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Prevention and treatment of infections in intensive care patients

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Prevention and treatment of infections in intensive care patients

Challenges and potential improvements

Heleen Aardema

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Prevention and treatment of infections in intensive care patients

Challenges and potential improvements

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Ter nagedachtenis aan mijn grootouders en mijn vader, Evert Aardema
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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

Infections are a major threat to the intensive care population¹⁻³, while the incidence of sepsis, defined by life-threatening organ dysfunction caused by a dysregulated host response to infection⁴, is rising over time⁵. Although overall sepsis-related mortality rates have declined over the years⁵, they are, despite an increased understanding of the pathophysiology of sepsis and therapeutic improvements⁶, still unacceptably high^{2,7}.

In this thesis, some aspects of three important topics with regard to the prevention and treatment of infections in intensive care patients are discussed. The first topic is antibiotic resistance, regarded by the World Health Organization as “one of the biggest threats to global health, food security, and development today”⁸. The second topic is pharmacokinetics of beta-lactams. In the recent international guidelines for sepsis and septic shock, it is recommended to optimise dosing of antimicrobials based on pharmacokinetic/ pharmacodynamic principles⁴. The third topic is the composition of intestinal microbiota, which is regarded as a key objective in sepsis research⁶. Within these three enormous themes, many questions are still unanswered.

To fill in gaps in the knowledge on these subjects, in this thesis, the following research **objectives** were defined:

- I. To investigate the burden and characteristics of highly resistant microorganisms in adult ICU patients in a Dutch university hospital (**Chapter 2**)
- II. To evaluate plasma concentrations of the beta-lactam antibiotics piperacillin and cefotaxime in critical care patients (**Chapter 3 and 4**)
- III. To understand the dynamics of intestinal microbiota in a critical care cohort admitted after planned major surgery (**Chapter 5**)

The goal of these objectives is to improve prevention and treatment of infections in the critical care setting. Below, the background and rationale behind the objectives are outlined, with references to respective chapters of this thesis.

BURDEN OF INFECTION AND MULTI-RESISTANT MICROORGANISMS IN THE CRITICAL CARE SETTING

Infections are a constant source of morbidity and mortality in critically ill patients^{1,9}. In a large prospective point prevalence study including Intensive Care Units (ICUs) in 75 countries (Extended Prevalence of Infection in Intensive Care II; EPIC II study), 51% of 12.796 participants were considered infected, either with community-acquired or with nosocomial infections¹. Because of this large burden of infection, consumption of antibiotics on ICUs is very high^{1,10}. In the EPIC II study, 71% of patients were receiving antibiotics (as prophylaxis or treatment) on the study day¹; in another large point-prevalence study evaluating antibiotic use throughout hospitals worldwide on a given day, 59% of 5184 ICU patients were treated with antibiotics on that day¹⁰. In

patients with sepsis or septic shock, timely treatment with an adequate antibiotic using optimal dosing is critical for a successful outcome^{4,11,12}. On the other hand, antibiotics are prescribed inappropriately for various reasons, such as a wrongly presumed infection diagnosis¹³⁻¹⁵ or non-adherence to guidelines, including use of prolonged surgical prophylaxis¹⁰. Benefits of adequate antimicrobial therapy notwithstanding, this large antibiotics consumption comes at a price; it may for instance induce overgrowth of resistant microorganisms through selection pressure¹⁶⁻¹⁸. This is not only disadvantageous for the individual patient, but also on group level. More resistance will lead to more inadequate antimicrobial therapy¹⁹ and infections with multi-resistant microorganisms lead to worse outcome^{20,21}. The vast consumption of antibiotics, both in hospital and in the community as well as in food production¹⁸, has contributed to the ever increasing prevalence of antimicrobial resistance, especially of multi-resistant Gram-negative pathogens^{18,22}. This increasing resistance necessitates moving towards the use of second-choice or ‘last resort’ antibiotics²³, while emergence of new-class antibiotics that have benefits over existing antibiotics and that can target Gram-negative hospital infections in ICU patients is only modest^{16,24}. In the need for standardised international terminology²³, on the initiation of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), an international group of experts introduced the now widely accepted definitions of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria²⁵ for public health use and epidemiological purposes. However, in clinical practice, national guidelines are not harmonised in terminology, testing for resistance and subsequent hygiene measures^{26,27}. In the Netherlands the term Highly Resistant Microorganism (HRMO) is well defined by the former Dutch Working Party on Infection Prevention²⁸ and implies specific recommendations for isolation precautions, active surveillance and contact tracing. This definition of HRMO is used as such throughout this thesis. According to European surveillance data, the Netherlands, along with Scandinavian countries, is considered an area with low incidence of antimicrobial resistance²⁹. For instance, prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) upon hospital admission in planned cardiothoracic surgery patients between 2010 and 2017 in the South of the Netherlands was 0.13%, with no changing trend over time³⁰. However, even in the Netherlands, prevalence of Extended Spectrum Beta-Lactamase (ESBL)-producing *Enterobacteriaceae* carriage in the community is emerging and no longer negligible^{31,32}. In a prevalence study among patients presenting with gastro-intestinal complaints to their general practitioner, 10.1 % of 720 stool samples were positive for ESBL-producing organisms³¹, while in a prevalence study in long-term care facilities including 385 residents ESBL-carriage was even 14.5%³².

The ICU is a so-called ‘epicenter’ of antimicrobial resistance^{9,19,33}, where antimicrobial resistance can be “created, disseminated, and amplified”⁹. In clinical practice, contributing factors include the high use of antimicrobial therapy, the density of a susceptible patient population, the

severity of illness, prolonged hospital stay with a higher chance of acquiring a multi-resistant strain through cross transmission or endogenous emergence of resistance, and flaws in infection control measures^{19,33,34}.

Highly resistant microorganisms can not only be acquired on the ICU, they can also be introduced through transfer of colonised patients from the wards or from the community reservoir^{9,34-38}.

Knowledge of local resistance and identification of patients at risk of colonisation or infection with an HRMO can help in optimising (empirical) antibiotic therapy and in applying optimal infection prevention measures. The burden of HRMOs in a typical ICU population including characterisation of these HRMOs was not well established in our region.

Thus, **objective I** was to investigate the burden and characteristics of highly resistant microorganisms in adult ICU patients in a Dutch university hospital. Hence, we assessed the incidence of HRMOs in the adult intensive care unit of our institution, including the evaluation of different categories of HRMOs, and the proportion of imported versus acquired HRMOs. Further, we analysed the outcome of patients with and without colonisation or infection with an HRMO. We also characterised this subpopulation of patients found positive for any HRMO (**Chapter 2**).

OPTIMAL TREATMENT OF INFECTIONS WITH BETA-LACTAMS

Besides adequate source control, as previously stated, timely administration of appropriate antibiotics using correct dosing is crucial in ICU patients with severe infections, to achieve the best possible clinical outcome and prevent emergence of antimicrobial resistance^{11,39-43}. For the selection of an appropriate antibiotic in the empiric setting, it is important to take into consideration possible causative pathogens, including their likely susceptibility patterns. Once a causative microorganism including its susceptibility to specific antibiotics is recognised, de-escalating is an important step in good antibiotic stewardship. Likewise, the duration of therapy should be established during treatment, and be as short as possible. Optimal antibiotic dosing is determined by pharmacokinetic (PK) and pharmacodynamic (PD) principles^{41,44-46}. Pharmacokinetics describes the rates and processes from drug absorption, to distribution, to elimination through metabolism or excretion^{41,46}; or “what the body does to the drug”⁴⁵. Pharmacodynamics describes the relationship between drug exposure and pharmacological (in antibiotics; microbiological or clinical) effects^{41,42,44-46}, or “what the drug does to the body”⁴⁵. Important factors that influence a drug’s pharmacokinetics are volume of distribution (Vd) and clearance (CL)⁴⁷. For antibiotics, PD characteristics are determined by inhibition of bacterial growth, rate and extent of bactericidal action and post-antibiotic effect⁴⁴. The antimicrobial activity is usually assessed by the Minimum Inhibitory Concentration (MIC) of a (suspected) causative pathogen. For different classes of antibiotics three different PK/PD indices are known: (a) the duration of time (T) the free (unbound) drug concentration remains above the MIC during a dosing interval ($fT_{>MIC}$) (e.g. beta-lactams); (b) the ratio of maximum drug concentration to MIC

(C_{max}/MIC) (e.g. aminoglycosides); and (c) the ratio of the area under the concentration-time curve during a 24-hour period to MIC (AUC_{0-24}/MIC) (e.g. glycopeptides and fluoroquinolones)^{41,47}. These different indices imply different optimal dosing schedules for different antibiotic classes.

Beta-lactams are the most prescribed class of antibiotics both in ward patients and in ICU patients^{10,48}. In critical care units throughout the Netherlands, beta-lactams are also widely employed in the context of Selective Decontamination of the Digestive tract (SDD), which includes four days of pre-emptive treatment with a cephalosporin, such as cefotaxime⁴⁸. In an environment with low levels of antimicrobial resistance, its use is associated with a reduction in ICU and hospital mortality and ICU-acquired bacteraemia⁴⁹.

Historically, the intermittent dosing of beta-lactams is derived from pharmacokinetic data obtained in healthy volunteers or non-severely ill patients^{50,51}. As the efficacy of beta-lactams is dependent on the time for which the antibiotic (unbound) plasma concentration remains above the MIC for a dosing period⁴⁷ and, except in the case of *Staphylococcus aureus*, there is no relevant post antibiotic effect^{50,52}, there is a risk of insufficient antibiotic exposure during the treatment period. This is unfavourable as it can lead to worse outcome and emergence of antimicrobial resistance^{42,43,53}. For beta-lactams a bactericidal effect is shown for a minimum value of $\%fT_{>MIC}$ between 50 and 70% in pre-clinical studies⁵⁴. However, clinical data involving the critically ill suggest a more aggressive approach to achieve a target of at least 100% $fT_{\geq MIC}$ is needed to pursue clinical cure, where a target of at least 100% $fT_{\geq 4 \times MIC}$ is considered optimal for ensuring clinical efficacy and prevention of selection of resistant subpopulations^{53,55,56}. In critical care patients, various physiologic alterations can result in an unpredictable pharmacokinetic profile. For instance, in a septic patient, for reasons not yet entirely understood^{57,58}, a state of augmented renal clearance can occur⁵⁹⁻⁶¹, while capillary leakage and an altered protein binding can both lead to a larger volume of distribution, all resulting in lower antibiotic plasma concentrations. However, emergence of organ dysfunction such as acute kidney failure or liver failure might lead to impaired clearance and hence higher antibiotic plasma concentrations^{47,60}. Furthermore, an altered pharmacodynamic profile should be anticipated compared with less severely ill patients, as in the critical care population causative pathogens with higher MICs should be taken into account⁶². Indeed, in critical care populations treated using a conventional (intermittent) dosing strategy, inadequate antibiotic exposure is often found^{44,51,63,64}. In a large prospective multicenter pharmacokinetic point prevalence study (“Defining Antibiotic Levels in Intensive Care Unit Patients- DALI”) including 8 beta-lactam antibiotics, a predefined PK/PD target of 100% $fT_{\geq MIC}$ was achieved in 60.4 % of patients⁵¹. Augmented renal clearance especially is a risk factor for target non-attainment^{44,64-67}. From a pharmacokinetic viewpoint continuous dosing in critical care patients thus seems a logical alternative. Several studies support this concept. For instance, Dulhunty *et al.*⁶⁸ compared plasma antibiotic concentrations in 60 patients treated either intermittently or continuously with piperacillin-tazobactam, meropenem, or ticarcillin-clavulanate in 5 ICUs. A predefined target of plasma concentration above the MIC (based on

breakpoints for *Pseudomonas aeruginosa*) on day 3 and 4 was met in 82 % of the continuously treated versus 29 % of the intermittently treated group. In another Australasian multicenter study comparing continuous versus intermittent dosing of beta-lactams (“Beta-lactam Infusion in Severe Sepsis – BLISS”) including 140 patients, a predefined PK/PD target attainment of 100% $fT_{\geq MIC}$ was met in 97 % of the continuous dosing versus 68 % of the intermittent dosing arm patients on day 3 of treatment⁶⁹. Both studies also report better clinical cure in the continuous dosing group^{68,69}. Better clinical outcome using prolonged or continuous infusion in the critically ill is suggested in several recent meta-analyses, as well⁷⁰⁻⁷³.

A large multicentre randomised controlled trial conducted by The Beta-Lactam Infusion Group (BLING) powered on mortality, comparing continuous with intermittent dosing of beta-lactams is currently recruiting patients⁷⁴. Although continuous dosing is suggested to have benefits in reviews⁷⁵ and is recommended in the guidelines published by the French Society of Anaesthesia and Intensive Care Medicine⁵⁵, it is not widely employed throughout ICUs as yet⁷⁶. Although many pharmacokinetic studies on beta-lactams have been published, no study involving a heterogeneous ICU population treated with continuous dosed piperacillin using dense sampling was known to us. Further, data on pharmacokinetics of cefotaxime in ICU patients, especially using continuous dosing, is scarce.

Consequently, **objective II** was to evaluate plasma concentrations of the beta-lactam antibiotics piperacillin and cefotaxime in critical care patients. We therefore described plasma concentrations of continuous dosed piperacillin in ICU patients, thereby assessing pharmacokinetic target attainment (**Chapter 3**). Also, we evaluated target attainment of either continuous or intermittent dosed cefotaxime in randomised critical care patients, treated in the context of SDD, throughout the treatment period (**Chapter 4**).

DYNAMICS OF INTESTINAL MICROBIOTA IN CRITICAL CARE PATIENTS; WHAT HAPPENS AND HOW CAN THIS BE RELEVANT?

The intestinal microbiota, or the microbes that collectively inhabit an ecosystem (such as the gut)⁷⁷, has been recognised as a true organ in recent years, playing a pivotal role in various important functions in the human body, including immune maturation and homeostasis, host cell proliferation, protection against pathogen overgrowth, regulation of intestine endocrine functions, biosynthesis of vitamins and neurotransmitters, and metabolisation of bile salts as well as branched-chain and aromatic amino acids⁷⁷⁻⁷⁹. The advent of culture-independent molecular techniques has allowed for in-depth analysis of the intestinal microbiota^{77,80}. The composition of intestinal microbiota is influenced in a complex way by numerous factors including genetics, disease states, diet, medication use and other environmental exposures^{77,81,82}. Although large interindividual variations exist, the composition in one individual is relatively stable over time^{81,83}. In the ‘normal’ gut microbiota, the phyla Firmicutes and Bacteroidetes account for over 90% of

the bacterial population⁸⁴. Among the Bacteroidetes phylum, the *Bacteroides* genus is one of the most prominent. These bacteria are known to digest complex polysaccharides into short-chain fatty acids (SCFAs), such as butyrate and propionate. SCFAs appear to have a role in the regulation of intestinal cell growth, and regulation of immune responses, amongst others⁸⁴. Some members within the *Bacteroides* genus, such as *Bacteroides fragilis*, can play a beneficial role in immune homeostasis within the intestinal lumen, but can also become pathogenic when they disseminate if intestinal perforation ensues⁸⁴. Within the Firmicutes phylum, several members of the *Clostridia* class exert beneficial roles in maintaining intestinal epithelial integrity and immune homeostasis. Other clusters of this class, however, include relevant pathogens such as *Clostridium perfringens* and *Clostridium difficile*. Further, the *Bacilli* class within the Firmicutes phylum contains pathobionts such as *Enterococcus* and *Streptococcus* species. Under normal conditions, these potential pathogens comprise only a small part of the intestinal microbiota, i.e. they exist in low abundance⁸⁴. A higher diversity of species (e.g. higher species richness) is generally associated with a healthy state of the gut^{81,85,86}. Although generally stable, the gut microbiota can be profoundly altered within days under exogenous influences such as diet, travel or antimicrobial consumption^{87,88}. For instance, treatment of healthy volunteers with five days of oral ciprofloxacin influenced the abundance of about a third of the bacterial intestinal taxa, with a decrease in taxonomic richness, diversity and evenness of the community. Recovery of the intestinal microbiota composition to the pre-treatment state took four weeks to up to six months⁸⁸. The perturbation of intestinal microbiota through antibiotic treatment causes loss of colonisation resistance, which implies that the ‘normal’ gut microbiota protects from invasion of pathogenic bacteria. This loss is clinically relevant, as it leads to a higher susceptibility to infections⁸⁹. Differences in intestinal microbiota composition, with loss of bacterial richness, have been linked with disease states such as obesity, inflammatory bowel disease and irritable bowel disease^{77,81,85}. During critical illness and subsequent admission to the ICU, the normal structure and function of the intestinal microbiota can become disturbed, leading to a state of ‘dysbiosis’ which in a complex way can be detrimental to the host^{79,90}. Many factors inherent to treatment in an intensive care environment contribute to this disruption of the intestinal microbiota. Antibiotic use is the most obvious intervention responsible for ablation of commensal microbiota (indigenous microbes considered beneficial to the host)⁷⁹. Other medications, such as gastric acid suppressants, sedatives, opiates, neuromuscular blockers and systemic catecholamines, as well as change in diet through enteral or parenteral feeding can also negatively influence the gut microbiota⁸⁰. Invasive procedures such as the placement of central lines and endotracheal intubations can disrupt natural barriers enabling microbial entry and proliferation⁷⁹. Also, the ICU environment itself can harbour microbes that can colonise patients through contamination⁷⁹. Moreover, various pathophysiological processes, including decreased oral intake, intestinal dysmotility, gut hypoperfusion and loss of gut integrity, endogenous catecholamine and inflammatory cytokine production, can disrupt the ‘normal’ intestinal microbiota⁸⁰. All these factors can give

way to the expansion of pathogens such as enterococci and invasion of *Enterobacteriaceae*⁹¹⁻⁹³. Although the role of intestinal microbiota in health and disease and, indeed, in critical illness, is increasingly recognised^{77,80}, relatively few studies on the dynamics of gut microbiota in critical care patients using culture-independent techniques have been published⁹⁴⁻¹⁰⁰. These studies generally involve acute care patients admitted with organ failure due to sepsis, trauma, or emergency surgery. Most of these studies⁹⁵⁻¹⁰⁰ utilised a case-control design and compared gut microbiota of ICU patients and healthy controls. All studies, smaller-scale pilots^{94,95,97,99} as well as larger studies^{96,98,100}, describe a similar pattern, with a rapid loss of diversity, including depletion of microorganisms considered beneficial for health, such as the butyrate-producing *Faecalibacterium*, and an increase in relative abundance of potential pathogens such as members of the *Enterobacteriaceae* family. The consequences of these perturbations in critical care patients, along with the interplay between gut epithelium, intestinal microbiota and immune system, are highly complex and only partially understood^{80,90,101}. Possibly disruption of the microbiota can directly contribute to a state of immunosuppression¹⁰². Also, changes in the gut microbiota can give way to a disturbed gut barrier and cellular apoptosis, which can lead to bacterial translocation and multiple organ dysfunction^{90,103}. Experimental models indicate a protective effect of short-chain fatty acids against the emergence of acute kidney failure after injury¹⁰⁴. Importantly, the virulence of a microorganism lies not only in the organism itself, but also in its surroundings; for example, *Pseudomonas aeruginosa* injected in the caecum of mice undergoing a sham operation and subsequently removed and then injected into the peritoneum of control mice does not lead to mortality, while *P. aeruginosa* injected into the caecum of mice undergoing non-fatal partial hepatectomy and subsequently removed and injected into the peritoneum of control mice leads to 100% mortality¹⁰⁵. The exact extent and role of dysbiosis in critical illness remain to be elucidated but hold promising insights in potential diagnostic and therapeutic possibilities in the prevention or cure of infections^{79,91}.

Ergo, **objective III** was to understand dynamics of intestinal microbiota in a critical care cohort admitted after planned major surgery. Thus, we described the dynamics of intestinal microbiota in planned cardio-surgical patients admitted to the ICU after surgery. We obtained fecal samples before, during and after admission, which meant that the patients were their own controls (**Chapter 5**).

A general discussion of results, followed by implications for further studies and a summary are outlined in **Chapter 6** and **7**, respectively.

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CHAPTER 2

BURDEN OF HIGHLY RESISTANT MICROORGANISMS IN A DUTCH INTENSIVE CARE UNIT

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ABSTRACT

BACKGROUND

The occurrence of highly resistant microorganisms (HRMOs) is a major threat to critical care patients, leading to worse outcomes, need for isolation measures, and demand for second-line or rescue antibiotics.

The aim of this study was to quantify the burden of HRMOs in an intensive care unit (ICU) for adult patients in a university hospital in the Netherlands. We evaluated local distribution of different HRMO categories and proportion of ICU-imported versus ICU-acquired HRMOs. Outcome of HRMO-positive patients versus controls was compared.

METHODS

In this prospective single centre study, culture results of all ICU patients during a four-month period were recorded, as well as APACHE scores, ICU mortality and length of stay (LOS) in the ICU.

RESULTS

58 of 962 (6.0 %) patients were HRMO positive during ICU stay. The majority (60 %) of those patients were HRMO positive on ICU admission. HRMO-positive patients had significantly higher APACHE scores, longer LOS and higher mortality compared with controls.

CONCLUSIONS

Our study suggests that a large part of antibiotic resistance in the ICU is imported. This underscores the importance of a robust surveillance and infection control program throughout the hospital, and implies that better recognition of those at risk for HRMO carriage before ICU admission may be worthwhile. Only a small minority of patients with HRMO at admission did not have any known risk factors for HRMO.

INTRODUCTION

Antibiotic resistance in the critical care population is an ever-increasing problem¹. The high use of antimicrobial therapy in the intensive care unit (ICU)², the large number of invasive procedures, the density of a susceptible patient population, the severity of underlying illness, and flaws in infection control measures are all contributing factors resulting in ICUs as ‘epicentres’ of antimicrobial resistance in hospitals^{3,4}. ICUs are considered generators of antimicrobial resistance³. In addition to acquisition of HRMOs in the ICU, part of the resistance problem is imported to the ICU through already colonised or infected patients admitted from other hospitals, general wards, or from the community⁵.

This continuous and rising threat of antimicrobial resistance is of relevance considering the outcome in patients infected with resistant rather than susceptible microorganisms is worse⁶. Measures to prevent cross-contamination include surveillance, barrier precautions and antibiotic stewardship. All preventive measures are labour intensive, costly and some are patient unfriendly. Resistance to first-line antibiotics urges the use of ‘rescue’ or second-line antibiotics with little hope of new effective alternatives in the near future⁷.

The incidence and characteristics of resistance can vary widely depending on local circumstances. According to European surveillance data, the Netherlands, along with Scandinavian countries, is considered an area with low incidence of antimicrobial resistance for Gram-positive as well as Gram-negative bacteria⁸. However, even in the Netherlands, prevalence of antimicrobial resistance in the community is not negligible, and is emerging^{9–11}.

We evaluated the incidence of HRMOs in our ICU to quantify the total burden of HRMOs, to clarify the local distribution of different categories of HRMOs and the proportion of imported versus acquired HRMOs in our ICU. Furthermore, we evaluated the outcome of patients affected by colonisation or infection with any HRMO vs. controls in terms of ICU mortality and length of stay (LOS) in the ICU. Finally, we wanted to characterise this subpopulation colonised or infected with an HRMO to enable better *a priori* recognition of affected patients, thus rendering better opportunities for adequate treatment and infection control.

MATERIALS AND METHODS

This is a single-centre study involving prospective data collection from 1 October 2009 to 31 January 2010 in an academic teaching hospital with 40 critical care beds distributed over 4 units (medical, cardiothoracic, neurological, and general surgery). All four units consist of a large multi-

bed floor combined with a few rooms for isolation. On the floors, standard hygienic procedures are maintained. Annually, between 2500 and 3000 patients are admitted. All patients admitted in the four-month study period were included for analysis. The study was approved by the ethics committee and the requirement of informed consent was waived. We recorded baseline characteristics including sex, age, referring speciality, unit of admission, APACHE II score, date and source of admission (emergency department or general ward vs. other hospital). APACHE II score was not recorded for cardiothoracic patients because this score is not validated for this subgroup. Of note, patients from various sources were admitted to the four separate units, e.g. the cardiothoracic unit did not only admit cardiothoracic surgery patients but other patients as well. Patients were considered referred from another hospital if they were transferred either directly to the ICU or indirectly through another ward in our hospital. The vast majority of this last group were admitted to the general ward for less than a week before admission to our ICU. All cultures taken either by indication or in the context of our structured surveillance program were evaluated. Surveillance screening included cultures from throat, nose, rectum, sputum and urine on admission, followed by cultures from throat, nose, rectum and sputum on day four and twice weekly thereafter during the stay in the ICU. Surveillance cultures were obtained from those patients with an anticipated stay of 48 hours or more on the day of admission. Patients referred from elsewhere were included in surveillance screening on the day of admission regardless of anticipated or actual length of stay.

Culture results were retrieved from the database of the department of medical microbiology. Susceptibility testing was done according to European guidelines (European Committee on Antimicrobial Susceptibility Testing, EUCAST).

HRMOs were defined by criteria issued by the Dutch Working Party on Infection Prevention¹² (table 1). All patients colonised or infected with an HRMO were placed in full contact isolation, as dictated by our protocol for infection prevention. A patient could be included only once in the study group; subsequent readmissions of the same patient were excluded from the study group but were analysed nevertheless. Only the first positive culture for any HRMO in an individual was recorded; subsequent cultures with the same organism were regarded as the same event. Different species of HRMOs within one patient were recorded as separate events. No distinction was made between either colonisation or infection with an HRMO. Of patients with an HRMO, further details such as antibiotic use during the hospital stay and medical history were retrieved from the patient's file. Other outcome measures for the entire study population included ICU mortality and LOS on the ICU.

We tried to identify clusters of HRMOs by analysing whether identical species of HRMOs were cultured in different patients during their ICU stay on the same unit.

Statistical methods included the χ^2 -test, Fisher's exact test, Student's t-test, Mann-Whitney U-test and Wilcoxon rank test, where appropriate, using Minitab ® Release 14.1 and Graph Pad Prism (Prism 5 for Windows, version 5.04, nov 6 2010) software.

Table 1. Definition of highly resistant microorganisms (HRMOs)¹²

	ESBL	Quinolones	Amino-glycosides	Carbapenems	Co-trimoxazole	Ceftazidime	Piperacillin	Penicillins	Glycopeptides	Oxacillin	Methicillin
Enterobacteriaceae											
<i>E.coli</i>	A	B	B	A	--	--	--	--	--	--	--
<i>Klebsiella</i> spp	A	B	B	A	--	--	--	--	--	--	--
Other	A	B	B	A	B	--	--	--	--	--	--
Non-fermenting gram-negative											
<i>Acinetobacter</i> spp.	--	B	B	A	--	B	--	--	--	--	--
<i>Stenotrophomonas</i> spp.				A		--	--	--	--	--	--
Other		C	C	C	C	C	C	--	--	--	--
Gram-positive											
<i>S. pneumoniae</i>	--	--	--	--	--	--	--	A	A	--	--
<i>Enterococcus</i> spp.	--	--	--	--	--	--	--	B	B	--	--
<i>S. aureus</i>	--	--	--	--	--	--	--	--	A	A	A

Resistance to one antibacterial agent in category A, to $\geq$ two in category B, or $\geq$ three in category C required to define microorganism as highly resistant microorganism (HRMO). ESBL = Extended beta-lactamase (resistance to any third-generation cephalosporin used as proxy in *E.coli*, *Klebsiella* spp., and *Proteus* spp.).

RESULTS

A total of 1061 admissions were recorded, 91 of which were re-admissions within the study period; hence 962 admitted patients were included in the study population (table 2). Baseline characteristics are presented in table 2 and 3. In 58 (6.0 %) patients an HRMO was found (in total 60 HRMOs; two patients had two different HRMOs). For distribution of HRMO species we refer to table 4. The distribution of these 58 patients according to unit and patient category is depicted in table 2.

Table 2. Patient characteristics

	Total	Without HRMO	With HRMO	P
Study population, n	962	904	58	
Male n (%)	595 (61.9 %)	556 (61.5 %)	39 (67.2 %)	0.38 (NS)
Age, years, median (range)	62 (12-91)	63 (12-91)	58 (16-82)	0.22 (NS)
APACHE II score, median (range)**(n)	13 (2 – 52) (532)	13 (2 – 44) (488)	18 (2 – 52) (44)	< 0.001
APACHE II > 20 (n =101)		83 (17.0 % of 488)	18 (40.9 % of 44)	< 0.001
APACHE II ≤ 20 (n = 431)		405	26	
Unit, n (%)				
Cardiopulmonary unit	390 (40.5 %)	381 (42.1 %)	9 (15.5 %)	< 0.001
Surgical unit	238 (24.7 %)	211 (23.3 %)	27 (46.6 %)	
Medical unit	181 (18.8 %)	168 (18.6 %)	13 (22.4 %)	
Neurosurgical unit	153 (15.9 %)	144 (15.9 %)	9 (15.5 %)	
Patient category, n (% of total)				
Cardiopulmonary surgery	405 (42.1 %)	392 (43.4 %)	13 (22.4 %)	
Medical	208 (21.6 %)	186 (20.6 %)	22 (37.9 %)	
Surgical	181 (18.8 %)	167 (18.5 %)	14 (24.1 %)	
Neurosurgical	103 (10.7 %)	101 (11.2 %)	2 (3.4 %)	
Trauma	44 (4.6 %)	38 (4.2 %)	6 (10.3 %)	
Neurological	17 (1.8 %)	16 (1.8 %)	1 (1.7 %)	
Gynaecological	4 (0.4 %)	4 (0.4 %)	0 (0 %)	

**APACHE II-score available for 532 (95.5 %) of non- cardiosurgical patients; HRMO = highly resistant microorganism.

Of 232 patients (24.1 %) referred from another hospital, 16 patients were colonised with an HRMO during their stay in our ICU (6.9 %), compared with 42 out of 730 patients (5.8 %) referred from our hospital (p= 0.52).

In those patients with any HRMO (n = 58), 47 patients (82.8 %) were found to have an HRMO within the first three days of ICU stay. Of these, 11 (23.4 %) were referred from another hospital; 36 (76.6 %) were admitted from a general ward of this hospital or from the emergency department. Of those not referred from elsewhere and found positive for an HRMO within three days (n = 36),

32 patients (88.9 %) had one or more comorbid conditions (table 4). In this group of 36 patients, 27 patients (75.0 %) had been admitted in the three months preceding current admission to the ICU; 12 patients (33.3 %) had received antibiotics in the months preceding ICU admission. Two patients (of 36, 5.6 %) were farmers working with livestock (pigs); both were found to be methicillin-resistant *Staphylococcus aureus* (MRSA)-positive. Two (of 36, 5.6 %) had no comorbid conditions, no recent hospital admission, no recent antibiotic treatment and no occupational exposure to HRMOs.

Median LOS for all ICU patients was 1 day (range 1 -130 days, mean 4 days). LOS in the ICU for HRMO-positive patients was significantly longer (median 5 days, range 1-130, mean 16 days) compared to HRMO-negative patients (median 1 day, range 1 -88, mean 3 days) (p = 0.000) (table 3).

Table 3. Patient characteristics and outcome

	Total	Without HRMO	With HRMO	P
Study population, n (%)	962	904 (94.0 %)	58 (6.0 %)	
Referral from other hospital, patients, n (%)	232 (of 962; 24.1 %)	216 (of 904; 23.9 %)	16 (of 58; 27.6 %)	0.52 (NS)
Admitted through general ward or emergency department, n (%)	730 (of 962; 75.9 %)	688 (of 904; 76.1 %)	42 (of 58; 72.4 %)	0.84 (NS)
Positive bloodcultures, patients,n ^{††}	28	25	3 (HRMO <i>E.coli</i> 2, MRSA 1)	
LOS ICU, days, median (range)	1; 4 (1-130)	1; 3 (1 - 88)	5; 16 (1 – 130)	< 0.001
ICU mortality, patients, n (%)	74 (7.7 %)	63 (7.0 %)	11 (19.0 %)	0.0031

^{††} Blood cultures with common skin contaminants (e.g. Coagulase-negative Staphylococci, viridans group Streptococci) had to be cultured on two or more separate occasions to be included (n = 23 cultures with positive culture with skin contaminant on one occasion excluded); HRMO = highly resistant microorganism; LOS = length of stay.

LOS in the ICU at the time of first positive culture for any HRMO was 1 day in 36 (60 %), 2-7 days in 12 (20 %), 8-14 days in 4 (7 %) and more than 14 days in 8 (13 %) (table 4). Patient categories with most HRMO-positive patients were medical (22 out of 208, 10.6 %), surgical (14 out of 181, 7.7 %) and trauma (6 out of 42, 13.6 %). Units with most HRMO-positive patients were the surgical unit (27 out of 218, 11.3 %) and the medical unit (13 out of 181, 7.2 %).

Of patients admitted to our ICU for more than 14 days, 18 of 57 (31.6 %) were found to have any HRMO during ICU stay vs. 31 of 840 (3.7 %) patients staying 7 days or less in our ICU (p < 0.0001). APACHE II score for HRMO-positive patients (available in 57 patients) was significantly higher (median 17, mean 19, range 2-52) compared with the APACHE II score for HRMO-negative patients (available in 875 patients) (median 13, mean 13, range, 2-44) (p = 0.000).

Overall ICU mortality was 74 (7.7 %); mortality was significantly higher in patients with HRMO

(11 out of 58, 19.0 %) than patients without HRMO (63 out of 904, 7.0 %) ($P = 0.0031$) (table 3). Further, 25 patients had a positive blood culture with a susceptible microorganism and three other patients had a positive blood culture with an HRMO (*E.coli* 2, MRSA 1).

Table 4. HRMO species and patient characteristics with HRMO

HRMO, patients, n (% of total patients)	58 (6.0 %)
HRMO, total, n*	60
Enterobacteriaceae	50
<i>E.coli</i>	40
<i>Klebsiella</i> spp.	2
Other†	8
Non-fermenting gram-negatives	5
<i>Pseudomonas</i> spp.	4
Other‡	1
Gram-positives	5
VRE	3
MRSA	2
LOS ICU on first day of positive HRMO culture	
Days, median (range)	1 (1-77)
1 day, n (% of 60)	36 (60 %)
2- 7 days, n (% of 60)	12 (20 %)
8 – 14 days, n (% of 60)	4 (6.7 %)
> 14 days, n (% of 60)	8 (13.3 %)
HRMO patients not referred from elsewhere and HRMO within three days (% of 58)	36 (62.1 %)
Admitted in preceding 3 months (n, % of 36)	27 (75.0 %)
Recent antibiotic-exposure (n, % of 36)	12 (33.0 %)
Co-morbid conditions (n, % of 36)	32 (88.9 %)
Cardiovascular (n, % of 36)	11 (30.6 %)
Malignancy (n, % of 36)	10 (27.8 %)
Organ transplantation (n, % of 36)	7 (19.4 %)
Pulmonary (n, % of 36)	6 (16.7 %)
Diabetes (n, % of 36)	4 (11.1 %)
Chronic hepatitis (HCV, HBV**) (n, % of 36)	2 (5.6 %)
Occupational exposure (pig farmer) (n, % of 36)	2 (5.6 %)
No known risk factor for HRMO (n, % of 36)	2 (5.6 %)

*2 patients had 2 HRMOs. †*E. cloacae* 4; *Citrobacter* spp. 3; *S. marcescens* 1. ‡*S. paucimobilis* 1; HRMO = highly resistant microorganism; LOS = length of stay; **HBV = Hepatitis B virus; HCV = Hepatitis C virus.

In the readmitted (excluded) patient group ($n = 99$), 16 patients (16.2 %) had any HRMO (*E.coli* 6, *Klebsiella* spp. 3, other *Enterobacteriaceae* 3, *Pseudomonas* spp. 1, other non-fermenting gram-negatives 1, vancomycin-resistant Enterococci 3). This percentage of HRMO-positive patients is significantly higher compared with the percentage of HRMO-positive patients in the study group (6.0 %, $P = 0.0002$).

In this study, we could not identify patient characteristics with sufficient specificity and sensitivity

to predict HRMO carriage. During the study period, we did not find clusters of identical HRMOs indicating an outbreak.

DISCUSSION

In this single-centre prospective study on the burden of HRMOs in critical care patients in an area where HRMOs are non-endemic^{8,13}, it is an important finding that more than half of HRMO-positive patients were identified from cultures taken on admission. This finding suggests that an important part of antibacterial resistance is imported to the ICU, rather than acquired during the ICU stay. Indeed, hospitalisation on a general ward prior to ICU admission is a recognised risk factor for HRMO acquisition¹⁴ and although the proportion of HRMOs introduced onto the ICU through already colonised or infected patients has been quantified in studies for MRSA¹⁵, its contribution for all HRMOs has, to the best of our knowledge, not been clearly elucidated as yet in our region. Of all patients admitted through the emergency department or general ward, 36 (out of total 932, 3.7 %) had an HRMO within three days of ICU stay. Two (of 36, 5.6 %) of these patients had no comorbid conditions, no recent hospital admission, no recent antibiotic treatment and no occupational exposure to HRMOs. Although a minority, this underscores the fact that HRMO is not restricted to the hospital, even in our area of low antibiotic resistance. Indeed, prevalence of antimicrobial resistance in the community, for instance carriage of extended-spectrum beta-lactamases-producing *Enterobacteriaceae*, is considerable, where contribution of contaminated foods - mainly poultry - and travel, remains to be elucidated⁹⁻¹¹.

Only three patients had a proven infection (bacteraemia) with any HRMO, therefore colonisation appears to be far more frequent than a serious infection. That HRMO-positive patients have a higher mortality might in part be explained by the fact that sicker patients are more often colonised with any HRMO. Indeed, in our study population those with an HRMO had a significantly higher APACHE-II score than those without HRMO (table 2).

In our cohort, Gram-negative bacteria comprised the largest part of all HRMOs. This is in line with the trend towards more Gram-negative antibiotic resistance in European ICUs, with a stabilisation or decrease in Gram-positive antibiotic resistance^{1,16}. Recently, others described cephalosporin and aminoglycoside resistance in a substantial number of critically ill patients colonised with *Enterobacteriaceae* (15 and 10 %, respectively) on ICU admission in a large Dutch multi-centre trial¹⁷.

We could not identify clusters of HRMO. Therefore, the hygiene measures set forth to contain the spread of HRMOs appeared sufficient in this study period.

Our finding that a substantial part of HRMOs are imported into the ICU underscores the imperative need to ensure strict application of hygienic practices, such as hand washing, as well as excellent use of antibiotic stewardship throughout medical care, inside and outside the hospital. Furthermore, this finding highlights the importance of a conscientious surveillance program on the ICU. Along this line, surveillance screening *before* possible ICU admission in specific populations on medical and surgical wards, or in patients with a high risk of community-acquired HRMO could be worthwhile in order to prevent cross-contamination on these wards and on the ICU. Indeed, in the Netherlands, a surveillance program comparable to our practice on the ICU is carried out on hemato-oncology and dialysis wards as well. Likewise, it is common practice to screen those with occupational exposure to livestock known to have a high carriage rate of MRSA prior to or at hospital admission¹⁸. Expanding surveillance to other high-risk populations, for instance those admitted to a surgical ward for an extended period, especially when receiving antibiotics, might be beneficial.

Some variables have been recognised as risk factors for carriage of HRMOs upon ICU admission, such as prior antimicrobial treatment, prior hospitalisation, and residence in a nursing home⁵. However, due to sample size, we could not extract patient characteristics from our study population to predict HRMO carriage. It might be helpful to further characterise those patients at risk for HRMO carriage in the context of a larger epidemiological study. This could lead to more differentiated isolation procedures and a more sophisticated choice of antibiotics in case of a proven or suspected infection. Further studies, however, do necessitate a uniform definition of 'highly resistant' enabling meaningful comparisons and data aggregation; currently, a wealth of definitions are being applied in the literature¹⁹.

There are some limitations to our study, the most important being that it is a single-centre study with a relatively small sample size. It is important to note that patients with an intended stay of two days or less are not included in the surveillance program, unless transferred from another hospital; positive cultures could thus have been missed in these patients. In this group of patients with a short LOS in the ICU, these potential false-negatives would contribute to our finding that a substantial part of HRMO-positive patients are found to be as such in the first days of admission. As our surveillance program is robustly implemented in our daily practice, it is unlikely that patients were missed out of this program for procedural reasons. Some patients underwent surveillance screening despite a short stay of 48 hours or less on the ICU. We did not differentiate between colonisation and infection with any HRMO. Causality between occurrence of an infection with an HRMO and worse outcome in the HRMO group can thus not be proven in this observational study. We did not analyse all known risk factors for HRMO carriage (referral from a nursing home was not recorded); due to sample size, subgroup analyses were not feasible. It could be of benefit to further characterise those at risk of harbouring infection or colonisation

with an HRMO, using a uniform international definition of HRMO, with expanding surveillance in high-risk groups outside the ICU, in order to enable maximum precautionary measures and give optimal antibiotic treatment. This combination of surveillance and timely isolation can prevent further spread of HRMOs, our biggest challenge in infection control in critically ill patients in the years ahead.

CONCLUSION

This observational study suggests that HRMOs on the ICU are quite often imported and not only acquired during the stay in the ICU. Gram-negative HRMOs were more abundant than Gram-positive and are of clinical significance even in a non-endemic area. Although most patients with any HRMO had comorbid conditions, were recently admitted to a hospital, had received antibiotics prior to ICU admission, or had occupational exposure to an HRMO, a small minority had no relevant history. Our findings underscore the importance of infection control and optimal surveillance on admission to the ICU.

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CHAPTER 3

TARGET ATTAINMENT WITH CONTINUOUS DOSING OF PIPERACILLIN/ TAZOBACTAM IN CRITICAL ILLNESS: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Optimal dosing of β -lactam antibiotics in critically ill patients is a challenge given the unpredictable pharmacokinetic profile of this population. Several studies have shown intermittent dosing to often yield inadequate drug concentrations. Continuous dosing is an attractive alternative from a pharmacodynamic point of view.

This study evaluated whether, during continuous dosing, piperacillin concentrations reached and maintained a pre-defined target in critically ill patients.

Adult patients treated with piperacillin by continuous dosing in the intensive care of a university medical centre in the Netherlands were prospectively studied. Total and unbound piperacillin concentrations drawn at fixed time points throughout the entire treatment course were determined by liquid chromatography-tandem mass spectrometry. A pharmacokinetic combined target of a piperacillin concentration ≥ 80 mg/L, reached within 1 h of starting study treatment and maintained throughout the treatment course, was set.

Eighteen patients were analysed. The median duration of monitored piperacillin treatment was 60 h (interquartile range, 33-96 h). Of the 18 patients, 5 (27.8 %) reached the combined target; 15 (83.3 %) reached and maintained a less strict target of > 16 mg/L.

In this patient cohort, this dosing schedule was insufficient to reach the pre-defined target. Depending on which target is to be met, a larger initial cumulative dose is desirable, combined with therapeutic drug monitoring.

INTRODUCTION

Infections, both community-acquired and nosocomial, are a constant source of morbidity and mortality in critically ill patients¹. β -Lactams, with or without a β -lactamase inhibitor, are the most prescribed group of antibiotics in this setting^{2,3}. Guidelines for the management of severe sepsis and septic shock advocate the initiation of antibiotics as soon as possible, using broad-spectrum antibiotics that penetrate in adequate concentrations at the presumed site of infection, ensuring optimal activity against all likely pathogens⁴. Choosing appropriate therapy is crucial, as inadequate antimicrobial treatment is an important determinant of poor outcome⁵. Optimal dosing is equally important because inadequate dosing leads to treatment failure and antibiotic resistance⁶.

Piperacillin/tazobactam (TZP) is a widely used β -lactam/ β -lactamase inhibitor combination. The effectiveness of piperacillin is determined by the time the unbound plasma concentration (fT) is higher than the minimum inhibitory concentration (MIC) of the causative bacteria ($fT_{>MIC}$)⁷. A maximum kill rate is achieved at a free drug concentration of ca. $4 \times \text{MIC}$ ⁸, with no additional effect above this concentration. There is no relevant post-antibiotic effect against Gram-negative microorganisms⁹. Dosing regimens have traditionally been based upon pharmacokinetics as tested *in vitro*, in animal models and in healthy volunteers^{6,10-12}. However, in critical illness, several complex mechanisms induce an altered pharmacokinetic profile owing to, for example, an increase in volume of distribution and an alteration in renal clearance¹¹. Numerous studies have shown inadequate drug concentrations in critically ill patients treated with β -lactams using conventional dosing regimens¹³⁻²⁰. In particular, augmented renal clearance, as might occur during the hyperdynamic stage of sepsis, appears to be a risk factor for failing to reach adequate β -lactam drug levels¹⁵⁻¹⁹.

From a pharmacodynamic point of view, continuous infusion is an attractive alternative to conventional intermittent dosing of β -lactams. This is also supported by clinical studies²¹⁻²⁵. The critical care population is likely to gain the most benefit from continuous dosing as this group tends to harbour pathogens with higher MICs²⁶ and to have an unpredictable PK profile¹¹. Although high-quality randomised trials showing a survival benefit are still lacking, in a recent meta-analysis of individual patient data from three randomised trials, treatment with β -lactam antibiotics by continuous infusion was associated with lower mortality compared with intermittent dosing in critically ill patients with severe sepsis²⁴. Continuous dosing of TZP however, is not yet widely employed in European intensive care units²⁷ (ICUs)²⁷.

This prospective study was conducted to evaluate whether, during continuous dosing, piperacillin concentrations reach and maintain a high target concentration in critically ill patients, likely to cover most problematic pathogens such as *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

STUDY DESIGN AND STUDY POPULATION

This prospective, observational, single-centre, cohort study was conducted in the Department of Critical Care of University Medical Center Groningen (UMCG) (Groningen, the Netherlands) between December 2013 and January 2015. The study was approved by the Medical Ethics Board of this hospital. Written informed consent was obtained from the patient or their next of kin. Patients were eligible for inclusion at the start of treatment with TZP for suspected or proven infection. Start of treatment was at the discretion of the treating physician. Inclusion criteria were: indication for treatment with TZP; admitted to the ICU; age ≥ 18 years; and able to give informed consent or legal representative able to give informed consent. All patients had an indwelling arterial line for reasons outside the study protocol. Exclusion criteria were: pregnancy; severe anaemia; use of renal replacement therapy; and contra-indications to continuous infusion. Patients already started on TZP by intermittent dosing (e.g. on the ward, before ICU admission) were included if no more than five doses had been given; continuous dosing was started directly after a next bolus.

All patients, regardless of kidney function, received a loading dose of 4g/0.5g TZP (Piperacillin/Tazobactam Fresenius Kabi 4g/0.5g powder for solution for infusion; LABESFAL Fresenius Kabi Group, Santiago de Besteiros, Portugal) infused over 20 min. Continuous dosing was started directly after the loading dose in all patients using a syringe pump (Alaris® GH-perfusor; CareFusion, Rolle, Switzerland). The first hour of starting treatment, including infusion of the loading dose over 20 min directly followed by continuous infusion, was considered the loading phase. The next phase, from 1 h after the start of treatment, was referred to as the maintenance phase. The sample drawn at 1 h after the start of treatment was considered as part of the maintenance phase. The dosing schedule for continuous infusion in the maintenance phase was adjusted to renal function [assessed by calculation of creatinine clearance (CL_{Cr}) over 24-h intervals using the equation: $\text{Urine creatinine (mmol/L)} \times \text{Urine volume (mL)} / \text{time (min)} \times \text{Serum creatinine (mmol/L)} / (\text{UCreat} \times \text{UVol} / \text{time} \times \text{SCreat})$; or, when parameters were not available, estimated using the Modification of Diet in Renal Disease (MDRD) formula for estimated glomerular filtration rate]. Renal function was recorded on the day of starting treatment with TZP in the context of the study.

Patients with a $CL_{Cr} > 40$ mL/min received a continuous infusion of 12/1.5g TZP every 24 h. Patients with a CL_{Cr} of 20-40 mL/min received a continuous dose of 8/1 g on Day 1 and of 12/ 1.5 g from Day 2 onwards. Patients with a $CL_{Cr} < 20$ mL/min received a continuous dose of 8/1 g from Day 1. Blood samples were drawn at the start of treatment in the context of the study on Day 1 and then at 20 min after the start of treatment (directly after the loading dose); subsequent samples were drawn at 40 min and at 1, 2, 4, 8, 12 and 24 h after the start of treatment; from

Day 2, samples were drawn every 12 h for a maximum period of 2 weeks or until treatment with TZP was stopped. Samples were centrifuged and frozen at -20°C , to be processed in batch by the Department of Clinical Pharmacy and Pharmacology of UMCG. Patient characteristics included demographic and clinical data, assessment of illness severity reflected by the Acute and Physiology and Chronic Health Evaluation (APACHE) IV Score, and laboratory investigations.

DEFINITION OF PK/PD TARGET

A 'strict' target was chosen based on the notion that for β -lactams, a maximum kill rate is achieved at a free (unbound) drug concentration of ca. $4 \times \text{MIC}$ of a causative organism, with no additional effect above this concentration^{8,28} and the absence of a relevant post-antibiotic effect against Gram-negative organisms⁹. *P.aeruginosa* was chosen as a possible causative microorganism in consideration of a 'worst case scenario', with a MIC clinical breakpoint of 16 mg/L (http://www.eucast.org/clinical_breakpoints; accessed 21 May 2016), to cover most problematic pathogens²⁹ in an empirical treatment setting.

The pre-defined PK/PD target was thus set at $100\% T_{\geq 5 \times \text{MIC}}$ (percentage of time of dosing interval during which the total concentration exceeds $5 \times \text{MIC}$), assuming 20-30 % protein binding^{30,31}; implying a target of $4 \times 16 = 64$ mg/L for unbound and $5 \times 16 = 80$ mg/L for total piperacillin concentration. This target is in line with targets set by other research groups considered experts in the field^{14,32} as well as reviews addressing the pharmacokinetics of beta-lactams^{8,33,34}. This target was to be met from 1 h after the start of treatment in the context of the study, i.e. during the maintenance phase; 1 h after start of the last bolus infusion directly followed by continuous infusion, and to be maintained thereafter; we will refer to this as a combined target (target reached within 1 h and maintained thereafter). Reaching a target of > 16 mg/L of piperacillin in the maintenance phase, i.e. at 1 h after the start of treatment and maintained thereafter, $100\% T_{> 1 \times \text{MIC}}$ was also determined. For unbound concentrations, a target of $\geq 4 \times \text{MIC}$ (64 mg/L) was set. Target attainment was evaluated at sample level as well as in individual patients. Whether the target concentration was reached at 1 h after start of treatment in the context of the study was also assessed.

BIOANALYSIS OF PIPERACILLIN SERUM CONCENTRATIONS

Total serum concentrations of piperacillin were determined at the Laboratory for Clinical Toxicology and Drugs Analysis of the Department of Clinical Pharmacy and Pharmacology of the UMCG using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. In brief, all analyses were performed on a triple quadrupole LC-MS/MS system (Thermo Scientific, San Jose, CA) with a Finnigan™ Surveyor® LC pump and a Finnigan™ Surveyor® autosampler (Thermo Scientific). The mobile phase consisted of an aqueous buffer (containing ammonium acetate 5 g/L, acetic acid 35 mL/L and trifluoroacetic acid 2 mL/L water), water and acetonitrile.

For chromatography, an Atlantis® HILIC Silica analytical column (2.1x100mm, 3µm) (Waters, Etten-Leur, the Netherlands) was used. A simple procedure for protein precipitation was used to prepare the samples. For piperacillin, the transition m/z 518.0 to 114.8 (collision energy 51 eV) was measured with a scan width of 0.5 m/z . The calibration curve ranged from 0.5 to 80 mg/L for piperacillin with a correlation coefficient of 0.99941. Within-run co-efficient of variation (CV) ranged from 2.5 to 12.9% and between-run CV ranged from 5.9 to 12.5%. Bias ranged from -13.4% at the lower limit of quantification (LLOQ) level to 10.1% at high level. Unbound piperacillin concentrations were determined in all patients at 1 h and 12 h after the start of treatment. Samples were prepared by ultrafiltration of the corresponding serum samples; 10 µL human serum was directly transferred into the upper reservoir of the centrifuge filters (Nanosep® 30 K Omega centrifugal device; Pall Corp.) and 200 µL of the internal standard solution was added. The centrifuge filters were closed and the samples were briefly homogenised using a vortex mixer. Filtration was done by centrifugation for 10 min at 12,000 × g.

STATISTICAL ANALYSIS

Target attainment was presented as a percentage; percentage of time at or above target per subject and percentage reaching target at group level. Continuous parameters were depicted in absolute numbers and either mean ± standard deviation (S.D.) or median [interquartile ranges (IQR)], depending on the distribution. Categorical data were depicted as percentage per/in category. Outliers in concentration data were investigated per patient in the maintenance phase (i.e. from 1 h after the start of treatment and onwards) and were defined as values outside the range of $3 \times \text{IQR} + Q3$ to $Q1 - 3 \times \text{IQR}$; these were subsequently excluded from the variability analysis and were assessed for exclusion in the target attainment analysis. In the boxplot, they were investigated per sampling period. To quantify within-patient variability, a CV per patient was calculated, defined as a patient's individual S.D. divided by the mean of this patient's concentrations, as measured during the maintenance phase, multiplied by 100%. The median, mean and range of these individual CVs were calculated. To quantify between-patient variability, we chose to calculate a CV for available concentrations measured at 40 min and at 12, 24, 48 and 60 h after the start of treatment in all patients, respectively. Median, mean and range of these five CVs were calculated. Statistical analyses were performed using SPSS Statistics for Windows v.22 (IBM Corp., Armonk, NY).

RESULTS

PATIENT CHARACTERISTICS

Twenty patients were included in the study; two patients were excluded because of breach of protocol. Baseline characteristics and clinical outcome data of this typical ICU population are presented in table 1. Almost all patients required vasopressors and mechanical ventilation (94.4 and 88.9 %, respectively). The median length of ICU stay was 9 days (IQR, 3 -13.3 days). Four patients (22.2 %) died in the ICU; none of the other patients died in hospital. Causes of death in the four patients were decompensated liver cirrhosis with subsequent multi-organ failure, severe traumatic brain injury, massive intrathoracic bleeding after oesophageal resection complicated by anastomotic leakage, and sepsis following chronic osteomyelitis.

Table 1. Baseline characteristics and clinical outcome data of patients included in the study (n=18)

Variable		Median [IQR]{range} or n (%)
Age (years)		61.5 [54-66.3]{19-72}
Male sex		14 (77.8)
BMI (kg/m ²)		25.1 [22.6–29.7]{21.6-41.2}
Patient category	Medical	6 (33.3)
	Surgery	10 (55.6)
	Trauma	2 (11.1)
Presumed/proven site of infection	Intra-abdominal	12 (66.7)
	Respiratory	3 (16.7)
	Skin/soft tissue	1 (5.6)
	Unknown	2 (11.1)
Co-morbidities ^a	None	5 (27.8)
	Solid malignancy	6 (33.3)
	Hemato-oncology	1 (5.6)
	Cardiovascular	6 (33.3)
	Chronic pulmonary	2 (11.1)
	Inflammatory bowel disease/diverticulitis	2 (11.1)
	Cushing's syndrome	2 (11.1)
	Liver Cirrhosis	1 (5.6)
	Diabetes	1 (5.6)
APACHE IV score ^b		64 [56-85]{24-128}
Mechanical ventilation		16 (88.9)
Use of Vasopressors		17 (94.4)
Measured CL _{CR} (mL/min) ^c		62.5 [26.8–116.8]{3.4-183.8}
ICU length of stay (days)		9 [3 – 13.3]{2-75}
ICU mortality		4 (22.2)
Hospital mortality		4 (22.2)

IQR, interquartile range; BMI, body mass index; APACHE, Acute Physiology and Chronic Health Evaluation; CL_{CR}, Creatinine clearance; ICU, intensive care unit. ^aMore than one variable per patient possible. ^bAvailable in 13 patients. ^cAvailable in 13 patients.

PIPERACILLIN CONCENTRATION DATA

In the 18 patients, 53 samples taken during the loading phase and 175 samples taken during the maintenance phase were available for analysis. The median follow-up (duration of piperacillin treatment including sampling of piperacillin concentrations) was 60 h (IQR, 33-96 h). Three outliers were excluded from the analysis. Two outliers were included as they represented very high values at the beginning of the sampling period most likely due to interindividual variability or due to procedural reasons.

Of 172 samples in the maintenance phase, 73 (42.4 %) were at or above the pre-defined target concentration of 80 mg/L; 168 (97.7 %) were > 16 mg/L. In 16 (88.9 %) of 18 patients a concentration of ≥ 80 mg/L was reached within 1 h after the start of treatment. However, in only 5 (27.8 %) of 18 analysed patients was a concentration of ≥ 80 mg/L maintained (i.e. reached the combined target). Two patients (11.1%) never reached a concentration ≥ 80 mg/L. On patient level, a median of 39.6 % of samples per patient in the maintenance phase was ≥ 80 mg/L [IQR, 15.5 - 100.0 mg/L].

All patients had a concentration of > 16 mg/L within 1 h after the start of treatment. In 15 patients (83.3 %), a concentration of > 16 mg/L was maintained. The data are summarised in table 2 and figure 1.

Table 2. Total piperacillin concentration (mg/L) from 1 h after the start of treatment (maintenance phase) and target attainment.

	N (% of 172 samples)	N (% of 18 patients)	Median	Interquartile range
Follow up* (h)			60	33-96
Concentration (n = 172 samples) (mg/L)			65.7	4.9-131.8 Total range, 8.7-284.9
C_{max} (n = 18 patients) (mg/L)			144.5	119.4-217.3
C_{min} (n = 18 patients) (mg/L)			46.2	28.7-87.2
$C \geq 80$ mg/L from 1 h after the start of treatment	73 (42.4 %)			
$C > 16$ mg/L from 1 h after the start of treatment	168 (97.7 %)			
% of samples with $C \geq 80$ mg/L from 1 h after the start of treatment, per patient (n = 18)			39.6	15.5-100
% of samples $C \geq 16$ mg/L from 1 h after the start of treatment, per patient (n = 18)			100	100-100
$C \geq 80$ mg/L reached within 1 h after the start of treatment		16 (88.9 %)		
$C > 16$ mg/L reached within 1 h after the start of treatment		18 (100 %)		
$C \geq 80$ mg/L reached within 1 h after the start of treatment and persistent $C \geq 80$ mg/L from 1 h after the start of treatment		5 (27.8 %)		
$C > 16$ mg/L reached within 1 h after the start of treatment and persistent $C \geq 16$ mg/L from 1 h after the start of treatment		15 (83.3 %)		

Table 2 continued.

	N (% of 172 samples)	N (% of 18 patients)	Median	Interquartile range
0 % of samples $C \geq 80$ mg/L from 1 h after the start of treatment, per patient (n = 18)		2 (11.1 %)		
0 % of samples $C \geq 16$ mg/L from 1 h after the start of treatment, per patient (n = 18)		0 (0%)		

C_{max} , maximum concentration ; C_{min} , minimum concentration ; C, concentration. *Duration of treatment with piperacillin, including sampling of piperacillin concentrations per protocol.

Figure 1. Total piperacillin concentration in time, from start of treatment, group level

Dark line middle of box = median; Whiskers represent 1.5 x InterQuartile Range (IQR); Circles represent outliers between 1.5 – 3 x IQR; Star represents outlier > 3 x IQR; Horizontal lines (16 mg/L and 80 mg/L) represent target concentrations discussed in text. Number of data that have been used per boxplots; above the respective boxplot in figure.

Two of the four deceased patients had piperacillin levels ≥ 80 mg/L at any time during treatment, from 1 h after the start of treatment.

All of the patients with a $CL_{Cr} < 50$ mL/min (7 patients) reached a piperacillin concentration of ≥ 80 mg/L within 1 h of starting treatment, and 3 (42.9%) of the 7 maintained a concentration of ≥ 80 mg/L. In patients with a $CL_{Cr} \geq 50$ mL/min (11 patients), 9 (81.8 %) reached a piperacillin concentration of ≥ 80 mg/L within 1 h of starting treatment and 2 (18.2%) of 11 maintained a concentration of ≥ 80 mg/L. Measurement of CL_{Cr} by $UCreat \times UVol / \text{time} \times SCreat$ was available in 13 of 18 patients; in 5 patients renal function was estimated using the MDRD formula.

Unbound piperacillin concentrations assessed at 1 h after the start of treatment (total, 18 samples in 18 patients) were ≥ 64 mg/L (4xMIC) in 16 (88.9 %) of 18 samples and were > 16 mg/L in all 18 samples (100%). Unbound piperacillin concentrations assessed at 12 h after the start of treatment (total, 18 samples in 18 patients) were ≥ 64 mg/L in 7 (38.9 %) of 18 samples and > 16 mg/L in all 18 samples (100 %). The median fraction unbound was 0.93 [IQR, 0.89 – 0.97].

VARIABILITY

The median within-patient CV was 32.3 % (mean, 39.7 %), with a range of 10.3 – 99.2 %. Concentrations analysed at 40 min and at 12, 24, 48 and 60 h after the start of treatment for between-patient variability were available for 18, 18, 15, 10 and 10 patients, respectively. Median CV for the five time-points was 71.8 % (mean, 78.7 %), with a range of 55.4 – 99.9 %. Five outliers were excluded from the calculation of the within-patient and between-patient CV.

DISCUSSION

To the best of our knowledge, this is the first observational study describing piperacillin concentrations over the entire treatment period in a heterogeneous group of adult ICU patients treated with continuous dosing. As expected, most patients showed a rapid rise in piperacillin concentration after receiving a loading dose; indeed, the vast majority (88.9 %) reached the pre-defined target concentration of ≥ 80 mg/L within 1 h after the start of treatment. Over the course of time, however, despite continuous administration, a large inter-individual and intra-individual variability in piperacillin concentrations was observed in this population, with a trend toward lower concentrations over time (figure 1). This large variability was also found in a recent study evaluating extended, i.e. prolonged but not continuous, infusion of piperacillin in ICU patients³⁵. Overall, the combined target of a total piperacillin concentration ≥ 80 mg/L reached within 1 h after starting study treatment and maintained throughout the treatment course was met in only 27.8 % of patients. Large recent studies analysing conventional dosing of β -lactams showed similar results, where pre-defined targets were not met in a large proportion of patients^{13,15}.

Total and unbound piperacillin concentrations were compared in a subset of samples; as expected, the difference between free and total concentrations was small. Because this difference is small, the cheaper and easier total concentration will suffice, as employed by others³⁶.

Toxic levels of piperacillin are not well defined in literature. No clinical signs of overdosing (convulsions) were seen in the current cohort.

Although a matter of debate, we chose an ‘aggressive’ combined target of 100 % $T_{\geq 5 \times \text{MIC}}$ for total

piperacillin concentration in the maintenance phase, with a presumed protein binding of ca. 20-30 % for piperacillin, using the piperacillin MIC breakpoint for *P.aeruginosa*. Within the set definition, the target concentration was to be met within a small timeframe, i.e. 1 h. PK/PD indices vary widely in the literature, ranging from 100 % $fT_{>\text{MIC}}$ to 40 – 100 % $fT_{>4 \times \text{MIC}}$ for unbound piperacillin concentrations³⁷. There is no conclusive evidence as to which target is required for an optimal therapeutic effect. Altered pharmacokinetics in the critical care patient and possible infection by pathogens with an MIC at or near the resistant breakpoint increase the risk of underdosing¹¹. We chose the strict combined target to ensure maximum killing of most problematic (Gram-negative) pathogens in a primarily empirical treatment setting. Supplementary table S1 illustrates the consequence of different target levels. Obviously, target attainment is influenced by the MIC judged to be relevant as dictated by local resistance patterns. A less strict target of 100 % $T_{>\text{MIC}}$ was still not met in 16.7 % of patients (table 2). Assuming the great majority of Gram-negatives to have an MIC < 16 mg/L would allow starting using the ‘one size fits all’-continuous dosing schedule, as was done in this study. Sampling for therapeutic drug monitoring (TDM) could then be done at any time during the maintenance phase to enable proper dose adjustment; in combination with culture results, assuming that these are available; this could mean lowering the dosing schedule in a substantial proportion of the population. However, If more resistant pathogens are cultured or if expected higher targets have to be met, the perspective changes. This would then make a case for TDM throughout the course of treatment. Piperacillin has a large therapeutic range. In this study, lower piperacillin concentrations were found over the course of treatment and a suboptimal target was attained, even when a lower target of 100 % $T_{>\text{MIC}}$ was set. Therefore, it seems safe and logical to start a larger cumulative dosing regimen, e.g. 16 g daily of piperacillin infused over 24 h, preceded by a loading dose of 4 g of piperacillin infused over 20 min, in a ‘hit fast, hit high’ strategy, followed, if possible, by downgrading based on TDM and cultured causative microorganism.

In our view, strengths of our study include it being the first observational study describing piperacillin concentrations in adult ICU patients treated with continuous dosing over the entire treatment period. Furthermore, total as well as unbound concentrations were assessed.

This study also has some limitations. Only piperacillin concentrations were analysed, not tazobactam. Piperacillin and tazobactam pharmacokinetics are not identical and in patients with renal function loss tazobactam overdose might occur³⁸. As outlined in the Materials and methods section, in several patients piperacillin treatment was started intermittently before the start of the study. As treatment was given in intervals of 8 h, in these patients it was still relevant to assess whether our target was met after the start of study treatment, i.e. continuous dosing directly after a bolus infused over 20 min.

Some samples were excluded from analysis as they were identified as outliers. Measurement

of CL_{Cr} by $UCreat \times UVol/time \times SCreat$ was not available in all patients; in 5 of 18 patients renal function was estimated using the MDRD formula. In this analysis, renal function measured/estimated on the first study day was used. In daily practice, however, in most patients CL_{Cr} was measured and no alteration in dosing due to significant changes in CL_{Cr} was needed. Patients with renal replacement therapy or other extracorporeal support were excluded because in these patients we considered kinetics to be so complicated that this deserves a separate study. The sample size was too small to identify subgroups that would benefit most from TDM.

CONCLUSIONS

These data show a large variability in piperacillin concentrations in critically ill patients treated with continuous dosing following a loading dose. With the dosing schedule used, the target set to reach 5 x MIC of *P. aeruginosa* during the entire continuous infusion from 1 h after the start of treatment could not be attained. Very low levels were rare. From a pharmacokinetic point of view, continuous dosing is more advantageous than intermittent dosing. However, optimising this dosing strategy merits further attention, as shown by the current data. Depending on which target is to be met, a larger initial cumulative dose is desirable, combined with TDM, to avoid subtherapeutic drug concentrations. Formal proof-of-effect of antibiotic concentrations on survival in randomised controlled studies is still lacking, but it seems both logical and feasible to try to achieve optimal dosing.

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CHAPTER 3

SUPPLEMENTARY MATERIAL

TARGET ATTAINMENT WITH CONTINUOUS DOSING OF
PIPERACILLIN/TAZOBACTAM IN CRITICAL ILLNESS:
A PROSPECTIVE OBSERVATIONAL STUDY

Supplementary Table S1. Probability of target attainment according to different targets and minimum inhibitory concentrations (MICs)

MIC (mg/L)	5 × MIC (mg/L)	N (%) ^a			
		100%T _{≥5xMIC}	100%T _{>MIC}	>50%T _{≥5xMIC}	>50%T _{>MIC}
1	5	18 (100)	18 (100)	18 (100)	18 (100)
2	10	16 (88.9)	18 (100)	18 (100)	18 (100)
4	20	15 (83.3)	18 (100)	17 (94.4)	18 (100)
8	40	10 (55.6)	18 (100)	15 (83.3)	18 (100)
16	80	5 (27.8)	15 (83.3)	7 (38.9)	18 (100)
32	160	2 (11.1)	12 (66.7)	3 (16.7)	16 (88.9)
64	320	0 (0)	6 (33.3)	0 (0)	9 (50.0)
128	640	0 (0)	3 (16.7)	0 (0)	3 (16.7)

%T_{≥5xMIC}, percentage of time of dosing interval during which the total concentration exceeds 5× MIC; %T_{>MIC}, percentage of time of dosing interval during which the total concentration exceeds the MIC.

^a From 1 h after start of treatment of 18 patients (172 samples).

CHAPTER 4

CONTINUOUS VERSUS INTERMITTENT INFUSION OF CEFOTAXIME IN CRITICALLY ILL PATIENTS: A RANDOMISED CONTROLLED TRIAL COMPARING PLASMA CONCENTRATIONS

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ABSTRACT

BACKGROUND

In critical care patients, reaching optimal β -lactam concentrations poses challenges, as infections are caused more often by microorganisms associated with higher MICs, and critically ill patients typically have an unpredictable pharmacokinetic/ pharmacodynamic profile. Conventional intermittent dosing frequently yields inadequate drug concentrations, while continuous dosing might result in better target attainment. Few studies address cefotaxime concentrations in this population.

OBJECTIVES

To assess total and unbound serum levels of cefotaxime and an active metabolite, desacetylcefotaxime, in critically ill patients treated with either continuously or intermittently dosed cefotaxime.

METHODS

Adult critical care patients with indication for treatment with cefotaxime were randomised to treatment with either intermittent dosing (1 g every 6 h) or continuous dosing (4 g/24h, after a loading dose of 1 g). We defined a preset target of reaching and maintaining a total cefotaxime concentration of 4 mg/L from 1 h after start of treatment. CCMO trial registration number NL50809.042.14, Clinicaltrials.gov NCT02560207.

RESULTS

Twenty-nine and 30 patients, respectively, were included in the continuous dosing group and the intermittent dosing group. A total of 642 samples were available for analysis. In the continuous dosing arm, 89.3% met our preset target, compared with 50% in the intermittent dosing arm. Patients not reaching this target had a significantly higher creatinine clearance on the day of admission.

CONCLUSIONS

These results support the application of a continuous dosing strategy of β -lactams in critical care patients and the practice of therapeutic drug monitoring in a subset of patients with higher renal clearance and need for prolonged treatment for further optimisation, where using total cefotaxime concentrations should suffice.

INTRODUCTION

Infection in ICUs is an important problem, leading to high antimicrobial consumption and substantial morbidity and mortality. In a large, international point-prevalence study, more than half of patients were considered to have an infection, while 71% were receiving antibiotics¹. In the critically ill, β -lactams are the most prescribed group of antibiotics².

To achieve the best clinical outcome, timely administration of appropriate antibiotics is critical in ICU patients with severe infections^{3–6}. To avoid treatment failure and emergence of antibiotic resistance, correct dosing is equally important^{7,8}. With β -lactams, the bactericidal effect depends on the time the unbound serum concentration exceeds the MIC of the causative microorganism⁹. Although, for cephalosporins, preclinical studies show a bactericidal effect for 60–70% $fT_{>MIC}$, clinical data involving the critically ill suggest a more aggressive approach to achieve a minimum target of 100% $fT_{\geq MIC}$ is needed to ensure optimal clinical cure in this vulnerable population^{9,10}. Optimal dosing in the individual ICU patient poses challenges as critical illness is associated with pharmacokinetic (PK) and pharmacodynamic (PD) differences compared with the non-critically ill^{9,11}. This patient group is typically prone to infections with microorganisms associated with higher MICs¹². Conventional dosing can lead to subtherapeutic levels due to augmented renal clearance in the case of renally cleared drugs and an increase of the patient's volume of distribution in the case of hydrophilic drugs, such as β -lactams⁹. Recently, a large multinational PK point-prevalence study including eight β -lactams showed that less than half of the patients reached a predefined preferred PK/PD target. Patients treated for infections in this study who did not achieve a target of 50% $fT_{>MIC}$ were 32% less likely to have a positive clinical outcome¹³. Conversely, renal dysfunction can result in elevated antibiotic concentrations and/or accumulation of metabolites¹⁴. Cefotaxime, however, seems to have a high threshold for (neuro)toxicity¹⁰. The complex PK changes in the critically ill are outlined in detail in several reviews^{9,14,15}.

Based on their time-dependent profile, continuous as opposed to intermittent dosing of β -lactams seems a logical alternative in the ICU population. This concept is supported by PK studies showing better target attainment using a continuous dosing approach^{16–18}.

In critical care units throughout the Netherlands, β -lactams are also widely employed in the context of selective decontamination of the digestive tract (SDD). In an environment with low levels of antimicrobial resistance, its use is associated with a reduction in ICU and hospital mortality and ICU-acquired bacteraemia¹⁹. The SDD approach includes 4 days of preemptive treatment with a cephalosporin, such as cefotaxime².

To date, only two observational studies on cefotaxime dosing in comparable critical care

populations are available, both of which evaluated intermittent dosing^{20,21}. As cefotaxime is widely prescribed, more knowledge about its PK in ICU patients is important to ensure best efficacy of the drug. Therefore, the aim of this study was to ascertain which dosing regimen of cefotaxime results in the most rapid and persistent target attainment in critically ill patients. We defined our target as a total (bound and unbound) cefotaxime concentration of at least 4 mg/L, to be reached within 1 h after start of treatment and to be maintained during treatment. Both total (bound and unbound) concentrations and unbound concentrations of cefotaxime and its active metabolite desacetylcefotaxime were evaluated.

PATIENTS AND METHODS

STUDY DESIGN AND PATIENT POPULATION

This randomised controlled single-centre study was conducted in a tertiary referral hospital in the Netherlands, between November 2015 and June 2016. The study was approved by the Medical Ethics Board of this hospital (ethics approval number METc 2014/468, CCMO trial registration number NL50809.042.14, Clinicaltrials.gov NCT02560207). Enrolment with deferred consent was used. Written consent was obtained from the patient or next of kin.

Patients aged ≥ 18 years were eligible for inclusion. It was possible to start cefotaxime (Sandoz B.V., Almere, the Netherlands) per protocol as part of SDD if a patient had an anticipated mechanical ventilation for > 48 h and ICU stay of > 72 h. The duration of treatment was 4 days, or shorter if the patient was discharged and transferred to a ward within that period, as SDD including cefotaxime was discontinued on discharge. Exclusion criteria were: inability to acquire written informed consent; contra-indication for cefotaxime, such as cephalosporin allergy; no indication for placement of an arterial line; and use of renal replacement therapy or extracorporeal life support. Patients were randomised by a research nurse using a secure web application service provided by the Trial Coordination Center of this hospital. After randomisation, patients were treated with either intermittent dosing (1 g every 6h) or continuous dosing (4 g/24h, after a loading dose of 1 g infused over 40 min) using a syringe pump (Alaris® GH perfusor; CareFusion, Rolle, Switzerland). Target attainment was the primary endpoint of this study and was based on the cefotaxime MIC breakpoint for Enterobacterales of 1 mg/L, as defined by EUCAST²². Consequently, we defined target unbound cefotaxime levels to be at least 1 mg/L. Since 25-40% of cefotaxime is bound to plasma proteins, and to allow for a safety margin due to variability in tissue penetration in ICU patients^{14,23}, total target (protein-bound and unbound) cefotaxime levels were defined as 4 mg/L and higher, at any given time point during treatment.

DATA COLLECTION

Blood samples were drawn from an indwelling arterial catheter, placed for routine monitoring. In patients randomised for continuous administration, 2 mL blood samples were drawn on Day 1 at 0 min, then at 40 min from start of infusion of the loading dose, i.e. immediately after completion of the loading dose. Subsequent samples were drawn at 1, 2, 4, 8, 12 and 24 h after start of administration on Day 1. During the subsequent days of continuous infusion, samples were drawn every 12 h, until the end of treatment on Day 4. In patients randomised for intermittent dosing, 2 mL blood samples were drawn on Day 1 at 0 min, directly after infusion at 40 min, 1, 2, 4, 8, 12 and 24 h after start of administration on Day 1. After that, trough and peak levels were obtained once daily just before and 40 min after bolus infusion, respectively, until the end of treatment. Samples were centrifuged and serum was frozen at -80°C , until analysis. Patient characteristics included demographic and clinical data, assessment of severity of illness reflected by the APACHE IV Score and laboratory investigations. Base line was considered start of cefotaxime treatment.

PK ANALYSIS

Plasma concentrations of cefotaxime (total and unbound) and both total and unbound concentrations of its active metabolite desacetylcefotaxime were determined at the laboratory of the Department of Clinical Pharmacy and Pharmacology of University Medical Center Groningen by means of a validated analytical method using LC-MS/MS. In brief, cefotaxime and desacetylcefotaxime were analysed by means of an isotope dilution method. As internal standard, a stable isotope of cefotaxime was used. LC-MS/MS equipment (Thermo Fisher Scientific, Waltham, USA) consisted of a Vanquish UPLC pump, autosampler, column compartment and Quantiva triple quadrupole mass spectrometer. Total cefotaxime and desacetylcefotaxime concentrations were measured after protein precipitation of the samples; free cefotaxime and desacetylcefotaxime were measured after temperature-controlled ultrafiltration of the samples using Nanosep 30K Omega Centrifugal Devices (Pall Life Sciences, Portsmouth, UK) and measuring cefotaxime and desacetylcefotaxime in the ultrafiltrate. The lower limit of quantitation of both cefotaxime and desacetylcefotaxime was 1 mg/L and the method was linear up to 200 mg/L for cefotaxime and up to 100 mg/L for desacetylcefotaxime. The assay complied with the criteria for bioanalytical method development as issued by the EMA²⁴. Target attainment was assessed by comparing measured concentrations with our preset target as described above; target attainment was thus defined by reaching a target of at least 4 mg/L for total cefotaxime and at least 1 mg/L for unbound cefotaxime within 1 h after start of treatment, and maintaining this target thereafter.

STATISTICAL ANALYSIS

Target attainment was presented as percentage of time above target per subject and the percentage reaching the target at group level. Continuous parameters such as age, weight, height, length of stay (LOS) and duration of mechanical ventilation were collected and depicted

in absolute figures and medians, including IQR. Non-normally distributed continuous variables were compared by Mann-Whitney *U*-test for unpaired data. Categorical data, which were depicted as proportions, were compared using the χ^2 square or Fisher's exact test (two-sided; type I, 5%). PK analysis was performed with and without correction for outliers and apparent permutations (trough level taken as peak level and vice versa). Outliers, assumed to have been caused by procedural shortcomings such as sampling during bolus infusion, were defined as higher than 3 x the IQR above Q3 and lower than 3 x below Q1. SPSS v 23.0 (IBM Corp., Armonk, NY) and Minitab® 18.1 (© 2017 Minitab, Inc.) were used for statistical analyses and graphics.

POWER CALCULATION

Based on available literature on β -lactam antibiotics, we expected continuous dosing to result in adequate drug levels in 80% of patients, compared with 40% of patients in the intermittent group¹⁷. Therefore, our sample size (taking into account an absolute effect size of 40%, an alpha of 0.05 and a beta of 0.8) was 23 patients per group. Correcting for potential dropout, we aimed for 30 patients per group.

RESULTS

DEMOGRAPHIC DATA AND CLINICAL CHARACTERISTICS

Two-hundred and eight patients were deemed eligible for inclusion. Of these, 128 were excluded from randomisation; 111 because admission occurred out of office hours and a research nurse was not available, 12 because inclusion criteria were not met and 5 were missed at screening. Eighty ICU patients were screened for eligibility and were randomised for treatment with continuous or intermittent dosing. Consent could not be obtained from 11 patients, 5 patients were excluded because of breach of protocol, such as wrong dosing, 2 patients died shortly after admittance, 1 patient had no indication for an arterial line, 1 patient received cefotaxime only very briefly and 1 patient did not receive cefotaxime. We thus included 59 patients for analysis; 29 in the continuous dosing arm and 30 in the intermittent dosing arm. Patient characteristics are shown in Table 1. Of the total group, most of the patients were middle-aged and male, with a median LOS in the ICU of 6 days and with a median APACHE IV score of 70. Weight and BMI were significantly different between the continuous and intermittent groups, with the heavier patients in the intermittent group.

Table 1 Demographic data and clinical characteristics

Variable	total	continuous	intermittent	P
Number of patients	59	29	30	
Male/Female <i>n/n</i> (%)	39/20 (66/34%)	20/9 (69/31%)	19/11 (63/37%)	0.648
Age (years), median (IQR)	67 (56-77)	67 (60.5-74)	66.5 (45.25-78.25)	0.808
Height (cm), median (IQR)	175 (170-185)	175 (171-185)	175 (168.25-185)	0.503
Weight (kg), median (IQR)	82 (74-97)	77 (70-93.50)	85.50 (75.75-101.25)	0.05
BMI (kg/m ²), median (IQR)	26.6 (24.5-30.9)	25.4 (22.7-28.9)	28.9 (24.5-32.2)	0.04
LOS ICU at the start of cefotaxime treatment (days), median (IQR)	1 (0-1)	1 (0-1.5)	1 (0-1)	0.315
Duration cefotaxime (days), median (IQR)	4 (3-5)	4 (3-5)	4 (3-5)	0.106
Patient category, <i>n</i> (%)	59 (100%)	29 (100%)	30 (100%)	0.362
Medical	17 (28.8%)	7 (24.1%)	10 (33.3%)	
Surgical	20 (33.9%)	9 (31%)	11 (36.7%)	
Trauma	4 (6.8%)	1 (3.4%)	3 (10%)	
Neurological	6 (10.2%)	5 (17.2%)	1 (3.3%)	
Other	12 (20.3%)	7 (24.1%)	5 (16.7%)	
Acute/planned admission, <i>n/n</i> (%)	15/44 (25.4/74.6%)	9/20 (31/69%)	6/24 (20/80%)	0.33
APACHE IV, median (IQR)	70 (53-93)	71 (57.5-95.5)	67.5 (49.5-90.75)	0.422
Vasopressor use - yes/no, <i>n/n</i> (%)	31/28 (53/47%)	16/13 (55/45%)	15/15 (50/50%)	0.446
Fluid resuscitation - yes/no, <i>n/n</i> (%)	35/24 (59/41%)	19/10 (66/34%)	16/14 (53/47%)	0.246
Mechanical ventilation -yes/no, <i>n/n</i> (%)	50/9 (85/15%)	24/5 (83/17%)	26/4 (87/13%)	0.478
Serum albumin (g/L), median (IQR)	30 (26-35)	30 (26-34)	30.5 (26-36)	0.470
Serum creatinine (μ mol/L), median (IQR)	81 (70-107)	84 (68-107)	80.5 (70-109)	0.617
Serum ALAT (U/L), median (IQR)	27 (13-57)	21 (11.5-51.5)	37.5 (20.75-63.75)	0.089
Urinary creatinine 24h (mmol/24h), median (IQR)	9 (7-13)	9 (6.1-12.5)	10 (7.75-14)	0.186
Creatinine clearance (mL/min), median (IQR)	80 (49-112)	75 (42.5-99.5)	84 (56.5-134.25)	0.214
LOS in the ICU (days), median (IQR)	6 (4-10)	6 (4-10.5)	6.5 (3-10.25)	0.483
ICU mortality, <i>n</i> (%)	10 (16.9%)	4 (13.8%)	6 (20%)	0.731
Hospital mortality, <i>n</i> (%)	11 (18.6%)	5 (17.2%)	6 (20%)	1.000

PK DATA

After correction for outliers (*n* = 15), 627 samples from 59 patients could be analysed (327 samples from 29 patients and 300 samples from 30 patients in the continuous group and the intermittent group, respectively); 271 and 247 samples were available from 1 h after start of treatment in the continuous group and intermittent group, respectively. The median number of samples per patient was 11 (IQR = 9-14) for the continuous group and 10 (IQR = 7-13) (not significant) for the intermittent group (Table S1). For total cefotaxime concentrations, the target of 4 mg/L was reached within 1 h after start of treatment and maintained thereafter in 89.3% of patients in the continuous versus 50% of patients in the intermittent arm (*P* = 0.003) (Figure 1 and Table S2). From 1 h after start of treatment, 266 of 271 (98.2%) available samples in the continuous group had a cefotaxime concentration $\geq$ 4 mg/L, versus 194 of 247 (78.5%) samples in the intermittent group (*P* < 0.0001). For unbound cefotaxime concentrations, the target of 1 mg/L was reached

and maintained in 96.4% of patients in the continuous arm versus 71.4% in the intermittent arm ($P = 0.025$) (Figure 2 and Table S3). Comparing all available concentration measurements from 1 h after start of treatment per group, median total cefotaxime, unbound cefotaxime, total desacetylcefotaxime and unbound desacetylcefotaxime concentrations were all significantly higher in the continuous group compared with the intermittent group (Figures 1-3 and Table S4). In patients not reaching our predefined target, creatinine clearance on ICU admittance was significantly higher than in patients who did reach this target. APACHE IV score, albumin concentration or BMI on ICU admittance were not associated with target attainment (Table 2).

Figure 1. Boxplot of total cefotaxime concentration, per timepoint, per treatment group

Figure 2. Boxplot of unbound cefotaxime concentration, per timepoint, per treatment group

Figure 3. Boxplot of total desacetylcefotaxime concentration, per timepoint, per treatment group

Table 2. Baseline characteristics in patients who did and did not reach and maintain a total cefotaxime target concentration of ≥ 4 mg/L

Baseline characteristic	Target reached (N =39)	Target not reached (N = 17)	P ^a
Albumin (g/L), median (IQR)	29 (26 – 34)	32 (28 – 39.5)	0.112
APACHE IV score, median (IQR)	73 (54 – 97)	61 (43.5 – 91.5)	0.121
BMI (kg/m ²), median (IQR)	25.7 (24.5 – 30.3)	27.5 (23.5 – 33.8)	0.354
Creatinine clearance (mL/min), median (IQR)	65 (30 – 99)	114 (84 – 173)	0.000

Data on target attainment available for 28 of 29 (96.6%) patients in continuous group and for 28 of 30 (93.3%) in intermittent group. ^aCalculated based on Mann Whitney *U*-test, two-sided

Figure 4. Target attainment, per MIC, per treatment group and target

DISCUSSION

Our randomised controlled study assessing total and unbound cefotaxime, as well as total and unbound desacetylcefotaxime concentrations in a heterogeneous group of ICU patients, showed that continuous dosing of cefotaxime in adult critical care patients will lead to a better PK target attainment compared with intermittent dosing.

Our results are in line with available literature^{17,25}. In a prospective, double-blind, randomised controlled trial, Dulhunty *et al.*¹⁷ compared PK and clinical outcome in 60 patients with severe sepsis allocated to treatment with a β -lactam antibiotic (piperacillin/tazobactam, meropenem or ticarcillin-clavulanate) through either continuous or intermittent dosing. Plasma antibiotic concentration exceeded a predefined MIC (based on breakpoints for *Pseudomonas aeruginosa*; free plasma antibiotic concentrations of 16 mg/L for piperacillin and ticarcillin, 2 mg/L for meropenem) in 82% of patients in the continuous arm versus 29% in the intermittent arm. Survival and ICU-free days did not significantly differ between the groups. As a wide array of targets and dosing schedules are employed, comparing PK studies on β -lactam dosing is complex. However, overall, as summarised in a recent review by Veiga and Paiva²⁵, continuous dosing seems to result in better PK results compared with intermittent dosing. Moreover, a better clinical outcome using prolonged or continuous infusion in the critically ill is suggested in several recent meta-analyses^{26–29}. A large multicentre randomised controlled trial powered on mortality comparing continuous with intermittent dosing of β -lactams is currently recruiting patients³⁰. To date, only a few studies on cefotaxime dosing in comparable cohorts of ICU patients have been published. Seguin *et al.*²⁰ assessed plasma and peritoneal levels of cefotaxime and its metabolite in 11 patients with secondary peritonitis treated with 4 g of cefotaxime daily through continuous infusion, following a bolus of 2 g. Although wide interpatient variation was found, this regimen provided a peritoneal concentration of $> 5 \times \text{MIC}$ for the recovered *Enterobacteriaceae* and the susceptibility breakpoint of cefotaxime for facultative Gram-negative microorganisms. In a prospective, open-label, non-randomised setting, Abhilash *et al.*²¹ examined plasma concentrations of cefotaxime in 30 critically ill patients treated with 1 g of cefotaxime three times daily infused over 30 min. Cefotaxime levels were found to be below the MIC and $< 5 \times \text{MIC}$ for the isolated microorganisms in 16.7% and 43.3% of patients, respectively.

The patients in our cohort who did not reach our target had higher creatinine clearance. Augmented renal clearance is a recognised risk factor for underdosing of β -lactams^{31,32}.

Strengths of our study are that we used a randomised controlled design and recruited typical 'real-life' ICU patients. We used dense sampling to allow for a precise assessment of the difference in target attainment. Furthermore, we also assessed unbound concentrations and

the active metabolite desacetylcefotaxime to explore differences in drug metabolism. However, as the antibacterial activity of desacetylcefotaxime is 4–8 fold less than cefotaxime and its contribution to the total concentration is low, we chose not to integrate the desacetylcefotaxime concentrations in the analysis of total cefotaxime concentration³³. In our cohort, we did not find accumulation of desacetylcefotaxime (Table S4). As expected, comparing the two treatment arms, results from the total and unbound concentrations of cefotaxime and desacetylcefotaxime were comparable, with higher median concentrations in the continuous dosing arm. As the free fraction percentage of cefotaxime appeared to have a low range in our cohort of heterogeneous critical care patients (Table S4), measurements of total cefotaxime concentrations for therapeutic drug monitoring (TDM) purposes should suffice. While not yet a standard procedure in many centres, the use of TDM in optimisation by personalising antibiotic dosing of β -lactams in the critical care population is gaining ground^{10,25,34,35}. Although evidence for a reduction in mortality is lacking thus far³⁴, the use of TDM has proven to lead to better PK target attainment³⁶ and might be especially useful in patients with high PK variability such as those with higher renal clearance^{10,25} who are to be treated for a longer period of time; in our cohort, with a median treatment period of 4 days, TDM would hardly be feasible. Higher dosing in this category could be an alternative strategy to obtain better target attainment when TDM is not available¹⁰.

This study also has some limitations. Although for β -lactams, a $\%fT_{>\text{MIC}}$ between 40 and 70% for a bactericidal effect is described in earlier *in vivo* studies⁷, and different targets have been assessed¹³, a target of an unbound concentration of at least 4 $\times$ the MIC for 100% of the time is considered optimal, and this target is advocated in several recent publications^{6,10,25}. Based on these recommendations, our target ($100\%fT_{>\text{MIC}}$) can be considered somewhat conservative. Applying the strictest target of $100\%fT_{>4 \times \text{MIC}}$ to our data, 82.4% versus 23.3% of patients would reach this target from 1 h after start of treatment in the continuous and intermittent arms, respectively (Table S5). Inclusion was feasible during office hours only. This might have created a selection bias for the study population, but not for allocation to the treatment arms. As this study was carried out in a single-centre setting and patients with renal replacement therapy or extracorporeal life support were excluded, our results might not be generalisable to all critical care patients. After careful consideration of the small sample size and heterogeneous nature of the population, we chose not to include clinical outcome, as we felt the results would not be supported by an adequately powered study. A large randomised controlled trial with clinical outcome as endpoint is on its way³⁰. Baseline characteristics such as creatinine clearance and serum albumin concentration were evaluated at start of treatment and not over time. The baseline weight and BMI were significantly higher in the intermittent-dosing treatment group. Obesity as a risk factor for underdosing is recognised in some studies^{37–39}, but not supported by other publications^{40,41}. In our study, we did not find such an association. Some results were excluded from analysis, as they were identified as outliers, and some results were apparent permutations. Results of an

analysis including these data points did not alter our main results (Tables S6-S10 and Figure S1). As cefotaxime was prescribed as preemptive antimicrobial treatment in the context of SDD, we used a presumptive MIC as issued by EUCAST. Target non-attainment would occur more often in the intermittent group at higher MIC targets (Tables S5 and S11 and Figure 4).

CONCLUSIONS

In our cohort of 59 patients, continuous dosing resulted in higher median total and unbound cefotaxime and desacetylcefotaxime levels, and our predefined target was met more often in the continuous dosing group. Patients who did not reach this target had higher creatinine clearance. Our study endorses a continuous dosing strategy of β -lactams in the challenge to optimise control of infectious problems in the vulnerable critical care population. In a selected patient subgroup with augmented renal clearance, higher dosing is indicated. TDM based on total cefotaxime concentrations could further optimise treatment in cases where prolonged treatment is indicated.

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CHAPTER 4

SUPPLEMENTARY MATERIAL

CONTINUOUS VERSUS INTERMITTENT INFUSION OF CEFOTAXIME
IN CRITICALLY ILL PATIENTS: A RANDOMISED CONTROLLED TRIAL
COMPARING PLASMA CONCENTRATIONS

Table S1. Number of samples per patient, no corrections (outliers, possible mix-ups);

Continuous group, N = 29, all samples N = 334; from t = 1 hour after start of treatment N = 277

Intermittent group, N=30, all samples N=308; from t = 1 hour after start of treatment N = 254

Number of samples after correction for outliers (see methods);

Continuous group, N = 29, all samples N = 327; from t = 1 hour after start of treatment N = 271

Intermittent group, N=30, all samples N=300; from t = 1 hour after start of treatment N = 247

Number of samples available for analysis, per patient, corrected

Number of samples	Continuous group	Intermittent group	P (Mann W U)
4	-	1	
5	-	-	
6	-	1	
7	1	7	
8	4	4	
9	3	1	
10	2	2	
11	6	1	
12	2	5	
13	3	3	
14	8	5	
total	29	30	
Median [IQR]	11 [9-14]	10 [7-13]	0.082

Number of samples per timepoint and per treatment group

Timepoint (hours after start of treatment)	Continuous (n = 29)	Intermittent (N = 30)
0	29 (100%)	27 (90%)
0.67	28 (96.6%)	27 (90%)
1	27 (93.1%)	28 (93.3%)
2	28 (96.6%)	28 (93.3%)
4	29 (100%)	28 (93.3%)
8	29 (100%)	28 (93.3%)
12	28 (96.6%)	28 (93.3%)
24	28 (96.6%)	23 (76.7%)
trough day 2	N/A	18 (60%)
36	24 (82.8%)	N/A
peak day 2	N/A	18 (60%)
48	25 (86.2%)	N/A
trough day 3	N/A	15 (50%)
60	20 (68.9%)	N/A
peak day 3	N/A	16 (53.3%)
72	16 (55.2%)	N/A
trough day 4	N/A	12 (40%)
84	12 (41.4%)	N/A
peak day 4	N/A	12 (40%)
96	11 (37.9%)	N/A

Table S2. Target total cefotaxime concentration ≥ 4 mg/L reached within 1 hour after start of treatment

AND on all (100%) available measurements from t = 1 hour onwards

Total cefotaxime concentration	Continuous	Intermittent	P*
≥ 4 mg/L within 1 hour after start of treatment, n, patients	27/28 (96.4%)	28/28 (100%)	1.000
AND 100% available samples ≥ 4 mg/L, n, patients	25/28 (89.3%)	14/28 (50%)	0.003

Available for analysis (t=0.67 and/or t = 1 available); Continuous 28 of 29 patients; Intermittent 28 of 30 patients. *Calculated based on Fisher's exact, 2-sided.

Table S3. Target unbound cefotaxime concentration ≥ 1 mg/L reached within 1 hour after start of treatment

AND on all (100%) available measurements from t = 1 hour onwards

Unbound cefotaxime concentration	Continuous	Intermittent	P*
≥ 1 mg/L within 1 hour after start of treatment, n, patients	27/28(96.4%)	28/28(100%)	1.000
AND 100% available samples ≥ 1 mg/L, n, patients	27/28(96.4%)	20/28 (71.4%)	0.025

Available for analysis (t=0.67 and/or t = 1 available); Continuous 28 of 29 patients; Intermittent 28 of 30 patients. *Calculated based on Fisher's exact, 2-sided.

Table S4. Concentrations from 1 hour after start of treatment

	Continuous	Intermittent	P*
Cefotaxime total concentration	18.46 [12.65 – 27.05]	12.18 [5.02 – 26.40]	0.000
Cefotaxime unbound concentration	12.92 [8.35-19.16]	8.18 [3.42-17.80]	0.000
Cefotaxime free fraction percentage	70.15 [66.29 – 74.17]	70.55 [65.22 – 75.00]	0.717
Desacetylcefotaxime total concentration	7.59 [5.60 – 12.43]	5.81 [3.61 – 9.49]	0.000
Desacetylcefotaxime unbound concentration	6.86 [5.09 – 11.14]	5.17 [3.19 – 8.28]	0.000
Desacetylcefotaxime free fraction percentage	88.98 [85.71 – 93.28]	88.13 [84.67 – 92.30]	0.020

Median [Interquartile range]. Number of available samples cefotaxime continuous group N= 271, intermittent group N=247; desacetylcefotaxime continuous group N =272; intermittent group N=249. *calculated based on Mann Whitney U, 2-sided

Table S5. Probability of target attainment according to different targets and MICs, free fraction cefotaxime

concentrations from t = 1, corrected data

MIC (mg/L)	4 x MIC (mg/L)	Target* ≥50%T _{≥MIC} cont N (%) [†]	Target* ≥50%T _{≥MIC} intermitt N (%) ^{††}	Target* 100%T _{≥MIC} cont N (%) [†]	Target* 100%T _{≥MIC} intermitt N (%) ^{††}	Target* ≥50%T _{≥4xMIC} cont N (%) [†]	Target* ≥50%T _{≥4xMIC} intermitt N (%) ^{††}	Target* 100%T _{≥4xMIC} cont N (%) [†]	Target* 100%T _{≥4xMIC} intermitt N (%) ^{††}
1	4	29(100%)	30(100%)	28(96.6%)	22(73.3%)	29(100%)	26(86.7%)	24(82.4%)	7(23.3%)
2	8	29(100%)	30(100%)	26(89.7%)	17(56.7%)	24(82.8%)	19(63.3%)	15(51.7%)	3(10.0%)
4	16	29(100%)	26(86.7%)	24(82.4%)	7(23.3%)	6(20.7%)	6(20.0%)	4(13.8%)	0(0.0%)
8	32	24(82.4%)	19(63.3%)	15(51.7%)	3(10.0%)	2(6.9%)	0(0.0%)	1(3.4%)	0(0.0%)
16	64	6(20.7%)	6(20%)	4(13.8%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
32	128	2(6.9%)	0(0.0%)	1(3.4%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

MIC = Minimum Inhibitory Concentration; Cont=continuous; intermit = intermittent. *From first hour after start of treatment [†]of 29 patients; ^{††}of 30 patients

Table S6. Target total cefotaxime concentration ≥ 4 mg/L reached within 1 hour after start of treatment AND all (100%) available measurements from t = 1 hour onwards total cefotaxime concentration ≥ 4 mg/L, uncorrected data

Total cefotaxime concentration	Continuous	Intermittent	P*
≥ 4 mg/L within 1 hour after start of treatment	27/28 patients (96.4%)	28/28 patients (100%)	1.000
AND 100% available samples ≥ 4 mg/L	25/28 patients (89.3%)	14/28 patients (50%)	0.003
Samples at t = 0.67, t = 1 h ≥ 4 mg/L	53/55 samples (96.4%)	55/55 samples (100%)	0.495
Samples from t = 1 ≥ 4 mg/L	272/277 samples (98.2%)	201/254 samples (79.1%)	0.000

Available for analysis (t=0.67 and/or t = 1 available); Continuous 28 of 29 patients; Intermittent 28 of 30 patients. *Calculated based on Fisher's exact, 2-sided.

Table S7 Target free fraction cefotaxime concentration ≥ 1 mg/L reached within 1 hour after start of treatment AND all (100%) available measurements from t = 1 hour onwards free fraction cefotaxime concentration ≥ 1 mg/L, uncorrected data

Free fraction cefotaxime concentration	Continuous	Intermittent	P*
≥ 1 mg/L within 1 hour after start of treatment	27/28(96.4%)	28/28(100%)	1.000
AND 100% available samples ≥ 1 mg/L	27/28(96.4%)	20/28 (71.4%)	0.025

Available for analysis (t=0.67 and/or t = 1 available); Continuous 28 of 29 patients; Intermittent 28 of 30 patients. *Calculated based on Fisher's exact, 2-sided.

Table S8 Concentrations from 1 hour after start of treatment, uncorrected data

	Continuous	Intermittent	P*
Cefotaxime total concentration	18.46 [12.65 – 27.05]	12.18 [5.02 – 26.40]	0.000
Cefotaxime free fraction concentration	13.09 [8.38 – 19.60]	9.20 [3.52 – 19.50]	0.000
Cefotaxime free fraction percentage	70.21 [66.40 – 74.40]	70.89 [65.50 – 75.00]	0.662
Desacetylcefotaxime total concentration	7.69 [5.63 – 13.81]	5.97 [3.63 – 9.71]	0.000
Desacetylcefotaxime free fraction concentration	6.95 [5.13 – 12.04]	5.31 [3.20 – 8.45]	0.000
Desacetylcefotaxime free fraction percentage	89.11 [85.75 – 93.42]	88.13 [84.77 – 92.37]	0.014

Median [Interquartile range]; Number of samples cefotaxime continuous 278, intermittent 255; number of samples desacetylcefotaxime continuous 277; intermittent 255. *Calculated based on Mann Whitney U, 2-sided

Table S9. Probability of target attainment according to different targets and MICs, total cefotaxime concentrations from t = 1, uncorrected data

MIC (mg/L)	4 x MIC (mg/L)	Target* $\geq 50\%T_{\geq MIC}$ cont N (%) [†]	Target* $\geq 50\%T_{\geq MIC}$ intermitt N (%) ^{††}	Target* 100%T _{$\geq MIC$} cont N (%) [†]	Target* 100%T _{$\geq MIC$} intermitt N (%) ^{††}	Target* $\geq 50\%T_{\geq 4xMIC}$ cont N (%) [†]	Target* $\geq 50\%T_{\geq 4xMIC}$ intermitt N (%) ^{††}	Target* 100%T _{$\geq 4xMIC$} cont N (%) [†]	Target* 100%T _{$\geq 4xMIC$} intermitt N (%) ^{††}
1	4	29 (100%)	30 (100%)	28(96.6%)	24 (80%)	29 (100%)	30 (100%)	26(89.6%)	15 (50%)
2	8	29 (100%)	30 (100%)	27(93.1%)	19(63.3%)	29 (100%)	23(76.7%)	24(82.8%)	6 (20%)
4	16	29 (100%)	30 (100%)	26(89.6%)	15 (50%)	17(58.6%)	16(53.3%)	10(34.5%)	1 (3.3%)
8	32	29 (100%)	23(76.7%)	24(82.8%)	6 (20%)	4 (13.8%)	2 (6.7%)	2 (6.9%)	0 (0%)
16	64	17(58.6%)	16(53.3%)	10(34.5%)	1 (3.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
32	128	4 (13.8%)	2 (6.7%)	2 (6.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

MIC = Minimum Inhibitory Concentration; Cont=continuous; intermitt = intermittent. *From first hour after start of treatment [†]of 29 patients; ^{††}of 30 patients

Table S10. Probability of target attainment according to different targets and MICs, free fraction cefotaxime concentrations from t = 1, uncorrected data

MIC (mg/L)	4 x MIC (mg/L)	Target* $\geq 50\%T_{\geq MIC}$ cont N (%) [†]	Target* $\geq 50\%T_{\geq MIC}$ intermitt N (%) ^{††}	Target* 100%T _{$\geq MIC$} cont N (%) [†]	Target* 100%T _{$\geq MIC$} intermitt N (%) ^{††}	Target* $\geq 50\%T_{\geq 4xMIC}$ cont N (%) [†]	Target* $\geq 50\%T_{\geq 4xMIC}$ intermitt N (%) ^{††}	Target* 100%T _{$\geq 4xMIC$} cont N (%) [†]	Target* 100%T _{$\geq 4xMIC$} intermitt N (%) ^{††}
1	4	29 (100%)	30 (100%)	28(96.6%)	22(73.3%)	29(100%)	28(93.3%)	24(82.8%)	13(43.3%)
2	8	29 (100%)	30 (100%)	26(89.7%)	17(56.7%)	24(82.8%)	19(63.3%)	15(51.7%)	3(10%)
4	16	29 (100%)	28(93.3%)	24(82.8%)	13(43.3%)	7(24.1%)	9(30%)	4(13.8%)	0(0%)
8	32	24(82.8%)	19(63.3%)	15(51.7%)	3(10%)	2(6.9%)	0(0%)	1(3.4%)	0 (0%)
16	64	7(24.1%)	9(30%)	4(13.8%)	0(0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
32	128	2(6.9%)	0(0%)	1(3.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

MIC = Minimum Inhibitory Concentration; Cont=continuous; intermitt = intermittent. *From first hour after start of treatment [†]of 29 patients; ^{††}of 30 patients

Table S11. Probability of target attainment according to different targets and MICs, total cefotaxime concentrations from t = 1, corrected data

MIC (mg/L)	4 x MIC (mg/L)	Target* $\geq 50\%T_{\geq MIC}$ cont N (%) [†]	Target* $\geq 50\%T_{\geq MIC}$ intermitt N (%) ^{††}	Target* 100%T _{$\geq MIC$} cont N (%) [†]	Target* 100%T _{$\geq MIC$} intermitt N (%) ^{††}	Target* $\geq 50\%T_{\geq 4xMIC}$ cont N (%) [†]	Target* $\geq 50\%T_{\geq 4xMIC}$ intermitt N (%) ^{††}	Target* 100%T _{$\geq 4xMIC$} cont N (%) [†]	Target* 100%T _{$\geq 4xMIC$} intermitt N (%) ^{††}
1	4	29(100%)	30(100%)	28(96.6%)	22(73.3%)	29(100%)	30(100%)	26(89.6%)	15(50.0%)
2	8	29(100%)	30(100%)	27(93.1%)	20(66.7%)	29(100%)	23(76.7%)	24(82.8%)	6(20.0%)
4	16	29(100%)	30(100%)	26(89.7%)	15(50.0%)	18(62.1%)	13(43.3%)	10(34.5%)	1(3.3%)
8	32	29(100%)	23(76.7%)	24(82.8%)	6(20.0%)	4(13.8%)	2(6.7%)	2(6.9%)	0(0.0%)
16	64	18(62.1%)	13(43.3%)	10(34.5%)	1(3.3%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
32	128	4(13.8%)	2(6.7%)	2(6.9%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

MIC = Minimum Inhibitory Concentration; Cont=continuous; intermitt = intermittent. *From first hour after start of treatment [†]of 29 patients; ^{††}of 30 patients

Figure S1. Boxplot of total cefotaxime concentration, per timepoint, per treatment group, uncorrected data

CHAPTER 5

MARKED CHANGES IN GUT MICROBIOTA IN CARDIO-SURGICAL INTENSIVE CARE PATIENTS: A LONGITUDINAL COHORT STUDY

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ABSTRACT

BACKGROUND

Virtually no studies on the dynamics of the intestinal microbiota in patients admitted to the intensive care unit (ICU) are published, despite the increasingly recognised important role of microbiota on human physiology. Critical care patients undergo treatments that are known to influence the microbiota. However, dynamics and extent of such changes are not yet fully understood. To address this topic, we analysed the microbiota before, during and after planned major cardio surgery that, for the first time, allowed us to follow the microbial dynamics of critical care patients. In this prospective, observational, longitudinal, single centre study, we analysed the fecal microbiota using 16S rRNA gene sequencing.

RESULTS

Samples of 97 patients admitted between April 2015 and November 2016 were included. In 32 patients, data of all three time points (before, during and after admission) were available for analysis. We found a large intra-individual variation in composition of gut microbiota. During admission, a significant change in microbial composition occurred in most patients, with a significant increase in pathobionts combined with a decrease in strictly anaerobic gut bacteria, typically beneficial for health. A lower bacterial diversity during admission was associated with longer hospitalisation. In most patients analysed at all three time points, the change in microbiota during hospital stay reverted to the original composition post-discharge.

CONCLUSIONS

Our study shows that, even with a short ICU stay, patients present a significant change in microbial composition shortly after admission. The unique longitudinal setup of this study displayed a restoration of the microbiota in most patients to baseline composition post-discharge, which demonstrated its great restorative capacity. A relative decrease in benign or even beneficial bacteria and increase of pathobionts shifts the microbial balance in the gut, which could have clinical relevance. In future studies, the microbiota of ICU patients should be considered a good target for optimisation.

INTRODUCTION

Infections are a major threat to critical care patients leading to increased morbidity and mortality and require high antibiotic consumption¹. Although the merits of antibiotics in this vulnerable population are undisputed, the impact of antibiotic use on the host's intestinal flora is an important concern². Selective digestive tract decontamination (SDD) is widely used as an infection prevention measure in Intensive Care Units (ICUs) in the Netherlands, leading to a mortality reduction compared to standard care³. Benefits notwithstanding³, SDD impacts the gut microbiota, by suppressing Gram-negative potential pathogens, *Staphylococcus aureus* (*S. aureus*) and yeasts, while maintaining anaerobic populations through selective use of antibiotics, with unclear clinical consequences⁴. Besides antibiotics, several other factors inherent to ICU stay are associated with a disturbance in the host's microbiota, also called "dysbiosis", where the balance between potentially pathogenic and beneficial bacteria is aberrant⁵. These factors include a change in nutrition, non-antibiotic pharmacological interventions (e.g. proton pump inhibitors and vasopressors), and various invasive procedures including endotracheal intubation and surgery^{5,6}. Beneficial roles of the healthy gut microbiota on human physiology are complex and include nutrient metabolism, modulation of host immune responses⁷ and protection against potential pathogens by competition⁸. In a state of dysbiosis, overgrowth of potential pathogens occurs which can lead to inflammation and infection⁷. Current culture-independent techniques allow for an in-depth analysis of the composition of gut microbiota^{7,8}.

Despite increased recognition of the important role of intestinal microbiota on health and disease⁸, few studies have been published on the dynamics of gut microbiota in ICU patients using 16S rRNA gene sequencing⁹⁻¹⁵, generally involving acute admissions for organ failure caused by infections, emergency surgery or trauma. Most of these studies¹⁰⁻¹⁵ utilise a case-control design and compare the gut microbiota composition of ICU patients and healthy controls. Small pilot studies^{9,10,12,14} as well as larger studies^{11,13,15} suggest the evidence of rapid disruption of gut microbiota during ICU stay associated with a loss of diversity and overgrowth of potentially pathogenic microorganisms, referred to here as pathobionts⁹. Previously published data show that antibiotic-associated disturbance of microbiota can take months to restore¹⁶.

The purpose of this study was to evaluate the dynamics of the composition of the gut microbiota in patients scheduled for cardiac surgery before, during and after hospital admission. Using 16S rRNA gene sequence analysis, we also evaluated a correlation between composition of intestinal microbiota and both baseline characteristics and clinical outcomes. We observed that, despite a large inter-individual variability in microbial composition at baseline, the composition of gut microbiota during hospital stay showed a concordant pattern towards decreasing microbial alpha diversity, with a relative preponderance of potentially pathogenic species. This shift was followed

by full or partial post-discharge restoration. These findings are in line with available literature^{7,9,10,17}.

MATERIALS AND METHODS

STUDY POPULATION AND STUDY DESIGN

In this study, one hundred patients undergoing planned cardiac surgery in the University Medical Center Groningen were recruited between April 2015 and November 2016. Adult patients, scheduled for cardiac surgery involving coronary artery bypass graft (CABG) and/or valve surgery, screened in our outpatient clinic preoperatively and admitted to the ICU after surgery, were eligible for inclusion. Patient characteristics and clinical data were recorded, including occurrence of complications during ICU and hospital stay. Duration of hospital stay included stay at peripheral hospitals for further recovery. Antibiotic consumption from 3 months prior to inclusion until 8 weeks after admission was documented. All patients received peri-operative prophylaxis for 24 h with cefazolin or clindamycin. On indication (expected length of stay (LOS) in ICU $\geq$ 72 h and/or expected duration of mechanical ventilation for $\geq$ 48 h), SDD was prescribed, consisting of cefotaxime 1 gram intravenously four times daily for 4 days and a topical application of tobramycin, colistin and amphotericin B into the oropharynx and stomach throughout ICU stay as described elsewhere³. Patients colonised with *Staphylococcus aureus* received intranasal mupirocin ointment pre-operatively according to national protocol. We defined the following as serious adverse outcomes: ICU- and in hospital-mortality, increased ($\geq$ 4 days) ICU length of stay (LOS), prolonged ($\geq$ 2 days) duration of mechanical ventilation, and occurrence of bacteraemia and post-operative wound infections, including mediastinitis. If a patient had one or more of these adverse outcomes, we defined this patient to have a combined adverse outcome (CAO). Fecal samples were obtained by a research nurse at ideally three time points: pre-admission on the day of pre-operative screening (baseline or Time point 1, T1), once during admission in the ICU or ward around day 4 (Time point 2, T2), and post-admission at a post-discharge routine visit or home visit (Time point 3, T3). All available samples were included for analysis. All samples were stored directly at 4 °C and transferred to -20 °C within 24 h; later, samples were stored at -80 °C until further processing. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethical approval by the Medical Ethics Committee of our institution was received (approval number METc2014/206). Informed consent was obtained from all individual participants included in the study.

MICROBIOTA ANALYSIS; 16S rRNA GENE SEQUENCING

DNA EXTRACTION AND MiSeq PREPARATION

From the stool sample, 0.25 g was used to extract the total DNA as previously described¹⁸. Subsequently, the amplification of the V3–V4 region of the 16S rRNA gene was performed using modified 341F and 806R primers (Supplementary material 1) containing a 6-nucleotide barcode and flow-cell adaptor on the 806R primer as described elsewhere¹⁹. A 2 x 300 cartridge (Illumina, Eindhoven, the Netherlands) was used to perform both MiSeq library preparation and sequencing. Supplementary material 2 represents a detailed description of the PCR protocol, DNA cleanup and the library preparation.

ANALYSIS OF SEQUENCE READS

The paired-end sequencing data received from Illumina software were processed by the software PANDAseq (version 2.5)²⁰ and QIIME (version 1.7.0)²¹. Readouts with a quality score below 0.9 were discarded by PANDAseq to increase the quality of the sequence readouts. De novo OTU-picking was performed without chimera filtering with Greengenes (version 13.5) as reference database and ARB software (version 5.5) using a SILVA database (version 123) was used to check for contamination by the “kitome” on OTU level, only negligible numbers were detected^{22,23}.

STATISTICAL ANALYSIS

The calculation of alpha diversity (Shannon index) and beta diversity was performed using R package ‘vegan’ (version 2.5-4) on the taxonomic level of genera. The associations of microbial beta diversity vs. baseline and outcome characteristics were calculated using *adonis* (permutational multivariate analysis of variance using distance matrices), stratifying permutations for multi-time points when comparing patient characteristics, or stratifying by patients when associating beta diversity to time.

The statistical analysis of associations of baseline and outcome patient characteristics vs. alpha diversity and microbial taxa were performed using linear regression (“base” package for R) and mixed models (package “lmerTest” v. 3.1-0), when including time point or patient as random effects. The correction for false discovery rate was applied considering the number of microbial taxa included into analysis at the level of 5%, and was calculated using the Benjamini-Hochberg method.

For each layer of microbiota (alpha diversity, beta diversity, bacterial taxa), the association analysis of microbiome features with baseline and clinical characteristics was performed on three levels. On level one, we associated the microbial composition against each of baseline and outcome characteristics adjusting for age and sex. For alpha diversity and bacterial taxa, a linear mixed-model approach was used with sampling time (before, during or after admission, encoded

as 3-level factor) as random effect. For bacterial beta diversity, we stratified permutations by time group using “*strata*” parameter for *adonis* function (R package “*vegan*” v. 2.5-4). On level two, we re-evaluated the associations between microbiota features with clinical outcomes (CAO, LOS at the hospital, antibiotic post discharge and therapeutic antibiotics), additionally adjusting for all the baseline characteristics (age, gender, co-morbidities, BMI, antibiotic pre-admission). On level three, we associated the outcome measures with microbiota composition in each time point separately.

The graphs were made with R package ‘ggplot2’ (version 3.1.1).

RESULTS

Inclusion of patients and collection of samples are summarised in Figure 1. Detailed baseline characteristics and outcome parameters are summarised in Table 1. Ninety-seven patients were included. Pre-admission fecal samples were obtained from 92 patients, during admission at a median of 4 days post-surgery (interquartile range (IQR), 3-5 days) from 53 patients; and post-admission at a median of 71 days post-surgery (IQR, 57-88 days) from 83 patients. From 32 patients, fecal samples obtained at all time points were available for analysis. Reasons for missing samples are shown in Figure 1. No patient withdrew consent. Details on antibiotic consumption are listed in Supplementary material 3.

LOWER ALPHA DIVERSITY ASSOCIATED WITH LONGER HOSPITAL STAY AND ANTIBIOTIC USE AFTER DISCHARGE

The microbial composition of fecal samples was determined using 16S rRNA gene analysis. Bacterial diversity, measured as Shannon index, significantly changed at T2 compared to baseline ($p = 0.02$). A linear mixed-effect model was used to determine associations between alpha diversity and patient baseline and outcome characteristics. First, we tested each variable separately. Length of hospital stay, use of SDD and use of antibiotics post-discharge were found to be significantly associated with the Shannon index ($p < 0.05$). When all these variables were combined together in a single model, only length of hospital stay and post-discharge antibiotic use remained significant; therefore, it was tested which time point they were associated to. A lower alpha diversity at T2 was associated with a longer hospital stay (Figure 2A) and at T3 with antibiotic use after discharge (Figure 2B).

Table 1. Baseline characteristics and outcome.

Variable	N = 97
Age, years	68 [62 – 73]
Sex (male)	73 (75%)
BMI, kg/m ²	27.5 ± 4.6
Planned operation	
Coronary Artery Bypass Graft (CABG), N (%)	39 (40.2%)
CABG + Valve repair, N (%)	17 (17.5%)
Valve repair, N (%)	41 (42.3%)
Co-morbidity	
Yes, N (%)	35 (36.1%)
Pulmonary disease	10 (10.3%)
Diabetes mellitus	22 (22.7%)
Chronic kidney failure	2 (2.1%)
Solid malignancy	4 (4.1%)
Hemato-oncologic malignancy	0 (0%)
Immunosuppression	2 (2.1%)
Alcohol/substance abuse	1 (1.0%)
Antibiotics in 3 months pre-admission	
Yes	12 (12.4%)
Unknown	8 (8.2%)
No	77 (79.4%)
Euroscore	1.77 [1.07 – 2.91]
APACHE IV	48 [39-57]
Exposure to antibiotics during admission	
24 hours of cefazolin ^a	96 (99.0%)
SDD ^b	7 (7.2%)
≥ 1 day antibiotics	14 (14.4%)
Length of stay (LOS) Intensive Care Unit (ICU), days	1 [1-2]
LOS hospital, days ^c	9 [8-13]
Need of vasopressors > 1 day	7 (7.2%)
Duration of mechanical ventilation	
< 24 hours (hrs)	92 (94.8%)
24 – 48 hrs	2 (2.1%)
2 – 5 days	1 (1.0%)
> 5 days	2 (2.1%)
Bacteraemia	3 (3.1%)
Peri-operative wound Infection	3 (3.1%)
Re-exploration	7 (7.2%)
Re-admission ICU	9 (9.3%)
Died during admission	1 (1.0%)
Combined adverse outcome ^d	13 (13.4%)
Antibiotics in 3 months post-discharge	
Yes	22 (22.7%)
Unknown	2 (2.1%)
No	73 (75.3%)

Data presented as median with [IQR], numbers with (%), or mean ± standard deviation, as appropriate.

^aOne additional patient received peri-procedural clindamycin due to penicillin allergy. ^bSDD denotes Selective Decontamination of the Digestive tract. ^cAvailable in 94 patients. ^dCombined adverse outcome defined as one or more of the following: ICU mortality, in-hospital mortality, length of stay in ICU ≥ 4 days, duration of mechanical ventilation ≥ 2 days, occurrence of Post-Operative Wound Infection (POWI), including mediastinitis, or bacteraemia.

Figure 1. Patient and sample flowchart.

Figure 2. The dot plots represent the association between the Shannon index at T2 and length of hospital stay (A) and the association between the Shannon index at T3 and antibiotic use after discharge (B).

THE MICROBIOTA COMPOSITION SHIFTS DURING ADMISSION BUT REVERTS BACK AFTER DISCHARGE

Principal Coordinate Analysis (PCoA) (Figure 3) was performed to identify a clustering pattern of the microbial compositions; the baseline (T1) and post-admission (T3) samples were spread over a large area in the PCoA plot, shifting from those taken days after surgery (T2) with an overall significant change in composition over time ($p < 0.001$). During admission, a shift in composition of gut microbiota from baseline was seen ($p < 0.001$), with an increase in principal component 1 and a decrease in principal component 2, away from the original profile. Plotted samples after discharge (T3) showed a difference with T2 ($p < 0.001$). At T3 the samples seem to return to the baseline situation in the PCoA plot indicating recovery of the microbial composition, although statistically, there is still a significant difference ($p = 0.021$). In order to determine how much of the variance observed among patients could be explained by the available baseline characteristics and outcomes, the PERMANOVA analysis (Supplementary material 4) was performed. We looked into each time point to investigate how the relative contribution of these characteristics would change during the study period. We found that treatment with SDD could explain almost 7% of the variation exhibited during admission (T2), while the combined adverse outcome alone was accountable for almost 5% at T2. The use of antibiotics during admission and post-discharge, collectively, would explain 6% of variation of the microbial composition after discharge (T3).

Figure 3. Principal coordinate analysis of the microbial composition of the samples from ICU patients significantly changed over the course of the study period ($p < 0.001$). The different time points are depicted in purple (T1), yellow (T2) and blue (T3). Patients who did (7) and did not (90) receive SDD treatment are indicated with open and closed symbols, respectively.

ASSOCIATIONS BETWEEN BACTERIAL TAXA AND PATIENT CHARACTERISTICS

Next, we performed the association study between bacterial genera with baseline characteristics and outcome parameters, results can be found in detail in Supplementary material 5. When estimating each of the baseline and outcome characteristics separately (level 1), we observed associations of several bacterial taxa with: (i) BMI, (ii) comorbidities (immunosuppression, solid malignancy and chronic kidney failure), (iii) therapeutic antibiotic use during admission and (iv) hospital length of stay. When adjusting for baseline characteristics (level 2), we observed associations with: (i) SDD, (ii) the use of antibiotics during admission and (iii) hospital length of stay.

BACTERIAL DYNAMICS OVER TIME

Figure 4 indicates that during the treatment timeline, strictly anaerobic short-chain fatty acid-producing gut bacteria typically beneficial for health, such as *Faecalibacterium*, *Anaerostipes*, *Blautia* and *Roseburia*^{24–26}, decreased significantly at T2. In contrast, pathobionts or members of oral microbiota such as *Enterococcus*, *Eggerthella*, *Peptococcus* and *Rothia*^{13,27–30}, showed a substantial increase at T2. Interestingly, the tendency to increase in T2 was also observed for the obligate anaerobic gut microbes, such as *Akkermansia*, *Bifidobacterium* and *Methanobrevibacter*, which are known to be the most stable in gut microbiota under external perturbations and assumed dependent on host genetic makeup³¹ (Supplementary material 6). Further, we specifically looked at the dynamics of *Enterobacteriaceae* in the SDD sub cohort. We found no consistent change over time, however, at T2 the relative contribution was lower than 1% (data not shown) in this group of patients.

CLINICAL COURSE AND GUT MICROBIOTA COMPOSITION OF PATIENTS WITH THE LOWEST SHANNON INDEX

The dynamics of the gut microbiota composition in four patients (#14, #40, #89, #98) who had the lowest Shannon index at T2 is depicted in Figure 5. Patient #14 had a complicated course after CABG with sternal dehiscence, mediastinitis, sternal closure with pectoralis major flaps, complicated by a wound infection, with cultures yielding *Enterobacter cloacae*, *S. aureus* and *Enterococcus faecium*, myocardial ischemia with decompensated heart failure and *S. aureus* bacteraemia, needing prolonged treatment with several classes of antibiotics (Supplementary material 3), also including treatment with SDD, a long hospital stay and readmission. In this patient, the relative abundance of *Enterococcaceae* was 82% at T2. In patient #40, who had an uneventful clinical course, 60% of the gut microbiota was dominated by *Bifidobacteriaceae* at T2, while at T1 and T3, *Bifidobacteriaceae*'s contribution to the total flora was 4%. Patient #89, who had a prolonged ICU stay due to shock caused by heart failure and subsequent renal failure, showed a relatively high abundance of *Bacteroidaceae* (57%) at T2, while this was 8 and 5% at T1 and T3, respectively. Likewise, there was a relatively high abundance of *Enterococcaceae* (18%) at T2; at T1 and T3 no *Enterococcaceae* were detected. Patient #98, with an uneventful course,

showed a remarkable relative abundance of *Enterobacteriaceae* (16%) at T1, with a decrease to 10% at T2, but an increase to 25% at T3, while at T2, a relative abundance of *Enterococcaceae* (24%) was detected; this was 0% at T1 and T3.

Figure 4. The boxplot shows the results of the association analysis of the gut microbiota in ICU patients over time. In the top row a selection of beneficial butyrate-producing gut bacteria (for details, cf. Supplementary material 6) show a significant decrease during the stay at the intensive care unit. In the bottom row we show bacteria that significantly increase during hospitalisation of which *Enterococcus* is considered a pathobiont while the other two are regarded as beneficial to gut health. In the analysis, only taxa remaining after application of FDR < 0.05 are considered significant and the *p*-value of the quadratic component of the model is depicted in the headings.

Figure 5. The bar chart of selected samples represents the relative abundance of the main taxa, expressed at family level, in the gut microbiota of ICU patients whose microbiota dramatically decreased according to their Shannon index during the stay at the ICU (**A**), and of ICU patients who had a stable Shannon index over time (**B**).

DISCUSSION

Only few studies on the dynamics of intestinal microbiota in critical care patients using culture-independent methods have been published^{9–15}, despite evolving interest in microbiota. Results provide a promising insight in potential diagnostic and therapeutic possibilities for the prevention or cure of infections^{5,8}. This is the first prospective, observational longitudinal study analysing the dynamics of microbiota in patients receiving major elective extra-intestinal surgery who post-

operatively were admitted to the ICU.

In order to analyse bacterial diversity, we included the Shannon index. As previously reported and in line with other studies¹⁵, we found a significantly lower Shannon index during than before admission. In our longitudinal setup, we found the Shannon index after discharge not to be significantly different from before admission. The main differences were observed between the first two time points, confirming that all interventions related to hospital admission for elective surgery have a major impact on a patient's microbiota. The length of stay at the hospital was associated with a lower Shannon index, especially in the microbiota sampled at T2, suggesting the potential use of the gut microbiota data for an intervention that might prevent post-surgery complications. A lower Shannon index at T3 was associated with use of antibiotics post-discharge indicating the strong effect that this therapy has on the microbial gut diversity.

The gut microbial composition of patients before, during and after admission showed a large intra-individual variability. These changes occurred not only in patients with a complicated course, but also in those with an uneventful post-operative recovery. Relatively few patients (13 individuals, i.e. 13.4%) had a predefined CAO. The median ICU stay was only 1 day, rendering this cohort to be different from the previous studies on dynamics of microbiota in ICU patients involving mainly acute admissions due to sepsis, surgery and trauma^{9–15}. During hospital stay, we observed a consistent pattern of changes in microbiota, characterised by: (i) a substantial drop in abundance of many bacteria from the Lachnospiraceae family, including the genera *Blautia*, *Roseburia* and *Dorea*; (ii) an increase in potential pathobionts, including enterococci; (iii) a relative increase in abundance of non-pathogenic gut commensal genera known to be stable over time, such as mucin-degrading *Akkermansia*, *Bifidobacterium* and the archaeon *Methanobrevibacter*. Many factors potentially responsible for variations in the microbiota composition may contribute to the observed dynamic pattern^{5,32}. All patients were exposed to multiple interventions including major surgery, dietary changes and antibiotic use for at least a short period. Some patients had more protracted courses with a longer ICU stay with use of SDD as an infection prevention measure, longer courses of antibiotics, and re-interventions. Although SDD is partially aimed at *Enterobacteriaceae*, no significant change in their abundance was seen in this small sub cohort likely due to sample size and possibly due to the low relative contribution of *Enterobacteriaceae* to the gut microbiota composition. The most pronounced dynamic change of the gut microbiota during hospital stay in our cohort is a decrease in abundance of bacterial members of healthy microbiota, mostly butyrate producers. Taking into account their roles as energy providers for colonocytes, together with their known anti-inflammatory properties¹⁶, we postulate that this decrease in butyrate producers may have a direct impact on clinical outcome. These results confirm and complement previously published studies in disease- and treatment-associated microbiota dynamics in different patient cohorts^{9–15}.

In most patients analysed at all time points, the change in microbiota during hospital stay reverted to the original composition post-discharge, showing the flexibility of the gut microbiota and its great restorative capacity. We observed the associations between microbial dynamics and clinical metadata but they appear not to be clinically relevant. Our data thus do not allow to state whether the microbial composition at baseline could influence outcome and whether a state of dysbiosis is a cause or an effect of critical illness. Dysbiosis was previously found to be associated with an increased risk for infection and adverse outcome. For instance, in a cohort of 301 medical ICU patients, disruption of microbiota with *Enterococcus* domination ($\geq 30\%$ relative abundance) as assessed by 16S rRNA gene sequencing at ICU admission was associated with a 22% increased risk for all-cause infection or death³³. Additionally, dysbiosis is associated with chronic disease states such as inflammatory bowel disease, obesity, diabetes and cardiovascular disease². In case of elective surgery, influencing or preventing a state of dysbiosis before surgery might lead to a better outcome. The use of novel probiotic strains to boost anaerobic butyrate-producing bacteria could improve resilience to the effect of surgery and hospital stay in general on the microbiota, or even simple nutritional advice or supplementation with vitamins could improve the condition of the patients' microbiota. A possible role for fecal microbiota transplantation (FMT) also merits consideration³⁴; from a recent study, autologous FMT showed a more rapid recovery than probiotics³⁵. Therefore, "profiling" the microbiota of a patient before admission could be of relevance. On the other hand, our data showed that microbiota profiling during admission is much more promising from a clinical perspective, due to its potential for predicting post-surgery clinical outcomes, such as length of hospital stay.

The strength of this study is its longitudinal design: ideally, patients were sampled three times, thereby serving as their own control. This study has several limitations. We had a relatively large number of missing fecal samples. Although in the context of mentioned previously published studies our cohort was relatively large, the sample size is still to be considered small. In future studies, as noted by others¹⁵, the use of rectal swabs for sequencing could be an attractive alternative especially in large-scale studies, with results similar to those from stool samples³⁶. Differentiating the individual factors responsible for changes in intestinal microbiota is not possible due to the omnipresent use of antibiotics. Also, no (control) patient is withheld from surgery. We did not assess the contribution of all factors potentially influencing the intestinal microbiota composition of the patient, e.g. the use of proton pump inhibitors was not evaluated⁵. Moreover, disentangling the many factors contributing to the disruption of the intestinal microbiota composition is limited by the inherent combination of some of these factors³⁷; a patient with a prolonged course due to infectious complications with hemodynamic instability will likely be treated with prolonged antibiotics, possibly from different antibiotic classes, for instance. How these individual factors contributed to the observed association between length of hospital stay and lower alpha diversity at T2, is therefore impossible to say in this study design.

Antibiotic use during stay in peripheral hospitals to which patients with uncomplicated courses were transferred was not documented. The use of 16S rRNA gene sequencing limits the resolution of microbial data to the level of genera and, for some taxonomic groups, species, and does not allow profiling bacterial strains or identifying abundances of metabolic functions. Viruses and fungi, although of presumed clinical relevance, were also not targeted by this method. As this study was carried out in a single centre setting involving cardiac surgery patients, our results might not be generalisable to all critical care patients. However, despite these limitations, our project is a step forward in understanding the dynamics of the microbiota and holds promising views on potential diagnostic and therapeutic options for ICU patients.

Our study shows that gut microbiota composition of elective surgery patients admitted to the ICU undergoes significant changes. The decrease in beneficial bacteria and the relative increase of specific pathobionts shift the microbial balance to dysbiosis in the gut, which has clinical relevance. Therefore, optimising the microbiota of patients admitted to the ICU should be considered in future studies.

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CHAPTER 5

SUPPLEMENTARY MATERIAL

MARKED CHANGES IN GUT MICROBIOTA IN
CARDIO-SURGICAL INTENSIVE CARE PATIENTS:
A LONGITUDINAL COHORT STUDY

5S

V3_F_modified	aatgatacggcagccacgcagatct	<u>acactctttccctacagacgctctccgatct</u>	NNNNCTACGGGAGGCAGCAG
V4_1R	caagcagaagcggcatacgcagat	ATCACG <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_2R	caagcagaagcggcatacgcagat	CGATGT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_3R	caagcagaagcggcatacgcagat	TTAGGC <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_4R	caagcagaagcggcatacgcagat	TGACCA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_5R	caagcagaagcggcatacgcagat	ACAGTG <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_6R	caagcagaagcggcatacgcagat	GCCAAT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_7R	caagcagaagcggcatacgcagat	CAGATC <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_8R	caagcagaagcggcatacgcagat	ACTTGA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_9R	caagcagaagcggcatacgcagat	GATCAG <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_10R	caagcagaagcggcatacgcagat	TAGCTT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_11R	caagcagaagcggcatacgcagat	GGCTAC <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_12R	caagcagaagcggcatacgcagat	CTTGTA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_13R	caagcagaagcggcatacgcagat	AGTACG <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_14R	caagcagaagcggcatacgcagat	TCAGTC <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_15R	caagcagaagcggcatacgcagat	TTGAGC <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_16R	caagcagaagcggcatacgcagat	AAGCGA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_17R	caagcagaagcggcatacgcagat	TCCTCA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_18R	caagcagaagcggcatacgcagat	GGTTGT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_19R	caagcagaagcggcatacgcagat	TGAGGT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_20R	caagcagaagcggcatacgcagat	TACCGT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_21R	caagcagaagcggcatacgcagat	CCAACT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_22R	caagcagaagcggcatacgcagat	AGAGAG <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_23R	caagcagaagcggcatacgcagat	CAC TTG <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_24R	caagcagaagcggcatacgcagat	TCAAGG <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_25R	caagcagaagcggcatacgcagat	AGTGGT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_26R	caagcagaagcggcatacgcagat	GACACT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_27R	caagcagaagcggcatacgcagat	CCTTCT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_28R	caagcagaagcggcatacgcagat	GGATAA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_29R	caagcagaagcggcatacgcagat	CCTTAA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_30R	caagcagaagcggcatacgcagat	CAAGAA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_31R	caagcagaagcggcatacgcagat	GTTGAA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_32R	caagcagaagcggcatacgcagat	TCACAA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_33R	caagcagaagcggcatacgcagat	AGTCAA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_34R	caagcagaagcggcatacgcagat	CGAATA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_35R	caagcagaagcggcatacgcagat	GCTATA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_36R	caagcagaagcggcatacgcagat	GAGTTA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_37R	caagcagaagcggcatacgcagat	TTGGTA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_38R	caagcagaagcggcatacgcagat	AACGTA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_39R	caagcagaagcggcatacgcagat	GTACTA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_40R	caagcagaagcggcatacgcagat	CATCTA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_41R	caagcagaagcggcatacgcagat	TGTAGA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_42R	caagcagaagcggcatacgcagat	ATCAGA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_43R	caagcagaagcggcatacgcagat	ACATGA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_44R	caagcagaagcggcatacgcagat	TAGACA <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_45R	caagcagaagcggcatacgcagat	GAGAA T <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_46R	caagcagaagcggcatacgcagat	CTCAAT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_47R	caagcagaagcggcatacgcagat	AGGTAT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT
V4_48R	caagcagaagcggcatacgcagat	TTGCAT <u>gtgactggagttcagacgtgtgctcttccgatct</u>	GGACTACHVGGGTWTC TAAT

[illegible]

Supplementary material 2

Reaction conditions consisted of an initial 94 °C for 3 min followed by 32 cycles of 94 °C for 45 sec, 50 °C for 60 sec, and 72 °C for 90 sec, and a final extension of 72 °C for 10 min. An agarose gel confirmed the presence of the product (band at ~465 base pairs) in successfully amplified samples. The remainder of the PCR product (~45 µl) of each sample was mixed thoroughly with 25 µl Agencourt AMPure XP magnetic beads (Beckman Coulter) and were incubated at room temperature for 5 minutes. Beads were subsequently separated from the solution by placing the tubes in a magnetic bead separator for 2 minutes. After discarding the cleared solution the beads were washed twice by suspending them in 200 µl freshly prepared 80% ethanol, incubating the tubes for 30s in the magnetic bead separator and subsequently discarding the cleared solution. The pellet was then air dried for 15 minutes and suspended in 52.5 µl 10 mM Tris HCl pH 8.5 buffer. Fifty µl of the cleared up solution are subsequently transferred to a new tube. The DNA concentration of each sample was done using a Qubit® 2.0 fluorometer (www.invitrogen.com/qubit) and the remainder of the sample was stored at -20 °C until library normalization. Library normalization was done the day before running samples on the MiSeq by making 2 nM dilutions of each sample. Samples were pooled together by combining 5 µl of each diluted sample. Ten µl of the pooled samples and 10 µl 0.2 M NaOH were subsequently combined and incubated for 5 minutes to denature the sample DNA. To this, 980 µl of the HT1 buffer from the MiSeq 2x300 kit is was subsequently added. A denatured diluted PhiX solution was made by combining 2 µl of a 10 nM PhiX library with 3 µl 10 mM Tris HCl pH 8.5 buffer with 0.1% Tween 20. These 5 µl were mixed with 5 µl 0.2 M NaOH and incubated for 5 minutes at room temperature. These 10 µl were subsequently mixed with 990 µl HT1 buffer. From the diluted sample pool, 150 µl were combined with 50 µl of the diluted PhiX solution and further diluted by adding 800 µl HT1 buffer. Finally, 600 µl of the prepared library were loaded into the sample loading reservoir of the MiSeq 2x300 cartridge.

Supplementary material 3. Classes of antibiotics received by each patient before (a), during (b) or after (c) hospital admission.

	Aminoglycosides	Carbapenems	Cephalosporins ²	Clavulanic acid	Co-trimoxazole	Macrolids	Metronidazole	Penicillins	Quinolones	Tetracyclines	Tazobactam	Topical A/P/T ¹	Vancomycin	Clindamycin ³	Linezolid	Rifampicin	Unknown ⁴
#1			b														
#2			b														c
#3			b														
#4			b														
#5			b														
#6			b	b					b								
#7			b														
#8			b														
#9			b														c
#10			b														
#11			b														
#12			b					c									
#13			b														
#14	c		b					c	b			b	b c	b	b		
#15			b														
#16			b														c
#17			b														
#19			b														
#20			b														
#21			b	c													
#22			b														a
#23			b														
#24			b								b						
#25			b					c									
#26			b					a									
#27			b														
#28			b														
#29			b														
#30			b														
#31			b														
#32			b														
#33			b														
#34			b					c									a
#35			b														
#36			b														
#37			b														
#38			b	a													
#39			b								b						
#40			b														
#41			b														
#42			b							a							

	Aminoglycosides	Carbapenems	Cephalosporins ²	Clavulanic acid	Co-trimoxazole	Macrolids	Metronidazole	Penicillins	Quinolones	Tetracyclines	Tazobactam	Topical A/P/T ¹	Vancomycin	Clindamycin ³	Linezolid	Rifampicin	Unknown ⁴
#43			b														
#44			b					a									
#45			b		b			b			b						c
#46			b														a
#47		c	b		c												
#48			b														c
#49			b	b				b c			b						a
#50			b														
#51			b														
#52			b							a							a
#53			b														c
#54			b														
#55			b														c
#56			b														
#57			b									b					
#58			b														
#59			b														
#60			b														
#61			b														a c
#62			b														a c
#63			b														
#64			b														
#65			b														
#66			b														
#67			b														
#68			b					c									
#69			b														
#70			b														
#71			b														
#72			b														
#73			b					a									
#74			b														
#76														b			
#77			b														
#78			b														
#79			b														
#80			b														
#81			b														
#82			b														
#83			b			b	b				b		b				
#84			b														
#86			b									b					
#87	b		b					b	b				b	b		b	
#88			b														

	Aminoglycosides	Carbapenems	Cephalosporins ²	Clavulanic acid	Co-trimoxazole	Macrolids	Metronidazole	Penicillins	Quinolones	Tetracyclines	Tazobactam	Topical A/P/T ¹	Vancomycin	Clindamycin ³	Linezolid	Rifampicin	Unknown ⁴
#89			b									b					
#90			b														
#91			b					b			b	b	b				
#92			b														
#93			b														
#94			b														
#95			b														c
#96			b														
#97			b														
#98			b							c							
#99			b														
#100			b														

¹Topical A/P/T denotes topical application of amphotericin B, polymyxin, tobramycin in the context of selective decontamination of the digestive tract, by protocol also including systemic application of a cephalosporin. ²Ninety-six patients received perioperative prophylaxis (a cephalosporin); ³one patient (#76) received clindamycin as perioperative prophylaxis. ⁴“Unknown” was registered when a patient stated to have received an antibiotic (before or after hospital stay), but its name could not be retrieved.

Supplementary material 4

Level 1			
Variable	R²	P-value	P-value adjusted
BMI	0,008420848	0,03449655	0,04785885
SDD	0,015861554	0,00089991	0,00649935
EUROSCORE	0,005207392	0,38276172	0,41010185
APACHE	0,002831469	0,91250875	0,91250875
AB pre admission	0,009825415	0,02519748	0,0419958
AB during admission	0,011442846	0,00289971	0,00869913
AB post admission	0,010997872	0,00429957	0,01074893
LOS	0,016584206	0,00059994	0,00649935
CAO	0,01334495	0,00129987	0,00649935
Pulmonary disease	0,005808058	0,22667733	0,26155077
Diabetes mellitus	0,008349447	0,03509649	0,04785885
Chronic kidney failure	0,009805689	0,0089991	0,01687331
Solid malignancy	0,011317233	0,00289971	0,00869913
Immunosuppression	0,012578389	0,0069993	0,0149985
Alcohol/illicit drug abuse	0,007252143	0,06139386	0,07674233

Level 1: Percentage of variance explained by each variable separately

Level 2		
Variable	R²	P-value
BMI	0,01009687	0,01529847
SDD	0,01591906	0,00089991
AB during admission	0,01428485	0,00089991
AB post admission	0,01318333	0,00149985
LOS	0,01505776	0,0019998
CAO	0,01180346	0,00649935

Level 2: Percentage of variance explained by all the variables together

Level 3						
Variable	R ² _T1	P-value_T1	R ² _T2	P-value_T2	R ² _T3	P-value_T3
BMI	0,0135391	0,46453546	0,02037377	0,646353646	0,02048294	0,141858142
SDD	0,01495817	0,33066933	0,0669292	0,005994006	0,01787005	0,234765235
AB during admission	0,01831876	0,18681319	0,04381511	0,05994006	0,03136523	0,014985015
AB post admission	0,01392735	0,42757243	0,0270532	0,35964036	0,03040475	0,008991009
LOS	0,02423924	0,05094905	0,0423465	0,072927073	0,02780047	0,03996004
CAO	0,01122007	0,63036963	0,04767171	0,045954046	0,02805348	0,017982018

Level 3: Percentage of variance explained by all the variables together at T2

Supplementary material 5

Level 1

Taxon	Variable	Coefficient	P-value adjusted
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Dorea	BMI	0.0018515	0.0450689
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Veillonellaceae.g_Acidaminococcus	BMI	0.001023	0.0492613
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Veillonellaceae.g_Dialster	BMI	-0.0034544	0.0450689
k_Bacteria.p_Firmicutes.c_Clostridia.o_SHA-98.f_g	BMI	-0.0001806	0.0106904
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Anaerostipes	AB during admission	-0.0146447	0.0491344
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Clostridiaceae.Other	LOS	0.0006735	0.039512
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g	LOS	-0.0016093	0.039512
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_[Tissierellaceae].g_Parvimonas	LOS	0.0002863	0.0000835
k_Bacteria.p_Proteobacteria.c_Gammaproteobacteria.o_Enterobacteriales.f_Enterobacteriaceae.g_Klebsiella	Chronic kidney failure	0.1204868	0.0000208
k_Bacteria.p_Proteobacteria.c_Gammaproteobacteria.o_Enterobacteriales.f_Enterobacteriaceae.g_Salmonella	Chronic kidney failure	0.0232286	0.0041082
k_Bacteria.p_Actinobacteria.c_Corinobacteria.o_Corinobacteriales.f_Corinobacteriaceae.Other	Solid malignancy	0.0199917	0.0149742
k_Bacteria.p_Actinobacteria.c_Corinobacteria.o_Corinobacteriales.f_Corinobacteriaceae.g	Solid malignancy	0.0444445	0.0149742
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_[Paraprevotellaceae].g_[Prevotella]	Solid malignancy	0.030854	0.0149742
k_Bacteria.p_Firmicutes.c_Bacilli.o_Lactobacillales.f_g	Solid malignancy	0.0129797	0.0302962
k_Bacteria.p_Actinobacteria.c_Corinobacteria.o_Corinobacteriales.f_Corinobacteriaceae.g_Stickia	Immunosuppression	0.0303001	0.0482438
k_Bacteria.p_Firmicutes.c_Bacilli.o_Lactobacillales.f_g	Immunosuppression	0.0332543	0.0000007
k_Bacteria.p_Firmicutes.c_Bacilli.o_Lactobacillales.f_Lactobacillaceae.g_Lactobacillus	Immunosuppression	0.1074336	0.0342592
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Peptococcaceae.g_Peptococcus	Immunosuppression	0.0370453	0.0328613
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.Other	Immunosuppression	0.0286804	0.0328613
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.g_Bulleidia	Immunosuppression	0.0407885	0.0328613
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.g_Catenibacterium	Immunosuppression	0.2118586	0.0000001
k_Bacteria.p_Proteobacteria.c_Gammaproteobacteria.o_Enterobacteriales.f_Enterobacteriaceae.g	Immunosuppression	0.1530103	0.0342592

Level 2

Taxon	Variable	Coefficient	P-value adjusted
k_Bacteria.Other.Other.Other.Other	SDD	0.0433318	0.0004437
k_Bacteria.p_Bacteroidetes.Other.Other.Other	SDD	0.0184851	0.0027675
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.Other.Other	SDD	0.03071	0.0431657
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_Prevotellaceae.Other	SDD	0.0035968	0.0431657
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_Rikenellaceae.Other	SDD	0.0051633	0.0425128
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_S24-7.g	SDD	0.0276482	0.0431657
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_[Paraprevotellaceae].g	SDD	0.0096511	0.0497072
k_Bacteria.p_Firmicutes.c_Bacilli.o_Lactobacillales.f_Carnobacteriaceae.g_Granulicatella	SDD	-0.0058821	0.0425128
k_Bacteria.p_Firmicutes.c_Clostridia.Other.Other	SDD	0.0087109	0.0431657
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Peptococcaceae.g_Peptococcus	SDD	0.0183843	0.0497072
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Ruminococcaceae.Other	SDD	-0.1082604	0.0425128
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Ruminococcaceae.g_Ruminococcus	SDD	-0.0776355	0.0497072
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.g_Bulleidia	SDD	0.0247354	0.0425128
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.g_Catenibacterium	SDD	0.0700017	0.0377014
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.g_Coprobacillus	SDD	-0.0267016	0.0227068
k_Bacteria.p_Proteobacteria.Other.Other	SDD	0.0066063	0.0425128
k_Bacteria.p_Proteobacteria.c_Betaproteobacteria.o_Burkholderiales.f_Oxalobacteraceae.g_Ralstonia	SDD	0.0053997	0.0442837
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfobivionales.f_Desulfobivriaceae.Other	SDD	0.005289	0.0425128
k_Bacteria.p_Proteobacteria.c_Gammaproteobacteria.o_Pseudomonadales.f_Moraxellaceae.g_Acinetobacter	SDD	0.0150882	0.0460991
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.g	AB post admission	0.0373258	0.0316047
k_Bacteria.Other.Other.Other	AB during admission	0.0288833	0.0199346
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Anaerostipes	AB during admission	-0.0175626	0.0199346
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Ruminococcaceae.g_Ruminococcus	AB during admission	-0.0839528	0.0199346
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Veillonellaceae.g_Megasphaera	AB during admission	0.0156027	0.0199346
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Clostridiaceae.Other	LOS	0.0009802	0.0021279
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g	LOS	-0.0021579	0.0091426
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Veillonellaceae.g_Veillonella	LOS	0.000529	0.0386213
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_[Tissierellaceae].g_Parvimonas	LOS	0.0004382	0.0000006

Supplementary material 6

Taxon	Linear component		Quadratic component	
	coefficient	p-value	coefficient	p-value
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Roseburia	-0.010814961	0.352175714	0.131724283	1.84847E-15
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.g_Coprobaecillus	0.002927002	0.226185358	-0.021636933	5.0746E-11
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Enterococcaceae.Other	-0.000324667	0.916808115	-0.024542753	1.25275E-09
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Christensenellaceae.g	-0.000702394	0.888984222	-0.037074313	7.25994E-08
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Blautia	-0.0013349	0.884036306	0.065901342	1.11686E-07
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Clostridiaceae.g_Clostridium	-0.001104506	0.747814719	0.024075272	1.32535E-07
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Aerococcaceae.g_Abiotrophia	-5.08628E-05	0.901435339	-0.002684981	3.30211E-07
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Christensenellaceae.g_Christensenella	2.8881E-05	0.973153215	-0.005654435	5.76676E-07
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Enterococcaceae.g_Enterococcus	-0.002010189	0.852529843	-0.068072716	7.79164E-07
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Streptococcaceae.g_Lactococcus	-0.000620758	0.664290189	-0.008825978	1.58016E-06
k_Bacteria.p_Actinobacteria.c_Corinobacteria.o_Corinobacteriales.f_Corinobacteriaceae.g_Fagerthella	0.002319367	0.109242102	-0.009041801	2.2437E-06
k_Bacteria.p_Actinobacteria.c_Actinobacteria.o_Bifidobacteriales.f_Bifidobacteriaceae.g_Bifidobacterium	0.009624585	0.423278559	-0.0753252	2.60585E-06
k_Archaea.p_Euryarchaeota.c_Methanobacteria.o_Methanobacteriales.f_Methanobacteriaceae.g_Methanobrevibacter	-0.000970765	0.292524203	-0.005402888	8.53159E-06
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g	-0.018573164	0.001474051	0.033884991	1.04352E-05
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Anaerostipes	-0.004990285	0.01401567	0.011070139	2.37767E-05
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Carnobacteriaceae.g_Granulicatella	0.000399485	0.542103974	-0.003586192	2.40013E-05
k_Bacteria.p_Actinobacteria.c_Actinobacteria.o_Actinomycetales.f_Micrococcaceae.g_Rothia	0.000305243	0.788739801	-0.006206001	3.47828E-05
k_Bacteria.p_Firmicutes.c_Bacilli.Other.Other.Other	0.001234206	0.428949911	-0.008187924	3.69267E-05
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_[Odoribacteriaceae].g_Butyricimonas	-0.000141694	0.882964888	-0.005107318	6.48255E-05
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Ruminococcaceae.g_Faecalibacterium	-0.00370653	0.738314647	0.058049224	7.509E-05
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Eubacteriaceae.g_Pseudoramibacter_Eubacterium	0.000845045	0.422901495	-0.00519389	0.000155269
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Veillonellaceae.g_Dialister	2.85281E-07	0.9999528	0.023729933	0.00024393
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Lachnospira	-0.006368446	0.123043475	0.01974404	0.000245398
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Leuconostocaceae.g_Leuconostoc	-0.00019705	0.719054032	-0.002590232	0.00030169
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Dorea	-0.00884678	0.016911592	0.016868535	0.00307814
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Lachnobacterium	-0.00400469	0.132964663	0.01227291	0.000374325
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Lachnospiraceae.g_Coproccoccus	-0.011650665	0.125598965	0.033989241	0.000427433
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Streptococcaceae.g	0.000741446	0.568621252	-0.00568806	0.000442484
k_Bacteria.p_Verrucomicrobia.c_Verrucomicrobiae.o_Verrucomicrobiales.f_Verrucomicrobiaceae.g_Akkermansia	-0.001500496	0.688897992	-0.01664749	0.000644471
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_Porphyromonadaceae.g_Parabacteroides	-0.001443458	0.761365683	-0.020863148	0.00071841
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Peptococcaceae.g_Peptococcus	-0.001776645	0.269331435	-0.006947401	0.001089955
k_Bacteria.p_Firmicutes.c_Erysipelotrichi.o_Erysipelotrichales.f_Erysipelotrichaceae.Other	0.001169462	0.374103354	-0.005515548	0.001524575
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_[Tissierellaceae].g_Finegoldia	-0.000196396	0.706984168	-0.002072275	0.001574015
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.Other.Other	0.003426616	0.155379439	-0.009422956	0.001802991
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_[Barnesiellaceae].g	0.000143855	0.951647994	-0.009380793	0.002656757
k_Bacteria.p_Actinobacteria.c_Corinobacteria.o_Corinobacteriales.f_Corinobacteriaceae.g_Collinsella	-0.002280028	0.633438288	0.018732329	0.00286587
k_Bacteria.p_Bacteroidetes.c_Bacteroidia.o_Bacteroidales.f_Rikenellaceae.g	0.004191353	0.339586339	-0.016723699	0.003329481
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Ruminococcaceae.g_Anaerotruncus	0.001246739	0.097478266	-0.002882021	0.006279703
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Eubacteriaceae.g_Anaerofustis	0.00472923	0.353065942	-0.001768914	0.00655898
k_Bacteria.p_Firmicutes.c_Clostridia.o_Clostridiales.f_Peptococcaceae.g	-0.000926676	0.028480596	0.001022389	0.038167095
k_Bacteria.p_Firmicutes.c_Bacillo_o_Lactobacillales.f_Lactobacillaceae.Other	0.002161628	0.000320197	0.000645383	0.377706633
k_Bacteria.p_Tenericutes.c_Mollicutes.o_RF39.f.g	-0.005967977	0.000542106	0.000726466	0.737776101

CHAPTER 6

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

BURDEN OF INFECTION AND MULTI-RESISTANT MICROORGANISMS IN THE CRITICAL CARE SETTING

Our main findings, when assessing the burden of Highly Resistant Microorganisms (HRMOs) in critical care patients in an area where HRMOs are non-endemic, were that a small but non-negligible proportion of patients was HRMO-positive on the ICU, and that the majority of these were found to be positive on admission; i.e. the majority of HRMOs were imported and not acquired on the ICU. The incidence of HRMOs in this cohort is in line with surveillance data throughout Europe, reflecting the Netherlands to be a country of low levels of antibiotic resistance, although these levels are rising¹. Also, the finding that Gram-negative HRMOs predominate over Gram-positives conforms to known trends throughout European ICUs². The higher mortality observed in HRMO-positive patients might be explained by two reasons. First of all, patients who are more severely ill, and thus have a greater risk of dying, are more likely to have known risk factors for HRMO-carriage, including antibiotic use and longer length of hospital stay³⁻⁷. Secondly, infections with a resistant microorganism pose an increased risk to mortality compared to infections with a susceptible microorganism^{8,9}. The significant finding of the majority of HRMOs being imported and not acquired on the ICU in this study underscores the need for strict standard precautions in all patients, including hand hygiene, and the use of a consistent surveillance program on the ICU, as well as in specific patient groups at risk for colonisation with any HRMO from outside the ICU, either from a hospital ward or from the community¹⁰. Indeed, in current practice, patients on the hemato-oncology ward and recipients of donor organs are screened sequentially in our and other institutions. Further, other at-risk patients such as those working with specific livestock¹¹ and patients who were recently admitted to a hospital abroad¹² are screened, as well. It could be of benefit to further characterise subgroups at risk of colonisation with an HRMO¹², to allow for optimal infection control and antibiotic stewardship. Further advantages of surveillance include knowledge of local epidemiology of antibiotic resistance, and of trends in resistance rates over time. Sharing data on resistance between local, national and international institutes does necessitate uniform definitions of antimicrobial drug-resistance, including the various degrees 'multi-drug'-resistance, 'extensive'-drug resistance, and 'pan-drug'-resistance, and of susceptibility and breakpoints as outlined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)^{13,14}.

A drawback of surveillance could be the overuse of last-resource antibiotics such as carbapenems¹⁵. When a carrier of an HRMO pathogen develops any nosocomial infection, treating physicians might be inclined to take into account this microorganism in their empirical antibiotic choice, while it might not be the causative microorganism of the infection. This choice is understandable because colonisation with for instance ESBL-producing *Enterobacteriaceae* is a risk factor for infection with the same microorganism¹⁶. On the other hand, only a minority of carriers of ESBL-producing *Enterobacteriaceae* will develop an infection with this pathogen^{17,18}. Further, once

broad empirical therapy has been started, carriage of an HRMO appears to be a reason for non-escalation^{19,20}. It would be of great value to be able to safely predict ensuing infection with this microorganism, as this would lead to a large decline in carbapenem use without compromising therapeutic efficacy. Thus far, studies assessing risk factors for developing an infection caused by an HRMO in HRMO-carriers have not yielded a reliable prediction rule^{18,21}. Hopefully, the advent of rapid diagnostics, such as PCR and MALDI-TOF, will prove to be an important development in adequate antimicrobial stewardship including prevention of overuse of broad-spectrum antibiotics in the years to come^{15,22}. In general, employing an active antimicrobial stewardship program with a multidisciplinary strategy is a prerequisite to ensure wise use of antimicrobial therapy, aiming to combat further emergence of antimicrobial resistance, or ideally, to reduce antimicrobial resistance⁶. Within such a multidisciplinary program, in some stable patients, a 'watchful waiting' strategy may be justified as reviewed by Denny *et al.*²³, while a potential infection source and causative microorganism are being evaluated and subsequent adequate source control is being ensured. Further research should evaluate selection of eligible subgroups, safety and benefit of such an antibiotic-sparing approach²³. In recent years the benefit of strict contact precautions for those with HRMO-carriage or -infection in the prevention of cross-transmission has been debated in several publications^{15,24-27}. In a European multi-centre study by Derde *et al.*, universal screening for multi-drug resistant *Enterobacteriaceae*-carriage with subsequent contact precautions for carriers did not appear to have a protective effect on acquisition rates. This intervention was done after a phase of implementing hand hygiene compliance (rising from 52% to 77%) and including use of chlorhexidine body-washing²⁴. In a recent study²⁶, an isolation strategy of contact precautions using a multiple-bed room appeared to be non-inferior to a strategy of contact precautions in a single-bed room for the prevention of transmission of ESBL-producing *Enterobacteriaceae*. This large multi-centre cluster-randomised, cross-over study was done on medical and surgical wards in the Netherlands. Notably, in both studies, cross transmission did occur, with risk of transmissibility dependent on the microorganism involved. For instance, in the cited cohorts non-*E.coli Enterobacteriaceae* (mainly *K.pneumoniae*) appeared to be 3 to 3.7 times more transmissible than *E.coli*^{26,28}. Despite controversies, contact precautions are consistently employed in Dutch hospitals as issued by a national guideline²⁹. It must be noted that the studies questioning contact precautions do assume or take into account adequate adherence to standard precautions. That said, while the acquisition and transmission of different HRMOs may be extremely complex¹⁰, one of the cornerstones of prevention seems to be the most simple of all, yet its application unfortunately appears not to be so; the compliance with handhygiene³⁰, despite all efforts, is still frighteningly low^{31,32}. Amidst all high-tech novelties, the optimal adherence to hand hygiene should be priority number one on the ICU.

Further studies on optimal use of contact precautions in, and cohorting of (ICU-)patients are warranted, also in the light of patient safety³³ and efficient use of the wards.

Further, because the intestinal microbiota is the main reservoir for multi-drug resistant Gram-negatives and vancomycin-resistant enterococci^{34,35}, influencing the composition of the intestinal microbiota, as is already done using non-absorbable antibiotics in the context of Selective Digestive tract Decontamination (SDD) in ICU patients^{36,37}, can be considered an important adjunctive to infection control³⁵. SDD is aimed at the prevention of secondary colonisation with Gram-negative bacteria, *S. aureus* and yeasts through application of nonabsorbable antimicrobial agents in the gastrointestinal tract and the oropharynx, the pre-emptive treatment of possible infections with commensal respiratory tract bacteria through systemic treatment with a cephalosporin for four days, while maintaining anaerobic gut bacteria through selective use of antibiotics³⁶. SDD was shown to reduce mortality compared to standard care in ICU patients in a setting of low prevalence for antimicrobial resistance³⁶ and is a widely employed infection-prevention measure in the Netherlands. In a large Dutch ICU cohort treated with SDD a reduction in rectal carriage of Gram-negatives was seen, as can be expected³⁶. In the same SDD-treated cohort, eradication of cephalosporin-resistant *Enterobacteriaceae* during ICU-stay was achieved in 73 % of carriers³⁷. In a post hoc analysis of a multi-centre cluster randomised trial involving 8,665 patients on 13 European ICUs with moderate-to high prevalence of antibiotic resistance (> 5 % of Gram-negative bacteraemia in ICU caused by ESBL in 2011), SDD (without systemic antibiotics) was associated with more eradication and less acquisition of third-generation cephalosporin-resistant Enterobacterales and carbapenem-resistant Gram-negative bacteria in the rectum than standard care³⁸. In this same cohort, however, the use of SDD, SOD or chlorhexidine mouthwash was not associated with reductions in ICU-acquired bloodstream infections caused by multi-drug resistant Gram-negatives compared with standard care³⁹. In contrast, in a prospective before-after study in Spain, a country with high prevalence of antimicrobial resistance, long-term use of SDD was associated with a reduction of infections caused by multi-drug resistant bacteria⁴⁰. In Israel, highly endemic for antimicrobial resistance, eradication of carbapenem-resistant *Klebsiella pneumoniae* rectal carriage was described in hospitalised patients⁴¹. Even Fecal Microbiota Transplantation (FMT), nowadays a well established treatment option in multiple recurrent *Clostridium difficile* infection (CDI)⁴², has been described as a means of decolonisation in HRMO carriers^{43,44}. Although routine decolonisation in carriers of multi-drug resistant bacteria is not advised by experts⁴⁵, some vulnerable subpopulations might benefit from such a strategy. More research is needed to further assess possible methods, target populations, benefits and risks of eradication of multi-drug resistant Gram-negative bacteria⁴⁵. It is an interesting finding that in some studies using sequencing methods to assess the composition of fecal microbiota, increased density, or relative abundance, of an HRMO, for instance through loss of colonisation resistance after antibiotic use, increases the risk of its spread in the environment, thus increasing risk of patient- to patient transmission⁴⁶, as well as increasing the likelihood of emergence of infection with this pathogen^{15,46,47}.

Another important realisation is that the impact of antibiotics on the composition of intestinal microbiota differs, even within the same antibiotic class, probably due to varying

pharmacokinetics^{46,48,49}. These findings might have consequences for antibiotic stewardship strategies.

OPTIMAL TREATMENT OF INFECTIONS WITH BETA-LACTAMS

The results of our studies on pharmacokinetics of piperacillin and cefotaxime are in line with and complementary to previous publications; continuous dosing yields higher plasma concentrations, although even when using this strategy there is a serious risk of target non-attainment, especially in those with normal or augmented renal clearance. Augmented renal clearance is a phenomenon often seen in the critical care population^{50,51}, but only partially understood^{50,52}.

It must be noted that in our PK studies, PK/PD targets were defined on rational grounds but not according to any international consensus complicating a comparison with results from other PK/PD studies on beta-lactams. In fact, no such consensus exists. In available literature, a wide array of PK/PD indices as target are used^{53,54}, ranging from $20\%fT_{>1 \times MIC}$ to $100\%fT_{>5 \times MIC}$ ⁵⁴, depending on the aim of respective studies; clinical cure and/or minimisation of risk of emergence of antibiotic resistance. Several experts now advocate a target of $100\%fT_{>4 \times MIC}$ in critical care patients to pursue both goals^{54,55}. Usually, a presumptive 'worst case' scenario MIC derived from the EUCAST data is used, as we did. To further complicate comparisons, very heterogeneous study populations are described in PK/PD studies⁵⁴.

In anticipation of results from a large multicentre trial comparing continuous versus intermittent dosing of beta-lactams in a critical care population powered on all-cause 90-day mortality which is currently recruiting patients⁵⁶, and assuming PK/PD targets to be a surrogate for clinical outcome, available literature as well as our own findings seem to endorse continuous dosing of beta-lactams in critical care patients. Furthermore, *a priori* higher dosing should be considered, especially in those with normal or augmented renal clearance, considering a high PK/PD target is to be met, as standard dosing administered by continuous infusion did not lead to adequate target attainment in our cohorts, nor in observations by others⁵⁷. Also, higher dosing might be considered in the obese; although we did not find such an association, several publications recognised obesity as a risk factor for underdosing⁵⁸⁻⁶⁰. However, even higher dosing does not preclude the risk of target non-attainment as shown very recently by Dhaese *et al.*⁶¹; in this study, plasma concentrations of piperacillin on the first two days of treatment were assessed in patients treated with 16 g/ 24h continuously dosed piperacillin, to be adjusted according to renal function. Achievement of target concentrations, defined as unbound piperacillin concentrations between 64 and 160 mg/L, was reached in only 76 of 205 (37.1%) patients. Therapeutic drug monitoring (TDM), in combination with modeling and dose adjustment, is an attractive tool to further optimise beta-lactam dosing⁶². When applying TDM for piperacillin or cefotaxime, based on our findings, measurement of total piperacillin or cefotaxime concentrations should

suffice, as there was a low variability in the free fraction percentage and for cefotaxime, no accumulation of its metabolite desacetylcefotaxime was found. The data from both of our studies using dense sampling could help refine modeling in a critical care cohort. This would not only apply for a local initiative, but also between centres; in the era of data-sharing, cohorts like ours can be used for further refinement and/or validation of models and in my opinion, this sharing should be implemented optimally in research communities. This applies especially to datasets of patients treated with continuously dosed beta-lactams, as PK parameters may be different when continuous dosing instead of intermittent dosing is used, and models based on intermittent dosing may not be representative for continuous dosing⁶³. In a publication by Dhaese *et al.*⁶⁴, saturation of piperacillin (renal) elimination was assessed in ICU patients receiving both continuous and intermittent dosing of piperacillin. In this study piperacillin elimination was found to be -partially- saturable and the population estimate for saturation appeared to be within the range of therapeutic plasma concentrations. This might imply that at the same daily cumulative piperacillin dose, continuous dosing leads to a lower total antibiotic exposure compared to intermittent dosing. This finding merits further evaluation.

Ideally, when using TDM, real-life MICs are taken into account for personalised dosing. To ensure fast results on finding a causative pathogen and its susceptibility, which is appropriate in the setting of critical illness, again, as mentioned above, the wider implementation of microbiological rapid diagnostics (e.g. multiplex PCR tests and rapid whole genome sequencing) could be advantageous in the near future^{65,66}. Both of these interventions, using TDM and delivering microbiological results, would require a multidisciplinary team effort between intensivist, pharmacist, and microbiologist.

Although underdosing of beta-lactams is the most common problem in the critical care population, and beta-lactams are generally considered to have a large therapeutic range, another motivation for TDM is the possibility of exposure to excessive drug levels, especially when using higher antibiotic dosing^{62,67}.

In the years to come, further studies and developments are needed to optimise beta-lactam dosing in the critical care population; first of all, the previously cited large multi-centre study comparing continuous to intermittent dosing of beta-lactams in ICU patients powered on 90-day mortality⁵⁶ is much awaited. Second, the shift in paradigm from 'one dose fits all' to highly individualised dosing⁶⁸ is now in its infancy. Many issues remain to be resolved and are suggestions for further research questions. These include: Which subgroups of ICU patients are most likely to benefit from TDM? What is the optimal *a priori* dosing of the different beta-lactams, taking patients characteristics such as creatinine clearance and body weight as well as dosing strategy into account? What is the exact mechanism of augmented renal clearance, and how can this phenomenon be anticipated on

in an optimal manner? How can the use of TDM using modeling be implemented in daily practice? Is the *summum* of personalised dosing, i.e. adaptive Target Controlled Infusion (aTCI)⁶⁹, feasible in the near future for optimal dosing of beta-lactams? How can scientific communities benefit most from data-sharing when building/ validating pharmacokinetic models? Can, or even should, we use real MICs in PK/PD target attainment and how can this be implemented in daily practice? Is the use of tissue sampling instead of plasma concentrations preferable and what PK/PD targets should be applied in that case?

In conclusion, a big challenge lies ahead to integrate all existing and future knowledge on PK/PD principles of beta-lactam dosing, including the use of TDM and integrated modeling using sophisticated software, to a pragmatic approach at the bedside. These developments hold a great promise for further optimisation of the treatment of infections in this vulnerable population, aiming to both improve clinical outcome and prevent emergence of antibiotic resistance.

DYNAMICS OF INTESTINAL MICROBIOTA IN CRITICAL CARE PATIENTS; WHAT HAPPENS AND HOW CAN THIS BE RELEVANT?

The findings of our study on the dynamics of fecal microbiota in cardiac surgery patients, with a decrease in relative abundance of strictly anaerobic bacteria and concurrent increase in relative abundance of potentially pathogenic bacteria, including enterococci, adds to the observations made by other research groups⁷⁰⁻⁷⁶, since our cohort represents planned surgery patients as opposed to acute admissions as included in other studies. The reversal of the microbiota in most patients to baseline composition after hospitalisation demonstrates its great restorative capacity. Although SDD is partially aimed at *Enterobacteriaceae*, no significant change in their abundance was seen in the patients treated with SDD. This is likely explained by the sample size in combination with the low relative contribution of *Enterobacteriaceae* to the gut microbiota composition in general.

The clinical consequences of the observed dynamics are not yet clear and should be assessed in further studies. We postulated the decrease in abundance of butyrate producers, such as *Faecalibacterium*, during hospital stay to have a direct impact on clinical outcome, considering their anti-inflammatory properties⁷⁷ and their role as energy provider for colonocytes⁷⁸. Dysbiosis was previously found to have an association with adverse outcome; in a cohort of medical ICU patients, *Enterococcus* domination ($\geq 30\%$ relative abundance) in the intestinal microbiota at ICU admission was associated with increased risk of infection and all-cause mortality, for instance⁷⁹. Similar associations were seen in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). In a cohort of 94 allo-HSCT patients, enterococcal domination increased the risk of vancomycin-resistant *Enterococcus* bacteraemia by 9-fold⁴⁷. Of note, this study had a longitudinal set-up comparable to ours, with base-line

sampling before transplantation occurred, followed by subsequent sampling until 35 days post-transplantation. The relevance of the composition of intestinal microbiota in this distinct patient group, challenged by long-lasting immunosuppression, mucosal damage and high vulnerability to infections, frequently necessitating ICU admission⁸⁰, and closely monitored before, during and after intervention, is now recognised^{81–84}. In the above-mentioned study by Taur *et al.* involving allo-HSCT patients⁴⁷, a very interesting finding is described: occurrence of intestinal domination by a pathogen preceding bloodstream infection by a mean of seven days. If the turn-around time of sequencing diagnostics would be fast, this could enable clinicians to anticipate or even intervene on these findings in the future. To investigate the relevance of duration of dysbiosis and possible associated infectious complications would be an interesting research objective in a larger-scale study including critical care patients followed longitudinally.

We hypothesise that, in case of elective surgery, influencing or preventing a state of dysbiosis before surgery might lead to a better outcome. From a mechanistic view^{77,78}, boosting anaerobic butyrate-producing bacteria by giving pre- or probiotics could leverage the composition of intestinal microbiota and this might prevent ensuing dysbiosis from interventions such as surgery itself and associated therapies including antibiotics, which, in turn, might be able to prevent infectious complications. Even optimising a patient's diet, by avoiding typical Western-style diet components such as sugar-sweetened soda and high-energy foods, while increasing dairy consumption, might ameliorate the composition of intestinal microbiota⁸⁵. Although more invasive, in theory, FMT is another way of optimising the composition of intestinal microbiota⁸⁶. However, our data do not allow to state whether baseline microbial composition could influence outcome or whether a state of dysbiosis is a cause or effect of illness.

Therefore, it is first necessary to better understand the complex interaction between the intestinal microbiota and the host. With many confounding factors in a highly heterogeneous ICU population, it is logical to perform conceptual studies in a laboratory setting using animal models or in an *in vivo* setting using healthy volunteers. Efforts to elucidate this complex interaction between host and its intestinal microbiota in the preclinical setting have led to very interesting insights as reviewed by Haak *et al.*⁸⁷, but many questions about the impact of dysbiosis in the critical care patient remain unanswered⁸⁸. For instance, a direct causality between dysbiosis and adverse outcome in critical care patients is not yet clear. In our cohort, we observed patients to develop dysbiosis while they had an uneventful clinical course. As mentioned above, we do not know whether the duration of disturbance in a patient's microbiota is an important factor as there are no large-scale studies in the clinical setting which have a longitudinal set-up. Most of our patients reverted to their base line composition post-discharge. Further, in our small cohort, we found no association between low bacterial diversity at baseline and adverse outcome. Second, it would therefore be relevant to know whether the intestinal microbiota could be used

as a prognostic marker and would thus enable 'profiling' patients before or during ICU admission to determine whether they are at risk of an adverse outcome in terms of infectious complications. To further assess the role of the composition of microbiota and possible contributing factors to the emergence of infections in critical care patients, it may be interesting to evaluate a larger cohort in a longitudinal set-up. For instance, data - including fecal samples - from the LifeLines cohort⁸⁵, a large cohort comprising participants from the general population in the northern Netherlands, could be used to further investigate the role of microbiota composition in a case-control design by selecting participants who develop sepsis leading to ICU admission and compare these with matched controls. Another study set-up could be to longitudinally follow participants with dysbiosis from LifeLines (or a comparable large population-based cohort⁸⁹) and compare those with matched controls defining infectious complications as endpoint, although for both set-ups a very large sample size would probably be needed. In further studies, however, the heterogeneity of both the sepsis syndrome⁹⁰ and the critical care population admitted with this diagnosis⁹¹ (or any critical care population) complicates studies evaluating causality. It might be of relevance to make a distinction between recently proposed sepsis phenotypes, based on host-response patterns⁹⁰ in studies on the composition of intestinal microbiota in sepsis patients on the ICU. Also, other specific patient characteristics could influence microbe-host interactions, and this needs to be evaluated in studies to come.

In an attempt to 'profile' patients for risk of infectious complications based on 'dysbiosis', it may also be of relevance to further define or subdivide 'dysbiosis'. In a study referred to above aiming to describe the clinical relevance of pathogen colonisation in ICU patients at admission, especially *Enterococcus* domination was associated with risk of death or all-cause infection⁷⁹. Further studies in critical care populations are warranted to enable further risk stratification and select those who might benefit from leverage of the intestinal microbiota composition in a personalised way. To this end, the use of rectal swabs instead of feces samples would, if handled and stored properly, simplify sampling options in large-scale studies, including planned and acute patients, without compromising quality of results⁹². Also, hopefully, developments of diagnostics will render sequencing less costly and faster in the near future. This would be particularly relevant when interventions based on findings from sequencing are considered.

Last, the next important and obvious research question would be whether optimisation of the composition of microbiota could be of benefit, indeed, and if so, to which subgroups this would apply and by what means this should be accomplished, as suggested above.

The use of SDD as a decolonisation strategy, thereby influencing microbiota composition, as described above, is an established infection-prevention measure already, at least in the Netherlands³⁶. The concept of SDD combined with observed benefits in ICU patients might imply

that other patient groups at high risk for infection could benefit from this strategy. Indeed, SDD is used on hemato-oncology wards in the Netherlands, as well. Further, use of SDD in elective gastro-intestinal surgery patients has been investigated^{93,94}. In a randomised controlled trial in the Netherlands, use of SDD in elective gastro-intestinal surgery patients showed less infectious complications in the SDD group compared to placebo⁹³. Further assessment of subpopulations who might benefit from SDD should follow. That said, paradoxically, the use of SDD was shown to significantly suppress the butyrate-producing *Faecalibacterium prausnitzii*, which plays an important role in maintaining colonisation resistance, without a compensating increase in other butyrate-producers⁹⁵. This finding suggests that more research on the exact impact of SDD on the intestinal microbiota is needed, and the composition of microbiota should thus be taken into account in further studies on SDD.

Many studies on probiotics (living non-pathogenic microorganisms) and synbiotics (probiotics combined with prebiotic fibres) in critical care patients in the prevention of infections have been conducted, as reviewed by Morrow *et al.*⁹⁶ and Manzanares *et al.*⁹⁷. A reduction in ventilator-associated pneumonia in a meta-analysis by Manzanares *et al.* was found⁹⁷, but without effect on mortality. Heterogeneity of populations, treatment modalities, sample size and publication bias hamper firm conclusions. Further studies should also evaluate the actual effect of probiotics on the intestinal microbiota, incorporating (longitudinal) sequencing of fecal material in the study set-up. Also, target subpopulations which could potentially benefit should be defined.

As previously mentioned, FMT as a means of optimising (or: replacing) the patient's intestinal microbiota should be noted. FMT treatment leading to a favourable outcome was described in some patients with sepsis-induced multiple organ failure^{98,99}.

May be, in the future, restoration of the intestinal microbiota using FMT could prove to be a treatment adjunctive in select patients who develop either severe distortion of the composition of intestinal microbiota during ICU stay or who are found to be carriers of multi-drug resistant bacteria³⁵, while at high risk of acquiring infections. Even an autologous FMT³⁵ could be an option when such dynamics in intestinal microbiota can be anticipated, such as in elective major surgery patients or allo-HSCT patients. In 2015, a Dutch stool bank was founded under strict regulations, aiming to provide a standardised product in the treatment of recurrent CDI¹⁰⁰. In this line, a stool bank for autologous FMT could be a future development as suggested by Tosh *et al.*³⁵, should FMT prove to be both beneficial and safe in hypothesised indications.

Answering all these questions on the composition of fecal microbiota and its dynamics holds very promising possibilities in the prevention and treatment of infections in ICU patients.

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CHAPTER 7

SUMMARY

Infections and related sepsis are an enormous burden on the intensive care¹⁻³, with substantial mortality⁴ and long-term sequelae in survivors^{5,6}.

In this thesis, some aspects of three large topics on the prevention and treatment of infections in intensive care patients were addressed. Despite a huge body of research on sepsis, defined as life-threatening organ dysfunction caused by a dysregulated host response to infection^{7,8}, many questions on optimal prevention and treatment of infections with related sepsis are still unanswered⁸. In this thesis, we aimed to evaluate three specific issues on antibiotic resistance, pharmacokinetics of beta-lactams, and the composition of intestinal microbiota in the critical care patient. The goal of these objectives is to improve prevention and treatment of infections in the critical care setting.

First, in **Chapter 2**, the burden of Highly Resistant Microorganisms (HRMOs) in an Intensive Care Unit (ICU) for adult patients in a university hospital in the Netherlands was assessed. We designed this single-centre prospective study as the exact burden of HRMOs in ICUs in our region was not known, while the occurrence of HRMOs is recognised as a major threat to critical care patients⁹, leading to worse outcomes, need for isolation measures, and demand for second-line or rescue antibiotics¹⁰⁻¹². The evaluation was done by analysing the local distribution of different HRMO-categories and proportion of ICU-imported versus ICU-acquired HRMOs, through collection of results from cultures taken by indication or in the context of a structured surveillance program over a four-month period. All patients with an anticipated stay of 48 hours or longer or those transferred from another medical centre are included in the surveillance program. This program comprises obtaining cultures from throat, rectum, and, when patients are mechanically ventilated, sputum twice weekly. Further, screening for VRE is performed by PCR-testing once every week, and screening for MRSA using PCR is done once on admission. When a urinary catheter is in place, a urine culture is obtained on admission, as well. Also, baseline characteristics and outcome of HRMO-positive patients versus patients without HRMO were compared.

We found that a small but relevant proportion of patients were HRMO-positive during ICU-stay. Most HRMOs were Gram-negative microorganisms. In this study population, the majority of HRMOs was imported and not acquired during ICU-stay. HRMO-positive patients had significantly higher APACHE scores, longer length of stay in the ICU and higher mortality compared to those without HRMO. Limitations of this study were that patients with a short stay were not enrolled in the surveillance program; in these patients, incidence of colonisation with HRMOs was thus not assessed. Further, no distinction was made between HRMO-colonisation and HRMO-infection. Finally, the fact that data of the study were collected 10 years ago and the single-centre set-up limit the study's generalisability, but the results were in line with current European surveillance

data, indicating the Netherlands to be a country with low levels of antibiotic resistance.

Second, we evaluated the pharmacokinetics of the beta-lactams piperacillin and cefotaxime in adult critical care patients. Adequate antibiotic dosing is crucial in critical care patients to pursue good clinical outcome and to prevent the emergence of antibiotic resistance¹³⁻¹⁵. Many publications on pharmacokinetics/ pharmacodynamics (PK/PD) of beta-lactams have shown better PK/PD target attainment when using prolonged or continuous dosing in ICU patients¹⁶⁻¹⁸, and better clinical outcome using this strategy compared to intermittent infusion is suggested in meta-analyses¹⁹⁻²¹.

Although literature on pharmacokinetics of beta-lactams is now abundant, studies involving a heterogeneous ICU-population treated with continuously dosed piperacillin or cefotaxime using dense sampling specifically are scarce.

In **Chapter 3**, we evaluated both total and unbound plasma concentrations of piperacillin in adult critical care patients. In this prospective single centre study, patients with proven or suspected infection with indication for treatment with piperacillin/tazobactam (P/T) as determined by the treating physician were included. All patients were treated with continuously dosed P/T. They all received a loading dose of 4/0.5 g P/T, immediately followed by 12/1.5 g/ 24 hours P/T using a syringe pump. Patients with renal dysfunction received an adjusted dosing schedule after 24 hours, based on available guidelines. Nine blood samples were obtained at scheduled time points in the first 24 hours of treatment; from day two, samples were taken twice daily at fixed intervals. All samples were analysed by liquid chromatography tandem mass spectrometry. Our primary endpoint was PK/PD target attainment. We predefined a PK/PD target of 100 % $T_{>5 \times MIC}$ for total piperacillin plasma concentrations, assuming a 20-30% protein binding, using a presumed 'worst case' MIC breakpoint for *P.aeruginosa*, which is, according to the EUCAST, 16 mg/L²². The target concentration was to be reached within one hour of starting treatment, to be maintained thereafter.

We found a large intra- and interindividual variability in piperacillin plasma concentrations. We further found that only a minority of patients reached our predefined target.

In **Chapter 4**, we assessed total and unbound cefotaxime plasma concentrations as well as total and unbound plasma concentrations of the active metabolite desacetylcefotaxime in adult ICU patients. In this randomised controlled single-centre study, patients treated with cefotaxime in the context of SDD at the discretion of the treating physician and who were included in the study were given either intermittently dosed (1 g every 6 h) or continuously dosed (4 g/24 h) cefotaxime, preceded by a loading dose of 1 g. Eight blood samples were obtained at scheduled

time points in the first 24 hours of treatment; from day two, samples were taken twice daily at fixed intervals. Plasma concentrations were analysed using liquid chromatography tandem mass spectrometry. Again, our primary endpoint was PK/PD target attainment. We based a preset target on the cefotaxime MIC breakpoint for *Enterobacterales* of 1 mg/L, as determined by the EUCAST²². We combined this with the assumption of a protein binding of 25-40% and allowed for a safety margin due to variability in tissue penetration in ICU patients^{23,24}; we therefore defined target attainment as reaching a total plasma cefotaxime concentration of at least 4 mg/L within 1 hour after start of treatment, maintaining this plasma concentration thereafter.

We found that significantly more patients from the continuous dosing group than from the intermittent dosing group reached the predefined target. Further, patients in this cohort who did not reach the predefined PK/PD target had significantly higher creatinine clearance compared to those who did reach this target.

In both studies, some limitations must be highlighted. Because of the single-centre design and the fact that patients receiving renal replacement therapy or extra corporeal life support were not included, results might not be generalisable to all ICU patients. Several experts now advocate a target of $100\%fT_{>4xMIC}$ (i.e. free plasma concentration > 4xMIC for 100% of the dosing interval) in critical care patients to pursue both clinical cure and suppression of antibiotic resistance^{25,26}. The target we set in the cefotaxime cohort can be considered somewhat conservative in that light. We therefore, upon reflection, assessed target attainment using the stricter PK/PD target of $100\%fT_{>5xMIC}$ as well. The target we set in the evaluation of piperacillin concentrations (100% $T_{>5xMIC}$ for total piperacillin plasma concentrations assuming a 20-30% protein binding) conforms to the target now recommended by experts^{25,26}. Most often, as we did, presumptive MICs are employed in PK/PD studies²⁶, and not actual MICs from pathogens found in patients' specimens, the latter often not being available. Without prior knowledge of the causative microorganism, it seems logical to anticipate a 'worst-case' scenario MIC, depending on expected pathogens and their respective local resistance patterns, based on epidemiological data. The target chosen in the study on piperacillin PK/PD target attainment was also used in most other pharmacokinetic studies of piperacillin in intensive care patients²⁶. Data on cefotaxime studies including comparable critical care cohorts are too scarce to enable such a comparison. The sample size of both studies did not allow for the evaluation of clinical outcome as an endpoint.

Third, we assessed the dynamics of intestinal microbiota in a critical care cohort admitted to the ICU after cardiac surgery. Beneficial roles of the healthy gut microbiota on human physiology are complex and include nutrient metabolism, modulation of host immune responses²⁷ and protection against potential pathogens by competition²⁸. Alterations in intestinal microbiota composition, with loss of bacterial diversity, have been linked with disease states such as obesity,

inflammatory bowel disease and irritable bowel disease²⁹⁻³¹. Benefits notwithstanding, antibiotic use has a large impact on the composition of intestinal microbiota³², while many other factors inherent to ICU stay, including change in diet, non-antimicrobial drugs and invasive procedures, can also lead to disruption of the intestinal microbiota, or 'dysbiosis'^{33,34}. Despite increased recognition of the important role of intestinal microbiota on health and disease²⁸, so far, only few studies on the dynamics of the intestinal microbiota in intensive care patients using 16S rRNA gene sequencing have been published. These studies mainly involved acute admissions for organ failure caused by infections, trauma or emergency surgery, and utilised a case-control design comparing the gut microbiota composition of ICU patients and healthy controls³⁵⁻⁴¹. Generally, as hypothesised, these studies showed a rapid disruption of the gut microbiota during ICU stay with a loss of diversity and overgrowth of potentially pathogenic microorganisms. The extent and dynamics of such changes are not yet fully understood. The dynamics of the composition of gut microbiota in planned surgery ICU patients, with samples collected before and after admission as controls, had not been described before.

In **Chapter 5**, we investigated the microbiota of patients planned for cardio surgery before, during and after hospital admission. We also evaluated a correlation between the composition of intestinal microbiota and both baseline characteristics and clinical outcomes. Fecal samples collected at these three time points were analysed using 16S rRNA gene sequencing. Also, baseline characteristics and clinical outcome were documented.

We found a large intra-individual variation in the composition of intestinal microbiota. In most patients, during admission, a significant change in microbial composition was observed. Bacterial diversity, measured as Shannon index, significantly decreased during admission compared to baseline. A significant decrease in strictly anaerobic intestinal bacteria, typically beneficial for health, and a concurrent increase in potentially pathogenic microorganisms, or 'pathobionts' was observed during admission.

Further, the dynamics of *Enterobacteriaceae* in the subgroup treated with SDD was evaluated, specifically. In this subgroup, we found no consistent change in relative contribution of *Enterobacteriaceae* over time. In most patients analysed at all three time points, the change in the composition of microbiota during hospital stay reverted to baseline post-discharge. In this study, a lower bacterial diversity during admission was associated with longer hospitalisation. Also, a lower diversity post-discharge was associated with antibiotic use post-discharge.

To our knowledge, this is the first study on the dynamics of intestinal microbiota performed on planned surgery patients including samples collected before, during and after admission, which meant that the patients were their own controls. With this set-up, we wanted to overcome the

bias of the large interindividual variability^{30,42,43}. Some limitations of this study should be noted. First, the collection of fecal samples proved to be challenging, resulting in a relatively large number of missing samples. The use of 16S rRNA gene sequencing precludes profiling of bacterial strains or detection of viruses and fungi, although these may be clinically important. Further, not all factors potentially influencing the composition of intestinal microbiota or shift thereof were evaluated; for instance, use of proton pump inhibitors was not recorded³⁴. Last, disentangling individual factors contributing to disturbances in the intestinal microbiota is difficult, as some are inherently combined; a patient with septic complications leading to hemodynamic instability and organ failure will likely receive antibiotics.

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APPENDICES

**SAMENVATTING IN HET NEDERLANDS/
SUMMARY IN DUTCH
LIST OF ABBREVIATIONS
DANKWOORD/
ACKNOWLEDGEMENTS
CURRICULUM VITAE
LIST OF PUBLICATIONS**

NEDERLANDSE SAMENVATTING

Infecties en gerelateerde sepsis vormen een enorme belasting voor de intensive care¹⁻³, met aanzienlijke sterfte⁴ en langdurige gevolgen voor patiënten die overleven^{5,6}.

In dit proefschrift werden enkele aspecten van drie belangrijke onderwerpen aangaande infecties bij patiënten op de intensive care behandeld. Ondanks een zeer grote hoeveelheid onderzoek naar sepsis, gedefinieerd als levensbedreigend orgaanfalen veroorzaakt door een ontregelde respons van de gastheer op een infectie^{7,8}, zijn nog steeds veel vragen over de optimale preventie en behandeling van infecties met gerelateerde sepsis onbeantwoord⁸. In dit proefschrift stelden we als doel om drie specifieke vraagstukken te evalueren die betrekking hebben op antibioticaresistentie, farmacokinetiek van beta-lactam antibiotica en de samenstelling van intestinale microbiota bij intensive care patiënten, ten einde met kennis van onderzoeksresultaten de preventie en behandeling van infecties bij IC-patiënten te kunnen verbeteren.

Ten eerste werd in **Hoofdstuk 2** de last van Bijzonder Resistente Microorganismen (BRMO's) op een Intensive Care Unit (ICU) voor volwassenen in een universitair ziekenhuis in Nederland geëvalueerd. We hebben deze single-center prospectieve studie opgezet omdat de exacte druk van BRMO's op IC's in onze regio niet bekend was, terwijl het vóórkomen van BRMO's wordt gezien als een grote bedreiging voor de intensive care patiënt⁹, leidend tot een slechtere klinische uitkomst, noodzaak tot het nemen van isolatiemaatregelen en tot uitwijken naar tweede keus- of reserve antibiotica¹⁰⁻¹². De evaluatie werd uitgevoerd door analyse van het vóórkomen van verschillende BRMO-categorieën in dit centrum en van het aandeel van ICU-geïmporteerde versus ICU-verworven BRMO's, door het verzamelen van resultaten van kweken die waren afgenomen op klinische indicatie of in de context van een gestructureerd surveillance programma gedurende een periode van vier maanden. Routine surveillance screening wordt toegepast bij alle patiënten met een verwacht IC-verblijf van 48 uur of langer of bij IC-patiënten die worden overgeplaatst vanuit een ander ziekenhuis. Deze screening omvat het tweemaal per week verkrijgen van kweken van keel, rectum en, bij beademde patiënten, van sputum. Tevens wordt eenmaal per week middels PCR op VRE gescreend, en eenmaal bij opname op vóórkomen van MRSA. Bij patiënten met een urinekatheter wordt bij opname een urinekweek ingezet. Ook werden in het onderzoek de patiëntkarakteristieken bij opname en de klinische uitkomst van BRMO-positieve patiënten versus patiënten zonder BRMO vergeleken.

We vonden dat een klein maar relevant deel van de patiënten BRMO-positief was tijdens verblijf op de intensive care. De meeste BRMO's waren Gram-negatieve microorganismen. In deze studiepopulatie bleek het merendeel van de BRMO's naar de intensive care geïmporteerd en niet verworven tijdens verblijf op de intensive care. BRMO-positieve patiënten hadden

significant hogere APACHE-scores, een langere verblijfsduur op de IC en een hogere mortaliteit in vergelijking met patiënten zonder BRMO.

Beperkingen van deze studie waren dat patiënten met een kort verblijf niet waren opgenomen in routine surveillance onderzoek; bij deze patiënten werd de incidentie van dragerschap met BRMO's dus niet beoordeeld. Verder werd geen onderscheid gemaakt tussen BRMO-dragerschap en -infectie. Tot slot maken het feit dat data van de studie 10 jaar geleden werden verzameld en de single-center opzet de generaliseerbaarheid van de studie beperkt, maar de resultaten waren in overeenstemming met huidige Europese surveillance gegevens, waaruit blijkt dat Nederland een land is met een lage antibioticaresistentie.

Ten tweede hebben we de farmacokinetiek van de beta-lactams piperacilline en cefotaxim geëvalueerd bij volwassen intensive care patiënten. Adequate dosering van antibiotica bij kritisch zieke patiënten is van groot belang om een goede klinische uitkomst te bewerkstelligen en om het in de hand werken van antibiotica-resistentie te voorkómen¹³⁻¹⁵. Veel publicaties over de farmacokinetiek/farmacodynamiek (PK/PD) van beta-lactams toonden een beter bereiken van een PK/PD-streefwaarde bij gebruik van verlengde of continue toediening bij IC-patiënten¹⁶⁻¹⁸ en een betere klinische uitkomst bij patiënten wanneer een verlengde- of continue infusie strategie werd toegepast in vergelijking met intermitterende infusie wordt gesuggereerd in meta-analyses¹⁹⁻²¹.

Hoewel inmiddels veel gepubliceerd is over de farmacokinetiek van beta-lactams, zijn er weinig studies bekend waarin een heterogene IC-populatie wordt geanalyseerd die wordt behandeld met continue gedoseerde piperacilline of cefotaxim en waarbij veel monsters per patiënt worden afgenomen.

In **Hoofdstuk 3** hebben we zowel de totale als de ongebonden plasma concentraties van piperacilline geëvalueerd bij volwassen intensive care patiënten. In dit prospectieve single-center onderzoek werden patiënten geïncludeerd met een bewezen of een vermoede infectie met indicatie voor behandeling met piperacilline/tazobactam (P/T), zoals gesteld door de behandelend arts. Alle patiënten werden behandeld met continue gedoseerde P/T. Ze ontvingen een oplaaddosis van 4/0,5 g P/T, onmiddellijk gevolgd door 12/1,5 g / 24 uur P/T, toegediend via een perfusor. Bij patiënten met een gestoorde nierfunctie werd na 24 uur de dosering aangepast op basis van vigerende richtlijnen. In de eerste 24 uur van behandeling werden op geplande tijdstippen negen bloedmonsters verkregen; vanaf dag twee werden monsters tweemaal daags met vaste intervallen afgenomen. Alle monsters werden geanalyseerd met vloeistofchromatografie met tandem-massaspectrometrie.

Ons primaire eindpunt was het behalen van een PK/PD-doel. We definieerden vooraf een PK / PD-doel van $100\%fT_{>5xMIC}$ voor totale piperacilline plasma concentraties, rekening houdend met 20-30 % eiwitbinding, waarbij werd uitgegaan van een verondersteld 'worst case' MIC-breekpunt voor *P.aeruginosa*, dat volgens de European Committee on Antimicrobial Susceptibility Testing (EUCAST)-database op 16 mg/L is vastgesteld²². De beoogde plasma concentratie diende binnen een uur bereikt te worden, om daarna gedurende de gehele behandelduur behouden te blijven. We vonden een grote intra- en interindividuele variabiliteit in plasma concentraties van piperacilline. We moesten verder vaststellen dat slechts een minderheid van de patiënten ons vooraf gedefinieerde doel bereikte.

In **Hoofdstuk 4** hebben we de totale en ongebonden plasma concentraties van cefotaxim en de totale en ongebonden plasma concentraties van de actieve metaboliet desacetylcefotaxim bij volwassen IC-patiënten bepaald. In dit gerandomiseerde gecontroleerde single-center onderzoek werden patiënten die startten met cefotaxim in het kader van SDD op indicatiestelling van de behandelend arts en die in het onderzoek waren geïncludeerd, behandeld met intermitterende (1 g elke 6 uur) of continue infusie (4 g / 24 uur) van cefotaxim, voorafgegaan door een oplaaddosis van 1 g. In de eerste 24 uur van behandeling werden acht bloedmonsters verkregen op geplande tijdstippen; vanaf dag twee werden monsters tweemaal daags met vaste intervallen afgenomen. Antibiotica plasma concentraties werden geanalyseerd met behulp van vloeistofchromatografie met tandem-massaspectrometrie. Ook hier was ons primaire eindpunt het bereiken van een PK/PD-doelstelling. We baseerden een vooraf gesteld doel op het cefotaxim MIC-breekpunt voor *Enterobacteriales* van 1 mg / L, zoals bepaald door de EUCAST²². We combineerden dit met de veronderstelling van een eiwitbinding van 25-40% en rekening houdend met een veiligheidsmarge vanwege variabiliteit in de weefselpenetratie bij IC-patiënten^{23,24}, definieerden we het PK/PD-doel als het binnen 1 uur na starten van behandeling bereiken en nadien behouden van een totale plasma cefotaxim concentratie van ten minste 4 mg/L gedurende de gehele behandelduur. We vonden dat significant meer patiënten uit de continue gedoseerde groep het vooraf gedefinieerde doel bereikten in vergelijking met patiënten uit de intermitterend gedoseerde groep. Verder bleken in deze studie die patiënten die het vooraf gedefinieerde PK/PD-doel niet bereikten een significant hogere creatinineklaring bij opname te hebben vergeleken met degenen die dit doel wel bereikten.

Enkele limitaties van beide studies verdienen aandacht. De single-center opzet en het feit dat patiënten die nierfunctie vervangende therapie of extra corporeal life support kregen niet werden geïncludeerd, maakt dat de resultaten van dit onderzoek waarschijnlijk niet geëxtrapoleerd kunnen worden naar alle IC-patiënten. Verschillende experts bevelen tegenwoordig een PK/PD-doelstelling aan van $100\%fT_{>4xMIC}$ (d.w.z. vrije plasma concentratie > 4xMIC gedurende 100 % van het doseringsinterval) bij intensive care patiënten om zowel een goede klinische uitkomst

als onderdrukking van antibioticaresistentie na te streven^{25,26}. Het doel dat we stelden in het cefotaxim-cohort kan in dat licht als enigszins conservatief worden beschouwd. Daarom hebben we bij nader inzien in de studie ook het behalen van de strengere PK/PD-doelstelling van $100\%fT_{4 \times MIC}$ geanalyseerd en beschreven. Het doel dat we stelden bij de evaluatie van piperacilline plasma concentraties ($100\%fT_{5 \times MIC}$ voor totale piperacilline plasma concentratie bij veronderstelde eiwitbinding van 20-30%) kwam overeen met de PK/PD-streefwaarde die door experts wordt aangeraden^{25,26}. Meestal worden, zoals wij ook deden, vermoedelijke MIC's gebruikt in PK/PD-onderzoeken²⁶, en geen MIC's van feitelijke ziekteverwekkers gevonden in kweekmateriaal van de patiënt; uitslagen hiervan zijn vaak niet beschikbaar. Zonder voorafgaande kennis van het veroorzakende microorganisme lijkt het logisch om uit te gaan van een 'worst case'-scenario MIC, afhankelijk van verwachte ziekteverwekkers en hun lokale resistentiepatronen op basis van epidemiologische gegevens. Het doel dat is gekozen in het onderzoek naar piperacilline PK/PD-streefwaarden werd ook toegepast in de meeste andere farmacokinetische onderzoeken met intensive care patiënten die met piperacilline werden behandeld²⁶. Het aantal studies waarin overeenkomstige cohorten van IC-patiënten behandeld met cefotaxim is beschreven is te klein om een dergelijk vergelijk tussen PK/PD-doelen mogelijk te maken. De steekproefomvang van beide studies liet een evaluatie op klinische uitkomst als eindpunt niet toe.

Ten derde hebben we de dynamiek van de intestinale microbiota geëvalueerd bij patiënten opgenomen op de IC na geplande hartchirurgie.

De gunstige rol van de 'gezonde' darmflora voor de fysiologie bij de mens is complex en omvat onder andere het metabolisme van voedingsstoffen, ontwikkeling van het immuunsysteem²⁷ en bescherming tegen potentiële pathogenen door competitie²⁸. Veranderingen in de samenstelling van de darmmicrobiota, met verlies van bacteriële diversiteit, zijn in verband gebracht met aandoeningen zoals obesitas, inflammatoire darmziekten en prikkelbare darmsyndroom²⁹⁻³¹. Afgezien van de voordelen heeft antibioticagebruik een drastische impact op de samenstelling van de darmmicrobiota³², terwijl vele andere factoren die onlosmakelijk verbonden zijn aan een verblijf op de IC, zoals verandering van dieet, geneesmiddelen anders dan antibiotica en invasieve procedures, eveneens kunnen leiden tot verstoring van de intestinale microbiota, ook 'dysbiosis' genoemd^{33,34}. Ondanks het feit dat er meer erkenning is gekomen voor de belangrijke rol van de intestinale microbiota in gezondheid en ziekte²⁸, zijn er tot nu toe slechts enkele studies gepubliceerd die de dynamiek van de darmmicrobiota bij patiënten op de intensive care, gebruikmakend van 16S rRNA gene sequencing, behandelen. Deze studies betroffen voornamelijk acute opnames in verband met orgaanfalen veroorzaakt door infecties, trauma of spoedchirurgie en gebruikten een case-control design waarbij de samenstelling van de darmmicrobiota van IC-patiënten en van gezonde controles werd vergeleken³⁵⁻⁴¹.

In het algemeen toonden deze onderzoeken, zoals verondersteld, een snelle verstoring van de darmmicrobiota tijdens opname op de IC met een verlies aan diversiteit en overgroei van potentiële pathogene microorganismen. De omvang en dynamiek van dergelijke veranderingen zijn nog niet volledig begrepen. De dynamiek van de samenstelling van darmmicrobiota bij geplande chirurgie patiënten opgenomen op de IC, waarbij monsters vóór en na opname als controles werden verzameld, was niet eerder beschreven.

In **Hoofdstuk 5** hebben we de samenstelling van darmmicrobiota voor, tijdens en na ziekenhuisopname onderzocht van patiënten die geplande hartchirurgie ondergingen. We hebben ook gekeken naar een eventuele correlatie tussen de samenstelling van de darmmicrobiota en zowel de patiëntkarakteristieken bij opname als de klinische uitkomst. Faeces monsters verzameld op beschreven drie tijdstippen werden geanalyseerd met behulp van 16S rRNA gene sequencing. Ook werden patiëntkarakteristieken en klinische uitkomstmaten gedocumenteerd. We vonden een grote intra-individuele variatie in de samenstelling van de intestinale microbiota. Bij de meeste patiënten werd tijdens opname een significante verandering in samenstelling van darmmicrobiota waargenomen. De bacteriële diversiteit, weergegeven als Shannon-index, toonde een significante daling gedurende de opname ten opzichte van vóór opname. We vonden tijdens opname een significante afname van strikt anaerobe darmbacteriën, beschouwd als onderdeel van 'gezonde' darmmicrobiota, en een gelijktijdige toename van potentieel pathogene microorganismen of 'pathobionten'.

Voorts werd specifiek naar de dynamiek van *Enterobacteriaceae* in de met SDD behandelde subgroep gekeken. In deze subgroep vonden we geen consistente verandering in de relatieve bijdrage van *Enterobacteriaceae* in de tijd. Bij de meeste patiënten die op alle drie de tijdstippen werden geanalyseerd, keerde na ontslag de tijdens het verblijf in het ziekenhuis ontstane verandering in de samenstelling van microbiota terug naar de situatie van vóór opname. In deze studie werd een lagere bacteriële diversiteit tijdens opname geassocieerd met een langere ziekenhuisopname. Ook werd een lagere bacteriële diversiteit na ontslag geassocieerd met antibioticagebruik na ontslag.

Voor zover wij weten, is dit de eerste studie die de dynamiek van darmmicrobiota bij geplande chirurgische patiënten beschrijft, waarbij afnames zijn gedaan vóór, tijdens en na de opname, zodat de patiënten hun eigen controle werden. Met deze studie-opzet wilden we voorbij kunnen gaan aan de bias die wordt gevormd door de grote interindividuele variabiliteit^{30,42,43}. Enkele beperkingen van dit onderzoek moeten worden opgemerkt. Ten eerste bleek het verzamelen van faeces-monsters een uitdaging te zijn, resulterend in een relatief groot aantal ontbrekende monsters. Het gebruik van 16S rRNA gene sequencing sluit determineren van specifieke bacteriestammen of detectie van virussen en schimmels uit, hoewel deze klinisch belangrijk

kunnen zijn. Verder werden niet alle factoren die mogelijk de samenstelling van de darmmicrobiota beïnvloeden of de dynamiek daarvan geëvalueerd; het gebruik van protonpompremmers bijvoorbeeld, werd niet geregistreerd³⁴. Ten slotte is het moeilijk om de bijdrage van individuele factoren die een rol spelen in verstoringen in de samenstelling van darmmicrobiota van elkaar te onderscheiden, omdat sommige factoren onlosmakelijk met elkaar zijn verbonden; een patiënt met septische complicaties met hemodynamische instabiliteit en orgaanfalen krijgt waarschijnlijk antibiotica.

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LIST OF ABBREVIATIONS

AB	Antibiotic(s)
allo-HSCT	allogenic Hematopoietic Stem Cell Transplantation
APACHE	Acute Physiology And Chronic Health Evaluation Score
aTCI	adaptive Target Controlled Infusion
AUC_{0-24}/MIC	The ratio of the area under the concentration-time curve during a 24-h period to MIC
BMI	Body Mass Index
C	Concentration
°C	degrees Celsius
CABG	Coronary Artery Bypass Graft
CAO	Combined Adverse Outcome
CDC	Centers for Disease Control and Prevention
CDI	<i>Clostridium difficile</i> Infection
CL	Clearance
CL_{Cr}	Creatinine Clearance
C_{max}	maximum Concentration
C_{max}/MIC	The ratio of maximum drug concentration to MIC
C_{min}	minimum Concentration
CV	Co-efficient of Variation
DNA	DeoxyriboNucleic Acid
ECDC	European Centre for Disease Prevention and Control
e.g.	Exempli gratia; for example
ESBL	Extended-Spectrum Beta-Lactamase
<i>et al.</i>	Et alii; and others
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FMT	Fecal Microbiota Transplantation
$fT_{>MIC}$	Time the free fraction of a drug is above the MIC
h	Hour(s)
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HRMO(s)	Highly Resistant Micro-Organism(s)
i.e.	Id est; that is
ICU(s)	Intensive Care Unit(s)
IQR	Inter Quartile Range
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LOS	Length Of Stay
MALDI-TOF	Matrix Assisted Laser Desorption/Ionisation Time-Of-Flight
MDRD	Modification of Diet in Renal Disease
METc	Medisch Ethische Toetsings Commissie (Medical Ethics Board)
MIC	Minimum Inhibitory Concentration
μL	microlitre(s)

(m)g	(milli-)gram(s)
(m)L	(milli-)Litre
min	minute(s)
mmol	millimole(s)
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
N/A	Not Applicable
OTU	Operational Taxonomic Unit
P/T	Piperacillin/Tazobactam
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic
PK	Pharmacokinetic
POWI	Post-Operative Wound Infection
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
SD	Standard Deviation
SCFAs	Short-Chain Fatty Acids
SCreat	Serum Creatinine
SDD	Selective Decontamination of the Digestive tract
T	Time point
TCI	Target Controlled Infusion
TDM	Therapeutic Drug Monitoring
TZP	Piperacillin/tazobactam
UCreat	Urine Creatinine
Vd	Volume of Distribution
VRE	vancomycin-resistant <i>Enterococcus</i>
Vs.	versus

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CURRICULUM VITAE

Heleen Aardema is geboren op 11 juni 1974, te Delfzijl. Zij groeide op in Leeuwarden, alwaar zij in 1992 haar eindexamen aan het Stedelijk Gymnasium behaalde.

Na te zijn uitgeloot voor de studie geneeskunde in Nederland werd deze studie aangevangen aan de Katholieke Universiteit Leuven, België. Vervolgens behaalde zij in 1994 de propedeuse farmacie aan de Rijksuniversiteit Groningen.

In 1994 startte zij alsnog haar studie geneeskunde aan diezelfde universiteit. De doctoraalfase werd in 1998 afgesloten met onderzoek aan het Hôpital Beaujon, Parijs, Frankrijk, naar uitkomsten van chronische hepatitis C virus infectie, onder supervisie van professoren P. Marcellin en C.H. Gips. Hierna werden co-schappen in het Medisch Spectrum Twente volbracht, gevolgd door een beweging naar het Westen, met een laatste keuze co-schap interne geneeskunde in het Bronovo Ziekenhuis, Den Haag, onder supervisie van wijlen dr. R. Bieger.

Na het behalen van het artsexamen in 2000 kon Heleen aan de slag als anios interne geneeskunde in hetzelfde ziekenhuis, en begon zij daar vanaf 2002 de opleiding tot internist, met als opleider dr. J.W. van 't Wout. De reeds bestaande interesse voor infectieziekten werd onder zijn inspirerend opleiderschap verder aangewakkerd.

Na een jaar in het LUMC (opleider: Prof. dr. J.A. Romijn) kroop het bloed toch waar het niet gaan kan en volgde een terugkeer naar het Noorden, waar in het Universitair Medisch Centrum Groningen een fellowship intensive care geneeskunde (opleider: Prof. dr. J.E. Tulleken) in 2008 werd afgesloten met een registratie als internist-intensivist, gevolgd door een Europese registratie in 2010.

Via de afdeling intensive care ontmoette zij vele gepassioneerde collega's uit diverse disciplines die eveneens geïnteresseerd zijn in de preventie en optimale behandeling van infecties, met wie zij de onderzoeken die leidden tot dit proefschrift mocht opzetten en uitvoeren.

Na jarenlang met passie en toewijding als intensivist te hebben gewerkt, besloot Heleen zich volledig op infectieziekten te gaan richten en startte zij in januari 2020 de opleiding tot arts-microbioloog, met als opleiders dr. G. A. Kampinga en prof. dr A.W. Friedrich.

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Aardema H, Lisotto P, Kurilshikov A, Diepeveen JR, Friedrich AW, Sinha B, de Smet AMGA, Harmsen HJM. Marked changes in gut microbiota in cardio-surgical intensive care patients: a longitudinal cohort study. *Front Cell Infect Microbiol* 2020;9:467. doi: 10.3389/fcimb.2019.00467.

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