resulted in low statistical power to detect differences between groups, particularly within the MIC categories around the breakpoint. In addition, I had planned to perform a subgroup analysis by organism and separately by empiric/definitive epochs of time, however the low number of events within the subgroups made this not feasible. In addition, the classification of empiric and definitive therapy was not recorded in BSI-FOO, and the time susceptibility results became available was also not recorded, I therefore decided to use an algorithm to define empirical therapy based on time from blood culture to start of treatment. This again may lead to miss-classification, however the algorithm used matched a clinicians "best guess" for approximately 75% of drugs administered in a subset of 75 patients examined.

Finally, I have only explored one outcome in this analysis (28-day mortality). Although we failed to show a relationship with mortality, other studies have found a relationship with treatment failure. This outcome was not measured in BSI-FOO but may be relevant for future studies.

6.4.4 Conclusions

It is unlikely that an RCT addressing this research question will take place since it is not possible to randomise participants to a specific MIC, therefore observational studies with a protocol designed to answer this question are needed to confirm the findings.

CHAPTER 7 SUMMARY OF FINDINGS AND FUTURE WORK

A detailed discussion of each topic is given within the respective chapters. In this chapter, I will summarise the main findings and implications from the research presented in this thesis as a whole. I will also discuss the overall strengths and limitations and areas for future research.

7.1 Summary of main findings

7.1.1 Key findings

In this thesis, I have used data from the BSI-FOO research programme to explore the impact of several modifiable risk factors for mortality in patients with a bloodstream infection. I specifically focussed on: (i) building a multivariable model of healthcare setting related factors, (ii) applying statistical methodology that accounts for immortal time bias to assess the effect of duration of therapy on mortality in patients with *S. aureus* BSI, (iii) using trial emulation methods to compare the results of the MERINO trial to published observational studies comparing treatments for ESBL-producing bacteraemia, and (iv) exploring the association between MIC to EUCAST breakpoint ratio and mortality in patients with a gram-negative BSI.

The main findings from this research are:

- In terms of healthcare setting related risk factors, I found that increased ward activity (admissions and discharges) was associated with increased hazard of death within medical wards and especially in critical care wards.
- Timely appropriate antimicrobial therapy was associated with reduced mortality over 28 days, and the effect of each day of delay was most marked in the first seven days.
- After accounting for immortal time bias, I found that duration of therapy ≥19 days was beneficial for the treatment of *S. aureus* BSI and I did not find any evidence to suggest reducing the duration to less than 18 days was safe.
- Using trial emulation methods, I found that the mortality rate in an emulated trial population was more than double the mortality rate in the MERINO trial. A smaller treatment effect was observed but it was in the same direction as the MERINO trial (favouring meropenem). Discrepancies between the MERINO trial and observational studies could be partly explained by differences in populations and from bias that arises in observational studies, such as immortal time bias and uncontrolled confounding.
- Finally, I did not find any evidence to suggest that MIC to EUCAST breakpoint ratio was associated with 28-day mortality in patients with a *E. coli* or *P. aeruginosa.*

7.1.2 Interpretation of findings

There are various non-modifiable patient factors that are known to be related to mortality. The literature review of reviews presented in Chapter 1 showed that age, comorbidities, severe sepsis, source of infection, neutropenia, type of infection and intensive care admission are known to be related with mortality. However, this review highlighted the need for research in modifiable risk factors – those that could be modified by changes in organisation or patient management - in this patient population. Such research could be used to inform potential intervention efforts, antimicrobial stewardship programmes and NHS guidelines.

In Chapter 3 I was able to relate a number of healthcare setting related risk factors to 28-day mortality, for the first time in this patient population. The results of this analysis highlighted that ward speciality, ward activity, ward movement within speciality, movements from critical care, and time to receipt of appropriate antibiotics were all risk factors associated with mortality within 28 days. In an adjusted model where ward activity was updated daily to reflect the ward activity where the patient spent most of the day, I found that increased ward activity was associated with an increased hazard of death within 28 days. A delay in starting effective antimicrobial therapy has previously been shown to be associated with higher mortality, and recent systematic reviews and meta-analyses have indicated that appropriate antimicrobial therapy has survival benefit[98, 116]. Similarly, our data shows that delays in administration of appropriate antimicrobials impact on mortality in BSI over days 0–6. Patients who received prompt appropriate antimicrobial therapy were less likely to die in the first week, and those patients who did survive the first week had apparently similar survival prospects over the next three weeks with or without the benefit of previous early appropriate therapy.

In addition to time to receipt of appropriate therapy, duration of therapy has also been shown to have an impact and shortening the duration of treatment could lower the risk of adverse effects of treatment and reduce the risk of antibiotic resistance development. I used a three-step procedure, cloning, censoring and weighting, proposed by Hernán to compare three treatment strategies of duration of active therapy: short therapy defined as <10 days, intermediate therapy defined as 10 – 18 days and long therapy defined as ≥19 days. After cloning, the weighted estimates of hazard of all-cause mortality for short therapy *vs* long therapy (reference category) was 1.74 (95% CI 1.36, 2.24) and for intermediate *vs* long therapy (reference category) was 1.09 (95% CI 0.98, 1.22), indicating that a long treatment duration reduces the risk of death compared to a short duration. The effect of short *vs* long and intermediate *vs* long therapy was strongest in the infections defined as complicated with an estimated hazard ratio of 3.04 (95% CI 1.32, 7.00) and 1.25 (95% CI 0.87, 1.79)

respectively compared to 1.70 (95% CI 1.26, 2.29) and 1.08 (95% CI 0.95, 1.22) in infections defined as non-complicated. This suggests that longer treatment is beneficial.

As well as timing and duration, choice of antibiotic may be of importance. I therefore looked at the choice of treatment for gram-negative BSI using trial emulation methods to compare the MERINO trial, a recent RCT which failed to demonstrate non-inferiority of Piperacillin-Tazobactam to Meropenem. However, a number of observational studies have shown that BLBLIs are an effective treatment for infections caused by ESBL producers. My findings suggest that the discrepancies between the MERINO trial and estimates from observational studies could be partly explained by differences in the sample population and also due to biases that arise from observational studies, e.g. survival bias and bias from unmeasured or uncontrolled confounding. The confidence intervals surrounding the estimates from my analysis are compatible with both lower and higher mortality in patients treated with Piperacillin-Tazobactam compared to Meropenem so further clinical trials with adequate power and refined eligibility criteria are required to determine efficacy due to the uncertainty around our estimates.

I also explored the association between MIC and mortality to investigate whether MIC would provide beneficial information in treatment choices, however I was not able to identify an association between MIC/EUCAST breakpoint and 28-day mortality.

7.1.3 Implication of findings

My research focused on patients with clinically significant pathogens that produce large numbers of infections that may be resistant to multiple drug treatments. The selected pathogens were chosen as they were highly unlikely to be contaminants but were also common causes of BSI, linked with significant mortality and remain a significant problem across the NHS. These findings can provide information on the management of BSI which may influence policy or recommendation in the NHS. For example, providing information for a BSI care bundle to be developed for the NHS. A care bundle could include recommended processes to limit ward activity as increases in admissions and discharges was shown to be associated with an increase in mortality.

7.2 Strengths and limitations

Strengths and limitations relevant to each topic are given in sections 3.5.3, 4.4.3, 5.4.3 and 6.4.3 respectively. I provide a summary of strengths and limitations of the analyses as a whole below.

BSI-FOO is one of the largest observational cohort studies of patients with BSI in the NHS which meant I was able to include a large number of patients in the analysis reducing the risk of

underpowered conclusions. A key strength of the research is that the observational study did not require individual patient consent therefore reducing the risk of selection bias. For example, less acutely ill patients may be more likely to be approached and provide consent which could undermine the scientific integrity and public value of the research. The National Information Governance Board approved the use of such routinely collected data without individual patient consent under section 251 of the NHS Act 2006.

Focussing on six key pathogens may limit the generalisability of the results. The pathogens were specifically focused on as they were common causes of BSI, linked with significant mortality and remain a significant problem across the NHS. Results may not be generalisable to other bloodstream infections and therefore conclusions drawn should be limited to the six key pathogens studied.

Another limitation is that the data collection was not designed specifically for these analyses but for a different study protocol which limited the data available, e.g. for the duration of therapy analysis there is the risk that unmeasured confounders such as CRP, which was not collected, may impact the results. For analyses which included both the BSI-FOO observational study and the RAPIDO trial, I could also only adjust for variables that were collected in both studies (please refer to chapters for relevant variables). It is important to consider severity of illness; I was able to partly control for this by adjusting for temperature and systolic blood pressure in most analyses, however more commonly used measures in the UK are national early warning score (NEWS). Unfortunately, at the time of the study this had only recently been introduced in the NHS, so I did not have reliable enough data to include.

I only looked at one outcome in these analyses – all-cause mortality within 28 days of blood culture. Other outcomes such as relapse of infection, complication rate and longer-term outcomes such as 90-day mortality may also be of importance, however such data were not collected in BSI-FOO so it was not possible to investigate these. Infection-related mortality may be considered a more appropriate outcome, however it was not possible to define this clearly enough to distinguish robustly from other causes of death as cause of death was not collected in BSI-FOO.

Finally, the research is limited to a single data source from a research programme that was conducted over 10 years ago. Since that time, the rate of MRSA bacteraemia has declined, however ESBL-producing *E. coli*, *P. aeruginosa* and MRSA BSI remain common and relevant in the NHS and I do not feel that the age of the dataset has an impact on the validity of the results. Performing further analysis using other data sources would provide external validity.

7.3 Future research

As mentioned previously, my research has focussed on short term outcomes, i.e. mortality within 28 days. However, longer term outcomes are also of importance, particularly in relation to duration of therapy. Future work could include obtaining the relevant approvals and running a mortality trace for this group of patients to investigate longer term outcomes, which could include cause of death as well as vital status. However, patient identifiable information was not made available to me so it is not possible to link to routine data to pick up subsequent hospital admissions. Another outcome which would be interesting to explore in future work is relapse of infection. Relapse of infection/repeat episodes were only recorded if the patient was re-screened for participation in the study, so it was not possible to reliably explore this outcome within the current dataset. Finally, C. difficile is an important outcome for treatment related risk factors, however it was not feasible to explore the impact in this dataset as only 5/587 (0.9%) of the duration of therapy analysis population developed C. difficile. Controlling the source of infection is also an important modifiable risk factor that could be explored using the BSI-FOO dataset, however this was beyond the scope of this thesis. It is also important to include patient and public involvement (PPI) in research. I attended a PPI group who focus on BSI as a guest and I had planned to present and discuss the findings from this research once complete, however due to the coronavirus pandemic it was not possible for me to return and present the results at this time. I plan to re-engage with the group and hope to present some of the results in the near future, possibly in an on-line meeting

7.4 Final conclusions

In conclusion, for all the pathogen groups studied, timely appropriate antimicrobial therapy was associated with improved clinical outcome as measured by mortality over 28 days, and the effect was most marked in the first seven days. Increased ward activity (admissions and discharges) was associated with worse outcomes (increased hazard of death) within medical wards and especially in critical care wards. Implementation of a novel approach to address the bias introduced by immortal time suggests that longer treatment (≥19 days) is beneficial. Further research is needed to validate the results and explore other outcomes including longer term mortality.

REFERENCES

1. PublicHealthEngland. Laboratory surveillance of polymicrobial bacteraemia and fungaemia in England, Wales and Northern Ireland: 2016. 2017.

2. Plowman R, Graves N, Griffin M, Roberts JA, Swan AV, Cookson B, et al. The socio-economic burden of hospital acquired infection. Public Health Laboratory Service,; 1999.

3. Public Health England. Annual Epidemiological Commentary: Mandatory MRSA, MSSA and *E. coli* bacteraemia and *C. difficile* infection data 2016/17. London, England2017. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/535635/AEC_final.pdf.

4. PublicHealthEngland. Laboratory surveillance of Pseudomonas and Stenotrophomonas spp bacteraemia in England, Wales and Northern Ireland: 2017. 2018.

5. PublicHealthEngland. Laboratory surveillance of candidaemia in England, Wales and Northern Ireland: 2018. 2019.

6. Lillie PJ, Allen J, Hall C, Walsh C, Adams K, Thaker H, et al. Long-term mortality following bloodstream infection. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2013;19(10):955-60.

7. Hounsom L, Grayson K, Melzer M. Mortality and associated risk factors in consecutive patients admitted to a UK NHS trust with community acquired bacteraemia. Postgraduate medical journal. 2011;87(1033):757-62.

8. Kao CH, Kuo YC, Chen CC, Chang YT, Chen YS, Wann SR, et al. Isolated pathogens and clinical outcomes of adult bacteremia in the emergency department: a retrospective study in a tertiary Referral Center. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 2011;44(3):215-21.

9. Griffiths P, Maruotti A, Recio Saucedo A, Redfern OC, Ball JE, Briggs J, et al. Nurse staffing, nursing assistants and hospital mortality: retrospective longitudinal cohort study. BMJ Quality & Safety. 2019;28(8):609.

10. Griffiths P, Ball J, Drennan J, Dall'Ora C, Jones J, Maruotti A, et al. Nurse staffing and patient outcomes: Strengths and limitations of the evidence to inform policy and practice. A review and discussion paper based on evidence reviewed for the National Institute for Health and Care Excellence Safe Staffing guideline development. International Journal of Nursing Studies. 2016;63:213-25.

11. Aiken LH, Clarke SP, Sloane DM, Sochalski J, Silber JH. Hospital nurse staffing and patient mortality, nurse burnout, and job dissatisfaction. Jama. 2002;288(16):1987-93.

12. National Institute for Health and Clinical Excellence. Healthcare-associated infections: prevention and control in primary and community care [updated February 2017] 2012.

13. Health Protection Agency. English National Point Prevalence Survey on Healthcare-associated Infections and Antimicrobial Use, 2011: preliminary data. London: Health Protection Agency,; 2012.

14. Health Protection Agency. Report for the National Audit Office: Trends in Rates of Healthcare Associated Infection in England 2004 to 2008.; 2009.

15. Public Health England. Annual epidemiological commentary: Gram-negative bacteraemia, MRSA bacteraemia, MSSA bacteraemia and C. difficile infections, up to and including financial year April 2019 to March 2020. London, England; 2020 03 December 2020.

16. National Institute for Health and Care Excellence. Urinary-tract infections: NICE; 2019 [14/08/2019]. Available from: https://bnf.nice.org.uk/treatment-summary/urinary-tract-infections.html.

17. Rottier WC, Ammerlaan HS, Bonten MJ. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum beta-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. The Journal of antimicrobial chemotherapy. 2012;67(6):1311-20.

18. Tumbarello M, Sanguinetti M, Montuori E, Trecarichi EM, Posteraro B, Fiori B, et al. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-beta-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. Antimicrob Agents Chemother. 2007;51(6):1987-94.

19. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. The Journal of antimicrobial chemotherapy. 2007;60(5):913-20.

20. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. Pseudomonas aeruginosa Bloodstream Infection: Importance of Appropriate Initial Antimicrobial Treatment. Antimicrobial Agents and Chemotherapy. 2005;49(4):1306-11.

21. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage Pseudomonas aeruginosa infections. Drugs in context. 2018;7:212527.

22. Rayner C, Munckhof WJ. Antibiotics currently used in the treatment of infections caused by Staphylococcus aureus. Intern Med J. 2005;35 Suppl 2:S3-16.

23. Nathwani D, Morgan M, Masterton R, Dryden M, Cookson B, French G, et al. Guidelines for UK practice for the diagnosis and management of methicillin-resistant Staphylococcus aureus (MRSA) infections presenting in the community. The Journal of antimicrobial chemotherapy. 2008;61:976-94.

24. Keighley C, Chen SC, Marriott D, Pope A, Chapman B, Kennedy K, et al. Candidaemia and a risk predictive model for overall mortality: a prospective multicentre study. BMC Infect Dis. 2019;19(1):445.

25. Weinstein RA, Fridkin SK. The Changing Face of Fungal Infections in Health Care Settings. Clinical Infectious Diseases. 2005;41(10):1455-60.

26. Cornely OA, Bassetti M, Calandra T, Garbino J, Kullberg BJ, Lortholary O, et al. ESCMID* guideline for the diagnosis and management of Candida diseases 2012: non-neutropenic adult patients. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012;18 Suppl 7:19-37.

27. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clinical Infectious Diseases. 2015;62(4):e1-e50.

28. Public Health England. Start Smart - Then Focus: Antimicrobial Stewardship Toolkit for English Hospitals London, England2015. Available from: https://www.gov.uk/government/publications/antimicrobial-stewardship-start-smart-then-focus.

29. World Health Organisation. Ten Threats to Global Health in 2019 2019 [Available from: https://www.who.int/news-room/feature-stories/ten-threats-to-global-health-in-2019.

30. SC D. Annual Report of the Chief Medical Officer. Infections and the rise of antimicrobial resistance. . London: Department of Health; 2013.

31. Macgowan A, Albur M. Frontline antibiotic therapy. Clinical medicine (London, England). 2013;13:263-8.

32. Davey P, Sneddon J, Nathwani D. Overview of strategies for overcoming the challenge of antimicrobial resistance. Expert review of clinical pharmacology. 2010;3(5):667-86.

33. Scottish Antimicrobial Prescribing Group, Scottish Medicines Consortium. 2008 [Available from: https://www.sapg.scot/.

34. Johnson AP, Ashiru-Oredope D, Beech E. Antibiotic Stewardship Initiatives as Part of the UK 5-Year Antimicrobial Resistance Strategy. Antibiotics (Basel, Switzerland). 2015;4(4):467-79.

35. Nathwani D, Sneddon J, Patton A, Malcolm W. Antimicrobial stewardship in Scotland: impact of a national programme. Antimicrobial Resistance and Infection Control. 2012;1(1):7.

36. Nathwani D, Sneddon J, Malcolm W, Wiuff C, Patton A, Hurding S, et al. Scottish Antimicrobial Prescribing Group (SAPG): development and impact of the Scottish National Antimicrobial Stewardship Programme. Int J Antimicrob Agents. 2011;38(1):16-26.

37. EUCAST. 2020 [cited 2021. Available from: https://www.eucast.org/clinical_breakpoints/.

38. Laupland KB, Gregson DB, Church DL, Ross T, Pitout JDD. Incidence, risk factors and outcomes of Escherichia coli bloodstream infections in a large Canadian region. Clinical Microbiology and Infection. 2008;14(11):1041-7.

39. Ortega M, Marco F, Soriano A, Almela M, Martínez JA, Muñoz A, et al. Analysis of 4758 Escherichia coli bacteraemia episodes: predictive factors for isolation of an antibiotic-resistant strain and their impact on the outcome. Journal of Antimicrobial Chemotherapy. 2009;63(3):568-74.

40. Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Ruíz M, Peña C, et al. Community-Onset Bacteremia Due to Extended-Spectrum β-Lactamase-Producing Escherichia coli: Risk Factors and Prognosis. Clinical Infectious Diseases. 2010;50(1):40-8.

41. Aliaga L, Mediavilla JD, Cobo F. A clinical index predicting mortality with *Pseudomonas aeruginosa* bacteraemia. Journal of Medical Microbiology. 2002;51(7):615-701.

42. van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of Mortality in Staphylococcus aureus Bacteremia. 2012;25(2):362-86.

43. Yahav D, Yassin S, Shaked H, Goldberg E, Bishara J, Paul M, et al. Risk factors for long-term mortality of Staphylococcus aureus bacteremia. 2016;35(5):785-90.

44. Colombo AL, Guimarães T, Sukienik T, Pasqualotto AC, Andreotti R, Queiroz-Telles F, et al. Prognostic factors and historical trends in the epidemiology of candidemia in critically ill patients: an analysis of five multicenter studies sequentially conducted over a 9-year period. 2014;40(10):1489-98.

45. Tang H-J, Liu W-L, Lin H-L, Lai C-C. Epidemiology and Prognostic Factors of Candidemia in Cancer Patients. PLOS ONE. 2014;9(6):e99103.

46. Thwaites GE, United Kingdom Clinical Infection Research G. The Management of Staphylococcus aureus Bacteremia in the United Kingdom and Vietnam: A Multi-Centre Evaluation. PLOS ONE. 2010;5(12):e14170.

47. Asgeirsson H, Thalme A, Weiland O. Staphylococcus aureus bacteraemia and endocarditis - epidemiology and outcome: a review. Infectious diseases (London, England). 2018;50(3):175-92.

48. Hassoun A, Linden PK, Friedman B. Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. Critical care (London, England). 2017;21(1):211.

49. Chatelier E, Mahieu R, Hamel JF, Chenouard R, Lozac'h P, Sallé A, et al. Pasteurella bacteraemia: Impact of comorbidities on outcome, based on a case series and literature review. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2020;92:89-96.

50. Jung N, Rieg S. Essentials in the management of S. aureus bloodstream infection. Infection. 2018;46(4):441-2.

51. Goodman AL, Cook GJ, Goh V. Imaging in the investigation and management of Staphylococcus aureus bacteraemia: a role for advanced imaging techniques. The Journal of hospital infection. 2020;105(2):234-41.

52. Mandolfo S, Anesi A, Maggio M, Rognoni V, Galli F, Forneris G. High success rate in salvage of catheter-related bloodstream infections due to Staphylococcus aureus, on behalf of project group of Italian society of nephrology. The journal of vascular access. 2020;21(3):336-41.

53. Kern WV, Rieg S. Burden of bacterial bloodstream infection-a brief update on epidemiology and significance of multidrug-resistant pathogens. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2020;26(2):151-7.

54. Levene I, Castagnola E, Haeusler GM. Antibiotic-resistant Gram-negative Blood Stream Infections in Children With Cancer: A Review of Epidemiology, Risk Factors, and Outcome. The Pediatric infectious disease journal. 2018;37(5):495-8.

55. Battaglia CC, Hale K. Hospital-Acquired Infections in Critically Ill Patients With Cancer. Journal of Intensive Care Medicine. 2018;34(7):523-36.

56. Kohler PP, Volling C, Green K, Uleryk EM, Shah PS, McGeer A. Carbapenem Resistance, Initial Antibiotic Therapy, and Mortality in Klebsiella pneumoniae Bacteremia: A Systematic Review and Meta-Analysis. Infection Control & Hospital Epidemiology. 2017;38(11):1319-28.

57. Righi E, Peri AM, Harris PN, Wailan AM, Liborio M, Lane SW, et al. Global prevalence of carbapenem resistance in neutropenic patients and association with mortality and carbapenem use: systematic review and meta-analysis. The Journal of antimicrobial chemotherapy. 2017;72(3):668-77.

58. Carrara E, Pfeffer I, Zusman O, Leibovici L, Paul M. Determinants of inappropriate empirical antibiotic treatment: systematic review and meta-analysis. International Journal of Antimicrobial Agents. 2018;51(4):548-53.

59. Phua AI, Hon KY, Holt A, O'Callaghan M, Bihari S. Candida catheter-related bloodstream infection in patients on home parenteral nutrition - Rates, risk factors, outcomes, and management. Clinical nutrition ESPEN. 2019;31:1-9.

60. Lewis PO, Heil EL, Covert KL, Cluck DB. Treatment strategies for persistent methicillin-resistant Staphylococcus aureus bacteraemia. Journal of clinical pharmacy and therapeutics. 2018;43(5):614-25.

61. Wang J, Pan Y, Shen J, Xu Y. The efficacy and safety of tigecycline for the treatment of bloodstream infections: a systematic review and meta-analysis. Annals of clinical microbiology and antimicrobials. 2017;16(1):24.

62. White BP, Barber KE, Stover KR. Ceftaroline for the treatment of methicillin-resistant Staphylococcus aureus bacteremia. American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists. 2017;74(4):201-8.

63. Ortwine JK, Bhavan K. Morbidity, mortality, and management of methicillin-resistant S. aureus bacteremia in the USA: update on antibacterial choices and understanding. Hospital practice (1995). 2018;46(2):64-72.

64. Vardakas KZ, Mavroudis AD, Georgiou M, Falagas ME. Intravenous colistin combination antimicrobial treatment vs. monotherapy: a systematic review and meta-analysis. Int J Antimicrob Agents. 2018;51(4):535-47.

65. Iosifidis E, Violaki A, Michalopoulou E, Volakli E, Diamanti E, Koliouskas D, et al. Use of Tigecycline in Pediatric Patients With Infections Predominantly Due to Extensively Drug-Resistant Gram-Negative Bacteria. Journal of the Pediatric Infectious Diseases Society. 2017;6(2):123-8.

66. Heidary M, Khosravi AD, Khoshnood S, Nasiri MJ, Soleimani S, Goudarzi M. Daptomycin. The Journal of antimicrobial chemotherapy. 2018;73(1):1-11.

67. Jacobs DM, Safir MC, Huang D, Minhaj F, Parker A, Rao GG. Triple combination antibiotic therapy for carbapenemase-producing Klebsiella pneumoniae: a systematic review. Annals of clinical microbiology and antimicrobials. 2017;16(1):76.

68. Li J, Echevarria KL, Traugott KA. beta-Lactam Therapy for Methicillin-Susceptible Staphylococcus aureus Bacteremia: A Comparative Review of Cefazolin versus Antistaphylococcal Penicillins. Pharmacotherapy. 2017;37(3):346-60.

69. Bartash R, Nori P. Beta-lactam combination therapy for the treatment of Staphylococcus aureus and Enterococcus species bacteremia: A summary and appraisal of the evidence. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2017;63:7-12.

70. Loubet P, Burdet C, Vindrios W, Grall N, Wolff M, Yazdanpanah Y, et al. Cefazolin versus anti-staphylococcal penicillins for treatment of methicillin-susceptible Staphylococcus aureus bacteraemia: a narrative review. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2018;24(2):125-32.

71. Beganovic M, Luther MK, Rice LB, Arias CA, Rybak MJ, LaPlante KL. A Review of Combination Antimicrobial Therapy for Enterococcus faecalis Bloodstream Infections and Infective Endocarditis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2018;67(2):303-9.

72. Branche A, Neeser O, Mueller B, Schuetz P. Procalcitonin to guide antibiotic decision making. Current opinion in infectious diseases. 2019;32(2):130-5.

73. Giannella M, Bartoletti M, Gatti M. Advances in the therapy of bacterial bloodstream infections. Clinical Microbiology and Infection. 2019;26.

74. Gudiol C, Cuervo G, Carratalà J. Optimizing therapy of bloodstream infection due to extended-spectrum β-lactamase-producing Enterobacteriaceae. Current opinion in critical care. 2019;25(5):438-48.

75. Pani A, Colombo F, Agnelli F, Frantellizzi V, Baratta F, Pastori D, et al. Off-label use of ceftaroline fosamil: A systematic review. Int J Antimicrob Agents. 2019;54(5):562-71.

76. Sakoulas G, Geriak M, Nizet V. Is a Reported Penicillin Allergy Sufficient Grounds to Forgo the Multidimensional Antimicrobial Benefits of β-Lactam Antibiotics? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019;68(1):157-64.

77. Sullivan RP, Marshall CS, Anstey NM, Ward L, Currie BJ. 2020 Review and revision of the 2015 Darwin melioidosis treatment guideline; paradigm drift not shift. PLoS neglected tropical diseases. 2020;14(9):e0008659.

78. Veganzones J, Montero A, Maseda E. New evidence on the use of fosfomycin for bacteremia and infectious endocarditis. Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia. 2019;32 Suppl 1(Suppl 1):25-9.

79. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nature reviews Microbiology. 2019;17(4):203-18.

80. Evans R, Pike K, Rogers C, Reynolds R, Stoddart M, Howe R, et al. Modifiable healthcare factors affecting 28-day survival in bloodstream infection: a prospective cohort study. BMC Infectious Diseases. 2020;20.

81. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID): Division of Healthcare Quality Promotion (DHQP)

2020 [Available from: https://www.cdc.gov/infectioncontrol/index.html.

82. CLSI. Methods for Dilution Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Eighth Edition. CLSI Document M07-A8. USA Clinical and Laboratory Standards Institute; 2009.

83. Arendrup MC, Cuenca-Estrella M, Lass-Florl C, Hope W. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012;18(7):E246-7.

84. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. 1973;60(8):646-9.

85. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. Journal of chronic diseases. 1987;40(5):373-83.

86. Royal College of Physicians. National Early Warning Score (NEWS) 2: Standardising the assessment of acute-illness severity in the NHS. Updated report of a working party. London: RCP; 2017.

87. MacGowan A, Grier S, Stoddart M, Reynolds R, Rogers C, Pike K, et al. Impact of rapid microbial identification on clinical outcomes in bloodstream infection: the RAPIDO randomized trial. Clinical Microbiology and Infection. 2020.

88. Griffiths P, Maruotti A, Recio Saucedo A, Redfern OC, Ball JE, Briggs J, et al. Nurse staffing, nursing assistants and hospital mortality: retrospective longitudinal cohort study. BMJ Qual Saf. 2018.

89. Rafferty AM, Clarke SP, Coles J, Ball J, James P, McKee M, et al. Outcomes of variation in hospital nurse staffing in English hospitals: cross-sectional analysis of survey data and discharge records. Int J Nurs Stud. 2007;44(2):175-82.

90. Needleman J, Buerhaus P, Pankratz VS, Leibson CL, Stevens SR, Harris M. Nurse Staffing and Inpatient Hospital Mortality. 2011;364(11):1037-45.

91. Sales A, Sharp N, Li Y-F, Lowy E, Greiner G, Liu C-F, et al. The Association Between Nursing Factors and Patient Mortality in the Veterans Health Administration: The View From the Nursing Unit Level. 2008;46(9):938-45.

92. Stone PW, Pogorzelska M, Kunches L, Hirschhorn LR. Hospital staffing and health care-associated infections: a systematic review of the literature. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;47(7):937-44.

93. Aiken LH, Clarke SP, Cheung RB, Sloane DM, Silber JH. Educational levels of hospital nurses and surgical patient mortality. Jama. 2003;290(12):1617-23.

94. Griffiths P, Renz A, Hughes J, Rafferty AM. Impact of organisation and management factors on infection control in hospitals: a scoping review. The Journal of hospital infection. 2009;73(1):1-14.

95. Kane RL, Shamliyan TA, Mueller C, Duval S, Wilt TJ. The association of registered nurse staffing levels and patient outcomes: systematic review and meta-analysis. Medical care. 2007;45(12):1195-204.

96. Khatib R, Saeed S, Sharma M, Riederer K, Fakih MG, Johnson LBJEJoCM, et al. Impact of initial antibiotic choice and delayed appropriate treatment on the outcome of Staphylococcus aureus bacteremia. 2006;25(3):181-5.

97. Paul M, Shani V, Muchtar E, Kariv G, Robenshtok E, Leibovici L. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. Antimicrob Agents Chemother. 2010;54(11):4851-63.

98. Kang C-I, Kim S-H, Kim H-B, Park S-W, Choe Y-J, Oh M-d, et al. *Pseudomonas aeruginosa* Bacteremia: Risk Factors for Mortality and Influence of Delayed Receipt of Effective Antimicrobial Therapy on Clinical Outcome. Clinical Infectious Diseases. 2003;37(6):745-51.

99. Buehrle DJ, Shields RK, Clarke LG, Potoski BA, Clancy CJ, Nguyen MH. Carbapenem-Resistant *Pseudomonas aeruginosa* Bacteremia: Risk Factors for Mortality and Microbiologic Treatment Failure. Antimicrobial Agents and Chemotherapy. 2017;61(1):e01243-16.

100. Zhou Z, Rahme E, Abrahamowicz M, Pilote L. Survival bias associated with time-to-treatment initiation in drug effectiveness evaluation: a comparison of methods. American journal of epidemiology. 2005;162(10):1016-23.

101. Suissa S. Effectiveness of inhaled corticosteroids in chronic obstructive pulmonary disease: immortal time bias in observational studies. American journal of respiratory and critical care medicine. 2003;168(1):49-53.

102. Corrao G, Rea F, Franchi M, Beccalli B, Locatelli A, Cantarutti A. Warning of Immortal Time Bias When Studying Drug Safety in Pregnancy: Application to Late Use of Antibiotics and Preterm Delivery. International journal of environmental research and public health. 2020;17.

103. Hines RB, Jiban MDJH, Specogna AV, Vishnubhotla P, Lee E, Troy SP, et al. Surveillance Colonoscopy in Older Stage I Colon Cancer Patients and the Association With Colon Cancer-Specific Mortality. Official journal of the American College of Gastroenterology | ACG. 2020;115(6).

104. Shintani AK, Girard TD, Eden SK, Arbogast PG, Moons KG, Ely EW. Immortal time bias in critical care research: application of time-varying Cox regression for observational cohort studies. Critical care medicine. 2009;37(11):2939-45.

105. Karim ME, Gustafson P, Petkau J, Tremlett H, Long-Term B, Adverse Effects of Beta-Interferon for Multiple Sclerosis Study G. Comparison of Statistical Approaches for Dealing With Immortal Time Bias in Drug Effectiveness Studies. American journal of epidemiology. 2016;184(4):325-35.

106. Gleiss A, Oberbauer R, Heinze G. An unjustified benefit: immortal time bias in the analysis of time-dependent events. 2018;31(2):125-30.

107. Cho IS, Chae YR, Kim JH, Yoo HR, Jang SY, Kim GR, et al. Statistical methods for elimination of guarantee-time bias in cohort studies: a simulation study. BMC Medical Research Methodology. 2017;17(1):126.

108. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. Journal of clinical epidemiology. 1995;48(12):1503-10.

109. Royston P, Sauerbrei W. Multivariable Model-Building. A Pragmatic Approach To Regression Analysis Based On Fractional Polynomials For Modelling Continuous Variables. Chichester, UK: Wiley; 2008.

110. Jann B. Stata tip 8: Splitting time-span records with categorical time-varying covariates. Stata J. 2004;4(2):221-2.

111. StataCorp. Stata 16 Base Reference Manual. College Station, TX: Stata Press; 2019.

112. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. 2011;30(4):377-99.

113. Rubin DB. Multiple Imputation for Nonresponse in Surveys. . New York: Wiley; 1987.

114. NHS England. NHS Services, Seven Days a Week Forum: Evidence base and clinical standards for the care and onward transfer of acute inpatients 2013. Available from: https://www.england.nhs.uk/wp-content/uploads/2013/12/evidence-base.pdf.

115. Leibovici L, Shraga I, Drucker M, Konigsberger H, Samra Z, Pitlik SD. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. Journal of Internal Medicine. 1998;244(5):379-86.

116. Coulter S, Roberts JA, Hajkowicz K, Halton K. The Use of Bloodstream Infection Mortality to Measure the Impact of Antimicrobial Stewardship Interventions: Assessing the Evidence. Infectious disease reports. 2017;9(1):6849.

117. Marquet K, Liesenborgs A, Bergs J, Vleugels A, Claes N. Incidence and outcome of inappropriate in-hospital empiric antibiotics for severe infection: a systematic review and meta-analysis. Critical Care. 2015;19(1):63.

118. Welch C, Bartlett J, Petersen I. Application of multiple imputation using the two-fold fully conditional specification algorithm in longitudinal clinical data. Stata J. 2014;14(2):418-31.

119. De Silva AP, Moreno-Betancur M, De Livera AM, Lee KJ, Simpson JA. A comparison of multiple imputation methods for handling missing values in longitudinal data in the presence of a time-varying covariate with a non-linear association with time: a simulation study. BMC Medical Research Methodology. 2017;17(1):114.

120. Huque MH, Carlin JB, Simpson JA, Lee KJ. A comparison of multiple imputation methods for missing data in longitudinal studies. BMC Medical Research Methodology. 2018;18(1):168.

121. Hernán MA. The hazards of hazard ratios. Epidemiology (Cambridge, Mass). 2010;21(1):13-5.

122. Holland TL, Arnold C, Fowler VG, Jr. Clinical management of Staphylococcus aureus bacteremia: a review. Jama. 2014;312(13):1330-41.

123. Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2011;52(3):e18-55.

124. Corey GR. Staphylococcus aureus Bloodstream Infections: Definitions and Treatment. Clinical Infectious Diseases. 2009;48(Supplement_4):S254-S9.

125. Fowler VG, Jr., Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, et al. Clinical identifiers of complicated Staphylococcus aureus bacteremia. Archives of internal medicine. 2003;163(17):2066-72.

126. Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. BMC Infect Dis. 2014;14:13.

127. Tamma PD, Avdic E, Li DX, Dzintars K, Cosgrove SE. Association of Adverse Events With Antibiotic Use in Hospitalized Patients. JAMA Internal Medicine. 2017;177(9):1308-15.

128. Suissa S. Immortal time bias in pharmaco-epidemiology. American journal of epidemiology. 2008;167(4):492-9.

129. Thorlacius-Ussing L, Sandholdt H, Nissen J, Rasmussen J, Skov R, Frimodt-Møller N, et al. Comparable Outcomes of Short-Course and Prolonged-Course Therapy in Selected Cases of Methicillin-Susceptible Staphylococcus aureus Bacteremia: A Pooled Cohort Study. Clinical Infectious Diseases. 2021.

130. Abbas M, Rossel A, de Kraker MEA, von Dach E, Marti C, Emonet S, et al. Association between treatment duration and mortality or relapse in adult patients with Staphylococcus aureus bacteraemia: a retrospective cohort study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2020;26(5):626-31.

131. Eichenberger EM, Fowler VG, Jr., Holland TL. Duration of antibiotic therapy for Staphylococcus aureus bacteraemia: the long and the short of it. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2020;26(5):536-8.

132. Berrevoets MAH, Kouijzer IJE, Slieker K, Aarntzen E, Kullberg BJ, Oever JT, et al. (18)F-FDG PET/CT-Guided Treatment Duration in Patients with High-Risk Staphylococcus Aureus Bacteremia: A Proof of Principle. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2019;60(7):998-1002.

133. Kim CJ, Song KH, Park KH, Kim M, Choe PG, Oh MD, et al. Impact of antimicrobial treatment duration on outcome of Staphylococcus aureus bacteraemia: a cohort study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019;25(6):723-32.

134. Thorlacius-Ussing L, Andersen CØ, Frimodt-Møller N, Knudsen IJD, Lundgren J, Benfield TL. Efficacy of seven and fourteen days of antibiotic treatment in uncomplicated Staphylococcus aureus bacteremia (SAB7): study protocol for a randomized controlled trial. Trials. 2019;20(1):250.

135. Kempley S, Kapellou O, McWilliams A, Banerjee J, McCorqodale A, Millar M. Antibiotic treatment duration and prevention of complications in neonatal Staphylococcus aureus bacteraemia. The Journal of hospital infection. 2015;91(2):129-35.

136. Chong YP, Moon SM, Bang KM, Park HJ, Park SY, Kim MN, et al. Treatment duration for uncomplicated Staphylococcus aureus bacteremia to prevent relapse: analysis of a prospective observational cohort study. Antimicrob Agents Chemother. 2013;57(3):1150-6.

137. Havey TC, Fowler RA, Pinto R, Elligsen M, Daneman N. Duration of antibiotic therapy for critically ill patients with bloodstream infections: A retrospective cohort study. The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale. 2013;24(3):129-37.

138. Asgeirsson H, Kristjansson M, Kristinsson KG, Gudlaugsson O. Staphylococcus aureus bacteraemia--Nationwide assessment of treatment adequacy and outcome. The Journal of infection. 2011;62(5):339-46.

139. Kreisel K, Boyd K, Langenberg P, Roghmann MC. Risk factors for recurrence in patients with Staphylococcus aureus infections complicated by bacteremia. Diagnostic microbiology and infectious disease. 2006;55(3):179-84.

140. Fätkenheuer G, Preuss M, Salzberger B, Schmeisser N, Cornely OA, Wisplinghoff H, et al. Long-term outcome and quality of care of patients with Staphylococcus aureus bacteremia. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2004;23(3):157-62.

141. Pigrau C, Rodríguez D, Planes AM, Almirante B, Larrosa N, Ribera E, et al. Management of catheter-related Staphylococcus aureus bacteremia: when may sonographic study be unnecessary? European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2003;22(12):713-9.

142. Chang FY, Peacock JE, Jr., Musher DM, Triplett P, MacDonald BB, Mylotte JM, et al. Staphylococcus aureus bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. Medicine. 2003;82(5):333-9.

143. Jensen AG, Wachmann CH, Espersen F, Scheibel J, Skinhøj P, Frimodt-Møller N. Treatment and outcome of Staphylococcus aureus bacteremia: a prospective study of 278 cases. Archives of internal medicine. 2002;162(1):25-32.

144. Zeylemaker MM, Jaspers CA, van Kraaij MG, Visser MR, Hoepelman IM. Long-term infectious complications and their relation to treatment duration in catheter-related Staphylococcus aureus bacteremia. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 2001;20(6):380-4.

145. Malanoski GJ, Samore MH, Pefanis A, Karchmer AW. Staphylococcus aureus Catheter-Associated Bacteremia: Minimal Effective Therapy and Unusual Infectious Complications Associated With Arterial Sheath Catheters. Archives of internal medicine. 1995;155(11):1161-6.

146. Jernigan JA, Farr BM. Short-course therapy of catheter-related Staphylococcus aureus bacteremia: a meta-analysis. Annals of internal medicine. 1993;119(4):304-11.

147. Raad, II, Sabbagh MF. Optimal duration of therapy for catheter-related Staphylococcus aureus bacteremia: a study of 55 cases and review. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 1992;14(1):75-82.

148. Ehni WF, Reller LB. Short-Course Therapy for Catheter-Associated Staphylococcus aureus Bacteremia. Archives of internal medicine. 1989;149(3):533-6.

149. Hernán MA. How to estimate the effect of treatment duration on survival outcomes using observational data. BMJ (Clinical research ed). 2018;360:k182.

150. Maringe C, Benitez Majano S, Exarchakou A, Smith M, Rachet B, Belot A, et al. Reflection on modern methods: trial emulation in the presence of immortal-time bias. Assessing the benefit of major surgery for elderly lung cancer patients using observational data. International journal of epidemiology. 2020;49(5):1719-29.

151. Williamson T, Ravani P. Marginal structural models in clinical research: when and how to use them? Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2017;32(suppl_2):ii84-ii90.

152. Hernán MA, Brumback B, Robins JM. Marginal Structural Models to Estimate the Joint Causal Effect of Nonrandomized Treatments. Journal of the American Statistical Association. 2001;96(454):440-8.

153. Hernán MA, Brumback BA, Robins JM. Estimating the causal effect of zidovudine on CD4 count with a marginal structural model for repeated measures. Statistics in medicine. 2002;21(12):1689-709.

154. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. American journal of epidemiology. 2008;168(6):656-64.

155. Harrell F. Regression Modeling Strategies. With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York: Springer; 2001.

156. D'Agostino RB, Lee ML, Belanger AJ, Cupples LA, Anderson K, Kannel WB. Relation of pooled logistic regression to time dependent Cox regression analysis: the Framingham Heart Study. Statistics in medicine. 1990;9(12):1501-15.

157. Hernán M, Robins J. Causal Inference: What If. Boca Raton: Chapman & Hall/CRC; 2020.

158. Petito LC, García-Albéniz X, Logan RW, Howlader N, Mariotto AB, Dahabreh IJ, et al. Estimates of Overall Survival in Patients With Cancer Receiving Different Treatment Regimens: Emulating Hypothetical Target Trials in the Surveillance, Epidemiology, and End Results (SEER)-Medicare Linked Database. JAMA network open. 2020;3(3):e200452.

159. Caniglia EC, Robins JM, Cain LE, Sabin C, Logan R, Abgrall S, et al. Emulating a trial of joint dynamic strategies: An application to monitoring and treatment of HIV-positive individuals. 2019;38(13):2428-46.

160. Dickerman BA, García-Albéniz X, Logan RW, Denaxas S, Hernán MA. Avoidable flaws in observational analyses: an application to statins and cancer. Nature Medicine. 2019;25(10):1601-6.

161. García-Albéniz X, Hernán MA, Logan RW, Price M, Armstrong K, Hsu J. Continuation of Annual Screening Mammography and Breast Cancer Mortality in Women Older Than 70 Years. Annals of internal medicine. 2020;172(6):381-9.

162. Encyclopedia of Research Design. 2010.

163. Yahav D, Franceschini E, Koppel F, Turjeman A, Babich T, Bitterman R, et al. Seven Versus 14 Days of Antibiotic Therapy for Uncomplicated Gram-negative Bacteremia: A Noninferiority Randomized Controlled Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019;69(7):1091-8.

164. Vardakas KZ, Tansarli GS, Rafailidis PI, Falagas ME. Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to Enterobacteriaceae producing extended-spectrum beta-lactamases: a systematic review and meta-analysis. The Journal of antimicrobial chemotherapy. 2012;67(12):2793-803.

165. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clinical Microbiology Reviews. 2005;18(4):657-86.

166. McLaughlin M, Advincula MR, Malczynski M, Qi C, Bolon M, Scheetz MH. Correlations of antibiotic use and carbapenem resistance in enterobacteriaceae. Antimicrob Agents Chemother. 2013;57(10):5131-3.

167. Harris PN, Tambyah PA, Paterson DL. beta-lactam and beta-lactamase inhibitor combinations in the treatment of extended-spectrum beta-lactamase producing Enterobacteriaceae: time for a reappraisal in the era of few antibiotic options? The Lancet Infectious diseases. 2015;15(4):475-85.

168. Tamma PD, Rodriguez-Bano J. The Use of Noncarbapenem beta-Lactams for the Treatment of Extended-Spectrum beta-Lactamase Infections. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;64(7):972-80.

169. Gudiol C, Royo-Cebrecos C, Abdala E, Akova M, Alvarez R, Maestro-de la Calle G, et al. Efficacy of beta-Lactam/beta-Lactamase Inhibitor Combinations for the Treatment of Bloodstream Infection Due to Extended-Spectrum-beta-Lactamase-Producing Enterobacteriaceae in Hematological Patients with Neutropenia. Antimicrobial Agents & Chemotherapy. 2017;61(8):08.

170. Gutierrez-Gutierrez B, Perez-Galera S, Salamanca E, de Cueto M, Calbo E, Almirante B, et al. A Multinational, Preregistered Cohort Study of beta-Lactam/beta-Lactamase Inhibitor Combinations for Treatment of Bloodstream Infections Due to Extended-Spectrum-beta-Lactamase-Producing Enterobacteriaceae. Antimicrobial Agents & Chemotherapy. 2016;60(7):4159-69.

171. Ko JH, Lee NR, Joo EJ, Moon SY, Choi JK, Park DA, et al. Appropriate non-carbapenems are not inferior to carbapenems as initial empirical therapy for bacteremia caused by extended-spectrum beta-lactamase-producing Enterobacteriaceae: a propensity score weighted multicenter cohort study. European Journal of Clinical Microbiology & Infectious Diseases. 2018;37(2):305-11.

172. Ng TM, Khong WX, Harris PN, De PP, Chow A, Tambyah PA, et al. Empiric Piperacillin-Tazobactam versus Carbapenems in the Treatment of Bacteraemia Due to Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae. PLoS ONE [Electronic Resource]. 2016;11(4):e0153696.

173. Rodriguez-Bano J, Navarro MD, Retamar P, Picon E, Pascual A. beta-Lactam/beta-lactam inhibitor combinations for the treatment of bacteremia due to extended-spectrum beta-lactamase-producing Escherichia coli: a post hoc analysis of prospective cohorts. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54(2):167-74.

174. Aslan AT, Akova M. Extended spectrum β-lactamase producing enterobacteriaceae: carbapenem sparing options. Expert review of anti-infective therapy. 2019;17(12):969-81.

175. Gutiérrez-Gutiérrez B, Rodríguez-Baño J. Current options for the treatment of infections due to extended-spectrum beta-lactamase-producing Enterobacteriaceae in different groups of patients. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2019;25(8):932-42.

176. Harris PNA, Tambyah PA, Lye DC, Mo Y, Lee TH, Yilmaz M, et al. Effect of Piperacillin-Tazobactam vs Meropenem on 30-Day Mortality for Patients With E coli or Klebsiella pneumoniae Bloodstream Infection and Ceftriaxone Resistance: A Randomized Clinical Trial. JAMA. 2018;320(10):984-94.

177. Henderson A, Paterson DL, Chatfield MD, Tambyah PA, Lye DC, De PP, et al. Association between minimum inhibitory concentration, beta-lactamase genes and mortality for patients treated with piperacillin/tazobactam or meropenem from the MERINO study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2020.

178. Ofer-Friedman H, Shefler C, Sharma S, Tirosh A, Tal-Jasper R, Kandipalli D, et al. Carbapenems Versus Piperacillin-Tazobactam for Bloodstream Infections of Nonurinary Source Caused by Extended-

Spectrum Beta-Lactamase-Producing Enterobacteriaceae. Infection Control & Hospital Epidemiology. 2015;36(8):981-5.

179. Tsai HY, Chen YH, Tang HJ, Huang CC, Liao CH, Chu FY, et al. Carbapenems and piperacillin/tazobactam for the treatment of bacteremia caused by extended-spectrum beta-lactamase-producing Proteus mirabilis. Diagnostic Microbiology & Infectious Disease. 2014;80(3):222-6.

180. Tamma PD, Han JH, Rock C, Harris AD, Lautenbach E, Hsu AJ, et al. Carbapenem therapy is associated with improved survival compared with piperacillin-tazobactam for patients with extended-spectrum beta-lactamase bacteremia. Clinical Infectious Diseases. 2015;60(9):1319-25.

181. Hernán MA, Sauer BC, Hernández-Díaz S, Platt R, Shrier I. Specifying a target trial prevents immortal time bias and other self-inflicted injuries in observational analyses. Journal of clinical epidemiology. 2016;79:70-5.

182. Hernán MA, Robins JM. Using Big Data to Emulate a Target Trial When a Randomized Trial Is Not Available. American journal of epidemiology. 2016;183(8):758-64.

183. Varas-Lorenzo C, García-Rodríguez LA, Perez-Gutthann S, Duque-Oliart A. Hormone replacement therapy and incidence of acute myocardial infarction. A population-based nested case-control study. Circulation. 2000;101(22):2572-8.

184. Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, et al. Estrogen plus progestin and the risk of coronary heart disease. The New England journal of medicine. 2003;349(6):523-34.

185. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Controlled clinical trials. 1998;19(1):61-109.

186. Hernán MA, Alonso A, Logan R, Grodstein F, Michels KB, Willett WC, et al. Observational studies analyzed like randomized experiments: an application to postmenopausal hormone therapy and coronary heart disease. Epidemiology (Cambridge, Mass). 2008;19(6):766-79.

187. Harris PNA, Tambyah PA, Lye DC, Mo Y, Lee TH, Yilmaz M, et al. Effect of Piperacillin-Tazobactam vs Meropenem on 30-Day Mortality for Patients With E coli or Klebsiella pneumoniae Bloodstream Infection and Ceftriaxone Resistance: A Randomized Clinical Trial. JAMA. 2018;320(10):984-94.

188. Cohen J. Statistical Power Analysis for the Behavioral Sciences. Hillsdale, New Jersey: Lawrence Erlbaum Associates; 1988.

189. Cole SR, Hernán MA. Adjusted survival curves with inverse probability weights. Computer methods and programs in biomedicine. 2004;75(1):45-9.

190. VanderWeele TJ, Shpitser I. A new criterion for confounder selection. Biometrics. 2011;67(4):1406-13.

191. Stel VS, Dekker FW, Zoccali C, Jager KJ. Instrumental variable analysis. Nephrology Dialysis Transplantation. 2012;28(7):1694-9.

192. Lousdal ML. An introduction to instrumental variable assumptions, validation and estimation. Emerging themes in epidemiology. 2018;15:1.

193. Stürmer T, Joshi M, Glynn RJ, Avorn J, Rothman KJ, Schneeweiss S. A review of the application of propensity score methods yielded increasing use, advantages in specific settings, but not substantially different estimates compared with conventional multivariable methods. Journal of clinical epidemiology. 2006;59(5):437.e1-.e24.

194. Braitman LE, Rosenbaum PR. Rare outcomes, common treatments: analytic strategies using propensity scores. Annals of internal medicine. 2002;137(8):693-5.

195. Nguyen T-L, Collins GS, Spence J, Daurès J-P, Devereaux PJ, Landais P, et al. Double-adjustment in propensity score matching analysis: choosing a threshold for considering residual imbalance. BMC Medical Research Methodology. 2017;17(1):78.

196. Rosenbaum PR, Rubin DB. The bias due to incomplete matching. Biometrics. 1985;41(1):103-16.

197. Bitterman R, Koppel F, Mussini C, Geffen Y, Chowers M, Rahav G, et al. Piperacillin-tazobactam versus meropenem for treatment of bloodstream infections caused by third-generation cephalosporin-resistant Enterobacteriaceae: a study protocol for a non-inferiority open-label randomised controlled trial (PeterPen). BMJ Open. 2021;11(2):e040210.

198. Defife R, Scheetz MH, Feinglass JM, Postelnick MJ, Scarsi KK. Effect of differences in MIC values on clinical outcomes in patients with bloodstream infections caused by gram-negative organisms treated with levofloxacin. Antimicrob Agents Chemother. 2009;53(3):1074-9.

199. Esterly JS, Wagner J, McLaughlin MM, Postelnick MJ, Qi C, Scheetz MH. Evaluation of clinical outcomes in patients with bloodstream infections due to Gram-negative bacteria according to carbapenem MIC stratification. Antimicrob Agents Chemother. 2012;56(9):4885-90.

200. Falagas ME, Tansarli GS, Rafailidis PI, Kapaskelis A, Vardakas KZ. Impact of antibiotic MIC on infection outcome in patients with susceptible Gram-negative bacteria: a systematic review and meta-analysis. Antimicrob Agents Chemother. 2012;56(8):4214-22.

201. Rhodes NJ, Liu J, McLaughlin MM, Qi C, Scheetz MH. Evaluation of clinical outcomes in patients with Gram-negative bloodstream infections according to cefepime MIC. Diagnostic microbiology and infectious disease. 2015;82(2):165-71.

202. O'Donnell JN, Rhodes NJ, Biehle LR, Esterly JS, Patel TS, McLaughlin MM, et al. Assessment of mortality stratified by meropenem minimum inhibitory concentration in patients with Enterobacteriaceae bacteraemia: A patient-level analysis of published data. Int J Antimicrob Agents. 2020;55(2):105849.

203. Shi C, Ye J, Xu R, Jin W, Xu S, Teng F, et al. Effect of the vancomycin minimum inhibitory concentration on clinical outcomes in patients with methicillin-susceptible Staphylococcus aureus bacteraemia: a systematic review and meta-analysis. BMJ Open. 2021;11(1):e040675.

204. Papadimitriou-Olivgeris M, Bartzavali C, Nikolopoulou A, Kolonitsiou F, Mplani V, Spiliopoulou I, et al. Impact of Tigecycline's MIC in the Outcome of Critically Ill Patients with Carbapenemase-Producing Klebsiella pneumoniae Bacteraemia Treated with Tigecycline Monotherapy-Validation of 2019's EUCAST Proposed Breakpoint Changes. Antibiotics (Basel, Switzerland). 2020;9(11).

205. Kagami K, Imai S, Tazawa Y, Iwasaki S, Fukumoto T, Akizawa K, et al. [Possibility of Poor Outcomes after Treatment Using Teicoplanin at the Minimum Inhibitory Concentration of >2 μg/mL

in Methicillin-resistant Staphylococcus aureus Bacteremia]. Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan. 2018;138(9):1181-9.

206. Abelenda Alonso G, Corbacho Loarte MD, Núñez Ramos R, Cervero Jiménez M, Jusdado Ruiz-Capillas JJ. Staphylococcus aureus bacteremia in a secondary level Spanish hospital: clinical implications of high vancomycin MIC. Revista espanola de quimioterapia : publicacion oficial de la Sociedad Espanola de Quimioterapia. 2018;31(4):353-62.

207. Ko JH, Peck KR, Jung DS, Lee JY, Kim HA, Ryu SY, et al. Impact of high MIC of fluconazole on outcomes of Candida glabrata bloodstream infection: a retrospective multicenter cohort study. Diagnostic microbiology and infectious disease. 2018;92(2):127-32.

208. Adani S, Bhowmick T, Weinstein MP, Narayanan N. Impact of Vancomycin MIC on Clinical Outcomes of Patients with Methicillin-Resistant Staphylococcus aureus Bacteremia Treated with Vancomycin at an Institution with Suppressed MIC Reporting. Antimicrob Agents Chemother. 2018;62(4).

209. Bouiller K, Laborde C, Aho SL, Hocquet D, Pechinot A, Le Moing V, et al. No effect of vancomycin MIC $\geqslant$ 1.5 mg/L on treatment outcome in methicillin-susceptible Staphylococcus aureus bacteraemia. Int J Antimicrob Agents. 2018;51(5):721-6.

210. Su TY, Ye JJ, Yang CC, Huang CT, Chia JH, Lee MH. Influence of borderline cefepime MIC on the outcome of cefepime-susceptible Pseudomonas aeruginosa bacteremia treated with a maximal cefepime dose: a hospital-based retrospective study. Annals of clinical microbiology and antimicrobials. 2017;16(1):52.

211. Yang YS, Wang YC, Kuo SC, Chen CT, Liu CP, Liu YM, et al. Multicenter Study of the Relationship between Carbapenem MIC Values and Clinical Outcome of Patients with Acinetobacter Bacteremia. Antimicrob Agents Chemother. 2017;61(9).

212. Hentzien M, Strady C, Vernet-Garnier V, Servettaz A, De Champs C, Delmer A, et al. Prognostic factors associated with 30-day in-hospital mortality in coagulase-negative Staphylococcus bacteraemia: no impact of vancomycin minimum inhibitory concentration. Infectious diseases (London, England). 2017;49(9):664-73.

213. Gentry CA, Williams RJ, 2nd. A propensity score-matched analysis of the impact of minimum inhibitory concentration on mortality in patients with Pseudomonas aeruginosa bacteraemia treated with piperacillin/tazobactam. Int J Antimicrob Agents. 2017;49(3):333-8.

214. Ratliff AR, Gentry CA, Williams RJ, 2nd. A propensity score-matched analysis of the impact of minimum inhibitory concentration on mortality in patients with Pseudomonas aeruginosa bacteremia treated with cefepime or ceftazidime. Diagnostic microbiology and infectious disease. 2017;87(4):376-81.

215. Song KH, Kim M, Kim CJ, Cho JE, Choi YJ, Park JS, et al. Impact of Vancomycin MIC on Treatment Outcomes in Invasive Staphylococcus aureus Infections. Antimicrob Agents Chemother. 2017;61(3).

216. Baxi SM, Clemenzi-Allen A, Gahbauer A, Deck D, Imp B, Vittinghoff E, et al. Vancomycin MIC Does Not Predict 90-Day Mortality, Readmission, or Recurrence in a Prospective Cohort of Adults with Staphylococcus aureus Bacteremia. Antimicrob Agents Chemother. 2016;60(9):5276-84.

217. Delgado-Valverde M, Torres E, Valiente-Mendez A, Almirante B, Gómez-Zorrilla S, Borrell N, et al. Impact of the MIC of piperacillin/tazobactam on the outcome for patients with bacteraemia due

to Enterobacteriaceae: the Bacteraemia-MIC project. The Journal of antimicrobial chemotherapy. 2016;71(2):521-30.

218. Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with Staphylococcus aureus bloodstream infections: a systematic review and meta-analysis. Jama. 2014;312(15):1552-64.

219. Castón JJ, González-Gasca F, Porras L, Illescas S, Romero MD, Gijón J. High vancomycin minimum inhibitory concentration is associated with poor outcome in patients with methicillin-susceptible Staphylococcus aureus bacteremia regardless of treatment. Scandinavian journal of infectious diseases. 2014;46(11):783-6.

220. Park SY, Oh IH, Lee HJ, Ihm CG, Son JS, Lee MS, et al. Impact of reduced vancomycin MIC on clinical outcomes of methicillin-resistant Staphylococcus aureus bacteremia. Antimicrob Agents Chemother. 2013;57(11):5536-42.

221. Hope R, Blackburn RM, Verlander NQ, Johnson AP, Kearns A, Hill R, et al. Vancomycin MIC as a predictor of outcome in MRSA bacteraemia in the UK context. The Journal of antimicrobial chemotherapy. 2013;68(11):2641-7.

222. Retamar P, López-Cerero L, Muniain MA, Pascual Á, Rodríguez-Baño J. Impact of the MIC of piperacillin-tazobactam on the outcome of patients with bacteremia due to extended-spectrum-β-lactamase-producing Escherichia coli. Antimicrob Agents Chemother. 2013;57(7):3402-4.

223. Holmes NE, Turnidge JD, Munckhof WJ, Robinson JO, Korman TM, O'Sullivan MV, et al. Vancomycin minimum inhibitory concentration, host comorbidities and mortality in Staphylococcus aureus bacteraemia. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2013;19(12):1163-8.

224. Jacob JT, DiazGranados CA. High vancomycin minimum inhibitory concentration and clinical outcomes in adults with methicillin-resistant Staphylococcus aureus infections: a meta-analysis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2013;17(2):e93-e100.

225. Woods CJ, Chowdhury A, Patel VM, Shorr AF. Impact of vancomycin minimum inhibitory concentration on mortality among critically ill patients with methicillin-resistant Staphylococcus aureus bacteremia. Infection control and hospital epidemiology. 2012;33(12):1246-9.

226. Mavros MN, Tansarli GS, Vardakas KZ, Rafailidis PI, Karageorgopoulos DE, Falagas ME. Impact of vancomycin minimum inhibitory concentration on clinical outcomes of patients with vancomycin-susceptible Staphylococcus aureus infections: a meta-analysis and meta-regression. Int J Antimicrob Agents. 2012;40(6):496-509.

227. Tamma PD, Turnbull AE, Milstone AM, Hsu AJ, Carroll KC, Cosgrove SE. Does the piperacillin minimum inhibitory concentration for Pseudomonas aeruginosa influence clinical outcomes of children with pseudomonal bacteremia? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;55(6):799-806.

228. Wi YM, Kim JM, Joo EJ, Ha YE, Kang CI, Ko KS, et al. High vancomycin minimum inhibitory concentration is a predictor of mortality in meticillin-resistant Staphylococcus aureus bacteraemia. Int J Antimicrob Agents. 2012;40(2):108-13.

229. Yeh YC, Yeh KM, Lin TY, Chiu SK, Yang YS, Wang YC, et al. Impact of vancomycin MIC creep on patients with methicillin-resistant Staphylococcus aureus bacteremia. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 2012;45(3):214-20.

230. van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in Staphylococcus aureus infections: a systematic review and meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2012;54(6):755-71.

231. Brown J, Brown K, Forrest A. Vancomycin AUC24/MIC ratio in patients with complicated bacteremia and infective endocarditis due to methicillin-resistant Staphylococcus aureus and its association with attributable mortality during hospitalization. Antimicrob Agents Chemother. 2012;56(2):634-8.

232. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant Staphylococcus aureus bacteremia treated with vancomycin. Antimicrob Agents Chemother. 2008;52(9):3315-20.

233. Soriano A, Marco F, Martínez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant Staphylococcus aureus bacteremia. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2008;46(2):193-200.

234. Maclayton DO, Suda KJ, Coval KA, York CB, Garey KW. Case-control study of the relationship between MRSA bacteremia with a vancomycin MIC of 2 microg/mL and risk factors, costs, and outcomes in inpatients undergoing hemodialysis. Clinical therapeutics. 2006;28(8):1208-16.

235. Sabu P, Elangovan D, Pragasam AK, Bakthavatchalam YD, Rodrigues C, Chitnis DS, et al. Efficacy ratio: A tool to enhance optimal antimicrobial use for intra-abdominal infections. Indian journal of pharmacology. 2018;50(6):332-5.