Gram-negative bacteraemia; a multi-centre prospective evaluation of empiric antibiotic therapy and outcome in English acute hospitals

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Abstract

Increasing antibiotic resistance makes choosing antibiotics for suspected Gram-negative infection challenging. This study set out to identify key determinants of mortality among patients with Gram-negative bacteraemia, focusing particularly on the importance of appropriate empiric antibiotic treatment.

We conducted a prospective observational study of 679 unselected adults with Gram-negative bacteraemia at ten acute English hospitals between October 2013 and March 2014. Appropriate empiric antibiotic treatment was defined as intravenous treatment, on the day of blood culture collection, with an antibiotic to which the cultured organism was sensitive *in vitro*. Mortality analyses were adjusted for patient demographics, co-morbidities and illness severity.

The majority of bacteraemias were community onset (70%); most were caused by *Escherichia coli* (65%), *Klebsiella* spp (15%) or *Pseudomonas* spp (7%). Main foci of infection were urinary tract (51%), abdomen/biliary tract (20%) and lower respiratory tract (14%). The main antibiotics used were co-amoxiclav (32%) and piperacillin-tazobactam (30%) with 34% receiving combination therapy (predominantly aminoglycosides). Empiric treatment was inappropriate in 34%. All-cause mortality was 8% at 7-days and 15% at 30-days. Independent predictors of mortality ($p<0.05$) included older age, greater burden of co-morbid disease, severity of illness at presentation and inflammatory response. Inappropriate empiric antibiotic therapy was not associated with mortality at either time point (adjusted OR=0.82 (95% CI 0.35-1.94) and 0.92 (0.50-1.66) respectively).

Although our study does not exclude an impact of empiric antibiotic choice on survival in Gram-negative bacteraemia, outcome is determined primarily by patient and disease factors.

INTRODUCTION

Bacteraemia is a common and severe systemic infection which affects approximately 600,000 people in the United States and 1.2 million people in Europe each year ; 15% of affected patients die within 30-days [1]. During the 1990s Gram-positive bacteria were the major pathogens causing bacteraemia but Gram-negative bacilli (GNB), particularly Enterobacteriaceae, are now re-emerging as the predominant pathogens isolated from blood [2-3].

Selection of appropriate empiric antibiotic treatment for suspected Gram-negative infection is particularly challenging because rates of resistance to the main antibiotic classes are increasing [4]; leading to enormous reliance on broad-spectrum agents [5]. The appropriateness of empiric antibiotic therapy for bacteraemia has been proposed as a performance measure for antimicrobial stewardship programmes [6.7]. However, the prognostic impact of empiric therapy in GNB bacteraemia is not established.

The impact of empiric antibiotic treatment on clinical outcome has been studied predominantly in critically ill patients with severe sepsis and septic shock. Among such patients delays in initiating active antibiotic treatment have been linked to increased risk of death [8,9]. However, these results may not be generalisable to all sepsis patients in whom other studies report benefit from delayed, focused treatment (10,11). Furthermore only around 50% of patients recruited to severe sepsis studies are bacteraemic and many studies investigating the impact of empiric antibiotic therapy specifically in bacteraemia have methodological limitations such as small sample size, heterogeneous patient populations and retrospective design [12-24]. A systematic review of these studies a published in 2007 found 'little evidence for or against recommendations regarding aggressive empiric therapy with broad-spectrum antibiotics' [25]. Two subsequent large, prospective studies have produced contrasting results among different patient populations (26,27). However, <50% of cases had GNB bacteraemia in these studies. In a retrospective study specifically in GNB bacteraemia Cain *et al* found an effect of empiric antibiotic therapy only among patients with a high prior probability of death(28).

The objective of this prospective, multi-centre observational cohort study was to identify the key determinants of mortality among unselected patients with GNB bacteraemia; focusing particularly on the importance of appropriate empiric antibiotic treatment.

PATIENTS AND METHODS

Setting and study population

We conducted this study at ten acute hospitals in England (see acknowledgements) including large (>1000 bed) tertiary hospitals and medium (500-1000 bed) district hospitals. Sites included cases for slightly different periods of 50-120 days depending on staff availability between November 2013 and March 2014, but at each site, while open medical microbiologists, recorded baseline clinical characteristics, management and outcome of consecutive adult patients fulfilling eligibility criteria at the time of routine clinical review. The co-primary outcomes were mortality at 7 and 30 days after blood was taken for culture, confirmed through each hospital's management information system which includes post-discharge deaths.

Patients were eligible for inclusion if they were ≥18 years, had one or more blood cultures showing a pure growth of either a lactose fermenting coliform (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Morganella* spp., *Citrobacter* spp., or *Proteus* spp.) or a *Pseudomonas* spp. Patients were excluded if the blood isolate was mixed with another pathogen. Only the first bacteraemia for each patient was included.

Organisms were identified and antibiotic sensitivity testing performed according to standard methods by each hospital's diagnostic laboratory.

Definitions

Bacteraemias were categorised as *nosocomial* if the first positive sample was taken ≥48 hours after hospital admission, otherwise they were categorised as *community-acquired.* Additionally, if the patient had been admitted to hospital in the preceding 30 days, had been transferred from another healthcare facility, was receiving chronic dialysis, immunosuppressive medication or had metastatic cancer, bacteraemia were considered *healthcare-associated* community-acquired.

Burden of co-morbid disease was assessed using an age-adjusted Charlson score. Severity of illness was assessed using the National Early Warning System (NEWS) Score which is widely used in English hospitals and assigns points for respiratory rate, oxygen saturation, need for supplemental oxygen, temperature, systolic blood pressure, heart rate and conscious level

(range 0-21) [29]. Patients scoring ≥5 should receive urgent medical review and ≥7 should be considered for escalation to high-dependency or intensive care.

Patients were considered to have received appropriate empiric antibiotic treatment if they were prescribed one or more intravenous doses of one or more antibiotics to which the organism cultured was sensitive *in vitro* on the day the blood culture was taken [13].

Ethics

Prior to the project starting the NHS Health Research Authority confirmed it constituted a service evaluation not requiring patient consent or formal review by a research ethics committee. Local research and development office approval was secured at each site.

Statistical analysis

Continuous and categorical baseline factors were compared using Kruskal-Wallis and χ^2 tests respectively. To account for vary amounts of missing data associations between baseline factors and 7- and 30-day mortality (binary outcome, logistic regression) were assessed univariably using both complete cases, and in multivariable models using 25 imputations with chained estimating equations [30] (see supplementary material for details). As the key exposure was empiric antibiotic therapy, patients who died on the day blood was taken for culture were excluded from the primary imputations and multivariable analysis because antibiotics may be futile so close to death. A sensitivity analysis included these patients in imputations and multivariable analyses. Final multivariable models were selected using backwards elimination (exit $p>0.05$) retaining empiric therapy as the key exposure of interest and including other significant factors to ensure control of confounding. See supplementary material for further details, including calculation of adjusted absolute mortality percentages and post-hoc sample size calculation. Analyses were performed using SPSS (IBM: Version 22) and Stata 13.1.

RESULTS

Study sites achieved a median of 96.5% recruitment of eligible patients (IQR 93.5-100%) obtaining prospective data on 679 adults with microbiologically confirmed GNB bacteraemia. Nine (1%) who died on the day blood was taken for culture were excluded from primary multivariable analyses, but included in sensitivity analyses. Data describing antimicrobial susceptibility or treatment were missing for 54 (8%) patients, leaving 616 (91%) with complete data on antibiotic treatment and 7-day mortality (figure 1). 30-day mortality data were missing on a further five.

Overall mortality was 8% (52/679) and 15% (101/674) at 7 and 30-days respectively. Table 1 shows the univariable associations between mortality and patient and disease factors and appropriateness of empiric antibiotic treatment for all 679 patients. In both complete-cases and multiple-imputations, patients who died within 7 days were older, had a greater burden of comorbid disease, were more acutely unwell as measured by NEWS score, more often had a non-urinary focus of infection and had higher levels of CRP and creatinine than patients who survived. Univariably Klebsiella and Pseudomonas *spp*. bacteraemia were also associated with higher 7-day mortality. The only additional factor consistently associated with higher 30-day mortality was nosocomial-onset bacteraemia. Among the 616 patients in whom appropriateness of empiric antibiotic therapy could be assessed, 210 (34%) received inappropriate treatment, 106 (17%) because the regimen used was not active *in vitro*, 104 (17%) because although active *in vitro* it was not given intravenously on the day of culture. Rates of inappropriate treatment were similar in survivors and non-survivors in both complete-cases and multiple imputations at both day-7 and day-30 ($p>0.2$).

Antibiotic resistance was most common to amoxicillin/ampicillin (64% for *E. coli)* and co-amoxiclav (36% overall). The most commonly used antibiotics were co-amoxiclav (32%) and piperacillin-tazobactam (30%) either alone or in combination with a second agent, usually an aminoglycoside (supplementary table 1). 34% of patients received combination therapy and this increased the activity of treatment against the organism cultured when the combination was with co-amoxiclav (27% vs 2%; $p<0.001$) and piperacillin-tazobactam (15% vs 6%; $p=0.05$).

As expected, significant potentially-confounding associations were present between patient, disease and treatment factors. Males were older (median (IQR) 73 (62-81) vs 71 (55-82) years p=0.03) and less likely to have *E. coli* bacteraemia (p<0.001). *E. coli* bacteraemias were less often nosocomial (24%), compared to *Klebsiella* spp. (40%), *Pseudomonas* spp (43%) and other Enterobacteriaceae (43%) (p<0.001). The commonest focus for *E. coli* bacteraemia was the urinary tract (58%) whereas for other GNB non-urinary foci predominated (*Klebsiella* spp. 63%, *Pseudomonas* spp. 67%. and other Enterobacteriaceae 62%) (p<0.001). At the time blood was taken for culture, median NEWS score was 4 (IQR 2-7;27% ≥7, when high-dependency transfer should be considered). Patients with *E. coli* bacteraemia had slightly lower NEWS score than other patients (median 4 (IQR 2-6) vs 5 (2-7), p=0.05). Patients with a urinary tract or line-related bacteraemia were less acutely unwell at presentation with 23% and 19% having NEWS ≥7 respectively, compared with 53% of patients with lower respiratory tract infection (p=0.006). Among baseline laboratory tests, only C-reactive protein (CRP) varied significantly by causative organism (p<0.001); being higher in patients with *Pseudomonas* spp. bacteraemia (median 180mg/dL (IQR 81-269) compared with 129mg/dL (IQR 58-202) for other bacteraemias (p=0.01). There was no evidence that appropriateness of treatment varied across species (p=0.7). NEWS score was slightly higher overall in those who received appropriate antibiotics (median (IQR) 4 (3-7) vs 4 (2-6) in those who did not (p=0.02). Among 143 patients who had a NEWS score ≥7, 7-day mortality was 12/100 (12%) for patients who received appropriate treatment and 4/43 (9%) for patients who did not (p=0.7); 30-day mortality was 23/113 (20%) versus 6/42 (14%) respectively (p=0.5).

In multivariable analysis adjusting for these inter-relationships, older age, higher NEWS score and higher CRP independently predicted greater 7-day and 30-day mortality (Table 2). In addition, patients with neutropenic sepsis were at increased risk of 7-day mortality. Higher Charlson score and neutrophil count, lower platelets, nosocomial onset, lower respiratory tract focus and onset of symptoms after blood cultures were taken also independently predicted a death at 30-days but not 7-days. Inappropriate empiric antibiotic therapy was not associated with mortality at either time point (adjusted OR=0.82 (95% CI 0.35-1.94) and 0.92 (0.50-1.66) respectively). There was no evidence of interactions between empiric therapy and other factors

for 7-day or 30-day mortality (p>0.08) except for 30-day mortality and neutrophils (interaction p=0.03); whereby risk of mortality at 30 days was higher in those receiving appropriate antibiotics with higher neutrophils. To assess the possibility that excluding the nine patients who died on the day of culture had obscured a benefit of early empiric therapy, a sensitivity analysis included these patients (two received appropriate therapy, seven died before initiating antibiotics classed as inappropriate therapy; Supplementary Table 2). Inappropriate empiric antibiotic therapy was still not associated with mortality at either time point (adjusted OR=1.24 (95% CI 0.62-2.49) and 1.15 (0.69-1.24) respectively).

DISCUSSION

We have undertaken a detailed prospective observational study of patients with GNB bacteraemia assessing the importance of appropriate empiric antibiotic treatment adjusted for confounding from patient and disease factors. 8% of our patients died within 7-days and 15% within 30-days. 34% did not receive an intravenous antibiotic with *in vitro* activity against the infecting pathogen on the day of blood culture. Mortality was not higher among these patients in any adjusted or unadjusted analysis using complete-cases or multiple-imputation. The main predictors of death were patient and disease factors, particularly older age, greater burden of disease, nosocomial acquisition and greater severity of acute illness.

Our findings contrast with several studies performed in the 1990s which reported that the appropriateness of empiric therapy is a key determinant of outcome in bacteraemia (12-14). It is notable that in these studies the main factor responsible for treatment being inappropriate was delay, measured in days, rather than resistance. Prompt review and treatment adjustment 24-48 hours after culture is standard practice in the NHS and may minimise the impact of inappropriate empiric therapy. Other studies demonstrating an impact of empiric therapy in bacteraemia have been performed in populations where multidrug resistance is common (16,19,23,24) or have included both Gram-positive and Gram-negative infections, sometimes along with fungaemia (17-19,27). We have studied GNB bacteraemia specifically and in a setting where multidrug resistance is uncommon. It may be that in our study patients in the 'inappropriate' group received therapy to which the organism was resistant *in vitro* but nevertheless had some activity *in vivo*. This may be particularly relevant for co-amoxiclav where the break-point (≤8mg/L) used to define susceptibility for systemic infections lies within the distribution of MICs for *E. coli* and disc testing may over-estimate resistance compared with broth microdilution methods. Some studies have considered quinolones, if active *in vitro* and given promptly as appropriate therapy in GNB bacteraemia. However, only four patients received ciprofloxacin by mouth on the day of blood culture in our study for a ciprofloxacin sensitive infection and re-categorising these cases does not alter our findings (data not shown).

Our findings are in keeping with several recent studies performed in different populations of bacteraemic patients, which have not demonstrated an impact of empiric antibiotic therapy on

outcome. Corona *et al* found no impact of empiric treatment on mortality in 1942 critical-care patients with bacteraemia (26). Anderson reported risk factors for inappropriate therapy among 1470 community-hospital bacteraemias but found no significant association with mortality (6). In a retrospective cohort study specifically in GNB bacteraemia Cain *et al* found an effect of empiric antibiotic therapy only among patients with a high probability of death. (28). This contrast with the older literature may reflect advances in supportive care, changes in patient mix and differences in the main antibiotic classes used.

Our study has limitations. We did not confirm antibiotic susceptibilities reported by diagnostic laboratories. However, variation between sites would not be expected to obscure an impact of antibiotic susceptibility across the whole study and should be small given that all the laboratories participate in national quality assessments and are accredited by the Royal College of Pathologists. A small number of patients were not recruited at some centres but there is no reason to think these were selected or will bias our findings. We used mortality as our primary outcome measure and have not studied other potential harms of inappropriate antibiotic therapy such as worsening of symptoms, necessitating for example escalation of care. Another important limitation is the varying amount of missing data in baseline factors; a generic challenge in such studies. We used multiple imputation to avoid loss of power from analyzing only (potentially unrepresentative) complete cases, a technique which is well recognised to produce unbiased estimates when missing data depend on other observed factors (including mortality), and enabling all patients to be included in multivariable models. Some potentially useful data were not collected, such as baseline albumin and rates of escalation to critical care.

Our study has notable strengths. It is one of the largest prospective multi-centre studies defining the determinants of mortality specifically in GNB bacteraemia and gathered data prospectively in clinical real-time. In line with previous recommendations [25], we have focused on empiric, as distinct from definitive therapy, accounted for the effects of confounding factors and controlled for severity of illness in our multivariable analysis. Our data show that patient and disease factors are the primary determinants of mortality. Antibiotic treatment algorithms for acutely unwell patients should incorporate patient factors with knowledge of local antibiotic resistance data to use broader-spectrum antibiotics for those patients who need them most.

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TRANSPARENCY DECLARATIONS

The authors have no potential conflicts of interest to declare.

REFERENCES

1. Goto M, Al-Hasan MN. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect 2013; 19: 501-9.

2. de Kraker ME, Jarlier V, Monen JC *et al*. The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. Clin Microbiol Infect 2013; 19: 860-8.

3. Laupland KB. Incidence of bloodstream infection: a review of population-based studies. Clin Microbiol Infect 2013; 19: 492-500.

4. Antimicrobial resistance surveillance in Europe 2013. European Centre for Disease Prevention and Control. Available at: http://ecdc.europa.eu/en/publications/Documents/antibiotic-resistance-in-EU-summary.pdf

5. Llewelyn MJ, Hand K, Hopkins S *et al*. Antibiotic policies in acute English NHS trusts: implementation of 'Start Smart-Then Focus' and relationship with *Clostridium difficile* infection rates. J Antimicrob Chemother. 2015; 70: 1230-5.

6. Anderson DJ, Moehring RW, Sloane R *et al*. Bloodstream Infections in Community Hospitals in the 21st Century: A Multicenter Cohort Study. PlosOne. 2014; 9: e91713.

7. Policy statement on antimicrobial stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). Infect Control Hosp Epidemiol 2012; 33: 322-7.

8. Dellinger P, Levy MM, Rhodes A et al Surviving Sepsis Campaign: International Guidelines for the Management of Severe Sepsis and Septic Shock 2012. Crit Care Med. 2013; 41:580-637.

9. Paul M, Shani V, Muchtar E *et al*. Systematic review and meta-analysis of the efficacy of appropriate empiric antibiotic therapy for sepsis. Antimicrob Agents Chemother 2010; 54: 4851-63.

10. de Groot… The association between time to antibiotics and relevant clinical outcomes in emergency department patients with various stages of sepsis: a prospective multi-center study Crit Care 2015

11. Heranjic T….. Aggressive versus conservative initiation of antimicrobial treatment in critically ill surgical patients with suspected intensive-care-unit-acquired infection: a quasi-experimental, before and after observational cohort study. Lancet Infect Dis 2012; ….

12. Pedersen G, Schonheyder HC, Sorensen HT. Antibiotic therapy and outcome of monomicrobial Gram-negative bacteraemia: a 3-year population-based study. Scand J Infect Dis 1997; 29: 601-6.

13. Leibovici L, Shraga I, Drucker M *et al*. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. J Intern Med 1998; 244: 379-86.

14. Ibrahim EH, Sherman G The Influence of Inadequate Antimicrobial Treatment of Bloodstream Infections on Patient Outcomes in the ICU Setting. Chest 2000

15. Micek ST, Lloyd AE, Ritchie DJ *et al*. *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother 2005; 49: 1306-11.

16. Scarsi KK, Feinglass JM, Scheetz MH *et al*. Impact of inactive empiric antimicrobial therapy on inpatient mortality and length of stay. Antimicrob Agents Chemother 2006; 50: 3355-60.

17. Cheong HS, Kang CI, Kwon KT *et al*. Clinical significance of healthcare-associated infections in community-onset *Escherichia coli* bacteraemia. J Antimicrob Chemother 2007; 60: 1355-60.

18. Lodise TP, Jr., Patel N, Kwa A *et al*. Predictors of 30-day mortality among patients with *Pseudomonas aeruginosa* bloodstream infections: impact of delayed appropriate antibiotic selection. Antimicrob Agents Chemother 2007; 51: 3510-5.

19. Peralta G, Sanchez MB, Garrido JC *et al*. Impact of antibiotic resistance and of adequate empirical antibiotic treatment in the prognosis of patients with *Escherichia coli* bacteraemia. J Antimicrob Chemother 2007; 60: 855-63.

20. Tumbarello M, Sanguinetti M, Montuori E *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: 1987-94.

21. Marschall J, Agniel D, Fraser VJ *et al*. Gram-negative bacteraemia in non-ICU patients: factors associated with inadequate antibiotic therapy and impact on outcomes. J Antimicrob Chemother 2008; 61: 1376-83.

22. Chen HC, Lin WL, Lin CC *et al*. Outcome of inadequate empirical antibiotic therapy in emergency department patients with community-onset bloodstream infections. J Antimicrob Chemother 2013; 68: 947-53.

23. Vazquez-Guillamet C, Scolari M, Zilberberg MD *et al*. Using the number needed to treat to assess appropriate antimicrobial therapy as a determinant of outcome in severe sepsis and septic shock. Crit Care Med 2014; 42: 2342-9.

24. Hernandez C, Feher C, Soriano A *et al*. Clinical characteristics and outcome of elderly patients with community-onset bacteremia. J Infect 2015; 70: 135-43.

25. McGregor JC, Rich SE, Harris AD *et al.* A Systematic Review of the Methods Used to Assess the Association between Appropriate Antibiotic Therapy and Mortality in Bacteremic Patients. Clin Infect Dis 2007; 45: 329-37.

26. Corona A, Bertolini G, Lipman J *et al*. Antibiotic use and impact on outcome from bacteraemic critical illness: the BActeraemia Study in Intensive Care (BASIC). J Antimicrob Chemother 2010; 65: 1276-85.

27 Retamar P…. Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: a propensity score-based analysis. Antimicrob Agent Chemother 2012

28 Cain SE, Portillo MM…. Stratification of the Impact of Inappropriate Empirical Antimicrobial Therapy for Gram-Negative Bloodstream Infections by Predicted Prognosis. Antimicrob Agent Chemother 2015

29. McGinley A, Pearse RM. A national early warning score for acutely ill patients. BMJ 2012; 345: e5310.

30. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. Stat Methods Med Res 2007; 16: 219-42.

Table 1. Baseline patient characteristics and empiric antibiotic treatment according to mortality among 679 patients with GNB bacteraemia. For each variable at each time point N=the number of patients for whom data were available. Percentages are column percentages and do not always add to 100% as a result of rounding. CC=complete case analysis (p-values from χ^2 or ranksum test for categorical and continuous baseline variables) MI=multiple imputation (p-values from logistic regression adjusted for the 25 multiple imputations; imputations based on all 679 patients, results similar excluding from imputations nine patients who died on the day blood was taken for culture).

	7-day all-cause mortality (N=679)				30-day all-cause mortality (N=674)[1]			
Clinical factor	**Survivors N=627 (92%)**	**Non-survivors N=52 (8%)**	**p-value (CC)**	**p-value (MI)**	**Survivors N=573 (85%)**	**Non-survivors N=101 (15%)**	**p-value (CC)**	**p-value (MI)**
Gender	**N=626**	**N=51**			**N=572**	**N=100**		
Male	335 (53%)	34 (67%)	0.02	0.07	304 (53%)	62 (62%)	0.1	0.09
Age	**N=627**	**N=52**			**N=572**	**N=101**		
Median (IQR)	71 (58-81)	79 (69.5-83)	**<0.001**	**0.002**	70 (57-81)	79 (69.5-85.5)	**<0.001**	**<0.001**
Co-morbidity score	**N=617**	**N=49**			**N=564**	**N=97**		
Median (IQR)	6 (4-8)	7 (5-10)	**<0.001**	**0.009**	6 (4-8)	7 (6-10)	**<0.001**	**<0.001**
Organism	**N=624**	**N=52**			**N=570**	**N=101**		
E. coli	409 (66%)	28 (54%)			375 (66%)	60 (59%)		
Klebsiella spp	92 (15%)	12 (23%)	**0.03**	**0.04**	86 (15%)	17 (17%)	**0.01**	**0.02**
Pseudomonas spp	42 (7%)	8 (15%)			35 (6%)	15 (15%)		
Others[2]	81 (13%)	4 (8%)			74 (13%)	9 (9%)		
Acquisition	**N=614**	**N=49**			**N=561**	**N=97**		
Community acquired	286 (47%)	20 (42%)			269 (48%)	34 (35%)		
Healthcare associated	148 (24%)	10 (20%)	0.4	0.4	134 (24%)	23 (24%)	**0.02**	**0.02**
Nosocomial	180 (29%)	19 (39%)			158 (28%)	40 (41%)		
Focus	**N=585**	**N=43**			**N=533**	**N=90**		
Urinary without device	223 (38%)	8 (19%)			207 (39%)	22 (10%)		
Urinary with device	83 (14%)	5 (12%)			75 (14%)	13 (15%)		
Abdominal/biliary	117 (20%)	10 (23%)			107 (20%)	19 (15%)		
Respiratory	35 (6%)	8 (19%)	**0.02**	**0.006[5]**	28 (5%)	14 (33%)	**<0.01**	**0.02[5]**
Neutropenic sepsis	16 (3%)	2 (5%)			16 (3%)	2 (11%)		
No clear source	34 (6%)	5 (12%)			30 (6%)	9 (23%)		
Vascular device	25 (4%)	1 (2%)			23 (4%)	3 (12%)		
Other [3]	52 (9%)	4 (9%)			47 (9%)	8 (15%)		
Duration of symptoms	**N=471**	**N=23**			**N=435**	**N=55**		
Symptoms post-culture only	10 (2%)	-			7 (2%)	3 (5%)		
Same day	143 (30%)	9 (39%)			134 (31%)	15 (27%)		
1 day	108 (23%)	4 (17%)	0.4	0.8	98 (23%)	14 (25%)	0.4	0.4
2-4 days	137 (29%)	9 (39%)			127 (29%)	19 (35%)		
5-7 days	32 (7%)	1 (6%)			30 (7%)	2 (4%)		
>7 days	41 (9%)	-			39 (9%)	2 (4%)		
Clinical disease severity								
NEWS score	**N=511**	**N=38**			**N=469**	**N=75**		
Median (IQR)	4 (2-7)	6.5 (4-9.3)	**<0.001**	**<0.001**	4 (2-6)	5 (3-8)	**<0.001**	**<0.001**
WCC	**N=613**	**N=50**			**N=560**	**N=98**		
($x10^9$/L) Median (IQR)	11.8 (7.7-16.8)	13 (7.4-20.7)	0.5	[6]	11.8 (7.6-16.3)	12.6 (8-22.5)	0.08	[6]

Neutrophil count	**N=589**	**N=49**			**N=539**	**N=34**		
($x10^9$/L) Median (IQR)	10.4 (6.4-14.8)	10.7 (5.3-18.9)	0.8	0.5	10.3 (6.2-14.6)	11.2 (6.7-19.5)	0.09	**0.002**
Platelet count	**N=610**	**N=50**			**N=558**	**N=97**		
($x10^9$/L) Median (IQR)	196 (134-273)	191 (109-286)	0.9	0.5	198 (134-271)	179 (109-291)	0.4	0.3
CRP	**N=590**	**N=47**			**N=539**	**N=93**		
(mg/dL) Median (IQR)	132 (56-205)	151 (81-287)	**0.04**	**0.003**	129 (55-202)	146 (71-261)	0.06	**0.009**
Creatinine	**N=609**	**N=50**			**N=556**	**N=98**		
(µmol/L) Median (IQR)	105 (74-163)	161 (91-246)	**<0.001**	**0.03**[7]	104 (73-161)	152 (87-225)	**<0.001**	**0.04**[7]
Initial antimicrobial therapy[4]	**N=582**	**N=34**			**N=532**	**N=79**		
Inappropriate	201 (35%)	9 (26%)	0.2	0.4	182 (34%)	26 (33%)	0.5	0.8

[1]Data for survival at 30 days were missing for five patients who are excluded from the CC analysis, but included in the MI analysis. [2]Including *Morganella* spp., *Serratia* spp., *Enterobacter* spp., *Proteus* spp. and *Citrobacter* spp. [3]Including any other focus. [4]Nine patients died on the day of blood culture collection and are excluded from comparisons of this factor; P=0.8 (7-day) and 0.6 (30-day) including these patients in MI analyses. [5]Focus considered with 6 categories in multiple imputation due to small numbers in individual categories leading to unstable imputations (urinary, abdominal/biliary, respiratory, neutropenic sepsis, no clear source, other). [6]Spearman correlation 0.96 between neutrophils and WCC so only neutrophils used in imputation models. [7] P=0.002 (7-day) and 0.001 (30-day) for inverse square-root transformed creatinine (the best-fitting univariable polynomial transformation).

Table 2: Independent (multivariable) predictors of all cause mortality at 7- and 30-days post GNB bacteraemia by multiple imputation (N=670).

Clinical factor	7-day all cause mortality		30-day all cause mortality	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Age (per 10 years older)	1.54 (1.11-1.97)	0.002	1.47 (1.15-1.80)	<0.001
Charlson score (per point higher)			1.13 (1.03-1.25)	0.01
NEWS score (per point higher)	1.26 (1.13-1.40)	<0.001	1.15 (1.05-1.25)	0.002
Neutrophil count (per 1 x 10^9/l higher)			1.05 (1.01-1.09)	0.009
CRP (per 10 mg/dl higher)	1.05 (1.02-1.08)	0.003	1.03 (1.01-1.06)	0.02
Platelet count (per 50 x 10^9/l higher)			0.86 (0.76- 0.97)	0.02
Acquisition:				
Community acquired			1.00	
Healthcare associated			1.37 (0.70-2.70)	0.36
Nosocomial			2.35 (1.24-4.43)	0.008
Focus:				
Urinary	1.00		1.00	
Abdominal/Biliary	2.07 (0.78-5.45)	0.14	1.37 (0.68-2.78)	0.38
Respiratory	2.90 (0.89-9.43)	0.08	3.32 (1.35-8.19)	0.009
No clear source	0.98 (0.18-5.33)	0.98	1.27 (0.42-3.81)	0.68
Neutropenic sepsis	8.29 (1.36-50.5)	0.02	3.17 (0.56-18.1)	0.19
Others[1]	2.66 (0.82-8.63)	0.10	2.05 (0.86-4.90)	0.11
Days from symptoms to blood culture:				
Symptoms after culture only			4.69 (1.01-21.8)	0.05
Same day			1.00	
1 day			1.34 (0.58-3.09)	0.49
2-4 days			1.32 (0.57-3.08)	0.51
5-7 days			0.66 (0.20-2.16)	0.49
Empiric therapy:				
Appropriate	1.00		1.00	
Inappropriate	0.82 (0.35-1.94)	0.66	0.92 (0.50-1.66)	0.77
Adjusted difference in the absolute percentage mortality between inappropriate vs appropriate empiric therapy (- means lower in inappropriate) [2]	-0.4% (-2.0%,+1.3%)		-0.3% (-2.5%,+1.9%)	

[1] Including any other focus. Note: Excluding nine patients who died on the day of blood culture (see Supplementary Table 2 for sensitivity analyses including these patients in the imputations and multivariable models). There was no independent impact on 7- or 30-day mortality of organism (p=0.4/0.7), gender (p=0.5/0.7), creatinine (p=0.1/0.2); and no independent impact of age-adjusted co-morbidity score (p=0.3), neutrophils (p=0.6), platelets (p=1.0), acquisition (p=0.6) or days of symptoms (p=0.8) on 7-day mortality. There was no evidence of interactions between empiric therapy and other factors for 7-day (p>0.15) or for 30-day mortality (p>0.08) except for 30-day mortality and neutrophils (interaction p=0.03); whereby risk of mortality at 30 days was higher in those receiving appropriate antibiotics if baseline neutrophils was >11, and higher in those receiving inappropriate antibiotics if baseline neutrophils was <11.

[2] Calculated from the coefficients of the regression model at the median/mode of other included factors, see supplementary material. Unadjusted difference in the absolute percentage mortality between inappropriate vs appropriate empiric therapy -2.0% (-6.5%,+2.4%) at 7-days and -0.6% (-6.6%,+5.4%) at 30 days.