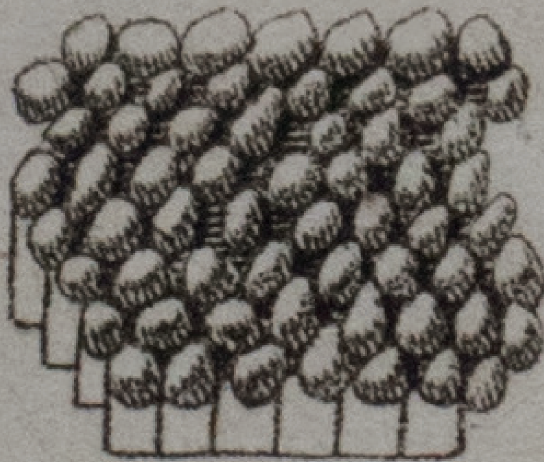
CONTROL OF MULTIDRUG-RESISTANT MICROORGANISMS IN DUTCH HEALTHCARE SETTINGS



VERONICA WETERINGS

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Control of Multidrug-Resistant Microorganisms In Dutch Healthcare Settings

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VOOR MIJN PA EN MA

Aut viam inveniam aut faciam

- Hannibal -

TABLE OF CONTENTS

Chapter 1	General introduction	11
Chapter 2	Prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* in patients at hospital admission in The Netherlands, 2010-2017: an observational study	23
Chapter 3	Next-generation sequence analysis reveals methicillin-resistance transfer to a methicillin-susceptible *Staphylococcus aureus* (MSSA) strain that subsequently caused a methicillin-resistant *Staphylococcus aureus* (MRSA) outbreak: a descriptive study	41
Chapter 4	Management of a hospital-wide vancomycin-resistant *Enterococcus faecium* outbreak in a Dutch general hospital, 2014-2017: successful control using a restrictive screening strategy	57
Chapter 5	An outbreak of colistin-resistant *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* in the Netherlands (July to December 2013), with inter-institutional spread	81
Chapter 6	Evaluation of an in vitro model with a novel statistical approach to measure differences in bacterial survival of extended-spectrum beta-lactamase-producing *Escherichia coli* on an inanimate surface	99
Chapter 7	Duration of colonization with extended-spectrum beta-lactamase-producing *Escherichia coli*: results of an open, dynamic cohort study in Dutch nursing home residents (2013 – 2019)	111
Chapter 8	Summary and general discussion	133
Closing pages	Nederlandse samenvatting	146
	Contributing authors	154
	Curriculum Vitae	157
	Publications	158
	Dankwoord	160

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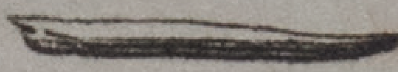

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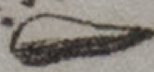
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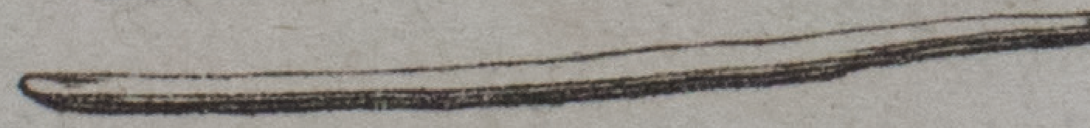
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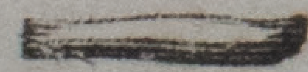
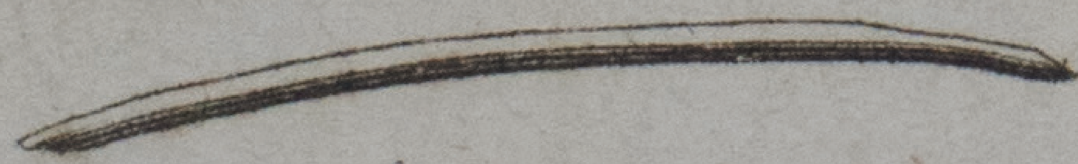

Chapter 1

GENERAL INTRODUCTION

"... But in great hospitals especially, it prevails at all times and is a real gangrene; it has been named the Hospital Gangrene. ... Let him bear this always in mind, that no dressings ever have been found to stop this ulcer; that no quantities of wine or bark which a man can bear have ever retarded this gangrene; let him bear in mind that this is a hospital disease; that without the circle of the infected walls the men are safe; let him, therefore, hurry them out of this house of death ... let him lay them in a schoolroom, a church, on a dunghill or in a stable ... let him carry them anywhere but to their graves."

John Bell (Principles of Surgery, 1801)

Outbreaks and (cross) infections have been a problem throughout the ages in healthcare settings [1]. In the old days, hospitals were crowded, dirty, and poorly ventilated. Hospital mortality was significant due to the high rate of (wound) infections, and in this period (before the introduction of antibiotics), there was no effective treatment for infections, and doctors could do little for patients but wait and hope.

In the period of late 1800s, more knowledge on the area of bacteriology came through, particularly by Koch and Pasteur. Based on this new knowledge, Lister developed the concept of surgical asepsis that decreased post amputation mortality rates. Moreover, with implementation of the Florence Nightingale's guidelines with recommendations on sanitation and hospital environment, cross-infection in most hospitals began to be controlled [2].

In the first half of the 20th century, there was a great turning point in medical history with the entrance to the antibiotic era. To begin with the discovered of penicillin by Fleming in 1928. In addition, the first successfully treated patient with penicillin in 1941 by Howard and colleagues. During the Second War, penicillin was mass-produced and referred to as 'the wonder drug' as it considerably increased the chance of recovery for wounded soldiers and lessened their suffering [3].

In the following years, many additional antibiotic agents, belonging to new classes, were discovered and introduced into daily clinical practice. This resulted in a major decline in bacterial infection-related mortality and morbidity and enabled the performance of complicated operations and other invasive procedures. Therefore, antibiotics were seen at first as to be the final answer to hospital (cross) infection [4].

ANTIBIOTIC RESISTANCE

Unfortunately, the high expectations of antibiotics could not be fulfilled as bacteria have evolved multiple mechanisms that cause resistance to antibiotics. Some of these main mechanisms are changes in the cell wall that decrease the penetration of antibiotics, creation of efflux pumps that transfer antibiotics outside the cell and the production of enzymes that degrade or modify antibiotics. These resistance mechanisms are spread among bacteria by processes such as genetic recombination and transfer of mobile genetic elements, including plasmids, integrons and transposons [5].

Antimicrobial resistance is rising to dangerously high levels in all parts of the world, leading to higher medical costs, prolonged hospital stays and is associated with increased morbidity and mortality [6]. Still, The Netherlands is one of the countries with the lowest antibiotic resistance rates in clinical isolates in Europe [7]. This is partly because of the prudent and restrictive use of antibiotics [8]. In addition, effective infection prevention policy in healthcare settings based on national guidelines.

MULTIDRUG-RESISTANT MICROORGANISMS (MDRO)

The criteria for defining MDRO can vary between countries [9]. In The Netherlands MDRO are defined as microorganisms which are known to cause disease, have acquired an antimicrobial resistance pattern that hampers (empirical) therapy and have the potential to spread if no transmission-based precautions are taken [10].

Examples of MDRO include Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E), and carbapenemase-producing *Enterobacteriaceae* (CPE).

Methicillin-resistant Staphylococcus aureus

Resistance to methicillin in *Staphylococcus aureus* is caused primarily by the *mecA* gene, which is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*) [11]. Horizontal transfer of this element is an important factor in the emergence of new clones of MRSA [12].

The first case of MRSA was described in the United Kingdom in 1961 [13], and since then MRSA has disseminated globally and become a leading cause of bacterial infections in both healthcare and community settings [14].

In 1970s, MRSA was responsible for large hospital outbreaks in Europe (so called healthcare-associated MRSA) and by the late 1980s in the United States [15]. In The Netherlands, after a series of MRSA outbreaks in Dutch hospitals in the early 1980s, the so-called Search and Destroy policy was implemented [16]. This policy relies on active screening of high-risk groups for MRSA carriage upon hospital admission and (pre-emptive) isolation and treatment of carriers. Studies have shown that this approach is highly successful and effective at maintaining a low prevalence of MRSA in Dutch hospitals [17,18].

In the 1990s, a substantial change in MRSA epidemiology was observed when the bacteria was detected in individuals without previous healthcare contact (community-associated MRSA) [19]. These cases affected younger individuals, ethnic minorities, and often involved severe skin or soft tissue infections [20]. Since the mid-2000s, MRSA has also been associated with livestock exposure (livestock-associated MRSA, LA-MRSA) [21]. This LA-MRSA was primarily detected in those who work with livestock, but in recent years LA-MRSA has also been observed among the general population [22–24].

Some reports suggest that the prevalence of MRSA patients without known risk factors in the community might be increasing [22]. Such an increase could result in undetected introduction of MRSA carriers into the hospital, and increase the risk of nosocomial spread to patients and healthcare workers because preventive measures are not taken.

Vancomycin-resistant enterococci

Vancomycin resistance in enterococci involve genetic elements, including *vanA*, *B*, *C*, *D*, *E*, *G*, *L*, *M* and *N*. The variants of *vanA*, and *vanB* are the most prevalent in clinically relevant isolated species [25] and are usually associated with mobile genetic elements such as transposons [26].

Initially enterococci have been considered low-virulent pathogens, however they have become an important cause of healthcare-associated infections especially in immunocompromised patients [27]. The first vancomycin-resistant enterococci was reported in Europe in 1988, and similar strains were later detected in the United States. Since then vancomycin-resistant enterococci (VRE) have been reported worldwide [28].

Transmission of VRE poses a problem, as only few second-line antibiotics are available for the treatment of VRE infections. Strategies to prevent transmission include screening of contact patients and isolation precautions of (suspected) VRE carriers [29,30]. The Netherlands has a national control strategy of highly resistant microorganisms including

VRE [31]. However, there is an ongoing debate about the general burden of VRE infections, the population at risk and the costs involved with the screening large number of patients.

Extended spectrum beta-lactamase-producing Enterobacteriaceae

Extended spectrum beta-lactamases (ESBL) are (usually plasmid-mediated) enzymes produced by a variety of Gram-negative bacteria. ESBLs inactivate beta-lactam antibiotics by hydrolysis and cause resistance to various types of beta-lactam antibiotics, including the expanded-spectrum (or third-generation) cephalosporins and monobactams [32].

The first plasmid-encoded ESBL was described in Germany in 1983 [33]. Since then more than 300 different ESBLs have been described, whereby *Klebsiella pneumoniae* and *Escherichia coli* remain the major ESBL-producing organisms isolated worldwide [34]. Most ESBLs can be divided into three groups: TEM, SHV, and CTX-M types. During the 1990s, TEM and SHV were dominant among ESBLs all over the world and largely found in *Klebsiella* spp. This situation has changed since the start of the 21st century, with CTX-M as the most prevalent ESBLs in many European countries, and with *Escherichia coli* in addition to *K. pneumoniae* as a major host [35].

The prevalence of ESBL-producing Enterobacteriaceae (ESBL-E) carriage has increased rapidly, even in countries known for prudent antibiotic use [8]. Consequently, ESBL-E has become a major concern worldwide, as treatment options are limited and many patients need the ‘last resort’ antibiotics treatment such as carbapenems to treat infection caused by ESBL-E.

The Dutch guideline for prevention of transmission of MDRO in hospitals recommends contact precautions for patients colonised or infected with ESBL-E [10]. However, the effectiveness of contact isolation for preventing transmission of ESBL-E has been questioned, especially in settings where ESBL-E is prevalent in the community and thus at hospital admission [36].

Carbapenemase-producing Enterobacteriaceae

Carbapenemases are a diverse group of beta-lactamases that are active against not only expanded-spectrum cephalosporins and monobactams, but also against the carbapenems. Carbapenemases are found on mobile genetic elements, such as plasmids, integrons, insertion sequences and transposons [37].

The first carbapenemase in Enterobacteriaceae was the chromosomally encoded Nmc that was identified in 1993 [38]. More recently, carbapenemase genes have emerged that are easily transferred on mobile elements among species. Carbapenemases are divided into different classes, depending on the structure of the enzyme and the hydrolytic mechanism at the active site [39]. Well-known carbapenemase are *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi Metallo- β-Lactamase (NDM) and oxacillinase (OXA).

CPE transmission may occur in both healthcare and community settings [41,42]. To control the spread of CPE in healthcare settings, the Dutch guidelines recommend risk-based screening and pre-emptive isolation (contact precautions in a single-bed room) for each patient upon admission [30].

Carbapenems are usually regarded as the last treatment option for serious infections caused by multidrug-resistant bacteria. However, with the widespread use of carbapenem antibiotics, the prevalence of CPE has increased rapidly and worldwide, and has become a serious and global threat as patients - infected with CPE - have limited treatment options and high mortality rates [40].

ANTIBIOTIC RESISTANCE AND INFECTION CONTROL

Antibiotic resistance is accelerated by the misuse and overuse of antibiotics, as well as poor infection prevention and control. The main purpose of infection prevention and control is to reduce the number of (nosocomial) infections, thereby reducing the need for antibiotics. The second purpose is to limit the spread of microorganisms including MDRO. Consequences of infections caused by MDROs can be severe, and include increased mortality and readmissions [43]. Effective infection control practices are crucial to limit emergence of antibiotic resistance and improve patient safety.

AIMS OF THESIS

The aim of this thesis is to provide an overview of the interventions that were implemented to investigate and control MRSA, VRE and CPE outbreaks in Dutch healthcare setting. It further aims to develop and evaluate a model to measure differences in bacterial survival of ESBL-producing *Escherichia coli* on an inanimate surface, and to assess whether survival depended on sequence type and suspension fluid. Finally, this thesis aims to assess the duration of rectal ESBL- producing *E. coli* colonisation, and compare duration of colonisation for ESBL-ST131 versus ESBL-non-ST131.

OUTLINE OF THESIS

Chapter 2 describes a study in a Dutch teaching hospital at which 30,000 people were screened for *Staphylococcus aureus* carriage upon hospital admission between 2010 and 2017.

Chapter 3 describes next generation sequence investigation of methicillin-resistance transfer to a methicillin-susceptible *Staphylococcus aureus* that resulted in a MRSA outbreak.

Chapter 4 describe the successful control of a vancomycin-resistant *Enterococcus faecium* outbreak in a Dutch general hospital using a more restrictive screening and isolation policy than recommended in the national guidelines.

Chapter 5 describes an outbreak of KPC-producing *Klebsiella pneumoniae* ST258 with inter-institutional spread between a hospital and a nursing home in the Netherlands.

Chapter 6 reports an in vitro model with a novel statistical approach to accurately measure differences in bacterial survival of extended-spectrum beta-lactamase-producing *Escherichia coli* on an inanimate surface, and assessed whether survival depended on sequence type (ST131 and ST10) and suspension fluid (water, saline and sheep blood).

Chapter 7 evaluates the median time to clearance of rectal extended-spectrum beta-lactamase-producing Escherichia coli carriage in Dutch residents over a period of six years, and estimates the influence of a possible bias when follow up is started during an outbreak

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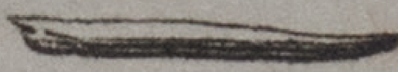

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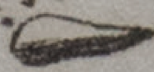

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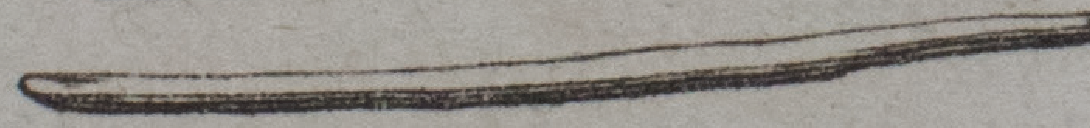

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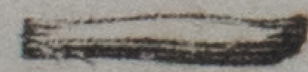
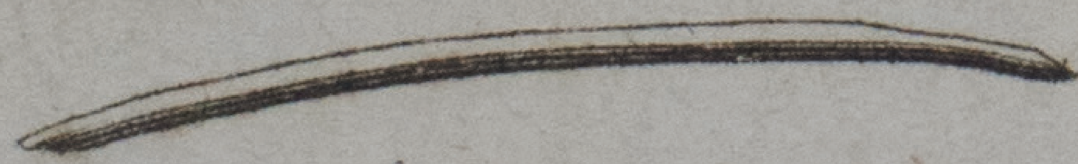



Chapter 2

PREVALENCE OF NASAL CARRIAGE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PATIENTS AT HOSPITAL ADMISSION IN THE NETHERLANDS, 2010-2017: AN OBSERVATIONAL STUDY

Veronica Weterings
Jacobien Veenemans
Miranda van Rijen
Jan Kluytmans

Clinical Microbiology and Infection 2019;25(11):1428.e1-1428.e5.

ABSTRACT

Objectives

We determined the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage upon hospital admission, among patients who were screened preoperatively for nasal *Staphylococcus aureus* carriage between 2010 and 2017. We also aimed to evaluate the prevalence of MRSA carriers without the standard risk factors for MRSA carriage.

Methods

We conducted an observational study to determine the prevalence of MRSA nasal carriage among patients who were screened preoperatively for nasal *S. aureus* carriage between 2010 and 2017. Samples of cardiothoracic patients were tested by polymerase chain reaction (PCR), other samples were cultured using chromogenic agar plates. A Poisson regression model with robust error variance was used to assess whether there was a trend in the prevalence of MRSA over time.

Results

In total, 31,093 nasal swabs were obtained from 25,660 patients. Three-hundred and seventy-five swabs (1.2%) had an invalid result. Therefore, 30,718 swabs (98.8%) were included in our analysis. Overall, *S. aureus* was detected in 7,981/30,718 patients (26.0%; 95% CI 25.5–26.5%) of whom 41 were MRSA (0.13%; 95% CI 0.10–0.18%). The MRSA prevalence varied from 0.03% to 0.17% over the years without evidence of a changing trend over time ($p = 0.40$). Results of the questionnaire revealed that 30 of the 41 patients (73.2%) had no known risk factors for MRSA carriage (0.10%; 95% CI 0.07–0.14%).

Conclusion

Our study revealed a sustained low prevalence of MRSA carriage upon hospital admission over 7 years. This supports the effectiveness of the Dutch Search and Destroy policy, in combination with a restrictive antibiotic prescription policy.

INTRODUCTION

Countries that have implemented the so-called Search and Destroy policy in combination with a prudent use of antibiotics have maintained low endemic levels of methicillin-resistant *Staphylococcus aureus* (MRSA) over the years [1–4]. This Search and Destroy policy relies on active screening of high-risk groups upon hospital admission and (pre-emptive) isolation and treatment of carriers [5] **(Supplementary Table S1)**.

In The Netherlands the Search and Destroy policy was implemented in the 1980s and studies conducted in the subsequent decades have shown that the proportion of Dutch patients colonised with MRSA upon hospital admission was low (0.03% in 1999 [6], and 0.11% in 2005-2007 [2]). Since then, several new risk groups have been added to the national guidelines. For example, livestock in 2006 and household members and partners/caretakers of MRSA carriers in 2012. It has been suggested that, in addition to the known risk groups, the prevalence of MRSA in the community might be increasing [7]. Also, the national report on the on the use of antibiotics and trends in antimicrobial resistance in The Netherlands from 2017 shows that in clinical samples, 82% (679/833) of all new MRSA cases registered at the National Reference Laboratory, none of the known risk factors for MRSA carriage could be identified [4]. Such an increase in community prevalence could result in undetected introduction of MRSA carriers into the hospital, and increase the risk of nosocomial spread to patients and healthcare workers because preventive measures are not taken.

The objective of our study was to determine the prevalence of MRSA nasal carriage upon hospital admission, among patients who were screened preoperatively for nasal *S. aureus* carriage between 2010 and 2017. We also aimed to evaluate the prevalence of MRSA carriers without the standard risk factors as described in the Dutch national guidelines [5].

METHODS

This observational study was performed in the Amphia Hospital Breda, a large teaching hospital in the south of The Netherlands. The study population consisted of patients who underwent a routine preoperative screening for *S. aureus* nasal carriage as part of the hospital's programme to prevent surgical site infections, between 1 January 2010 and 31 December 2017. Screening was performed on the day of admission in all patients undergoing cardiothoracic surgery, and at the outpatient clinic in those undergoing orthopaedic or vascular surgery, interventional cardiology and those receiving a spinal cord stimulator (SCS). All *S. aureus* carriers received treatment with mupirocin nasal ointment and chlorhexidine soap perioperatively [8].

Nasal swabs were collected during preoperative assessments using the eSwab medium (Copan, Murrieta, CA). Samples of interventional cardiologic, orthopaedic, vascular patients and patients receiving an SCS were cultured using chromogenic agar plates (CHROMagar *S. aureus*, bioMérieux, France) and 5% sheep blood agar. For cardiothoracic patients, samples were tested by polymerase chain reaction (PCR) (GeneXpert SA Nasal Complete, Cepheid, CA, USA), as rapid results were required in this population. When PCR indicated an MRSA-positive result, the original nasal swabs were cultured on 5% sheep blood agar to obtain the *S. aureus* isolate.

Presumptive *S. aureus* colonies were confirmed with a latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd, UK), and DNase (DNase agar; Oxoid Ltd, UK). Antibiotic susceptibility testing was performed using an automated system (Vitek 2, bioMérieux, France). Resistance to cefoxitin was measured using the cefoxitin disk diffusion method according to EUCAST standards [9] and MRSA confirmation was completed by detecting staphylococcal protein A (*spa*), the gene for methicillin resistance (*mec*A), and staphylococcal cassette chromosome (SCC*mec*) by PCR (Xpert SA Nasal Complete, Cepheid, CA, USA).

Detection of the Pantone Valentine leukocidin (PVL) gene and molecular typing using Multiple Loci Variable Number Tandem Repeat Analysis (MLVA) were performed by The Netherlands National Institute for Public Health and the Environment, which functions as the Dutch national reference centre for MRSA.

When nasal carriage of MRSA was detected, a questionnaire was conducted by .the infection control team to assess the presence of potential risk factors for MRSA carriage, as defined in the national MRSA guidelines by the Dutch Working party on Infection Prevention (WIP) [5].

For each year, prevalence and confidence intervals (CI) were calculated in Stata software (vs 15, StataCorp, College Station, TX, USA). To assess whether there was a trend in the prevalence of MRSA over time, we used a Poisson regression model with robust error variance [10] in which we adjusted for the test method used.

RESULTS

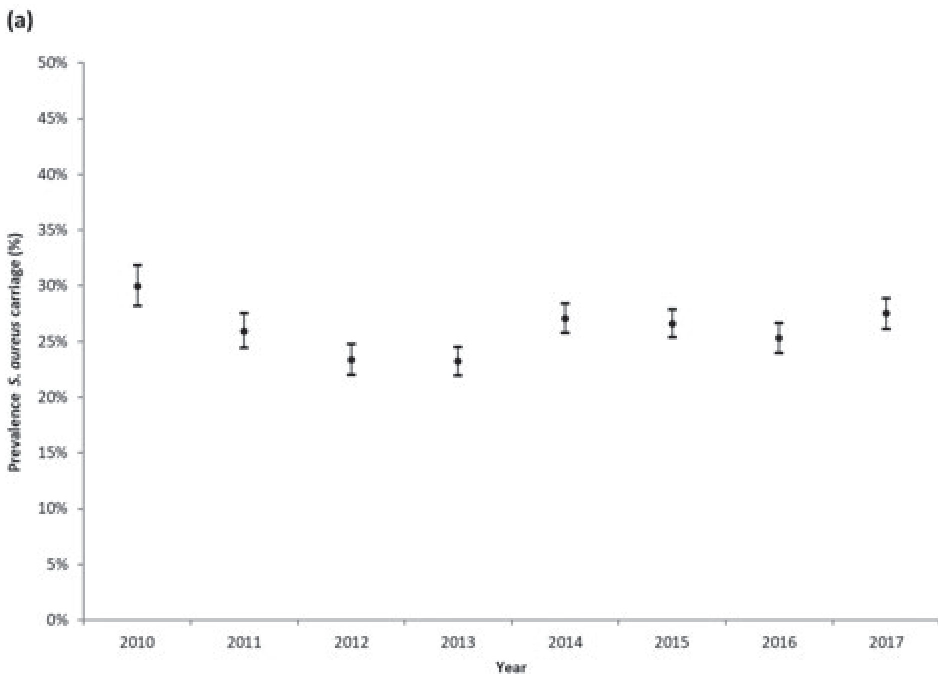
In total, MRSA carriage was assessed in 31,093 admissions (of 25,660 patients). In 375 tests (1.2%) no result could be obtained because either the Xpert gave an invalid result (n = 359) or no growth was observed on the sheep blood agar (n = 16). Therefore, 30,718 admissions (98.8%) were included in our analysis **(Table 1)**. The number of operations, and thus the number patients screened, was highest for cardiothoracic surgery/interventional cardiology and orthopaedics. The proportion of patients tested by PCR decreased over the years from 100% in 2010 to 39% in 2017.

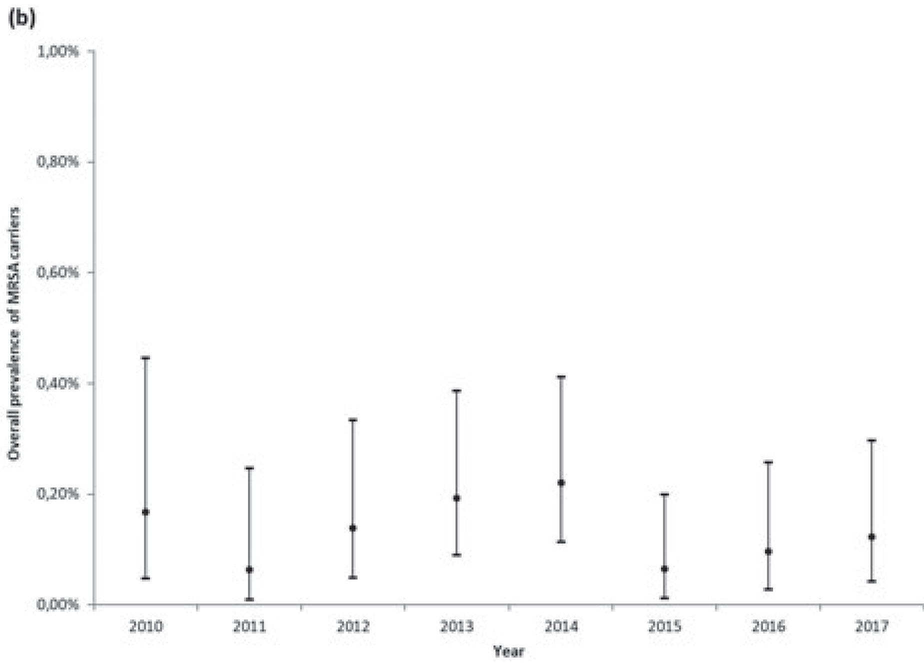
Table 1: sample distribution and characteristics of the preoperative nasal screening for *S. aureus* nasal carriage, 2010-2017

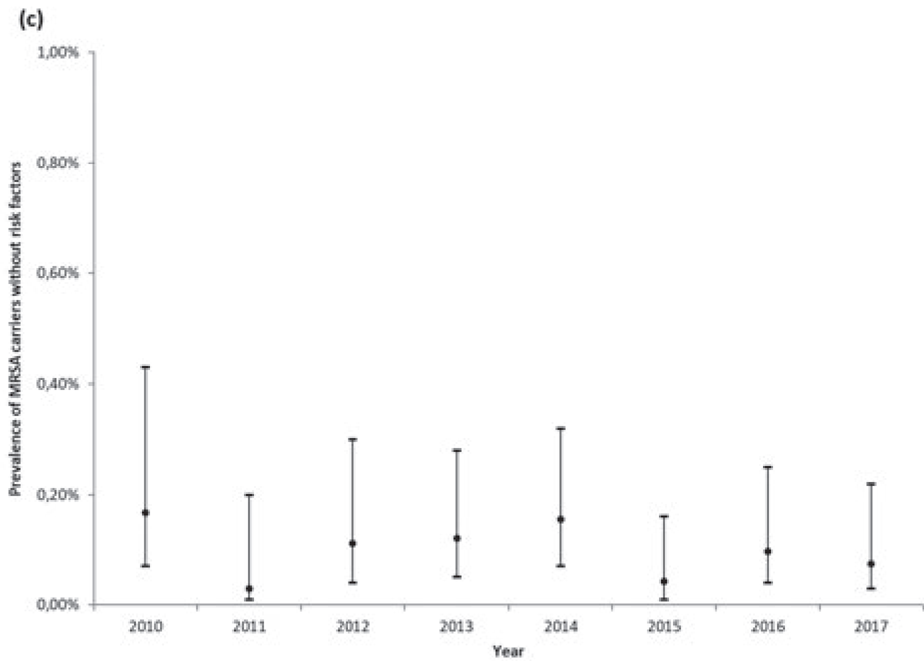
	Year								
	2010	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2010-2017**
No. of screening samples	2619	3190	3630	4182	4569	4663	4170	4070	31093
No. of screening samples excluded (%)	228 (8.7)	23 (0.7)	18 (0.5)	23 (0.5)	41 (0.9)	25 (0.5)	12 (0.3)	5 (0.1)	375 (1.2)
No. of screening samples included (%)	2391 (91.3)	3167 (99.3)	3612 (99.5)	4159 (99.5))	4528 (99.1)	4638 (99.5)	4158 (99.7)	4065 (99.9)	30718 (98.8)
No. of unique patients	2308	2984	3407	3903	4286	4387	3918	3811	25660
- *Age in years, median (range)*	69 (13-94)	68 (13-99)	69 (15-102)	69 (10-99)	69 (11-98)	69 (13-99)	70 (9-100)	71 (15-98)	69 (9-102)
- *No. of male (%)*	1385 (57.9)	1608 (50.8)	1873 (51.9)	2286 (55.0)	2411 (53.2)	2493 (53.8)	2209 (53.1)	2241 (55.1)	16506 (53.7)
Method									
- *PCR (%)*	2308 (100)	1711 (57.3)	1428 (41.9)	1021 (26.2)	884 (20.6)	561 (12.8)	454 (11.6)	396 (10.4)	8763 (30.2)
- *Culture (%)*	0 (0)	1273 (42.7)	1979 (58.1)	2882 (73.8)	3402 (79.4)	3826 (87.2)	3464 (88.4)	3415 (89.6)	20241 (69.8)
No. of patients included per discipline (%)									
- *Cardiothoracic surgery/ interventional cardiology*	1186 (51.4)	1420 (47.6)	1556 (45.7)	1835 (47.0)	1997 (46.6)	2076 (47.3)	1721 (44.0)	1723 (45.2)	13514 (46.6)
- *Orthopaedic*	852 (36.9)	1527 (51.2)	1778 (52.2)	1723 (44.1)	1932 (45.1)	1911 (43.6)	1814 (46.3)	1719 (45.1)	13256 (45.7)
- *Vascular surgery*	270 (11.7)	37 (1.2)	10 (0.3)	178 (4.6)	216 (5.0)	257 (5.9)	256 (6.5)	260 (6.8)	1484 (5.1)
- *Spinal cord stimulator*	0 (0.0)	0 (0.0)	63 (1.8)	167 (4.3)	141 (3.3)	143 (3.4)	127 (3.2)	109 (2.9)	750 (2.6)
MRSA carriers, total	4	2	5	8	10	3	4	5	41
- *With risk factors*	0	1	1	3	3	1	0	2	11
- *Without known risk factors*	4	1	4	5	7	2	4	3	30

Overall, upon hospital admission *S. aureus* was detected in 7,981/30,718 patients (26.0%; 95% CI 25.5 - 26.5%) of whom 41 were MRSA (0.13%; 95% CI 0.10 - 0.18%) **(Figure 1 a, b)**. The proportion of MRSA among *S. aureus* isolates was 0.50% (95% CI 0.37 - 0.70%). The MRSA prevalence varied from 0.03% to 0.17% over the years, without evidence of a changing trend in MRSA prevalence over time (p = 0.40). There was no evidence of a difference in prevalence between the departments **(Supplementary Figure S2)**. Of the 41 MRSA carriers who were identified, 30 (73.2%) had no known risk factor for MRSA carriage as defined in the national guidelines [5]. The overall prevalence of MRSA carriers without risk factors upon hospital admission was 0.10% (95% CI 0.07 - 0.14%) **(Figure 1c)**.

Figure 1: prevalence of *Staphylococcus aureus* (a), methicillin-resistant *Staphylococcus aureus* (MRSA) (b) and MRSA without risk factors (c) nasal carriage upon hospital admission between 2010 and 2017 with 95% confidence interval.




(b)
1,00%
0,80%
0,60%
0,40%
0,20%
0,00%
Overall prevalence of MRSA carriers
2010
2011
2012
2013
2014
2015
2016
2017
Year


(c)
1,00%
0,80%
0,60%
0,40%
0,20%
0,00%
Prevalence of MRSA carriers without risk factors
2010
2011
2012
2013
2014
2015
2016
2017
Year

Among the patients without risk factors molecular typing grouped the MRSA isolates into seven MLVA clonal complexes: eight isolates (26.7%) belonged to MC0005; five (16.7%) to MC0398, representing livestock-associated MRSA; four (13.3%) to MC0022; four (13.3%) to MC0045; three (10.0%) to MC0030; two (6.7%) to MC0008; one (3.3%) to MC0001 and MC0621 each. The remaining two isolates were MLVA singletons (MCnone). Three isolates were positive for the PVL gene (7.3%) **(Supplementary Table S3)**.

DISCUSSION

In this large study among more than 30,000 patients were screened upon hospital admission between 2010 and 2017, the overall prevalence of nasal carriage of MRSA was 0.13%. We found no evidence of an increase of this prevalence over the years. This prevalence is lower than the reported MRSA prevalence at admission in several reports from other countries (range 1.1 - 7.3%) [11–16] and similar to the prevalence reported in a study by Bode et al. who screened all patients (surgical and non-surgical, n = 6000) prior to admission in five Dutch hospitals in 2005-2007, and reported a prevalence of 0.11% [2].

Our study showed that the majority of the MRSA carriers (30/41 patients; 73.2%) had no known risk factor for MRSA carriage (overall prevalence of 0.10%), whereby the prevalence of MRSA carriers without risk factors remained constant over the years. More than 10 years ago the above-mentioned study by Bode et al. found a similar proportion of carriers without risk factors for MRSA carriage (five out of seven MRSA carriers; 71%) [2], thus this proportion remained stable over the last decade. It should be noted that the MRSA risk profile from our study population (elderly patients screened upon admission pre-operatively in a rural area in The Netherlands) might differ from that of the general population in The Netherlands, and from other hospital settings including those reported in above-mentioned studies. Our study included predominantly patients undergoing cardiothoracic or orthopaedic procedures, whereas the study by Wertheim et al. screened all patients upon admission to non-surgical departments [6].

Molecular typing grouped the 41 MRSA isolates in to seven different MLVA complexes, with MC0005 and MC0398 as the predominant complexes. The distribution of the MLVA complexes are in line with the distribution found in the Dutch national MRSA surveillance program [4] **(Supplementary figure S4**), and include widely disseminated international clones [17,18]. Although MLVA complex 5 (MC0005) is traditionally hospital-associated, eight of 30 patients without known risk factors carried this complex without having been admitted to a foreign hospital in the past 2 months. Whether MRSA carriage in these

patients resulted from a visit to a foreign hospital longer than 2 months ago (MRSA colonisation can persist for months to years) or was acquired in the community, could not be established. Five of 30 patients without risk factors carried livestock-associated MC0398, an MRSA clade that has been the predominant clade in The Netherlands since 2007 [4,19]. Carriage of livestock-associated MRSA in patients without direct contact to pigs or veal calves has been reported before [7, 20,21], but a clear reservoir or transmission route has not yet been revealed. The Amphia Hospital serves a population in an area with a high pig density [22]. Density of livestock has been identified as a risk factor for nasal carriage of livestock-associated MRSA for persons without direct contact with livestock [20,23,24]. Of the five patients, one patient indicated that he had an allotment garden next to a piggery, and three patients had (indirect) work-related contact with dairy cattle (dairy cattle is not included as risk factors in the national MRSA guideline). The presence of livestock-associated MRSA in the food chain (meat) [20,25,26], in diverse animal sources[27] or indirect animal-to-human transmission through the environment have been described [28,29], hampering the detection of these carriers using targeted screening.

The remaining MLVA complexes MC0022 (also known as EMRSA-15) and MC0045 (found in eight patients without risk factors) are major healthcare-associated MRSA clones in Europe [30–32]. MC0001, MC0008 and MC0030 are common community-associated MRSA clones [33–35]. PVL is a molecular marker associated with CA-MRSA worldwide [36], in our study the three PVL positive strains belonged to MLVA complex MC0001 and MC0008. Our data are in line with the observation that distinction between community-associated MRSA and healthcare-associated MRSA is fading, as patients with MRSA infections due to community-associated strains have also been observed with increasing frequency among patients in hospital settings, and vice versa [30,32,37].

There are several limitations of this study. First, it is possible that some known MRSA carriers were excluded from the routine nasal screening because the carrier status was already known, or because they were following after MRSA eradication treatment. This may have resulted in an underestimate of the overall MRSA prevalence. We believe, however, that this group was not structurally excluded as three of the 41 MRSA patients were known MRSA carriers **(Supplementary table S3)**. Moreover, exclusion of these patients would not have affected the prevalence of MRSA among patients without the standard risk factors.

Second, the MRSA prevalence may also have been underestimated because only the anterior nares were used as sampling sites. Although up to 28% of carriers may carry

S. aureus (including MRSA) on other sites than the nose (throat, perineum), the anterior nares are the most frequent carriage site for *S. aureus* [38], and we believe that a substantial underestimate of the MRSA prevalence is unlikely. Our findings are further supported by the fact that during the study period, there has been no increase in the number of (community-acquired) invasive infections caused by MRSA in the Amphia Hospital: the proportion of MRSA among all cases of *S. aureus* bacteraemia was very low (6/678; 0.9%, data not shown). Of these, only four (0.6%) were community-onset (one in 2012, two in 2015 and one in 2016).

In summary, our study showed a sustained low prevalence of MRSA carriage upon hospital admission over a period of 7 years. This indicates that, despite a (constant) presence of MRSA carriers outside the standard risk groups, there is no evidence of an increase in the overall prevalence of MRSA, and that the search and destroy policy is still effective.

Transparency declaration

The authors declare no competing interests. No funds were provided for this study.

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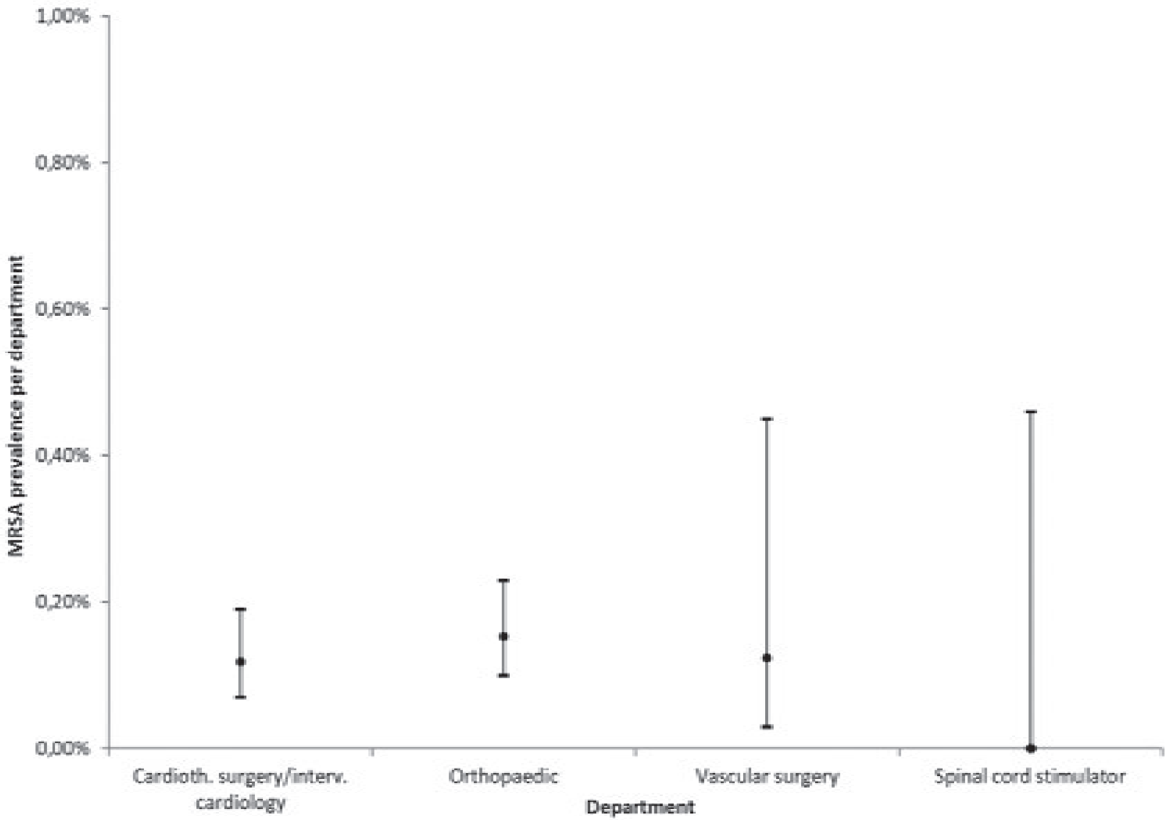
SUPPLEMENTARY MATERIAL

Supplementary table S1: risk categories of patients for MRSA carriage as defined by Working Group on Infection Prevention (WIP) "Guideline for MRSA (hospitals) 2012".

Risk category	Discription
Category I (proven MRSA carriage)	The patient is proven MRSA positive.
	The MRSA-positive patient received MRSA eradication therapy but did not yet have three consecutive negative MRSA tests at intervals of at least seven days.
Category II (high risk of MRSA carriage)	The patient has had unprotected contact with an MRSA-positive patient in the past two months: within the hospital (contact tracing); outside the hospital (roommates, partners or carers of MRSA-positive patients).
	In the past two months, the patient stayed at another Dutch healthcare institution in a department or unit with an ungoing MRSA outbreak.
	The patient stayed in a foreign health care institution for more than 24 hours in the past two months.
	The patient stayed in a foreign care institution for less than 24 hours in the past two months and has at least one risk factor for MRSA carriage: (a) an invasive intervention in a foreign hospital; (b) chronic infections or persistent skin lesions ans/or infection such as abscesses or furuncles that are present when admitted to a Dutch hospital.
	Foreign dialysis patient
	The patient had contact with industrial, live pigs / veal calves / broilers regardless whether this contact was professional or not and / or lives on such farm.
	Children who have been adopted from abroad and live in the Netherlands.
Category III (increased risk of MRSA carriage)	The patient has had unprotected contact with an MRSA-positive healthcare worker in the past two months
	The patient from the Netherlands underwent hemodialysis in a foreign healthcare institution in the past two months
	The patient stayed in a foreign care institution longer than two months ago and has at least one risk factor for MRSA carriage: (a) an invasive intervention in a foreign hospital; (b) chronic infections or persistent skin lesions ans/or infection such as abscesses or furuncles that are present when admitted to a Dutch hospital.
	The MRSA-positive patient has three consecutive negative MRSA tests at intervals of at least seven days and is still in the follow-up period of 1 year after the first negative test.
Category IV	The patient does not belong to category I, II or III.

Supplementary figure S2: overall prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) per department - with 95% confidence interval.

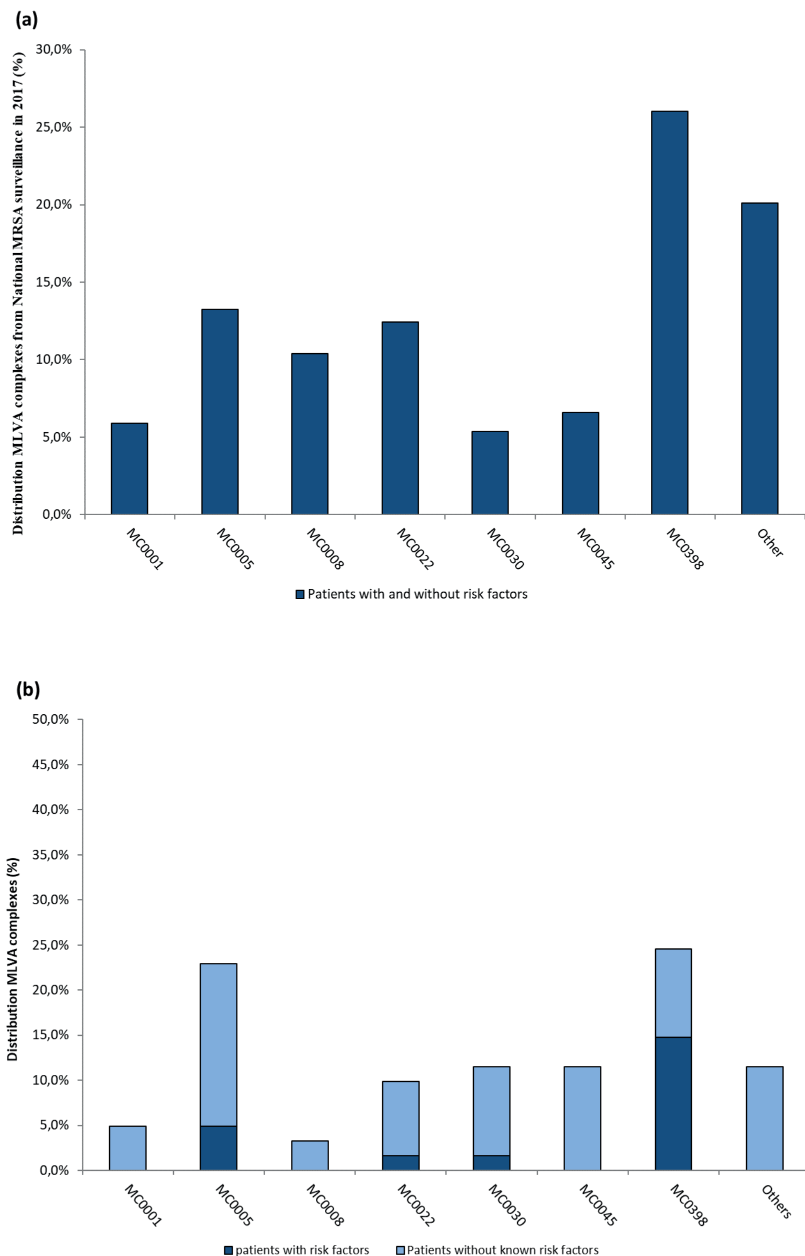


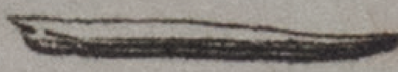
Supplementary table S3: Characteristics of MRSA carriers and strains. M: male; F: female; Neg: negative; Pos: positive; IN-CARD/CARD-TH: interventional cardiology/cardiothoracic surgery; ORTH: orthopaedic; VASC: vascular surgery; MLVA: Multiple-Locus Variable number tandem repeat Analysis; PVL: Panton-Valentine Leukocidin; CIP: ciprofloxacin, TRI: trimethoprim, CLI: clindamycin, CLA: Clarithromycin, MUP: mupirocin, RIF: rifampin, DOX: doxycycline, FUS: fusidic acid. Antimicrobial susceptibility according to the criteria of the European Committee on Antimicrobial Susceptibility Testing, *a*: patient has an allotment garden next to a piggery, *b*: daughter/son has a dairy farm, *c:* patient is a dairy farmer.

Year	Patient	Gender	Age	Department	MRSA risk factor	MLVA		PVL	Antibiotic susceptibility patterns							
						Type	Complex		CIP	TRI	CLI	CLA	MUP	RIF	DOX	FUS
2010	1	M	61	IN-CARD/CARD-TH	No	MT0549	MC0045	Neg	S	S	S	S	S	S	-	S
	2	M	65	ORTH	No	MT0067	MC0005	Neg	S	S	S	S	S	S	-	S
	3	M	75	IN-CARD/CARD-TH	No	MT0512	MC0005	Neg	R	S	R	R	S	S	-	S
	4	M	77	ORTH	No	MT2857	MC0030	Neg	S	S	S	S	S	S	-	S
2011	5	M	68	ORTH	No [a]	MT0398	MC0398	Neg	S	R	R	R	S	S	-	S
	6	M	76	IN-CARD/CARD-TH	Yes (proven MRSA carrier)	MT0512	MC0005	Neg	R	S	R	R	S	S	-	S
2012	7	M	69	ORTH	No	MT0491	MC0022	Neg	S	R	S	S	S	S	-	S
	8	M	74	ORTH	No	MT0398	MC0398	Neg	S	S	S	S	S	S	-	S
	9	F	80	ORTH	Yes (proven MRSA carrier)	MT0393	MC0030	Neg	S	-	S	S	S	S	-	S
	10	F	45	ORTH	No	MT0237	MC0008	Pos	S	S	S	S	S	S	-	S
	11	M	74	ORTH	No	MT0023	MC0005	Neg	S	S	S	S	S	S	-	I
2013	12	M	65	ORTH	Yes (contact with pigs/meat calves)	MT0398	MC0398	Neg	S	S	R	R	S	S	R	S
	13	M	67	IN-CARD/CARD-TH	No	MT4499	MCnone	Neg	R	S	R	R	S	S	S	S
	14	M	50	IN-CARD/CARD-TH	Yes (proven MRSA carrier)	MT0589	MC0398	Neg	S	-	S	S	S	S	-	S
	15	F	84	IN-CARD/CARD-TH	Yes (admission foreign hospital)	MT0602	MC0005	Neg	S	S	S	S	S	S	S	S
	16	F	78	IN-CARD/CARD-TH	No[b]	MT0398	MC0398	Neg	S	S	S	S	S	S	-	S
	17	F	81	ORTH	No[b]	MT0398	MC0398	Neg	S	S	S	S	S	S	-	S
	18	M	80	IN-CARD/CARD-TH	No	MT0602	MC0005	Neg	S	S	R	R	S	S	-	S
	19	M	73	VASC	No	MT0023	MC0005	Neg	S	S	S	S	S	S	S	R

Year	Patient	Gender	Age	Department	MRSA risk factor	MLVA		PVL	Antibiotic susceptibility patterns							
						Type	Complex		CIP	TRI	CLI	CLA	MUP	RIF	DOX	FUS
2014	20	F	43	ORTH	No	MT0491	MC0022	Neg	S	R	S	S	S	S	S	S
	21	M	63	IN-CARD/CARD-TH	No	MT2172	MC0030	Neg	S	S	S	S	S	S	S	S
	22	F	83	ORTH	Yes (contact with pigs/meat calves)	MT0398	MC0398	Neg	R	R	R	R	S	S	R	S
	23	F	68	ORTH	Yes (contact with pigs/meat calves)	MT0398	MC0398	Neg	S	S	S	S	S	S	R	S
	24	M	68	ORTH	Yes (contact with pigs/meat calves)	MT0398	MC0398	Neg	S	S	S	S	S	S	R	S
	25	M	38	ORTH	No	MT0503	MC0045	Neg	S	S	S	S	S	S	S	S
	26	F	54	IN-CARD/CARD-TH	No	MT1980	MC0001	Pos	S	S	S	R	S	S	R	S
	27	F	74	IN-CARD/CARD-TH	No	MT0128	MC0005	Neg	S	S	S	S	S	S	S	S
	28	M	41	IN-CARD/CARD-TH	No	MT0393	MC0030	Neg	S	S	S	S	S	S	S	S
	29	F	94	VASC	No	MT0005	MC0005	Neg	S	S	S	S	S	S	S	R
2015	30	M	42	ORTH	Yes (contact with pigs/meat calves)	MT0398	MC0398	Neg	S	R	S	S	S	S	R	S
	31	M	56	ORTH	No	MT0936	MC0045	Neg	S	S	S	S	S	S	S	R
	32	M	72	IN-CARD/CARD-TH	No	MT2578	MC0005	Neg	S	S	S	S	S	S	S	S
2016	33	M	62	ORTH	No	MT2055	MC0022	Neg	S	-	S	S	I	S	S	S
	34	F	75	ORTH	No	MT1760	MC0008	Pos	R	S	R	S	S	R	S	S
	35	F	30	IN-CARD/CARD-TH	No	MT5302	MCnone	Neg	S	R	R	S	S	S	R	S
	36	M	51	IN-CARD/CARD-TH	No	MT0462	MC0022	Neg	S	S	S	S	S	S	S	S
2017	37	M	39	ORTH	Yes (MRSA positive family member)	MT0869	MC0005	Neg	S	R	S	S	S	S	S	S
	38	M	76	IN-CARD/CARD-TH	Yes (contact with pigs/meat calves)	MT0572	MC0398	Neg	S	-	S	S	S	S	R	S
	39	M	71	ORTH	No[c]	MT0398	MC0398	Neg	S	S	S	S	S	S	R	S
	40	M	77	ORTH	No	MT0710	MC0621	Neg	S	S	S	R	S	S	S	S
	41	V	88	IN-CARD/CARD-TH	No	MT0497	MC0045	Neg	R	-	S	S	I	S	S	S

Supplementary figure S4: (a) Distribution MLVA complexes from National MRSA surveillance in 2017; dark blue: patients with and without standard risk factors for MRSA carriage (source: Dutch Institute for Public Health and Environment (RIVM), SWAB. NethMap 2018, Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. 2018.). **(**b): Distribution MLVA complexes at hospital admission 2010-2017; dark blue: patients without standard risk factors for MRSA carriage, light blue: with standard risk factors for MRSA carriage according to the national guideline.



A. 

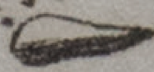
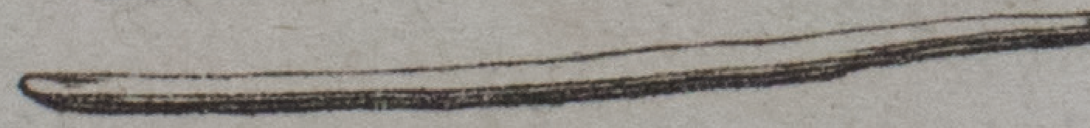
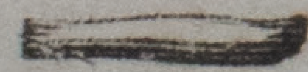
B. C. 

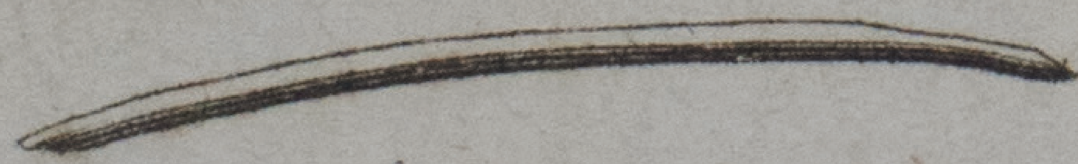
Fig. 3.

E.

F.







Chapter 3

NEXT-GENERATION SEQUENCE ANALYSIS REVEALS TRANSFER OF METHICILLIN RESISTANCE TO A METHICILLIN-SUSCEPTIBLE STAPHYLOCOCCUS AUREUS STRAIN THAT SUBSEQUENTLY CAUSED A METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS OUTBREAK: A DESCRIPTIVE STUDY

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ABSTRACT

Background

Resistance to methicillin in *Staphylococcus aureus* is caused primarily by the *mecA* gene, which is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). Horizontal transfer of this element is supposed to be an important factor in the emergence of new clones of methicillin-resistant *Staphylococcus aureus* (MRSA) but has been rarely observed in real time.

In 2012, an outbreak occurred involving a healthcare worker (HCW) and three patients, all carrying a fusidic acid-resistant MRSA strain. The husband of the HCW was screened for MRSA carriage, but only a methicillin-susceptible *S. aureus* (MSSA) strain, which was also resistant to fusidic acid, was detected. Multiple-locus variable-number tandem repeat analysis (MLVA) typing showed that both the MSSA and MRSA isolates were MT4053-MC0005. This finding led to the hypothesis that the MSSA strain acquired the SCC*mec* and subsequently caused an outbreak. To support this hypothesis, next generation sequencing of the MSSA and MRSA isolates was performed.

Results

This study showed that the MSSA isolate clustered closely with the outbreak isolates based on whole-genome multilocus sequence typing (wgMLST) and single-nucleotide polymorphism (SNP) analysis, with a genetic distance of 17 genes and 44 SNPs, respectively. Remarkably, there were relatively large differences in the mobile genetic elements in strains within and between individuals.

Conclusion

The limited genetic distance between the MSSA and MRSA isolates in combination with a clear epidemiologic link supports the hypothesis that the MSSA isolate acquired a SCC*mec* and that the resulting MRSA strain caused an outbreak.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial and community-acquired infections [1]. The methicillin resistance gene *mecA* encodes an additional penicillin-binding protein (PBP), PBP2, which has a low affinity for all beta-lactam antibiotics and is not present in susceptible strains [2,3]. *MecA* is located on a mobile element called staphylococcal cassette chromosome *mec* (SCC*mec*). To date, 11 main types of SCC*mec* (types I to XI) have been defined, and they differ in both size and composition (http://www.sccmec.org/Pages/SCC_TypesEN.html). SCC*mec* also carries genes that are responsible for the regulation of *mecA* transcription, i.e., those encoding the repressor *MecI* and the transmembrane beta-lactam-sensing signal transducer *MecRI* [4]. For transfer, SCC*mec* carries specific genes called *ccr* genes, which encode recombinases of the invertase/resolvase family. Currently, three phylogenetically distinct *ccr* genes, *ccrA*, *ccrB*, and *ccrC*, have been reported [3].

The first MRSA strain emerged when a SCC*mec* with the *mecA* gene was integrated into the chromosome of a susceptible *S. aureus* strain. The mechanism responsible for the transfer of methicillin resistance has not been well understood. It has been suggested that SCC*mec* can be transferred between staphylococci and that *mecA* positive coagulase-negative staphylococci may be a potential reservoir for these elements [3]. This phenomenon has not been observed frequently, possibly due to the fact that clonal lineages have restriction barriers that control horizontal transfer between lineages [5,6]. However, some studies have reported the horizontal transfer of SCC*mec.* Berglund et al. described the transfer of SCC*mec* from a methicillin-resistant *Staphylococcus haemolyticus* strain to a methicillin-sensitive *S. aureus* (MSSA) strain, creating a new clone of MRSA that caused an outbreak on a neonatal ward [7]. Bloemendaal et al. described a transfer of SCC*mec* from *Staphylococcus epidermidis* to a MSSA in an infant during antibiotic treatment [8].

This report describes a MRSA outbreak on the oncology ward in a Dutch hospital that was caused by a healthcare worker (HCW) carrying a methicillin-susceptible *S. aureus* strain, which acquired a SCC*mec* cassette and which was subsequently transmitted to patients.

METHODS

Microbiology

For contact screening, swabs of the nose, throat, and perineum were collected using eSwab medium (Copan, Murrieta, CA, USA). The swabs were inoculated on chromID MRSA agar plates (bioMérieux, La Balme, France). The remaining eSwab fluid was transferred in

Mueller-Hinton broth supplemented with 6.5% sodium chloride. The overnight Mueller-Hinton broth was subcultured onto both a chromID MRSA agar plate and sheep blood agar [9]. Presumptive *S. aureus* colonies were confirmed with a latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd., Dartford, United Kingdom) and DNase (DNase agar; Oxoid Ltd., Basingstoke, United Kingdom). Antibiotic susceptibility testing was performed using an automated system (Vitek 2; bioMérieux, Marcy l'Etoile, France). Resistance to cefoxitin was measured using the cefoxitin disk diffusion method according to EUCAST standards [10].

Molecular typing

Confirmation of methicillin resistance and *S. aureus* species identification were performed by a PCR for detection of the staphylococcal protein A (*spa*) gene, the gene for methicillin resistance (*mecA*), and staphylococcal cassette chromosome *mec* (SCC*mec*) (GeneXpert SA nasal complete; Cepheid, CA, USA). Further genotyping was performed using MLVA for all isolates in this study [11].

Next-generation sequencing (NGS) was performed on six isolates, which comprised the first MRSA isolate from patients 1, 2, and 3 and the HCW, as well as the MSSA isolate from the husband of the HCW, and one additional MRSA isolate from the HCW isolated in November 2014 after three MRSA treatment cycles. To provide epidemiological and genetic context, 52 MRSA isolates yielding MC0005 were randomly selected from the national MRSA surveillance in 2016 and included in the analysis.

A complete reference genome of the isolate from the first patient (accession number CP015173) was obtained by a hybrid approach combining PacBio (Pacific Biosciences, Menlo Park, CA, USA) and Illumina HiSeq (Illumina, San Diego, CA, USA) data. For the five remaining isolates, NGS was performed using Illumina HiSeq. The NGS data were used for multilocus sequence typing (MLST) and whole-genome MLST (wgMLST) using the available wgMLST scheme in SeqSphere software version 2.3.0 (Ridom GmbH, Münster, Germany). Single-nucleotide polymorphism (SNP) analysis was performed by mapping the Illumina reads of the isolates against the consensus sequence of the reference genome obtained from the first patient. SNP identification was performed with CLC Genomics workbench version 9.5.3 (Qiagen, Hilden, Germany) using the criteria of a frequency of 75% and a forward/reverse read balance of 5%. In addition, the SCC*mec* types were identified using the consensus sequence of the reference genome in the CLC Genomics workbench version 9.5.3 (Qiagen, Hilden, Germany) and a blast search in the NCBI database. The plasmids and bacteriophages were identified by performing a blast

search of the contigs of the de novo assembly of the unmapped reads of the reference sequence against the NCBI database. All resulting data were imported into BioNumerics version 7.6 for comparative analyses (Applied Maths, Sint-Martens-Latem, Belgium).

RESULTS

Outbreak description

In September 2012, a patient who had been hospitalized for 5 weeks on the oncology ward developed a MRSA bacteraemia. Contact tracing for MRSA revealed three other (asymptomatic) MRSA carriers: two patients and one HCW. No known risk factors for MRSA acquisition were present in the three patients or the HCW. The HCW was temporarily removed from patient care and started on decolonisation treatment with mupirocin nasal ointment, chlorhexidine wash, oral rifampin, and co-trimoxazole. After initially successful decolonisation, recolonization was documented after 1 month. A second decolonisation treatment was prescribed but failed after 3 months. After the second failure episode, MRSA contact tracing was expanded to the husband, pets (dog and cat), three family members, and three close friends of the HCW, but all were found MRSA negative. In the end, decolonisation of the HCW was achieved after four MRSA decolonisation treatment courses. Remarkably, before final decolonisation, the HCW tested MRSA positive exclusively in one culture site, the perineum. The index patient and second patient were not decolonised due to the medical condition of these two patients; the third patient lost colonisation spontaneously.

Previous bacteriological findings in the HCW and the husband of the HCW.

In March 2011 (18 months prior to the outbreak), a screening sample from the throat, nose, and perineum had been obtained from the involved HCW upon her new employment at the hospital, and a fusidic acid-resistant MSSA strain was detected. We hypothesized that the fusidic acid-resistant MSSA strain from the HCW acquired a SCC*mec* and subsequently caused an outbreak. Unfortunately, the fusidic acid-resistant MSSA strain was not available for further testing. However, in September 2012, prior to the first decolonisation attempt of the HCW, the husband of the HCW was tested for MRSA carriage, and a fusidic acid-resistant MSSA strain was detected in the nose, leading to the hypothesis that this MSSA isolate might be identical to the MRSA isolate from the HCW. To test our hypothesis, the MRSA outbreak isolates (from patients and the HCW) and the MSSA strain of the husband were further genotyped. The sequence of events is indicated in **Figure 1.**

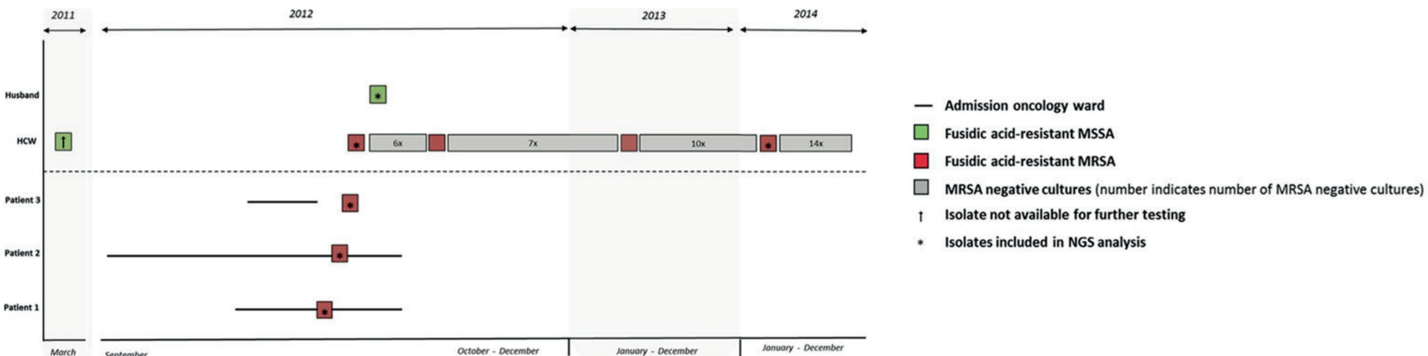


Figure 1: timeline of the MRSA outbreak on the oncology ward and previous bacteriological findings in the HCW and the husband of the HCW. In September 2012, an outbreak occurred on the oncology ward involving an HCW and three patients, all carrying a fusidic acid-resistant MRSA strain. In March 2011, a screening sample had been obtained from the HCW, and a fusidic acid-resistant MSSA strain was detected. Unfortunately, this strain was not available for further testing. Prior to the first decolonisation attempt of the HCW, the husband of the HCW was tested for MRSA carriage, and a fusidic acid-resistant MSSA strain was detected in the nose. Next-generation sequencing was performed on six isolates: the first MRSA isolate from patients 1, 2, and 3 and the HCW, the MSSA isolate from the husband, and one additional MRSA isolate from the HCW isolated in November 2014 after three MRSA treatment cycles.

Microbiology and molecular typing.

All isolates (MSSA and MRSA) were resistant to fusidic acid (MIC, 32 mg/litre). This is a relatively rare antibiogram in our setting; in the past 5 years, only 1% of all clinical isolates of *S. aureus* in our laboratory have been found resistant to fusidic acid. Multiple-locus variable-number tandem-repeat analysis (MLVA) typing showed that all the isolates involved were MT4053-MC0005, a type which had not been found before or since in the Netherlands.

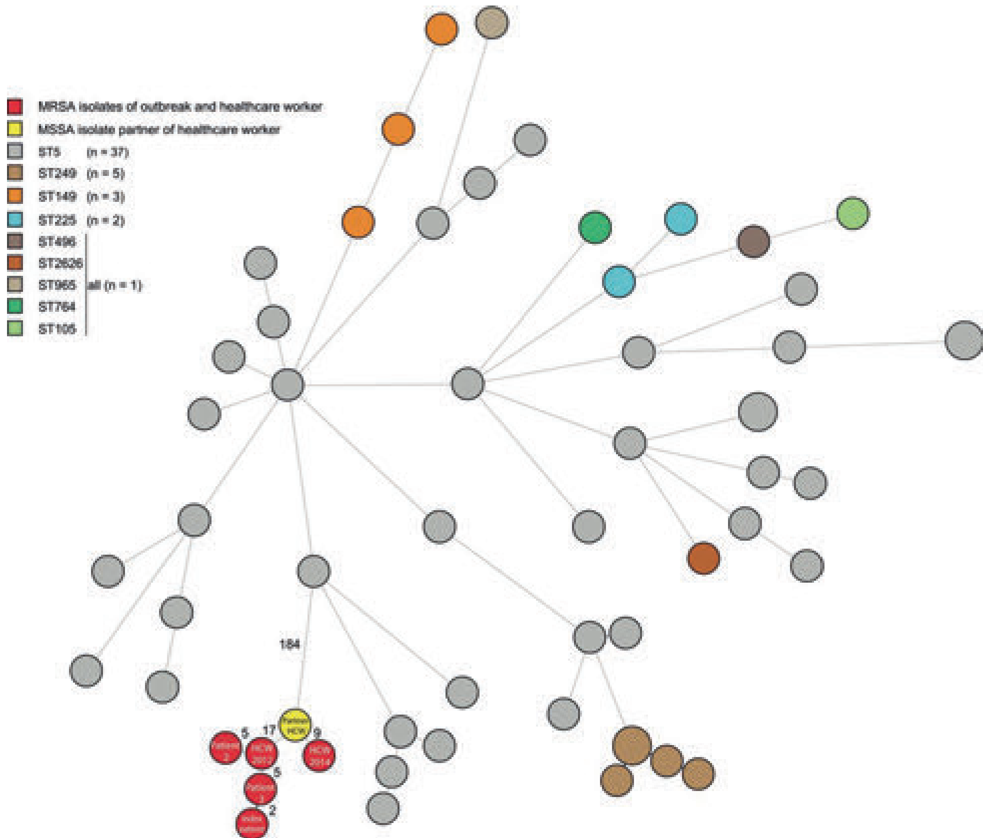
Classical multilocus sequence typing (MLST) based on the next-generation sequencing (NGS) data revealed that all outbreak isolates yielded sequence type 5 (ST5), a sequence type belonging to clonal complex 5 (CC5). The 52 context isolates were also mainly ST5 (n=37), but eight other STs were also found, albeit all belonging or closely related to CC5.

The minimum spanning tree based on all 1,861 genes of the whole-genome MLST (wgMLST) scheme showed that all outbreak isolates, the two MRSA isolates from the HCW, and the MSSA isolate from the partner of the HCW clustered closely together, with a maximum genetic distance of 17 alleles between two neighbouring isolates **(Figure 2)**. The distance from one of the isolates in this cluster to the nearest unrelated context isolate was 184 alleles.

Single-nucleotide polymorphism (SNP) analysis showed that the outbreak isolates and the isolate from the HCW from 2012 had limited variation, with a maximum of 17 SNPs between the isolates. In comparison to the reference sequence, the MSSA isolate from the partner of the HCW had 44 SNPs while the isolate from the HCW obtained in 2014 had 52 SNPs. The genetic distance between the MSSA isolate and the MRSA isolate from the HCW in 2014 was 27 SNPs **(Table 1)**.

Comparative genomics showed two deletions, of 87 bp and 818 bp, in the MSSA chromosome compared to the MRSA outbreak isolates and the MRSA isolate from the HCW in 2014. The 87-bp deletion sequence encodes the YbaK/EbsC protein, a transcriptional regulation protein. The 818-bp deletion sequence, located in two adjacent genes encoding serine proteases, caused an in-frame fusion of the two genes, creating a new serine protease-encoding open reading frame. No other deletions were found in the isolates. SCC*mec* analysis showed that all four outbreak isolates carried a SCC*mec* type V (5C2&5C). However, compared to the SCC*mec* reference strain (AB512767), there was an 8.5-kb deletion in the region where the second *ccrC* gene was located in all outbreak isolates. In the MSSA isolate, the complete 38.8-kb SCC*mec* region was absent. Interestingly, the MRSA isolate from the HCW cultured in 2014 carried the same SCC*mec* type as the outbreak isolates but without the 8.5-kb deletion.

Figure 2: minimum spanning tree based on next-generation sequencing of 58 MLVA complex 5 (MC5) *S. aureus* isolates. The minimum spanning tree was based on 1,861 genes. The size of each circle indicates the number of isolates. Colours represent MRSA isolates from the outbreak and the HCW (red) and the MSSA isolate from the partner of the HCW (yellow), while the other colours indicate the sequence types (STs) of the MC5 context isolates from the Dutch MRSA surveillance. The lengths of the lines between isolates represent the numbers of different alleles.



Analysis of the NGS data showed the presence of two plasmids with lengths of 4.3 kb (pRIVM6519-1, accession number CP015174) and 3.1 kb (pRIVM6519-2, accession number CP015175) in all four outbreak isolates. No plasmids were detected in the MSSA isolate, whereas the isolate from the HCW from 2014 contained only the pRIVM6519-2 plasmid. Furthermore, an additional (identical) bacteriophage was present in the isolate from the HCW from 2014 and in the MSSA isolate from the husband of the HCW, but this bacteriophage was absent from the four outbreak isolates (**Table 1**).

Table 1: microbiology and molecular data of the MSSA and MRSA isolates. * Isolates included in NGS analysis, ref reference genome

No.	Isolate	MRSA/MSSA positive sample sites	Isolation date	MLVA	NGS					
					MLST	**SNP**	**Missing regions**	**SCCmec**	**Plasmids**	**bacteriophage**
P1	MRSA	Nose, throat, **blood culture***	June 24, 2012	MT4053-MC0005	CC5	*ref*	*Ref*	V(5C2&5); ccrC1 deletion	4,3 kb; 3,1 kb	Absent
P2	MRSA	Nose, **throat***	September 17, 2012	MT4053-MC0005	CC5	17	none	V(5C2&5); ccrC1 deletion	4,3 kb; 3,1 kb	Absent
P3	MRSA	Nose, **throat***, perineum	October 4, 2012	MT4053-MC0005	CC5	5	None	V(5C2&5); ccrC1 deletion	4,3 kb; 3,1 kb	Absent
HCW	MRSA	**Perineum***	September 21, 2012	MT4053-MC0005	CC5	11	None	V(5C2&5); ccrC1 deletion	4,3 kb; 3,1 kb	Absent
	MRSA	**Perineum***	November 19, 2014	MT4053-MC0005	CC5	44	None	V(5C2&5)	Absent	Additional phage
Husband	MSSA	**Throat***	September 25, 2012	MT4053-MC0005	CC5	52	87bp; 818bp	absent	3.1 kb	Additional phage

DISCUSSION

We hypothesized that a fusidic acid-resistant MSSA strain acquired a SCC*mec* and subsequently caused an MRSA outbreak on the oncology ward in a Dutch hospital. The hypothesis was initially based on a rare antibiogram (*S. aureus* resistant to fusidic acid with a priori chance of 1% in our setting) and was further supported by the unique MLVA type MT4053-MC0005, a type which had not been found before or since in the Netherlands. NGS analysis together with a clear epidemiological link confirmed our hypothesis.

Whole-genome sequencing can provide improved resolution to define transmission pathways and characterize outbreaks [12]. Other studies using next-generation sequencing of *S. aureus* isolates from outbreaks have been published, and most of these studies determined relatedness by the number of SNPs between genomes [13–16]. In the present study, wgMLST typing was also applied. This gene-by-gene approach has the advantage that both SNPs and single recombination events are treated as one evolutionary event. WgMLST showed that all outbreak isolates, the MSSA isolate, and the MRSA isolates of the HCW in 2014 clustered closely together in the minimum spanning tree with a large distance to the neighbouring epidemiologically unrelated CC5 isolates.

To date, for wgMLST of *S. aureus*, no clear minimum genetic diversity (in order to exclude a transmission event) has been established. Recently, this has been determined for Gram-negative bacteria in a large epidemiological study in hospitals [13], but for *S. aureus* this has not been done. However, considering the epidemiological linkage in a setting with hardly any circulation of MRSA [17] and in the context of epidemiologically unrelated CC5 strains, this study showed that the MRSA and MSSA isolates are almost certainly related to each other. SNP analysis also showed that the outbreak strains were nearly identical. However, the numbers of SNPs found within the MSSA isolate and the MRSA isolate from the HCW in 2014 were higher than the distances observed with wgMLST and were above the threshold observed by Golubchik et al. of 40 SNPs or fewer to define recent transmission [12]. The observed differences between the methods can be explained by the fact that the SNP analysis also takes into account the variation between genes. Furthermore, more SNPs could have been identified, since we used one of the outbreak isolates as a reference chromosome for SNP analysis, whereas the wgMLST scheme has a fixed reference that is used for analysis of all *S. aureus* isolates.

The higher number of SNPs present in the MSSA isolate could be explained by a different evolutionary path, since the time of transmission of the MSSA isolate between the husband of the HCW and the HCW is unknown. The differences in number of SNPs

between the MRSA isolates from the HCW in 2012 and 2014 might be explained by the fact that the HCW underwent multiple MRSA eradication therapies that could have caused the observed changes in the chromosome. When bacteria are exposed to environmental conditions [18], or when a mismatch repair system becomes disrupted [16], higher rates of point mutations can occur.

We found multiple variations in the compositions of the mobile genetic elements within (MRSA isolates from the HCW) and between (MSSA strain from the husband of the HCW versus the MRSA outbreak strain) individuals. A recent study suggested that the acquisition and loss of mobile genetic elements within nasal colonisation populations are common phenomena [19]. Also, individuals can harbour multiple variants of isolates, a phenomenon termed as within-host diversity [20]. In the case of *S. aureus*, where long-term carriage is common, within-host diversity can be substantial. Longitudinal studies revealed that the size of population diversity can fluctuate over time, and relatively large differences have been detected within individuals [12,21].

Our study has some limitations. First, only single colonies were sampled from each host, and therefore the diversity within the individuals was not taken into account. Second, the reference genome in this study was derived from a clinical sample (patient with bacteraemia), in contrast to the other genomes, which were derived from asymptomatic carriers. Young et al. showed that *S. aureus* strains can acquire mutations during disease progression [21]. However, except for the MSSA isolate, no deletions were found when the genomes of the outbreak isolates were compared to the reference genome. Furthermore, in our study, a fusidic acid-resistant MSSA was detected first, followed by fusidic acid-resistant MRSA cultures from the HCW. This led to the hypothesis that the MSSA strain acquired the SCC*mec*. In contrast, some studies have reported (partial) excision of the SCC*mec* resulting in the conversion of MRSA to MSSA [22,23]. Chlebowicz et al. described an in vivo loss of type V (5C2&5)-like SCC*mec* in *S. aureus* ST398, due to recombination between two *ccrC* genes [22]. The fact that the HCW was found MRSA positive at multiple times, over a long period of time, possibly indicates that the type V (5C2&5) SCC*mec* in the outbreak strain is relatively stable. In addition, the SCC*mec* region in the MSSA isolate was entirely absent, which supports our hypothesis of acquisition rather than loss of the SCC*mec* cassette.

Lastly, this report describes a MRSA outbreak caused by an HCW. While screening of patients at risk of MRSA carriage is generally accepted, screening of HCWs remains controversial [24,25]. Several guidelines, mostly from regions with low MRSA prevalence,

advocate screening of HCWs inter alia after exposure to MRSA-positive patients [26]. On the other hand, the guidelines of several European countries and American professional societies advocate screening of HCWs only in selected situations, such as epidemiological outbreaks or when transmission continues despite implementation of basic control measures [27,28]. Furthermore, the Dutch national guideline recommends sampling all sites for patients but is inconclusive for perineal samples in HCWs [9]. Here, the HCW tested MRSA positive exclusively in one culture site, the perineum. This extranasal carriage could be an explanation for the repeated treatment failure in the HCW, as perineal carriage is associated with treatment failure [29]. This outbreak shows the potential role of HCWs as a cause of MRSA outbreaks. It underlines the importance of screening HCWs as part of a comprehensive infection control policy, and sampling all sites should be recommended for patients and HCWs.

In conclusion, the limited differences in the wgMLST and SNP analysis in combination with a clear epidemiologic link support the hypothesis that the MSSA isolate acquired a SCC*mec* and that the resulting MRSA strain caused an outbreak. Remarkably, relatively large differences in mobile genetic elements were found between the two MRSA isolates of the HCW. More research is needed to establish how much variation in genomes occurs during colonisation in individuals, over long periods, and the variations in response to different hosts.

Ethics statement.

The data used in this study were part of routine practices in The Netherlands and did not require approval from an ethics committee. The data were anonymized and analyzed according to the local regulations and laws that apply to medical information.

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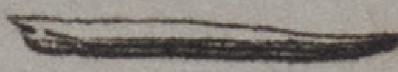

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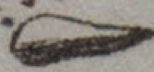

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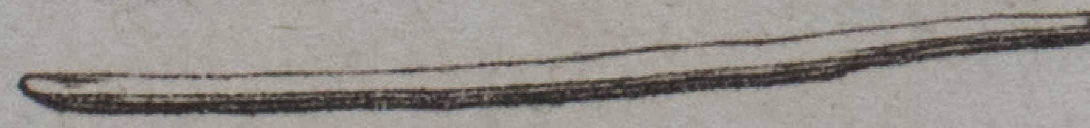

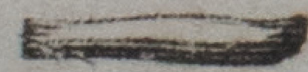

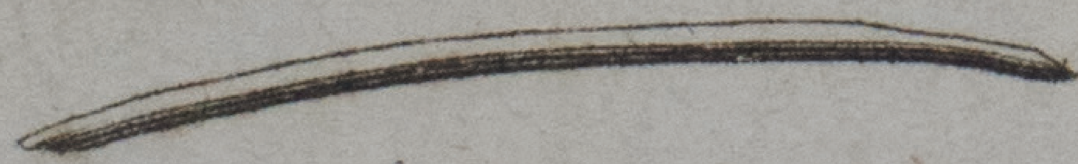

Chapter 4

MANAGEMENT OF A HOSPITAL-WIDE VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECIUM OUTBREAK IN A DUTCH GENERAL HOSPITAL, 2014-2017: SUCCESSFUL CONTROL USING A RESTRICTIVE SCREENING STRATEGY

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ABSTRACT

Background

The emergence of vancomycin resistant enterococci poses a major problem in healthcare settings. Here we describe a hospital-wide outbreak of vancomycin-resistant *Enterococcus faecium* in a general hospital in The Netherlands in the period December 2014 – February 2017.

Due to late detection of the outbreak, a large cohort of approximately 25,000 (discharged) patients was classified as 'VRE suspected'. Hereupon a mitigated screening and isolation policy, as compared with the national guideline, was implemented to control the outbreak.

Methods

After the outbreak was identified, a screening policy consisting of a single rectal swab culture (with enrichment broth) to discontinue isolation and removing 'VRE suspected' label in the electronic patient files for readmitted VRE suspected patients, was implemented.

In addition to the on admission screening, periodic hospital-wide point prevalence screening, measures to improve compliance with standard infection control precautions and enhanced environmental cleaning were implemented to control the outbreak.

Results

Between September 2014 and February 2017, 140 patients were identified to be colonised by *vanA* mediated vancomycin-resistant *Enterococcus faecium* (VRE*fm*). Two of these patients developed bacteraemia. AFLP typing showed that the outbreak was caused by a single clone. Extensive environmental contamination was found in multiple wards. Within nine months after the detection of the outbreak, no new VRE cases were detected.

Conclusion

We implemented a control strategy based on targeted screening and isolation in combination with implementation of general precautions and environmental cleaning. The strategy was less stringent than the Dutch national guideline for VRE control. This strategy successfully controlled the outbreak, while it was associated with a reduction in the number of isolation days and the number of cultures taken.

BACKGROUND

In line with the increased global spread of multi-drug resistant microorganisms, the prevalence of vancomycin resistance among enterococci is rising. The European Antimicrobial Resistance Surveillance Network (EARS-Net) reported a significant increase of number of vancomycin-resistant *Enterococcus faecium* in invasive isolates from 2015 (10.5%) to 2018 (17.3%) in Europe [1]. In the Netherlands, however, the proportion of VRE in infection-related isolates remained stable and slightly lower than 1% over the past 5 years [2].

Despite this low and stable prevalence, there has been an increase in the number of VRE outbreaks. Whereas vancomycin-resistant enterococci (VRE) were detected only sporadically in the Netherlands before 2012, an increasing number of VRE outbreaks have required considerable time and resources to contain over the past eight years [2]. In 2018 and 2019, VRE outbreaks were among the largest and the most frequently reported in the Netherlands (period 2018 – June 2019: VRE 682 patients in 24 outbreaks versus MRSA 93 patients in 20 outbreaks) [3].

Due to the low virulence of VRE and its ability to survive on hospital environmental surfaces, VRE outbreaks have the potential to become substantial in size before they are detected by routine clinical cultures. Strategies to prevent VRE transmission include screening of contact patients and isolation precautions of (suspected) VRE carriers [4,5]. The Netherlands has a national control strategy of highly resistant micro-organisms including VRE [6]. During outbreaks, contact patients (those admitted to the same room or ward as the index patient) are pre-emptively isolated while awaiting test results to prevent further spread. In this context, there is ongoing discussion about the number of rectal swabs to be tested - with culture considered as gold standard - before a VRE suspected patient can be declared VRE negative. The negative predictive value of 1 negative rectal swab is considered insufficient, and the Dutch national guideline advocates taking 3-5 rectal cultures on separate days [6]. The combination of late outbreak detection and multiple cultures per contact-patient before VRE carriage can be excluded can result in very large numbers of patients to be isolated and screened, hence it is a substantial burden for hospital infection control departments, leading to significant laboratory costs, and exhaust hospital isolation facilities.

This paper describes an outbreak of VRE*fm* in a general hospital in The Netherlands that was detected in December 2015, following an alert from a neighbouring hospital where VRE*fm* carriage was detected in four recently transferred patients. A hospital-wide screening of all patients admitted for more than 48 hours for VRE carriage indicated the

spread of VRE among patients admitted to various departments at one of two hospital locations. The outbreak was successfully controlled slightly more than a year after its detection (February 2017).

This outbreak report provides an overview of the interventions that were implemented to control the outbreak, including a screening strategy that was more restrictive than the Dutch national guideline recommends.

METHODS

Design

We retrospectively describe the interventions that were implemented to control an outbreak of VRE*fm* that occurred between September 2014 and February 2017 in a 364-bed general hospital in a non-endemic setting in in the South West of the Netherlands.

Setting

The Admiraal De Ruyter Hospital (ADRZ) has approximately 25,000 admissions per year at two different locations (Goes and Vlissingen), and a catchment area of 248,000 inhabitants, and supplies 85% to 90% of the requested hospital care in this area. Tertiary care patients are referred to the surrounding academic centres. Most patients are admitted to an acute admission unit (AAU), consisting of six single rooms and eight multi-bed room with five beds each (total 46 beds), before being transferred to their specific wards (on average after 48 hours).

VRE bloodstream infections are very rare in the hospital: in the past 6 years, no VRE bloodstream infections have been detected outside the outbreak period **(Supplementary material S1).** The antibiotic consumption rate in the hospital is relatively stable and low over the years: in 2016, the overall antibiotic use was 75.2 defined daily doses (DDD) per 100 patient days.

The internal infection control protocol for VRE was based on the Dutch national guideline for (targeted) screening and control measures [6]. This included contact isolation for VRE-positive patients; pre-emptive isolation and screening for patients with previous admission to foreign healthcare facilities and screening of roommates following an unexpected case of VRE. After discharge of a VRE positive patient, routine cleaning and disinfection of patient rooms with 250 ppm chlorine was performed.

Participants

Participants were all patients admitted to the Admiraal De Ruyter Hospital (ADRZ) between September 1, 2014 and February 5, 2017. Patients were categorised into three groups according to their VRE status and potential risk of VRE carriage: (1) VRE carrier: VRE-positive patients; (2) VRE suspected patients: all patients with prior hospitalisation in the ADRZ hospital location Goes from September 1, 2014. In the beginning of the outbreak, it was unclear whether the outbreak included neighbouring nursing homes or rehabilitation centres, therefore patients transferred from these institutions were also categorised as VRE suspected in the first phase of the outbreak.

Patients with no prior hospitalisation in the ADRZ hospital in the outbreak period, nursing home or rehabilitation centre were categorised as (3) low-risk patients.

Interventions

Upon detection of the outbreak in December 2015, an outbreak management team was initiated consisting of a clinical microbiologist, an infection control specialist, representatives of the hospital management and of the medical staff, the manager of housekeeping and logistics, and a member of the communication department.

The control measures can be divided into three phases, accompanied by the different screening and isolation policies. **Table 1** summarizes the dates, isolation and screening policies per phase and risk group. In **Table 2**, an overview of the implemented control measures during the outbreak is shown.

VRE suspected patients were automatically labelled in the electronic patient system. The decision to pre-emptively isolate these patients, and the number of cultures required to remove the 'VRE suspected' label were adjusted in time over the course of the outbreak, and with the risk profile of the patients involved, as indicated in **table 1** and further specified in the results section below.

To detect unnoticed VRE transmission in the hospital, regular screening of all patients hospitalised for more than 48 hours, and those admitted to high-risk units (ICU, dialysis) was implemented during the entire outbreak (**Table 1**).

Table 1: overview of dates, isolation and screening policies per phase and risk group during VREfm outbreak period

Risk group	Definition	Phase 1 Dec 14, 2015 – Mar 20, 2016		Phase 2 Mar 21 , 2016 – Nov 13, 2016		Phase 3 Nov 14, 2016 – Feb 5, 2017	
		Identification strategy	**Contact precautions**	**Identification strategy**	**Contact precautions**	**Identification strategy**	**Contact precautions**
VRE carrier	Patient infected or colonised with VRE*fm*	N/A	Yes [a]	N/A	Yes [a]	N/A	Yes [a]
VRE-suspected	All patients with prior hospitalisation in the ADRZ hospital location Goes from September 1, 2014 until start of zero transmission period	Single rectal swab on admission	Yes, while awaiting result of single admission screening	Single rectal swab on admission	No	Single rectal swab on admission	No
	Patients transferred from nursing homes or rehabilitation centres	Single rectal swab on admission	Yes, while awaiting result of single admission screening	Single rectal swab on admission	No	No	No
	Roommate of unexpected VRE carrier	N/A	Yes[c]	3 separate rectal cultures (collection at day 3, 5 and 7 after the last exposure)	Yes [a]	3 separate rectal cultures (collection at day 3, 5 and 7 after the last exposure)	Yes [a]
All patients (incl. low risk)	Hospital wide surveillance	Weekly screening of all patients hospitalised > 2 days[b]	N/A	Weekly screening of all patients hospitalised > 2 days	N/A	Monthly screening of all patients hospitalised > 2 days	N/A

[a] Single room or cohorting with other VRE carriers if insufficient availability of single rooms. [b]In high risk departments (dialysis and ICU) all admitted patients are screened, regardless of the admission time. [c]After cleaning of the ward was completed, patients were separated int two cohorts: clean (new admissions not fulfilling the criteria of 'VRE suspected'), and VRE suspected, each with dedicated staff. Contact precaution (CP) policy: Single room or—if single room was not available—cohort CP of patients with same VRE status on multi occupancy room. Healthcare workers wear gown with long sleeves and gloves before each contact with the patient or the patient environment. Rooms were daily cleaned, medical devices were cleaning and disinfected (alcohol 70%) before leaving the room, and after patient discharge, the room is cleaned and disinfected (250 ppm chlorine).

Table 2: overview of the implemented control measures during VRE*fm* outbreak period

Table 2 Overview of infection control measures during outbreak:		
2015	December (*Phase I*)	Detection of the outbreak Single hospital-wide screening limited to patients admitted for at least 48h
2016	January	Initiation of Outbreak Management Team (OMT)
		Reporting outbreak to the national Early warning and response meeting of Hospital-acquired Infections and AntiMicrobial Resistance (SO-ZI/AMR)
		Electronic labelling of VRE-positive patients (confirmed label) and patients with prior hospitalisation in het ADRZ hospital in from September 1, 2014, or prior hospitalisation in a nursing home or rehabilitation centre ('VRE suspected' label)
		Informing local hospital personnel and patients, and surrounding hospitals and nursing homes
		Introducing screening for VRE carriage on admission for all patients with electronic 'VRE suspected' label and patients from nursing homes or rehabilitation centres
		Weekly hospital-wide VRE rectal screening limited to patients admitted for at least 48h. In high risk departments (dialysis and ICU) all admitted patients are screened, regardless of the admission time
		Start of cleaning and disinfection (250 ppm chlorine) of the entire hospital: wards are cleaned one by one, whereby patients are temporarily transferred to other (not yet cleaned) parts of the same or other wards
		Introduction of disinfectant wipes for contact surfaces in patient rooms and general areas
		Start of mandatory plenary training sessions for all healthcare workers on general precautions and cleaning issues
		Clear division of cleaning tasks for healthcare workers and cleaning personnel
	February	After cleaning of the ward: release rooms previously occupied by VRE positive patients after cleaning and disinfection based on environmental cultures
	March *(Phase II)*	Audits of adherence to infection control and cleaning protocols by infection control department
		Implementing screening and isolation protocol for 'high risk' patients (direct contacts of VRE carriers, mostly roommates)
		Reintroducing of cleaning and disinfection (250 ppm chlorine) of the entire hospital: departments are cleaned one by one, whereby patients are temporarily transferred to other (not yet cleaned) departments
		Intensifying communication to healthcare workers and managers
	November *(Phase III)*	Screening for VRE carriage upon admission limited to only patients with prior hospitalisation in het ADRZ hospital in period December 1, 2015 – November 14, 2016
		Monthly hospital-wide VRE rectal screening limited to patients admitted for at least 48h. In high risk departments (dialysis and ICU) all admitted patients are screened, regardless of the admission time
2017	February *(End of the outbreak)*	Removing all outbreak related 'VRE suspected' labels in the electronic patient system
		Start hospital-wide VRE rectal screening limited to patients admitted for at least 7 days

Audits, cleaning and education

Cleaning tasks had to be performed by nurses or by dedicated cleaning personnel depending on the objects. During audits it became clear that the tasks had not been defined clearly and consequently some items were not always cleaned. As an intervention the tasks were specified in writing and subsequently the cleaning responsibilities were clearly defined. Also, damaged hospital equipment and furniture were repaired or replaced, and the cleaning frequency of sanitary facilities was increased. Audits on implementation of infection control measures and cleaning practises were performed, including adenosine triphosphate (ATP) measurements [7] performed by the infection prevention department. The ATP measurements were performed in patient rooms and common areas and to control cleaning after discharge (data not shown). All healthcare workers and cleaning personnel received mandatory training on standard infection control and cleaning policies. The number of alcohol-based hand rub (ABHR) dispensers was increased so ABHR was available at 'point of care' in all wards.

CULTURING AND TYPING

Environmental sampling

To assess the extent of environmental contamination in general (surveillance) and after cleaning and disinfection of the patient rooms, a range of high touch surfaces and (medical) equipment were sampled using 10 cm x 10 cm sterile gauzes moistened with sterile saline [8]. See **Supplementary material S2** for an overview of the most frequently sampled surfaces and equipment.

Microbiology

Rectal swabs or feces was collected by nursing staff using the eSwab medium (Copan, Murrieta, USA). In total, 100 µL eSwab transport medium was transferred to a brain heart infusion broth containing 4 mg/L amoxicillin. After an overnight incubation at 35-37°C, 10 µL of the broth was transferred to a Columbia colistin nalidixic acid-agar with 5% Sheep Blood and vancomycin (6 µg/mL) and grown overnight at 35-37°C. For all suspected colonies growing on the selective media, species identification and susceptibility testing was performed using automated systems (Vitek MS and Vitek 2) (bioMérieux, Marcy l'Etoile, France) and E-test (bioMérieux, Marcy l'Etoile, France).

Resistance genes were detected using an in house *vanA*/*vanB* duplex polymerase chain reaction (Elisabeth-TweeSteden Hospital, Tilburg, The Netherlands, see **Supplementary material S3**).

Multilocus sequence typing (MLST) [9] and Amplified fragment Length Polymorphism (AFLP) [10] was used to type VRE isolates. During the peak of the outbreak, typing was not performed when isolates carried a *VanA* gene and patients involved shared a clear epidemiological link to avoid unnecessary costs. Hence, further typing was performed for 62 isolates of VRE*fm* positive patients (55 isolates of rectal screenings samples and 7 isolates of clinical samples) and three environmental isolates. An overview of the AFLP results is presented in **Supplementary material S4_A** and **S4_B**.

RESULTS

Outbreak overview

From September 2014 to February 2017, a total of 140 vancomycin-resistant *Enterococcus faecium* (VRE*fm*) cases were identified. **Figure 1** shows the epidemic curve of detection of new VRE*fm*-positive patients per week.

The VRE*fm* strain found during this outbreak expressed a high level of resistance to vancomycin (MIC > 256 mg/L) and teicoplanin (MIC > 32 mg/L) and carried the *vanA* gene. VRE typing of one of the first isolates showed MLST ST117, designated AFLP type AT14181; subsequent AFLP analysis of 64 isolates in the course of the outbreak period revealed a single AFLP clone **(Supplementary material S4)**.

The outbreak was detected in December 2015, where VRE*fm* carriage was found in four patients who had been transferred from location Goes. On December 14, 2015, following the alert from a neighbouring academic hospital, all patients admitted for more than 48 hours were screened for VRE carriage: of the 158 patients screened, 13 patients (8%) were carriers of VRE*fm*. The VRE*fm* positive patients were all admitted to various departments at location Goes (none at location Vlissingen), including the Intensive Care Unit, indicating that VRE*fm* had spread throughout the entire location (with the exception of the AAU, and the children's and maternity ward).

Retrospectively, the start of the outbreak was set at September 1, 2014, as on this date the first VRE*fm was* detected in a clinical specimen of a patient admitted to the surgical ward at location Goes. In the months following September 2014, VRE*fm* strains had been isolated in clinical materials from five patients during their admission to location Goes (**Figure 1**). These had been at the time considered to be incidental findings without a clear epidemiological link. AFLP typing in December 2015 showed that these isolated belonged to a single AFLP clone (**Supplementary material S4**).

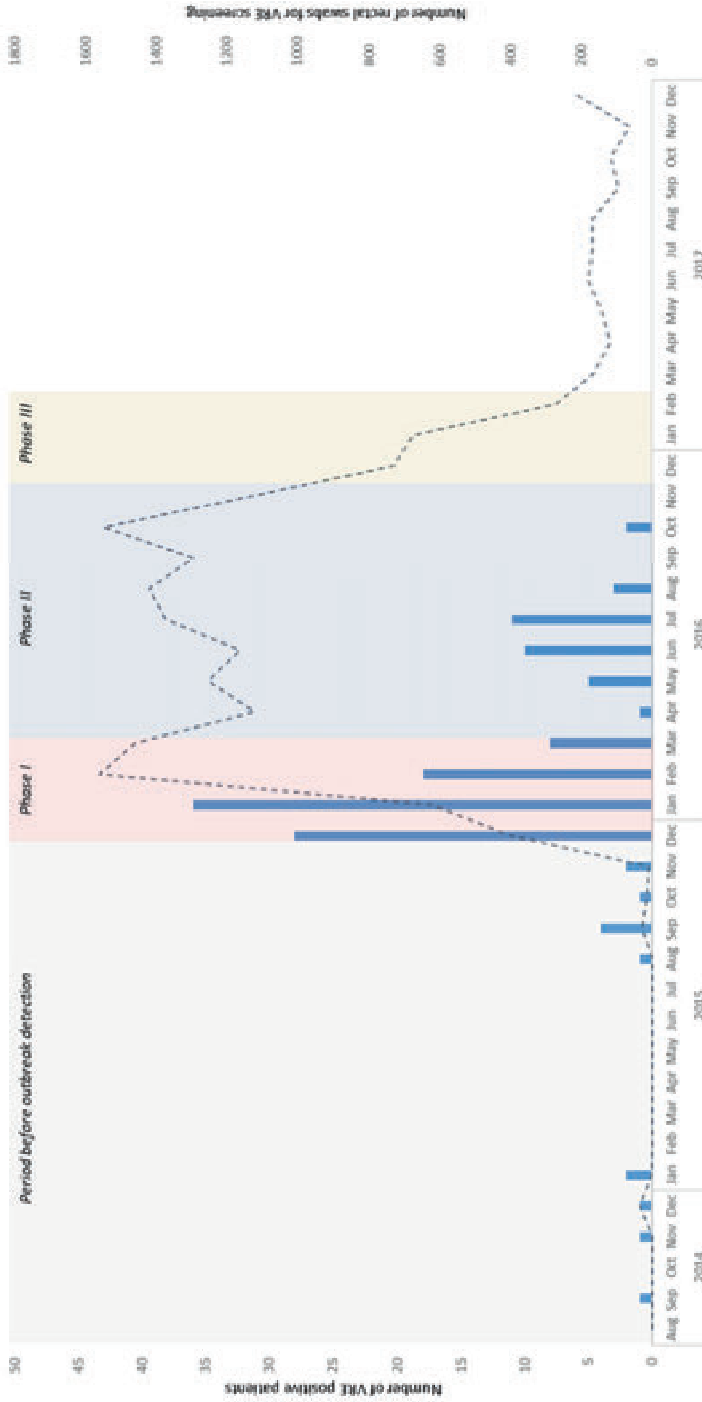
In the first phase out the outbreak from January 2016, VRE suspected patients were isolated with contact precautions (preferably in single rooms) upon (re)admission. Isolation measures were discontinued and the 'VRE suspected' label was removed based on a single negative rectal culture. Due to the large number of VRE suspected patients and insufficient availability of single rooms, separate cohorts were formed for VRE positive and VRE suspected patients, each with dedicated rooms, staff and (medical) equipment. Two extra five-bed-rooms were opened on the AAU to accommodate these cohorts by mid-January 2016. On the other wards, VRE suspected or positive patients were placed in a single room or cohorted with other (suspected) VRE carriers if there was insufficient availability of single rooms.

Screening of VRE suspected patients upon admission between January and March 2016 (phase I), showed that only 2 of 647 patients (0.31%) were positive for VRE*fm*. None of these patients were residing in a nursing home or rehabilitation facility. Given the low VRE prevalence in the cohort of patients admitted between September 2014 and December 2015 and the absence of VRE carriage among patients transferred from other institutions, patients from this cohort were labelled 'medium risk' and no longer pre-emptively isolated upon admission starting from March 21, 2016 (phase II). Screening of this cohort by performing a single rectal culture upon admission remained unchanged.

In this second phase of the outbreak, patients who had been in contact with a VRE*fm*-positive patient (roommates of VRE carriers) were labelled 'high risk', isolated and screened using 3 separate rectum cultures on day 3,5 and 7 after last exposure (**table 1**).

After an initial decline in the number of new VRE*fm* findings in February and March 2016 – there was a second peak in the number of VRE*fm* positive patients in May – June 2016. On-site audits performed during phase II showed shortcomings in infection control preconditions on several wards in the hospital: wards were cluttered, the separation of dirty and clean areas was not clear, and the replacement of damaged hospital equipment and furniture had not been implemented. The division of labour with regards to cleaning responsibilities between cleaning personnel and healthcare workers was further emphasized in this phase, and environmental sampling increased in frequency (see below).

Figure 1 epidemiological curve of new VRE-positive patients (blue bar, n = 135) and number of rectal screening cultures on admission, prevalence and contact tracing (dotted line) in ADRZ. Patients who were transferred to other healthcare settings when found to be VRE positive (n = 5) are not shown in this graph.



As of November 2016, there were no new VRE*fm* findings in the preceding three months and therefore screening for VRE carriage on admission was limited to only patients with prior hospitalisation in het ADRZ hospital in the period December 2015 – November 2016 (phase III). Furthermore, the frequency of hospital-wide screening of patients with >48 hours length of stay was from then on performed monthly instead of weekly.

Control of the outbreak

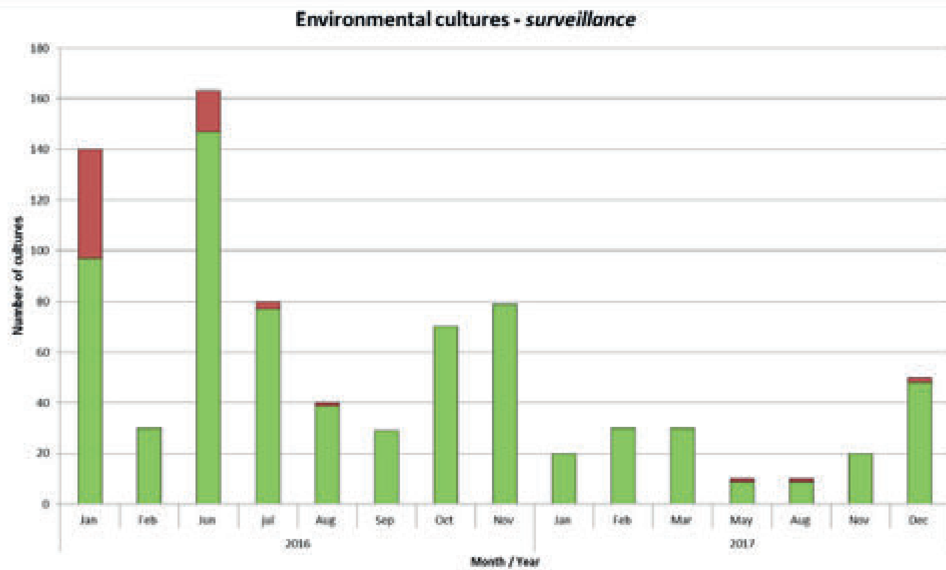
No further cases occurred over a three months period and control measures were terminated in February 2017. Admission and prevalence surveillance cultures were discontinued and all outbreak related 'VRE suspected' labels in the electronic patient system were removed. Furthermore, a hospital-wide VRE rectal screening limited to patients admitted for at least 7 days was implemented as a standard surveillance form that moment on.

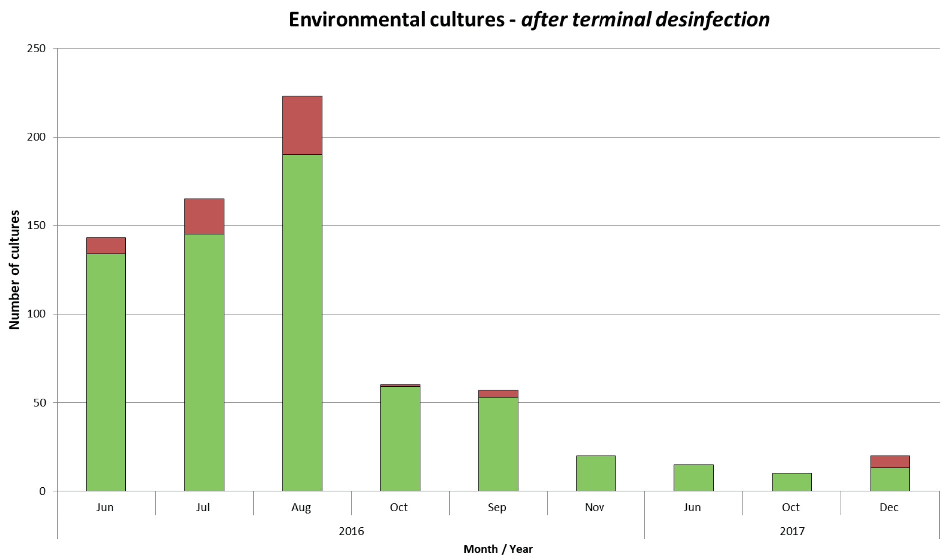
Environmental cultures

In January 2016, environmental cultures were obtained throughout the hospital to assess the extent of environmental contamination. The cultures showed extensive VRE contamination on the surgical, internal, pulmonary and neurology wards (43/80 samples VRE positive; 53.7%). Environmental samples of the AUU, ICU and dialysis department were VRE negative (0/60 samples) **(Figure 2a)**.

In June 2016, environmental screening was repeated on multiple wards (n = 130 samples), and again extensive VRE contamination was found in the surgical ward (19/20 samples, 95.0%) and to a lesser extent on the cardiology ward (4/20 samples; 20.0%). Consequently, stepwise cleaning and disinfection (250 ppm chlorine) of these wards was performed. After cleaning these wards were closed pending VRE negative environmental results. Following a peak in VRE transmission, environmental surveillance was continued and intensified: from June 2016, rooms previously occupied by VRE-positive patients were only released after cleaning and negative environmental cultures. Ten percent (74/713 culture) of the room surfaces remained VRE*fm* positive after terminal disinfection. **(Figure 2b)** In some cases, VRE*fm* was still detected after two rounds of terminal disinfection on e.g. patient bed, infusion pole and pull-up bar.

Figure 2 Environmental cultures of the patient rooms, a range of high touch surfaces and (medical) equipment's at random times (surveillance) **(a)** and after disinfection **(b)**. Red bar: VREfm positive cultures; green bar: VRE negative cultures.





Infections during the outbreak period

Eight (5.7%) patients developed a *VREfm* infection, of whom two (1.4%) had bacteraemia. Two patients, with extensive co-morbidities, died shortly after detection. One patient, also with extensive co-morbidities (including renal failure, haemodialysis and vascular disease) developed a severe osteomyelitis following a surgical procedure, which eventually led to amputation of her left hand.

DISCUSSION

Here we describe the successful control of a VRE*fm* outbreak in a hospital using a more restrictive screening and isolation policy than recommended in the national guidelines [6]. With this approach, within nine months after the detection of the outbreak, no new VRE*fm* cases were detected and after twelve months, the outbreak was considered fully controlled. In addition to the targeted screening and isolation there was an intensive focus on optimisation of environmental cleaning procedures.

In general, there is no consensus on the optimal VRE screening, isolation and surveillance protocol, reflected by the variation in infection control approaches within and between countries [11–13]. The number of rectal cultures required to consider a known carrier or contact patient VRE-negative, is unclear. Studies show that the sensitivity of a single rectal swab is low, ranging from 42,5% to 79% [14–17], and this increases when taking multiple swabs: Pearman et al. showed that on average four rectal swabs, collected on separate days, were needed to detect 95% of carriers compared to 56% with one rectal swab [15]. Explanations for the increase in sensitivity when taking multiple rectal swabs include a fluctuation in faecal excretion of VRE, and/or the presence of an intestinal transit time after VRE is transmitted (time between transmission event and detectable VRE levels in the faeces). It should be noted that in most of these studies the rectal swabs were inoculated directly on selective media. Addition of a broth enrichment step (as done in our study) increases the yield of a rectal swab culture substantially [18]. Lastly, sensitivity may depend on the load of VRE in faeces [17]; a high VRE load in faeces also results in a higher sensitivity of a rectal swab, whereby patients with lower faecal loads probably contribute less to transmission.

In 2015, a Dutch guideline was published which recommends taking 3-5 rectal cultures on separate days to reliably exclude carriage in a suspected VRE carrier [6]. This guideline makes no specific recommendation about the timing of the 3-5 separate rectal culture collection, apart from recommending that the last culture is ideally performed at least seven days after the last exposure. During our outbreak, the screening policy consisting of

a single rectal swab culture (with enrichment broth) upon re-admission for excluding VRE carriage in the majority of VRE suspected patients in the first phase of the outbreak, and a maximum of three culture in the second phase for 'high risk' VRE suspected patients.

In this outbreak, there was a long period of time between the first clinical VRE finding (September 2014) and the detection of the outbreak (December 2015), resulting in a large cohort of approximately 25,000 discharged and potentially exposed patients, hence classified as a 'VRE suspected'. Readmission of these patients occurred frequently and it was estimated, based on historical data, that approximately 4,700 admissions / year would be categorised as 'VRE suspected'. In case of limiting the screening to those who were re-admitted (approx. 4,700/year), isolating all 'VRE suspected' patients in a single room upon admission pending at least 3 rectal screening cultures on separate days would place a large burden on hospital room capacity, and still require a very large number of cultures. With the assumption that the sensitivity of a single swab is substantially higher when performed > 7 days after the last exposure, and because a substantial proportion of exposed patients was expected to have lost VRE carriage within 6 months [19], we decided to perform screening for VRE by taking a single swab, and limit screening to patients who were re-admitted to the hospital. By ending pre-emptive contact precautions after a single VRE-negative rectal swab, most patients were isolated only the first two days of admission. With this strategy, that was less stringent than the Dutch national recommendations for VRE control, a total of 19,677 rectal swabs (of 9,279 patients) were collected during the entire outbreak (admission-, prevalence- and contact surveillance cultures together), thereby significantly cutting administrative effort, time of isolation and laboratory costs.

Though it can be argued that we have not detected all VRE*fm* carriers (and underestimated the size of the outbreak) due to suboptimal sensitivity of our screening policy, our experience shows that VRE transmission from undetected carriers was, even if present, insufficient to caused sustained transmission. Whether this strategy would have been effective in settings with higher complexity of care (where patients probably have longer duration of carriage, longer admissions, and a higher transmission potential and infection risk) is unclear. In such settings, even a small loss in sensitivity may lead to ongoing transmission. In a recent paper, Frakking et al. describe a successful control of a large VRE outbreak (n = 242 patients) in a Dutch teaching hospital and tertiary referral centre [4]. The outbreak lasted 18 months and was eventually controlled after major efforts, including twice-weekly screening of all admitted patients, environmental decontamination with hydrogen peroxide vapour, strict isolation precautions (in isolation

rooms with anteroom), a VRE quarantine ward and abandoning the use of ciprofloxacin prophylaxis during neutropenic fever. The authors advocate a screening policy with at least 4-5 rectal swabs before excluding VRE carriage.

It should be noted that in our study, for the majority of the cohort of suspected VRE patients the last exposure was > 7 days ago (for many patients even several months), which (in combination with intensive focus on general precautions and environmental cleaning) may have been an important reason for the success of this strategy. In addition, the prevalence among re-admitted patients was <1%, indicating a low background risk of undetected introductions of VRE in the hospital.

As described, we are careful to generalize our findings to other settings. Our results suggest, however, that the current general Dutch recommendations to take 3-5 cultures to exclude VRE carriage in all exposed patients may be reconsidered for centres with lower complexity of care, especially when the last exposure was > 7 days ago. This lowers the costs and limits the duration of pre-emptive isolation.

CONCLUSION

To conclude, we describe a large VRE outbreak in a general hospital in The Netherlands that was successfully controlled, while substantially reducing the number of cultures to be taken and the number of isolation days, and thereby cutting laboratory costs.

ACKNOWLEDGEMENTS

We would like to acknowledge the healthcare workers, colleagues of the department of Infection control and Laboratory for Microbiology of the Admiraal De Ruyter Hospital— who contributed to the investigation and the control of this outbreak. Furthermore, the authors thank the department of Medical Microbiology and Infection Control of Amsterdam UMC for the AFLP analysis and dendrogram construction.

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SUPPLEMENTARY MATERIAL

Supplementary table S1: number of new cases of VRE bacteraemia and VRE bacteraemia per 100.000 patient days in The Admiraal De Ruyter Hospital, 2014 - 2020

	2014	**2015**	**2016**	**2017**	**2018**	**2019**	**2020***
Number of new cases of VRE bacteraemia	0	2	0	0	0	0	0
VRE bacteraemia per 100.000 patient days	0	4,4	0	0	0	0	0

***** *only data until December, 28, 2020 were included*

Supplementary table S2: overview of the items sampled during the outbreak, indicating which items were tested VRE positive at least once.

Item	**Tested VRE positive at least once**
Patient room	
Hospital bed	**Yes**
Pillow	**Yes**
Mattress	**Yes**
Pull-up bracket	**Yes**
Paging system at bed	No
Nightstand	**Yes**
Telephone patient room	**Yes**
Electricity bar in patient room	**Yes**
Sink	No
Shower	**Yes**
Toilet	**Yes**
Doorknob bathroom	No
Chair	**Yes**
Table	No
Medical devices	
Glucose meter	No
Blood pressure meter	**Yes**
Ear thermometer	No
Stethoscope	No
Infusion pomp	No
Ward bounds materials	
Computer in teampost	No
Telephone teampost	No
Paper medical records	**Yes**
Door handles sterile storage	No
Pneumatic tube system	**Yes**
Computer on wheels	**Yes**
Commode chair	**Yes**
Corridor wall hand rail	**Yes**
Walker	No
storage trays	No
Others	No

Supplementary material S3: protocol of the in house vanA/vanB duplex polymerase chain reaction performed at the Elisabeth-TweeSteden Hospital, Tilburg, The Netherlands

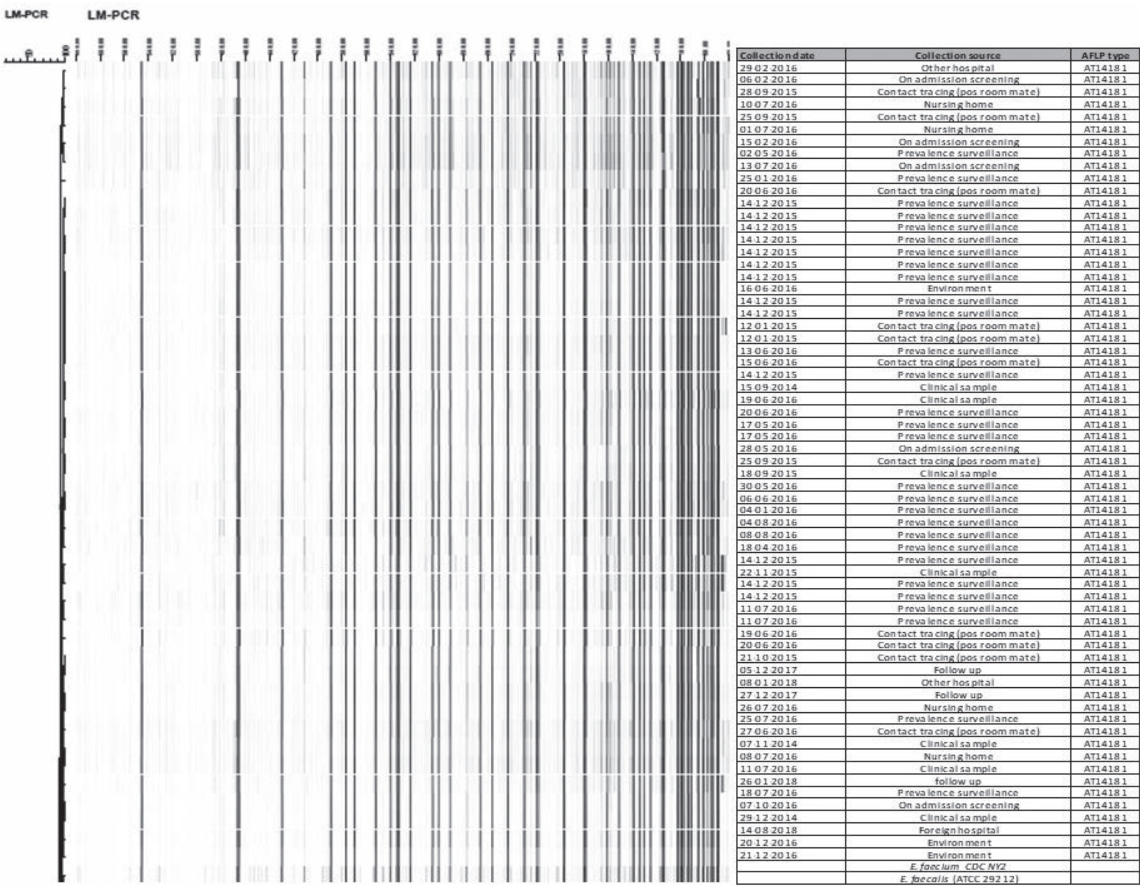
DNA was extracted using the QIAsymphony DSP virus/pathogen midi kit and pathogen complex 400 protocol of the QIAsymphony Sample Processing (SP) system (Qiagen, Hilden, Germany). Amplification reactions were performed in a volume of 25 µL with PCR mastermix (QuantiTect Multiplex PCR NoROX Kit, QIAgen) and 10 µL DNA sample. A multiplex PCR using *vanA*-, *vanB*-, and *E. faecium*-specific primers and probes (**table A**) was performed. For the amplification and detection of the internal control PCR primers and detection probe specific for PhHV-1 were used (Niesters HGM. Clinical virology in real time. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2002 Dec;25 Suppl 3:S3-12.). The amplification reaction was performed using 200 nM of each *vanA*, *vanB* and *E. faecium*-specific primers, 100 nM of each PhHV-1-specific primer, and 100 nM of each *vanA*-, *vanB*-, *E. faecium*- PhHV-specific probe.

Amplification consisted of 15 minutes at 95°C followed by 45 cycles of 15 seconds at 95°C, 30 seconds at 60°C, and 15 seconds at 72°C. Amplification, detection, and analysis were performed with the Rotor-gene real-time detection system (QIAgen). Negative and positive control samples were included in each amplification run. PCR output from this system consists of a Ct-value, representing the amplification cycle in which the level of fluorescent signal exceeds the background fluorescence, and reflecting the target-specific DNA load in the sample tested.

Table A primer and sequences for multiplex PCR of vancomycin resistance genotyping and enterococci species identification among vancomycin-resistant enterococci

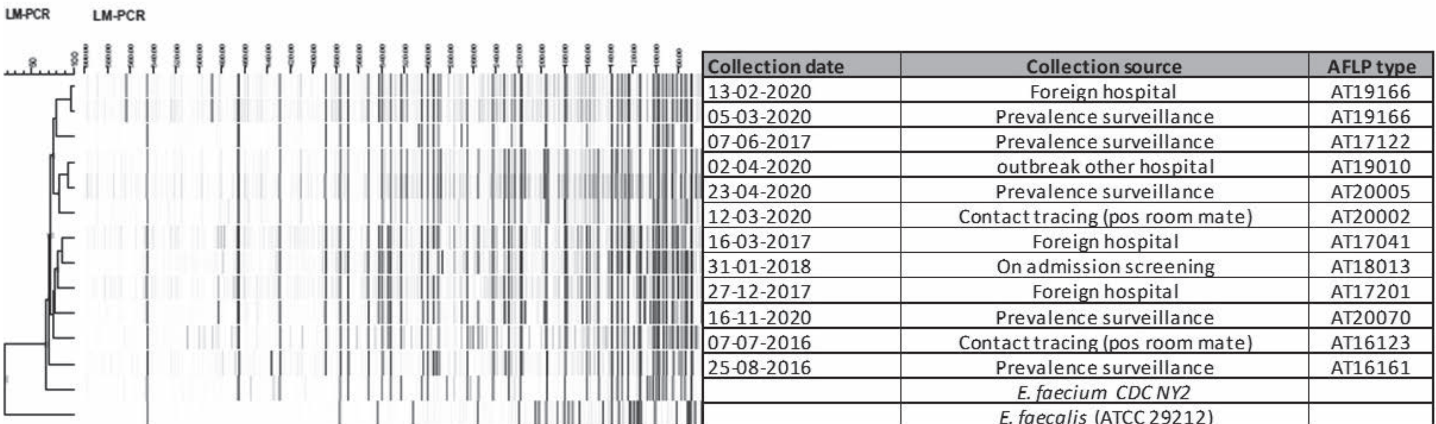
Primer name	Primer sequence	Target gene
vanA-F1	GCCGGAAAAAGGCTCTGAA	*vanA gene*
vanA-R1	TCCTCGCTCCTCTGCTGAA	*vanA gene*
vanA-1-FAMBhq1	ACGCAGTTATAACCGTTCCCGCAGACC	*vanA gene*
vanB-F1	CGCAGCTTGCATGGACAA	*vanB gene*
vanB-R1	GGCGATGCCCGCATT	*vanB gene*
vanB-1-VIC (MGB)	TCACTGGCCTACATTC	*vanB gene*
Efa-F1	GGAATGGCGCAAAACTTAGA	*atpA gene*
Efa-R1	AGGCCTCTCCAACTGGAACT	*atpA gene*
Efa-1-TRBhq2	TGGCGATTTCGAGTCCATTCG	*atpA gene*

Supplementary material S4_A: dendrogram based on Amplified fragment length polymorphism (AFLP) of 65 VRE strains identified during the outbreak. *E. faecium* (CDC NY2) and *E. faecalis* (ATCC 29212) were included in the analysis as reference strains. The cut-off value for identical strains was 90% relative similarity.



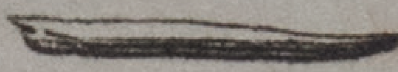
Collection date	Collection source	AFLP type
29 02 2016	Other hospital	AT14181
06 02 2016	On admission screening	AT14181
28 09 2015	Contact tracing (pos room mate)	AT14181
10 07 2016	Nursing home	AT14181
25 09 2015	Contact tracing (pos room mate)	AT14181
01 07 2016	Nursing home	AT14181
15 02 2016	On admission screening	AT14181
02 05 2016	Prevalence surveillance	AT14181
13 07 2016	On admission screening	AT14181
25 01 2016	Prevalence surveillance	AT14181
20 06 2016	Contact tracing (pos room mate)	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
16 06 2016	Environment	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
12 01 2015	Contact tracing (pos room mate)	AT14181
12 01 2015	Contact tracing (pos room mate)	AT14181
13 06 2016	Prevalence surveillance	AT14181
15 06 2016	Contact tracing (pos room mate)	AT14181
14 12 2015	Prevalence surveillance	AT14181
15 09 2014	Clinical sample	AT14181
19 06 2016	Clinical sample	AT14181
20 06 2016	Prevalence surveillance	AT14181
17 05 2016	Prevalence surveillance	AT14181
17 05 2016	Prevalence surveillance	AT14181
28 05 2016	On admission screening	AT14181
25 09 2015	Contact tracing (pos room mate)	AT14181
18 09 2015	Clinical sample	AT14181
30 05 2016	Prevalence surveillance	AT14181
06 06 2016	Prevalence surveillance	AT14181
04 01 2016	Prevalence surveillance	AT14181
04 08 2016	Prevalence surveillance	AT14181
08 08 2016	Prevalence surveillance	AT14181
18 04 2016	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
22 11 2015	Clinical sample	AT14181
14 12 2015	Prevalence surveillance	AT14181
14 12 2015	Prevalence surveillance	AT14181
11 07 2016	Prevalence surveillance	AT14181
11 07 2016	Prevalence surveillance	AT14181
19 06 2016	Contact tracing (pos room mate)	AT14181
20 06 2016	Contact tracing (pos room mate)	AT14181
21 10 2015	Contact tracing (pos room mate)	AT14181
05 12 2017	Follow up	AT14181
08 01 2018	Other hospital	AT14181
27 12 2017	Follow up	AT14181
26 07 2016	Nursing home	AT14181
25 07 2016	Prevalence surveillance	AT14181
27 06 2016	Contact tracing (pos room mate)	AT14181
07 11 2014	Clinical sample	AT14181
08 07 2016	Nursing home	AT14181
11 07 2016	Clinical sample	AT14181
26 01 2018	follow up	AT14181
18 07 2016	Prevalence surveillance	AT14181
07 10 2016	On admission screening	AT14181
29 12 2014	Clinical sample	AT14181
14 08 2018	Foreign hospital	AT14181
20 12 2016	Environment	AT14181
21 12 2016	Environment	AT14181
	E. faecium CDC NY2	
	E. faecalis (ATCC 29212)	

Supplementary material S4_b: dendrogram based on Amplified fragment length polymorphism (AFLP) of 12 VRE strains identified between 2016 and 2020 in ADRZ - NOT belonging to the outbreak cluster. *E. faecium* (CDC NY2) and *E. faecalis* (ATCC 29212) were included in the analysis as reference strains. The cut-off value for identical strains was 90% relative similarity.



Collection date	Collection source	AFLP type
13-02-2020	Foreign hospital	AT19166
05-03-2020	Prevalence surveillance	AT19166
07-06-2017	Prevalence surveillance	AT17122
02-04-2020	outbreak other hospital	AT19010
23-04-2020	Prevalence surveillance	AT20005
12-03-2020	Contact tracing (pos room mate)	AT20002
16-03-2017	Foreign hospital	AT17041
31-01-2018	On admission screening	AT18013
27-12-2017	Foreign hospital	AT17201
16-11-2020	Prevalence surveillance	AT20070
07-07-2016	Contact tracing (pos room mate)	AT16123
25-08-2016	Prevalence surveillance	AT16161
	E. faecium CDC NY2	
	E. faecalis (ATCC 29212)	

A.



B.

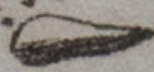
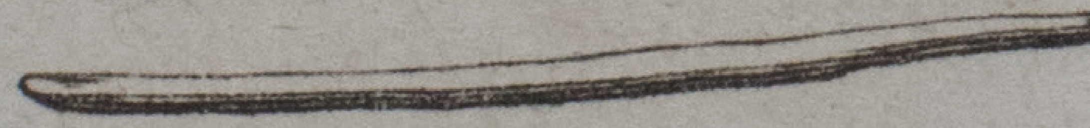
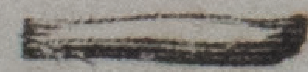


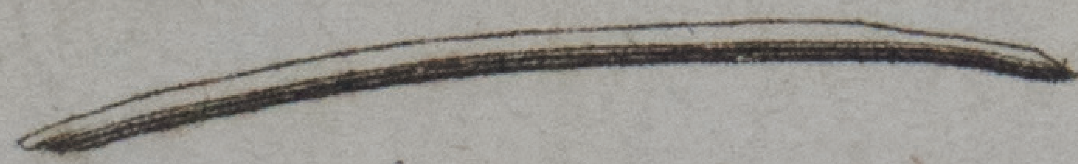
Fig. 3.

E.

F







Chapter 5

AN OUTBREAK OF COLISTIN-RESISTANT KLEBSIELLA PNEUMONIAE CARBAPENEMASE-PRODUCING KLEBSIELLA PNEUMONIAE IN THE NETHERLANDS (JULY TO DECEMBER 2013), WITH INTER-INSTITUTIONAL SPREAD

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ABSTRACT

Objective

We describe an outbreak of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* (KPC-KP) ST258 that occurred in two institutions (a hospital and a nursing home) in the Netherlands between July and December 2013.

Methods

In total, six patients were found to be positive for KPC-KP. All isolates were resistant to colistin and exhibited reduced susceptibility to gentamicin and tigecycline. In all settings, extensive environmental contamination was found. Whole genome sequencing revealed the presence of *bla*KPC-2 and *bla*SHV-12 genes, as well as the close relatedness of patient and environmental isolates. In the hospital setting, one transmission was detected, despite contact precautions. After upgrading to strict isolation, no further spread was found. After the transfer of the index patient to a nursing home in the same region, four further transmissions occurred. The outbreak in the nursing home was controlled by transferring all KPC-KP-positive residents to a separate location outside the nursing home, where a dedicated nursing team cared for patients.

Conclusion

This outbreak illustrates that the spread of pan-resistant Enterobacteriaceae can be controlled, but may be difficult, particularly in long-term care facilities. It, therefore, poses a major threat to patient safety. Clear guidelines to control reservoirs in and outside the hospitals are urgently needed.

INTRODUCTION

Up to now, hospital outbreaks with carbapenemase-producing Enterobacteriaceae (CPE) occur sporadically in Northern and Western Europe, and most reports concern isolates that are introduced by patients from high prevalence areas. However, the frequency of such outbreaks is increasing, and regional spread has been reported [1]. Information about the modes of transmission and potential reservoirs are urgently needed in order to design strategies to control the spread of these bacteria.

Hospital infections caused by CPE, and, in particular, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumonia* (KPC-KP), have been associated with increased cost and length of hospital stay, frequent treatment failures, and death [2]. Few antimicrobial agents exist to treat infections with these bacteria, and colistin is one of the last treatment options. Although resistance to colistin is still rare, there have been several reports of colistin-resistant KPC-KP in the past few years [3–5].

Although the KPC gene has been identified in several other Gram-negative bacteria, it is still most commonly found in Klebsiella species. One specific sequence type (ST), ST258, is particularly successful and represents the vast majority of KPC-KP isolates found globally. ST258 isolates have the ability to rapidly disseminate in healthcare settings and are frequently associated with outbreaks in hospitals and long-term care facilities [3,6–8]. Analysis of the ST258 genome may provide further insight into factors related to the epidemiological success of this clone [9–11].

In this paper, we report an outbreak of KPC-KP ST258 that occurred in the Netherlands between July and December 2013 and involved two institutions (a hospital and a nursing home). We describe the control measures taken to prevent further spread, the results of the microbiological and molecular investigations, and the results of the environmental screening cultures taken during the outbreak.

METHODS

Detection of the outbreak

The outbreak was recognized on July 13, 2013, when a KPC-KP isolate was detected in the pleural fluid of a 65-year-old man (patient 2) admitted to the pulmonary ward of a 1,370-bed teaching hospital in Breda, the Netherlands. This event followed the earlier repatriation of a 69-year-old woman (the index patient) from an intensive care unit in a Greek hospital on June 25, 2013, who was found to be colonised with KPC-KP two days after admission to

the pulmonary ward. The index patient had been nursed using contact precautions since admission to the hospital. Because carbapenem-resistant Enterobacteriaceae have been practically absent in this hospital, the finding was considered indicative of nosocomial transmission, and an outbreak management team was formed.

Twelve weeks after the transfer of the index patient to a nursing home in the same region (on August 5, 2013), another KPC-KP isolate was found in a rectal screening culture of one of the nursing home residents (patient 3) during a short admission to the previously mentioned hospital (November 5, 2013). This initiated an investigation to assess the extent of transmission in the nursing home.

Setting

The hospital has approximately 40,000 admissions/year at three separate locations. Carbapenems are rarely used (< 2 defined daily doses/100 admissions) and, although patients colonised with CPE had been detected previously, no cases of nosocomial transmission had occurred. The infection control department consists of six infection control practitioners (ICPs), headed by a clinical microbiologist, and is part of the microbiology laboratory. The pulmonary ward has five single, three double, and two four-patient bedrooms. The mean length of stay is 7.3 days.

The nursing home consists of four departments (a total of 150 beds), each with specific patient populations, including a 34-bed rehabilitation ward for patients with neurological diseases (mostly cerebrovascular accidents), and patients who underwent orthopaedic or surgical operations. The rehabilitation ward has four single, five double, and five four-patient bedrooms, and a shared area for social activities. Patients reside in this ward for periods of up to 6 months. Before the outbreak, diagnostic cultures were sent to a commercial laboratory, and there was no dedicated ICP. On-site or telephone consultations by a self-employed ICP were requested by the nursing home ad hoc.

Infection control measures

Hospital

Because the index patient was transferred from a country where multidrug-resistant organisms (MDROs) are highly endemic [12], isolation measures were implemented while awaiting the results of screening cultures, in accordance with national guidelines [13]: the patient was placed in a single room, and healthcare workers (HCWs) donned gloves and

gowns before entering the patient room. Following the detection of KPC-KP carriage of the index patient, an ICP visited the pulmonary ward on a daily basis to educate staff and clarify practical infection control issues.

When, despite these measures, a second patient colonised with a KPC-KP was found, isolation measures were increased: both patients were transferred to a single room with anteroom and negative air pressure, and HCWs donned gloves, gowns, face masks, and caps before entering the patient room. In addition, a dedicated nursing team was appointed (cohorting) for the two patients. Both rooms were cleaned daily at the end of the cleaning shift. On August 5, 2013, the index patient was discharged to the rehabilitation ward of a nursing home and patient 2 was discharged home. The patient rooms were disinfected with a quaternary ammonium compound. To guarantee direct isolation measures upon future readmission, KPC-colonised patients were flagged in the electronic patient record system. At the time of transfer to the nursing home, information regarding the carriage of a pan resistant KPC-KP and the fact that transmission had occurred despite standard isolation measures was provided to a nurse at the rehabilitation ward by telephone.

Nursing home

Upon transfer to the rehabilitation ward of the nursing home, the index patient was admitted to a single room with private sanitary facilities. HCWs wore gloves and gowns during care moments. There were no restrictions for social activities and the patient had unrestricted access to communal areas in the facility.

On November 5, 2013, when transmission on the rehabilitation ward was detected, isolation measures were intensified: the KPC-positive patients were cared for in single rooms with the door closed, and were no longer allowed to leave their rooms. HCWs donned gloves, gowns, and face masks before entering the patient room, and were instructed to remove personal protective equipment and rub their hands with 70 % alcohol before leaving the patient room. Frequent audits of hand hygiene compliance were performed by an ICP and direct feedback was given to the HCWs and cleaning staff. Both rooms were cleaned daily at the end of the cleaning shift.

Contact screening

In the hospital, contact screening for the carriage of KPC-producing Gram-negative bacteria was performed for all patients who had been hospitalized for at least 48 h on the pulmonary ward since June 25, 2013, including those already discharged. Prospective

screening was performed twice weekly on all hospitalized patients of the pulmonary ward, until 2 weeks after the discharge of both KPC-positive patients.

In the nursing home, contact screening for KPC carriage was initiated after the third KPC-positive patient was identified on November 5, 2013, among all patients discharged from the rehabilitation ward since August 5, 2013. Prospective screening of all patients on the rehabilitation ward was performed once a week from November 8. All nursing home residents (including those on other wards) were screened for KPC carriage twice at a 6-week interval on November 27, 2013 and January 7, 2014.

HCWs in the hospital and nursing home were not tested for KPC-producing Gram-negative bacteria carriage.

Case definition

A case was defined as any patient infected or colonised with KPC-producing Gram-negative bacteria. Swabs from the rectum, sputum (in case of cough), insertion sites, sites of infection, and urine (in case of an indwelling catheter) were collected by nursing staff using the eSwab medium (Copan, Murrieta, CA, USA).

Sedimentation cultures and environmental sampling

Air sedimentation cultures were taken by placing solid agar plates (n=9) in the hall, anteroom, next to the bed, on the window sill, and on the bathroom sink. The agar plates were opened for 1.5 h, during the time that the index patient received physiotherapy and tracheostomy care, which included suction of secretions and rinsing of the inner cannula of the trachea cannula in the bathroom.

In the rehabilitation ward of the nursing home, a range of high-touch surfaces (e.g., night cabin, door knob) and equipment (e.g., glucose meter, patient lift) were sampled using 10- cm×10-cm sterile gauzes moistened with sterile saline.

Microbiological methods

Patient swabs were inoculated on the Colorex KPC plate (CHROmagar, bioTRADING, the Netherlands) and EbSA plates (Cepheid, Ledeberg, Belgium). The remaining eSwab fluid was transferred in 5 mL of tryptic soy broth with vancomycin (8 mg/L) and cefotaxime (0.25 mg/L) (TSB-VC; Cepheid), which was inoculated on the same media overnight. Gauzes used for environmental sampling were immediately placed in 10 mL of selective broth

(TSB-VC) and grown overnight at 35-37 °C. Ten microliters of the broth was transferred to a Colorex KPC plate and an EbSA plate and grown overnight at 35-37 °C.

For all oxidase-negative Gram-negative rods growing on the selective media, species identification and susceptibility testing was performed using automated systems (Vitek MS and Vitek 2; bioMérieux, Marcy-l'Étoile, France) and the Etest (bioMérieux, Marcy-l'Étoile, France).

Molecular methods

Resistance genes were detected using a commercial microarray (Check-MDR CT103, Check-Points, Wageningen, the Netherlands) that can detect the β-lactamase genes of TEM, SHV, and CTX-M, pAmpC (CMY-2, DHA, FOX, ACC-1, ACT/MIR, and CMY-1/MOX), and carbapenemase genes (KPC, OXA-48-like, VIM, IMP, and NDM).

Typing was performed for every first unique KPC- producing isolate in each patient, and for four isolates found in the environment of the rehabilitation ward. *K. pneumoniae* isolates were typed using both amplified fragment length polymorphism (AFLP) as described by Mohammadi et al. [14] and multilocus sequence typing (MLST), whereas other species were typed using AFLP only.

Whole-genome sequencing (WGS) and subsequent MLST+ typing and resistome analysis was performed on the same isolates, as well as one additional isolate of the index patient (isolated on December 9, 2013). Genomic DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. The DNA concentration was measured using the Qubit dsDNA HS and BR Assay Kit (Life Technologies, Carlsbad, CA, USA). The DNA library was prepared using the Nextera XT v2 kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions, and then run on the MiSeq system (Illumina) for generating paired-end 250-bp reads. De novo assembly was performed by the CLC Genomics Workbench v7.0.4 (Qiagen, Hilden, Germany) after quality trimming (Qs≥20) with optimal word sizes. The assembled genomes were uploaded to a webtool called ResFinder v2.1 (http://cge.cbs.dtu.dk/services/ResFinder/) for identifying the acquired resistance genes with default settings. Assembled genomes were typed by MLST+ using SeqSphere v1.0 (Ridom GmbH, Münster, Germany). Briefly, an in-house defined MLST+ scheme including 3,102K. pneumoniae genes was used as a reference for extracting open reading frames (ORFs) from the genome of each isolate by SeqSphere. Only the ORFs shared by all samples analyzed here without premature stop codons and ambiguous nucleotides

were kept for MLST+ typing and, further, for generating the minimum spanning tree. This Whole 201 Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under accession numbers JTFU00000000, JTFV00000000, JTFW00000000, JTFX00000000, JTFY00000000, JTFZ00000000, and JTGA00000000.

RESULTS

Outbreak investigation and control

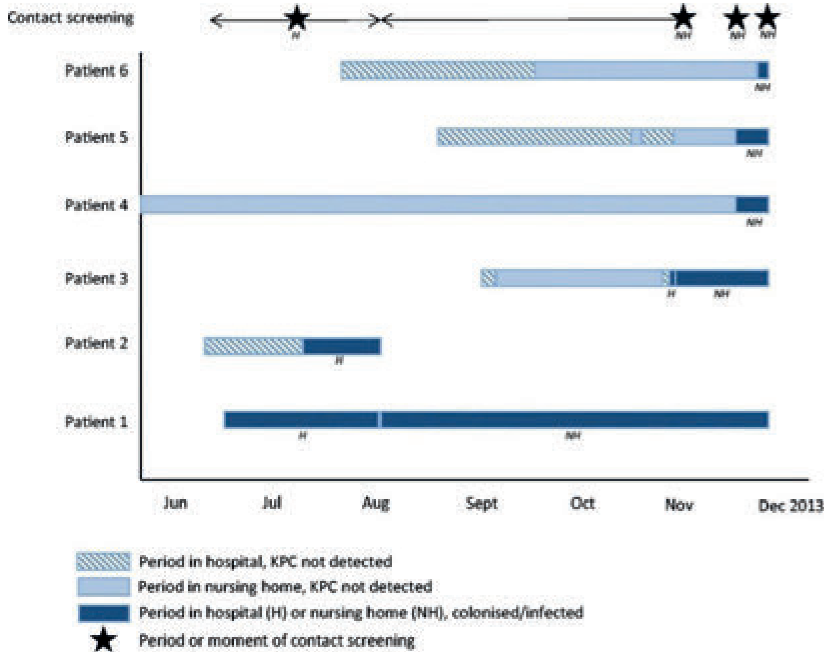
From June through November 2013, a total of six patients were found to be colonised by colistin-resistant KPC-KP. The characteristics of the patients with KPC-producing Enterobacteriaceae are shown in **Table 1**. The sequence of events (including time points of contact screening) is indicated in **Figure 1**.

In the hospital, contact screening (n=114) up to 2 weeks after the discharge of both KPC carriers revealed no further cases. In the nursing home, transmission occurred from the index patient to four additional patients. The second patient in the nursing home (patient 3) suffered from severe diarrhoea upon admission to the rehabilitation ward in September, and, therefore, staff had worn gloves and gowns during care moments since admission. Despite these measures, she was found to carry the outbreak strain on November 5, 2013, and isolation measures were upgraded as described above. Following this event, contact screening (n=29) of discharged patients and the first of three prospective contact screening rounds (November 8, 2013, n= 23) in the rehabilitation ward revealed no secondary cases. However, the second (November 18, 2013, n=22) and third (November 22, 2013, n=19) contact screenings revealed two and one additional KPC-KP carriers, respectively (patients 4, 5, and 6). After the detection of patients 4 and 5, cohorting of all KPC-positive patients to one side of the ward with dedicated nursing staff was implemented on November 20, 2013. Despite these stringent measures, a sixth patient was found to be colonised by the outbreak strain. Subsequently, all KPC-positive patients were transferred to a separate, empty location outside the main building of the nursing home, with dedicated nursing staff, on November 27, 2013. After this transfer, the rehabilitation ward was disinfected with hydrogen peroxide vapor. Surveillance cultures taken on November 27, 2013 and January 7, 2014 in all nursing home patients (n=270) and the environment revealed no further transmission or environmental contamination.

Table 1: Characteristics of patients with KPC-producing Klebsiella pneumoniae*. Several recognized risk factors for KPC colonisation and infection were present in all KPC-positive patients, such as previous use of carbapenem, chronic obstructive pulmonary disease and poor functional status*

Pat.	Sex	Age	Comorbidities	Antibiotic use last 30 days	Colonisation/ infection	Medical devices	Duration of carriage in days
1	F	69	Cerebrovascular accident	Meropenem, Mycamine	Colonisation	Trachea stoma, urinary catheter, percutaneous endoscopic gastrostomy catheter	226
2	M	65	Chronic obstructive pulmonary disease, Morbus Crohn	Clindamycine, Cefuroxime, Ceftazidim, Meropenem, Metronidazol, Penicillin, Tobramycin, Piperacillin/ Tazobactam.	Colonisation (pleura drain fluid)	Pleural drain	320
3	F	74	Recurrent *Clostridium difficile* infections	Vancomycine	Infection (urine)	urinary catheter	97
4	M	70	Cerebrovascular accident	None	Colonisation	urinary catheter, percutaneous endoscopic gastrostomy catheter	26 (died, death unrelated to KPC)
5	M	78	Diabetes mellitus (leading to an amputated leg)	None	Infection (urine)	urinary catheter	34 (died, death associated with KPC)
6	M	49	Postanoxic encephalopathy after cardiopulmonary resuscitation, comatose	None	Colonisation	urinary catheter, percutaneous endoscopic gastrostomy catheter	3

Figure 1. sequence of patient detection in the hospital and nursing home



Adherence to infection control

Audits in the nursing home revealed several shortcomings in the infection control measures during the outbreak period. Among these were unnecessary and inappropriate glove use and poor hand hygiene compliance by HCWs and cleaning staff, whereby personal protection was mentioned as the mean reason for wearing gloves. During several days in September, a shared storage of gowns was installed in the room of the index patient. Gowns were retrieved from the index patient's room for use for patient 3, who resided in a nearby room. A single glucose meter was available on the rehabilitation ward that was used for the index patient and for patients 5 and 6. In addition, patients were frequently transferred to different rooms within the ward: patient 4 was relocated four times in 3 weeks, once to a room that had previously been occupied by the index patient and cleaned afterwards.

In the separate facility, all five KPC-positive patients had a single room with private sanitary facilities. They had unrestricted access to communal areas (where standard precautions were employed), but only after adequate hand hygiene before leaving the

patient room. HCWs only donned gloves and gowns before entering the patient room and removed them before leaving the room. Staff working on this separate facility was not screened for KPC carriage. In March 2014, the separate location was closed because patients were found to be KPC-negative (n=3) or died (n=2, of which one associated to KPC-KP infection).

Environmental contamination

One day after the KPC-positive patients were cohorted to one side of the ward in the nursing home, KPC-KP was identified in 4/24 samples (taken from night cabins, door knob, and the glucose meter). No KPC-producing isolates were found in the environmental cultures (n=24) from the non-cohorting side of the ward.

In the separate facility, environmental contamination was monitored by taking weekly environmental cultures from predefined items **(Table 2)**. A high level of environmental contamination of KPC-producing Gram-negative bacteria was found in the patient rooms and on the two patient lifts, and less frequently in communal/kitchen areas. In addition to KPC-KP, KPC-*producing Enterobacter aerogenes* (room of index patient and patient 5, kitchen worktop) and KPC-producing *Acinetobacter baumannii complex* (glucose meter) were detected in the environment. In contrast to the *E. aerogenes* strains, the KPC-producing *A. baumannii complex* strain was not detected in cultures of the KPC-positive patients.

Sedimentation cultures

In the hospital, KPC-KP was detected in the room of the index patient on 3 out of 9 sedimentation agar plates placed on the left and right sides of the footboard of the bed, and on the sink. Similarly, KPC-KP was detected on 2 of 20 solid agar plates that were placed in the direct patient environment during routine care of the patients admitted to the separate location (on the right side of the headboard of the night cabin of patient 5).

Microbiology and molecular typing

All KPC-KP isolates were multidrug-resistant and exhibited intermediate levels of resistance to gentamicin (4 mg/L) and tigecycline **(Table 3)**. The microarray revealed the presence of KPC, SHV, and (non-ESBL) TEM.

Table 2: environmental cultures of separate location. Environmental cultures of the separate location from three items per patients room (bed including bed controller, door (knobs) bathroom and door (knob) wardrobe) and six items in the common areas (kitchen worktop, table, handle and keypad of bedpan washer, computer keyboard and mouse in nursing office, dECT telephones (4x) and the glucose meter). In 5 occasions, the two patient lifts were also cultured. Patient behaviour appeared associated with the degree of in environmental contamination. For patient 3, who was immobile and bed bound, KPC-KP was detected on the bed/bed controller, but not on other sites in the patient room. By contrast, for patient 5 (who exhibited unhygienic behaviour with urine and faeces) all items in the patient room were positive for KPC-KP.

Date	Kitchen worktop	table	Bedpan washer	Keyboard and mouse	dECT phones (4x)	glucosemeter	P1: bed and bed controller	P1: door (knob) bathroom	P1: door (knob) wardrobe	P3: bed and bed controller	P3: door (knob) bathroom	P3: door (knob) wardrobe	P4: bed and bed controller	P4: door (knob) bathroom	P4: door (knob) wardrobe	P5: bed and bed controller	P5: door (knob) bathroom	P5: door (knob) wardrobe	P6: bed and bed controller	P6: door (knob) bathroom	P6: door (knob) wardrobe	Patient lift patient 5	patient lift patient 3 and 4
5-12-2013	green	green	green	green	green	green	red	green	red	green	green	green	red	green	green	red	red	red	green	green	green	red	red
10-12-2013	green	green	green	green	green	red	red	green	red	red	green	green	green	green	green	red	red	red	green	green	green	red	red
20-12-2013	green	red	green	green	green	green	green	green	green	red	green	green	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	red
23-12-2013	red	green	green	green	green	grey	red	red	red	red	green	green	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey
3-1-2014	green	green	green	green	green	green	green	green	green	green	green	green	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	green
10-1-2014	green	green	green	red	green	red	green	green	green	red	green	green	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	red
21-1-2014	green	green	green	green	green	green	green	green	green	red	green	green	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey
7-2-2014	green	green	green	green	green	green	green	green	red	red	green	green	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey
12-2-2014	green	green	green	green	green	green	green	green	green	green	green	green	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey	grey

(green) CPE not detected

(red) KPC-producing Enterobacteriaceae detected

(grey) Not tested

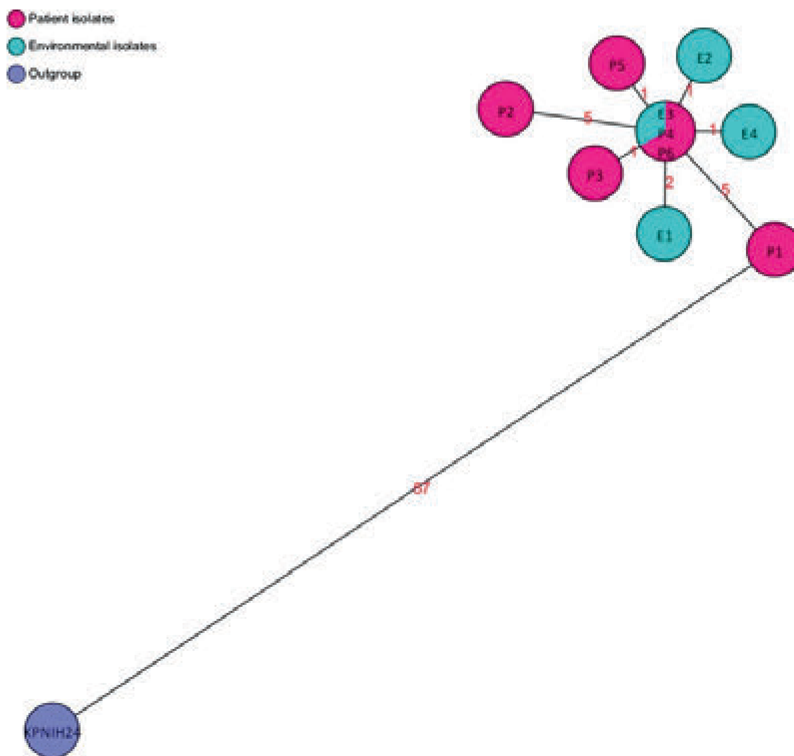
Table 3: Susceptibility and molecular profile of every first unique carbapenemase-producing isolate. H=hospital, NH=nursing home, SL=separate location *Isolate detected in hospital, acquisition occurred during *admission nursing home*

No.	Isolate	date first isolate (2013)	Samples	Institute	MIC (mg/L)				Bla genes	ST	AFLP
					IPM	MER	TIG	COL			
P1 (Index)	*K. pneumoniae*	June 24	Rectal, urine, decubitus wound	H	3	6	2	6	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	Ent. aerogenus	November 8	Rectal	NH	24	>32	ND	ND	KPC-2	ND	B
P2	*K. pneumoniae*	July 5	Pleural fluid, rectal.	H	32	32	1.5	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
P3	*K. pneumoniae*	October 31	Rectal, urine (catheter)	H*	4	6	1	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	Ent. aerogenus	October 31	Rectal, urine (catheter)	H*	12	4	1.5	ND	KPC-2, TEM *wildtype*	ND	B
	E. coli	December 30	Rectal	SL	1	0.38	ND	ND	KPC-2, TEM *wildtype*	ND	ND
P4	*K. pneumoniae*	November 18	Rectal	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	Ent. aerogenus	December 9	Rectal	SL	6	>32	ND	ND	KPC-2	ND	B
P5	*K. pneumoniae*	November 18	Rectal, urine (catheter), wound foot	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
	Ent. aerogenus	December 9	Rectal	SL	12	1.5	ND	ND	KPC-2, TEM *wildtype*	ND	C
P6	*K. pneumoniae*	November 22	Rectal	NH	8	4	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E1	*K. pneumoniae*	November 21	Night cabin (room patient 1)	NH	3	3	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E2	*K. pneumoniae*	November 21	Door knob (room patient 1)	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E3	*K. pneumoniae*	November 21	Night cabin (room patient 4/5)	NH	32	6	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A
E4	*K. pneumoniae*	November 21	Glucose meter	NH	>32	>32	ND	ND	KPC-2, OXA-9, SHV-12, TEM-1	258	A

5

MLST indicated that the isolates belonged to the pandemic sequence type ST258 and were clustered together by AFLP. In addition to *K. pneumoniae*, KPC-producing *E. coli* and KPC-producing *E. aerogenes* were detected in the index patient, patient 3, patient 4, and patient 5. The KPC-producing *E. aerogenes* strains of the index patient, patient 3, and patient 4 belonged to the same AFLP cluster, and the strain of patient 5 belonged to a different genotype.

Figure 2. Core genome typing (MLST+) of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* (KPC-KP) outbreak strains. This minimum spanning tree was generated by SeqSphere, which was based on comparing 3,042 alleles that were defined as the core genome of the strains analyzed in this study. The values indicate the number of different alleles. Different colours represent different epidemic groups, as shown in the legend. An ST258 strain *K. pneumoniae* KPNIH24 (GenBank accession number CP008797) was included in the analysis as the outgroup



The MLST+ result was concordant with that of AFLP. Of the 3,102 target genes included in the scheme, 3,042 were present in all isolates and were subsequently used in the MLST+ typing. The minimum spanning tree shows that all patient and environmental isolates were tightly clustered, exhibiting only up to seven different alleles (a seven-allele difference was found between P1 and E1, not indicated in **Figure 2**). Isolates of patients

4 and 6 (P4 and P6) were identical to that of environmental isolate E3, which had 1–5 different alleles compared with the other isolates **(Figure 2)**. This clearly suggests that all isolates were related to the outbreak and that transmissions occurred between patients and the environment. The gene-by-gene approach was confirmed by a single nucleotide polymorphism (SNP) analysis. In total, 26 SNPs were detected among the 11 isolates (Table 4). Remarkably, the two strains that were epidemiologically most related (P1 and P2) had the largest number of SNP differences (n=10).

In accordance with the microarray results, the resistome analysis of all KPC-KP isolates showed the presence of *bla*OXA-9, *bla*SHV-12, *bla*TEM-1, and *bla*KPC-2. Moreover, the aminoglycoside resistance gene *aac(6')Ib*, fluoroquinolone resistance genes *oqxA* and *oqxB*, and fosfomycin resistance gene *fosA* were found in all isolates. All isolates, except the isolate of patient 2, also harbored resistance genes *aadA2* and *aph(3')-Ia* for aminoglycoside, *mph(A)* for macrolide, *catA1* for phenicol, *sul1* for sulfonamide, and *dfrA12* for trimethoprim.

DISCUSSION

In the outbreak described here, nosocomial transmission of KPC-KP occurred despite the implementation of contact precautions following the national guideline on the control of multidrug-resistant microorganisms for the index patient. Although the outbreak could be controlled relatively easily in the hospital setting by transferring patients to an isolation room with anteroom and negative air pressure and appointing a dedicated nursing team, inter-institutional spread occurred after the index patient was transferred to a nursing home in the same region. This was detected only after one of the nursing home residents was admitted to the hospital, and further transmission in the nursing home was difficult to control.

During this outbreak, we found extensive environmental contamination in all settings. The last transmission occurred after separating all positive patients and appointing a dedicated nursing team, with the most probable route of transmission being a shared device (glucose meter). Our findings are consistent with recent studies showing that *Klebsiella spp*. are found in the environment more frequently than other coliforms [15,16], and add to growing evidence that the environment can act as an important reservoir for transmission during KPC-KP outbreaks. The results of the air sedimentation cultures suggest that HCWs and the patient's environment may not only be contaminated by direct contact, but also by droplets or other (airborne) particles. To what extent this contributes to transmission is, as yet, unclear, and it should be noted that bacteria may survive longer on the sedimentation agars than on other

inanimate surfaces. It is, however, certainly an aspect that is not taken into consideration in many infection control guidelines and questions the effectiveness of contact precautions on multiple bed wards. Taken together, the ability to contaminate and persist in the environment may explain the high transmission potential of this clone, and must be taken into account in environmental cleaning procedures. Other factors explaining the success of this clone, such as colonisation potential and duration of carriage, need to be investigated.

Adherence to infection control measures is generally more difficult in long-term care facilities than in hospitals, due to differences in population characteristics, length of stay, staff education level, setup, and the level of social interaction between patients/ residents. In addition, the diagnostic sampling frequency is generally low. As a result, detecting and preventing the transmission of highly resistant microorganisms in these facilities is particularly challenging, as illustrated by this report. Once a reservoir has been established, this will result in frequent (and potentially unnoticed) introductions into surrounding hospitals. The monitoring of patient movements and direct communication between hospitals and long-term care facilities is of utmost importance to prevent inter-institutional spread, and future efforts should focus on optimizing these processes.

This report also illustrates the possible applications of WGS during outbreak investigations. WGS has the potential to become an important molecular epidemiological tool by obtaining almost all available DNA information in one single method. It could, thus, replace multiple conventional (molecular) methods used in current routine laboratories. Its high typing resolution combined with detailed epidemiological data will allow the identification of transmission chains during outbreaks.

Lastly, this outbreak raises the important question of how the spread of KPC (and other MDROs) in nursing homes and other long-term care facilities can be controlled. Possible solutions include the design of an effective decolonisation strategy (for example, selective gut decontamination followed by a fecal transplant) or cohorting of KPC-positive patients in dedicated (regional) locations with highly trained personnel. Information about the effectiveness of decolonisation strategies is urgently needed.

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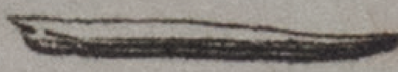

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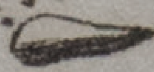

Fig. 3.

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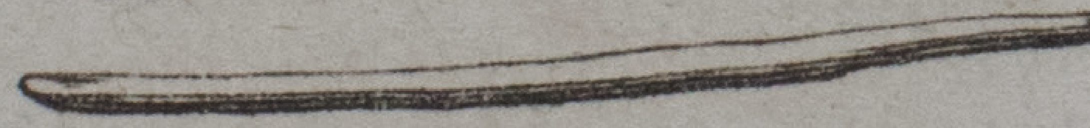

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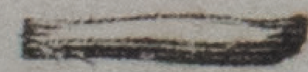

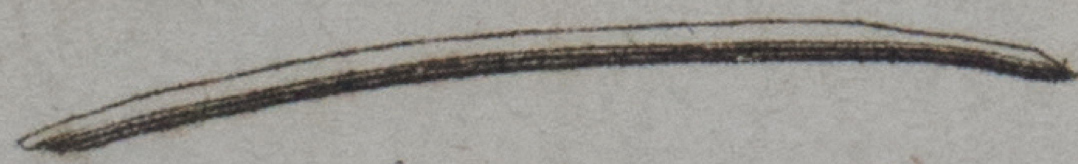

Chapter 6

EVALUATION OF AN IN VITRO MODEL WITH A NOVEL STATISTICAL APPROACH TO MEASURE DIFFERENCES IN BACTERIAL SURVIVAL OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ESCHERICHIA COLI ON AN INANIMATE SURFACE

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Antimicrobial Resistance & Infection Control 2019; 8 (106)

ABSTRACT

Background

The role of environmental contamination in the transmission of Enterobacteriaceae is increasingly recognized. However, factors influencing the duration of survival in the environment have not yet been extensively studied. In this study, we developed and evaluated an in vitro model with a novel statistical approach to accurately measure differences in bacterial survival that can be used to model the effects of multiple factors/conditions in future experiments.

Methods

Two extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) isolates were used for this in vitro experiment: a CTX-M-15-producing *E. coli* sequence type (ST) 131 and a CTX-M-1-producing *E. coli* ST10 isolate. Each strain was 1:1 diluted in sterile water, sterile saline or sheep blood. Cover glasses (18 × 18 mm) were inoculated with the dilution and subsequently kept at room temperature. Bacterial survival on the glasses was determined hourly during the first day, once daily during the following 6 days, and from day 7 on, once weekly up to 100 days. The experiment was repeated six times for each strain, per suspension fluid.

Results

Viable bacteria could be detected up to 70 days. A biphasic survival curve for all suspension fluids was observed, whereby there was a rapid decrease in the number of viable bacteria in the first 7 h, followed by a much slower decrease in the subsequent days.

Conclusions

We found a difference in survival probability between *E. coli* ST10 and ST131, with a higher proportion of viable bacteria remaining after 7 h for ST131, particularly in sheep blood.

BACKGROUND

The important role of the environment in the nosocomial transmission of microorganisms is increasingly recognized [1–9]. As an example, several studies have demonstrated that prior room occupation by patients carrying *Acinetobacter baumannii*, vancomycin-resistant *enterococci* or methicillin-resistant *Staphylococcus aureus* increases the risk of nosocomial acquisition of these bacteria [3,7–9].

A critical factor for transmission of a microorganism via the environment is its ability to survive on environmental surfaces. The ability of Gram-positive pathogens to survive on (hospital) inanimate surfaces for long periods of time has frequently been addressed [10–12]. Where the survival of Enterobacteriaceae on dry surfaces was long believed to be limited, recent studies have shown that environmental sources may contribute to the transmission of multidrug-resistant Enterobacteriaceae [13–15]. Havill et al. showed that carbapenem-resistant Enterobacteriaceae survived for extended periods of time on metal discs [16]. Also, other factors beside the ability to survive on dry surfaces have been reviewed [17], revealing that high humidity, low temperature, a higher inoculum and the presence of protein were associated with longer survival.

In infection control, it is important to investigate what exactly are factors that support or hinder survival. The downside is that it is difficult to accurately measure differences in bacterial survival that can be used to model the effects of multiple factors or conditions (e.g. biobased materials, antibacterial coatings) simultaneously.

We developed and evaluated a model to measure differences in bacterial survival of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* on an inanimate surface, and assessed whether survival depended on sequence type and suspension fluid.

METHODS

Two ESBL-producing *E. coli* isolates from a well-documented Dutch collection of ESBL-producing Enterobacteriaceae were used for this in vitro experiment: a CTX-M-15-producing *E. coli* ST131 cultured from a patient during a large *E. coli* ST131 outbreak in a Dutch nursing home [18], and a CTX-M-1-producing *E. coli* ST10 isolate cultured from chicken meat [19].

E. coli isolates were retrieved from − 80 °C Microbank vials (ProLab Diagnositcs, Ontario, Canada), and grown overnight at 35 to 37 °C on sheep blood agar (SBA) plates. For

both *E. coli* isolates a one McFarland suspension was 1:1 diluted in sterile water, sterile saline and sterile sheep blood and the initial concentration of viable bacteria in these suspensions at time zero (t = 0) was determined for each material and strain separately following procedures described below. Cover glasses (18 × 18 mm) were inoculated with 20 μL of the bacterial suspensions. After inoculation, cover glasses were kept at room temperature. Bacterial survival on the cover glasses was determined hourly during the first 7 h, once daily during the first 7 days, and weekly from day seven until day 100. The cover glasses were placed into 2 mL brainheart infusion (BHI) broth and vortexed for 30 s to suspend all viable bacteria present on the glasses. At each time point, bacterial survival was assessed for six cover glasses for each of the six bacterial suspensions.

For all bacterial suspensions (sheep blood, water and saline at t = 0 and BHI broth at the subsequent time intervals), a series of sheep blood agar (SBA) plates were inoculated with 20 μL of a 10-fold dilution series of the BHI-suspension. The number of colony-forming units (CFU) on the SBA plates was counted after overnight incubation at 35 to 37 °C. To enable accurate counting, the SBA plates with a colony count in the range of 30 to 300 CFU were used to estimate the number of viable bacteria in each suspension and on the cover glasses, and results were expressed as CFU per mL. When the colony count of all dilutions were below 30 CFU per plate, the remaining 1800 μL of BHI-broth was filtered over a sterile 0.45 μm Millipore filter that was placed on a SBA plate. CFU on this plate were counted after overnight incubation at 35 to 37 °C. The detection limit of the assay was 56 CFU/mL.

Statistical analysis

The dataset used for the statistical analysis consisted of a time-series of surviving concentrations of viable bacteria for each of 36 combinations formed by six repetitions, two strains and three suspension fluids. For each time-series the first concentration at t = 0 was deleted as the number of bacteria was too high to reliably measure (36 observations) and also concentrations equal to zero mainly in the upper tail of the time-series were deleted (233 observations). The number of remaining non-zero concentrations as of t = 1 (0.042 days) was 913 in total and ranged per time-series from 16 to 36 (average 25.4) across the 36 combinations. The day of the last non-zero concentration measured per time-series ranged from 18 to 70 (average 56.6) across the 36 combinations.

The number (Ct) of viable bacteria surviving over time (t) was assumed to follow the exponential model $Ct = C0*exp.(-rt)$ complying with a negative exponential distribution

of survival time (days) with daily death rate(r). C0 denotes the concentration measured at 1 h (0.042 days). The model was reformulated after natural logarithmic transformation as ln(Ct/C0) = −rt. The daily death rate r was assumed to change from r1 to a lower value r2 after 7 h (0.25 days) according to a broken-stick survival model with one change point set at 0.25 days. The effects of repetition, strain and suspension on r were estimated using linear mixed modelling through the origin. The effect of repetition (6 levels) was assumed to be random, whereas the effects of strain (2 levels) and suspension (3 levels) were assumed to be fixed and allowed to be different between r1 and r2. The within-repetition (co)variance matrix was assumed to have a first-order autoregressive structure.

The survival probability at 7 h was calculated as exp.(− 0.25*r1). During the first 7 h an hourly survival probability was calculated as exp.(−r1/24). After 7 h a daily survival probability was calculated as exp.(−r2).

Differences in r between strains and suspensions were estimated using the linear mixed model and exponentially transformed so as to be interpretable as ratios of hourly or daily survival probabilities. As both strain and suspension were in the model, their effects on survival were assessed independently. The statistical analyses were performed in SAS, version 9.2 (SAS Institute Inc., Cary, North Carolina, USA), and SPSS, version 23 (SPSS Inc., Chicago, Illinois).

RESULT

Survival curves of *E. coli* ST10 and ST131 in water, saline and sheep blood are shown in **Figure 1**. A biphasic survival curve for all materials was observed, i.e. a rapid decrease in the number of viable bacteria was observed in the first 7 h, followed by a much slower decrease in the subsequent days. In the first 7 h of the experiment, the hourly survival probability of *E. coli* ST131 was higher than that of *E. coli* ST10 (survival probability ratio 1.14 [95%CI: 1.05– 1.25]) **(Table 1)**.

Also, the proportion of surviving bacteria in the first 7 h strongly depended on the suspension fluid used: the proportion surviving per hour was substantially higher in sheep blood than in the other media. **(Table 1)** In **Table 2** the proportion of viable bacteria after 7 h is presented for all strain-suspension combinations. An adjusted survival probability ratio of 2.17 [95% CI: 1.37–3.44] was observed for E. coli ST131 as compared to E. coli ST10. The absolute difference in the proportion of viable bacteria after 7 h between ST types was largest for sheep blood (44.3%). After the first 7 h, the absolute number of viable bacteria remaining was very low (e.g. for ST131 in water, only 0,03% of the initial number of viable

bacteria remained). At this time, the decline in the number of viable bacteria was less pronounced, and a difference between the strains no longer detectable **(Table 1).**

Figure 1. Observed (circle ST10; triangle ST131) and predicted survival of ST10 (solid line) or ST131(dotted line) in water, saline and sheep blood in the first 7 h (I) and total study period (II)

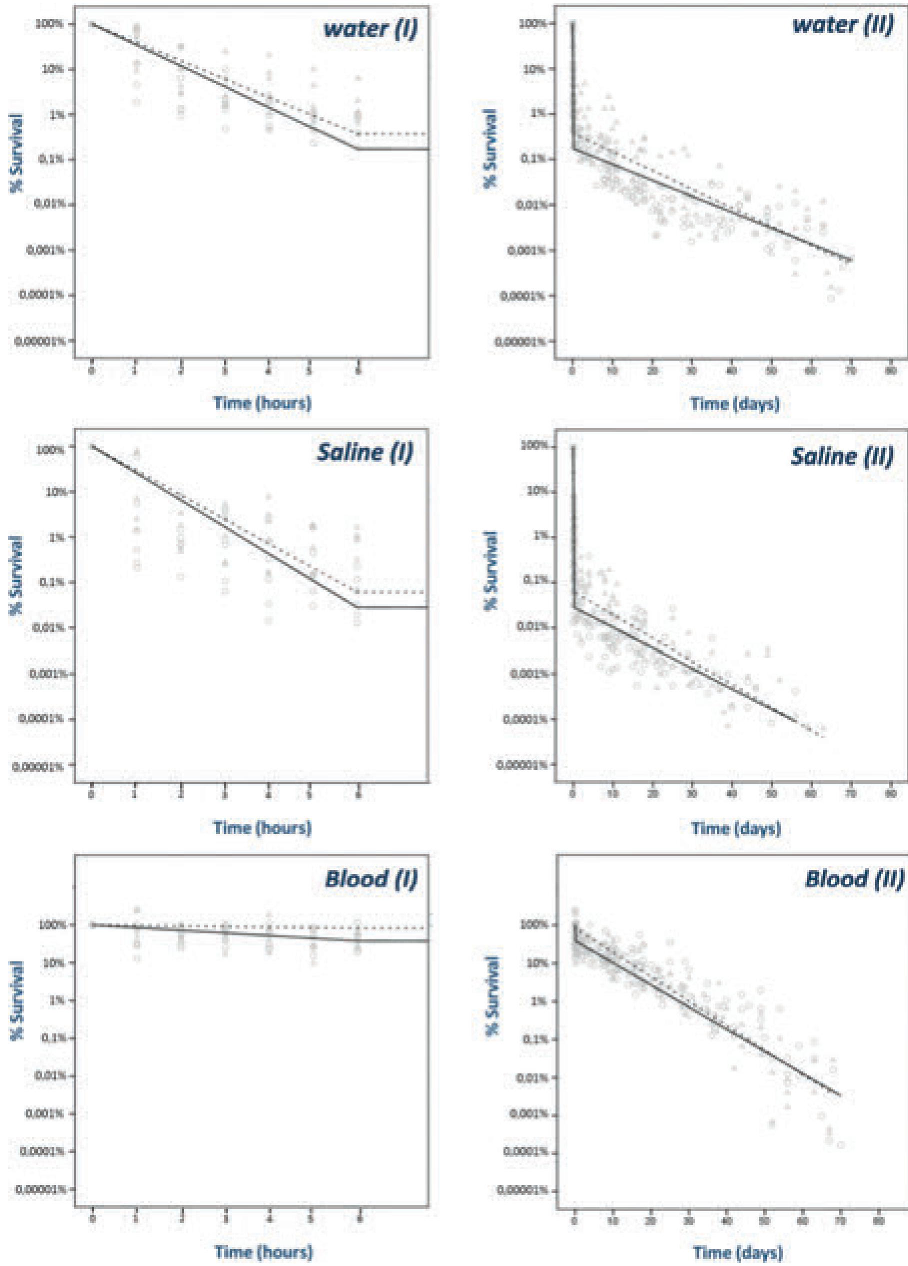

Table 1. survival probabilities per hour (for time period t < 7h) and per day (for time period t > 7h) for *E. coli* ST10 and ST131 per suspension fluid. The survival probability ratio indicates the relative difference in survival between ST131 versus ST10

	t < 7h			**t > 7h**		
	Proportion surviving per hour (% [95% CI])		**Survival probability ratio [95% CI]**	**Proportion surviving per day (% [95% CI])**		**Survival probability ratio [95% CI]**
	ST10	*ST131*		*ST10*	*ST131*	
Water	25.6 [23.6 – 27.7]	29.1 [26.9 – 31.5]	1.14 [1.05 – 1.23]	90.2 [88.2 – 92.3]	89.0 [87.0 – 90.9]	0.99 [0.97 – 1.00]
Saline	34.6 [32.0 – 37.3]	39.3 [36.4 – 42.5]		92.2 [90.5 – 94.0]	91.0 [89.3 – 92.7]	
Blood	85.1 [78.8 – 91.9]	96.8 [89.6 – 104.5]		87.5 [85.8 – 89.1]	86.3 [84.6 – 87.9]	

Table 2. proportion surviving after 7h for *E. coli* ST10 and ST131 per suspension fluid. The survival probability ratio indicates the relative difference in survival probability between ST131 versus ST10.

	Proportion surviving after 7 hour (% [95% CI])		**Survival probability ratio [95% CI]**
	ST10	*ST131*	
Water	0.03 [0.02 – 0.05]	0.06 [0.04 – 0.10]	2.17 [1.37 – 3.44]
Saline	0.17 [0.11 – 0.27]	0.37 [0.23 – 0.59]	
Blood	37.9 [23.9 – 60.1]	82.3 [51.9 – 100]	

DISCUSSION

The role of environmental contamination in the transmission of Enterobacteriaceae is increasingly recognized. However, factors influencing the duration of survival in the environment have not yet been extensively studied.

Our study showed that *E. coli* bacteria remained viable on dry inanimate surface up to 70 days and that survival of these bacteria, particularly in the first 7 h of the experiment, was influenced by the type of suspension fluid used. Survival was significantly prolonged in sheep blood as compared to water and saline. In addition, we showed a difference in survival probability between *E. coli* ST10 and ST131, particularly in sheep blood: at 7 h, the survival probability of ST131 was more than twice that of ST10 (82% versus 38%).

The enhanced survival in sheep blood is probably due to the presence of proteins and other nutrients creating an optimal environment for survival. This finding supports the notion that bacterial survival can be influenced by the degree of environmental contamination [20], and emphasises the importance of environmental cleaning to not only remove bacteria but also to get rid of nutrients for bacteria, as part of an effective infection control policy. Other studies previously reported extended survival times for *E. coli* [17,21–23]. Neely showed that *E. coli* survived for extended periods of time (up to 15 days) on different hospital fabrics and plastic [22]. Starlander et al. reported extended survival (up to 28 days) and found a major difference in survival in the environment between different *E. coli* strains, whereby the ESBL-producing *E. coli* isolates tended to survive much longer than the AmpC-producing isolates. An explanation for this difference in survival was not described [21]. Also, in previous studies a range of materials was used to determine bacterial survival, e.g. Havill et al used metal disk to determine bacterial survival over time [16]. In our in vitro model, we used an inert material to avoid any (chemical) influence of the material on the bacterial survival.

Our study has some limitations. First, a relatively high inoculum was applied on the cover glasses (~ 3 × 106 CFU). Weber et al. and Neely both reported that bacterial survival can be affected by inoculum size [22,23]. Neely suggested that bacteria in a nutrient-limiting situation can live on dying bacteria nearby, and therefore longer survival is expected in a more concentrated bacterial population. Secondly, the experiment was performed with only one isolate per ST type. Still, our results support the hypothesis that an enhanced environmental survival of ST131 may contribute to its potential to spread via (contaminated) environments more successfully than other clones. A recent study, however, describing an outbreak of ST131 in a Dutch nursing home did not find evidence

of an increased acquisition risk of ST131 as compared to other ESBLs [24], and further work is required to investigate this hypothesis. Specifically, the in vitro experiment should be repeated and extended to include several different isolates, sequence types and varied inoculum sizes.

In our experiment, survival of ESBL-producing *E. coli* ST10 and ST131 followed a biphasic pattern, with a rapid decrease in the number of viable bacteria during the first 7 h, followed by a much slower decrease in the subsequent 70 days. The biphasic character of the survival curve may be explained by the fact that part of the suspended bacterial population consisted of so-called 'persisters'. Bacterial persisters are rare, transient phenotypic variants in a non-growing state that can tolerate environmental stresses (e.g. starvation, pH, antibiotics) and survive longer than the normal phenotypic variants. As mentioned above, this persister phenotype may explain the biphasic kill curve, whereby the first phase represents the rapid death of normal cells and the second phase indicates the remaining presence of persisters that are characterized by a slower cell death [25,26]. Alternatively, the effect of environmental factors, and particularly the effect of drying, might be most pronounced during the first days of the experiment and contribute to the change in survival rate over time.

The ability of both strains to remain viable for prolonged periods of time on dry inanimate surfaces, underlines the importance that environmental cleaning is part of a comprehensive infection control policy. In recent years, several novel products have been developed to reduce microbial contamination of the (hospital) environment, such as antimicrobial or "self-disinfecting" surfaces. Still, more research is necessary to determine their effectiveness in reducing microbial contamination. In this study, we developed a model with a novel statistical approach to accurately measure differences in bacterial survival, that can be used to model the effects of multiple factors/conditions simultaneously in future experiments.

CONCLUSION

ESBL-producing *E. coli* ST10 and ST131 can survive on dry inanimate surfaces for long periods of time, where bacterial survival was increased in sheep blood as compared to water and saline. We showed a difference in survival probability between *E. coli* ST10 and ST131, with a higher proportion of viable bacteria remaining after 7 h for ST131, particularly in sheep blood.

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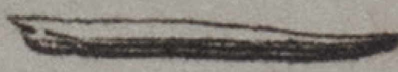
A.



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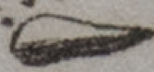

Fig. 3.

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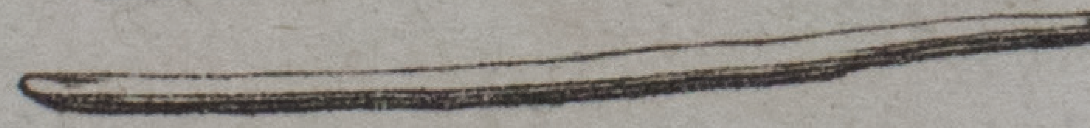

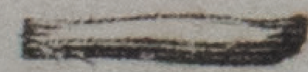

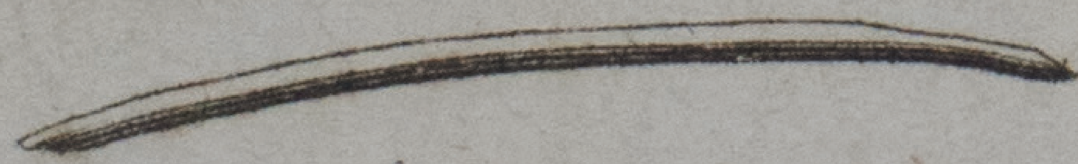

Chapter 7

DURATION OF RECTAL COLONIZATION WITH EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ESCHERICHIA COLI: RESULTS OF AN OPEN, DYNAMIC COHORT STUDY IN DUTCH NURSING HOME RESIDENTS (2013 – 2019)

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ABSTRACT

Background

In 2016, a study in a Dutch nursing home showed prolonged colonization duration of extended-spectrum beta-lactamase-producing (ESBL)-ST131 compared to ESBL-non-ST131. In this study, we assessed the duration of rectal ESBL-producing *E. coli* (ESBL-EC) colonization in residents in the same nursing home for an extended period of six years. We aimed to estimate the influence of a possible bias when follow up is started during an outbreak.

Methods

Between 2013 and 2019, repetitive point prevalence surveys were performed by culturing rectal or faecal swabs from all residents. Kaplan-Meier survival analysis was performed to calculate the median time to clearance of ESBL-EC with a log-rank analysis to test for differences between ESBL-ST131 and ESBL-non-ST131.

Results

The study showed a median time to clearance of 13,0 months (95%CI 0,0 – 27,9) for ESBL-ST131 compared to 11,2 months (95%CI 4,8 – 17,6) for ESBL-non-ST131 (p = 0,044). In the subgroup analysis of residents who were ESBL-EC positive in their first survey, the median time to clearance for ST131 was 59,7 months (95%CI 23,7 – 95,6) compared to 16,2 months (95%CI 2,1– 30,4) for ESBL-non-ST131 (p = 0,036). In the subgroup analysis of residents who acquired ESBL-EC, the median time to clearance for ST131 was 7,2 months (95%CI 2,1 – 12,2) compared to 7,9 months (95%CI 0,0 – 18,3) for ESBL-non-ST131 (p = 0,718). The median time to clearance in the ESBL-ST131 group was significantly longer in residents who were ESBL-ST131 colonised upon entering the study than in residents who acquired ESBL-ST131 during the study (p = 0,001).

Conclusion

A prolonged colonization with ESBL-ST131 was only found in the subgroup who was ESBL-EC positive upon entering the study. The prolonged duration with ESBL-ST131 in the previous study was probably biased by factors that occurred during (the start of) the outbreak.

BACKGROUND

Escherichia coli (*E. coli*) sequence type (ST) 131 is an extraintestinal pathogenic *E. coli* (ExPEC) [1,2], and is nowadays the predominant *E. coli* lineage among ExPEC isolates worldwide [3]. Moreover, ST131 is associated with the worldwide spread of the CTX-M-15 extended spectrum *β*-lactamase (ESBL) resistance gene [3]. ESBL-producing ST131 (ESBL-ST131) is a major contributor to hospital- and community-acquired infections, such as urinary tract infections and bloodstream infections [4,5]. ESBL-ST131 infections are most common among elderly people and ESBL-ST131 carriage is particularly prevalent in nursing homes and long-term care facilities [6,7].

Despite many studies examining the epidemiology of ESBL-ST131, the reason why this clone is so successful is still not fully understood. In 2016, Overdevest et al. evaluated the duration of colonization with ESBL-ST131 in residents of a Dutch nursing home where ST131 had spread extensively [8]. Between March 2013 and April 2014, six point prevalence surveys were performed at intervals of three months by culturing faeces or rectal swabs. The study showed a prolonged colonization duration of residents with ESBL-ST131, with a median time to clearance of 13 months compared to two to three months for other ESBL-producing *E. coli* (ESBL-non-ST131) ($p < 0.001$).

Overdevest et al. started the study after the detection of an ESBL-ST131 outbreak. They calculated the duration of colonization from the first positive culture within the study period. With this approach, ~50% of the cases were already colonized at the first screening (onset date of colonization unknown). This approach (outbreak setting and unknown onset date) may introduce bias, as it is more likely to start the observation period of a case shortly after the time of acquisition for ESBL-ST131 compared to ESBL-non-ST131. Unknown factors (e.g. resident's characteristics, institutional characteristics and/or environmental factors) may have contributed to the difference observed in duration of colonization. The point prevalence surveys in the Dutch nursing home of the aforementioned study were continued until June 2019. In this study, we evaluated the duration of rectal ESBL-EC colonization of residents in the same nursing home over an extended period of 6 years (March 2013 to June 2019).

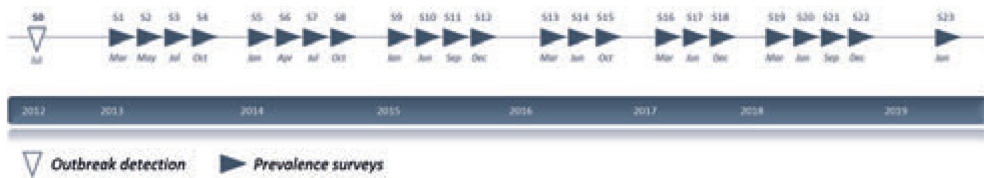
The objective of this study was to compare the duration of rectal ESBL-EC colonization for ESBL-ST131 versus ESBL-non-ST131. In addition to the overall comparison, we performed a comparison between residents who acquired ESBL-EC during the study and those who were colonized upon entering the study. We aimed to estimate the influence of a possible bias when follow up is started during an outbreak.

METHODS

Detection of ST131 outbreak and setting

As part of the standard infection control policy, a prevalence survey was performed in a Dutch nursing home **(Figure 1)** in 2012, showing a high prevalence (20,6%) of rectal ESBL colonization [7]. Strain typing showed the extensive presence of ESBL-ST131, among smaller clusters and unique strains of other sequences types. Outbreak containment measures were implemented, including repetitive prevalence surveys at intervals of three to six months by culturing faeces or rectal swabs from all residents. In June 2019, the prevalence surveys were ended.

Figure 1: Overview of the outbreak detection and subsequent prevalence studies in the nursing home.



Study design and study population

We conducted an open, dynamic cohort study based on data generated by repeated point prevalence surveys from March 2013 to June 2019 as part of the existing infection control policy in a nursing home in the Netherlands. Residents with at least one ESBL-EC positive rectal or faecal swab obtained in a prevalence survey during the study period were eligible for inclusion. Residents acquiring ESBL-EC in the final prevalence survey (June 2019) were not included.

Detection of ESBL-producing E. coli

The intended sampling scheme consisted of a quarterly screening of all residents of the wards involved. Throughout all point prevalence surveys colonization of ESBL-EC was determined by culture of rectal or faecal samples collected using Eswab (Copan, Italy). Swabs were inoculated directly on extended-spectrum β-lactamase screening agar (EbSA) (AlphaOmega,’s-Gravenhage, Netherlands) and 5% Sheep blood agar (growth control). The remaining Eswab fluid was transferred in selective enrichment broth consisting of 5 mL tryptic soy broth containing cefotaxime (0,25 mg/L) and vancomycin (8 mg/L) (TSB-VC). After 18–24 hours of incubation (35–37°C), the TSB-VC was subcultured on an EbSA

plate. For all Gram-negative rods growing on the EbSA, either directly or after overnight culture in enrichment broth, species identification and susceptibility testing was performed by MALDI-TOF (bioMérieux, Marcy l'Etoile, France) and VITEK 2 (bioMérieux, Marcy l'Etoile, France), respectively. Phenotypic ESBL production was confirmed by double disk method [9].

Genotyping and strain typing

All phenotypically confirmed ESBL-EC underwent an O25:ST131-specific PCR [10]. ESBL genotyping was performed using a micro-array (CheckPoints, Wageningen, the Netherlands) [11,12] and strain typing by using amplified fragment length polymorphism (AFLP) [13]. An AFLP cluster was assigned based on both visual and computerised interpretation of AFLP patterns.

ESBL genotyping and strain typing were performed for the first ESBL-EC from each resident and for subsequent ESBL-EC strains that were not similar to the first strain. Similarity was defined as identical species and O25:ST131 status and absence of major differences in susceptibility (susceptible vs. resistant) for all antibiotics tested.

Definitions

Rectal colonization with ESBL-EC was defined as detection of ESBL-EC in at least one rectal or faecal swab. Acquisition of ESBL-EC was defined as a resident with at least one ESBL-EC negative culture before the first ESBL-EC positive culture. A resident was considered no longer colonized (loss of colonization) when, after a previous ESBL-EC positive culture, at least one rectal or faecal swab did not reveal ESBL-EC or when strain typing showed a different MLST or cluster type than found in the previous ESBL-EC positive culture of the resident **(Supplementary figure 1)**. Residents were only included in the study once, i.e. the first time ESBL-EC carriage was detected in the study period.

Additional analyses were performed whereby loss of colonization status was reached when at least two (instead of one) rectal or faecal swabs no longer yielded ESBL-EC or when two swabs showed a different sequence type and/or cluster type than found in the previous ESBL-EC positive culture of the resident **(Supplementary figure 2)**.

Variables

Data concerning gender, date of birth, and if applicable date of discharge from nursing home or date of death were obtained from the nursing homes records.

Duration of colonization

For each resident, the duration of rectal colonization with ESBL-EC was calculated as the time from the first ESBL-EC positive sample until the last ESBL-EC positive sample plus half the time between the last ESBL-EC positive culture and the first ESBL-EC negative culture **(Supplementary figure 1a)** or the first ESBL-EC positive culture with a different cluster/sequence type **(Supplementary figure 1b).** Both situations were labelled as an 'event' in the Kaplan-Meier survival analysis.

In residents whose last sample was still ESBL-EC positive (no loss of colonization) at the end of the study period, the time of rectal colonization of ESBL-EC was calculated as the time from the first ESBL-EC positive sample until the last ESBL-EC positive sample **(Supplementary figure 1c)**. For residents who died or were discharged before the end of the study, the time of rectal colonization of ESBL-EC was calculated as the time from the first ESBL-EC positive sample until time of death or discharge **(Supplementary figure 1d)**. These cases were labelled as 'censored' in the Kaplan-Meier survival analysis.

Statistical analysis

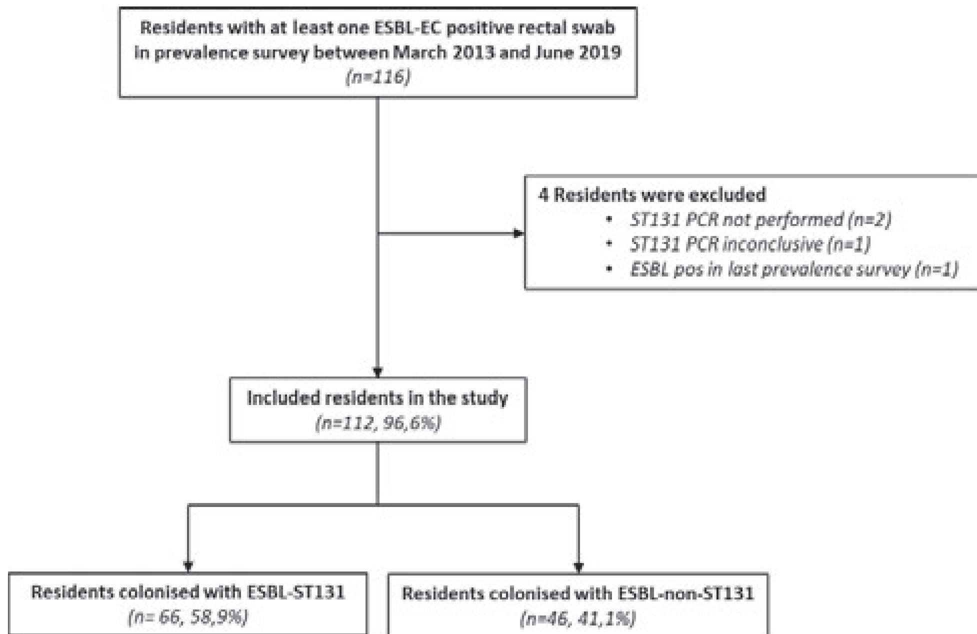
Data analysis was performed using statistical package for social science (SPSS) version 25. The prevalence of carriage was calculated as the percentage of carriers among all residents. Comparisons of categorical variables between the ESBL-ST131 and ESBL-non-ST131 group were performed with Fisher's exact test and continuous variables were compared by the Mann-Whitney *U* test. Kaplan-Meier survival analysis was performed to calculate the median time to clearance for ESBL-EC with a Log-Rank analysis to test for differences between ESBL-ST131 and ESBL-non-ST131 and between ESBL-ST131 positive at start and ESBL-ST131 acquisition during study. A p-value < 0.05 was considered as statistically significant.

RESULTS

Between March 2013 and June 2019, 23-point prevalence surveys were performed at intervals of three to six months **(Figure 1).** A total of 4.980 samples were cultured from 1.806 unique residents. In total, 116 residents (6,4%) had at least one ESBL-EC positive sample in any prevalence survey **(figure 2)**. Of these, four residents were excluded from the study because O25:ST131-specific PCR was not performed (*n=2*), the result of the O25:ST131-specific PCR was inconclusive (*n=1*) or the resident acquired ESBL-EC in the final prevalence survey (*n=1*).

Therefore, 112 residents were included: 66 residents (58,9%) with ESBL-ST131 rectal colonization and 46 residents (41,1%) with ESBL-non-ST131 rectal colonization **(Figure 2)**. The median age on the day of the first ESBL-EC positive rectal sample was 82 years (interquartile range (IQR): 75 – 88) and 63/112 (56,3%) of the residents were female. The median follow-up time (period between first positive ESBL-EC culture and last culture, regardless of culture result of the last culture) was 17,9 months (IQR: 6,0 – 40,6) for ESBL-ST131 and 10,1 months (IQR: 2,8 – 30,2) for ESBL-non-ST131 (*p = 0,106).*

Figure 2: Flowchart for inclusion



In total 55 of 112 residents (49,1%) were already colonized with ESBL-EC at the time they first participated in any prevalence survey: 33/66 (50,0%) for ESBL-ST131 and 22/46 (47,8%) for ESBL-non-ST131 (*p* = 0,850)., Of these 55, 32 (58,2%) were colonized in the first prevalence survey in March 2013 (ESBL-ST131: 23/32 (71,9%); ESBL-non-ST131: 9/32 (28,1%)). Furthermore, 49/112 (43,8%) were newly admitted to the nursing home after the first prevalence survey in March 2013. The age at time of first ESBL-EC positive sample and gender for ESBL-ST131 and ESBL-non-ST131 per analysis are presented in **table 1.**

Table 1: age on the day of the first ESBL-EC positive rectal sample and gender characteristics of the residents for ESBL-ST131 and ESBL-non-ST131, per analysis.

	All residents with at least one positive ESBL-EC culture (*n*=112)		**Residents who were ESBL positive in their first prevalence survey (n = 55)**		**Residents who acquired ESBL-EC during the study (n=57)**	
MLST group	**ESBL-ST131 (*n* =66)**	**ESBL-non-ST131 (*n*=46)**	**ESBL-ST131 (*n* =33)**	**ESBL-non-ST131 (*n*=22)**	**ESBL-ST131 (*n* =33)**	**ESBL-non-ST131 (*n*=24)**
Age in years, median (IQR)	82 (76 – 88)	82 (74 – 87)	83 (74 – 88)	84 (76 – 87)	82 (77,5 – 88,5)	81 (73,0 – 86,5)
Female gender, *n* (%)	37 (56,0%)	26 (56,5%)	19 (57,6%)	11 (50,0%)	18 (54,5%)	15 (62,5%)

Molecular characterization of ESBL genes

The predominant ESBL genotype in the ESBL-ST131 group was *bla*CTX-M-15 (n = 57; 86,4%), followed by *bla*CTX-M-9 (n = 9; 13,6%). The ESBL genes in the non-ESBL-ST131 group were more diverse, the predominant ESBL genotype was *bla*CTX-M-9 (n = 13; 28,3%), followed by *bla*CTX-M-15 (n = 11; 23,9%), *bla*CTX-M-1 (n = 8; 17,4%), *bla*CTX-M-3 (n = 8; 17,4%), *bla*TEM (n = 3; 6,5%) and *bla*SHV (n = 3; 6,5%).

Duration of colonization

During the study period loss of colonization of ESBL-EC (based on one ESBL-EC negative swab) or colonization with a different cluster type than in previous cultures (based on one swab with a different cluster type) was observed in 31/66 ESBL-ST131 carriers (47,0%) and in 26/46 ESBL-non-ST131 carriers (56,5%) (p = 0,343). Three of 112 residents (2,7%) with an ESBL-negative sample became ESBL-positive again in the sample following the first ESBL-EC negative sample. An overview of the proportion and reasons for events or censoring for the Kaplan-Meier survival analyses per selection group is shown in **Supplementary table 1-3**.

An overview of the proportion and reasons for events or censoring for the Kaplan-Meier survival analyses per selection group based on at least two (instead of one) rectal or faecal swabs is shown in **Supplementary table 4-6.**

Figure 3A shows the Kaplan-Meier survival curve of ESBL-EC colonization over time for all included residents. The median time to clearance for ESBL-ST131 was 13,0 months (95%CI 0,0 – 27,9) compared to 11,2 months (95%CI 4,8 – 17,6) for ESBL-non-ST131 (p = 0,044). **Figure 3B** shows the Kaplan-Meier survival curve of ESBL-EC colonization over

time for residents who were ESBL-EC positive in their first prevalence survey in the study. The median time to clearance for ESBL-ST131 was 59,7 months (95%CI 23,7 – 95,6) compared to 16,2 months (95%CI 2,1– 30,4) for ESBL-non-ST131 (*p* = 0,036). **Figure 3C** shows the Kaplan-Meier survival curves of ESBL-EC colonization over time for residents who acquired ESBL-EC during the study. The median time to clearance for ESBL-ST131 was 7,2 months (95%CI 2,1 – 12,2) compared to 7,9 months (95%CI 0,0 – 18,3) for ESBL-non-ST131 (*p* = 0,718).

Kaplan-Meier curves of survival probability of residents colonised with ESBL-producing *E. coli*, by ST131 and non-ST131 with two (instead of one) ESBL-EC negative cultures/other strain type to consider a resident no longer colonised are shown in the **Supplementary figure 2-5.** In addition, no significant difference was found between the median time to clearance for ESBL-ST131 and ESBL-non-ST131.

To further investigate the influence of ESBL-EC colonization status upon inclusion in the study (meaning ESBL-EC positive in first prevalence survey versus acquisition during study period), an additional Kaplan-Meier survival analysis was performed. In this analysis we first stratified ESBL-ST131 and ESBL-non-ST131. Within these two groups we compared the patients who were positive in the first culture and those who had at least one negative before the first positive culture. In the ESBL-ST131 group, the median time to clearance was significantly longer in residents who were ESBL-EC colonised upon entering the study than in residents who acquired ESBL-EC (*p* = 0,001) **(Figure 4A)**. In the subgroup ESBL-non-ST131 no significant differences were found between the two groups (*p* = 0,399) **(Figure 4B)**.

Figure 3: Kaplan-Meier curves of survival probability of residents colonised with ESBL-producing *E. coli*, by ST131 and non-ST131 for all residents **(A) ,** for residents who were ESBL-EC positive in their first prevalence survey in the study **(B)**, and for residents who acquired ESBL-EC during the study **(C)**. Values below the survival graphs indicate the number of residents per MLST group.

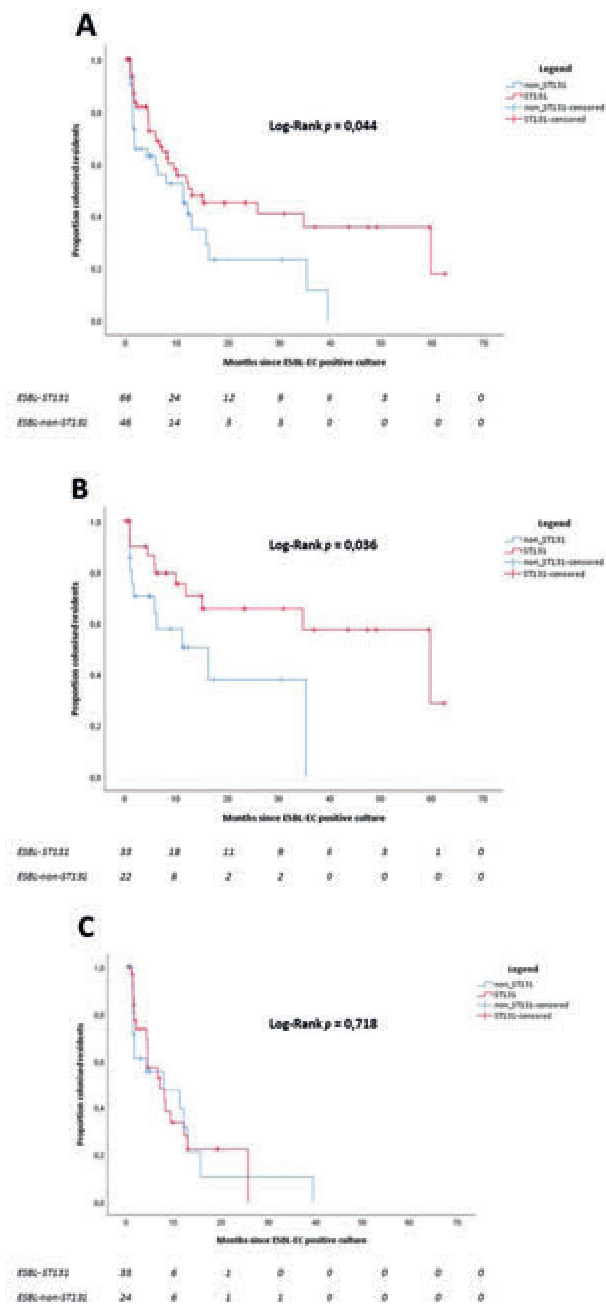
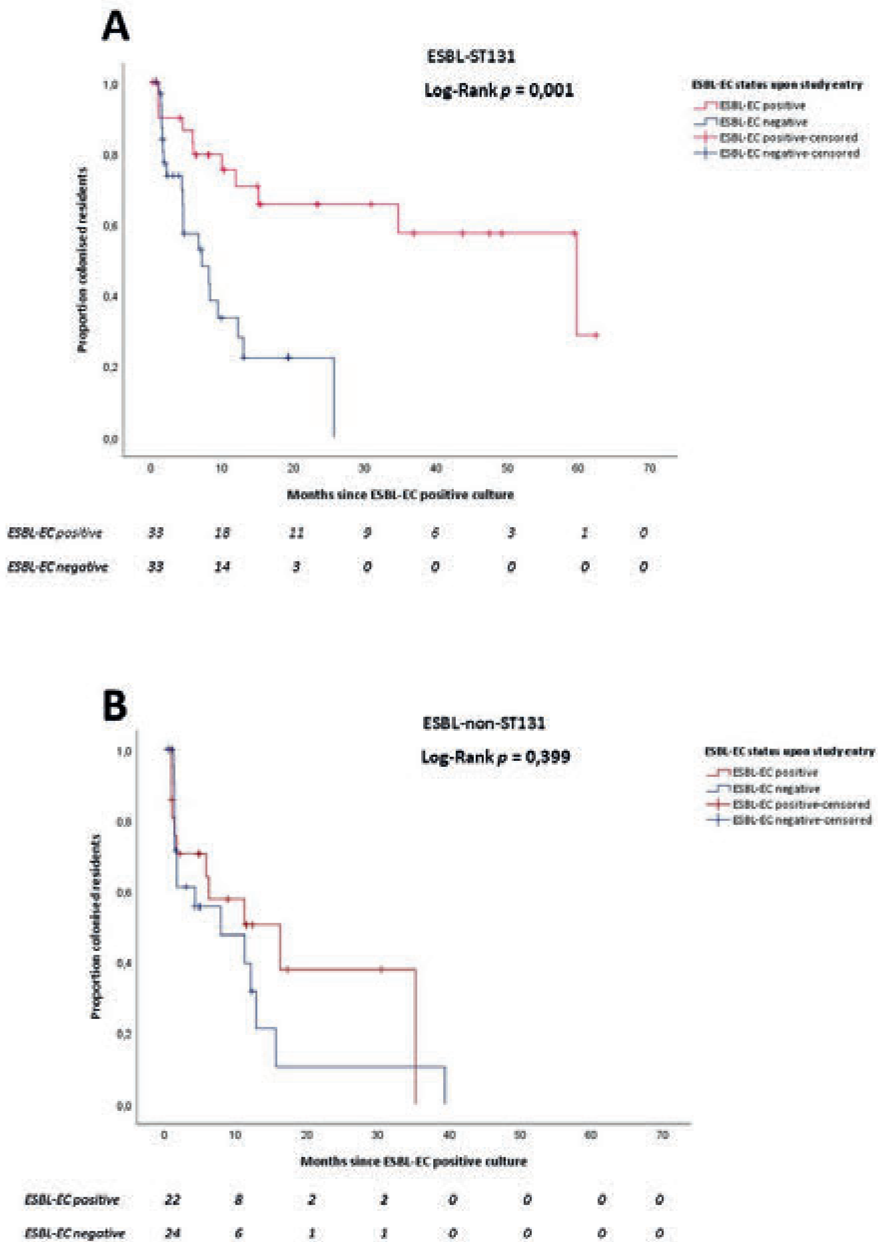


Figure 4: Kaplan-Meier survival curve of survival probability of residents colonised with ESBL-ST131 **(A)** and ESBL-non-ST131 **(B)** by ESBL-EC status (positive or negative) upon entry of the study



DISCUSSION

In this study, the median time to clearance for ESBL-ST131 was significant longer for residents who were already ESBL-EC colonised upon entry in the study, whereas this difference was not observed in residents who acquired ESBL-EC during the follow up period. This difference was not found for other sequence types.

We evaluated the median time to clearance of rectal ESBL- EC colonization in anursing home over a period of six years in all residents, and performed a subgroup analysis for residents who were already ESBL-EC colonised upon study entry and compared it to those who acquired ESBL-EC during their stay at the nursing home. In the primary analysis (all residents) we found a significant prolonged median time to clearance for ESBL-ST131. For residents who were ESBL-EC positive upon study entry this difference was much more pronounced, while no difference was observed for residents who acquired ESBL-EC during the study.

A further analysis was done based on a stratification of the ESBL-ST131 and ESBL-non-ST131 groups. Per group, we compared those who were already colonised in the first culture with those who acquired ESBL-EC during the follow-up period. This analysis revealed a significantly longer time to clearance in residents who were colonised with ESBL-ST131 upon entry in the study compared to those who acquired ESBL-ST131 during the study. This difference was not found for other sequence types. These results indicate that the prolonged duration of colonization with ESBL-ST131 found in the initial study in 2016 [8], was caused by the residents who were already colonized with ESBL-ST131 when the follow-up was started. It is not clear what the underlying reasons are for this phenomenon. A possible explanation could be that the residents who were colonized with ESBL-ST131 during the outbreak period, were more susceptible to long-term colonization and that this group was overrepresented. However, many new residents entered the cohort in the following years and some acquired ESBL-ST131. If there was a group of individuals who were more likely to be colonized for longer periods, there must have been some of those in the group with acquired ESBL-ST131 as well. This was not the case and we consider this explanation unlikely. Another explanation for the longer colonization but also for the observed higher mortality in the ESBL-ST131 group that was colonized upon study entry could be that this prolonged colonization was limited to a subgroup of ESBL-ST131 strains. A follow-up study based on whole-genome sequencing may be able to provide more clarity on this. The observed difference in mortality cannot be explained by a difference in the duration of follow up because it was only observed in

the group that was positive upon entry in the study, and both groups (ESBL-ST131 and ESBL-non-ST131) have the same follow up duration.

Other studies that examined the duration of colonization for ESBL-ST131 compared to other ESBL-EC also found a longer duration of carriage for ESBL-ST131. Van Duijkeren et al. found that ESBL-EC colonization persisted for >8 months in one-third of the ESBL-positive persons from the Dutch general population and found that longer colonization was statistically significant associated with the detection of phylogenetic group B2 and ST131, among others [14]. Titelman et al. included patients after a first-time ESBL-producing Enterobacteriaceae infection and showed that colonization with *E. coli* was still apparent after 12 months in 64% (n=26), and 40% (n=14) of those carrying *E. coli* ST131 or other STs, respectively (p = 0.12) [15]. Ismail et al. investigated the incidence and duration of colonization of ciprofloxacin-resistant *E. coli* in nursing homes in Michigan. The study showed that ST131 strains in residents were carried significantly longer when compared to non-ST131 strains (10 months versus 3 months) [16]. In all of the above studies, an ESBL positive culture (prevalence or clinical) was the starting point for inclusion in the studies, which could potentially influence the outcome of the studies.

In some studies on duration of colonization, ESBL-EC clearance is defined as two consecutive negative samples [17]. In this study, we defined ESBL-EC clearance when one sample no longer yielded ESBL-EC or when strain typing showed a different cluster type than found in the previous ESBL-EC positive culture of the resident. This approach was chosen to keep the results comparable to Overdevest et al. Furthermore, only a small proportion of the residents (2,7%) became ESBL-EC positive after a negative ESBL-EC culture. Moreover, using two negative cultures did not significantly alter the results.

This study has some limitations. First, there are several known risk factors for (prolonged) ESBL colonization duration, such as antibiotic use, proton pump inhibitor use or variables associated with higher need for care [14, 17]. Unfortunately, such clinical information was not acquired in the study and therefore we cannot further investigate the possible influence of (specific) resident factors.

Another limitation is the setting. The study was performed in a Dutch nursing home during an outbreak situation, which may reduce generalizability for other settings and/or patient populations. The major strengths of our study include the standardised cultures taken at defined intervals and most important the long follow-up period up to six years.

CONCLUSION

A longer duration of colonization with ESBL-ST131 compared to ESBL-non-ST131 was found in the group who was ESBL-EC positive upon entering the study. In the subgroup of residents who acquired ESBL-EC during the study period, no difference in the duration of colonization between ESBL-ST131 and ESBL-non-ST131 was found. We conclude that the longer duration of colonization with ESBL-ST131 was caused by factors that occurred during the outbreak that preceded the observation period. In newly colonized individuals there was no difference in duration of colonization between ESBL-ST131 and other sequence types.

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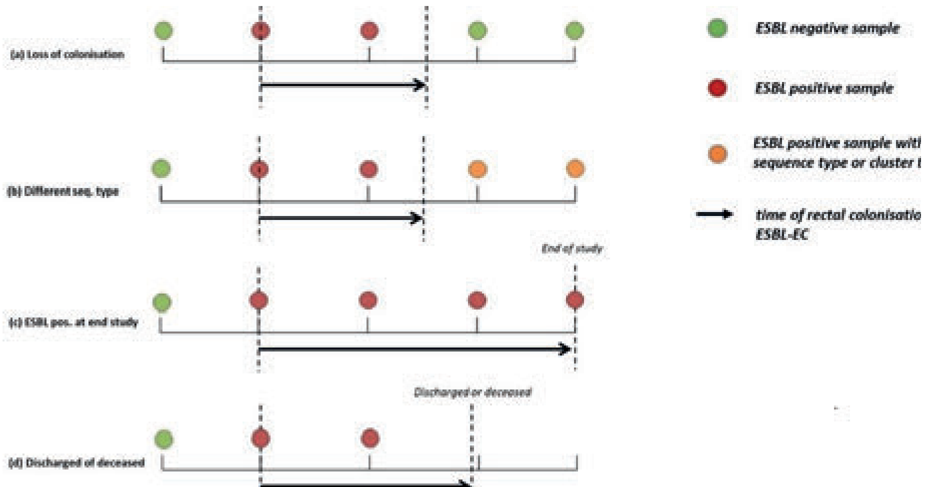
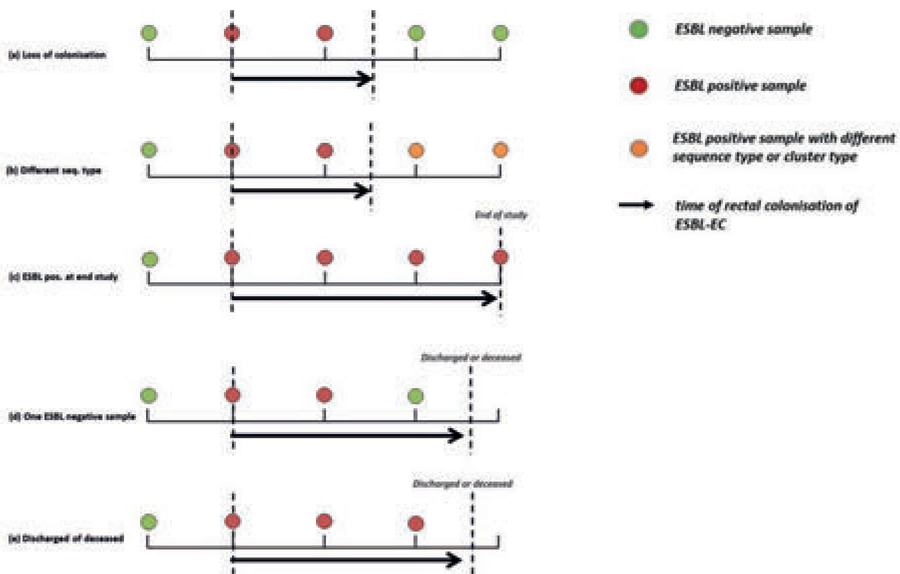
SUPPLEMENTARY MATERIAL

Supplementary figure 1: schematic representation of determination colonisation duration in different situations whereby 'loss of colonisation' is reached with at least **one** sample no longer yielded ESBL-EC or when a sample showed a different cluster type than found in the previous ESBL-EC positive culture of the resident



Supplementary figure 2: schematic representation of determination colonisation duration in different situations whereby 'loss of colonisation' is reached with at least **TWO** (instead of one) samples no longer yielded ESBL-EC or when two samples showed a different cluster type than found in the previous ESBL-EC positive culture of the resident



Supplementary table 1: reasons for event or censoring in Kaplan Meier analysis for all residents with at least one ESBL-EC positive culture (n=112) - with **ONE** ESBL-EC negative cultures/other strain type to consider a resident no longer colonised.

Reasons for event of censoring in Kaplan Meier analysis	**ESBL-ST131 (n=66)**	**ESBL-non-ST131 (n=46)**	***p***
Loss of colonisation (event), n (%)	31 (47.0)	22 (47.8)	*1.00*
Acquisition of other strain type (event), n (%)	0 (0.0)	4 (8.7)	*0.03*
Still ESBL-EC positive at end of study (censored), n (%)	2 (3.0)	0 (0.0)	*0.51*
Discharged in study period (censored), n (%)	2 (3.0)	5 (10.9)	*0.12*
Deceased in study period (censored), n (%)	31 (47.0)	15 (32.6)	*0.17*

Supplementary table 2: reasons for event or censoring in Kaplan Meier analysis for residents who were ESBL-EC positive in their first prevalence survey in the study (n = 55) - with **ONE** ESBL-EC negative culture/other strain type to consider a resident no longer colonised.

Reasons for event of censoring in Kaplan Meier analysis	**ESBL-ST131 (n=33)**	**ESBL-non-ST131 (n = 22)**	***p***
Loss of colonisation (event), n (%)	11 (33.3)	9 (40.9)	*0.58*
Acquisition of other strain type (event), n (%)	0 (0.0)	2 (9.1)	*0.16*
Still ESBL-EC positive at end of study (censored), n (%)	1 (3,0)	0 (0.0)	*1.00*
Discharged in study period (censored), n (%)	1 (3,0)	4 (18.2)	*0.14*
Deceased in study period (censored), n (%)	20 (60.6)	7 (31.8)	*0.05*

Supplementary table 3: reasons for event or censoring in Kaplan Meier analysis for residents who acquired ESBL-EC colonisation during the study (n=57) - with **ONE** ESBL-EC negative culture/other strain type to consider a resident no longer colonised.

Reasons for event of censoring in Kaplan Meier analysis	**ESBL-ST131 (n=33)**	**ESBL-non-ST131 (n=24)**	***P***
Loss of colonisation (event), n (%)	20 (60.6)	13 (54.2)	*0.79*
Acquisition of other strain type (event), n (%)	0 (0.0)	2 (8.3)	*0.17*
Still ESBL-EC positive at end of study (censored), n (%)	1 (3.0)	0 (0.0)	*1.00*
Discharged in study period (censored), n (%)	1 (3.0)	1 (4.2)	*1.00*
Deceased in study period (censored), n (%)	11 (33.3)	8 (33.3)	*1.00*

Supplementary table 4: reasons for event or censoring in Kaplan Meier analysis for all residents with at least one ESBL-EC positive culture (n=112) - with **TWO** ESBL-EC negative cultures/other strain type to consider a resident no longer colonised.

Reasons for event of censoring in Kaplan Meier analysis	**ESBL-ST131 (n=66)**	**ESBL-non-ST131 (n=46)**	***p***
Loss of colonisation (event), n (%)	26 (39.4)	19 (41.3)	*0.84*
Acquisition of other strain type (event), n (%)	0 (0.0)	1 (2.2)	*0.41*
Still ESBL-EC positive at end of study (censored), n (%)	2 (3.0)	0 (0.0)	*0.51*
Discharged in study period (censored), n (%)	2 (3.0)	5 (10.9)	*0.12*
Deceased in study period (censored), n (%)	31 (47.0)	15 (32.6)	*0.11*
Only one negative culture/culture with other strain type (censored), n (%)	5 (7.6)	6 (13.0)	*0.35*

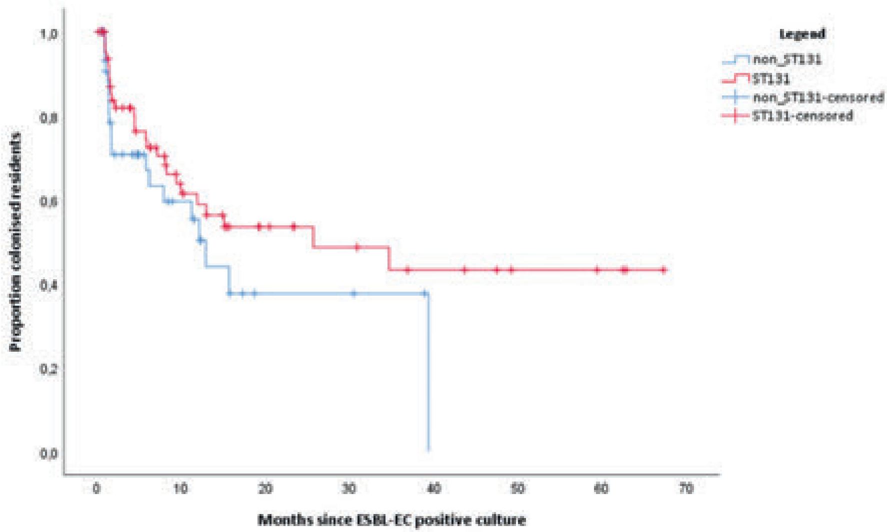
Supplementary table 5: reasons for event or censoring in Kaplan Meier analysis for residents who were ESBL-EC positive in their first prevalence survey in the study (n = 55) - with **TWO** ESBL-EC negative culture/other strain type to consider a resident no longer colonised.

Reasons for event of censoring in Kaplan Meier analysis	**ESBL-ST131 (n=33)**	**ESBL-non-ST131 (n=22)**	***p***
Loss of colonisation (event), n (%)	9 (27.3)	9 (40.9)	*0.38*
Acquisition of other strain type (event), n (%)	0 (0.0)	0 (0.0)	*1.00*
Still ESBL-EC positive at end of study (censored), n (%)	1 (3.0)	0 (0.0)	*1.00*
Discharged in study period (censored), n (%)	1 (3.0)	4 (18.2)	*0.14*
Deceased in study period (censored), n (%)	20 (60.6)	7 (31.8)	*0.05*
Only one negative culture/culture with other strain type (censored), n (%)	2 (6.1)	2 (9.1)	*1.00*

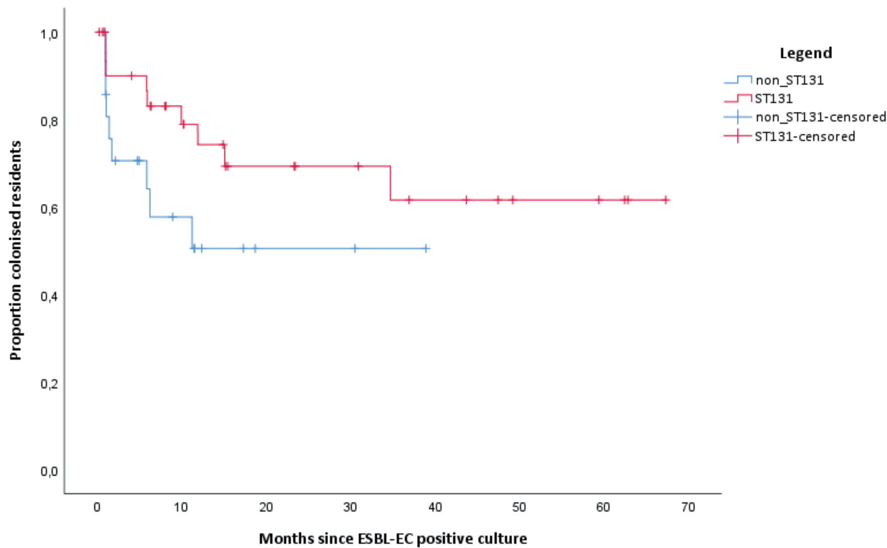
Supplementary table 6: reasons for event or censoring in Kaplan Meier analysis for residents who acquired ESBL-EC colonisation during the study (n=57) - with **TWO** ESBL-EC negative culture/other strain type to consider a resident no longer colonised.

Reasons for event of censoring in Kaplan Meier analysis	**ESBL-ST131 (n=33)**	**ESBL-non-ST131 (n=24)**	***p***
Loss of colonisation (event), n (%)	17 (51.5)	10 (41.7)	*0.66*
Acquisition of other strain type (event), n (%)	0 (0.0)	1 (4.2)	*0.41*
Still ESBL-EC positive at end of study (censored), n (%)	1 (3.0)	0 (0.0)	*1.00*
Discharged in study period (censored), n (%)	1 (3.0)	1 (4.2)	*1.00*
Deceased in study period (censored), n (%)	11 (33.3)	8 (33.3)	*1.00*
Only one negative culture/culture with other strain type (censored), n (%)	3 (9.1)	4 (16.7)	*0.44*

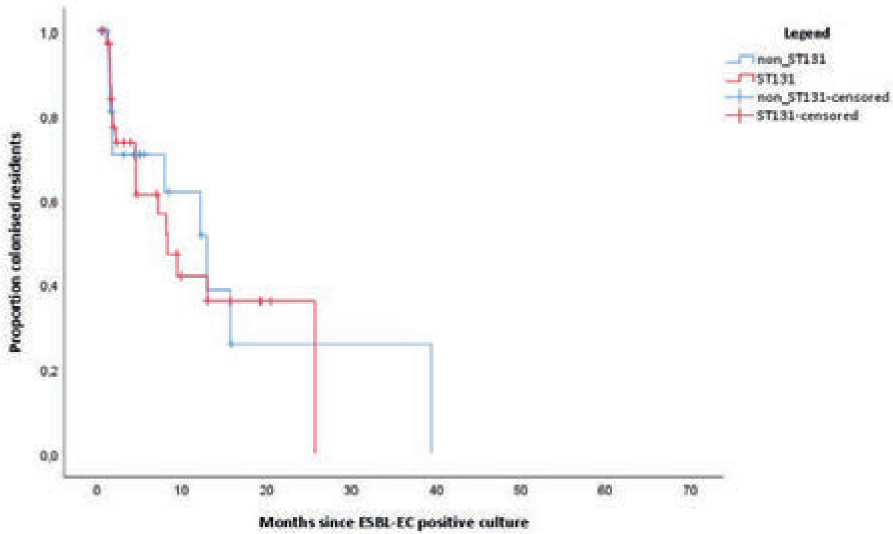
Supplementary figure 3: Kaplan-Meier curve of ESBL-EC colonisation over time for all residents (n = 112) when considering a resident no longer colonised with TWO ESBL-EC negative culture/other strain type (p = 0.181).

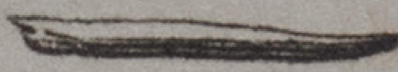


Supplementary figure 4 Kaplan-Meier curve of ESBL-EC colonisation over time for residents who were ESBL-EC positive in their first prevalence survey the study (n = 55) when considering a resident no longer colonised with TWO ESBL-EC negative culture/other strain type (p = 0.101).



Supplementary figure 5 Kaplan-Meier curve of ESBL-EC colonisation over time for residents who acquired ESBL-EC colonisation during the study when considering a resident no longer colonised with TWO ESBL-EC negative culture/other strain type (p = 0.815).



A. 

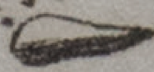
B. C 

Fig. 3.

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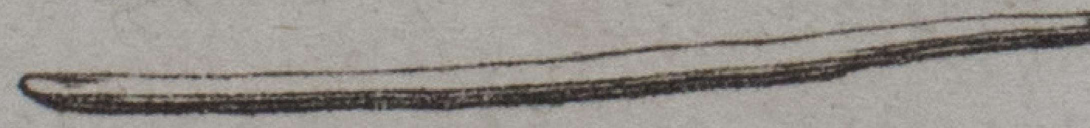
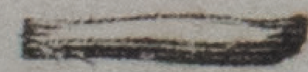
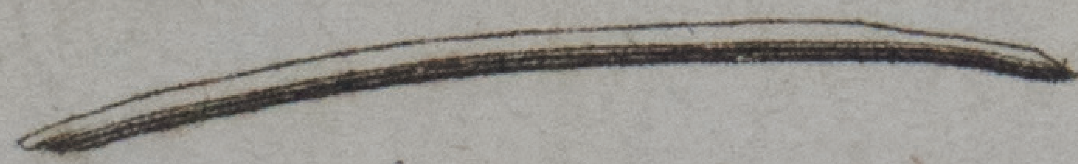


F 



Chapter 8

SUMMARY AND GENERAL DISCUSSION

The Netherlands is a small country located in Northwest Europe with a relatively high population density of more than 500 inhabitants per square kilometre. The Netherlands is famous for its bulb fields, windmills and innovative water-management – and is also known for the successful management of antibiotic resistance.

HISTORY OF INFECTION CONTROL (PRACTITIONERS) IN THE NETHERLANDS

In 1966, a commission of the Dutch Health Care Council stated that healthcare institutions were required to create infection control committees (ICC). The task of these committees was to advise hospital directors and healthcare workers about infection control policies [1, 2]. After the realisation of these ICC, it was recognized that implementation of the ICC guidelines and compliance monitoring was difficult. Therefore, a new nursing specialty was established, and in 1968 the first four infection control practitioners began the practice of infection control in the Netherlands [3]. In 1973, 32 ICP founded the Dutch Society of Infection Prevention in Healthcare ('Vereniging voor Hygiene en Infectiepreventie in de Gezondheidszorg', VHIG) with the primary aim of translating ICC guidelines into practice on the wards [2]. Today, the VHIG has 500 members working in various fields of healthcare, including hospitals, nursing and care homes, private clinics and public healthcare.

Although the fact that clinical microbiology laboratory is an essential part of an effective infection control program (e.g. surveillance, typing) [4], the collaboration structure between the medical microbiologist and infection control practitioner in the early years was insufficiently defined, not transparent or even absent in some hospitals. Nowadays it is clear that controlling outbreaks and controlling particularly multidrug-resistant microorganisms require a multidisciplinary strategy. Where, in particular, the collaboration between medical microbiologists and infection control practitioner is essential for an effective and efficient infection prevention control program [5].

NATIONAL GUIDELINES ON INFECTION CONTROL

Over the years, the need arose among infection prevention professionals for uniform national guidelines in their field. Therefore, in 1980 a national Working Party on Infection Control ('Werkgroep Infectie Preventie', WIP) was founded by four Dutch societies, which were involved in infectious diseases and infection control. Over the years, the WIP issued many national guidelines for infection prevention for healthcare institutions and also answered infection prevention questions from the professional field [6]. Unfortunately, in 2017, all activities of the WIP were discontinued - putting an end to an internationally renowned icon in healthcare [7].

Fortunately, there is a new initiative: the Partnership for Infection Prevention Guidelines ('Samenwerkingsverband Richtlijnen Infectiepreventie', SRI) was launched in 2021. The SRI is an initiative of the Ministry of Health, Welfare and Sport in which eight organizations work together to continue the work of the former Working Group Infection Prevention.

MULTIDRUG-RESISTANT ORGANISMS (MDRO)

The criteria for defining MDRO can vary between countries [8]. In the Netherlands MDRO are defined as microorganisms which are known to cause disease, have acquired an antimicrobial resistance pattern that hampers (empirical) therapy and has the potential to spread if no transmission-based precautions are taken [9]. Examples of MDRO include Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E), and carbapenemase-producing Enterobacteriaceae (CPE).

Certain successful MDRO clones, so-called multidrug-resistant high-risk clones, have played an essential role in the global emergence of antibiotic resistance among Gram-negative organisms (especially the Enterobacteriaceae) and Gram-positive organisms (*S. aureus* and enterococci). High-risk clones have advantages over other clones in the ability to colonise, spread, and persist in hosts and have enhanced pathogenicity, fitness and survival skills **(chapter 6)** [10]. Examples of these high-risk clones are *Escherichia coli*-ST131 **(chapter 7)** and *Klebsiella pneumoniae*-ST258 **(chapter 5)** which are associated with the global dissemination of *bla*CTX-M-15 and *bla*KPC respectively [11].

ANTIMICROBIAL RESISTANCE

Antimicrobial resistance is rising to dangerously high levels in all parts of the world, leading to higher medical costs, prolonged hospital stays and is associated with increased morbidity and mortality [12]. Still, The Netherlands is one of the countries with the lowest antibiotic resistance rates in clinical isolates in Europe [13]. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) prevalence in blood cultures, for example, are substantially lower at respectively 1,4% and 0.9% than the European Union average at 15,5% and 18,3% in 2019 [13,14]. The prevalence of carbapenemase-producing Enterobacteriaceae (CPE) in the Netherlands remains low in blood cultures (0,0% for *E. coli* and *K. pneumoniae* versus respectively 0.3% and 10% in European Union) [13,14]. Only the prevalence of Extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in blood cultures is increasing in the Netherlands in the recent years (2019: *E. coli* 7,5% and *K. pneumoniae* 9,6%) but remains below the average

of the European Union (2019: *E. coli* 15,1% and *K. pneumoniae* 31,3%) [13,14].

This low antibiotic resistance prevalence is partly because of the prudent and restrictive use of antibiotics (not further discussed in this thesis) [14]. In addition, effective infection prevention policy in healthcare settings is based on national guidelines as mentioned above. This thesis describes, among other things, several practical aspects of the implementation of the national guidelines to control multidrug-resistant organisms (MDRO) outbreaks in Dutch healthcare settings.

SUCCESSFUL INFECTION PREVENTION POLICY

Probably one of the most well-known and successful infection prevention policies is the so-called Search and Destroy policy [15], which was implemented after a series of MRSA outbreaks in Dutch hospitals in the early 1980s [2]. The policy relies on active screening of high-risk groups for MRSA carriage upon hospital admission and (pre-emptive) isolation and treatment of carriers. In the first years, the emphasis was mainly on patients from foreign hospitals. However, in the years since several new risk groups have been added, for example livestock in 2006 and household members and partners/caretakers of MRSA carriers in 2012. Since 2005, the active screening and pre-emptive isolation of high-risk groups (except for livestock) have been extended to control other MDROs [16, 17]. The compliance of all Dutch hospitals with these guidelines is one of the keys to the success of this strategy. In **Chapter 2** we describe an observational study to determine the prevalence of MRSA nasal carriage among patients who were screened preoperatively for nasal *S. aureus* carriage between 2010 and 2017 [18]. The study showed a sustained low prevalence of MRSA carriage upon hospital admission over 7 years (0.13%; 95% CI 0.10–0.18%). The MRSA prevalence varied from 0.03% to 0.17% over the years without evidence of a changing trend over time (p = 0.40). These findings support the effectiveness of the Dutch Search and Destroy policy, in combination with a restrictive antibiotic prescription policy.

CONTROVERSIES IN INFECTION CONTROL

In addition to the positive effects of the Dutch national policy (low prevalence of MDRO), there is also a considerable controversy with regards to this high-risk screening and (pre-emptive) isolation strategy. In general, isolation measures are associated with adverse effects in terms of patient satisfaction and level of care provided by healthcare workers and healthcare workers experience a heavier workload while caring for a patient in isolation [19,20].

In addition, in a low-endemic setting such as in the Netherlands, many assessments, cultures and pre-emptive isolation days are needed to detect a small number of patients

with a MDRO. Van Hout et al. evaluated the risk assessment for identifying MDRO carriers upon hospitalisation in a Dutch tertiary hospital and showed that 1,778 MDRO risk assessments needed to be performed to detect one new MDRO (PPV 1,8%; 95%CI 1,5 – 2,2). The authors then estimate that at least two working weeks are spent by healthcare workers per newly identified MDRO carrier [21].

Furthermore, active surveillance of MDRO on admission or during an outbreak is associated with significant costs. Several cost-benefit studies that were carried out in Dutch hospitals showed that the MRSA Search and Destroy policy is cost-effective [22,23]. To date, no cost-benefit studies have been conducted with regard to ESBL, CPE or VRE policy in Dutch hospitals. In fact, the balance between costs and benefits is a major point of discussion in the case of outbreak management of the low virulent pathogen VRE. VRE outbreaks have the potential to become substantial in size before they are detected by routine clinical cultures. In **chapter 4** we describe an outbreak where, if the national guideline [17] was followed, would result in 75,000 – 125,000 surveillance cultures to detect all carriers [24]. This would have required considerable administrative effort and laboratory costs. Therefore, in this outbreak a less stringent control strategy than the national guideline was implemented - based on targeted screening and isolation in combination with the implementation of general precautions and environmental cleaning. This strategy successfully controlled the outbreak, while it was associated with a reduction in the number of isolation days and the number of cultures taken.

TIMES ARE CHANGING.

The single most important remaining question now is: how long will the Dutch MDRO approach of Search (in high-risk groups) and Destroy (if possible) remain successful? The epidemiology of MDRO has changed in recent years: MRSA is no longer confined to hospitals and long-term care facilities with patients belonging to known risk groups, with the emergence of the community-acquired MRSA[25]. The majority of the MRSA carriers (30/41 patients; 73.2%) found upon hospital admission in our study (**chapter 2**) had no known risk factor for MRSA carriage (overall prevalence of 0.10%) [18]. These numbers suggest that one in 1000 admissions, in the Amphia hospital is an MRSA positive patient which is not screened and hence, no extra precautionary measures are taken beyond the standard care.

A similar change has taken place in epidemiology for ESBL-producing Enterobacteriaceae whereby the resistance mechanisms were first reported in nosocomial pathogens, followed by the appearance in the community [26]. Recent studies show that the community reservoir of ESBL is probably the most important driver for the emergence and spread of

ESBL-producing Enterobacteriaceae [27,28] and that they are most probably imported from this community reservoir into the hospital settings [26]. Targeted screening of high-risk patients on admission might prove impractical since risk factors for ESBL carriage are too general (e.g. travelling, previous antibiotic use [29]) or difficult to quantify in an assessment (e.g. household overcrowding [30]). A recent cluster-randomised trial in 14 Dutch centres showed that 95% of the ESBL carriers in the study were not detected by routine clinical cultures at any time during admission [31]. In accordance, the authors of a cross-sectional single-centre study estimated a proportion of >99% of undetected ESBL carriers in a Dutch tertiary hospital despite a risk assessment for MDRO carriage [21].

In addition, more alarming is, the changing epidemiology of carbapenemase-producing Enterobacteriaceae. Recently, cases of community-onset CPE infection in patients without recent healthcare contact have been reported in the EU [32]. Also, acquisition of CPE during travel by healthy travellers without healthcare contact during travel has been found in a Dutch study among Dutch travellers [33]. Furthermore, the national report on the use of antibiotics and trends in antimicrobial resistance in the Netherlands from 2019 also shows that in 38% (100/260) of all new CPE cases of which epidemiological questionnaire data were available, none of the known risk factors could be identified [14]. This makes preventative measures to stop hospital spread very difficult or very costly.

There is also serious concern about the introduction of CPE into the food chain. Fortunately, no CPE has been detected in farmed animals in the Netherlands the recent years [14] However, in other countries in the EU as in non-EU countries, CPE had been reported in food-producing animals and their environment [34,35]. This is troublesome since transmission via the food chain

has the potential to spread CPE to of the intestinal flora of (healthy) persons who have not been exposed to healthcare [32] and reflecting the spread of CTX-M producing microorganisms in the community since the 2000s [36]. In particular, the spread of CPE-producing *E. coli* is worrying as it could result in community-acquired urinary tract infections for which the therapies are not as effective or are associated with more side effects. [37].

THE IMPORTANCE OF THE BASICS

The basis of the successful national Search and Destroy policy is becoming uncertain: the current increase of MDRO carriers without known risk factors will result in the undetected introduction in healthcare settings, and increase the risk of nosocomial spread to patients and healthcare workers because preventive measures are not taken. This emphasizes the importance of high compliance with standard precautions e.g. hand hygiene compliance

and a clean environment in the healthcare setting. Unfortunately, standard precautions are not always properly implemented in Dutch healthcare settings. A mixed-methods study conducted in the intensive care unit and surgical ward of 24 Dutch hospitals in 2007 showed an overall hand hygiene compliance below 20% [38]. In 2017, a cluster randomized controlled trial in 33 Dutch nursing homes showed an overall compliance of 12% [39]. Furthermore, lapses in cleaning and disinfection procedures could make equipment and the environment become a reservoir for transferring (multidrug-resistant) microorganisms. In **chapter 6** we showed that ESBL-producing *E. coli* ST10 and ST131 remained viable on dry inanimate surfaces up to 70 days and that the survival of these bacteria was influenced by the degree of environmental contamination [40]. In 2017, the Netherlands Health and Youth Care Inspectorate (IGJ) carried out thematic monitoring of compliance with the infection prevention guidelines in 21 Dutch hospitals and concluded that the total process of cleaning and disinfection, including the use of resources and materials, and the training of employees was assessed insufficient or poor in 44% of the hospitals [41].

Based on these results, it is likely that the current level of standard precautions in many Dutch healthcare settings is not sufficient to control the high-risk clones. In fact, we are already dealing with outbreaks of those clones in hospitals and nursing homes. For example, in **chapter 5** we describe an outbreak of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* ST258 that occurred in a hospital and subsequently in a nursing home. In both settings, extensive environmental contamination was found. The outbreak illustrates that the spread of pan-resistant Enterobacteriaceae can be controlled, but may be difficult, particularly in long-term care facilities. In **chapter 4** we describe the control of a large VRE CC17 outbreak in a Dutch hospital with extensive environmental contamination in multiple wards. In addition to the targeted screening and isolation, the outbreak was controlled with focus on optimisation of the standard precautions in particular the environmental cleaning procedures.

FUTURE PERSPECTIVES

In summary, with regard to MDRO in the Netherlands, we can state that the water is rising, but the dikes are just holding up for now. To keep our feet dry, we will have to closely monitor the development of MDRO by surveillance and molecular typing, monitor (objective) outcome indicators such as the incidence of hospital-acquired bacteraemia caused by MDRO [42] to evaluate the quality of infection prevention. In addition, if necessary, adjust the current national control measures or structure in the Dutch healthcare settings.

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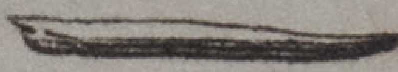
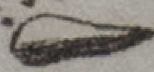
A. 

B. C 

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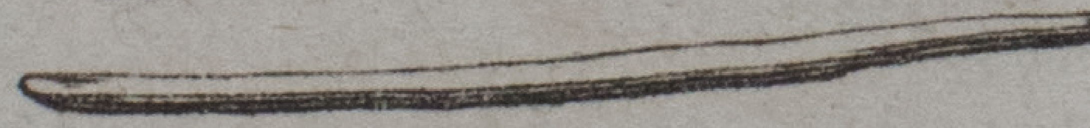
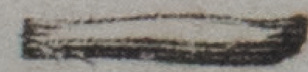
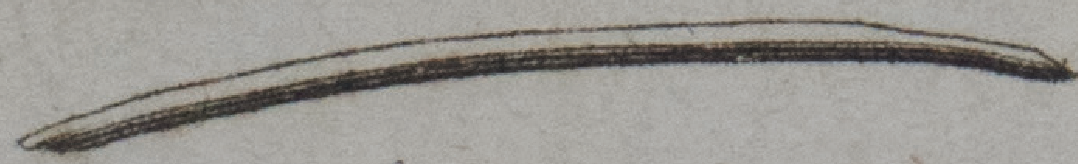
F 





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NEDERLANDSE SAMENVATTING

Nederland is een klein land in Noordwest-Europa met een relatief hoge bevolkingsdichtheid van meer dan 500 inwoners per vierkante kilometer. Nederland staat bekend om zijn bollenvelden, windmolens en innovatief waterbeheer, maar staat ook bekend om het succesvolle beheer van antibioticaresistentie.

Geschiedenis van (deskundige) infectiepreventie in Nederland

In 1966 stelde een commissie van de Raad voor de Gezondheidszorg dat zorginstellingen verplicht waren om een infectiecommissie in te stellen. De taak van deze commissie was het adviseren van ziekenhuisdirecteuren en zorgmedewerkers op het gebied van infectiepreventie [1,2]. Na de realisatie van deze commissies werd duidelijk dat de implementatie van infectiepreventie richtlijnen en het toezicht op de naleving ervan moeilijk was. Daarom werd een nieuw vakgebied opgericht en begonnen in 1968 de eerste vier hygiënisten in Nederland met het uitvoeren van infectiebestrijding [3]. In 1973 richtten 32 hygiënisten de Vereniging voor Hygiënisten Intramurale Gezondheidszorg (VHIG) op met als primair doel het vertalen van richtlijnen van de infectiecommissie naar de werkvloer [2]. Tegenwoordig heeft de VHIG 500 leden en is de naam hygiënist aangepast naar deskundige infectiepreventie. Tegenwoordig zijn deskundigen infectiepreventie werkzaam in de verschillende gebieden van de gezondheidszorg, waaronder ziekenhuizen, verpleeg- en verzorgingshuizen, privéklinieken en openbare gezondheidszorg.

Ondanks het feit dat het klinisch microbiologisch laboratorium een essentieel onderdeel is van een effectief infectiepreventiebeleid (bijvoorbeeld surveillance, typeren) [4], was de samenwerkingsstructuur tussen de medisch microbioloog en de deskundige infectiepreventie in de beginjaren onvoldoende gedefinieerd, niet transparant of zelfs afwezig in sommige ziekenhuizen. Tegenwoordig is duidelijk dat het beheersen van uitbraken en het bestrijden van met name multiresistente micro-organismen een multidisciplinaire strategie vergen. Waarbij met name de samenwerking tussen medisch microbiologen en deskundige infectiepreventie essentieel is voor een effectief en efficiënt infectiepreventie beleid [5].

Nationale richtlijnen voor infectiepreventie

In de loop der jaren ontstond bij infectiepreventie-professionals de behoefte aan uniforme richtlijnen in hun vakgebied. Daarom werd in 1980 een landelijke Werkgroep Infectiepreventie (WIP) opgericht door vier Nederlandse verenigingen die zich bezighielden

met infectieziekten en infectiebestrijding. De WIP heeft in de loop der jaren veel richtlijnen voor zorginstellingen uitgebracht en beantwoordde ook vragen uit het werkveld [6]. Helaas zijn in 2017 alle activiteiten van de WIP stopgezet, waarmee een einde komt aan een internationaal gerenommeerd icoon in de zorg [7]. Gelukkig volgde een nieuw initiatief: in 2021 is het Samenwerkingsverband Richtlijnen Infectiepreventie (SRI) gestart. Het SRI is een initiatief van het ministerie van Volksgezondheid, Welzijn en Sport (VWS) waarin acht organisaties samenwerken om het werk van de voormalige WIP voort te zetten.

Bijzonder resistente micro-organismen (BRMO)

De criteria voor het definiëren van BRMO kunnen per land verschillen [8]. In Nederland worden BRMO gedefinieerd als een micro-organismen waarvan bekend is dat ze ziekten veroorzaken, een antimicrobieel resistentiepatroon hebben dat (empirische) therapie belemmert en zich kan verspreiden als er geen voorzorgsmaatregelen worden genomen [9]. Voorbeelden van BRMO zijn methicilline-resistente *Staphylococcus aureus* (MRSA), vancomycine-resistente enterokokken (VRE), extended spectrum beta-lactamase-producerende Enterobacteriaceae (ESBL-E) en Enterobacteriaceae die carbapenemase (CPE) produceren.

Bepaalde succesvolle BRMO-klonen, zogenaamde hoog-risico klonen, hebben een essentiële rol gespeeld in de wereldwijde opkomst van antibioticaresistentie bij Gram-negatieve organismen (vooral de Enterobacteriaceae) en Grampositieve organismen (*S. aureus* en enterococci). Hoog-risico klonen hebben voordelen ten opzichte van andere klonen wat betreft het vermogen om te koloniseren, zich te verspreiden en in gastheren te persisteren en hebben verbeterde pathogeniciteit, fitheid en overlevingsvaardigheden **(hoofdstuk 6)** [10]. Voorbeelden van deze hoog- risico klonen zijn *Escherichia coli*-ST131 of *Klebsiella pneumoniae*-ST258 geassocieerd met de wereldwijde verspreiding van respectievelijk *bla*CTX-M-15 en *bla*KPC11

Antimicrobiële resistentie

Antimicrobiële resistentie stijgt in alle delen van de wereld tot gevaarlijk hoge niveaus, wat leidt tot hogere medische kosten, langere ziekenhuisopnames en wordt geassocieerd met verhoogde morbiditeit en mortaliteit [12]. Toch is Nederland één van de landen met de laagste antibioticaresistentie in klinische isolaten in Europa [13]. De prevalentie van MRSA en VRE in bloedkweken is bijvoorbeeld aanzienlijk lager met respectievelijk 1,4% en 0,9% dan het gemiddelde van de Europese Unie met 15,5% en 18,3% in 2019

[13,14]. De prevalentie van CPE in Nederland blijft laag in bloedkweken (0,0% voor *E. coli* en *K. pneumoniae* versus respectievelijk 0,3% en 10,0% in de Europese Unie (EU)) [13,14]. Alleen de prevalentie van extended spectrum beta-lactamase (ESBL) producerende Enterobacteriaceae in bloedkweken neemt de afgelopen jaren in Nederland toe (2019: *E. coli* 7,5% en *K. pneumoniae* 9,6%), maar nog steeds laag in vergelijking met de gemiddelde prevalentie van de Europese Unie (2019: *E. coli* 15,1% en *K. pneumoniae* 31,3%) [13,14].

Deze lage prevalentie van antibioticaresistentie is deels te wijten aan het behoedzame en restrictieve gebruik van antibiotica (niet verder besproken in dit proefschrift) [14] met daarbij een effectief infectiepreventiebeleid op basis van landelijke richtlijnen zoals hierboven genoemd. Dit proefschrift beschrijft onder meer een aantal praktische aspecten bij de implementatie van de landelijke richtlijnen voor de bestrijding van multiresistente micro-organismen uitbraken in Nederlandse zorginstellingen.

Succesvol infectiepreventiebeleid

Een van de bekendste en meest succesvolle infectiepreventiebeleidsmaatregelen is het zogenoemde Search and Destroy-beleid [15], dat begin jaren tachtig werd ingevoerd na een reeks MRSA-uitbraken in Nederlandse ziekenhuizen. Het beleid bestaat uit het screenen van patiënten met een verhoogd risico op MRSA-dragerschap bij ziekenhuisopname, (preventieve) isolatie en behandeling van MRSA-dragers. In de eerste jaren lag de nadruk vooral op het screenen van patiënten na een opname in een buitenlandse zorginstelling. De laatste 20 jaar zijn hier verschillende nieuwe risicogroepen aan toegevoegd, zoals veehouders in 2006 en huisgenoten en partners/verzorgers van MRSA-dragers in 2012. Sinds 2005 is het actief screenen en preventief isoleren van risicogroepen (met uitzondering van veehouders) uitgebreid naar andere BRMO's [16,17]. De goede naleving van deze richtlijnen door alle Nederlandse ziekenhuizen is een van de sleutels tot het succes van deze strategie. **In Hoofdstuk 2** beschrijven we een observationele studie om de prevalentie van MRSA-neusdragerschap te bepalen bij patiënten die preoperatief werden gescreend op *S. aureus*-neusdragerschap tussen 2010 en 2017 [18]. De studie toonde een structurele lage prevalentie van MRSA-dragerschap bij ziekenhuisopname gedurende 7 jaar (0,13%; 95% BI 0,10-0,18%). De MRSA-prevalentie varieerde van 0,03% tot 0,17% door de jaren heen zonder aanwijzingen voor een veranderende trend in de tijd (p = 0,40). Deze bevindingen onderschrijven de effectiviteit van het Nederlandse Search and Destroy-beleid, in combinatie met een restrictief antibioticabeleid.

Controverses in infectiepreventie

Naast de positieve effecten van het Nederlandse nationale beleid (lage prevalentie van BRMO) is er ook controverse rondom deze screening en (preventieve) isolatiestrategie. Over het algemeen kunnen isolatiemaatregelen gepaard gaan met nadelige effecten op de tevredenheid van patiënten, niveau van zorg en ervaren gezondheidswerkers een zwaardere werkdruk bij de zorg voor een patiënt in isolatie [19,20].

Daarnaast zijn in een laag-endemische setting zoals in Nederland veel anamnesevragen, kweken en preventieve isolatiedagen nodig om een klein aantal patiënten met een BRMO op te sporen. Van Hout et al. evalueerde recent de risicobeoordeling voor het identificeren van BRMO-dragers bij ziekenhuisopname in een Nederlands tertiair ziekenhuis en toonde aan dat 1.778 BRMO-risicobeoordelingen moesten worden uitgevoerd om één nieuwe BRMO te detecteren (PPV 1,8%; 95% BI 1,5 – 2,2). De auteurs schatten in dat per nieuw geïdentificeerde BRMO-drager minimaal twee werkweken worden besteed door zorgmedewerkers [21].

Bovendien gaat actieve surveillance op BRMO bij opname of tijdens een uitbraak gepaard met aanzienlijke kosten. Uit meerdere kosten-baten studies die zijn uitgevoerd in Nederlandse ziekenhuizen blijkt dat het MRSA Search and Destroy-beleid kosteneffectief is [22,23]. Tot op heden zijn geen kosten-baten studies gedaan met betrekking tot het ESBL, CPE of VRE-beleid in Nederlandse ziekenhuizen. De balans tussen kosten en baten is een belangrijk discussiepunt, zeker in het geval van uitbraakbeheersing van de laag virulente ziekteverwekker VRE. VRE-uitbraken hebben het potentieel om aanzienlijk in omvang te worden, voordat ze worden gedetecteerd door klinische kweken. In **hoofdstuk 4** beschrijven we een uitbraak waarbij, als de landelijke richtlijn [17] zou zijn gevolgd, 75.000 – 125.000 surveillance kweken nodig waren geweest [24]. Dit zou aanzienlijke administratieve inspanningen en laboratoriumkosten met zich meegebracht hebben. Daarom is bij deze uitbraak een minder stringente bestrijdingsstrategie dan de landelijke richtlijn geïmplementeerd - gebaseerd op gerichte screening en isolatie in combinatie met implementatie van algemene voorzorgsmaatregelen en schoonmaak van de omgeving. Deze strategie heeft de uitbraak met succes onder controle gebracht, terwijl het gepaard ging met een vermindering van het aantal isolatiedagen en het aantal afgenomen kweken.

Tijden veranderen

De allerbelangrijkste vraag die nu overblijft is: hoe lang blijft de Nederlandse BRMO-aanpak van Search (in risicogroepen) and Destroy (indien mogelijk) succesvol? De epidemiologie van BRMO's is de afgelopen jaren veranderd: MRSA is niet langer beperkt

tot zorginstellingen met patiënten die tot bekende risicogroepen behoren, met de opkomst van de gemeenschap verworven MRSA [25]. De meerderheid van de MRSA-dragers (30/41 patiënten; 73,2%) in onze studie **(hoofdstuk 2)** had geen bekende risicofactor voor MRSA-dragerschap (totale prevalentie van 0,10%) [18]. Dit betekent dat een op de 1000 opnames, een MRSA-positieve patiënt niet wordt gescreend of geïsoleerd bij opname in het Amphia ziekenhuis.

Een vergelijkbare verandering heeft plaatsgevonden in de epidemiologie voor ESBL-producerende Enterobacteriaceae, waarbij de resistentiemechanismen voor het eerst werden gemeld bij nosocomiale pathogenen, gevolgd door het verschijnen in de algemene bevolking [26]. Recente studies tonen aan dat het ESBL reservoir in de algemene bevolking waarschijnlijk de belangrijkste drijfveer is voor de opkomst en verspreiding van ESBL-producerende Enterobacteriaceae [27,28] en dat ze hoogstwaarschijnlijk uit dit reservoir worden geïntroduceerd in de ziekenhuisomgeving[26]. Gerichte screening van hoog-risico patiënten bij opname is lastig, aangezien risicofactoren voor ESBL-dragerschap te algemeen zijn (bijv. reizen of eerder antibioticagebruik [29]) of moeilijk te kwantificeren (bijv. overbevolking van huishoudens [30]). Uit een recent clustergerandomiseerd onderzoek in 14 Nederlandse centra bleek zelfs dat 95% van de ESBL-dragers in het onderzoek op geen enkel moment tijdens de opname was gedetecteerd door klinische kweken [31]. Dit komt overeen met een andere cross-sectionele studie in een Nederlands tertiair ziekenhuis waarbij er sprake was van >99% niet-gedetecteerde ESBL-dragers, ondanks een risicobeoordeling voor BRMO-dragerschap [21] bij opname.

Nog alarmerender is de veranderende epidemiologie van carbapenemase-producerende Enterobacteriaceae. Onlangs zijn in de EU 32 gevallen van CPE-infecties gemeld bij patiënten zonder recent contact met de gezondheidszorg. Ook is het verwerven van CPE tijdens het reizen door gezonde reizigers zonder zorgcontact aangetoond in een Nederlands onderzoek onder Nederlandse reizigers [33]. Verder blijkt uit het nationale rapport over het antibioticagebruik en trends in antimicrobiële resistentie in Nederland van 2019 dat in 38% (100/260) van alle nieuwe CPE-gevallen waarvan epidemiologische gegevens beschikbaar waren, geen van de bekende risicofactoren konden worden geïdentificeerd [14].

Er is ook grote bezorgdheid over de introductie van CPE in de voedselketen. Gelukkig werd de afgelopen jaren in Nederland geen CPE aangetoond bij landbouwhuisdieren [14]. In andere EU-landen, maar ook in niet-EU-landen, is CPE gemeld bij voedselproducerende dieren en hun omgeving [34,35]. Dit is zorgwekkend aangezien de overdracht via de voedselketen

het potentieel heeft om CPE te verspreiden naar de darmflora van (gezonde) personen die niet zijn blootgesteld aan gezondheidszorg [32] en weerspiegelt de verspreiding van CTX-M-producerende micro-organismen in de algemene bevolking sinds het jaar 2000 [36]. Met name de verspreiding van CPE-producerende *E. coli* is zorgwekkend, omdat deze kan leiden tot door de algemene bevolking verworven urineweginfecties waarvoor geen effectieve therapie bestaat [37].

Het belang van de basis

De basis van het succesvolle nationale Search and Destroy-beleid wordt onzeker: de huidige toename van BRMO-dragers zonder bekende risicofactoren zal leiden tot onopgemerkte introductie in zorginstellingen en het risico op nosocomiale verspreiding naar patiënten en zorgmedewerkers vergroten, omdat preventieve maatregelen niet worden genomen. Dit benadrukt het belang van een hoge mate van naleving van standaard voorzorgsmaatregelen, bijvoorbeeld het naleven van de handhygiënerichtlijn en een schone omgeving in de gezondheidszorg. Helaas blijkt in de praktijk, dat de standaard voorzorgsmaatregelen niet altijd goed worden uitgevoerd. Een groot onderzoek, uitgevoerd op de intensive care en chirurgische afdeling van 24 Nederlandse ziekenhuizen in 2007, toonde aan dat de algehele naleving van handhygiëne onder de 20% ligt [38]. In 2017 toonde een gerandomiseerde, gecontroleerde clusterstudie in 33 Nederlandse verpleeghuizen een algehele naleving van handhygiëne van 12% aan [39]. Bovendien kunnen door fouten in de reinigings- en desinfectieprocedures, apparatuur en de omgeving een reservoir worden van (multiresistente) micro-organismen. In **hoofdstuk 6** hebben we aangetoond dat ESBL-producerende *E. coli* ST10 en ST131 tot 70 dagen levensvatbaar bleven op een droog levenloos oppervlak en dat de overleving van deze bacteriën werd beïnvloed door de mate van verontreiniging [40]. In 2017 heeft de Inspectie Gezondheidszorg en Jeugd (IGJ) in 21 Nederlandse ziekenhuizen thematisch toezicht gehouden op de naleving van de infectiepreventie richtlijnen en kwam tot de conclusie dat het totale proces van reiniging en desinfectie (inclusief gebruik van middelen en materialen, en het opleiden van medewerkers) in 44% van de ziekenhuizen onvoldoende of slecht was [41].

Op basis van deze resultaten is het aannemelijk dat het huidige niveau van standaard voorzorgsmaatregelen in veel Nederlandse zorginstellingen niet voldoende is om de hoog risico klonen te bestrijden. In feite hebben we al te maken met uitbraken van deze klonen in ziekenhuizen en verpleeghuizen. In **hoofdstuk 5** beschrijven we bijvoorbeeld een uitbraak van *Klebsiella pneumoniae* carbapenemase-producerende *Klebsiella pneumoniae*

ST258 die optrad in een ziekenhuis en vervolgens in een verpleeghuis. In beide instellingen werd uitgebreide omgevingscontaminatie gevonden. De uitbraak illustreert dat de verspreiding van panresistente Enterobacteriaceae onder controle kan worden gebracht, maar moeilijk kan zijn, vooral in instellingen voor langdurige zorg. In **hoofdstuk 4** beschrijven we de beheersing van een grote VRE CC17-uitbraak in een Nederlands ziekenhuis met uitgebreide omgevingscontaminatie op meerdere afdelingen. Naast de gerichte screening en isolatie, werd de uitbraak gecontroleerd door optimalisatie van de standaard voorzorgsmaatregelen, met name de schoonmaakprocedures.

Toekomstperspectieven

Samenvattend kunnen we met betrekking tot BRMO in Nederland stellen dat het water stijgt en dat de dijken voorlopig standhouden. En om onze voeten droog te houden, zullen we de ontwikkelingen rondom BRMO nauwlettend moeten monitoren door middel van surveillance, moleculaire typering, en (objectieve) uitkomstindicatoren zoals de incidentie van in het ziekenhuis opgelopen bacteriëmie veroorzaakt door BRMO[42] om de kwaliteit van infectiepreventie te evalueren. En zo nodig de huidige landelijke beheersmaatregelen in de Nederlandse zorginstellingen aanpassen.

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CURRICULUM VITAE

Veronica was born on October 27, 1978 in Raamsdonk, the Netherlands. After graduating the HU University of Applied Sciences Life Sciences in Utrecht in 2002, she was employed as a lab technician at the Amphia Hospital in Breda.

In 2010, she had the opportunity to begin her training to be an Infection control practitioner at the department of infection control at the Amphia hospital. In 2012, she completed her training and received her Postgraduate Infection Control Expert Diploma from the Amphia Academy Infectious Disease Foudation.

In 2015, she started her PhD with Prof. Dr. J. Kluytmans and Prof. Dr. A. Voss as her supervisors and Dr. J. Veenemans as her co-supervisor. After her PhD, she will continue to work in the infection prevention department of the Amphia hospital as a Consultant Infection control.

Veronica lives together with Jurgen van Strien, and their sons Robbie (2007) and Lars (2010).

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D

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Microbiologen, arts-assistenten, medisch moleculair biologen, analisten en alle andere medewerkers van Microvida bedankt voor jullie input en leerzame discussies op refereeravonden, congressen en andere bijeenkomsten. In het bijzonder wil ik nog mijn **(oud-)collega's bij Microvida locatie Molengracht** bedanken. Ik wil jullie bedanken voor al die duizenden prevalentie, neus- en uitbraak kweken uit dit proefschrift die jullie hebben ingezet en uitgewerkt. In het bijzonder wil ik nog **Carlo** bedanken. Carlo, je bent een wandelende (en zingende) kennisbank op het gebied van microbiologie. Dank voor alle uitleg over remmingszones, ESBLs, cAmpC, enzovoorts. En **Angela** en **Stijn,** dank voor al jullie werk m.b.t. WGS-analyses, waarvan er een aantal ook in dit proefschrift staan. Ik hoop dat we in de toekomst nog aan veel leuke projecten kunnen samenwerken. En **Ingrid** bedankt voor het draaien, bijschaven, aanpassen en weer draaien van alle GLIMS query's met jouw bijbehorende engelengeduld.

Mijn (oud)collega's van de Kenniskern Infectiepreventie: **Anja, Ada, Annemarie, Bianca, Diana, Esther, Gonny, Heidi, Inge, Irma, Jannie, Laura, Linda, Mark, Melanie, Miranda, Natalie, Suzanne, Tineke, Titia, Thera, Wouter en Yvonne.** Ik ben trots op onze afdeling, op wat we de afgelopen jaren bereikt hebben en hoe we als afdeling altijd voor elkaar klaar staan. In het bijzonder wil ik nog **Miranda en Wouter** bedanken: bedankt voor alle mogelijkheden die ik krijg binnen onze afdeling om te groeien. En **Gonny**, zoals Carlo een

wandelde microbiologie kennisbank is, ben jij dat voor de infectiepreventie. Jij bent mijn werkbegeleider geweest tijdens de opleiding en hebt mij wegwijs gemaakt in dit moeilijke vak, veel dank hiervoor. Nu nog steeds ben ik blij dat ik regelmatig bij je kan aankloppen voor advies.

De KPC-uitbraak in 2013 was een van de eerste grote uitbraken waar ik bij betrokken was als deskundige infectiepreventie. Ik heb door deze uitbraak veel geleerd over CPE's, outbreakmanagement en de bijbehorende uitdagingen, met name in de verpleeghuissetting. Maar ik heb hierbij ook van dichtbij gezien wat voor impact een dergelijke (BRMO) uitbraak heeft op patiënten/bewoners, familieleden en medewerkers. Deze uitbraak heeft mij hierdoor zowel op het werk- als persoonlijk vlak gevormd. Ik wil graag bewoners, familieleden, medewerkers van De Riethorst (nu Mijzo) en andere betrokkenen bedanken voor hun openheid en samenwerking. In het bijzonder wil ik nog een aantal medewerkers van het eerste uur bedanken: **Adrie de Laat, Bert Vos, Eliane Thewessen, Désirée van Stenis, Liesbeth van Boxel**, **Stijn Raven (GGD)** en **Kimberly Verwoert.**

De I-4-1-Health werkgroep. Binnen dit project heb ik mogen ervaren hoe leuk en inspirerend het is om met mensen samen te werken, die het vak infectiepreventie net zo leuk vinden als mijzelf. Met als extra bonuspunten een kijkje in de (indrukwekkende) kennis en werkwijze van de Belgische afdelingen infectiepreventie. **Annette, Ellen, Helen, Ina, Isabelle, Martine** en **Pascal**, ik heb genoten en veel geleerd van onze overleggen, bezoeken aan de ziekenhuizen, stadtours, etc. Dank hiervoor en ik hoop op nog mooie samenwerkingen in de toekomst.

De 'donderdagochtend'-projectgroep **Andreas**, **Annemieke, Anouk, Carlo, Esther, Jaco, Jean-Luc, Joep, Karen, Karlijn**, **Mark, Martine, Nathalie, Rik, Robbert, Suzan, Thera, Vivian** en **Wouter.** De quote van Oliver Wendell Holmes "*Many ideas grow better when transplanted into another mind than the one where they sprang up*" omschrijft het projectoverleg mijn inziens goed. Het is altijd inspirerend om bij dit overleg aan te sluiten, waarbij iedereen vanuit zijn/haar eigen vakgebied een bijdrage levert om stappen te maken in mooie onderzoek(voorstell)en, publicaties en promoties. In het bijzonder wil ik nog **Suzan** bedanken voor alle uitleg over WGS, de leuke sparmomenten over WGS en infectiepreventie en gewoon de gezellige gesprekken. Iedereen dank voor jullie input en leerzame discussies! Dat er nog veel cakes en koffiekoeken uitgedeeld mogen worden.

Mijn "medeslachtoffers" **Andreas, Evert, Joep** en **Pepijn**:

Jongens, ik wil jullie heel erg danken voor alle steun, humor en gezelligheid. Onze app-groep waarin reviewer 2 altijd met rotte tomaten werd afgeschoten heeft me door vele dipjes heen geholpen.

Andreas, het is altijd gezellig om (bij) te kletsen met je. Ook binnen het i-4-1Health project was een leuke samenwerking waarbij ik je wat beter heb leren kennen. En hopelijk kunnen we snel op je ATP (Andreas' Top Proefschrift) proosten!

Evert, ik heb de afgelopen jaren heb ik ontzettend gelachen om je (gort)droge humor en positiviteit. Daarnaast blijf je natuurlijk bij mij bekend als de Amp-C-ostel man. Ik vond/vind het altijd erg leuk om je zo enthousiast te horen praten over hairpins en mutaties. Nu ga je een nieuwe, gave, jaloersmakende uitdaging aan. Hopelijk houd je ons op de hoogte van bezigheden en maakt je ergens ook nog een keer tijd voor een biertje om bij te kletsen.

Joep, ook met jou heb ik de afgelopen veel gelachen maar dan om je heerlijke sarcastische humor. Ook je 'voorblad' figuur tijdens een promotie overleg vond ik geweldig… maar ik bleek de enige te zijn. Plasmiden – geen idee.

Nu gaan we beginnen aan een heel gaaf project/projecten. Ik verwacht weer veel van je te leren, leuk te discussiëren en mooie stappen te maken in dit vakgebied.

Pepijn naast veel humor ben je ook iemand die feilloos aanvoelt of iemand even wat steun nodig heeft of behoefte heeft aan een luistert oor. Dank voor al je hulp, gezelligheid en leerzame gesprekken (m.u.v. de overleggen waarin DAPC werd besproken… die vond ik vreselijk ;-)).

Naast collega's zijn er ook veel mensen die op andere wijze hebben bijgedragen aan dit proefschrift. Mijn **lieve vrienden en familie** wil ik bedanken voor hun steun, interesse en afleiding als dat nodig was. Ook meteen excuses voor alle vergeten afspraken, het opstarten van WhatsApp gesprekken en dan … * krekelgeluid*…, wel beloofde maar niet uitgevoerde terugbelgesprekken, etc. Na het afsluiten van dit promotie traject zijn timemanagement en agendabeheer de volgende uitdagingen waar ik mee aan de slag ga. Een aantal personen wil ik graag in het bijzonder bedanken:

Diana en Rainier, jullie hebben jarenlang opgepast op Robbie en Lars. En dit ging op zo'n goede/fijne manier, dat onze jongens het nu soms nog over hebben hoe leuk het was om bij molen de herten eten te geven, met Bo en later Luna te wandelen, bij opa op de koffie te gaan. Dank voor alle hulp! En nu lekker genieten van oppassen, maar dan op

jullie schattige kleindochter Jackie.

Ellis, ondanks dat we elkaar tegenwoordig niet heel vaak live zien, ben je iemand die altijd een mooi onderdeel bent geweest in de afgelopen +40 jaar van mijn leven. Of het nu samen op de lagere school zitten is, VHS-banden van Tour of Duty kijken (mooooove, moooovve…), flauwvallen bij een Take That concert, gillen naar Robbie Williams als volwassen vrouwen zonder enige schaamte of gewoon afspreken om bij te kletsen. Op een of andere manier voelt het altijd alsof we elkaar gisteren nog hebben gesproken. Ik ben trots op hoe en waar je nu in het leven staat! Een inspiratie voor mij en anderen. Én ik ben zo blij dat ík nu eindelijk eens een boekje kan geven tijdens onze etentjes met Danny!

Frøken en Suzan, we kennen elkaar van HLO Utrecht en hebben genoten van het studentenleven aldaar. Maar ook na het HLO nog contact gehouden met gezellige uitstapjes en bijzondere persoonlijke momenten. Dank hiervoor!

Carola en Pascal, vrienden van het eerste uur, die altijd voor ons klaar staan! Carola, jij bent iemand wie ik dag en nacht mag bellen, altijd klaar staat met goede raad, er niet om heen draait en bovenal een heel lieve vriendin is. Ik ben blij met een vriendin zoals jij, dank!

Mijn paranimfen **Ina** en **Esther**

Ina: van (oud)collega naar vriendin/ helft van het duo Martha & Mira. Ik heb de afgelopen jaren veel van je geleerd op het gebied van microbiologie, infectiepreventie en onderzoek, maar ook op het persoonlijke vlak. Heel veel dank hiervoor. Ik geniet ervan dat we nu ook dit project samen tot een mooi einde hebben weten te brengen.

Esther: van familie - uitbreidend naar collega - naar vriendin. Het was de afgelopen jaren een feestje om je kamergenoot te zijn, zoals onze buren (en de buren van de buren) ook konden horen. Het is fijn om kletsen over zaken die ik weer niet begreep op het project overleg, maar ook over persoonlijke zaken. Heel veel dank hiervoor. Nu helaas geen kamergenoten meer, maar nog genoeg momenten en stof om bij te kletsen, al dan niet op de 'what happens@…'app (#secured with end-to-end encryption#).

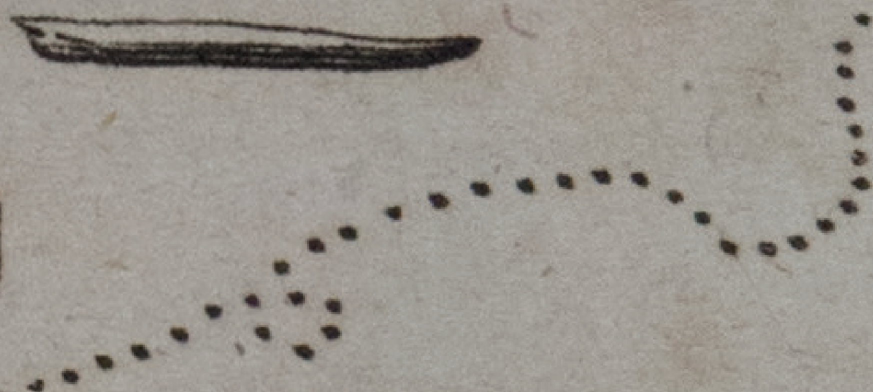
Lieve **Pa en Ma.** Dankzij jullie sta ik hier nu! Het gezegde 'alles voor het jong' is denk ik ooit bedacht naar jullie voorbeeld. Want zo is het: altijd hard gewerkt, zodat ik niks te kort kwam/kom en alle kansen kon pakken die op mijn pad kwamen. Dank voor alle hulp en steun. En dank voor alle goede zorg en liefde voor Robbie en Lars. Met heel veel liefde draag ik dit boekje aan jullie op.

Lieve **Jurgen**. Van met je Honda MT brommer aan de Juliana MAVO mij opwachten na schooltijd - naar deze dag die we samen vieren met onze twee mannen. Vooraf hebben we niet helemaal goed kunnen inschatten hoeveel tijd deze promotie zou gaan kosten. Ook de planning van dit proefschrift was ook niet helemaal te volgen voor je, vrees ik. Mogelijk kwam dit ook doordat ik meerdere keren heb aangeven 'dat het einde nu echt inzicht was'. Waarbij achteraf dan weer bleek dat het inderdaad het einde inzicht was, maar alleen als je door een elektronenmicroscoop keek. Bedankt voor alle steun, liefde en geduld van de afgelopen jaren. En nu tijd voor mooie en nieuwe uitdagingen!

En uiteindelijk kom ik bij de twee belangrijkste twee mensen in mijn leven. Lieve **Robbie** en **Lars**, ik ben trots op jullie! Trots op wie jullie zijn en wat jullie doen. Het is heerlijk om te zien hoe jullie van kleine jongens - nu opgroeien tot leuke, lieve en stoere pubers. De komende jaren staat in het teken van verder groeien, ontdekken, beleven en verwonderen. Weet dat papa en ik er altijd voor jullie zijn.

Veronica

A.



B. C.

Fig.

E.

F.