

Decontamination Strategies in ICU - a European Perspective

Decontaminatie strategieën op de intensive care

Bastiaan Wittekamp

ISBN: 978-94-6332-353-6

Cover design by: Loes Kema, GVO

Cover image: Harry Hadders

Layout by Ferdinand van Nispen, *my-thesis.nl*

Printed by GVO drukkers & vormgevers B.V.

All rights reserved. No parts of this thesis may be reproduced, stored or transmitted in any form or by any means, without the permission of the author.

Financial support by Biomerieux for the publication of this thesis is gratefully acknowledged.

Decontamination Strategies in ICU

a European Perspective

Decontaminatie strategieën op de intensive care
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. H.R.B.M. Kummeling, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 19 juni 2018 des middags te 4.15 uur

door

Bastiaan Hendrik-Jan Wittekamp

geboren op 28 april 1984
te Huizen

Promotor: Prof. dr. M.J.M. Bonten

Table of contents

Chapter 1	Introduction	9
Chapter 2	Associations between enteral colonization with Gram-negative bacteria and subsequent ICU-acquired infections and colonization of the respiratory tract	21
Chapter 3	Nystatin versus amphotericin B to prevent and eradicate Candida colonization during selective digestive tract decontamination in critically ill patients	43
Chapter 4	Regulatory obstacles affecting ecological studies in the ICU	57
Chapter 5	Decontamination strategies in Intensive Care Units: a cluster-randomized cross-over study	67
Chapter 6	Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU	101
Chapter 7	The effects of topical antibiotics on carriage with 3rd-generation cephalosporin and carbapenem resistant gram-negative bacteria in ICU patients	111
Chapter 8	Colistin and tobramycin resistance during long-term use of selective decontamination strategies in the intensive care unit: a post-hoc analysis	131
Chapter 9	General discussion	145

Closing pages	157
Summary	159
Summary in Dutch – Nederlandse samenvatting	163
Dankwoord	171
Curriculum vitae	174
List of publications	175

Chapter 1

Introduction

Based on
Antibiotic prophylaxis in the era of multidrug-resistant bacteria
Expert Opinion on Investigational Drugs 2012; 21(6): 767-772

Bastiaan H.J. Wittekamp ¹
Marc J.M. Bonten ^{1,2}

¹ Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht,
The Netherlands

² Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands

Introduction

Critically ill patients admitted to the intensive care unit (ICU) have an increased risk for infections due to their underlying illness and associated deprived immune state. The ICU environment enhances this risk as the high level of care requires frequent contact moments between health care workers and patients with the risk of transmitting pathogens. Natural barriers for pathogenic micro organisms, such as the skin, are often breached by devices used for hemodynamic monitoring or administration of medication. Mechanical ventilation through an endotracheal tube facilitates colonization and infection of the lower respiratory tract.

Decontamination strategies aim to prevent infections by eliminating pathogenic micro-organisms in the gut and oropharynx, before they can spread to other body sites, a process called translocation (1).

This introduction will review three decontamination strategies for ICU patients: selective digestive tract decontamination (SDD), selective oropharyngeal decontamination (SOD) and chlorhexidine mouthwash (CHX). The history and background of these strategies will be described, followed by a summary of the available evidence on the effects on patient centered outcomes, identification of the gaps in knowledge and the aim of this thesis.

History and background of decontamination strategies

Decontamination strategies exist in two forms; strategies with antibiotics and strategies with antiseptic agents.

Strategies with antibiotics

Among strategies with antibiotics are SDD and SOD. SDD was introduced to the intensive care unit in 1984 (2). SDD consists of an antimicrobial mouth paste and gastro-enteral suspension and targets the oropharynx and the gut. The first and to date most often used SDD and SOD regimen consists of colistin, tobramycin and an antifungal agent, amphotericin B (2). These antimicrobial agents are not absorbed from the gut and target aerobic potential pathogenic micro-organisms, such as *Enterobacteriaceae*, *S. Aureus* and yeasts that colonize the oropharynx and gut. The term 'selective' refers to the preservation of anaerobic flora which prevents overgrowth and colonization by potential pathogens, as was discovered in animal

studies in the nineteen-eighties (3). In addition to topical antibiotics, systemic cephalosporins during the first four days in ICU have been part of the original SDD regimen to prevent early infections that could be caused by endogenous colonizers (2).

SOD consists of the same mouth paste with topical antibiotics that is used in SDD with the aim to prevent upper respiratory tract infections, which are among the most common infections in ICU patients (4). Systemic antibiotics and the gastro-enteral suspension are not part of SOD.

Strategies with antiseptics – chlorhexidine mouthwash

Among decontamination strategies with antiseptics is chlorhexidine digluconate mouthwash. Chlorhexidine is not selective in its effects and acts against a broad range of micro-organism, including Gram-positive and -negative bacteria and yeasts. It has an initial bactericidal effect, followed by a prolonged bacteriostatic effect (5) and can be applied in the form of an oral gel or solution. Often used in dentistry in concentrations up to 0.2%, it is commercially available and has a marketing authorization for oral care.

Current practice – evidence translated in clinical care

SDD

There are multiple studies showing that SDD improves survival of ICU patients (6-9) and prevents infections in ICU patients (6, 7, 10, 11), including bacteremia with highly resistant micro-organisms (HRMO) (12). In the largest study comparing SDD to standard care thus far, among 5.939 patients in the Netherlands, SDD reduced mortality on day 28 after ICU admission with 3.5% (from 27.5% to 24.0%, relative risk reduction 13%, number needed to treat 29), compared to standard care without decontamination. Apart from a survival benefit, there was a trend towards a shorter length of ICU stay associated with SDD, for which a statistically significant difference was found in another Dutch study from 2003 among 934 patients (8). In this study, SDD also improved survival in the ICU and hospital, compared to standard care without decontamination.

Several other studies have been performed before the turn of the century, but most of these studies had small sample sizes and -- sometimes -- poor methodological quality and there was considerable heterogeneity in decolonization regimens, outcome definitions, and patient populations that were studied, limiting generalizability of results (10). The largest studies with an effect on survival (6, 13) were cluster randomized studies in which the order of study periods (standard care, SDD and SOD) was randomized per ICU (cluster), rather than randomizing a treatment to individual patients as in preceding randomized controlled trials. In cluster randomized trials, one intervention can be administered to all eligible patients in the ICU during a pre-determined study period, mimicking the situation in which interventions would be applied to all patients as part of standard care. This creates the possibility to study the ICU ecology, in contrast to classical RCTs in which different decontamination strategies may be used simultaneously during the study.

The effect of SDD and SOD on the prevalence of antimicrobial resistance among bacteria has been a concern since their introduction to the ICU. After conflicting findings in early studies (14-16), a meta-analysis published in 2013 including 35 studies with data on antimicrobial resistance found no evidence for an increase in antimicrobial resistance associated with the use of SDD and SOD (17). Moreover, during SDD in the Dutch cluster study the prevalence of highly resistant microorganisms in the ICU was lower than during standard care, indicating that SDD (and SOD) reduce, rather than increase the prevalence of resistant bacteria (6, 12).

Nevertheless, the largest cluster studies with a beneficial effect on survival and ICU ecology (6, 8, 13) were performed in the Netherlands with relatively low levels of antimicrobial resistance. This limits generalizability to settings with higher levels of antimicrobial resistance (18) and feeds skepticism about the safety of SOD and SDD with respect to the prevalence of antimicrobial resistance and their effectiveness (19). As a result, SDD is sporadically used in ICUs outside the Netherlands (20). In contrast, SDD and SOD have been recommended as standard care in the Netherlands for ventilated ICU patients with an expected length of stay of more than 48 hours, or non-ventilated patients who are expected to stay in ICU for more than 72 hours (21). The SDD regimen that is most often used is identical to the original regimen from 1984 (2) (Table 1).

SOD

SOD has been shown to reduce the incidence of ventilator associated pneumonia (VAP) among ventilated patients (22, 23), but also mortality on day 28 after ICU admission and bacteremia acquired during ICU stay, compared to standard care without decontamination (6). Compared to SDD, SOD was less effective in preventing bacteremia in recent comparative studies (6, 7). In addition, SDD was more effective than SOD in preventing mortality measured on day 28 after ICU admission as well as in preventing ICU and hospital mortality (7, 13).

As with SDD, there is no evidence supporting the fear that SOD would lead to an increase in the prevalence of antimicrobial resistance and SOD has been associated with decreasing antimicrobial resistance (17, 24). The total amount of administered antibiotics used in SOD is smaller compared to SDD and it is therefore cheaper than SDD. In the Netherlands, SOD is currently being used as alternative for SDD in some ICUs, although this may change after SDD was found to be superior to SOD in preventing bacteremia and improving survival (21). In other European countries, SOD seems to be used infrequently, although there are no exact numbers support this.

CHX mouthwash

Meta-analyses demonstrated a reduction in respiratory tract infections with the use of CHX mouthwash among ICU patients (25, 26), although in a recent meta-analysis the protective effect of CHX mouthwash on the incidence of respiratory tract infections was limited to ICU patients who underwent cardiac surgery and the reduction on the incidence of VAP among non-cardiac surgery patients was not statistically significant (26). Moreover, CHX mouthwash was associated with a trend towards increased mortality in this and another meta-analysis (26, 27) and although the underlying mechanisms remain to be determined, these results have increased awareness for potential harmful effects of chlorhexidine.

Resistance to chlorhexidine has been reported among *Enterobacter* spp., *Pseudomonas* spp., *Proteus* spp., *Providencia* spp. and *Enterococcus* spp. (28). So far this has not led to a practice of restricted use in the ICU and chlorhexidine is nowadays a commonly used antiseptic agent for oral care in ICUs across Europe (29). There are numerous different regimens, although mouthwash with chlorhexidine digluconate in a concentration of 0.12% to 0.2% is most frequently used and often

applied 3-4 times daily in addition to regular oral care (e.g., tooth brushing). More recently, chlorhexidine 2% was found to be more effective in preventing respiratory tract infections than lower concentrations (30-32). Until now, no single prospective study has compared the effectiveness of chlorhexidine 2% to lower concentrations and decontamination strategies with antibiotics.

Table 1. Components of SDD and SOD and chlorhexidine mouthwash as often used in clinical practice

	Oral agent*	Gastro-enteral suspension*	Systemic antibiotics
SDD	Mouth paste: Tobramycin 2%, amphotericin 2%, colistin 2%	Aminoglycoside (i.e. tobramycin 80mg) Polymyxin (i.e. colistin or polymyxin E 100mg 4 times daily) Antifungal agent (i.e. amphotericin B 500mg)	3rd Generation cephalosporin, (i.e. cefotaxime 1,000mg*) during the first 4 days of admission
SOD	Mouth paste: Tobramycin 2%, amphotericin B 2%, colistin 2%;		
CHX	Mouthwash: Chlorhexidine digluconate 0.12 – 0.2%		

* Administered 4 times daily

Abbreviations: SDD: selective digestive tract decontamination; SOD: selective oropharyngeal decontamination; CHX: chlorhexidine mouthwash.

Aim of this thesis

The prophylactic use of antibiotics can only be justified when clinical benefits on relevant patient outcomes, such as morbidity or mortality and the absence of emergence of antibiotic resistance have been unequivocally demonstrated. This is not the case for ICU settings with higher antibiotic resistance levels than in ICUs in the Netherlands, as large studies comparing the effect of SDD, SOD and CHX mouthwash to standard care are lacking. An overview of current available evidence on aspects of decontamination strategies in settings with low and higher resistance levels is presented in table 2.

This leads to the current practice in which SDD and SOD are considered to be beneficial and therefore standard care for ICU patients in the Netherlands, but not for patients in other European countries. The aim of this thesis is, therefore, to compare the effectiveness and ecological safety of three decontamination strategies, SDD, SOD and CHX mouthwash, for ICU patients in other European countries with higher endemicity of antibiotic resistance.

Outline of this thesis

This thesis starts with testing the hypothesis that colonization of the gut and the upper respiratory tract and infections acquired during ICU stay are interdependent, in chapter 2. In chapter 3, the effectiveness of SDD with nystatin in preventing candida colonization is compared to SDD with amphotericin B, as nystatin could be a cheaper alternative for amphotericin B.

In preparation for a cluster-randomized study comparing SDD, SOD and CHX mouthwash, chapter 4 comments on the European trial directive (2001/20/EC) on which national legislation regarding clinical trials in European countries is based. The 2001 directive has no provision for waiving informed consent while this is required for cluster randomized studies on the ecological effects of decontamination strategies. In chapter 5 the results of the R-GNOSIS study are reported: a cluster randomized study on the effectiveness and ecological safety of SDD, SOD and CHX mouthwash that was performed in 13 ICUs in 6 countries. In chapter 6, side effects of CHX 2% mouthwash are reported that occurred during cluster randomized study.

The hypothesis that decontamination strategies can eradicate ESBL and carbapenem resistant GNB from the gut and respiratory tract is tested In chapter 7 with data from the cluster randomized study. Long-term effects of decontamination strategies in Dutch ICUs are evaluated in chapter 8, using data from two previous Dutch cluster randomized trials.

Finally, the main findings of this thesis are summarized and translated into possible clinical implications in the discussion section.

Table 2. Requirements for prophylactic use of antibiotics in decolonization strategies and available evidence

Requirement	Low resistance level*	Moderate-high resistance level*	Remark
Effectiveness demonstrated in individual studies	Yes	No	Convincing evidence from 4 studies in settings with low levels of resistance (6, 8, 9). Aggregated data from other settings do suggest an effect on mortality, without evidence from individual trials (10). SDD is more effective than SOD in preventing bacteremia and mortality in the most recent comparative study (13).
Short term ecological safety demonstrated	Yes	No	No evidence of increased resistance in the short term in settings with low levels of resistance (6, 8, 9). An increase in aminoglycoside resistance among Gram-negative bacteria in the rectum was found during SDD in the most recent Dutch cluster study (13). Increased prevalence of colonization with gram positives and MRSA and resistance to cephalosporins during SDD in settings with moderate-high levels of antibiotic resistance (14, 33-36).
Long-term ecological safety demonstrated	No	No	No increase in resistance rates during SDD in two studies with follow-up for up to 5 years (33, 37). Increased point-prevalence of resistance to ceftazidime after discontinuation of SDD in one multi-center study (38), which was not observed in another study (15).
Ecological safety after ICU discharge demonstrated	No	No	No data available
Ecological safety in non-culturable flora demonstrated	No	No	Limited data available. One study showed an increase in (mainly aminoglycoside) resistance genes associated with SDD (39).
Cost effectiveness demonstrated	Yes	No	Cost-effectiveness analysis based on Dutch cluster randomized study (de Smet, 2009 (6)) (40). An updated analysis will follow in 2018.

Abbreviations: SDD: selective digestive tract decontamination; MRSA: methicillin-resistant *Staphylococcus aureus*.

* Low resistance level is defined as: <5% of bacteremia episodes caused by MRSA for *S. aureus*, VRE for enterococci and extended spectrum beta lactamase or carbapenamase producing strains for Enterobacteriaceae (6, 8, 9). Moderate-high resistance level is defined as: settings not meeting the criteria for low levels of resistance.

References

1. Sertaridou E, Papaioannou V, Kolios G, Pneumatikos I. Gut failure in critical care: old school versus new school. *Annals of gastroenterology*. 2015;28(3):309-22.
2. Stoutenbeek CP, van Saene HK, Miranda DR, Zandstra DF. The effect of selective decontamination of the digestive tract on colonisation and infection rate in multiple trauma patients. *Intensive care medicine*. 1984;10(4):185-92.
3. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk L-v. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *The Journal of hygiene*. 1971;69(3):405-11.
4. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. *Jama*. 2009;302(21):2323-9.
5. Jenkins S, Addy M, Wade W. The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo. *Journal of clinical periodontology*. 1988;15(7):415-24.
6. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *The New England journal of medicine*. 2009;360(1):20-31.
7. Oostdijk EA, Kesecioglu J, Schultz MJ, Visser CE, de Jonge E, van Essen EH, et al. Effects of decontamination of the oropharynx and intestinal tract on antibiotic resistance in ICUs: a randomized clinical trial. *Jama*. 2014;312(14):1429-37.
8. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet (London, England)*. 2003;362(9389):1011-6.
9. Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ, et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. *American journal of respiratory and critical care medicine*. 2002;166(8):1029-37.
10. Liberati A, D'Amico R, Pifferi S, Torri V, Brazzi L, Parmelli E. Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care. *The Cochrane database of systematic reviews*. 2009(4):Cd000022.
11. Camus C, Sauvadet E, Tavenard A, Piau C, Uhel F, Bouju P, et al. Decline of multidrug-resistant Gram negative infections with the routine use of a multiple decontamination regimen in ICU. *The Journal of infection*. 2016;73(3):200-9.
12. de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernards AT, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. *The Lancet Infectious diseases*. 2011;11(5):372-80.
13. Oostdijk EAN, Kesecioglu J, Schultz MJ, Visser CE, de Jonge E, van Essen EHR, et al. Notice of Retraction and Replacement: Oostdijk et al. Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. *JAMA*. 2014;312(14):1429-1437. *Jama*. 2017;317(15):1583-4.
14. Lingnau W, Berger J, Javorsky F, Fille M, Allerberger F, Benzer H. Changing bacterial ecology during a five-year period of selective intestinal decontamination. *The Journal of hospital infection*. 1998;39(3):195-206.
15. Hammond JM, Potgieter PD. Long-term effects of selective decontamination on antimicrobial resistance. *Critical care medicine*. 1995;23(4):637-45.
16. Verwaest C, Verhaegen J, Ferdinande P, Schetz M, Van den Berghe G, Verbist L, et al. Randomized, controlled trial of selective digestive decontamination in 600 mechanically ventilated patients in a multidisciplinary intensive care unit. *Critical care medicine*. 1997;25(1):63-71.
17. Daneman N, Sarwar S, Fowler RA, Cuthbertson BH. Effect of selective decontamination on antimicrobial resistance in intensive care units: a systematic review and meta-analysis. *The Lancet Infectious diseases*. 2013;13(4):328-41.
18. Cuthbertson BH, Campbell MK, MacLennan G, Duncan EM, Marshall AP, Wells EC, et al. Clinical stakeholders' opinions on the use of selective decontamination of the digestive tract in critically ill patients in intensive care units: an international Delphi study. *Critical care (London, England)*. 2013;17(6):R266.
19. Bastin AJ, Ryanna KB. Use of selective decontamination of the digestive tract in United Kingdom intensive care units. *Anaesthesia*. 2009;64(1):46-9.

20. Reis Miranda D, Citerio G, Perner A, Dimopoulos G, Torres A, Hoes A, et al. Use of selective digestive tract decontamination in European intensive cares: the ifs and whys. *Minerva anesthesiologica*. 2015;81(7):734-42.
21. Oostdijk EADJ, E.; Kullberg, B.J.; Natsch, S.; De Smet, A.M.G.A.; Vandenbroucke--Grauls, C.M.J.E.; Van Der Vorm, E.; Bonten, M.J.M. SWAB-Richtlijn: selectieve decontaminatie bij patiënten op de intensive care. Stichting Werkgroep Antibioticabeleid, 2014.
22. Schultz MJ, Haas LE. Antibiotics or probiotics as preventive measures against ventilator-associated pneumonia: a literature review. *Critical care (London, England)*. 2011;15(1):R18.
23. Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van der Geest S, van Tiel FH, et al. Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. *American journal of respiratory and critical care medicine*. 2001;164(3):382-8.
24. de Smet AM, Bonten MJ, Kluytmans JA. For whom should we use selective decontamination of the digestive tract? *Current opinion in infectious diseases*. 2012;25(2):211-7.
25. Chan EY, Ruest A, Meade MO, Cook DJ. Oral decontamination for prevention of pneumonia in mechanically ventilated adults: systematic review and meta-analysis. *BMJ (Clinical research ed)*. 2007;334(7599):889.
26. Klompas M, Speck K, Howell MD, Greene LR, Berenholtz SM. Reappraisal of routine oral care with chlorhexidine gluconate for patients receiving mechanical ventilation: systematic review and meta-analysis. *JAMA internal medicine*. 2014;174(5):751-61.
27. Price R, MacLennan G, Glen J. Selective digestive or oropharyngeal decontamination and topical oropharyngeal chlorhexidine for prevention of death in general intensive care: systematic review and network meta-analysis. *BMJ (Clinical research ed)*. 2014;348:g2197.
28. Kampf G. Acquired resistance to chlorhexidine - is it time to establish an 'antiseptic stewardship' initiative? *The Journal of hospital infection*. 2016;94(3):213-27.
29. Rello J, Koulenti D, Blot S, Sierra R, Diaz E, De Waele JJ, et al. Oral care practices in intensive care units: a survey of 59 European ICUs. *Intensive care medicine*. 2007;33(6):1066-70.
30. Derde LP, Bonten MJ. Oropharyngeal decontamination in intensive care patients: less is not more. *Critical care (London, England)*. 2009;13(5):183.
31. Labeau SO, Van de Vyver K, Brusselaers N, Vogelaers D, Blot SI. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. *Lancet Infect Dis* 2011;11:845-54.
32. Zand F, Zahed L, Mansouri P, Dehghanrad F, Bahrani M, Ghorbani M. The effects of oral rinse with 0.2% and 2% chlorhexidine on oropharyngeal colonization and ventilator associated pneumonia in adults' intensive care units. *Journal of critical care*. 2017;40:318-22.
33. Heining A, Meyer E, Schwab F, Marschal M, Unertl K, Krueger WA. Effects of long-term routine use of selective digestive decontamination on antimicrobial resistance. *Intensive care medicine*. 2006;32(10):1569-76.
34. Leone M, Albanese J, Antonini F, Nguyen-Michel A, Martin C. Long-term (6-year) effect of selective digestive decontamination on antimicrobial resistance in intensive care, multiple-trauma patients. *Critical care medicine*. 2003;31(8):2090-5.
35. Nardi G, Valentini U, Proietti A, De Monte A, Di Silvestre A, Muzzi R, et al. Epidemiological impact of prolonged systematic use of topical SDD on bacterial colonization of the tracheobronchial tree and antibiotic resistance. A three year study. *Intensive care medicine*. 1993;19(5):273-8.
36. Sanchez Garcia M, Cambronero Galache JA, Lopez Diaz J, Cerda Cerda E, Rubio Blasco J, Gomez Aguinaga MA, et al. Effectiveness and cost of selective decontamination of the digestive tract in critically ill intubated patients. A randomized, double-blind, placebo-controlled, multicenter trial. *American journal of respiratory and critical care medicine*. 1998;158(3):908-16.
37. Ochoa-Ardila ME, Garcia-Canas A, Gomez-Mediavilla K, Gonzalez-Torralba A, Alia I, Garcia-Hierro P, et al. Long-term use of selective decontamination of the digestive tract does not increase antibiotic resistance: a 5-year prospective cohort study. *Intensive care medicine*. 2011;37(9):1458-65.
38. Oostdijk EA, de Smet AM, Blok HE, Thieme Groen ES, van Asselt GJ, Benus RF, et al. Ecological effects of selective decontamination on resistant gram-negative bacterial colonization. *American journal of respiratory and critical care medicine*. 2010;181(5):452-7.
39. Buelow E, Gonzalez TB, Versluis D, Oostdijk EA, Ogilvie LA, van Mourik MS, et al. Effects of selective digestive decontamination (SDD) on the gut resistome. *The Journal of antimicrobial chemotherapy*. 2014;69(8):2215-23.
40. Oostdijk EA, de Wit GA, Bakker M, de Smet AM, Bonten MJ. Selective decontamination of the digestive tract and selective oropharyngeal decontamination in intensive care unit patients: a cost-effectiveness analysis. *BMJ open*. 2013;3(3). DOI: 10.1136/bmjopen-2012-002529.

Chapter 2

Associations between enteral colonization with Gram-negative bacteria and ICU-acquired infections and colonization of the respiratory tract

Clinical infectious diseases 2018; 66(4): 497-503

Jos F. Frencken^{1,2*}

Bastiaan H.J. Wittekamp^{1*}

Nienke L. Plantinga¹

Cristian Spitoni³

Kirsten van de Groep^{1,2}

Olaf L. Cremer²

Marc J.M. Bonten^{1,4}

* both authors contributed equally to this manuscript

¹ Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands

² Department of Intensive Care Medicine, University Medical Center Utrecht, Utrecht, The Netherlands

³ Department of mathematics, Utrecht University, Utrecht, The Netherlands

⁴ Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands

Abstract

Background: Enteral and respiratory tract colonization with Gram-negative bacteria may lead to subsequent infections in critically ill patients. We aimed to clarify the interdependence between gut and respiratory tract colonization and their associations with ICU-acquired infections in patients receiving selective digestive tract decontamination (SDD).

Methods: Colonization status of the rectum and respiratory tract was determined using twice weekly microbiological surveillance in mechanically ventilated subjects receiving SDD between May 2011 and June 2015 in a tertiary medical-surgical ICU in The Netherlands. Acquisition of infections was monitored daily by dedicated observers. Marginal structural models were used to determine the associations between Gram-negative rectal colonization and respiratory tract colonization, ICU-acquired Gram-negative infection, and ICU-acquired Gram-negative bacteremia.

Results: Among 2,066 ICU admissions, 1,157 (56.0%) ever had documented Gram-negative carriage in the rectum during ICU-stay. Cumulative incidences of ICU-acquired Gram-negative infection and bacteremia were 6.0% (n=124) and 2.1% (n=44), respectively. Rectal colonization was an independent risk factor for both respiratory tract colonization (cause specific hazard ratio (CSHR) 2.93, 95% CI 2.02-4.23) and new Gram-negative infection in the ICU (CSHR 3.04, 95% CI 1.99-4.65). Both rectal and respiratory tract colonization were associated with bacteremia (CSHR 7.37, 95% CI 3.25-16.68 and CSHR 2.56, 95% CI 1.09-6.03, respectively). Similar associations were observed when Enterobacteriaceae and glucose non-fermenting Gram-negative bacteria were analyzed separately.

Conclusions: Gram-negative rectal colonization tends to be stronger associated with subsequent ICU-acquired Gram-negative infections than Gram-negative respiratory tract colonization. Gram-negative rectal colonization seems hardly associated with subsequent ICU-acquired Gram-negative respiratory tract colonization.

Introduction

Disruption of the normal microbiota during critical illness can lead to overgrowth of aerobic Gram-negative bacteria (GNB) in the gut and respiratory tract, which may lead to subsequent infectious complications, such as bacteremia (1). This hypothesis is supported by previous findings that Gram-negative (GN) colonization of the gut was associated with ICU-acquired GN bacteremia (2). Moreover, Selective Digestive tract Decontamination (SDD), was associated with a lower incidence of respiratory tract colonization with antibiotic resistant bacteria than Selective Oropharyngeal Decontamination (SOD), also suggesting that the intestinal tract serves as a reservoir for GNB colonizing the respiratory tract during ICU-stay (3). The potential pathophysiological mechanisms leading to bacteremia include direct translocation of bacteria from the gut into the bloodstream (systemic translocation) and indirect transmission via the mesenteric lymph nodes (gut-lymph translocation), which may be facilitated by altered intestinal permeability and possibly changes in virulence of the gut microbiota in critically ill patients (1, 4). Alternatively, bacteremia may result from aspiration of gastric contents leading to respiratory tract colonization and subsequent pneumonia. Indeed, SOD (without affecting the intestinal flora) has also been associated with reduced incidences of ventilator associated pneumonia (VAP) (5) and ICU-acquired bacteremia with Enterobacteriaceae (6). However, the relative importance of respiratory tract and intestinal colonization for ICU-acquired infections is unknown, as is the association between rectal colonization and acquisition of respiratory tract colonization.

We aimed to clarify the interdependence between gut and respiratory tract colonization and their associations with ICU-acquired infections and ICU-acquired bacteremia. To this end, we quantified the associations between gut colonization with GNB and the occurrence of ICU-acquired infections, between the occurrence of gut and respiratory tract colonization and ICU-acquired bacteremia and between gut colonization with GNB and subsequent respiratory tract colonization in a large cohort of patients admitted to the ICU (Figure 1).

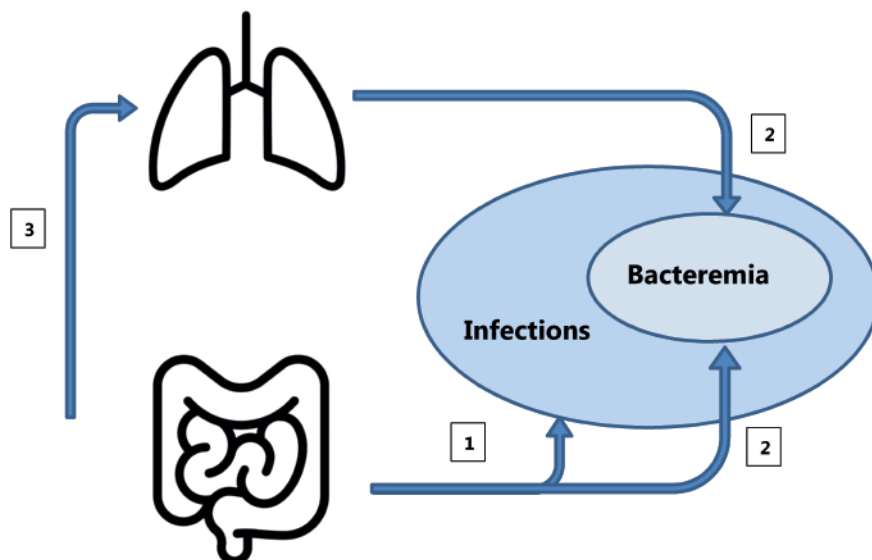


Figure 1: Possible associations between rectal and respiratory tract colonization and infections

1. First objective: the association between rectal colonization with Gram-negative bacteria and ICU-acquired infections with Gram-negative bacteria.
2. Second objective: The association between rectal and/or respiratory tract colonization with Gram-negative bacteria and ICU-acquired bacteremia.
3. Third objective: The association between rectal colonization with Gram-negative bacteria and acquisition of respiratory tract colonization.

Materials and methods

Study design

We studied consecutive patients admitted to the ICU of the University Medical Center Utrecht between May 2011 and June 2015, who were mechanically ventilated, had a length of stay of at least two days, and received SDD. The SDD regimen has been described previously (6) and consisted of three components: 1) a gastro intestinal suspension containing colistin, tobramycin and amphotericin B, administered four times daily via a nasogastric tube, 2) a paste with the same antimicrobials, applied four times daily in the mouth and 3) cefotaxime administered four times daily intravenously for a duration of four days. As part of the SDD-protocol, surveillance cultures of the rectum and respiratory tract were performed twice weekly; patients without rectum or respiratory tract samples were excluded from this analysis. Patients with persistent or newly-acquired GNB colonization in their second or later surveillance samples received an intensified

SDD regimen, with eight times daily application of the antibiotic suspension or paste and nebulization with tobramycin or colistin.

Clinical data were prospectively collected as part of the Molecular diagnosis And Risk stratification of Sepsis (MARS) project (7), including information on patient demographics, medication use, physiological parameters and organ failure scores. Patients were screened daily for the presence of infections during their ICU stay by dedicated physician observers. Infection and bacteremia events were considered ICU-acquired if they occurred two days or more after ICU admission. The likelihood of infection was defined according to adapted CDC and International Sepsis Forum criteria based on a post hoc review of all available clinical data (8). For the current analysis we included all infections where the likelihood was rated as at least possible (7). The medical ethical committee approved an opt-out consent procedure (protocol number 10-056C).

Study objectives

The first objective was to determine the association between rectal colonization with GNB and development of an ICU-acquired infection with GNB (Figure 1). Infections were considered to be associated with colonization if the diagnosis was established on a day with GNB colonization. We did not determine the association between respiratory tract colonization and ICU-acquired infections, because colonization of the respiratory tract is a diagnostic criterium for VAP and this would, therefore, introduce bias.

The second objective was to determine the association between both respiratory tract and rectal colonization with GNB and ICU-acquired bacteremia due to GNB.

The third objective was to determine the association between rectal colonization with GNB and subsequent acquisition of respiratory tract colonization with GNB. This analysis was limited to subjects having respiratory cultures yielding no GNB growth during the first 2 days in ICU, and at least one complete set of rectum and respiratory surveillance cultures available later. As sensitivity analyses, all objectives were also studied for Enterobacteriaceae and glucose non-fermenting (GNF) GNB separately.

Colonization status

Surveillance samples from the respiratory tract (consisting of throat swabs and/or endotracheal aspirates) as well as the rectum were taken upon ICU-admission and twice weekly thereafter, and inoculated on MacConkey, blood agar and Mout media. Cultures were analyzed semi-quantitatively for growth of GNB, followed by species identification using MALDI-TOF and susceptibility testing according to local protocols. GNB colonization was defined as the presence one or more GNB species in a single surveillance sample. Changes in colonization status could thus occur if a positive culture was followed by a negative culture or vice versa, in which case the switch was assumed to have occurred in the middle of the interval between two subsequent cultures (See supplement, figure S1, for a detailed explanation of colonization definition).

For the third objective, only complete surveillance culture sets (i.e., concomitant rectum and respiratory surveillance samples) were analyzed. Respiratory cultures performed at discretion of the treating physician were excluded to prevent information bias (as rectal cultures were only performed for surveillance purposes). Acquisition of GNB respiratory tract colonization was attributed to rectal colonization if the rectum was colonized with any GNB *prior* to the first positive respiratory culture.

Statistical analyses

We performed Cox regression analyses with marginal structural models (MSM) to assess the associations of interest. A MSM was used because time-varying confounders, such as antibiotic exposure, may influence both the outcome (infection) and the determinant (GNB colonization), but in addition, the inverse relation is also present (GNB colonization may influence future antibiotic use). An explanation of MSMs can be found in the supplement material (Supplement: rationale for using MSM analyses). We estimated the cause specific hazard ratio (CSHR) for the endpoints of interest and competing events. This hazard ratio can be interpreted as the (relative) change in the instantaneous probability of acquiring an infection on a colonized vs. a non-colonized day. Death and discharge from ICU were both considered competing endpoints, since they preclude the later occurrence of colonization and infection in the ICU.

Confounders were selected a priori using clinical judgment. Age, medical or surgical admission type, the presence of immune deficiency, comorbidities and infection upon ICU admission were treated as baseline (i.e., time-fixed) confounders, whereas Sequential Organ Failure Assessment (SOFA) score, use of antibiotics with an effect on GNB and mechanical ventilation status were included as time-varying confounders (i.e., values could vary per day) in all models. In addition, the presence of surgical drains was included as a time-varying confounder in the model for the outcomes ICU-acquired bacteremia and infection. Multiple ICU admissions were analyzed separately if patients had been readmitted to the ICU during the study period, therefore a random effect was added to the Cox regression models to account for correlation caused by readmission of patients. SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 3.2.2 (R foundation for Statistical Computing, 2015) were used to perform statistical analyses.

Results

Of 3,184 admissions during which SDD was used, 2,066 ICU episodes (in 1,874 unique patients) were included for the analysis of objectives 1 and 2 (all having one or more rectum and respiratory samples and a length of stay >2 days) and 1,457 (in 1,345 patients) for objective 3 (having both a rectal and respiratory surveillance sample on admission and during follow-up) (Figure 1 and Supplement figure S2, inclusion flow chart). The median length of stay was 7 days (interquartile range (IQR) 4-13). A total of 8,465 rectum and 10,087 respiratory tract cultures were taken during 25,766 ICU days, averaging 1.0 and 1.2 culture every three days, respectively. In 97% and 99% of ICU episodes a first rectum and respiratory tract culture had been obtained within 4 days. There was a decrease in the proportion of rectal cultures yielding GNB growth from 45.4% at admission, to 9.3% by day 10 in the ICU. For respiratory samples, this was 28.9% and 17.2%, respectively.

In 1,157 (56.0%) ICU episodes at least one rectum culture grew GNB during ICU-admission, and 804 (38.9%) had one or more respiratory cultures with GNB. The median duration of colonization was 4 (IQR 3-8) and 4 (IQR 3-6) days in patients who (ever) had respiratory and/or rectal GNB colonization, respectively, whereas the median number of non-colonized days under observation in these patients was 7 (IQR 4-13) and 7 (IQR 4-14). Patients without GNB in any rectum culture,

referred to as 'never colonized', stayed in ICU for a median of 8 days (IQR 6-14 days) and more frequently had a history of hematologic malignancy and immune deficiency or an infection upon ICU admission, and were less often admitted to ICU for a surgical reason than ever colonized patients (Table 1).

Table 1. Characteristics of ICU admissions that were non-colonized versus colonized with gram-negative bacteria in the rectum during intensive care admission (n=2,066).

Patient characteristics	Rectum colonization with GNB		p-value
	Never colonized (n=909) ^a n (%) / median (IQR) ^c	Ever colonized (n=1,157) ^b n (%) / median (IQR) ^c	
Age (years)	61 (48-71)	62 (50-71)	0.056
Male	598 (66)	744 (64)	0.562
Chronic renal insufficiency	63 (7)	102 (9)	0.111
Congestive heart failure	85 (9)	162 (14)	0.001
Myocardial infarction	88 (10)	139 (12)	0.087
COPD	102 (11)	136 (12)	0.683
Diabetes mellitus	146 (16)	202 (18)	0.378
Immune deficiency	152 (17)	150 (13)	0.018
Hematologic malignancy	63 (7)	34 (3)	<0.001
Cerebrovascular disease	99 (11)	149 (13)	0.158
Charlson comorbidity index	1 (0-2)	1 (0-2)	0.71
ICU admission characteristics			
Surgical admission	308 (34)	506 (44)	<0.001
Readmission	177 (20)	139 (12)	<0.001
Infection at admission	491 (54)	508 (44)	<0.001
APACHE IV Score	76 (61-98)	78 (62-99)	0.322

^a 11,242 patient days at risk.

^b 16,648 patient days at risk, of which 5,513 colonized.

^c Data are shown as absolute number (%) or median (Q1-Q3).

Abbreviations: n, number; IQR, interquartile range; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation.

Rectal colonization and ICU-acquired infections with GNB

There were 124 ICU-acquired GNB infections during 27,890 observation days (incidence rate 4.4 per 1,000 days). The majority were respiratory tract infections (n=70, 57%), followed by primary or catheter-related bloodstream infections (n=28, 23%), abdominal infections (n=8, 7%) and other (n=18, 15%). Of these, 45 (36%) were diagnosed during simultaneous rectal GNB colonization, and in 32 (71%) of these episodes the species causing infection and colonization were identical. The incidence rate of infections was 8.2/1,000 colonized days, versus 3.5/1,000 non-

colonized days. The most common causative pathogens were *P. aeruginosa* (29%), *E. cloacae* (14%), and *S. marcescens* (14%).

After adjustment for confounders in a MSM, the estimated CSHR for developing new infection during rectal colonization with GNB was 3.04 (95% CI 1.99-4.65, p -value <0.001) (Table 2). The CSHR for Enterobacteriaceae and GNF GNB separately were similar (Supplement, table S1, result of MSM analyses for Enterobacteriaceae and GNF GNB). The increased CSHR for ICU discharge and death indicate that being colonized increased the rate of being discharged from ICU or dying in the ICU, leading to shorter length of stay and shorter exposure to the risk of infection. Nevertheless, colonization was associated with an increased hazard for infection, as expressed by the increased CSHR (Table 2).

Table 2. Results of marginal structural model analyses for the association between rectum colonization with Gram-negative bacteria and ICU-acquired infections (n=2,066).

Model	Outcome					
	ICU-acquired GNB infection		ICU death		ICU discharge	
	CSHR (95%CI)	<i>p</i> -value	CSHR (95%CI)	<i>p</i> -value	CSHR (95%CI)	<i>p</i> -value
Crude ^a	4.38 (2.92-6.57)	<0.001	2.05 (1.56-2.69)	<0.001	1.12 (0.98-1.28)	0.085
MS model ^b	3.04 (1.99-4.65)	<0.001	1.73 (1.26-2.36)	0.001	1.39 (1.16-1.55)	<0.001

Abbreviations: ICU intensive care unit, CSHR cause-specific hazard rate, CI confidence interval; GNB Gram-negative bacteria, MS marginal structural

^a Results from cox regression model with colonization as a time-dependent variable (not adjusted for confounders).

^b Results from MS model with correction for time-fixed and time-dependent confounders (age, immune deficiency, Charlson comorbidity index, surgical admission, infection at admission, mechanical ventilation, SOFA score, SOFA change, gram negative antibiotic, surgical drains).

Respiratory tract and rectal colonization and bacteremia with GNB

We observed 44 episodes of ICU-acquired GNB-bacteremia during 27,890 observation days (incidence rate 1.6/1000 days) with 45 species. Of the 44 episodes, 20 (46%) occurred during concurrent rectal colonization (incidence rate 3.6/1,000 colonized days, versus 1.1/1,000 non-colonized days), with the same species causing infection and colonization in 18/20 cases. For respiratory tract colonization, 16 bacteremia episodes (36%) occurred during concurrent respiratory tract colonization (same species in 15/16). The incidence rate was 2.7/1,000 colonized days, versus 1.3/1,000 non-colonized days in the respiratory tract. Bacteremia was most frequently caused by *P. aeruginosa* (15, 33%), *E.coli* (8,

18%), and *S. marcescens* (5, 11%). The adjusted CSHR for bacteremia with GNB was 7.37 (95% CI 3.25-16.68, p-value <0.001) for GNB rectal colonization; 2.56 (95% CI 1.09-6.03, p-value 0.032) for GNB respiratory tract colonization; and 10.84 (95% CI 4.23-27.77, p-value <0.001) for concurrent GNB colonization at both sites (Table 3). Results were similar for Enterobacteriaceae and GNF GNB when analyzed separately (Supplement, table S2, result of MSM analyses for Enterobacteriaceae and GNF GNB).

Table 3. Association between rectum or respiratory tract colonization with Gram-negative bacteria and ICU-acquired bacteremia (n=2,066)

Model	Outcome					
	ICU-acquired bacteremia		ICU death		ICU discharge	
	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value
Rectum colonization						
<i>Crude</i> ^a	9.07 (3.94-20.86)	<0.001	1.86 (1.38-2.52)	<0.001	1.12 (0.97-1.30)	0.013
<i>MS model</i> ^b	7.37 (3.25-16.68)	<0.001	1.78 (1.29-2.46)	<0.001	1.18 (1.01-1.39)	0.039
Respiratory tract colonization						
<i>Crude</i> ^a	2.63 (1.14-6.03)	0.023	0.93 (0.68-1.28)	0.657	0.84 (0.72-0.79)	0.020
<i>MS model</i> ^b	2.56 (1.09-6.03)	0.032	0.90 (0.64-1.27)	0.552	0.87 (0.74-1.02)	0.090
Rectum and respiratory tract colonization						
<i>Crude</i> ^a	11.13(4.50-27.58)	<0.001	2.32 (1.54-3.50)	<0.001	0.87 (0.68-1.14)	0.270
<i>MS model</i> ^b	10.84 (4.23-27.77)	<0.001	2.24 (1.43-3.51)	<0.001	0.95 (0.72-1.24)	0.685

Abbreviations: ICU, intensive care unit; CSHR, cause-specific hazard rate; CI, confidence interval; GNB, Gram-negative bacteria; MS, marginal structural

a) Results from cox regression model with colonization as a time-dependent variable (not adjusted for confounders).

b) Results from MS model with correction for time-fixed and time-dependent confounders (age, immune deficiency, Charlson comorbidity index, surgical admission, infection at admission, mechanical ventilation, SOFA score, SOFA change, gram negative antibiotic, surgical drains).

Rectal colonization and acquisition of respiratory tract colonization

There were 999 ICU episodes with respiratory cultures not growing GNB on ICU-admission, who thus remained at risk for subsequent colonization. (Supplement, figure S2, inclusion flow chart). Baseline characteristics can be found in the supplement (Supplement, table S3, characteristics of ICU admissions for objective 3).

There were 119 episodes of ICU-acquired GNB respiratory tract colonization during 13,389 observation days (incidence rate 8.9 per 1,000 days); 31 (26%) of these occurred during concurrent rectal GNB colonization, with identical species present

in 11/31 (35%) episodes. There were 61 episodes of ICU-acquired respiratory tract colonization with Enterobacteriaceae, of which 19 (31%) occurred during rectal colonization, with identical species in eight episodes (42%). For GNF GNB, there were 104 episodes of ICU-acquired respiratory tract colonization of which six (6%) occurred during GNF GNB rectum colonization, in five episodes (83%) with *P. aeruginosa* carriage at both sites.

The CSHR for acquiring GNB respiratory tract colonization was 2.93 (95% CI 2.02-4.23, p-value <0.001) and similar for Enterobacteriaceae and GNF GNB separately. (Table 4 and Supplement, table S4, result of MSM analyses for Enterobacteriaceae and GNF GNB).

Table 4. Association between rectum colonization with Gram-negative bacteria and acquisition of respiratory tract colonization

Model	Outcome					
	ICU-acquired respiratory tract colonization		ICU death		ICU discharge	
	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value
Crude ^a	2.28 (1.44-3.63)	<0.001	2.83 (1.78-4.50)	<0.001	0.91 (0.71-1.17)	0.461
MS model ^b	2.93 (2.02-4.23)	<0.001	1.92 (1.10-3.35)	0.021	1.17 (0.91-1.51)	0.218

Abbreviations: GNF, glucose non-fermenting; ICU, intensive care unit; CSHR, cause-specific hazard rate; CI, confidence interval; GNB, Gram-negative bacteria; MS, marginal structural

a) Results from MS model (cox model accounting for repeated admission by a random nested effect, but not for confounders).

b) Results from MS model with time-fixed and time-dependent confounders (age, immune deficiency, Charlson comorbidity index, surgical admission, infection at admission, mechanical ventilation, SOFA score, SOFA change, gram negative antibiotic, inhalation antibiotics).

Discussion

In this study among patients receiving SDD, colonization of the gut with GNB was associated with an increased risk of ICU-acquired infection. These findings confirm and extend previously reported associations between gut colonization and ICU-acquired GNB bacteremia (2), and between gut colonization and infection with *P. aeruginosa*, *K. pneumoniae* and *A. baumannii* (9-11). Mechanisms that may explain these associations include bacterial translocation from the gut, exogenous translocation via the fecal patina leading to respiratory tract colonization, vascular-

catheter associated bacteremia through the colonized skin, and micro-aspiration of gut flora colonizing the stomach (12).

In our study, both rectal and respiratory carriage were associated with an increased risk of ICU-acquired bacteremia. However, the association for rectal carriage was stronger than for respiratory tract carriage, indicating that the gut is a more important reservoir for GNB bacteremia. Yet, simultaneous colonization at *both* body sites had the highest hazard rate for acquiring bacteremia. This is in line with clinical observations that SDD, which targets carriage in the respiratory tract and in the gut, was more effective in reducing the incidence of ICU-acquired GNB bacteremia than SOD, which only targets carriage in the respiratory tract (6, 13).

Migration of GNB from the gut to the respiratory tract may occur through endogenous and exogenous routes. Indeed, rectal carriage with GNB in our study was associated with subsequent acquisition of GNB respiratory tract colonization. However, where congruence between species colonizing rectum and causing ICU-acquired infections was 71%, congruence was only 35% for rectal and respiratory tract colonization. This low congruence at the species level questions a causal relationship between rectal colonization and acquisition of respiratory tract colonization, and suggests unmeasured confounding. The size of our study precluded species-specific analyses, which could have resulted in more accurate estimates of associations between rectal and respiratory tract colonization. Furthermore, colonization at other body sites or exogenous sources may also be associated with respiratory tract colonization and ICU acquired infections. For instance, 38 of 48 ICU-acquired infections caused by *Pseudomonas* and *Stenotrophomonas* species occurred without documented rectal colonization with GNB, suggesting the gut was not the source of these infections.

In our cohort of mechanically ventilated patients treated with SDD, the incidence rate of ICU acquired GN infections (4.4/1,000 days) was low compared to other cohorts. For example, in a French cohort without SDD the incidence of all cause ICU acquired GN infections in mechanically ventilated patients was 14/1,000 patient days (14). Also, the incidence rate of ICU acquired bacteremia (1.6/1000 days) was low compared to a Taiwanese study including mechanically ventilated ICU patients (15), and similar to the rate observed in a meta-analysis on the effect of CHX bathing (1.1/1,000 days), although also non-ventilated ICU patients were included in the latter study (16).

In some patients treated with SDD intestinal colonization with GNB persisted, despite application of laxative agents and intensified administration of SDD. Apparently, achieved concentrations of topical antibiotics in the distal parts of the gastrointestinal tract remained too low. It is unknown whether further increasing the frequency of SDD administration (to more than eight times per day) is safe. Absorption of tobramycin leading to toxic levels has been described in patients receiving CVWH (17).

Strengths of this study include the cohort size, the protocolized surveillance for carriage, the prospective adjudication of infections by independent research physicians, and the use of advanced methodology to account for time-dependent confounders. Limitations of this study include absence of screening for GNB carriage at other body sites, and the absence of molecular typing of bacteria, which precludes assessment of genotypic relatedness of species colonizing and infecting patients. Our assumption of relatedness based on species identification most likely overestimated associations. In a recently published study, though, it was concluded based on whole genome sequencing results that in 80% of infections the genome of *K. pneumoniae* was identical to that of the *K. pneumoniae* carried in the rectum (10). Finally, misclassification may have occurred (most notably falsely categorizing patients as non-colonized) as the sensitivity of rectal swabs and perianal swabs to detect GNB colonization is less than 100% (18, 19). This may have influenced the strength and accuracy of the associations.

Conclusion

Rectal colonization with GNB is associated with an increased risk of ICU-acquired GNB infection and respiratory tract colonization. Future studies are warranted to determine whether the protective effects of SDD on the occurrence of ICU-acquired bacteremia can be safely augmented in patients with persistent intestinal carriage.

Acknowledgements

We thank P.M.C. Klein Klouwenberg, D.S.Y. Ong, D. Verboom, and all trial nurses for their participation in the data collection.

References

1. Sertaridou E, Papaioannou V, Kolios G, Pneumatikos I. Gut failure in critical care: old school versus new school. *Ann Gastroenterol*. 2015;28(3):309-22.
2. Oostdijk EA, de Smet AM, Kesecioglu J, Bonten MJ. The role of intestinal colonization with gram-negative bacteria as a source for intensive care unit-acquired bacteremia. *Critical care medicine*. 2011;39(5):961-6.
3. de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernards AT, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. *The Lancet Infectious diseases*. 2011;11(5):372-80.
4. Dickson RP, Singer BH, Newstead MW, Falkowski NR, Erb-Downward JR, Standiford TJ, et al. Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nature microbiology*. 2016;1(10):16113.
5. Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van der Geest S, van Tiel FH, et al. Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. *Am J Respir Crit Care Med*. 2001;164(3):382-8.
6. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *N Engl J Med*. 2009;360(1):20-31.
7. Klein Klouwenberg PM, Ong DS, Bos LD, de Beer FM, van Hooijdonk RT, Huson MA, et al. Interobserver agreement of Centers for Disease Control and Prevention criteria for classifying infections in critically ill patients. *Critical care medicine*. 2013;41(10):2373-8.
8. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Critical care medicine*. 2005;33(7):1538-48.
9. Gomez-Zorrilla S, Camoerz M, Tubau F, Canizares R, Periche E, Dominguez MA, et al. Prospective observational study of prior rectal colonization status as a predictor for subsequent development of *Pseudomonas aeruginosa* clinical infections. *Antimicrobial agents and chemotherapy*. 2015;59(9):5213-9.
10. Gorrie CL, Mirceta M, Wick RR, Edwards DJ, Thomson NR, Strugnell RA, et al. Gastrointestinal carriage is a major reservoir of *K. pneumoniae* infection in intensive care patients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2017; doi: 10.1093/cid/cix270.
11. Latibeaudiere R, Rosa R, Laowansiri P, Arheart K, Namias N, Munoz-Price LS. Surveillance cultures growing carbapenem-Resistant *Acinetobacter baumannii* predict the development of clinical infections: a retrospective cohort study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2015;60(3):415-22.
12. Donskey CJ. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2004;39(2):219-26.
13. Oostdijk EAN, Kesecioglu J, Schultz MJ, Visser CE, de Jonge E, van Essen EHR, et al. Notice of Retraction and Replacement: Oostdijk et al. Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. *JAMA*. 2014;312(14):1429-1437. *Jama*. 2017;317(15):1583-4.
14. Camus C, Salomon S, Bouchigny C, Gacouin A, Lavoue S, Donnio PY, et al. Short-term decline in all-cause acquired infections with the routine use of a decontamination regimen combining topical polymyxin, tobramycin, and amphotericin B with mupirocin and chlorhexidine in the ICU: a single-center experience. *Critical care medicine*. 2014;42(5):1121-30.
15. Ko HK, Yu WK, Lien TC, Wang JH, Slutsky AS, Zhang H, et al. Intensive care unit-acquired bacteremia in mechanically ventilated patients: clinical features and outcomes. *PloS one*. 2013;8(12):e83298.
16. Afonso E, Blot K, Blot S. Prevention of hospital-acquired bloodstream infections through chlorhexidine gluconate-impregnated washcloth bathing in intensive care units: a systematic review and meta-analysis of randomised crossover trials. *Euro surveillance*. 2016;21(46); doi: 10.2807/1560-7917.es.2016.21.46.30400.

17. Mol M, van Kan HJ, Schultz MJ, de Jonge E. Systemic tobramycin concentrations during selective decontamination of the digestive tract in intensive care unit patients on continuous venovenous hemofiltration. *Intensive care medicine*. 2008;34(5):903-6.
18. Lautenbach E, Harris AD, Perencevich EN, Nachamkin I, Tolomeo P, Metlay JP. Test characteristics of perirectal and rectal swab compared to stool sample for detection of fluoroquinolone-resistant *Escherichia coli* in the gastrointestinal tract. *Antimicrobial agents and chemotherapy*. 2005;49(2):798-800.
19. Dyakova E, Bisnauthsing KN, Querol-Rubiera A, Patel A, Ahanonu C, Tosas August O, et al. Efficacy and acceptability of rectal and perineal sampling for identifying gastrointestinal colonization with extended spectrum beta-lactamase Enterobacteriaceae. *Clin Microbiol Infect*. 2017; doi: 10.1016/j.cmi.2017.02.019.
20. Daniel RM, Cousens SN, De Stavola BL, Kenward MG, Sterne JA. Methods for dealing with time-dependent confounding. *Stat Med*. 2013;32(9):1584-618.
21. Bailly S, Pirracchio R, Timsit JF. What's new in the quantification of causal effects from longitudinal cohort studies: a brief introduction to marginal structural models for intensivists. *Intensive care medicine*. 2016;42(4):576-9.

Supplement

Appendix: Rationale for using a marginal structural model

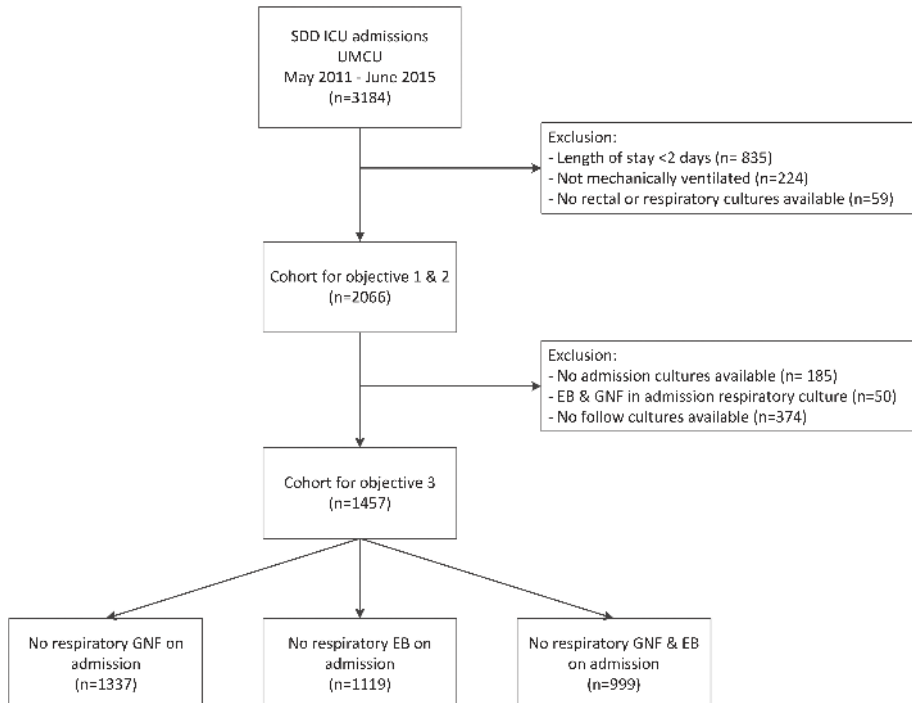
The marginal structural model (MSM) survival-analysis technique was chosen over a regular time-dependent Cox-regression analysis given the presence of both time-varying exposure and time-varying confounders. To estimate a causal effect of GN colonization (time-varying exposure) on a certain endpoint it is necessary to correct for both time fixed (e.g. age) and time-varying confounders (e.g. antibiotic use). Time-varying confounders such as antibiotic use may influence the outcome (infection) and may influence the determinant (GN colonization), but the inverse relation is also present (GN colonization may influence future antibiotic use). Correcting for antibiotic use through regular time-dependent Cox regression analysis would therefore eliminate part of the association between GN colonization and infection since it behaves as an intermediate on the causal pathway. In addition, this might introduce collider stratification bias (20). To overcome these problems a MSM can be used. For patients sharing a set of confounders, the proportion of patients that is colonized is calculated per day, thereby obtaining the theoretical probability of being colonized during ICU stay for each combination of (time-varying) confounders present during ICU stay. A weight is calculated from the inverse of these probabilities, allowing re-weighting of all cases based on the distribution of confounders, thus creating a pseudo population as if the determinant 'GN colonization status' had been randomized (20, 21). The weights attributed to cases are subsequently used in a Cox regression analysis to model the association between GN colonization and the chosen endpoints. To further reduce the chance of correcting for intermediates we used lagged values of the preceding day for all time-dependent confounders. We adjusted for disease severity (as measured by the sequential organ failure assessment (SOFA) score) in two ways: (1) we used the lagged value of the total SOFA score of two days before and (2) we calculated the difference between the 2 day lagged SOFA score and 1 day lagged value (lag 1 – lag 2) in order to incorporate the trajectory of the SOFA score as a confounder.

Supplement figure S1. Definition of colonization with Gram-negative bacteria

Days	1	2	3	4	5	6	7	8	9	10
Cultures example 1		+		+			-			+
Colonization example 1										
Cultures example 2	+			-			-			+
Colonization example 2										
Cultures example 3				-			+			-
Colonization example 3										

A culture positive for Gram-negative bacteria is depicted by a '+', whereas a '-' indicates a culture that did not grow Gram-negative bacteria. The subsequent colonization status per day based on these cultures is shown by the red (not colonized with Gram-negative bacteria) and green (colonized with Gram-negative bacteria) squares. Colonized patients could switch to non-colonized if a positive culture result was followed by a negative result. Changing of colonization status was assumed to occur in the middle of an interval between two subsequent culture results. Colonization status on admission was based on the first available culture result.

Supplement figure S2. Inclusion flow chart



n: number of admissions

Abbreviations: *UMCU* University Medical Center Utrecht, *SDD* selective digestive tract decontamination, *ICU* intensive care unit, *GNF* Glucose non-fermenting, *EB* enterobacteriaceae.

Supplement table S1. Results of marginal structural model analyses for objective 1: the association between rectum colonization with Enterobacteriaceae and Glucose non-fermenting Gram-negative bacteria and ICU-acquired infections.

	Outcome					
	ICU-acquired GNB infection		ICU death		ICU discharge	
	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value
<i>Enterobacteriaceae</i>						
<i>Crude^a</i>	7.30 (4.21-12.57)	<0.001	1.86 (1.40-2.47)	<0.001	1.19 (1.04-1.36)	0.011
<i>MS model^b</i>	2.93 (2.03-4.23)	<0.001	1.56 (1.13-2.15)	0.007	1.42 (1.22-1.64)	<0.001
<i>Glucose non-fermenting GNB</i>						
<i>Crude^a</i>	5.84 (2.63-12.97)	<0.001	1.97 (1.19-3.25)	0.008	0.58 (0.40-0.86)	0.006
<i>MS model^b</i>	3.76 (1.39-10.16)	0.009	1.44 (0.77-2.67)	0.253	0.85 (0.59-1.20)	0.351

Abbreviations: ICU, intensive care unit; CSHR, cause-specific hazard rate; CI, confidence interval; GNB, Gram-negative bacteria; MS, marginal structural

^a Results from cox regression model with colonization as a time-dependent variable (not adjusted for confounders)

^b Results from MS model with correction for time-fixed and time-dependent confounders (age, immune deficiency, Charlson comorbidity index, surgical admission, infection at admission, mechanical ventilation, SOFA score, SOFA change, gram negative antibiotic, surgical drains)

Supplement table S2. Results of marginal structural model analyses for objective 2: The association between rectum or respiratory tract colonization with Enterobacteriaceae or Glucose non-fermenting Gram-negative bacteria and ICU-acquired bacteremia.

	Outcome					
	ICU-acquired bacteremia		ICU death		ICU discharge	
	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value
<i>Enterobacteriaceae</i>						
Rectum colonization						
<i>Crude^a</i>	25.36 (7.58-84.77)	<0.001	1.79 (1.32-2.44)	<0.001	1.20 (1.03-1.39)	0.019
<i>MS model^b</i>	19.41 (5.77-65.30)	<0.001	1.68 (1.20-2.33)	0.002	1.26 (1.07-1.48)	0.005
Respiratory tract colonization						
<i>Crude^a</i>	5.32 (1.04-27.25)	0.045	0.98 (0.63-1.52)	0.929	1.00 (0.82-2.21)	0.952
<i>MS model^b</i>	5.64 (1.12-28.40)	0.036	0.97 (0.61-1.55)	0.970	1.00 (0.81-1.23)	0.963
Rectum and respiratory tract colonization						
<i>Crude^a</i>	24.85 (5.69-108.53)	<0.001	1.91 (1,15-3,17)	0.012	1.01 (0.76-1.33)	0.960
<i>MS model^b</i>	21.28 (4.70-96.40)	<0.001	1.71 (0.97-3.00)	0.065	1.10 (0.81-1.48)	0.555
<i>Glucose non-fermenting GNB</i>						
Rectum colonization						
<i>Crude^a</i>	18.93 (5.61-63.91)	<0.001	2.01 (1.16-3.47)	0.013	0.68 (0.46-0.99)	0.045
<i>MS model^b</i>	15.33 (4.17-56.43)	<0.001	1.68 (0.89-3.19)	0.112	0.81 (0.54-1.22)	0.313
Respiratory tract colonization						
<i>Crude^a</i>	4.95 (1.89-12.99)	0.001	0.94 (0.66-1.33)	0.719	0.79 (0.66-0.94)	0.010
<i>MS model^b</i>	5.53 (2.12-14.41)	<0.001	1.00 (0.68-1.46)	0.972	0.80 (0.65-0.97)	0.027
Rectum and respiratory tract colonization						
<i>Crude^a</i>	30.23 (8.92-102.51)	<0.001	2.48 (1.06-5.80)	0.037	0.38 (0.14-1.01)	0.052
<i>MS model^b</i>	41.43 (10.0-170,9)	<0.001	2.83 (1.11-7.23)	0.030	0.42 (0.15-1.17)	0.097

Abbreviations: ICU, intensive care unit; CSHR, cause-specific hazard rate; CI, confidence interval; GNB, Gram-negative bacteria; MS, marginal structural

^a Results from cox regression model with colonization as a time-dependent variable (not adjusted for confounders)

^b Results from MS model with correction for time-fixed and time-dependent confounders (age, immune deficiency, Charlson comorbidity index, surgical admission, infection at admission, mechanical ventilation, SOFA score, SOFA change, gram negative antibiotic, surgical drains)

Chapter 2

Supplement table S3. Characteristics of ICU admissions without respiratory tract colonization with Gram-negative bacteria at admission (objective 3, n=999).

	Rectum colonization		p-value
	Never colonized (n=454) n (%) / median (IQR) ^a	Ever colonized (n=545) n (%) / median (IQR) ^a	
<i>Patient characteristics</i>			
Age (years)	60 (46-70)	60 (49-70)	0.450
Male	301 (66)	350 (64)	0.492
Chronic renal insufficiency	31 (7)	43 (8)	0.523
Congestive heart failure	52 (12)	75 (14)	0.041
Myocardial infarction	45 (10)	54 (10)	0.998
COPD	53 (12)	52 (10)	0.274
Diabetes mellitus	61 (13)	96 (18)	0.071
Immune deficiency	80 (18)	76 (14)	0.111
Hematologic malignancy	33 (7)	18 (3)	0.005
Cerebrovascular disease	46 (10)	69 (13)	0.212
Charlson comorbidity index	0 (0-2)	0 (0-2)	0.94
<i>ICU admission characteristics</i>			
Surgical admission	135 (30)	239 (44)	<0.001
Readmission	91 (20)	48 (9)	<0.001
Infection at admission	246 (54)	234 (43)	<0.001
APACHE IV Score	76 (61-99)	78 (61-97)	0.730

Abbreviations: n, number; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation.

^a Data are shown as absolute number (%) or median (Q1-Q3).

Supplement table S4. Results of marginal structural model analyses for objective 3: Association between rectum colonization with Enterobacteriaceae or Glucose non-fermenting Gram-negative bacteria and acquisition of respiratory tract colonization.

	Outcome					
	ICU-acquired respiratory tract colonization		ICU death		ICU discharge	
	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value	CSHR (95%CI)	p-value
<i>Enterobacteriaceae</i>						
Crude ^a	3.23 (1.63-6.03)	<0.001	2.85 (1.85-4.37)	<0.001	0.90 (0.70-1.16)	0.419
MS model ^b	3.00 (1.57-5.71)	0.001	1.66 (0.93-2.95)	0.085	1.19 (0.92-1.54)	0.177
<i>Glucose non-fermenting GNB</i>						
Crude ^a	2.75 (1.23-6.13)	0.013	2.95 (1.62-5.38)	<0.001	0.68 (0.41-1.13)	0.136
MS model ^b	2.60 (1.12-6.03)	0.027	2.19 (1.03-4.66)	0.042	0.99 (0.62-1.60)	0.980

Abbreviations: GNF, glucose non-fermenting; ICU, intensive care unit; CSHR, cause-specific hazard rate; CI, confidence interval; GNB, Gram-negative bacteria; MS, marginal structural

^a Results from MS model (cox model accounting for repeated admission by a random nested effect, but not for confounders)

^b Results from MS model with time-fixed and time-dependent confounders (age, immune deficiency, Charlson comorbidity index, surgical admission, infection at admission, mechanical ventilation, SOFA score, SOFA change, gram negative antibiotic, inhalation antibiotics)

Chapter 3

Nystatin versus amphotericin B to prevent and eradicate Candida colonization during selective digestive tract decontamination in critically ill patients

Intensive care medicine 2015; 41(12): 2235-2236

Bastiaan H.J. Wittekamp ^{*1}

David S.Y. Ong ^{*1,2,3}

Olaf L. Cremer ²

Marc J.M. Bonten ^{1,3}

* both authors contributed equally to this work

¹ Julius Center for Health Sciences and Primary Care

² Department of Intensive Care Medicine

³ Department of Medical Microbiology

All at University Medical Center Utrecht, Utrecht, The Netherlands

Abstract

Purpose: To compare the effectiveness of topical application of nystatin and amphotericin B for prevention of *Candida* colonization in the intestinal and respiratory tract of critically ill patients.

Methods: In a before-after study in a 32-bed mixed ICU in a tertiary care center, we determined colonization with *Candida* species in the intestinal and respiratory tracts of patients receiving one of three regimens of Selective Digestive tract Decontamination (SDD): SDD with amphotericin B as antifungal component in both oropharyngeal paste and enteral suspension (Am/Am, 16 months); amphotericin B in oropharyngeal paste and nystatin in enteral suspension (Am/Nys, 17 months); and nystatin in both oropharyngeal paste and enteral suspension (Nys/Nys, 10 months). Acquisition of *Candida* colonization in the rectum and respiratory tract and decolonization of *Candida* from the rectum was based on rectum and respiratory tract surveillance cultures.

Results: During the three periods 867, 849 and 489 patients were enrolled, respectively. Rectal colonization rates were 1.9 (95% CI 1.5-2.4), 1.3 (95% CI 1.0-1.7) and 1.0 (95% CI 0.6-1.3) events per 100 days for subsequent periods. Nys/Nys was independently associated with less rectal acquisition of *Candida* compared to Am/Am (adjusted hazard ratio (HR) 0.52, 95% CI 0.33-0.83) and associated with faster rectal decolonization (adjusted HR 1.70, 95%CI 1.18-2.45) in 373 patients who were already colonized at admission. Acquisition rates in the respiratory tract were not significantly different in the three study periods.

Conclusion: Nystatin was more effective than amphotericin B in both preventing and eradicating *Candida* colonization of the rectum during SDD, and had comparable effects in preventing respiratory tract carriage with *Candida*.

Introduction

The use of selective digestive tract decontamination (SDD) and selective oropharyngeal decontamination (SOD) in intensive care units (ICUs) with low levels of antibiotic resistance has been associated with absolute reductions in day-28 mortality of 3.5% and 2.9%, respectively, compared to standard care without decontamination strategies, corresponding to a number needed to treat of 34 and 29 (1). SDD has been shown to reduce intensive care unit (ICU) length of stay, ICU mortality and hospital mortality (2, 3). Both strategies aim to eliminate potential pathogenic microorganisms, such as *Staphylococcus aureus*, Gram-negative bacteria and yeasts from the oropharynx and digestive tracts of ICU patients while preserving the anaerobic flora to prevent colonization, overgrowth and subsequent infections such as ventilator associated pneumonia.

Fungal colonization, most often by *Candida* species, occurs frequently in ICU patients who are admitted for 7 days or more (4). In these patients *Candida* colonization was present in 21-29% of rectal surveillance samples and 18-26% of bronchial aspirate surveillance samples (4). *Candida* colonization was an independent risk factor for subsequent yeast infection (5).

The antimicrobial regimen most often used in SDD consists of three components: a topical mouth paste, gastro- enteral suspension, and systemic antimicrobial prophylaxis with a broad-spectrum antimicrobial agent, such as a third generation cephalosporin. The mouth paste and suspension usually contain colistin, tobramycin and amphotericin B. The latter is to eradicate yeast and prevent yeast colonization and infection. In some studies nystatin has been used instead of amphotericin B (6-8). Nystatin and amphotericin B are both effective against a broad range of fungi and are not absorbed from the digestive tract (9). The effects of amphotericin B and nystatin, as used in SDD regimens, on *Candida* colonization and eradication have never been compared. Yet, amphotericin B is currently more expensive than nystatin and periodically has been difficult to acquire due to scarce of its raw materials, fuelling initiatives to substitute one drug for the other. We, therefore, prospectively compared the efficacy of nystatin and amphotericin B as intestinal and oropharyngeal components of SDD with respect to prevention of *Candida* colonization in the respiratory tract and eradication of *Candida* from the rectum and respiratory tract.

Methods

We performed a before-after study evaluating subsequent changes to an SDD regimen in a 32-bed medical-surgical ICU of a tertiary care hospital in the Netherlands between June 2011 and December 2014. All patients who were admitted for 48 hours or longer (and thus eligible to receive SDD until discharge from the ICU) were included in the study. We excluded patients who received concurrent systemic antifungal treatment during their ICU stay. The local ethics committee waived the need for informed consent.

SDD treatment

There were three study periods: (1) from June 2011 till October 1st 2012 (16 months) SDD treatment included four times daily (q.d.s.) application of a mouth paste containing 2% polymyxin E, 2% tobramycin and 2% amphotericin B, administration (q.d.s.) of a suspension with the same components (100 mg polymyxin E, 80 mg tobramycin and 500 mg amphotericin B) through the nasogastric tube, and the systemic administration (q.d.s.) of cefotaxime during the first four days of ICU admission (Am/Am); (2) from October 2012 till March 1st 2014 (17 months) nystatin (2×10^6 units per dose) replaced amphotericin B in the enteral suspension only (Am/Nys); (3) from March 2014 till January 1st 2014 (10 months) nystatin replaced amphotericin B in both the oropharyngeal paste and enteral solution (Nys/Nys). All other components of the SDD protocol, including infection surveillance methods, isolation measures and indications for treatment of infections remained unchanged during the three study periods.

Microbiological cultures

Microbiological screening samples, including rectal swabs and respiratory tract samples (sputum, endotracheal aspirate or broncho-alveolar lavage (BAL) samples when available), were routinely collected in all patients on admission and twice weekly thereafter as part of the SDD protocol (1). For *Candida* detection, samples were inoculated on malt extract agar plates, and *Candida* load was semi-quantitatively determined (i.e. classified as <10, 10-100, and >100 colony forming units). Culture results were used to modify the SDD regimen, for instance by adding nebulization with amphotericin B in case of persistent carriage of *Candida* in respiratory tract samples despite SDD (10).

Outcome

The primary outcome measures were the acquisition rates of *Candida* colonization in the rectum and respiratory tract in each study period. Secondary outcomes were the prevalence of *Candida* in the rectum and respiratory tract during the three study periods and eradication of rectal carriage with *Candida* in patients who were colonized with *Candida* at the time of ICU admission.

Colonization was defined as the presence of *Candida* species in two or more consecutive samples from one body site (rectal or respiratory tract) obtained on different days in the ICU or the presence of *Candida* in the last available sample of that body site. Patients were decolonized if two or more consecutive rectal samples showed no growth of *Candida* in a patient previously identified as colonized, or the absence of *Candida* in the last available rectum sample.

The effect of nystatin and amphotericin B on decolonization of the respiratory tract was not studied, as we hypothesized that the SDD mouth paste would not eradicate *Candida* from the lower respiratory tract, but rather prevent colonization.

Data analysis

Cox survival regression was used to study the associations between the SDD regimens and acquisition of *Candida* colonization in the rectum and respiratory tract. For this analysis we considered patients who had at least two rectal or two respiratory tract samples available and were not colonized by *Candida* in the rectum or respiratory tract, respectively, based on the first two available surveillance cultures since admission. We calculated the proportion of patients who acquired *Candida* colonization and the corresponding acquisition rates in the rectum and respiratory tract. In our multivariable models we adjusted for all co-variables that changed the crude effect estimate of either one of the SDD regimens by more than 10% as well as all patient characteristics that were imbalanced between the groups at baseline (p value <0.20).

For the secondary analysis on decolonization of rectal carriage Cox survival regression was used. We included patients who had at least two rectal samples available and with documented rectal colonization in the first two surveillance cultures since admission. All data were analyzed with SAS 9.2 (Cary, NC).

Results

A total of 2,456 patients were admitted for 48 hours or longer during the entire study period. After exclusion of patients who received concurrent systemic antifungal treatment, 2,205 patients remained. The median length of ICU stay was 5.6 (inter quartile range (IQR) 3.3-10.0), 5.8 (IQR 3.2-10.8) and 5.8 days (IQR 3.4-11.7) for the Am/Am, Am/Nys and Nys/Nys periods, respectively ($p=0.71$). A median of 2 (IQR 1-4) rectum surveillance and 2 (IQR 1-4) respiratory surveillance cultures were available per patient. *Candida* was present in 23.3%, 21.2% and 18.4% of rectum surveillance cultures and in 34.7%, 42.4% and 33.0% of respiratory surveillance cultures, for study period 1 to 3, respectively (Table 1). Of the 2,205 included patients, 1,468 patients had at least two rectum surveillance cultures available, and 1,378 patients had at least two sputum surveillance cultures available.

Table 1. Timeline of the study

Period	1 (Am/Am)	2 (Am/Nys)	3 (Nys/Nys)
Follow up (months)	16	17	10
Date	June 2011 - October 2012	October 2012 - March 2014	March 2014 - January 2015
Mouth paste ^a	Amphotericin B	Amphotericin B	Nystatin
Gastro-enteral Suspension ^a	Amphotericin B	Nystatin	Nystatin
Number of patients ^b	867	849	489
Number of:			
Rectum surveillance cultures	2,277	2,584	1,486
Candida positive rectum cultures (%)	531 (23.3%)	548 (21.2%)	274 (18.4%)
Median number of cultures / patient (IQR)	2 (1-3)	2 (1-4)	2 (2-4)
Respiratory surveillance cultures ^c	2,430	2,671	1,569
Candida positive respiratory cultures (%)	844 (34.7%)	1,133 (42.4%)	518 (33.0%)
Median number of respiratory cultures / patient (IQR)	2 (1-4)	2 (1-4)	2 (1-4)

(a) in combination with colistin and tobramycin

(b) all patients with stay >48 hours (excluding systemic antifungal therapy)

(c) sputum, endotracheal aspirate or broncho-alveolar lavage (BAL) samples

Acquisition of Candida colonization in the rectum

Of the 1,468 patients who had at least two rectum surveillance cultures available, 1,095 cases (75%) were not colonized at the start of ICU admission and included in the analysis on Candida acquisition in the rectum.

Intestinal Candida colonization occurred in 80 of 441 (18%), 62 of 415 (15%), and 24 of 239 (10%) patients during period 1 (Am/Am), period 2 (Am/Nys) and period 3 (Nys/Nys) ($p=0.02$), respectively. Acquisition rates were 1.9 (95% confidence interval (CI) 1.5-2.4), 1.3 (95% CI 1.0-1.7) and 1.0 (95% CI 0.6-1.3) events per 100 patient days, respectively. The time until colonization was comparable: 6 (IQR 4-10), 6 (IQR 4-12) and 7 (IQR 3-12) days ($p=0.58$), respectively. Compared to the reference period (i.e., Am/Am) and after adjustment for baseline imbalances (Table 2), no significant difference was found for Am/Nys (adjusted HR 0.74, 95% CI 0.53-1.03), whereas Nys/Nys was associated with prevention of Candida colonization in the intestinal tract (adjusted HR 0.52, 95% CI 0.33-0.83) (Table 3).

Acquisition of Candida colonization in respiratory samples

Of 1,378 patients from whom at least two screening sputum cultures were available, 702 were not colonized in the sputum with Candida at admission. Respiratory tract colonization occurred in 61 of 295 (21%), 79 of 215 (31%), and 31 of 156 (20%) patients during period 1 to period 3, respectively ($p<0.01$). Acquisition rates were 2.5 (95% CI 1.8-3.1), 2.7 (95% CI 2.1-3.3) and 2.1 (95% CI 1.3-2.8) events per 100 patient days, respectively. After adjustment for baseline imbalances (Table 2), neither Am/Nys nor Nys/Nys was associated with altered acquisition rates of Candida in the lower respiratory tract (Table 3).

Candida decolonization in the intestinal tract

Among a total of 373 patients who were already colonized with Candida in the rectum at admission, decolonization occurred in 59 of 127 (46%), 84 of 150 (56%), and 57 of 96 (59%) patients during period 1 to period 3 ($p=0.12$), respectively. Decolonization rates were 6.7 (95% CI 5.0-8.4), 8.2 (95% CI 6.5-10.0) and 10.8 (95% CI 8.0-13.6) events per 100 patient days, respectively. After adjustment for baseline imbalances, Nys/Nys was associated with faster decolonization of Candida in the rectum (adjusted HR 1.70, 95% CI 1.18-2.45) (Table 3).

Table 2. Characteristics of patients that were not colonized on admission

Characteristic	Rectum analysis (n=1095) ^a		Respiratory tract analysis (n=702) ^a			
	Period		Period			
	1 (n=441)	2 (n=415)	3 (n=239)	1 (n=295)	2 (n=251)	3 (n=156)
Age - years	59 (48-71)	61 (50-70)	61 (48-72)	58 (48-69)	60 (49-69)	58 (40-73)
Male sex	302 (68)	291 (70)	159 (67)	189 (64)	168 (67)	104 (67)
Body Mass Index	25 (23-28)	26 (23-30)	26 (23-29)	25 (23-28)	26 (23-30)	26 (23-30)
Chronic obstructive pulmonary disease	50 (11)	46 (11)	26 (11)	29 (10)	27 (11)	12 (8)
Diabetes mellitus	74 (17)	66 (16)	23 (10)	34 (12)	32 (13)	21 (13)
Hypertension	157 (36)	142 (34)	64 (27)	94 (32)	83 (33)	45 (29)
Chronic dialysis	4 (1)	7 (2)	1 (0)	3 (1)	6 (2)	1 (1)
Cancer	60 (14)	66 (16)	31 (13)	38 (13)	35 (14)	23 (15)
Immune deficiency ^b	29 (7)	41 (10)	25 (10)	20 (7)	19 (8)	15 (10)
Corticosteroids ^c	37 (8)	31 (7)	15 (6)	23 (8)	13 (5)	10 (6)
Medical reason for admission	13 (3)	13 (3)	10 (4)	11 (4)	9 (4)	5 (3)
Other immunosuppressive medication	246 (56)	233 (56)	135 (56)	165 (56)	132 (53)	95 (16)
Prior ICU admission during hospital stay	59 (13)	58 (14)	28 (12)	39 (13)	34 (14)	14 (9)
APACHE IV score	73 (58-90)	73 (60-96)	76 (67- 89)	73 (58-91)	71 (60-97)	76 (63-88)
ICU length of stay	8 (5-13)	9 (5-15)	9 (5-14)	8 (5-13)	10 (6-17)	10 (6-16)
ICU mortality	52 (12)	51 (12)	39 (16)	44 (15)	37 (15)	25 (16)

Data are presented as medians (IQR) or absolute numbers (%).

(a) patients with 2 or more culture results available, not colonized on admission

(b) Immune deficiency was defined as use of immunosuppressive medication (prednisone 0.1 mg/kg for at least 3 months, prednisone 75 mg/day during at least one week or equivalent), chemotherapy/radiotherapy in the year preceding intensive care unit admission, and a known humoral or cellular immune deficiency

(c) Corticosteroid use was defined as a daily dose > 100 mg hydrocortisone or equivalent.

Table 3. Effectiveness of nystatin versus amphotericin B in preventing *Candida* acquisition and achieving decolonization

	Crude Hazard Ratio [95% CI]	Adjusted Hazard Ratio [95% CI]
Primary analysis		
Candida acquisition in rectum		
Am/Am (reference group , period 1)	1	1
Am/Nys (period 2)	0.75 (0.54-1.05)	0.74 (0.53-1.03) ^a
Nys/Nys (period 3)	0.53 (0.33-0.83)	0.52 (0.33-0.83) ^a
Candida acquisition in sputum		
Am/Am (reference group , period 1)	1	1
Am/Nys (period 2)	1.23 (0.88-1.72)	1.21 (0.86-1.70) ^b
Nys/Nys (period 3)	0.87 (0.57-1.33)	0.85 (0.55-1.31) ^b
Secondary analysis		
Candida decolonization in rectum		
Am/Am (reference group , period 1)	1	1
Am/Nys (period 2)	1.23 (0.88-1.72)	1.22 (0.87-1.70) ^c
Nys/Nys (period 3)	1.71 (1.19-2.48)	1.70 (1.18-2.45) ^c

(a) Covariables selected for multivariable analysis were APACHE IV score, diabetes mellitus, hypertension, immune deficiency and body mass index.

(b) Covariables selected for multivariable analysis were body mass index and COPD.

(c) Covariables selected for multivariable analysis were gender, body mass index and corticosteroid use (a daily dose > 100 mg hydrocortisone or equivalent).

Discussion

This study evaluated the microbiological effects of changing the antifungal component of SDD from amphotericin B to nystatin. Less patients acquired intestinal *Candida* colonization when nystatin was used in the topical components of SDD, compared to amphotericin B. Nystatin was not more effective than amphotericin B in preventing acquisition of *Candida* in the lower respiratory tract.

Although amphotericin B and nystatin for decolonization purposes have never been compared head to head, nystatin has been used in previous SDD regimens (6-8). The optimal dose is unknown, however, most studies use 1-2 x 10⁶ units per dose, similar to the dose used in this study.

In our study, SDD could not prevent intestinal acquisition of *Candida* in 10-18% of patients. In a previous Dutch multicentre study, SDD with amphotericin B

led to increased acquisition of *Candida* and other yeasts in the respiratory tract, compared to standard care without SDD (Odds Ratio (OR) 1.59; 95% CI 1.31-1.93)(11). In contrast, in the same study the odds of candidemia with *Candida* or other yeasts was lower in SDD, compared to standard care (OR 0.33; 95% CI 0.13-0.82)(11). Furthermore, two meta-analyses found a beneficial effect of SDD with amphotericin B or nystatin on the odds of yeast colonization in general (OR 0.12; 95% CI 0.05-0.29 and OR 0.32; 95% CI 0.19-0.53) (12, 13) and fungal infections (OR 0.29; 95% CI 0.18-0.45 and OR 0.30; 95% CI 0.17-0.53)(12).

Although this is an observational study with a before-after design, randomization of individual patients could introduce bias. SDD has been shown to change the ICU ecology (1, 2), creating dependency of patients. Contamination of the treatment effect would occur if patients admitted to the same ward would use different decolonization strategies simultaneously. Therefore, unit wide application of the intervention is the most appropriate design, allowing for unbiased evaluation of ICU ecology. The three phases of the study with sequential changes of antifungal components allowed an estimation of the effect per component change, during periods of at least 10 months. All patients followed a protocolized surveillance scheme with obtaining respiratory tract and rectal samples on admission and twice weekly thereafter, minimizing the risk for information bias. Treatment indications and surveillance for *Candida* did not change during study period.

This study has some limitations. We did not evaluate resistance of fungal species against amphotericin B or nystatin. However, resistance to nystatin among *Candida* species is rare (9). Furthermore, we did not evaluate whether there was any effect of the intervention on the bacterial colonization status. This could be subject of further research, as could be the optimal dose of nystatin and frequency of application.

Amphotericin B and nystatin are similar, as both belong to the class of polyene antifungal agents. Yet, in our hospital amphotericin B is nowadays five times more expensive than nystatin. This is largely due to a global-wide shortness of raw materials of amphotericin. Since nystatin seems to be at least as effective as amphotericin B in prevention of colonization, nystatin will improve the cost-effectiveness of SDD.

Conclusion

In SDD, nystatin was more effective than amphotericin B in eradicating *Candida* from the rectum and preventing rectal *Candida* colonization, and is not inferior to amphotericin B in preventing *Candida* respiratory tract colonization. Nystatin use will improve the cost-effectiveness of SDD and SOD.

References

1. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *N Engl J Med*. 2009;360(1):20-31.
2. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet*. 2003;362(9389):1011-6.
3. Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ, et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. *American journal of respiratory and critical care medicine*. 2002;166(8):1029-37.
4. Leon C, Alvarez-Lerma F, Ruiz-Santana S, Leon MA, Nolla J, Jorda R, et al. Fungal colonization and/or infection in non-neutropenic critically ill patients: results of the EPCAN observational study. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*. 2009;28(3):233-42.
5. Pittet D, Monod M, Suter PM, Frenk E, Auckenthaler R. Candida colonization and subsequent infections in critically ill surgical patients. *Annals of surgery*. 1994;220(6):751-8.
6. Wiener J, Itokazu G, Nathan C, Kabins SA, Weinstein RA. A randomized, double-blind, placebo-controlled trial of selective digestive decontamination in a medical-surgical intensive care unit. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1995;20(4):861-7.
7. Cockerill FR, 3rd, Muller SR, Anhalt JP, Marsh HM, Farnell MB, Mucha P, et al. Prevention of infection in critically ill patients by selective decontamination of the digestive tract. *Annals of internal medicine*. 1992;117(7):545-53.
8. Flaherty J, Nathan C, Kabins SA, Weinstein RA. Pilot trial of selective decontamination for prevention of bacterial infection in an intensive care unit. *The Journal of infectious diseases*. 1990;162(6):1393-7.
9. Eggimann P, Wolff M, Garbino J. Oral nystatin as antifungal prophylaxis in critically ill patients: an old SDD tool to be renewed? *Intensive care medicine*. 2005;31(11):1466-8.
10. Ong DS, Klein Klouwenberg PM, Spitoni C, Bonten MJ, Cremer OL. Nebulised amphotericin B to eradicate Candida colonisation from the respiratory tract in critically ill patients receiving selective digestive decontamination: a cohort study. *Critical care (London, England)*. 2013;17(5):R233.
11. de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernardts AT, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. *The Lancet Infectious diseases*. 2011;11(5):372-80.
12. Silvestri L, van Saene HK, Milanese M, Gregori D. Impact of selective decontamination of the digestive tract on fungal carriage and infection: systematic review of randomized controlled trials. *Intensive care medicine*. 2005;31(7):898-910.
13. van Till JO, van Ruler O, Lamme B, Weber RJ, Reitsma JB, Boermeester MA. Single-drug therapy or selective decontamination of the digestive tract as antifungal prophylaxis in critically ill patients: a systematic review. *Critical care (London, England)*. 2007;11(6):R126.

Chapter 4

Regulatory obstacles conducting studies on ecological interventions in intensive care: the example of decontamination strategies

Lancet Infectious Diseases 2014; 14(10): 913-915

Bastiaan H.J. Wittekamp¹

Matt P. Wise²

Christian Brun-Buisson³

Marc J.M. Bonten^{1,4}

¹ Julius Center for Health Sciences and Primary care, University Medical Center Utrecht, The Netherlands

² Adult Critical Care, University Hospital of Wales, Cardiff, UK

³ Assistance Publique-Hôpitaux de Paris, Groupe Henri Mondor; Université Paris-Est, Creteil, France

⁴ Medical Microbiology, University Medical Center Utrecht, The Netherlands

Introduction

Healthcare-associated bacterial infections are an important cause of morbidity and mortality in critically ill patients, especially those requiring mechanical ventilation. Decolonization with topical antibiotics, such as selective digestive tract decontamination (SDD) or selective oropharyngeal decontamination (SOD), eradicates potentially pathogenic bacteria preventing ventilator-associated pneumonia and bacteremia. In two Dutch studies SDD and SOD reduced mortality, intensive care unit (ICU) length of stay, ICU-acquired bacteremia and carriage with antibiotic-resistant bacteria (1, 2). Accordingly, both measures were considered cost-effective (3). Currently, only studies that evaluated the unit-wide implementation of SDD or SOD provide evidence of a survival benefit, with relative and absolute mortality reductions of 10% and 3%, respectively (3).

The beneficial effects of decolonization were demonstrated in the Netherlands with low levels of antibiotic resistance, consequently these measures have not been widely adopted elsewhere because of uncertainty over the long-term effects on resistance (4, 5). Evaluation of decolonization strategies in other countries is advocated, and could save many thousands of lives if similar results are obtained (4, 6, 7). Since decolonization strategies represent a population-based intervention, and affect the microbiological ecology of the whole ICU population and not just individuals, recent studies adopted a cluster randomized trial (CRT) design where the unit of randomization is the ICU population. Moreover, if SDD or SOD were adopted as the standard of care it would be applied as a unit-wide intervention. The CRT is therefore the optimal design for evaluating decolonization strategies. As part of the European Union seventh Framework-funded project "Resistance in Gram-Negative Organisms: Studying Intervention Strategies" (RGNOSIS; HEALTH.2011.2.3.1-3), a CRT is planned to evaluate the ecological effects of SDD and SOD in 11,000 patients in countries outside the Netherlands. Yet, the preparation of this CRT has been confronted with a number of ethical and regulatory issues.

The requirement for patients' individual informed consent

According to the European Clinical Trials Directive 2001/20/EC, Good Clinical Practice guidelines and the Law in many countries, individual consent is required in clinical trials involving medications, unless administered as an emergency when a consent waiver may be obtained (8). Currently SDD and SOD are used in some units throughout Europe as unit-wide interventions according to clinicians' preference, despite equipoise over clinical efficacy outside the Netherlands. However, it might only be possible to evaluate these measures in a CRT at the same ICU if all patients provide informed consent. Failure to obtain consent in a substantial proportion of patients would compromise the clustered study design and introduces bias into the estimation of treatment effects (9).

There is currently no provision for waiving informed consent in the European Clinical Trials Directive 2001/20/EC on which national legislation regarding clinical trials in European countries is based and it is unclear if this will change under the new European "Clinical Trials Regulation" which will replace it. In this respect, the current European directive is more conservative than other international directives. In the United States, the 'Common Rule' which applies to research on human subjects, states that informed consent may be waived provided:

- Research involves no more than minimal risk to subjects;
- The waiver will not adversely affect the rights and welfare of subjects;
- Research could not practicably be carried out without the waiver or alteration;
- Whenever appropriate, subjects will be provided with additional pertinent information after participation (10, 11).

Similarly, the Council for International Organizations of Medical Sciences has a provision for waiving some or all elements of informed consent under similar exceptional circumstances (12). Indeed, several recent studies adapted the informed consent procedure accordingly (Table 1).

Table 1. Interventional studies in ICU settings with a waiver for informed consent

Source	Year	Title	Reason for waiver
Pubmed.com*			
Clifton <i>et al.</i> (13)	2011	Very early hypothermia induction in patients with severe brain injury (the National Acute Brain Injury Study: Hypothermia II): a randomised trial	<i>Not reported</i>
Huang <i>et al.</i> (14)	2013	Targeted versus Universal Decolonization to Prevent ICU Infection	<i>Not reported</i>
Climo <i>et al.</i> (15)	2013	Effect of daily chlorhexidine bathing on hospital-acquired infection	Minimal risk
Caserta <i>et al.</i> (16)	2012	A program for sustained improvement in preventing ventilator associated pneumonia in an intensive care setting	QIP
Huskins <i>et al.</i> (17)	2011	Intervention to Reduce Transmission of Resistant Bacteria in Intensive Care	<i>Not reported</i>
de Smet <i>et al.</i> (1)	2009	Decontamination of the Digestive Tract and Oropharynx in ICU Patients	<i>Not reported</i>
Pronovost <i>et al.</i> (18)	2006	An Intervention to Decrease Catheter-Related Bloodstream Infections in the ICU	QIP, considered exempt from review*
Schulman <i>et al.</i> (19)	2006	The Effect of Antipyretic Therapy upon Outcomes in Critically Ill Patients: A Randomized, Prospective Study	<i>Not reported</i>
Clinicaltrialregister.eu			
EudraCT number 2010-024263-40		Plasma exchange as therapy for disseminated intravascular coagulation (DIC)- a randomized-controlled trial	<i>Not reported</i>
Clinicaltrials.gov			
		<i>None</i>	<i>None</i>

Abbreviations: ICU: intensive care unit; QIP: quality improvement program

* Search terms: waive* informed consent AND intensive care

** This study led to a discussion on the need for informed consent for 'quality improvement' studies or studies evaluating a change in regular care. The SQUIRE standards for reporting of QIP provide insight in this matter (20).

This table is not meant to be exhaustive, rather illustrative.

Based on experience with SDD and SOD, which is considered standard care for ICU patients in the Netherlands, and data from >60 interventional and observational studies, the risks associated with this therapy appears minimal as adverse events have seldom been reported. While conducting new trials, the safety and welfare of patients can be assured by monitoring serious adverse events and continuous monitoring of antibiotic resistance through surveillance, with an independent Data Safety Committee and predefined stopping rules. Future ecological studies on the effects of SDD and SOD could be permitted with a waiver for informed consent under these regulations in some national jurisdiction.

Conclusion

The current European Clinical Trial Directive impedes rigorous scientific evaluation of SDD and SOD in CRTs where a waiver for informed consent is needed. A provision in the European Clinical Trials Regulations allowing the possibility to alter the informed consent procedure when this is not in conflict with ethical standards and essential for the methodological validity, is needed.¹ This could resolve clinical uncertainties from which the (ICU) population as a whole could benefit.

¹ Update 2017: The new Clinical Trials Regulation (CTR) EU No 536/2014 has been adopted by the European Commission and will become effective in 2018/2019. This regulation and has a provision for altered informed consent for cluster randomized trials and is available on: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2014_536/reg_2014_536_en.pdf

References

1. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *The New England journal of medicine*. 2009;360(1):20-31.
2. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet*. 2003;362(9389):1011-6.
3. Oostdijk EA, de Wit GA, Bakker M, de Smet AM, Bonten MJ. Selective decontamination of the digestive tract and selective oropharyngeal decontamination in intensive care unit patients: a cost-effectiveness analysis. *BMJ open*. 2013;3(3).
4. Bastin AJ, Ryanna KB. Use of selective decontamination of the digestive tract in United Kingdom intensive care units. *Anaesthesia*. 2009;64(1):46-9.
5. Cuthbertson BH, Campbell MK, MacLennan G, Duncan EM, Marshall AP, Wells EC, et al. Clinical stakeholders' opinions on the use of selective decontamination of the digestive tract in critically ill patients in intensive care units: an international Delphi study. *Critical care (London, England)*. 2013;17(6):R266.
6. Daneman N, Sarwar S, Fowler RA, Cuthbertson BH. Effect of selective decontamination on antimicrobial resistance in intensive care units: a systematic review and meta-analysis. *The Lancet Infectious diseases*. 2013;13(4):328-41.
7. Walden AP, Bonten MJ, Wise MP. Should selective digestive decontamination be used in critically ill patients? *BMJ (Clinical research ed)*. 2012;345:e6697.
8. Directive 2001/20/EC of the European Parliament and the Council of 4 Apr 2001 on the approximation of laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. *Official J Eur Community*2001. p. 34-44.
9. Roberts I, Prieto-Merino D, Shakur H, Chalmers I, Nicholl J. Effect of consent rituals on mortality in emergency care research. *Lancet (London, England)*. 2011;377(9771):1071-2.
10. McClure KB, Delorio NM, Schmidt TA, Chiodo G, Gorman P. A qualitative study of institutional review board members' experience reviewing research proposals using emergency exception from informed consent. *Journal of medical ethics*. 2007;33(5):289-93.
11. Protection of human subjects: Council for International Organizations of Medical Sciences; 2009 [cited 2014 19 July]. Available from: <https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html#46.116>.
12. Sciences CfIOoM. International ethical guidelines for biomedical research Geneva: Council for International Organizations of Medical Sciences 2002 [cited 1 November 2013]. Available from: http://www.cioms.ch/publications/layout_guide2002.pdf.
13. Clifton GL, Valadka A, Zygun D, Coffey CS, Drever P, Fourwinds S, et al. Very early hypothermia induction in patients with severe brain injury (the National Acute Brain Injury Study: Hypothermia II): a randomised trial. *Lancet neurology*. 2011;10(2):131-9.
14. Huang SS, Septimus E, Kleinman K, Moody J, Hickok J, Avery TR, et al. Targeted versus universal decolonization to prevent ICU infection. *The New England journal of medicine*. 2013;368(24):2255-65.
15. Climo MW, Yokoe DS, Warren DK, Perl TM, Bolon M, Herwaldt LA, et al. Effect of daily chlorhexidine bathing on hospital-acquired infection. *The New England journal of medicine*. 2013;368(6):533-42.
16. Caserta RA, Marra AR, Durao MS, Silva CV, Pavao dos Santos OF, Neves HS, et al. A program for sustained improvement in preventing ventilator associated pneumonia in an intensive care setting. *BMC infectious diseases*. 2012;12:234.
17. Huskins WC, Huckabee CM, O'Grady NP, Murray P, Kopetskie H, Zimmer L, et al. Intervention to reduce transmission of resistant bacteria in intensive care. *The New England journal of medicine*. 2011;364(15):1407-18.
18. Pronovost P, Needham D, Berenholtz S, Sinopoli D, Chu H, Cosgrove S, et al. An intervention to decrease catheter-related bloodstream infections in the ICU. *The New England journal of medicine*. 2006;355(26):2725-32.
19. Schulman CI, Namias N, Doherty J, Manning RJ, Li P, Elhaddad A, et al. The effect of antipyretic therapy upon outcomes in critically ill patients: a randomized, prospective study. *Surgical infections*. 2005;6(4):369-75.
20. Ogrinc G, Mooney SE, Estrada C, Foster T, Goldmann D, Hall LW, et al. The SQUIRE (Standards for Quality Improvement Reporting Excellence) guidelines for quality improvement reporting: explanation and elaboration. *Quality & safety in health care*. 2008;17 Suppl 1:i13-32.

Appendix

Update on European trial regulations (CTR EU No 536/2014)

Article 30

Informed consent in cluster trials

1. Where a clinical trial is to be conducted exclusively in one Member State, that Member State may, [...] allow the investigator to obtain informed consent by the simplified means set out in paragraph 2 of this Article, provided that all of the conditions set out in paragraph 3 of this Article are fulfilled.

2. For clinical trials that fulfil the conditions set out in paragraph 3, informed consent shall be deemed to have been obtained if: (a) the information required under points (a), (b), (d) and (e) of Article 29(2) is given, in accordance with what is laid down in the protocol, prior to the inclusion of the subject in the clinical trial, and this information makes clear, in particular, that the subject can refuse to participate in, or withdraw at any time from, the clinical trial without any resulting detriment; and (b) the potential subject, after being informed, does not object to participating in the clinical trial.

3. Informed consent may be obtained by the simplified means set out in paragraph 2, if all the following conditions are fulfilled: (a) the simplified means for obtaining informed consent do not contradict national law in the Member State concerned; (b) the methodology of the clinical trial requires that groups of subjects rather than individual subjects are allocated to receive different investigational medicinal products in a clinical trial; (c) the clinical trial is a low-intervention clinical trial and the investigational medicinal products are used in accordance with the terms of the marketing authorization; (d) there are no interventions other than the standard treatment of the subjects concerned; (e) the protocol justifies the reasons for obtaining informed consent with simplified means and describes the scope of information provided to the subjects, as well as the ways of providing information.

4. The investigator shall document all refusals and withdrawals and shall ensure that no data for the clinical trial are collected from subjects that refuse to participate in or have withdrawn from the clinical trial.

Available on:

[https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2014_536/
reg_2014_536_en.pdf](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2014_536/reg_2014_536_en.pdf)

accessed 26-7-2017

Chapter 5

Decontamination strategies in Intensive Care Units

a cluster-randomized cross-over study

JAMA, Published online October 22, 2018.
doi:10.1001/jama.2018.13765

Bastiaan H.J. Wittekamp^{1*}

Nienke L. Plantinga^{1*}

Ben S. Cooper²

Christian Brun-Buisson³

Marc J.M. Bonten^{1,4}

On behalf of the R-GNOSIS ICU study group

* both authors contributed equally to this work

¹ Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht,
The Netherlands

² Nuffield Department of Clinical Medicine, University of Oxford, UK

³ CHU Henri Mondor & Univ. Paris Est Créteil, Creteil, France

⁴ Medical Microbiology, University Medical Center Utrecht, The Netherlands

Abstract

Background: Selective digestive tract decontamination (SDD), oropharyngeal decontamination (SOD) and chlorhexidine mouthwash have been associated with improved patient outcomes in Intensive Care Units with low levels of antibiotic resistance. Evidence is lacking in settings with higher prevalence of antibiotic resistance.

Methods: Following a baseline period which included universal daily body washings with chlorhexidine, ICUs were cluster randomized to three six-month intervention periods with chlorhexidine 1% oral care, SOD or SDD (order randomized). Patients with an expected length of mechanical ventilation of >24 hours were included to determine the occurrence of bloodstream infection with highly resistant micro-organisms acquired in the intensive care unit (primary endpoint), survival (at three time points) and unit-wide prevalence of antibiotic resistance. Cox-proportional hazard and mixed-effect logistic regression modelling were used with propensity scores.

Results: 8,665 patients were included from 13 intensive care units in 6 countries. Adjusted hazard ratios (95% confidence interval) for ICU-acquired HRMO bloodstream infection were 1.06 (0.58-1.99), 0.83 (0.46-1.51) and 0.76 (0.38-1.52) during chlorhexidine mouthwash, SOD and SDD versus baseline, respectively. Adjusted odds ratios (95% confidence interval) for day 28 mortality were 1.07 (0.86-1.32), 1.05 (0.85-1.29) and 1.03 (0.80-1.32) for chlorhexidine mouthwash, SOD and SDD versus baseline, respectively. Unit-wide prevalence of carriage with highly resistant micro-organisms in respiratory tract and rectum remained stable during interventions.

Conclusions: In ICUs with moderate-to-high antibiotic resistance prevalence, use of chlorhexidine 1% oral care, SOD or SDD were not associated with reductions in ICU-acquired bloodstream infections or mortality, as compared with standard care including chlorhexidine body washings. (ClinicalTrials.gov NCT02208154)

Introduction

Care of Intensive Care Unit (ICU) patients is frequently complicated by infections, which are associated with increased morbidity, mortality and healthcare costs (1, 2). Selective digestive tract decontamination (SDD) and selective oropharyngeal decontamination (SOD) consist of topical antimicrobial agents targeting aerobic Gram-negative pathogens, *S. aureus* and yeasts in the gastro-intestinal tract (SDD) and oropharynx (SDD/SOD) to prevent infections. In ICUs with low levels of antimicrobial resistance SDD and SOD have been associated with improved patient outcome (3, 4), with SDD being more efficacious than SOD (5, 6). Currently, SDD or SOD is routinely used in ICUs in the Netherlands, but its use has not been widely adopted in other countries (7), mainly because of a paucity of efficacy data in settings with higher levels of antibiotic resistance and fear of emergence of antibiotic resistance, although the latter is not supported by meta-analyses (8). In contrast, chlorhexidine (CHX) mouthwash is widely used in ICU patients and its use has been associated with a lower incidence of ventilator associated pneumonia (9, 10), with CHX 2% being more efficacious than lower concentrations (9). Yet, in meta-analyses CHX mouthwash was associated with higher mortality in ICU patients (11, 12). SDD and SOD have never been compared head-to-head to CHX mouthwash in ICU patients.

Given the equipoise on the effectiveness and ecological safety of these decontamination strategies in ICUs with moderate to high levels of antibiotic resistance, we conducted a cluster-randomized cross-over trial in six European countries to quantify the effects of SDD, SOD and CHX mouthwash on ICU-acquired bloodstream infections (BSI) with highly-resistant micro-organisms (HRMO), patient survival and the unit-wide prevalence of antimicrobial resistance.

Methods

Study design

We conducted a multicenter cluster-randomized study with crossover of interventions in 13 ICUs from six European countries between December 2013 and May 2017 (Supplement Table S1). Only ICUs with a prevalence of at least 5% of extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae

among BSI were eligible. Following a baseline period with standard care for at least six months, SDD, SOD and CHX mouthwash were implemented in a sequential computer generated randomized order in each participating center. All study periods were intended to last six months and were separated by a one month wash-out/in period. Standard care in all study periods consisted of universal use of daily chlorhexidine-digluconate 2% body washing (CHX-BW) for all ICU patients until ICU discharge and implementation of the World Health Organization hand hygiene protocol, including weekly observations (13). CHX mouthwash (0.12% or 0.20%) was allowed as part of standard care if this was part of regular care before the study. All hospitals obtained a waiver for individual patient informed consent from the relevant local and/or national ethical committee.

Patients

Patients with an expected duration of invasive mechanical ventilation of at least 24 hours were eligible. Exclusion criteria included age below 18 years, pregnancy and allergy to any study intervention component. Eligible patients admitted during the first two weeks of the wash-out/in period received the new intervention but were not part of the study population.

Objectives

The primary endpoints were 1) the incidence of ICU-acquired BSI with HRMO in study patients and 2) the unit wide prevalence of HRMO, which was measured by monthly point prevalence surveys of all patients in ICU. Secondary endpoints were survival at the 28th day of ICU admission, at ICU discharge and at hospital discharge, and the unit wide use of systemic antibiotics (expressed as defined daily doses per patient day). ICU-acquired BSI was defined as bacteremia or candidemia diagnosed from day 2 of ICU stay onwards, with the initial day of ICU-admission being designated as day 0. Definitions of HRMO are listed in Supplement Table S2 and mainly include Enterobacteriaceae resistant to third generation cephalosporins and Gram-negative bacteria (GNB) resistant to carbapenems, colistin or three or more antibiotics (14).

Interventions

SDD, SOD and CHX 2% mouthwash were manufactured by the pharmacy of the University Medical Center Utrecht, the Netherlands. The gastro-intestinal suspension contained 1.9 million units colistin sulphate, 80 mg tobramycin

sulphate and 2.0 million units nystatin per dosage (10 ml, 4 times daily during SDD, through a nasogastric tube) and the oropharyngeal paste contained 0.19 million units colistin sulphate, 10 mg tobramycin sulphate and 0.1 million units nystatin per dosage (0.5 g, 4 times daily during SDD/SOD). The SDD regimen did not include a four-day course of intravenous cephalosporin, as this was not considered appropriate in settings with a high prevalence of HRMO infection and carriage. CHX 2% mouthwash was replaced by CHX 1% oral gel (GSK) after the reporting of oral mucosal side-effects in 29 of 295 (9.8%) patients treated in two hospitals (15). SDD, SOD and CHX mouthwash were initiated after study inclusion and applied after regular oral care until mechanical ventilation was stopped. Adherence to decontamination strategies was monitored with monthly compliance measurements and recording of interruptions in individual patients. Rectum and respiratory surveillance samples (endotracheal aspirate when possible, or throat swabs) were obtained twice weekly from all study patients, and once monthly from all patients present in the unit on that day for point prevalence surveys. Microbiology methods are described in the supplementary appendix. A safety committee consisting of three independent experts reviewed the results of monthly point prevalence samples at three-month intervals. The committee members were blinded to the interventions applied and could recommend interruption of the study in a participating ICU if an increase in antimicrobial resistance was apparent.

Sample size and statistical analyses

The sample size was based on the secondary endpoint of mortality on day-28 of ICU admission. To demonstrate a 10% relative difference in 28-day mortality for each intervention, 10,800 patients were required (using a baseline 28-day mortality of 27.5%, alpha 0.05, beta 0.80, intra-cluster correlation coefficient 0.010). The required sample size to detect a 50% reduction of ICU-acquired BSI due to HRMO for each intervention (from a baseline incidence of 1.4%) was 6,700.

Three cohorts were created for the analyses of clinical outcomes: unique ICU-admissions for ICU-survival and ICU-acquired BSI, unique hospital-admissions for hospital survival, and unique ICU admissions with no prior ICU-admission within 30 days for 28-day survival. All analyses were performed on cases without missing covariates or endpoints and according to the intention-to-treat principle. To adjust for differences in patient characteristics between study periods, propensity scores

were calculated using generalized boosted methods (16) and inverse probability weighting was used to balance the distribution of center, age, gender, the Charlson comorbidity index (17), disease severity, admission type, antibiotic use on ICU admission and location before ICU admission (confounders). ICU-acquired BSI and ICU and hospital survival were analyzed with Cox-proportional hazard analyses stratified for center, with discharge and death as competing events where applicable. For the analysis of 28-day mortality a mixed-effect logistic regression model was used with a fixed effect for center and a random effect for the 52 center-period combinations. All models were adjusted for the confounders and mean hand hygiene compliance per study period per center. A sensitivity analysis was performed excluding patients that stayed less than three days in ICU, because they might have been overrepresented in the baseline period.

The prevalence of HRMO carriage based on point prevalence surveys was analyzed separately for rectum and respiratory tract, with logistic regression models (log link) for each endpoint. Each model included correction for underlying time trends per ICU and estimated a mean time trend per study period.

SPSS (IBM, version 21) and R software, version 3.3.2 (R Project for Statistical Computing) were used for data preparation and statistical analyses, respectively. The full statistical analysis plan can be found on ClinicalTrials.gov (www.clinicaltrials.gov, NCT02208154).

Results

Between 1st December 2013 and 31st May 2017 32,933 ICU-admissions were screened, of which 8,665 were included, yielding 8,509 unique hospital admissions and 8,496 inclusions for 28-day mortality (Figure 1, see Supplement Table S3 for baseline characteristics of screened patients). There were 23 ICU-admissions with missing covariates and one, 37 and 56 patients with a missing ICU-, hospital- and 28-day survival status, respectively. On average, 26.3% of screened patients were included, ranging from 12.8% to 76.4% for individual ICUs.

Figure 1. Flowchart and cohorts for analyses

Patients screened	baseline 8,106	CHX 7,898	SOD 8,407	SDD 8,522
Patients excluded (% of screened)	5,855 (72.2%)	5,790 (73.3%)	6,183 (73.5%)	6,440 (75.6%)
<u>Reasons for exclusion (% of excluded)</u>				
no MV	4,212 (71.9%)	3,963 (68.4%)	4,166 (67.3%)	4,460 (69.2%)
estimated length of MV<24	1,483 (25.3%)	1,642 (28.4%)	1,758 (28.4%)	1,725 (26.8%)
age <18 years	84 (1.4%)	102 (1.8%)	93 (1.5%)	100 (1.6%)
other reason	107 (1.8%)	124 (2.1%)	131 (2.1%)	139 (2.2%)
no reason given / not screened in time	104 (1.8%)	100 (1.7%)	165 (2.7%)	145 (2.3%)
Patients included (% of screened)	2,251 (27.8%)	2,108 (26.7%)	2,224 (26.5%)	2,082 (24.4%)
<u>Analysis Cohorts</u>				
ICU mortality	2,251	2,108	2,224	2,082
Hospital mortality	2,214	2,066	2,185	2,044
28-day mortality ^a	2,206	2,067	2,185	2,038

Abbreviations: CHX, chlorhexidine mouthwash; ICU, intensive care unit; MV, mechanical ventilation; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

a) 169 (1.9%) of patients had been included in the prior 30 days and were excluded from this analysis.

Patient characteristics differing between baseline and intervention periods included the mean APACHE II and SAPS II score and the proportion of patients receiving antibiotics at ICU-admission (Table 1, Supplement Table S4). Among included patients, the average proportion receiving decontamination according to protocol, determined by monthly compliance measurements, was 92.5%, 92.4% and 94.2% during the CHX, SOD and SDD periods, respectively (Supplement Table S5). Average hand hygiene compliance was 64.1% during the baseline period and ranged from 72.2% to 72.5% during intervention periods (Supplement Table S6).

ICU-acquired bloodstream infections

Overall, 573 patients had 713 episodes of ICU-acquired BSI, most frequently caused by *Enterococcus* spp. (N=125), *Klebsiella* spp. (N=121), *Candida* spp. (N=69), *S. aureus* (N=67) and *Pseudomonas* spp. (N=61) (Table 2). As compared to baseline, the adjusted hazard ratios (with 95% confidence intervals (CI)) for the study interventions were 1.08 (0.85-1.39), 0.94 (0.76 – 1.17) and 0.79 (0.60 – 1.05) for CHX, SOD and SDD, respectively (Table 3).

Table 1. Baseline table (study population)

	baseline	CHX	SOD	SDD
	2251	2108	2224	2082
Age (mean, SD)	62.0 (15.6)	61.4 (15.7)	61.6 (15.7)	62.8 (15.5)
Male gender (%)	1,420 (63.1%)	1,358 (64.4%)	1,439 (64.7%)	1,344 (64.6%)
APACHE II (mean, SD) - 5 hospitals	20.3 (8.6)	19.8 (8.2)	20.5 (9.3)	21.8 (8.7)
SAPS II (mean, SD) - 8 hospitals	53.0 (18.0)	54.8 (17.9)	54.4 (17.5)	55.0 (18.0)
Type of ICU-admission				
medical	1,464 (65.3%)	1,323 (63.0%)	1,442 (64.9%)	1,385 (66.6%)
trauma with surgery	138 (6.2%)	142 (6.8%)	156 (7.0%)	115 (5.5%)
trauma, no surgery	113 (5.0%)	88 (4.2%)	104 (4.7%)	88 (4.2%)
surgical, scheduled	198 (8.8%)	173 (8.2%)	173 (7.8%)	178 (8.6%)
surgical, unscheduled	328 (14.6%)	374 (17.8%)	346 (15.6%)	314 (15.1%)
surgical, unspecified	10 (0.4%)	8 (0.4%)	3 (0.1%)	2 (0.1%)
Location before ICU admission				
Same hospital	1,020 (45.3%)	1,032 (49.0%)	1,025 (46.1%)	1,035 (49.7%)
Another hospital or long term care facility	400 (17.8%)	312 (14.8%)	316 (14.2%)	301 (14.5%)
Home (directly or via emergency room)	831 (36.9%)	764 (36.2%)	883 (39.7%)	746 (35.8%)
Antibiotic at the time of ICU admission	943 (41.9%)	832 (39.5%)	992 (44.6%)	744 (35.8%)
Sites of organ failure				
Respiratory illness	1,023 (45.5%)	990 (47.0%)	998 (44.9%)	985 (47.3%)
Cardiovascular illness	828 (36.8%)	811 (38.5%)	835 (37.5%)	792 (38.0%)
Neurologic illness	686 (30.5%)	674 (32.0%)	615 (27.7%)	603 (29.0%)
Other illness (renal, hepatic, metabolic, hematologic and/or other)	633 (28.1%)	617 (29.3%)	742 (33.4%)	676 (32.5%)
Charlson comorbidity Index (mean, SD)	2.15 (2.42)	2.38 (2.49)	2.35 (2.42)	2.42 (2.56)

Abbreviations: AIDS/HIV, acquired immunodeficiency syndrome/human immunodeficiency virus; CHX, chlorhexidine mouthwash; DM, diabetes mellitus; ICU, intensive care unit; SD, standard deviation; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

ICU-acquired BSI with HRMO occurred in 169 patients (182 episodes), most frequently with *K. pneumoniae* (N=56), *Enterobacter cloacae* (N=20), Methicillin-resistant *S. aureus* (MRSA) (N=18), *Pseudomonas aeruginosa* (N=17) and *Escherichia coli* (N=15) (Table 2). As compared to baseline, adjusted hazard ratios and 95% CI of HRMO BSI during study interventions were 1.07 (0.58-1.99), 0.83 (0.46-1.51) and 0.77 (0.38-1.52) during CHX, SOD and SDD, respectively (Table 3). There were no statistically significant associations between interventions and competing endpoints ICU discharge and death (data not shown). Micro-organisms not included in the definition of BSI are listed in Supplement Table S7.

Table 2. ICU acquired bloodstream infections per study period

Study period	baseline		CHX		SOD		SDD	
	n	%	n	%	n	%	n	%
Number of patients	2251		2108		2224		2082	
ICU acquired bloodstream infections with HRMO ^{a,b}								
HRMO bacteremia episodes (no. of patients)	58 (53)		49 (44)		40 (38)		35 (34)	
Enterobacteriaceae	39	67.2	29	59.2	26	65.0	24	68.6
Resistant to 3 rd generation-cephalosporins	35		25		24		24	
Resistant to colistin	2		2		5		5	
Glucose non-fermenting Gram-negative bacteria	9	15.5	10	20.4	5	12.5	3	8.6
Pseudomonas species	4		9		3		2	
Other glucose non-fermenting Gram-negative bacteria ^c	4	6.9	2	4.1	3	7.5	0	0.0
Gram-positive micro-organisms	6	10.3	8	16.3	6	15.0	8	22.9
Vancomycin resistant enterococci	3		4		3		0	
Methicillin resistant Staphylococcus aureus	3		4		3		8	
ICU acquired bloodstream infections with relevant micro-organisms ^{a,d}								
Bacteremia episodes (no. of patients)	199 (154)		201 (156)		172 (140)		141 (123)	
Enterobacteriaceae	99	49.7	90	44.8	77	44.8	51	36.2
Intrinsic colistin resistant	30		13		14		10	
Glucose non-fermenting Gram-negative bacteria	31	15.6	19	9.5	20	11.6	15	10.6
Pseudomonas species	21		16		15		9	
Gram-positive micro-organisms	43	21.6	61	30.3	47	27.3	50	35.5
Enterococcus faecium / faecalis	27		32		34		32	
Staphylococcus aureus	13		25		12		17	
Yeasts	15	7.5	22	10.9	23	13.4	18	12.8
Other	11	5.5	9	4.5	5	2.9	7	5.0

Abbreviations: CHX, chlorhexidine mouthwash; HRMO, highly resistant micro-organism; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

a) Bloodstream infection defined as first occurrence of unique species on day 2 of ICU stay onwards, with the initial day of ICU-admission being designated as day 0

b) HRMO according to definition in Supplement Table S2, in brief HRMO include: Enterobacteriaceae resistant to third generation cephalosporins, Gram-negative bacteria resistant to carbapenems, colistin or three or more antibiotics from separate classes and Gram-positive micro-organisms classified as VRE or MRSA

c) Stenotrophomonas spp., Burkholderia spp. and Achromobacter spp.

d) Excluding coagulase negative Staphylococcus, Micrococcus and Clostridium species and Non-pneumococcal Streptococci; also including HRMOs

Survival

As compared to baseline, adjusted hazard ratios (95%-CI) were 1.03 (0.92-1.16), 1.00 (0.89-1.14) and 0.95 (0.81-1.11) during CHX, SOD and SDD, respectively, for ICU mortality; 0.97 (0.85-1.11), 1.00 (0.87-1.14) and 0.96 (0.82-1.12) during CHX, SOD and SDD, respectively, for hospital mortality, and adjusted odds ratios (95%-CI) were 1.07 (0.86-1.32) 1.05 (0.85-1.29) and 1.03 (0.80-1.32) during CHX, SOD and SDD, respectively, for 28-day mortality (Table 3). Sensitivity analyses restricted to patients with an ICU stay of at least three days lead to similar results for all survival endpoints (data not shown).

Antimicrobial use and resistance

The unit wide consumption of systemic antibiotics was 1.1, 1.0, 1.0 and 1.1 defined daily doses (DDD) per patient day during the baseline, CHX, SOD and SDD study period, respectively (Supplement Table S8).

In total, 5,536 respiratory and 5,441 rectal samples were obtained from 5,706 survey participants during 329 point prevalence surveys (Table 4 & Supplement Table S9). Completeness of susceptibility testing was >95% (Supplement Table S10). The overall prevalence of carriage with Gram-negative HRMO ranged from 17.1% to 25.3% in rectum samples and from 10.2% to 15.2% in respiratory tract samples, without statistically significant differences between study periods. The prevalence of colistin resistance (in non-intrinsically resistant Gram-negative bacteria) ranged from 0.7% (CHX) to 2.3% (SDD) in the rectum and was <1.0% in the respiratory tract during all study periods (Supplement Table S11).

Results from the twice weekly surveillance cultures (study patients) indicate a gradually lower carriage rate of antibiotic-resistant GNB in the rectum during SDD and the respiratory tract during SDD/SOD, in comparison with other study periods. (Supplement Figure S1). On day 14 of ICU stay the proportion of rectal cultures growing GNB from selective media was 14.8% during SDD and 28.3% during baseline period.

Table 3. Effects of interventions on ICU-acquired bloodstream infection (BSI) and patient survival.

	baseline	CHX	SOD	SDD	CHX vs. baseline	SOD vs. baseline	SDD vs. baseline
	2251	2108	2224	2082			
Patients with ICU acquired BSI with HRMO	53 (2.4%)	44 (2.1%)	38 (1.7%)	34 (1.6%)			
<i>Rate (per 1,000 patients days at risk)</i>	1.84	1.56	1.32	1.24	1.07 (0.58-1.99)	0.83 (0.46-1.51)	0.77 (0.38-1.52)
Patients with ICU acquired BSI (any)	154 (6.8%)	156 (7.4%)	140 (6.3%)	123 (5.9%)			
<i>Rate (per 1,000 patients days at risk)</i>	5.69	5.95	5.12	4.67	1.09 (0.85-1.39)	0.94 (0.76-1.17)	0.79 (0.60-1.05)
Death - no. (%)							
in ICU ^b	691/2,251 (30.7%)	664/2,107 (31.5%)	685/2,224 (30.8%)	645/2,082 (31.0%)	1.03 (0.92-1.16)	1.00 (0.89-1.14)	0.95 (0.81-1.11)
in hospital ^c	839/2,206 (38.0%)	782/2,055 (38.1%)	845/2,184 (38.7%)	816/2,027 (40.3%)	0.97 (0.85-1.11)	1.00 (0.87-1.14)	0.96 (0.82-1.12)
at 28th day of ICU-admission ^d	701/2,198 (31.9%)	675/2,049 (32.9%)	703/2,171 (32.4%)	689/2,022 (34.1%)	1.07 (0.86-1.32)	1.05 (0.85-1.29)	1.03 (0.80-1.32)
days in ICU (median, IQR)	10 [5 - 18]	10 [6 - 19]	10 [6 - 18]	11 [6 - 18]			
days in hospital (median, IQR)	23 [11 - 45]	24 [12 - 45]	23 [12 - 43]	24 [12 - 44]			
days on MV (in ICU) (median, IQR)	6 [3 - 13]	7 [4 - 13]	6 [3 - 12]	7 [3 - 12]			

Abbreviations: CHX, chlorhexidine; CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; MV, mechanical ventilation; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination.

a) all models accounted for clustering using a fixed effect on ICU and a random effect on study period (13 ICUs x 4 study periods) and were adjusted for age, gender, the Charlson comorbidity index, APACHE II or SAPS II score, admission type, antibiotic use on ICU admission, location before ICU admission (in both propensity score and final models) and mean hand hygiene compliance per study period (only in final models)

b) 1 missing outcome

c) 37 missing outcomes

d) 56 missing outcomes

Table 4. Prevalence of unit-wide carriage of highly resistant micro-organisms (HRMO) in rectum and respiratory tract

	Baseline		CHX		SOD		SDD	
	prev.	aRR *	prev.	aRR *	prev.	aRR *	prev.	aRR *
Descriptive statistics point prevalence surveys								
Proportion of patients in the unit screened	93.1%	94.3%	92.2%	92.3%	92.3%	92.3%	92.3%	92.3%
No. of patients sampled	1456	1424	1469	1407	1407	1407	1407	1407
Included in study population (% of sampled)	63.0%	61.7%	60.7%	59.8%	59.8%	59.8%	59.8%	59.8%
No. of rectal samples (% of patients sampled)	1392 (95.6%)	1370 (96.2%)	1419 (96.6%)	1355 (96.3%)	1355 (96.3%)	1355 (96.3%)	1355 (96.3%)	1355 (96.3%)
No. of respiratory samples (% of patients sampled)	1381 (94.8%)	1333 (93.6%)	1408 (95.8%)	1319 (93.7%)	1319 (93.7%)	1319 (93.7%)	1319 (93.7%)	1319 (93.7%)
Rectum	prev.	aRR *	prev.	aRR *	prev.	aRR *	prev.	aRR *
HRMO Enterobacteriaceae	16.1%	1.07 (0.99-1.16)	21.7%	1.07 (0.99-1.16)	19.7%	1.04 (0.96-1.13)	13.9%	1.05 (0.95-1.16)
3rd generation cephalosporin resistance	15.8%	1.07 (0.99-1.16)	21.5%	1.07 (0.99-1.16)	19.2%	1.04 (0.96-1.13)	13.7%	1.07 (0.97-1.18)
carbapenem resistance	3.2%	0.68 (0.54-0.86)	3.1%	0.68 (0.54-0.86)	2.9%	0.85 (0.71-1.03)	2.6%	0.8 (0.64-1.01)
MDR	10.8%	1.07 (0.97-1.19)	15.5%	1.07 (0.97-1.19)	14.2%	1.06 (0.96-1.17)	10.0%	1.1 (0.97-1.24)
colistin resistance**	0.5%	0.81 (0.54-1.21)	1.6%	0.81 (0.54-1.21)	1.8%	0.97 (0.65-1.45)	1.3%	0.96 (0.60-1.54)
HRMO Glucose non-fermenting GNB	3.2%	0.77 (0.62-0.95)	3.2%	0.77 (0.62-0.95)	3.3%	0.93 (0.76-1.14)	2.3%	0.81 (0.63-1.04)
HRMO GNB, regardless of antibiotic susceptibility	1.0%	0.80 (0.50-1.27)	1.5%	0.80 (0.50-1.27)	1.1%	0.8 (0.49-1.30)	1.6%	1.01 (0.64-1.58)
Any HRMO GNB	19.3%	1.03 (0.96-1.11)	25.3%	1.03 (0.96-1.11)	23.0%	1.03 (0.96-1.11)	17.1%	1.04 (0.96-1.14)
VRE	2.2%	0.96 (0.74-1.24)	1.5%	0.96 (0.74-1.24)	1.8%	0.94 (0.73-1.21)	4.2%	1.03 (0.84-1.27)
HRMO Enterobacteriaceae	6.6%	0.94 (0.81-1.09)	7.6%	0.94 (0.81-1.09)	4.2%	0.93 (0.8-1.09)	4.7%	0.94 (0.78-1.13)
3rd generation cephalosporin resistance	6.4%	0.95 (0.82-1.10)	7.4%	0.95 (0.82-1.10)	4.2%	0.93 (0.80-1.09)	4.5%	0.94 (0.78-1.13)
carbapenem resistance	1.4%	0.71 (0.47-1.07)	1.1%	0.71 (0.47-1.07)	0.9%	0.68 (0.48-0.94)	0.5%	0.59 (0.37-0.97)
MDR	4.0%	1.02 (0.84-1.23)	5.2%	1.02 (0.84-1.23)	3.3%	0.92 (0.76-1.12)	3.5%	1.04 (0.83-1.31)
colistin resistance**	0.1%	0.57 (0.29-1.14)	0.8%	0.57 (0.29-1.14)	0.9%	0.66 (0.36-1.21)	0.3%	0.61 (0.30-1.22)
HRMO Glucose non-fermenting GNB	3.4%	0.8 (0.64-1.00)	2.9%	0.8 (0.64-1.00)	3.8%	0.84 (0.7-1.00)	2.7%	0.75 (0.58-0.96)
HRMO GNB, regardless of antibiotic susceptibility	3.8%	1.16 (0.94-1.44)	5.2%	1.16 (0.94-1.44)	3.2%	0.97 (0.77-1.22)	3.6%	1.04 (0.83-1.31)
Any HRMO GNB	12.9%	0.98 (0.88-1.08)	15.2%	0.98 (0.88-1.08)	10.3%	0.93 (0.84-1.04)	10.2%	0.94 (0.83-1.06)
MRSA	1.7%	0.95 (0.66-1.36)	1.1%	0.95 (0.66-1.36)	1.3%	0.77 (0.59-1.00)	1.7%	0.73 (0.54-0.97)

Abbreviations: CHX, chlorhexidine; GNF-GNB, glucose non-fermenting gram-negative bacteria; HRMO, highly resistant micro-organism; MDR, multidrug resistant (resistance to

3 or more antibiotics/classes of antibiotics [Supplement Table 2]); MRSA, methicillin resistant *S. aureus*; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination; VRE, vancomycin-resistant *E. faecium*/*E. faecalis*

* aRR; adjusted relative risk per month, all models were corrected for underlying time trends per centre

** excluding Enterobacteriaceae with intrinsic colistin resistance (*Proteus* spp., *Morganella* spp., *Serratia* spp., *Providencia* spp., *Hafnia alvei*)

*** for *Pseudomonas* spp., resistance to at least one other antibiotic was required to be reported as carbapenem-R

Deviations from study protocol

There were three major deviations from the study protocol. CHX 2% mouthwash was replaced by CHX 1% oral gel after adverse events, mainly consisting of oromucosal lesions, recorded in a total of 29 patients in the two centers that first implemented CHX 2% (15). No serious adverse events were reported during the use of CHX 1%, SOD and SDD.

The study was temporarily interrupted in two centers. In one center, an increased prevalence of colistin-resistant *K. pneumoniae* was identified by the safety committee which led to the identification of a clonal outbreak after SOD had been used for 3.5 months. After a 7-month period of outbreak containment, SOD was reintroduced. In another center, the hospital infection control committee interrupted the study after SOD had been used for 5 months, pending evaluations of an increased prevalence of carbapenem-resistant Enterobacteriaceae. Further investigation revealed that the outbreak was polyclonal and occurring in multiple hospital wards simultaneously. After an interruption of seven months the next randomized study phase (being SDD) was introduced after IRB approval.

Discussion

In this cluster-randomized multicenter study in 13 European ICUs, decontamination strategies with either antibiotics (SDD, SOD) or CHX mouthwash did not reduce ICU-acquired BSI due to antibiotic resistant bacteria or any pathogen (including yeasts), nor mortality, in ventilated ICU patients when compared to standard care, which included universal daily body washings with chlorhexidine during ICU stay and a hand hygiene program. Furthermore, the unit-wide prevalence of carriage with HRMO did not change during the interventions.

SDD has not been widely adopted outside of the Netherlands because of concerns about accelerating antimicrobial resistance and skepticism regarding its effectiveness (18, 19), especially in healthcare systems with a higher level of antimicrobial resistance, even though two large Dutch studies demonstrated improved patient outcomes (3, 4).

Several aspects of the current study may explain the discrepant findings, compared to similar studies in Dutch centers (3-5). First, the current study was designed to test the efficacy of decontamination regimens in ICUs with higher prevalence of antibiotic resistance. Indeed, the prevalence of carriage with HRMO in rectum and respiratory tract and the 25.5% proportion of ICU-acquired BSI episodes caused by HRMO are clearly higher than those reported from Dutch ICUs in previous studies (3-5). Decontamination strategies using conventional SDD or SOD regimens may be less effective in this context, especially in areas with high prevalence of resistance to aminoglycosides or colistin among GNB. The unit wide prevalence of colonization with gentamicin-resistant GNB was 8.3% in the rectum and 4.5% in the respiratory tract, which is twice as high as in the de Smet study (4), but comparable to the more recent study by Oostdijk et al. (5).

Second, SDD did not include a four-day course of intravenous third generation cephalosporins, which might have reduced the effects of SDD. In fact, during SDD there were 48 episodes of ICU-acquired BSI occurring within the first four days of inclusion, 17 of which involved pathogens susceptible to third-generation cephalosporins. Naturally, absence of cefotaxime during SDD cannot explain the discrepant findings for SOD, which was also associated with a reduction in mortality and ICU-acquired BSI in the study by de Smet et al. (4).

Third, interventions were discontinued at the end of mechanical ventilation, instead of at ICU discharge. In the de Smet study, SDD/SOD were administered during >95% of patients days (4, 5), whereas in the current study mechanical ventilation days accounted for 69.2% of all days in ICU, reflecting the maximum proportion of time during which patients received study interventions. In fact, during CHX, SOD and SDD there were 32, 23 and 33 ICU-acquired BSI episodes that occurred on days without mechanical ventilation. In a post-hoc sensitivity analysis where all SDD-treated patients with ICU-acquired BSI caused by a pathogen susceptible to third-generation cephalosporins during the first four days and/or with ICU-

acquired BSI with any pathogen in the absence of mechanical ventilation, were considered as alive at all mortality endpoints, HRs (95%-CI) for SDD (compared to baseline) would be 0.93 (0.79-1.08) and 0.93 (0.79-1.10) for ICU and hospital mortality, respectively, and the OR (95%-CI) for 28-day mortality would be 1.01 (0.78-1.29). We, therefore, conclude that it is unlikely that these protocol variations explain the discrepant findings with regard to SDD efficacy for patient outcome compared to previous studies.

Fourth, standard care in the current study included strategies which may have influenced carriage and transmission of HRMO and were not implemented in the Dutch studies, such as oral care with antiseptics (CHX mouthwash 0.12 or 0.2%) in 11 of 13 centers, implementation of the WHO hand hygiene protocol and universal daily CHX 2% body washings for all patients in the ICU until discharge. Although the effects of these strategies on colonization and infection with GNB cannot be assessed within the current study, they may have reduced the potential of the three interventions to offer additional benefits (20).

Infections and carriage with bacteria with acquired resistance to colistin occurred sporadically and prevalence did not increase during SOD and SDD, which both contained topical application of colistin. Although the use of E-testing in eleven hospitals might have underestimated the prevalence of colistin resistance, there was no evidence of increased colistin resistance prevalence in the centralized prevalence surveys in which colistin susceptibility was tested using automated testing (BD Phoenix, BD Diagnostic Systems) of strains isolated from non-selective media.

Strengths of this study include its sample size and selection of ICUs in six European countries, with resistance rates that better reflect the average European or American setting than Dutch ICUs, thereby improving external validity and generalizability of findings, as well as the detailed unit-wide resistance monitoring with monthly point prevalence studies. Study limitations include the inherent risk of (selection) bias due to cluster randomization and the fixed start with the baseline period, precluding adjustment for changes in ICU organization, ecology or unmeasured patient characteristics over time. Furthermore, the originally targeted sample size of 10,800 patients was not reached. Yet, the absence of a statistically significant effect on the incidence of ICU-acquired BSI was not related to a lack of power of

the study, since there were more patients included than the anticipated sample size for this primary endpoint and the incidence of ICU-acquired BSI due to HRMO was higher than expected. In addition, a larger study population would probably not have resulted in a statistically significant effect for any of the survival endpoints with the observed effect sizes for SDD, SOD and CHX having odds ratios close to, or even above, 1. Finally, monitoring of carriage with antibiotic resistant bacteria ended at ICU-discharge, precluding evaluation of long-term effects of the interventions.

Acknowledgements

The authors would like to thank the research nurses for collection of the data, prof. A.M.G.A. de Smet, prof. J. Chastre and prof. A. Andremont for their participation in the data safety committee; Miranda Hopman for her support in data collection and study logistics; Raween Kalicharan and the pharmacy of the University Medical Center Utrecht for the production of study medication.

List of investigators

Bastiaan H Wittekamp, MD, and Nienke L Plantinga, MD, University Medical Center Utrecht; Ben S Cooper, PhD, Nuffield Department of Clinical Medicine, University of Oxford; Joaquin Lopez-Contreras MD/PhD, Hospital de Sant Pau-Universitat Autònoma de Barcelona; Prof. Pere Coll, Hospital de Sant Pau-Universitat Autònoma de Barcelona; Prof. Jordi Mancebo, Hospital de Sant Pau-Universitat Autònoma de Barcelona; Matt P Wise, MD/PhD, University Hospital of Wales, Cardiff; Matt PG Morgan, MD/PhD, University Hospital of Wales, Cardiff; Prof. Pieter Depuydt, Ghent University Hospital; Jerina Boelens, MD/PhD, Ghent University Hospital; Thierry Dugernier, MD/PhD, Clinique Saint Pierre, Ottignies; Valérie Verbelen, PhD, Clinique Saint Pierre, Ottignies; Prof. Philippe G Jorens, Antwerp University Hospital, University of Antwerp; Walter Verbrugghe, MD, Antwerp University Hospital, University of Antwerp; Prof. Surbhi Malhotra-Kumar, University of Antwerp; Prof. Pierre Damas, CHU Liège; Cécile Meex, PhD, CHU Liège; Kris Leleu, MD, AZ Sint Jan Bruges; Anne-Marie van den Abeele, MD, Saint-Lucas Hospital Ghent, Francisco Esteves, MD, Centro Hospitalar de Trás-os-Montes os Montes e Alto Douro, Vila Real; Ana Filipa Gomes Pimenta de Matos, Centro Hospitalar de Trás-os-Montes os Montes e Alto Douro, Vila Real; Prof. A. Torres, Hospital Clinic of Barcelona; Sara Fernández Méndez, MD, Hospital Clinic of Barcelona; Andrea Vergara Gomez, Msc, Hospital Clinic of Barcelona; Viktorija Tomic, MD/ PhD, University Clinic of Respiratory and Allergic Diseases, Golnik; Franc Sifrer, MD, University Clinic of Respiratory and Allergic Diseases Golnik, Esther Villarreal Tello, MD, Hospital Universitario La Fe, Valencia; Jesus Ruiz Ramos, PhD, Hospital Universitario La Fe, Valencia; Irene Aragao, MD, Hospital Santo Antonio - Centro Hospitalar do Porto (CHP); Claudia Santos, MD, Hospital Santo Antonio - Centro Hospitalar do Porto (CHP); Roberta HM Sperning; Msc, Azienda Ospedaliera San Camillo Forlanini, Rome, Patrizia Coppadoro, Azienda Ospedaliera San Camillo Forlanini, Rome; Giuseppe Nardi, MD, Ospedale Infermi RIMINI – AUSL della Romagna, Prof. Christian Brun-Buisson, CHU Henri Mondor & Univ. Paris Est Créteil, Paris; Prof. Marc J.M. Bonten, University Medical Center Utrecht.

References

1. Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009;302:2323-9.
2. Scott RD. The Direct medical costs of Healthcare-Associated Infections in U.S. Hospitals and the Benefits of Prevention. Center for disease control and prevention, 2009. (Accessed January 4, 2017, at https://www.cdc.gov/hai/pdfs/hai/scott_costpaper.pdf.)
3. de Jonge E, Schultz MJ, Spanjaard L, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet* 2003;362:1011-6.
4. de Smet AM, Kluytmans JA, Cooper BS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *N Engl J Med* 2009;360:20-31.
5. Oostdijk EAN, Kesecioglu J, Schultz MJ, et al. Notice of Retraction and Replacement: Oostdijk et al. Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. *JAMA* 2014;312:1429-1437. *JAMA* 2017;317:1583-4.
6. Plantinga NL, de Smet A, Oostdijk EAN, et al. Selective digestive and oropharyngeal decontamination in medical and surgical ICU patients: individual patient data meta-analysis. *Clin Microbiol Infect* 2017; DOI: 10.1016/j.cmi.2017.08.019.
7. Bastin AJ, Ryanna KB. Use of selective decontamination of the digestive tract in United Kingdom intensive care units. *Anaesthesia* 2009;64:46-9.
8. Daneman N, Sarwar S, Fowler RA, Cuthbertson BH. Effect of selective decontamination on antimicrobial resistance in intensive care units: a systematic review and meta-analysis. *Lancet Infect Dis* 2013;13:328-41.
9. Labeau SO, Van de Vyver K, Brusselaers N, Vogelaers D, Blot SI. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. *Lancet Infect Dis* 2011;11:845-54.
10. Hua F, Xie H, Worthington HV, Furness S, Zhang Q, Li C. Oral hygiene care for critically ill patients to prevent ventilator-associated pneumonia. *Cochrane Database Syst Rev* 2016;10:Cd008367.
11. Klompas M, Speck K, Howell MD, Greene LR, Berenholtz SM. Reappraisal of routine oral care with chlorhexidine gluconate for patients receiving mechanical ventilation: systematic review and meta-analysis. *JAMA Intern Med* 2014;174:751-61.
12. Price R, MacLennan G, Glen J. Selective digestive or oropharyngeal decontamination and topical oropharyngeal chlorhexidine for prevention of death in general intensive care: systematic review and network meta-analysis. *BMJ* 2014;348:g2197.
13. WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care. Geneva: World Health Organization, 2009. (Accessed January 4, 2017, at http://apps.who.int/iris/bitstream/10665/44102/1/9789241597906_eng.pdf.)
14. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268-81.
15. Plantinga NL, Wittekamp BH, Leleu K, et al. Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU. *Intensive Care Med* 2016;42:620-1.
16. McCaffrey DF, Griffin BA, Almiraal D, Slaughter ME, Ramchand R, Burgette LF. A tutorial on propensity score estimation for multiple treatments using generalized boosted models. *Stat Med* 2013;32:3388-414.
17. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;40:373-83.
18. Duncan EM, Cuthbertson BH, Prior ME, et al. The views of health care professionals about selective decontamination of the digestive tract: an international, theoretically informed interview study. *Crit Care* 2014;29:634-40.
19. Cuthbertson BH, Campbell MK, MacLennan G, et al. Clinical stakeholders' opinions on the use of selective decontamination of the digestive tract in critically ill patients in intensive care units: an international Delphi study. *Crit Care* 2013;17:R266.
20. Derde LP, Cooper BS, Goossens H, et al. Interventions to reduce colonisation and transmission of antimicrobial-resistant bacteria in intensive care units: an interrupted time series study and cluster randomised trial. *Lancet Infect Dis* 2014;14:31-9.

Supplement

Appendix: microbiology methods

Surveillance cultures

Twice weekly (Monday/Thursday) rectum and respiratory surveillance samples were collected from study patients and inoculated on ESBL-selective agar (chromID, bioMérieux). Species determination and antibiotic susceptibility testing were performed according to local protocols, with a minimum common set of antibiotics tested for GNB (Supplement Table S2). Five centers used automated antibiotic susceptibility testing (AST), five relied on disk diffusion and three used both methods (Supplement Table S1). Colistin testing was performed at least once per species per tractus (rectum or respiratory) per patient, by automatic testing in five centers and by E-testing (bioMérieux) in eleven centers; three centers used both approaches.

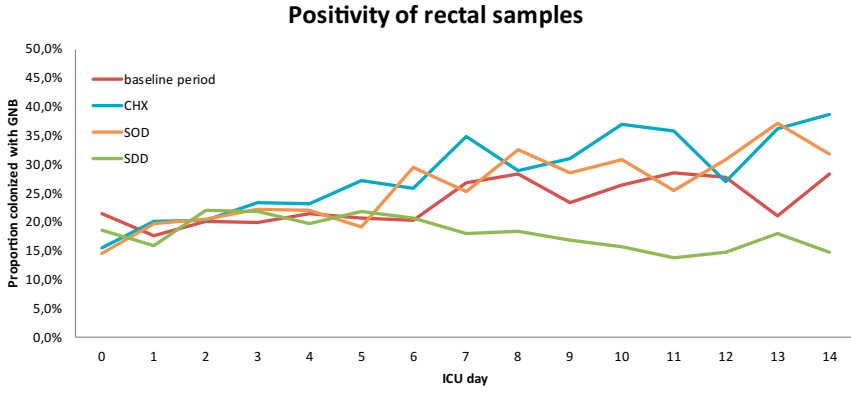
Point prevalence cultures

Rectal swabs and respiratory tract secretions were obtained for point prevalence surveys once monthly, from all patients present in the unit on that day, and inoculated on ESBL- (both samples), VRE- (rectal swabs) and MRSA- selective agar (respiratory tract samples, all chromID, bioMérieux). Growing isolates were processed using the laboratory procedures described above. Once every three months, these samples were also inoculated on plain MacConkey agar, from which a maximum of three morphologically distinct colonies were selected for species determination (MALDI-TOF, Bruker) and automated susceptibility testing (BD Phoenix, BD Diagnostic Systems) at the University Medical Center Utrecht to determine the prevalence of colistin susceptibility among GNB isolated from non-selective media.

Clinical cultures

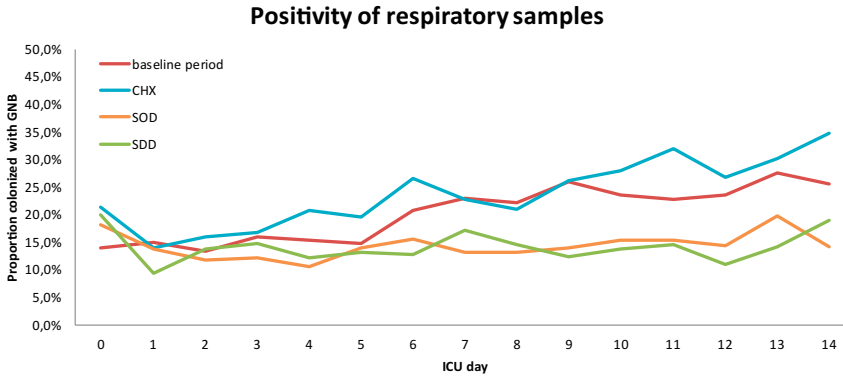
Results from clinical respiratory and blood samples were collected for all study patients. These samples were processed according to local microbiological procedures.

Supplement figure S1. Proportion of surveillance samples positive for Gram-negative bacteria on day 1 – day 14 of ICU-admission (ESBL-selective media)



nr. of rectum samples

baseline period	275	558	465	438	378	396	345	299	279	232	238	196	212	180	159
CHX	239	458	444	444	366	361	322	299	281	226	225	179	185	174	163
SOD	178	476	474	469	368	381	373	341	246	246	234	197	185	186	148
SDD	236	437	446	430	382	304	359	321	246	227	229	202	157	178	169



nr. of respiratory samples

baseline period	251	560	471	458	379	396	338	287	276	235	234	190	212	182	164
CHX	207	458	455	447	368	362	327	303	268	229	219	185	183	176	164
SOD	187	481	487	459	368	357	354	329	238	244	223	196	174	177	148
SDD	210	439	435	437	386	296	354	316	240	218	226	200	157	171	158

Supplement Table S1. Characteristics of participating centers

Center	Country	No. of beds	Type of ICU (medical/ surgical/ mixed)	CHX oral care in standard care	Duration standard care period (months)	Randomization sequence	No. of patients included (% of screened)	Prevalence of colonization with 3GC-R in rectum	Susceptibility testing GNB surveillance cultures	
									disk diffusion	automated
1	B	36	mixed	0.12%	6	CHX - SOD - SDD	900 (14.9%)	19.0%	x	
2	B	24	mixed	0.20%	5.9	CHX - SDD - SOD	317 (12.8%)	13.6%		phoenix
3	S	12	medical	none	5.9	SOD - SDD - CHX	168 (22.0%)	7.5%	x	
4	B	42	mixed	0.20%	6	SDD - CHX - SOD	1231 (19.4%)	7.2%	x	
5	ES	30	mixed	0.12%	6	SOD - CHX - SDD	1043 (32.7%)	30.0%	x	sensititre
6	IT	12	mixed	0.20%	6	SDD - SOD - CHX	598 (76.4%)	23.2%	x	vitek / phoenix
7	P	10	mixed	0.12%	14.5	SDD - CHX - SOD	639 ^(a)	5.7%		vitek/microscan
8	B	15	mixed	0.20%	6	SOD - SDD - CHX	337 (20.9%)	15.2%	x	vitek
9	B	42	mixed	0.20%	5.9	CHX - SOD - SDD	1297 (28.0%)	18.8%		vitek
10	ES	24	medical	0.12%	8.1	SDD - SOD - CHX	375 (18.3%)	35.2%	x	
11	ES	8	mixed	0.12%	9	CHX - SDD - SOD	237 (23.1%)	11.3%		phoenix
12	UK	22	mixed	none	8	SOD - CHX - SDD	1109 (44.1%)	8.9%	x	
13	P	9	mixed	0.20%	7.1	SDD - SOD - CHX	414 (53.1%)	0.0% ^(b)		microscan

Abbreviations: 3GC-R, resistance to third generation cephalosporins (Cefotaxime or Ceftriaxone or Cefazidime); B, Belgium; CHX, chlorhexidine mouthwash; GNB, Gram-negative bacteria; ES, Spain; IT, Italy; P, Portugal; UK, United Kingdom; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

a) Only excluded patients with point prevalence data were registered as part of the screened population

b) 31/31 rectal samples were negative for 3GC-R; in the respiratory tract the prevalence of 3GC-R Enterobacteriaceae was 2/31 (6.5%)

Supplement table S2. Antibiotic susceptibility testing and definition of highly resistant micro-organism

Minimum required antibiotic susceptibility testing list	HRMO criteria ¹
Enterobacteriaceae	
Imipenem or Meropenem	A
Colistin (for species other than naturally resistant)*	A*
Cefotaxime or Ceftriaxone or Ceftazidime	A and B****
Ciprofloxacin**	B
Gentamicin	B
Amikacin	B
Piperacillin or Pip/tazobactam	B
Trimethoprim-Sulfamethoxazole	B
Glucose non-fermenting Gram-negative bacteria	
Imipenem or Meropenem or Doripenem	A***
Colistin	A
Ceftazidime	B
Ciprofloxacin**	B
Gentamicin	B
Amikacin	B
Piperacillin or Pip/tazobactam or Ticarcillin	B
Trimethoprim-Sulfamethoxazole	B
Gram-negative bacteria, considered HRMO regardless of susceptibility testing result	
<i>Stenotrophomonas spp.</i>	
<i>Achromobacter spp.</i>	
<i>Burkholderia spp.</i>	
Gram-positive HRMO	
Methicillin-resistant <i>S. aureus</i> (MRSA)	
Vancomycin resistant <i>E.faecium/E.faecalis</i> (VRE)	

Legend

- A** intermediate/resistant result for an antibacterial agent from any one of the indicated groups of this category is sufficient to define the micro-organism as HRMO
- B** intermediate/resistant result for antibacterial agents from at least three of the indicated groups in this category is required to define the micro-organism as HRMO
- *** except intrinsically resistant *Proteus spp.*, *Providencia spp.*, *Morganella spp.*, *Serratia spp.* and *H. alvei*; these species are defined as a HRMO when they meet resistance criteria for the other antibiotics listed
- **** result from ofloxacin or levofloxacin was used as alternative if ciprofloxacin was not available
- ***** For *Pseudomonas aeruginosa*, resistance to one of the other antibiotic groups was necessary to be reported as carbapenem-R HRMO.
- ****** Resistance to third generation cephalosporins directly qualified for HRMO *and* was counted as one of three antibiotic classes for criterium B.

Abbreviations: HRMO: highly resistant micro-organism; spp.: species

Modified from: A.P. Magiorakos et al., Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, Clinical Microbiology and Infection, April 2011

Supplement table S3. Baseline characteristics of screened population

	baseline	CHX	SOD	SDD
	8106	7898	8407	8522
ICU-admission in prior 30 days	356 (4.4%)	442 (5.6%)	441 (5.2%)	493 (5.8%)
Age (mean, SD)	61.3 (17.7)	60.7 (18.6)	61.3 (18.1)	60.9 (18.6)
Sex (male)	4996 (61.6)	4928 (62.4)	5238 (62.3)	5264 (61.8%)
APACHE II (mean, SD) - 5 hospitals*	16.1 (7.9)	15.4 (7.6)	16.3 (7.9)	16.6 (8.0)
SAPS II (mean, SD) - 8 hospitals**	42.4 (18.6)	42.3 (18.9)	43.8 (18.9)	43.8 (18.7)
ICU-LOS (median, IQR)	4 [2 – 7]	4 [2 – 7]	4 [3-7]	4 [2-7]

Abbreviations: CHX, chlorhexidine mouthwash; ICU, intensive care unit; LOS, length of stay; SD, standard deviation; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination. * 137 missing values, ** 2306 missing values (within these selected hospitals)

Supplement table S4. All baseline characteristics (study population)

	baseline	CHX	SOD	SDD
	2251	2108	2224	2082
ICU-admission in prior 30 days <i>with inclusion</i>	45 (2.0%)	41 (1.9%)	39 (1.8%)	44 (2.1%)
Age (mean, SD)	62.0(15.6)	61.4 (15.7)	61.6 (15.7)	62.8 (15.5)
Male gender (%)	1420 (63.1%)	1358 (64.4%)	1439 (64.7%)	1344 (64.6%)
APACHE II (mean, SD) - 5 hospitals	20.3 (8.6)	19.8 (8.2)	20.5 (9.3)	21.8 (8.7)
SAPS II (mean, SD) - 8 hospitals	53.0 (18.0)	54.8 (17.9)	54.4 (17.5)	55.0 (18.0)
Type of ICU-admission				
medical	1464 (65.3%)	1323 (63.0%)	1442 (64.9%)	1385 (66.6%)
trauma with surgery	138 (6.2%)	142 (6.8%)	156 (7.0%)	115 (5.5%)
trauma, no surgery	113 (5.0%)	88 (4.2%)	104 (4.7%)	88 (4.2%)
surgical, scheduled	198 (8.8%)	173 (8.2%)	173 (7.8%)	178 (8.6%)
surgical, unscheduled	328 (14.6%)	374 (17.8%)	346 (15.6%)	314 (15.1%)
surgical, unspecified	10 (0.4%)	8 (0.4%)	3 (0.1%)	2 (0.1%)
Location before ICU admission				
Same hospital	1020 (45.3%)	1032 (49.0%)	1025 (46.1%)	1035 (49.7%)
Another hospital or long term care facility	400 (17.8%)	312 (14.8%)	316 (14.2%)	301 (14.5%)
Home (directly or via emergency room)	831 (36.9%)	764 (36.2%)	883 (39.7%)	746 (35.8%)
If admitted in a hospital or long-term care facility, prior location				
Operating room	379 (27.1%)	409 (30.9%)	371 (28.4%)	360 (27.8%)
Other ICU	229 (16.4%)	221 (16.7%)	221 (16.9%)	176 (13.6%)
Acute care ward	726 (52.0%)	656 (49.5%)	642 (49.1%)	710 (54.8%)
Rehabilitation or long term care facility	63 (4.5%)	38 (2.9%)	74 (5.7%)	49 (3.8%)
Antibiotic at the time of ICU admission	943 (41.9%)	832 (39.5%)	992 (44.6%)	744 (35.8%)
Acute illness on ICU-admission	1999 (88.9%)	1888 (89.6%)	2007 (90.2%)	1932 (92.8%)
Sites of organ failure				
Respiratory illness	1023 (45.5%)	990 (47.0%)	998 (44.9%)	985 (47.3%)
Cardiovascular illness	828 (36.8%)	811 (38.5%)	835 (37.5%)	792 (38.0%)
Neurologic illness	686 (30.5%)	674 (32.0%)	615 (27.7%)	603 (29.0%)
Renal illness	232 (10.3%)	203 (9.6%)	220 (9.9%)	201 (9.7%)
Hepatic illness	148 (6.6%)	122 (5.8%)	124 (5.6%)	104 (5.0%)
Metabolic illness	128 (5.7%)	146 (6.9%)	145 (6.5%)	106 (5.1%)
Hematologic illness	135 (6.0%)	111 (5.3%)	155 (7.0%)	109 (5.2%)
Other illness	210 (9.3%)	253 (12.0%)	345 (15.5%)	344 (16.5%)
Charlson comorbidity Index (mean, SD)	2.15 (2.42)	2.38 (2.49)	2.35 (2.42)	2.42 (2.56)
Charlson comorbidity Index (categories)				
0	738 (32.8%)	631 (29.9%)	653 (29.4%)	626 (30.1%)
1-2	759 (33.7%)	674 (32.0%)	718 (32.3%)	654 (31.4%)
3-4	399 (17.7%)	398 (18.9%)	461 (20.7%)	410 (19.7%)
>4	355 (15.8%)	405 (19.2%)	392 (17.6%)	392 (18.8%)

Supplement table S4. continued

	baseline	CHX	SOD	SDD
	2251	2108	2224	2082
Comorbidities				
Any malignancy (including leukemia and lymphoma)	319 (14.2%)	296 (14.0%)	329 (14.8%)	289 (13.9%)
Metastatic solid tumor	108 (4.8%)	129 (6.1%)	104 (4.7%)	143 (6.9%)
Hematologic cancer	71 (3.2%)	66 (3.1%)	80 (3.6%)	61 (2.9%)
Immunodepression (incl steroids/immunesuppression)	190 (8.4%)	223 (10.6%)	193 (8.7%)	206 (9.9%)
Peripheral vascular disease	260 (11.6%)	254 (12.0%)	262 (11.8%)	325 (15.6%)
Myocardial infarction	232 (10.3%)	230 (10.9%)	219 (9.8%)	209 (10.0%)
Cerebrovascular disease	225 (10.0%)	200 (9.5%)	177 (8.0%)	199 (9.6%)
Congestive heart failure	340 (15.1%)	425 (20.2%)	469 (21.1%)	443 (21.3%)
Pulmonary disease / chronic respiratory failure	384 (17.1%)	398 (18.9%)	400 (18.0%)	395 (19.0%)
DM without chronic complication	269 (12.0%)	298 (14.1%)	251 (11.3%)	280 (13.4%)
DM with chronic complication	187 (8.3%)	147 (7.0%)	180 (8.1%)	148 (7.1%)
Chronic renal failure	257 (11.4%)	278 (13.2%)	337 (15.2%)	303 (14.6%)
Peptic ulcer disease	139 (6.2%)	168 (8.0%)	189 (8.5%)	145 (7.0%)
Dementia	43 (1.9%)	52 (2.5%)	54 (2.4%)	55 (2.6%)
Hemiplegia / paraplegia	50 (2.2%)	47 (2.2%)	56 (2.5%)	53 (2.5%)
Moderate / Severe liver disease	132 (5.9%)	124 (5.9%)	119 (5.4%)	88 (4.2%)
Mild liver disease	56 (2.5%)	87 (4.1%)	58 (2.6%)	67 (3.2%)
Rheumatologic disease	63 (2.8%)	102 (4.8%)	91 (4.1%)	76 (3.7%)
AIDS/HIV infection	20 (0.9%)	17 (0.8%)	29 (1.3%)	21 (1.0%)

Abbreviations: AIDS/HIV, acquired immunodeficiency syndrome/human immunodeficiency virus; CHX, chlorhexidine mouthwash; DM, diabetes mellitus; ICU, intensive care unit; SD, standard deviation; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

Supplement table S5. Compliance measures

	CHX	SOD	SDD
Protocol compliance (monthly unit-wide point prevalence screening)			
Patients admitted	1,667	1,693	1,588
Ventilated (% of admitted) - <i>representing % eligible</i>	722 (43.1%)	708 (41.8%)	640 (40.3%)
Included	693	682	604
Patient received 4 doses of study intervention in past 24h (% of included)	641 (92.5%)	630 (92.4%)	569 (94.2%)
Treatment compliance (amongst included patients)	2,108	2,224	2,082
Treatment compliance (% of included)			
Intervention interrupted for more than > 24h (4 doses or more) before extubation	54 (2.6%)	82 (3.7%)	100 (4.8%)
Type of medication interrupted			
CHX	54 (2.6%)	2 (0.1%)	0 (0.0%)
SOD	0 (0.0%)	75 (3.4%)	0 (0.0%)
SDD, paste only	0 (0.0%)	0 (0.0%)	13 (0.6%)
SDD, suspension only	0 (0.0%)	1 (0.0%)	54 (2.6%)
SDD, both paste and suspension	0 (0.0%)	2 (0.1%)	33 (1.6%)
Duration of interruption			
1-3 days	28 (1.3%)	47 (2.1%)	58 (2.8%)
4-7 days	17 (0.8%)	25 (1.1%)	30 (1.4%)
8-14 days	1 (0.0%)	2 (0.1%)	7 (0.3%)
15 days or more	1 (0.0%)	4 (0.2%)	2 (0.1%)
Reason for interruption			
Intolerance	15 (0.7%)	2 (0.1%)	1 (0.0%)
Pt colonized with HRMO, only sensitive to colistin	0 (0.0%)	1 (0.0%)	0 (0.0%)
Pt colonized with HRMO resistant to carbapenems AND tobramycin	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gastro-enteral contra-indication	2 (0.1%)	0 (0.0%)	33 (1.6%)
Doctor's decision	4 (0.2%)	17 (0.8%)	8 (0.4%)
Patient's (or proxy) decision	10 (0.5%)	4 (0.2%)	10 (0.5%)

Supplement table S6. Average hand hygiene compliance per study period

center	baseline period	CHX	SOD	SDD
1	45.3%	59.1%	60.3%	54.7%
2	66.0%	74.8%	66.2%	66.3%
3	76.2%	80.7%	71.9%	78.8%
4	67.0%	71.2%	74.1%	76.1%
5	61.4%	70.3%	75.0%	73.7%
6	74.1%	73.3%	64.2%	75.2%
7	64.9%	86.5%	93.6%	65.0%
8	66.3%	70.4%	70.3%	74.6%
9	61.1%	70.3%	75.5%	64.3%
10	58.4%	72.7%	70.6%	65.0%
11	61.7%	67.3%	74.0%	76.6%
12	32.6%	61.7%	48.7%	78.0%
13	86.5%	86.4%	91.1%	87.7%
Overall	64.1%	72.4%	72.5%	72.2%

Abbreviations: CHX, chlorhexidine mouthwash; SOD, selective oropharyngeal decontamination; SDD, selective digestive tract decontamination.

Supplement table S7. Micro-organisms in positive blood cultures that are not included in the study definition of BSI, per study period (first occurrence of unique species on day 2 of ICU admission onwards)

Study period	baseline		CHX		SOD		SDD	
	n	%	n	%	n	%	n	%
Number of patients	2251		2108		2224		2082	
Other ICU acquired positive blood cultures^(a)	106		136		142		148	
Coagulase negative staphylococcus spp.	97	91.5%	117	86.0%	127	89.4%	135	91.2%
Clostridium spp.	0	0.0%	1	0.7%	1	0.7%	1	0.7%
Non-pneumococcal streptococcus spp.	5	4.7%	13	9.6%	8	5.6%	8	5.4%
Micrococcus spp.	0	0.0%	0	0.0%	2	1.4%	1	0.7%
Other	4	3.8%	5	3.7%	4	2.8%	3	2.0%

Abbreviations: BSI, Bloodstream infection; CHX, chlorhexidine mouthwash; SOD, selective oropharyngeal decontamination; SDD, selective digestive tract decontamination; ICU, intensive care unit; spp., species

a) Excluding species considered contaminants (Bacillus, Atopobium, Corynebacterium and Propionibacterium species)

Supplement table S8. Systemic antibiotics used

Study period	Baseline	CHX	SOD	SDD
% Patient days included	54%	56%	54%	52%
Antibiotic type (DDD per 1000 patient days)				
Penicillin + beta-lactamase inhibitor	402	355	371	422
Cephalosporins	176	160	180	188
Carbapenems	179	177	172	186
Fluoroquinolones	104	99	85	94
Aminoglycosides	30	26	24	23
Colistin	24	25	38	36
Tetracyclins	9	16	25	15
Macrolides	53	54	58	68
Lincosamides	25	22	23	23
Others	56	66	54	60
Mean DDD per day	1,1	1,0	1,0	1,1

Abbreviations: CHX, chlorhexidine mouthwash; DDD, defined daily dose; SOD, selective oropharyngeal decontamination; SDD, selective digestive tract decontamination.

Supplement table S9. Complete point prevalence results

	standard care		CHX		SOD		SDD		
Rectum	prev.	prev.	aRR *	prev.	aRR *	prev.	aRR *	prev.	aRR *
<u>Enterobacteriaceae</u>	16.1%	21.7%	1.07 (0.99-1.16)	19.7%	1.04 (0.96-1.13)	13.9%	1.05 (0.95-1.16)		
3rd generation cephalosporin resistance	15.8%	21.5%	1.07 (0.99-1.16)	19.2%	1.04 (0.96-1.13)	13.7%	1.07 (0.97-1.18)		
carbapenem resistance	3.2%	3.1%	0.68 (0.54-0.86)	2.9%	0.85 (0.71-1.03)	2.6%	0.8 (0.64-1.01)		
MDR	10.8%	15.5%	1.07 (0.97-1.19)	14.2%	1.06 (0.96-1.17)	10.0%	1.1 (0.97-1.24)		
colistin resistance**	0.5%	1.6%	0.81 (0.54-1.21)	1.8%	0.97 (0.65-1.45)	1.3%	0.96 (0.6-1.54)		
Glucose non-fermenting GNB	3.2%	3.2%	0.77 (0.62-0.95)	3.3%	0.93 (0.76-1.14)	2.3%	0.81 (0.63-1.04)		
carbapenem resistance ***	2.9%	2.9%	0.75 (0.6-0.93)	2.7%	0.95 (0.76-1.18)	1.8%	0.8 (0.6-1.06)		
colistin resistance	0.0%	0.1%		0.1%		0.3%			
MDR	2.4%	2.0%	0.66 (0.5-0.89)	2.7%	0.82 (0.65-1.05)	1.7%	0.71 (0.52-0.98)		
HRMO GNB, regardless of antibiotic susceptibility	1.0%	1.5%	0.8 (0.5-1.27)	1.1%	0.8 (0.49-1.3)	1.6%	1.01 (0.64-1.58)		
Any HRMO GNB	19.3%	25.3%	1.03 (0.96-1.11)	23.0%	1.03 (0.96-1.11)	17.1%	1.04 (0.96-1.14)		
<u>VRE</u>	2.2%	1.5%	0.96 (0.74-1.24)	1.8%	0.94 (0.73-1.21)	4.2%	1.03 (0.84-1.27)		
<i>Enterobacteriaceae</i> or <i>GNF-GNB</i> with gentamicin resistance	8.3%	9.0%	0.95 (0.84-1.08)	10.4%	0.99 (0.89-1.1)	7.2%	1 (0.87-1.15)		
Respiratory tract									
<u>Enterobacteriaceae</u>	6.6%	7.6%	0.94 (0.81-1.09)	4.2%	0.93 (0.8-1.09)	4.7%	0.94 (0.78-1.13)		
3rd generation cephalosporin resistance	6.4%	7.4%	0.95 (0.82-1.10)	4.2%	0.93 (0.80-1.09)	4.5%	0.94 (0.78-1.13)		
carbapenem resistance	1.4%	1.1%	0.71 (0.47-1.07)	0.9%	0.68 (0.48-0.94)	0.5%	0.59 (0.37-0.97)		
MDR	4.0%	5.2%	1.02 (0.84-1.23)	3.3%	0.92 (0.76-1.12)	3.5%	1.04 (0.83-1.31)		
colistin resistance**	0.1%	0.8%	0.57 (0.29-1.14)	0.9%	0.66 (0.36-1.21)	0.3%	0.61 (0.30-1.22)		
Glucose non-fermenting GNB	3.4%	2.9%	0.8 (0.64-1.00)	3.8%	0.84 (0.7-1)	2.7%	0.75 (0.58-0.96)		
carbapenem resistance ***	3.1%	2.8%	0.8 (0.63-1.00)	3.4%	0.83 (0.69-1)	2.4%	0.80 (0.62-1.04)		
colistin resistance	0.3%	0.0%		0.1%		0.2%			
MDR	2.5%	1.7%	0.75 (0.55-1.01)	2.6%	0.83 (0.67-1.03)	2.2%	0.76 (0.57-1.01)		
HRMO GNB, regardless of antibiotic susceptibility	3.8%	5.2%	1.16 (0.94-1.44)	3.2%	0.97 (0.77-1.22)	3.6%	1.04 (0.83-1.31)		
Any HRMO GNB	12.9%	15.2%	0.98 (0.88-1.08)	10.3%	0.93 (0.84-1.04)	10.2%	0.94 (0.83-1.06)		
<u>MRSA</u>	1.7%	1.1%	0.95 (0.66-1.36)	1.3%	0.77 (0.59-1)	1.7%	0.73 (0.54-0.97)		
<i>Enterobacteriaceae</i> or <i>GNF-GNB</i> with gentamicin resistance	4.5%	4.3%	0.84 (0.69-1.02)	4.4%	0.86 (0.72-1.02)	4.0%	0.85 (0.68-1.06)		

Abbreviations: CHX, chlorhexidine; GNF-GNB, glucose non-fermenting gram-negative bacteria; HRMO, highly resistant micro-organism; MDR, multidrug resistant (resistance to 3 or more antibiotics/classes of antibiotics [Supplement Table S2]); MRSA, methicillin resistant *S. aureus*; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination; VRE, vancomycin-resistant *E. faecium*/*E. faecalis*.

* aRR; adjusted relative risk per month, all models were corrected for underlying time trends per centre* * excluding Enterobacteriaceae with intrinsic colistin resistance (*Proteus* spp., *Morganella* spp., *Serratia* spp., *Providencia* spp., *Hafnia* spp.

*** for *Pseudomonas* spp., resistance to at least one other antibiotic was required to be reported as carbapenem-R

Supplement table S10. Compliance with antibiotic susceptibility testing in point prevalence samples

<u>Antibiotics for which AST was requested</u>	rectum		respiratory tract	
	Enterobacteriaceae (N=1,153)	GNF-GNB (N=467)	Enterobacteriaceae (N=559)	GNF-GNB (N=399)
colistin	96.9%	93.8%	97.3%	92.0%
meropenem or imipenem	98.0%	n/a	98.0%	n/a
meropenem or imipenem or doripenem	n/a	97.2%	n/a	94.0%
piperacillin or piperacillin/tazobactam	97.9%	n/a	98.7%	n/a
piperacillin or piperacillin/tazobactam or ticarcillin	n/a	95.9%	n/a	89.2%
cefotaxim or ceftriaxone or ceftazidime	98.0%	n/a	98.6%	n/a
ceftazidime	n/a	95.9%	n/a	89.0%
gentamicin	97.2%	97.0%	98.4%	93.5%
amikacin	98.0%	97.4%	98.7%	93.7%
fluoroquinolone	98.2%	97.2%	98.7%	94.0%
sulfamethoxazole/trimethoprim	97.7%	n/a	98.2%	n/a
no. of AST results missing	204	119	69	218
no. of AST results expected	9183	3736	4362	3192
Completeness of AST	97.8%	96.8%	98.4%	93.2%
Completeness of AST per tractus	97.5%		96.2%	

Abbreviations: AST, antibiotic susceptibility testing; GNF-GNB, glucose non-fermenting gram-negative bacteria (including *Pseudomonas* spp.)

Supplement table S11. Prevalence of colistin-resistant GNB in 3 monthly point prevalence surveys (10/13 centers)

	baseline	CHX	SOD	SDD
No. of point prevalence surveys	19	19	18	22
Number of point prevalence samples				
Rectum	360	409	354	402
Respiratory tract	360	396	337	377
Colonization with GNB				
Rectum	256 (71.1%)	289 (70.7%)	253 (71.5%)	245 (60.9%)
Respiratory tract	112 (31.1%)	146 (36.9%)	85 (25.2%)	105 (27.9%)
Colonization with colistin-R GNB (excl. intrinsically colistin resistant species*)				
Rectum	4 (1.1%)	3 (0.7%)	8 (2.0%)	8 (2.3%)
Respiratory tract	1 (0.2%)	3 (0.8%)	3 (0.9%)	0 (0.0%)
Colonization with <i>intrinsically</i> colistin-R GNB*				
Rectum	38 (10.6%)	43 (10.5%)	24 (6.8%)	36 (9.0%)
Respiratory tract	20 (5.6%)	23 (5.8%)	13 (3.9%)	23 (6.1%)

Abbreviations: CHX, chlorhexidine mouthwash; GNB, Gram-negative bacteria; SOD, selective oropharyngeal decontamination; SDD, selective digestive tract decontamination;

Methods: Once every three months, rectum and respiratory samples obtained during point prevalence surveys were inoculated on plain MacConkey agar from which a maximum of three morphologically distinct colonies were selected in the local laboratory. These isolates were shipped to the University Medical Center of Utrecht for species determination (MALDI-TOF, Bruker) and automated susceptibility testing (BD Phoenix, BD) to determine the prevalence of colistin susceptibility among GNB isolated from non-selective media.

* intrinsically colistin resistant species included *Proteus* spp., *Morganella* spp., *Serratia* spp., *Providencia* spp., *Hafnia alvei*

Chapter 6

Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU

Intensive Care Medicine 2016; 42 (4): 620-621

Bastiaan H.J. Wittekamp^{1*}

Nienke L. Plantinga^{1*}

Kris Leleu²

Pieter Depuydt³

Anne-Marie Van den Abeele⁴

Christian Brun-Buisson⁵

Marc J.M. Bonten^{1,6}

* both authors contributed equally to this work

¹ Julius Center for Health Sciences and Primary care, University Medical Center Utrecht, The Netherlands

² Department of Intensive Care, AZ St. Lucas Hospital, Ghent, Belgium

³ Department of Intensive Care, Ghent University Hospital, Ghent, Belgium

⁴ Clinical Microbiology Laboratory, AZ St. Lucas Hospital, Ghent, Belgium

⁵ Assistance Publique-Hôpitaux de Paris, Groupe Henri Mondor; Université Paris-Est, Creteil, France

⁶ Medical Microbiology, University Medical Center Utrecht, The Netherlands

Introduction

Oral care using a chlorhexidine solution is commonly used as infection prevention measure in European ICUs (1). The preventive effects of different decontamination strategies, amongst which mouthwash with chlorhexidine digluconate 2% (CHX 2%), on the incidence of ICU-acquired bacteremia with multidrug-resistant bacteria was investigated in a multicenter cluster-randomized study in 13 ICUs in 6 European countries (chapter 5). An unexpected high incidence of oral mucosal lesions was observed in ICU patients receiving CHX 2%.

Methods

In the multicenter study ICUs started with a baseline period of 6 months, during which oral care included chlorhexidine 0.12% or 0.2% mouthwash when this was part of routine oral care. Subsequently, oral decontamination with CHX 2%, SOD and SDD were implemented in a randomized order per ICU, each intervention being applied for a 6-month period. Adult patients were eligible for inclusion if they had an expected length of mechanical ventilation of 24 hours or more. After inclusion, patients were treated according to the study protocol until extubation. Because of the ecological nature of the study and the perceived minimal risks associated with the interventions studied, a waiver for informed consent - or permission to perform the study with the possibility for patients or legal representatives to sign informed refusal - was obtained from the local institutional review boards for all participating hospitals and, in countries where this was required, from the national regulatory authorities (2).

During the CHX intervention, 10 mL of a 2% chlorhexidine digluconate mouthwash solution was applied to the oral mucosa four times daily after performing standard oral care. ICUs were instructed to administer CHX 2% by soaking a toothette (sponge) in the solution and gently applying it to the surface of the teeth, gumline and tongue. Excess chlorhexidine remaining in the oral cavity was to be removed by suctioning.

CHX 2% was produced according to GMP guidelines by the department of Clinical Pharmacy of the University Medical Center Utrecht (Sponsor of the study). The composition of the solution is shown in textbox 1. Excipients used in the solution include sorbitolum liquidum cristallisabile, purified water, alcohol 96%

Chapter 6

v/v (7g/100ml) and Peppermint oil Ph. Eur. All these substances are also used in the production of the commercially available chlorhexidine digluconate 0.12% and 0.20% solutions (e.g. Corsodyl[®], Farmaclair, Hérouville St Clair, France), and no other substance was added to the solution.

Textbox 1. Chlorhexidine 2% mouthwash formulation (p. 100 mL)

Chlorhexidine digluconate solution 200 g/L Ph.Eur	10.7	gr	Active ingredient
Sorbitolum liquidum cristallisabile Ph.Eur.	53.5	gr	Taste adjuster
Peppermint oil Ph.Eur.	150	µl	Taste adjuster
Alcohol 96% v/v Ph.Eur.	7	gr	pH adjuster
Purified water Ph.Eur	100	ml	Solvent

Abbreviations: ml, milliliter; gr, gram; µl, microliter; L, liter; Ph.Eur, European Pharmacopoeia (European quality standard); v/v, volume per volume

Formal recording and reporting of adverse events was not part of the study protocol, but left to the discretion of investigators, unless such events met the definition of suspected unexpected serious adverse reactions (SUSAR). These are serious adverse events (i.e., events that result in either death, are life threatening, require hospitalization or prolongation of hospitalization and/or result in persistent or significant disability or incapacity) that are reasonably related to the intervention under study. The adverse events described here did not meet this definition and were therefore reported spontaneously by two participating sites and then analyzed.

Patient characteristics were compared between the baseline and CHX 2% periods and between patients with and without adverse events using Pearson's Chi-square test for categorical variables and the two-sided independent samples t-test for continuous variables. Furthermore, associations between respiratory tract colonization with *Candida* species and adverse events were assessed.

Results

Oral mucosal lesions, including erosive lesions, ulcerations, white/yellow plaque formation and bleeding mucosa were observed in 29 of 295 patients (9.8%) that had received CHX 2% in the first two hospitals using this intervention (Table 1). The

median time to onset of oral lesions was 8.0 days (IQR 4.5 – 11.0) in the 24 patients in whom duration of exposure could be ascertained. CHX 2% was discontinued prematurely in 16/29 cases and oral mucosal lesions disappeared after cessation of CHX 2% in all patients.

Table 1. Line-listing of patients who experienced adverse events during the use of CHX 2%

	Symptoms as described in medical file (remarks and consultations)	Days of onset adverse events ^a	CHX stopped prematurely?	Candida ^b	Herpes simplex ^c
Hospital A					
1	bleeding gums	2	yes	no	NT
2	yellow viscous plaque on whole lower lip, lesions in both cheeks at the height of the teeth, yellow +++, severe +++	1	yes	no	NT
3	bleeding gums and white lesions in the entire mouth	24	yes	yes	NT
4	crustae lips, yellow lesion under the tongue, viscous plaque in throat, non-painful	19 ^d	no	no	NT
5	diffuse white plaques in the pharynx and cheeks	8	yes	yes	NT
6	swelling of lips and mucosa, spontaneous bleeding lips, white plaque and starting white lesions of both cheeks at the height of the teeth	4	yes	no	NT
7	white tongue and 'atonic' color of the gums	10	yes	no	NT
8	bleedy mouth	6	yes	no	NT
9	bleeding gums, one zone yellow plaque in mouth, white plaques on tongue	8	yes	yes	NT
10	bleeding gums, zones with yellow plaque in mouth	2	no	no	NT
11	yellow plaques with slight bleeding on inside of lips	8	no	no	NT
12	bleeding gums, no distinct ulcers, swelling mucosae and lips (pt with thrombocytopenia, $10 \times 10^3 / \text{mm}^3$)	2	no	yes	NT
13	tiny white bleeding ulcers near teeth pockets	12	yes	no	Positive (oral lesion)
14	bleeding ulcerations gingival pockets ischemic lesions uvula palatum molle mucosal edema (consult stomatology: no necrotizing gingivitis)	11	yes	yes	Positive (nose)
15	bleeding gums near teeth pocket (consult stomatology: tiny ulcerations, no necrotizing gingivitis lesions caused by friction during mouth care)	11	no	no	Positive (ETA)
16	excessive mouth bleeding with clotting (consultation with ENT physician: no clear lesions in mouth. laryngoscopic examination by intensive care physician: palatum molle ulcerations matching endotracheal tube position lesions cauterized, bleeding stopped)	10	yes	yes	low positive (ETA)
17	bleeding ulcerations gingival pockets (consult stomatology: no necrotizing gingivitis)	12	no	no	low positive (ETA)

Table 1. continued

Symptoms as described in medical file (remarks and consultations)	Days of onset adverse events ^a	CHX stopped pre-maturely?	Candida ^b	Herpes simplex ^c
Hospital B^e				
1 dry tongue, "aphthous lesions", thickening	<i>unknown</i>	no	<i>unknown</i>	NT
2 white plaque	<i>unknown</i>	no	<i>unknown</i>	NT
3 white plaque	<i>unknown</i>	no	<i>unknown</i>	NT
4 yellow plaque	<i>unknown</i>	no	<i>unknown</i>	NT
5 white plaque	<i>unknown</i>	no	<i>unknown</i>	NT
6 white plaque tongue	6	no	no	NT
7 white plaque tongue	8	yes	no	NT
8 white plaque tongue, gums and lips	11	yes	yes	NT
9 "aphthous lesions" lower lip + gums	14	yes	yes	NT
10 white plaque tongue	2	no	no	NT
11 white plaques tongue and (open) «aphthous lesions" lower lip	6	yes	no	NT
12 white plaque tongue, open wound lower lip	6	yes	no	NT

Abbreviations: ETA, endotracheal aspirate; NT, not tested.

a) Time between inclusion date and occurrence of adverse events. b) Defined by the presence or absence of *Candida* spp. in any respiratory culture result (including oropharyngeal swabs), taken at least 1 day prior to the onset of adverse events. c) Result from Herpes Simplex q-PCR (body site), histology was not performed. d) Adverse events occurred during the 2nd episode of mechanical ventilation, "days of onset" include 3 ventilator-free days. e) The first 5 cases from hospital B were reported retrospectively, the date of onset of adverse events could therefore not be retrieved.

Table 2. Baseline characteristics of patients included in the baseline period and the chlorhexidine 2% period (CHX 2%)

	Baseline period (N=310)	CHX 2% period (N=295)	Pearson Chi-Square / Indep. T-test
Male gender	192 (61.9%)	184 (62.4%)	P = 0.912
Admission type			P = 0.344
Medical	188 (60.6%)	166 (56.3%)	
Trauma	18 (5.8%)	25 (8.5%)	
Surgical	104 (33.5%)	104 (35.3%)	
Acute illness (y/n)	251 (81.0%)	222 (75.3%)	P = 0.089
Antibiotic at ICU admission (y/n) ^a	55/144 (38.2%)	134/288 (46.5%)	P = 0.100
Age (SD)	60.0 (15.0)	60.1 (15.5)	P = 0.899
APACHE II, mean (SD)	21.6 (8.8)	20.3 (8.8)	P = 0.056
ICU-LOS, median (IQR)	12 (6-22)	12 (7-21)	
geometric mean (SD)	11.5 (2.4)	11.6 (2.3)	P = 0.870 (LN)
Length of MV, median (IQR)	6 (3-14)	7 (3-13)	
geometric mean (SD)	6.9 (2.5)	6.9 (2.4)	P = 0.966 (LN)
Candida-positive respiratory culture during ICU-admission	143 (48.1%)	120 (41.7%)	P = 0.115

Abbreviations: CHX, chlorhexidine; SD, standard deviation; IQR, interquartile range; LN, log-transformed variable; N, number of patients.

^a Differentially missing data; in one hospital, this variable was missing in 73.8% of baseline patients and 3.0% of CHX 2% patients.

Patient characteristics were comparable for the baseline (n=310) and CHX 2% periods (n=295) for the two ICUs (Table 2). During the baseline period CHX 0.20% and 0.12% were used for oral care in hospital A and B, respectively, without evidence of oral lesions in any patient. All other procedures related to oral care remained identical per hospital during both periods.

Amongst the CHX 2% treated patients, occurrence of side-effects was associated with male gender, APACHE II score, length of stay in the ICU and duration of mechanical ventilation, suggesting a dose-relationship, with increasing risks of oral mucosal lesions for the more severely ill patients, undergoing mechanical ventilation and receiving CHX 2% for longer periods (Table 3). This hypothesis is supported by the localization of the lesions in the oral cavity; most lesions occurred where stasis of the CHX 2% mouthwash might have occurred – despite suctioning after administration – such as below the tongue and in the buccal pockets.

Mechanical stress during application of CHX 2% may have played a role in hospital A, where the solution was initially applied using Kocher's forceps with gauzes and where the incidence seemed to have reduced after changing to application using a syringe. Hospital B had applied CHX 2% with a gauze wrapped around a gloved finger.

In 12 patients symptoms predominantly consisted of pronounced white plaques at the tongue and other localizations in the mouth, in some resembling *Candida* infection. Yet, the incidence rate ratio between prior respiratory tract colonization with *Candida* spp. (monitored twice weekly as part of the study protocol and in clinical cultures) and the occurrence of side-effects was 0.94 (95%-confidence interval 0.09 – 1.79) (Table 4). An association with herpes reactivation could not be determined as reactivation was investigated in five affected patients only (Table 1).

Table 3. Characteristics of CHX 2% treated patients with and without adverse events

	adverse events (N=29)	no adverse events (N=266)	Pearson Chi-Square / Indep. T-test
Male gender	23 (79.3%)	161 (60.5%)	P = 0.047
Admission type			P = 0.155
Medical	13 (44.8%)	153 (57.5%)	
Trauma	5 (17.2%)	20 (7.5%)	
Surgical	11 (37.9%)	93 (35.0%)	
Acute illness (y/n)	24 (82.8%)	198 (74.4%)	P = 0.324
Antibiotic at ICU admission (y/n)	11/29 (37.9%)	123/259 (47.5%)	P = 0.328
Age, mean (SD)	60.4 (13.3)	60.1 (15.7)	P = 0.921
APACHE II, mean (SD)	26.7 (8.0)	19.6 (8.6)	P < 0.0005
ICU-LOS, median (IQR)	28 (21 – 41.5)	10.5 (6-19)	
mean (SD)	27.2 (1.8)	10.6 (2.2)	P < 0.0005 (LN)
Length of MV, median (IQR)	19 (14.5 – 28.5)	6 (3-11)	
mean (SD)	18.8 (1.8)	6.2 (2.3)	P < 0.0005 (LN)

Abbreviations: SD, standard deviation; IQR, interquartile range; LN, log-transformed variable; N, number of patients.

Table 4. Association between respiratory tract colonization with *Candida spp.* and the occurrence of adverse events*

	Patient days at risk (PD)	Adverse events (cases)	Incidence rate (1000 PD ⁻¹)
Candida detected in respiratory tract	879	8	9.10
No candida detected in respiratory tract	1,651	16	9.69
	2,530	24	9.49

* Patients were considered at risk for adverse events from the start of CHX 2% until a) two days after extubation

or b) if prior to that date: until ICU-discharge. The first five cases from hospital B were excluded from this analysis, because the start date of adverse events was unknown.

The study safety committee recommended to replace CHX 2% mouthwash by a CHX 1% oral gel in the remaining hospitals. Since then, CHX 1% was withdrawn for reasons of intolerance in 2 of 419 (0.5%) patients in 4 hospitals, after 12 and 30 days of use.

Conclusion

Based on these findings we recommend against the use of 2% chlorhexidine digluconate mouthwash in ICU patients.

References

1. Rello J, Koulenti D, Blot S, Sierra R, Diaz E, De Waele JJ, et al. Oral care practices in intensive care units: a survey of 59 European ICUs. *Intensive care medicine*. 2007;33(6):1066-70.
2. Wittekamp BH, Wise MP, Brun-Buisson C, Bonten MJ. Regulatory obstacles affecting ecological studies in the ICU. *The Lancet Infectious diseases*. 2014;14(10):913-5.

Chapter 7

The effects of topical antibiotics on carriage with 3rd-generation cephalosporin and carbapenem resistant gram-negative bacteria in ICU patients

Manuscript in preparation

Bastiaan H.J. Wittekamp¹

Nienke L. Plantinga¹

Christian Brun-Buisson²

Marc J.M. Bonten^{1,3}

for the R-GNOSIS ICU study group

¹ Julius Center for Health Sciences and Primary care, University Medical Center Utrecht,
The Netherlands

² Assistance Publique-Hôpitaux de Paris, Groupe Henri Mondor; Université Paris-Est, Creteil,
France

³ Medical Microbiology, University Medical Center Utrecht, The Netherlands

Abstract

Objectives: To quantify the effects of selective digestive tract decontamination (SDD), selective oropharyngeal decontamination (SOD) and chlorhexidine (CHX) mouthwash on rectum and respiratory tract carriage of third generation cephalosporin resistant Enterobacteriaceae (3GCR-E) and carbapenem resistant Gram-negative bacteria (CR-GNB) in ICU patients.

Methods: Within a European cluster-randomized cross-over trial carriage with 3GCR-E or CR-GNB in the rectum and respiratory tract was determined at least twice weekly. Mechanically ventilated patients with 3GCR-E or CR-GNB carriage within the first 5 days of ICU-stay and at least one follow-up culture were included. Persistence of carriage was compared to standard care (SC) and analyzed using Cox-regression.

Results: Among 8,665 patients in the trial, 643 and 154 patients were included for the analysis on rectal carriage of 3GCR-E and CR-GNB, respectively, and 291 and 143 for respiratory tract carriage of 3GCR-E and CR-GNB, respectively. 3GCR-E and CR-GNB present during initial ICU stay were more frequently eradicated in rectum samples during SDD with respective cause specific hazard ratios (CSHR) of 1.76 (95%CI 1.31-2.36) and 3.17 (95% CI 1.60-6.29) for SDD, versus SC. For 3GCR-E there was a tendency towards eradication in respiratory samples during SDD (CSHR 1.47 (95% CI 0.98-2.20)) and SOD (CSHR 1.38 (95% CI 0.92-2.06)), versus SC, which was not observed during CHX or for CR-GNB.

Conclusions: SDD was associated with eradication of CR-GNB and 3GCR-E colonization in the rectum, and SDD and SOD were associated with a tendency towards eradication of 3GCR-E from the respiratory tract. These effects might prevent the occurrence of ICU-acquired infections and cross transmission of such bacteria.

Introduction

The incidence of infections caused by extended spectrum beta-lactamases (ESBL) producing or carbapenem resistant Gram-negative bacteria (CR-GNB) is rising in ICU patients. Reducing carriage of these antibiotic resistant bacteria during ICU stay could prevent infections and cross-transmission. Decontamination regimens such as Selective Digestive Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) have been associated with reduced prevalence of carriage with antibiotic resistant bacteria in settings with low resistance levels (1). These strategies have been applied as part of control strategies for outbreaks of ESBL and CR-GNB, as well as for targeted treatment of patients colonized with such bacteria (2-4), with different results (5-9). There is, however, considerable heterogeneity across these studies in terms of study populations, clinical settings, definitions of decolonization, length of follow-up, and decontamination regimens applied. Moreover, the efficacy of CHX mouthwash without concomitant topical antibiotics for reducing carriage in the respiratory tract has not been determined. As a consequence, the effectiveness of different decontamination regimens and their use for eradication of antibiotic resistant Gram-negative bacteria (GNB) in ICU patients remain controversial. We, therefore, used data recorded within an international cluster-randomized cross-over study (chapter 5) comparing SDD, SOD and CHX mouthwash with standard care (SC) to determine the efficacy of these regimens in eradication of 3rd-generation cephalosporin resistant Enterobacteriaceae (3GCR-E) and CR-GNB in the respiratory tract and rectum during ICU stay.

Methods

We analyzed data from 13 European ICUs that participated in a cluster randomized cross-over trial between December 2013 and May 2017, in which the effect of SDD, SOD and CHX mouthwash on survival, ICU acquired bacteremia and antibiotic resistance were compared to a baseline period of SC. (www.clinicaltrials.gov, NCT02208154). Each study period lasted six months and all ICUs started with the SC period. The order of the three intervention periods was randomized per ICU and study periods were separated by a one month wash-out/in period. Patients with an expected duration of invasive mechanical ventilation of 24 hours or longer

were included in the main study and eligible to receive the study intervention. The need for informed consent was waived by the local ethics committees.

For the current analysis we included all patients with documented carriage of 3GCR-E or CR-GNB in the rectum or respiratory tract at any time during the first 5 days in ICU, labelled as “initial ICU stay”, and from whom at least one rectum or respiratory culture following the index culture was available. 3GCR-E was defined as any species of *Enterobacteriaceae* with resistance to cefotaxime, ceftriaxone and/or ceftazidime, whichever antibiotic was tested. CR-GNB was defined as any species of *Enterobacteriaceae* or glucose non-fermenting Gram-negative bacteria (NF-GNB) with resistance to imipenem, meropenem or doripenem.

SDD consisted of a gastro-intestinal suspension with 1.9 million units colistin sulphate, 80 mg tobramycin sulphate and 2.0 million units nystatin per dosage (10 ml, 4 times daily through a nasogastric tube) and a mouthpaste with 0.19 million units colistin sulphate, 10 mg tobramycin sulphate and 0.1 million units nystatin per dosage (0.5 g, 4 times daily during). SOD consisted of the mouthpaste only. Systemic broad spectrum antibiotics were not part of SDD or SOD. CHX mouthwash consisted of chlorhexidine digluconate 1% (11/13 hospitals) or 2% (2/13 hospitals) (10). All decontamination strategies were applied at 6 hours intervals until extubation. No other decontamination strategies were used during the SC period, except for chlorhexidine 0.12% and 0.2% mouthwash in 6 and 5 of 13 ICUs, respectively, since it was part of regular care before the start of the study.

The World Health Organization hand hygiene protocol (11) and daily Chlorhexidine 2% body washes were implemented before the start of the study as standard care for all patients in the ICU.

Rectum (rectal swabs) and respiratory tract surveillance samples (endotracheal aspirates or throat swabs) were obtained twice weekly (on Monday and Thursday) and plated on selective chromogenic media (ESBL chrom-ID, bioMérieux). Results from rectum and respiratory samples obtained for clinical reasons were also included. Species identification and resistance determination was done by disk diffusion or automated methods using VITEK (bioMérieux) or Phoenix (BD diagnostics) according to local protocols. The methods of susceptibility testing per hospital did not change during the study.

Species of 3GCR-E and CR-GNB that were identified during initial ICU stay were followed until the last available sample in ICU. Eradication was defined as the absence of the species identified as 3GCR-E or CR-GNB in one and all following available samples from the respective body site until ICU discharge. Crude incidence rate ratio's (IRR) for eradication per 1,000 days at risk and 95% confidence intervals (CI) were calculated to compare eradication rates of 3GCR-E and CR-GNB per study period. In addition, CHX and SC were combined as a reference group for the comparison of eradication from the respiratory tract with SDD and SOD, which both include the same mouthpaste of topical antibiotics. A survival analysis was performed using a mixed-effect Cox regression with a random intercept on ICU (cluster) to correct for patient dependency within clusters, since patients within clusters may be more similar than patients between clusters. ICU-discharge and death were considered competing events, since these may have prevented the observation of the outcome of interest. Left truncated data was taken into account. We adjusted for disease severity, antibiotic use on admission and the Charlson comorbidity index (CCI) (12). Two different disease severity scores were used by ICUs, either APACHE II score or SAPS II score. We standardized both scores and included an interaction term between the standardized score and a dummy variable for ICU-using-APACHE or ICU-using-SAPS in the cox model. For statistical analyses we used IBM SPSS statistics version 21 and R software version 3.2.2 (R project for Statistical Computing).

Results

Patients

Among the 8,665 ICU admissions enrolled in the main study, there were 922 unique patients eligible for the current analyses (Table 1). Their median durations of mechanical ventilation and stay in ICU were 10 (range 1-149) and 15 days (range 2-121), respectively and the median number of rectum and respiratory tract samples was 4 (range 2-49) and 5 (range 2-80), respectively. Among these 922 patients there were no apparent differences in patient characteristics per study period (Table 2).

Table 1. Number of patients (cases) and species included in each cohort per study period

Analysis cohort	SC		CHX		SOD		SDD		Total ^a	
	Cases	Species ^b	Cases	Species ^b	Cases	Species ^b	Cases	Species ^b	Cases	Species
	n	n	n	n	n	n	n	n	n	n
3GCR-E rectum	163	177	156	168	165	180	159	177	643	702
3GCR-E respiratory	76	79	73	77	72	75	70	72	291	303
CR-GNB rectum	41	44	35	36	38	38	40	46	154	164
CR-GNB respiratory	37	37	31	32	41	42	34	34	143	145

Abbreviations: SC: standard care; CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract; n: number; 3GCR-E: third generation cephalosporin resistant enterobacteriaceae; CR-GNB: carbapenem resistant Gram-negative bacteria

(a) 8,665 admissions were included in the main study, patients carrying 3GCR-E and / or CR-GNB were included in each respective cohort

(b) number of unique micro-organisms identified during initial ICU stay, included for all analyses

Table 2. Baseline characteristics of unique patients per study period (total n=922)

	SC (n=232)		CHX (n=227)		SOD (n=245)		SDD (n=218)	
Female, n (%)	89	38,4%	66	29,1%	78	31,8%	62	28,4%
Age, median (IQR)	66	(55-75)	65	(54-74)	66	(51-76)	65	(54-74)
APACHEII, median (IQR) ^a	23	(19-30)	22	(16-29)	23	(17-29)	24	(20-32)
SAPSII score, median (IQR) ^b	53	(41-67)	55	(43-67)	52	(42-65)	52	(42-72)
Medical admission, n (%)	170	73,3%	150	66,1%	166	67,8%	154	70,6%
Comorbidity index, median (IQR) ^c	2	(1-4)	2	(1-5)	2	(1-5)	2	(1-5)
Antibiotic use on admission, n (%)	126	54,3%	106	46,7%	142	58,0%	112	51,4%

Abbreviations: n: number; IQR: inter-quartile range; SC: standard care; CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; APACHE: acute physiology and chronic health evaluation, SAPS: Simplified Acute Physiology Score

(a) among 321 patients who had an APACHEII score available

(b) among 601 patients who had a SAPSII score available

(c) Charlson co-morbidity index (12)

Micro-organisms

In total, there were 1,314 species of antibiotic resistant GNB included in the four analysis cohorts (Table 1). The species of antibiotic resistant GNB per tractus can be found in table 3. Enterobacteriaceae that were resistant to 3rd-generation cephalosporins were most often *E. coli* (346/702 (49%)) in the rectum and Klebsiella species (91/303 (30%)) in the respiratory tract. The most frequent CR-GNB were Pseudomonas species (72/164 (44%) and 79/145 (54%) in the rectum and respiratory tract, respectively) (Table 3).

Table 3. Number of MDR-GNB species per tractus

Third generation cephalosporin resistant Enterobacteriaceae	Number	%
Rectum		
Citrobacter freundii	27	4
Enterobacter species	88	13
Escherichia coli	346	49
Hafnia alvei	5	1
Klebsiella oxytoca	25	4
Klebsiella pneumoniae	157	22
Morganella morganii	3	0
Proteus species	8	1
Serratia marcescens	1	0
Other	42	6
Total	702	
Respiratory tract		
Citrobacter freundii	13	4
Enterobacter species	56	18
Escherichia coli	73	24
Hafnia alvei	12	4
Klebsiella oxytoca	10	3
Klebsiella pneumoniae	91	30
Morganella morganii	4	1
Proteus species	2	1
Serratia marcescens	13	4
Other	29	10
Total	303	
Carbapenem resistant Gram-negative bacteria		
	Number	%
Rectum		
Acinetobacter baumannii	12	7
Citrobacter freundii	3	2
Enterobacter species	11	7
Escherichia coli	5	3
Klebsiella oxytoca	4	2
Klebsiella pneumoniae	44	27
Morganella morganii	2	1
Proteus mirabilis	2	1
Pseudomonas aeruginosa	72	44
Other	9	5
Total	164	
Respiratory tract		
Acinetobacter baumannii	20	14
Citrobacter freundii	1	1
Enterobacter species	6	4
Klebsiella pneumoniae	23	16
Morganella morganii	1	1
Pseudomonas aeruginosa	79	54
Serratia marcescens	1	1
Other	14	10
Total	145	

Rectal carriage

During SC, rectal carriage of 3GCR-E and CR-GNB became undetectable in respectively 43% and 36% of species identified during initial ICU-stay (Supplement, table S1). For SDD these proportions were 59% and 67% for 3GCR-E and CR-GNB, respectively, yielding IRR for rectal eradication of 1.71 (95% confidence interval (CI) 1.26-2.33) for 3GCR-E and 3.12 (95% CI 1.66-6.11) for CR-GNB (Supplement table S1 and table 4). IRRs for SOD and CHX were not statistically significantly different from SC (Table 4). The proportion of follow-up cultures with 3GCR-E and CR-GNB species that were first identified during initial ICU stay in the rectum and respiratory tract declined faster during SDD, compared to other study periods (Supplement, figure S1 and S2). The adjusted cause specific hazard rates (CSHR) for eradication of 3GCR-E and CR-GNB species from the rectum during SDD were 1.76 (95% CI 1.31-2.36) and 3.17 (95% CI 1.60-6.29), respectively (Table 5).

Table 4. Crude incidence rate ratios of eradication compared to standard care per study period

	SC		CHX		SOD		SDD		SDD + SOD	
	rate ratio	rate ratio ^a	(95% CI)	rate ratio ^a	(95% CI)	rate ratio ^a	(95% CI)	rate ratio ^b	(95% CI)	
Third generation cephalosporin resistant Enterobacteriaceae										
Rectum	1.00	0.70	(0.50, 0.99)	1.01	(0.72, 1.41)	1.71	(1.26, 2.33)			
Respiratory	1.00	0.81	(0.54, 1.23)	1.68	(1.12, 2.54)	1.67	(1.11, 2.52)	1.87	(1.41, 2.49)	
Carbapenem resistant GNB										
Rectum	1.00	1.27	(0.59, 2.75)	1.36	(0.64, 2.90)	3.12	(1.66, 6.11)			
Respiratory	1.00	0.55	(0.26, 1.10)	0.82	(0.43, 1.53)	0.89	(0.46, 1.71)	1.11	(0.69, 1.79)	

Abbreviations: CI: confidence interval; GNB: Gram-negative bacteria; SC: standard care; CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination

(a) incidence rate ratios compared to SC, rate per 1000 days at risk

(b) incidence rate ratios compared to composite reference (SC, CHX), rate per 1000 days at risk

Respiratory tract carriage

During SC, respiratory tract carriage became undetectable in 62% and 65% of the 3GCR-E and CR-GNB identified during initial ICU-stay, respectively (Supplement, table S1). For SDD these proportions were 74% and 53% for 3GCR-E and CR-GNB, respectively, yielding incidence rate ratios (IRR) for eradication of the respiratory tract of 1.67 (95% CI 1.11-2.52) for 3GCR-E and 0.89 (95% CI 0.46-1.71) for CR-GNB (Supplement table S1 and table 4). For SOD, the proportions of 3GCR-E and CR-GNB

that disappeared from respiratory tract cultures were 69% and 50%, respectively, yielding incidence rate ratios (IRR) for respiratory tract eradication, compared to SC, of 1.68 (95% CI 1.12-2.54) for 3GCR-E and 0.82 (95% CI 0.43-1.53) for CR-GNB (Table 4). In the Cox regression analysis, which adjusted for possible confounders and the clustered nature of the data, SDD and SOD were not associated with a statistically significant higher hazards for eradication of 3GCR-E or CR-GNB species from the respiratory tract, compared to SC, although there was a trend towards more eradication of 3GCR-E species with SDD (CSHR 1.47, 95% CI 0.98-2.20) and SOD (CSHR 1.38, 95%CI 0.92-2.06) (Table 5). The combined SDD and SOD group, representing the two study interventions consisting of topical oral antibiotics, had a higher hazard for eradication of 3GCR-E from the respiratory tract compared to the combined reference group of CHX and SC, in which no topical oral antibiotics were used (CSHR 1.54 (95% CI 1.16-2.05). For the competing events death and ICU discharge the CSHRs were not statistically significantly different from SC (Table 5).

For CHX, IRRs and CSHRs for eradication were not statistically significantly different from SC for 3GCR-E and CR-GNB (Table 4 and 5). The proportions of 3GCR-E and CR-GNB species identified during initial ICU stay persisting in respiratory tract cultures during each study period are depicted in the supplement (Supplement, figure S3 and S4).

Discussion

In this study, where patients with an expected duration of mechanical ventilation of 24 hours or more were treated with SDD, SOD or CHX mouthwash, or received none of these interventions, SDD was associated with more eradication of both 3GCR-E (a proxy for ESBL), and CR-GNB from the rectum than SC. This may reduce the risk of subsequent infection for the individual patient (13, 14). Indeed, within the current analysis, we found that the crude incidence rate of ICU-acquired bacteremia with multidrug-resistant Gram-negative bacteria (MDR-GNB) after clearance of initial colonization with 3GCR-E in the rectum was 1.58 per 1,000 ICU days, compared to 3.26 per 1,000 ICU days during rectal 3GCR-E colonization. In addition, eradication may reduce the risk of transmission of MDR-GNB or plasmids encoding for resistance to other patients.

Table 5. Cause specific hazard ratios for eradication and competing endpoints discharge and death

Third generation cephalosporin resistant Enterobacteriaceae										
	CSHR Eradication			P-value	CSHR Discharge			CSHR Death		
Rectum										
CHX	0,82	0,58	1,14	0,24	0,87	0,61	1,24	0,88	0,54	1,44
SOD	0,95	0,68	1,33	0,76	1,11	0,80	1,55	0,95	0,58	1,56
SDD	1,76	1,31	2,36	<0,001	0,90	0,62	1,31	0,86	0,49	1,50
Respiratory tract										
CHX	0,86	0,57	1,30	0,60	0,71	0,34	1,50	0,94	0,39	2,27
SOD	1,38	0,92	2,06	0,17	1,28	0,57	2,86	2,01	0,87	4,67
SDD	1,47	0,98	2,20	0,08	0,78	0,33	1,85	1,57	0,65	3,79
SOD-SDD vs. other ^a	1,54	1,16	2,05	<0,001	1,17	0,66	2,07	1,83	1,00	3,36
Carbapenem resistant Gram-negative bacteria										
Rectum										
CHX	1,12	0,51	2,45	0,78	0,82	0,36	1,84	1,06	0,41	2,77
SOD	1,09	0,51	2,34	0,81	1,09	0,50	2,38	0,93	0,35	2,48
SDD	3,17	1,60	6,29	<0,001	1,31	0,60	2,87	0,42	0,11	1,57
Respiratory tract										
CHX	0,61	0,31	1,20	0,16	0,81	0,33	1,98	1,51	0,51	4,44
SOD	0,81	0,44	1,51	0,50	1,16	0,50	2,68	1,81	0,62	5,32
SDD	0,77	0,41	1,45	0,41	0,96	0,40	2,28	0,93	0,27	3,20
SOD-SDD vs. other ^a	0,97	0,61	1,55	0,85	1,19	0,65	2,18	1,08	0,51	2,27

Abbreviations: CSHR: cause specific hazard ratios; CI: confidence interval; CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; vs.: versus; ref.: reference

a) SDD and SOD versus composite reference (Standard care plus CHX)

The observed 59% eradication of 3GCR-E from the rectum with SDD during ICU-stay is in line with previous studies reporting 42% clearance of ESBL producing Enterobacteriaceae with enteral colistin (8), 54% clearance of ESBL-producing GNB with a combination of polymyxin and tobramycin (15) and 73% clearance of 3GCR-E with a similar SDD regimen including systemic cephalosporins during initial ICU stay (16). The 67% clearance rate of CR-GNB from the rectum with SDD is higher than two studies reporting 37% and 43% clearance with gentamycin and colistin (4, 6) and comparable to the 61% reported by an Israeli non-ICU study using polymyxin and gentamycin (3). Yet, these studies differed in definitions used for decolonization, duration of follow-up and antibiotic regimens, precluding any meaningful comparison.

One of these studies reported an increase in resistance against colistin and gentamycin post-treatment among KPC-2-producing *Klebsiella pneumoniae*

isolates (6). In the main study described in chapter 5 of this thesis the prevalence of colistin resistance was low during all study periods and there were no increases in antimicrobial resistance (chapter 5). In the current cohort, amongst patients in whom decolonization was achieved, the prevalence of carriage with intrinsically colistin resistant GNB was comparable for SC, SOD and SDD (supplement, table S2).

The current study allowed the determination of the natural history of bacterial carriage during ICU-stay, i.e., in the absence of interventions that aim to modulate bacterial carriage. During SC, initial colonization with 3GCR-E or CR-GNB in the respiratory tract disappeared in more than half of all unique species. For rectal carriage these percentages were 43% and 36% for 3GCR-E and CR-GNB, respectively. Naturally, carriage may have been affected by intravenous antibiotics, but this information was not available.

The effects of SDD and SOD on respiratory tract carriage of MDR-GNB were smaller than the effects of SDD on rectal carriage. Although crude incidence rates for eradication of 3GCR-E from the respiratory tract were higher (and statistically significantly different) for SDD and SOD than for SC, only a statistically non-significant trend in favor of SDD and SOD remained after adjustment for confounding, clustering and competing events. Combining the interventions that both used topical antibiotics (i.e. SOD and SDD) increased the cohort size and narrowed the confidence interval, resulting in a statistically significant increased hazard rate for eradication of 3GCR-E compared to SC and CHX. The absence of an effect of SDD and SOD on eradication of CR-GNB in the respiratory tract might be explained by differences in micro organisms in the CR-GNB and 3GCR-E groups, as 55% of the CR-GNB in the respiratory tract were *Pseudomonas* spp. which may be less susceptible to topical antibiotics than *Enterobacteriaceae* representing the 3GCR-E group.

CHX mouthwash was not associated with decontamination of 3GCR-E or CR-GNB from the respiratory tract (or intestinal tract), compared to SC which included oral care with CHX 0.2% or 0.12% in most participating ICUs. Chlorhexidine gluconate is widely used as standard oral care in intensive care patients and has been associated with reduced occurrence of ventilator associated pneumonia (17), especially in patients undergoing cardiac surgery, but surprisingly also with increased mortality in meta-analyses (18, 19). There are no previous reports on

the effect of chlorhexidine mouthwash on eradication of MDR-GNB from the oropharynx or respiratory tract. In our study, CHX 2% mouthwash was associated with oral mucosal lesions (10). Serious adverse events were not reported after replacement with CHX 1% oral gel.

Strengths of this study are its sample size, a standardized surveillance protocol reducing information bias, and the presence of a valid control group of patients not receiving any intervention. Study limitations include the absence of confirmation of carbapenemase or ESBL-production in phenotypically resistant bacteria, as this was not routine practice in most participating microbiology laboratories. Naturally, screening cultures do not have optimal sensitivity, even though ESBL chromogenic agar was used in all study sites. However, local screening procedures did not change during the course of the study. In addition, acquisition of colonization with antibiotic resistant bacteria was not taken into account and this will be subject of future analyses. Furthermore, the duration of follow-up was limited to ICU-stay. In three other studies investigating the effects of topical antibiotics on carriage with MDR-GNB in ICU patients follow-up ranged from 2 to 7 weeks and carriage tended to reappear after initial decontamination (2, 3, 8). Nevertheless, even temporary suppression of colonization with HMRO during ICU-stay may be beneficial for individual patients and may reduce colonization pressure in ICU.

Conclusion

The current findings provide further evidence that SDD reduces rectal carriage with 3GCR-E and CR-GNB present during initial ICU stay, compared to SC. Oral decontamination with topical antibiotics, as in SOD and SDD, may shorten duration of initial colonization with 3GCR-E in the respiratory tract, although carriage with CR-GNB persisted. Chlorhexidine mouthwash had no effect on decontamination of 3GCR-E and CR-GNB.

Acknowledgements

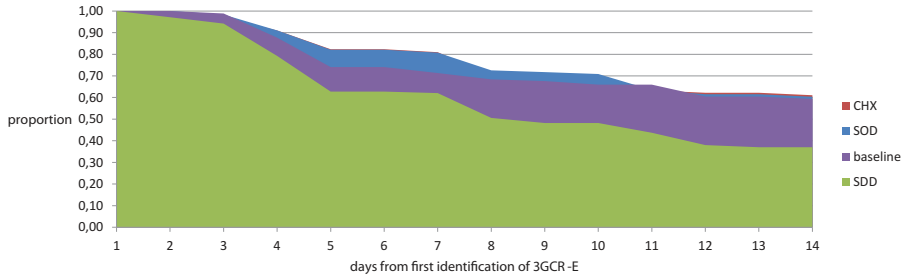
The authors thank the R-GNOSIS ICU-study group: Joaquin Lopez-Contreras MD/PhD, Hospital de Sant Pau-Universitat Autònoma de Barcelona; Prof. Pere Coll, Hospital de Sant Pau-Universitat Autònoma de Barcelona, Prof. Jordi Mancebo, Hospital de Sant Pau-Universitat Autònoma de Barcelona; Matt P Wise, MD/PhD, University Hospital of Wales, Cardiff; Matt PG Morgan, MD/PhD, University Hospital of Wales, Cardiff; Prof. Pieter Depuydt, Ghent University Hospital; Jerina Boelens, MD/PhD, Ghent University Hospital; Thierry Dugernier, MD/PhD, Clinique Saint Pierre, Ottignies; Valérie Verbelen, PhD, Clinique Saint Pierre, Ottignies; Prof. Philippe G Jorens, Antwerp University Hospital, University of Antwerp; Walter Verbrugghe, MD, Antwerp University Hospital, University of Antwerp; Prof. Surbhi Malhotra-Kumar, University of Antwerp; Prof. Pierre Damas, CHU Liège; Cécile Meex, PhD, CHU Liège; Kris Leleu, MD, AZ Sint Jan Bruges; Anne-Marie van den Abeele, MD, Saint-Lucas Hospital Ghent, Francisco Esteves, MD, Centro Hospitalar de Trás-os-Montes os Montes e Alto Douro, Vila Real; Ana Filipa Gomes Pimenta de Matos, Centro Hospitalar de Trás-os-Montes os Montes e Alto Douro, Vila Real; Prof. A. Torres, Hospital Clinic of Barcelona; Sara Fernández Méndez, MD, Hospital Clinic of Barcelona; Andrea Vergara Gomez, Msc, Hospital Clinic of Barcelona; Viktorija Tomic, MD/ PhD, University Clinic of Respiratory and Allergic Diseases, Golnik; Franc Sifrer, MD, University Clinic of Respiratory and Allergic Diseases Golnik, Esther Villarreal Tello, MD, Hospital Universitario La Fe, Valencia; Jesus Ruiz Ramos, PhD, Hospital Universitario La Fe, Valencia; Irene Aragao, MD, Hospital Santo Antonio - Centro Hospitalar do Porto (CHP); Claudia Santos, MD, Hospital Santo Antonio - Centro Hospitalar do Porto (CHP); Roberta HM Sperring; Msc, Azienda Ospedaliera San Camillo Forlanini, Rome, Patrizia Coppadoro, Azienda Ospedaliera San Camillo Forlanini, Rome; Giuseppe Nardi, MD, Ospedale Infermi RIMINI – AUSL della Romagna.

References

1. de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernards AT, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. *The Lancet Infectious diseases*. 2011;11(5):372-80.
2. Huttner B, Hausteiner T, Uckay I, Renzi G, Stewardson A, Schaeffer D, et al. Decolonization of intestinal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae with oral colistin and neomycin: a randomized, double-blind, placebo-controlled trial. *The Journal of antimicrobial chemotherapy*. 2013;68(10):2375-82.
3. Saidel-Odes L, Polachek H, Peled N, Riesenberk K, Schlaeffer F, Trabelsi Y, et al. A Randomized, Double-Blind, Placebo-Controlled Trial of Selective Digestive Decontamination Using Oral Gentamicin and Oral Polymyxin E for Eradication of Carbapenem-Resistant *Klebsiella pneumoniae* Carriage. *Infection control and hospital epidemiology*. 2012;33(1):14-9.
4. Oren I, Sprecher H, Finkelstein R, Hadad S, Neuberger A, Hussein K, et al. Eradication of carbapenem-resistant Enterobacteriaceae gastrointestinal colonization with nonabsorbable oral antibiotic treatment: A prospective controlled trial. *Am J Infect Control*. 2013;41(12):1167-72.
5. Brun-Buisson C, Legrand P, Rauss A, Richard C, Montravers F, Besbes M, et al. Intestinal decontamination for control of nosocomial multiresistant gram-negative bacilli. Study of an outbreak in an intensive care unit. *Annals of internal medicine*. 1989;110(11):873-81.
6. Lubbert C, Fauchoux S, Becker-Rux D, Laudi S, Durrbeck A, Busch T, et al. Rapid emergence of secondary resistance to gentamicin and colistin following selective digestive decontamination in patients with KPC-2-producing *Klebsiella pneumoniae*: a single-centre experience. *Int J Antimicrob Agents*. 2013;42(6):565-70.
7. Paterson DL, Singh N, Rihs JD, Squier C, Rihs BL, Muder RR. Control of an outbreak of infection due to extended-spectrum beta-lactamase-producing *Escherichia coli* in a liver transplantation unit. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2001;33(1):126-8.
8. Rieg S, Kupper MF, deWith K, Serr A, Bohnert JA, Kern WV. Intestinal decolonization of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBL): a retrospective observational study in patients at risk for infection and a brief review of the literature. *BMC infectious diseases*. 2015;15:475.
9. Taylor ME, Oppenheim BA. Selective decontamination of the gastrointestinal tract as an infection control measure. *The Journal of hospital infection*. 1991;17(4):271-8.
10. Plantinga NL, Wittekamp BH, Leleu K, Depuydt P, Van den Abeele AM, Brun-Buisson C, et al. Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU. *Intensive care medicine*. 2016;42(4):620-1.
11. WHO Guidelines Approved by the Guidelines Review Committee. WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care. Geneva: World Health Organization World Health Organization.; 2009.
12. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *Journal of chronic diseases*. 1987;40(5):373-83.
13. Frencken JF, Wittekamp BHJ, Plantinga NL, Spitoni C, van de Groep K, Cremer OL, et al. Associations Between Enteral Colonization With Gram-Negative Bacteria and Intensive Care Unit-Acquired Infections and Colonization of the Respiratory Tract. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2017.
14. Oostdijk EA, de Smet AM, Kesecioglu J, Bonten MJ. The role of intestinal colonization with gram-negative bacteria as a source for intensive care unit-acquired bacteremia. *Critical care medicine*. 2011;39(5):961-6.
15. Abecasis F, Sarginson RE, Kerr S, Taylor N, van Saene HK. Is selective digestive decontamination useful in controlling aerobic gram-negative bacilli producing extended spectrum beta-lactamases? *Microbial drug resistance*. 2011;17(1):17-23.
16. Oostdijk EA, de Smet AM, Kesecioglu J, Bonten MJ. Decontamination of cephalosporin-resistant Enterobacteriaceae during selective digestive tract decontamination in intensive care units. *The Journal of antimicrobial chemotherapy*. 2012;67(9):2250-3.

17. Hua F, Xie H, Worthington HV, Furness S, Zhang Q, Li C. Oral hygiene care for critically ill patients to prevent ventilator-associated pneumonia. *The Cochrane database of systematic reviews*. 2016;10:Cd008367.
18. Price R, MacLennan G, Glen J. Selective digestive or oropharyngeal decontamination and topical oropharyngeal chlorhexidine for prevention of death in general intensive care: systematic review and network meta-analysis. *BMJ (Clinical research ed)*. 2014;348:g2197.
19. Klompas M, Speck K, Howell MD, Greene LR, Berenholtz SM. Reappraisal of routine oral care with chlorhexidine gluconate for patients receiving mechanical ventilation: systematic review and meta-analysis. *JAMA internal medicine*. 2014;174(5):751-61.

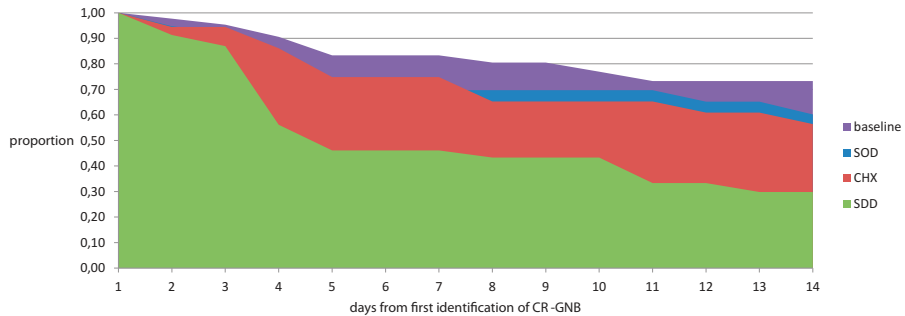
Supplement



Supplement figure S1. Proportion of third generation cephalosporin resistant Enterobacteriaceae* persisting in rectum samples during ICU stay

Abbreviations: CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; 3GCR-E: third generation cephalosporin resistant Enterobacteriaceae

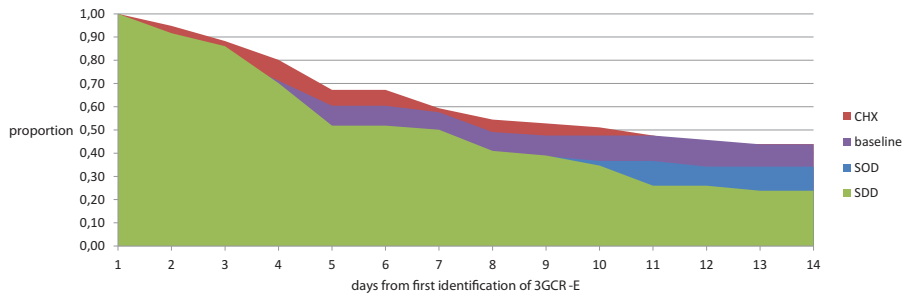
* identified during initial ICU stay



Supplement figure S2. Proportion of carbapenem resistant Gram-negative bacteria* persisting in rectum samples during ICU stay

Abbreviations: CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; CR-GNB: carbapenem resistant Gram-negative bacteria

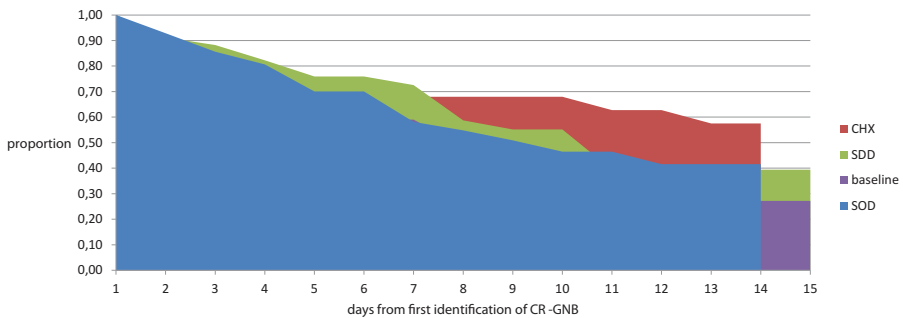
* identified during initial ICU stay



Supplement figure S3. Proportion of third generation cephalosporin resistant Enterobacteriaceae* persisting in respiratory tract samples during ICU stay

Abbreviations: CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; 3GCR-E: third generation cephalosporin resistant Enterobacteriaceae

* identified during initial ICU stay



Supplement figure S4. Proportion of carbapenem resistant Gram-negative bacteria* persisting in respiratory tract samples during ICU stay

Abbreviations: CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; CR-GNB: carbapenem resistant Gram-negative bacteria

* identified during initial ICU stay

Supplement table S1. Number of MDR-GNB during initial ICU-stay and proportion eradicated during ICU stay

	SC			CHX			SOD			SDD		
	eradicat		%	eradicat		%	eradicat		%	eradicat		%
	species	n		species	n		species	n		species	n	
Third generation cephalosporin resistant Enterobacteriaceae												
Rectum	177	76	43	168	69	41	180	74	41	177	105	59
Respiratory	79	49	62	77	49	64	75	52	69	72	53	74
Carbapenem resistant GNB												
Rectum	44	16	36	36	15	42	38	16	42	46	31	67
Respiratory	37	24	65	32	14	44	42	21	50	34	18	53

Abbreviations: MDR-GNB: multi-drug resistant Gram-negative bacteria; SC: standard care; CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; n: number, GNB: Gram-negative bacteria

Supplement table S2. Number of patients colonized with intrinsic colistin resistant GNB among patients with eradication of MDR-GNB

Study period	SC			CHX			SOD			SDD		
	eradicat		%	eradicat		%	eradicat		%	eradicat		%
	species	n		species	n		species	n		species	n	
Rectum	3GCR-E eradicated	76		69		74		105				
	colonized with intrinsic colistin R	4	5,3	4	5,8	1	1,4	6	5,7			
Respiratory	3GCR-E eradicated	49		49		52		53				
	colonized with intrinsic colistin R	7	14,3	11	22,4	7	13,5	5	9,4			

Abbreviations: GNB: Gram-negative bacteria; MDR-GNB: multidrug-resistant Gram-negative bacteria; SC: standard care; CHX: chlorhexidine mouthwash; SOD: selective oropharyngeal decontamination; SDD: selective digestive tract decontamination; n: number

Chapter 8

Colistin and tobramycin resistance during long-term use of selective decontamination strategies in the intensive care unit: a post-hoc analysis

Critical Care 2015; 19(1): 113

Bastiaan H. Wittekamp¹

Evelien A.N. Oostdijk^{2,3}

Anne Marie G.A. de Smet⁴

Marc J.M. Bonten^{1,3}

¹ Julius Center for Health Sciences and Primary care, University Medical Center Utrecht, The Netherlands

² Department of Intensive Care Medicine, University Medical Center Utrecht, The Netherlands

³ Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands

⁴ CAPE, Critical Care, Anesthesiology, Peri-operative and Emergency Medicine Research Program, University of Groningen, University Medical Center Groningen, The Netherlands

Abstract

Introduction: Selective decontamination of the digestive tract (SDD) and selective oropharyngeal decontamination (SOD) have been shown to improve intensive care unit (ICU) patients' outcomes. The aim of this study was to determine the effects of long-term use of SDD and SOD on colistin and tobramycin resistance among Gram-negative bacteria.

Methods: We performed a post-hoc analysis of two consecutive multicentre cluster-randomized trials with crossover of interventions. SDD and SOD were alternately but continuously used during 7 years in five Dutch ICUs participating in two consecutive cluster-randomized trials. In both trials, to measure colistin and tobramycin resistance among Gram-negative bacteria, rectal and respiratory samples were obtained monthly from all patients present in the ICU.

Results: The prevalence of tobramycin resistance in respiratory and rectal samples decreased significantly during long-term use of SOD and SDD (rectal samples risk ratio (RR) 0.35 (95%CI 0.23-0.53); respiratory samples RR 0.48 (95%CI 0.32-0.73), SDD compared to standard care). Colistin resistance in rectal and respiratory samples did not change (rectal samples RR 0.63 (95%CI 0.29-1.38); respiratory samples RR 1.26 (95%CI 0.35-4.57), SDD compared to standard care).

Conclusions: In this study, in a setting with low antimicrobial resistance rates, the prevalence of resistance against colistin and tobramycin among Gram-negative bacteria did not increase during a mean of 7 years of SDD or SOD use.

Introduction

Selective digestive tract decontamination (SDD) and selective oropharyngeal decontamination (SOD) aim to eradicate potential pathogenic microorganisms from the digestive tract to prevent infections in intensive care patients. The most commonly used SDD regimen consists of a non-absorbable antimicrobial mouth paste and gastro-enteral suspension containing colistin, tobramycin and amphotericin B. In addition, systemic broad-spectrum antibiotics are administered during the first four days in the intensive care unit (ICU). SOD consists of the mouth paste only. Both strategies have been associated with lower mortality, shortened length of stay in hospital and ICU, and less ICU-acquired infections such as bacteremia (1-3). Routine use of SDD and SOD has remained controversial, mainly because of the fear that (long-term) use will increase antibiotic resistance (4, 5). A recent systematic review and meta-analysis failed to demonstrate such an association, but also concluded that more evidence is needed regarding the long-term effects of SDD/SOD on ICU ecology (5). We, therefore, measured the prevalence of colistin and tobramycin resistance in five ICUs that have continuously been using SDD or SOD for 6 years or longer.

Methods

The effects of SOD and SDD were evaluated in a cluster-randomized cross-over study between 2004 and 2006 (study I). Each of 13 participating ICUs used SDD, SOD and standard care (no SDD/SOD), as unit-wide measures for 6 months, with the order of the three periods randomized per ICU. Methodological details and results of the study have been published previously (3). A second cluster-randomized cross-over study (study II) evaluated the effects of SOD and SDD (without standard care period) when applied as unit-wide interventions during 12 months in 16 Dutch ICUs between 2009 and 2013 (6). Five ICUs participated in both studies, and continued to use SDD as standard care in the interval between studies (2006 to 2009). These ICUs were contacted to verify that no changes in infection control strategies had taken place for the duration of both studies. For both studies, the need for informed consent was waived by the institutional review board.

During both studies, monthly point prevalence surveys were performed, in which rectal swabs and throat swabs or endotracheal aspirates (respiratory samples) were obtained from all patients present in the ICU on the day of the survey. This included patients who did not receive SDD or SOD at the time of the point prevalence survey. Where possible endotracheal aspirates were obtained, with throat swabs regarded as the best option in non-intubated patients.

Microbiology methods

Samples were plated on selective agar, including media containing polymyxin and tobramycin in local microbiology laboratories. Screening for colistin resistance was done using plates containing polymyxin B (5 mg/l) in study period I and polymyxin E (4 mg/l) in study period II. Cultures were analyzed semi-quantitatively for growth of Gram-negative bacteria. Minimum inhibitory concentration (MIC) values for colistin and tobramycin were determined using automated testing. EUCAST cutoff values were used to determine antibiotic resistance to colistin and tobramycin. Bacteria with susceptibility reported as intermediate (I) or resistant (R) were considered resistant. Species with intrinsic resistance to colistin, such as *Morganella*, *Citrobacter* and *Serratia* spp. were excluded from the analysis for colistin resistance. Additional information on the characteristics of the two studies, including microbiology methods used, can be found in the supplement, table S1.

Statistical analysis

Prevalence for colistin and tobramycin resistance were calculated separately per intervention period by dividing the number of patients with one or more resistant isolates per intervention period by the total number of patients included in the surveys of that intervention period. Patients could participate in multiple sequential surveys during one intervention period. The prevalence of antibiotic resistance is given as percentage with 95% confidence intervals (CI). Relative risks (RR) and 95% CI were calculated to compare the prevalence of resistance between the two study periods.

Results

The average duration of SDD/SOD use per ICU was 7.05 years (range 6.8 to 7.5 years), excluding the 6-month standard care period of study period I. A timeline of the two studies can be found in Figure 1. During study period I, 1,007 respiratory and 1,093 rectal samples were obtained from 1,189 patients in the five participating ICUs. During study period II, 1,755 respiratory and 1,808 rectal samples were obtained from 1,865 patients.

	Study I*				Interval	Study II*					
	2004		2006			2009		2013			
Hospital						Strategy (months)					
A		SOD	Control	SDD		SDD (44)	SOD	SDD			
B			SDD	SOD	Control	SDD (42)	SDD	SOD			
C			SOD	Control	SDD	SDD (44)		SDD	SOD		
D			SDD	Control	SOD	SDD (47)			SDD	SOD	
E	Control	SOD	SDD			SDD (51)				SOD	SDD

Figure 1. Timeline of the two consecutive studies and interval period

(*) In both studies interventions were separated by a one month wash-in wash-out period (not shown) (3, 6). The baseline period is the control period in which ICUs used standard care, not including SDD or SOD. All centers continued SDD in the interval period (yellow). The end of the intervention period marks the end of the study.

Abbreviations: ICU, intensive care unit; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

The prevalence for colistin resistance in rectal samples ranged from 1.2% (during SOD) to 2.8% (during SDD) in study period I, and were 1.1% and 1.7% during SOD and SDD, respectively in study period II (Figure 2). In respiratory tract samples, the prevalence for colistin resistance ranged from 0.88% (standard care) to 2.1% (SDD) in study period I and were 1.1% and 0.6% during SDD and SOD in study period II (Figure 3). There were no statistically significant differences between study periods or between intervention periods, except for a significant decrease in colistin resistance in rectal samples during the SOD period of study period II compared to the standard care period of study period I (RR 0.41 (95%CI 0.17-0.98)) (Table 1).

The prevalence of tobramycin resistance in rectal samples in study period I was lowest during SDD (6.6%) (Figure 4). Relative risks were 0.54 (95%CI 0.34-0.87) and 0.46 (95%CI 0.29-0.72), for SDD I and SOD I, compared to standard care, respectively.

In study period II, the prevalence was 4.2% during SDD (RR 0.64 (95%CI 0.40-1.04), as compared to SDD in study period I) and 8% during SOD (RR 0.56 (95%CI 0.39-0.78) as compared to SOD in study period I) (Table 1). The prevalence for tobramycin resistance in respiratory samples during SDD in study period I was (6.7%) (Figure 5), which was lower than during standard care and SOD (RR 0.61 (95%CI 0.38-1.00) and 0.71 (95%CI 0.42-1.18), respectively). In study period II, the prevalence was 5.3% during SDD (RR 0.78 (95%CI 0.49-1.25) as compared to SDD in study period I) and 4.5% during SOD (RR 0.48 (95%CI 0.30-0.76), as compared to SOD in study period I) (Table 1 and Figure 5).

Table 1. Relative risk of colistin and tobramycin resistance

	Relative risk (95% CI)			
	SDDII vs standard care	SDD II vs I	SOD II vs standard care	SOD II vs I
Colistin				
Rectum	0.63 (0.29-1.38)	0,62 (0,29 - 1,33)	0.41 (0.17-0.98)	0,94 (0,30 - 2,97)
Respiratory tract	1.26 (0.35-4.57)	0,52 (0,21 - 1,31)	0.66 (0.16-2.73)	0,34 (0,10 - 1,18)
Tobramycin				
Rectum	0.35 (0.23-0.53)	0,64 (0,40 - 1,04)	0.66 (0.47-0.95)	0,56 (0,39 - 0,78)
Respiratory tract	0.48 (0.32-0.73)	0,78 (0,49 - 1,25)	0.42 (0.27-0.64)	0,48 (0,30 - 0,76)

Abbreviations: CI, confidence interval; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

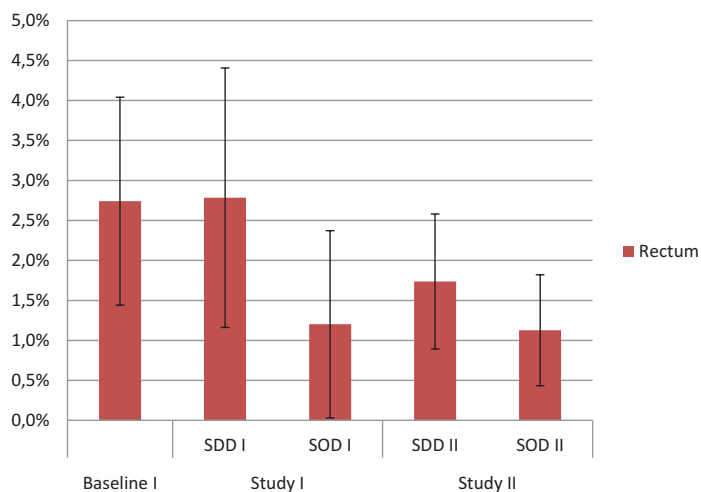


Figure 2. Colistin resistance in rectal samples

Prevalence of Gram-negative bacteria with intermediate susceptibility (I) or resistant (R) to colistin in rectal samples obtained during study period 1 and 2 respectively.

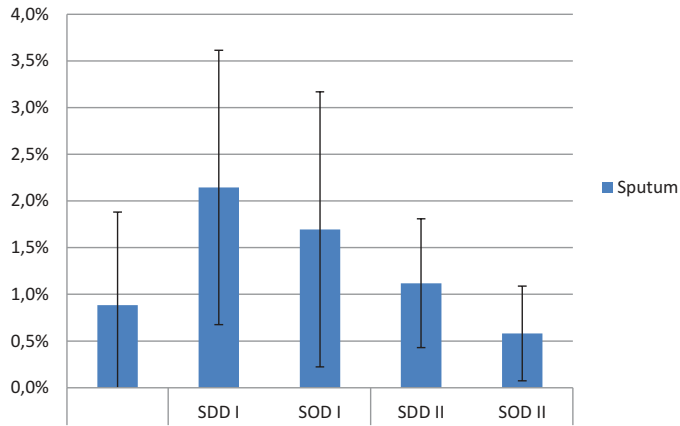


Figure 3. Colistin resistance in respiratory samples

Prevalence of Gram-negative bacteria with intermediate susceptibility (I) or resistant (R) to colistin in respiratory samples obtained during study period 1 and 2 respectively.

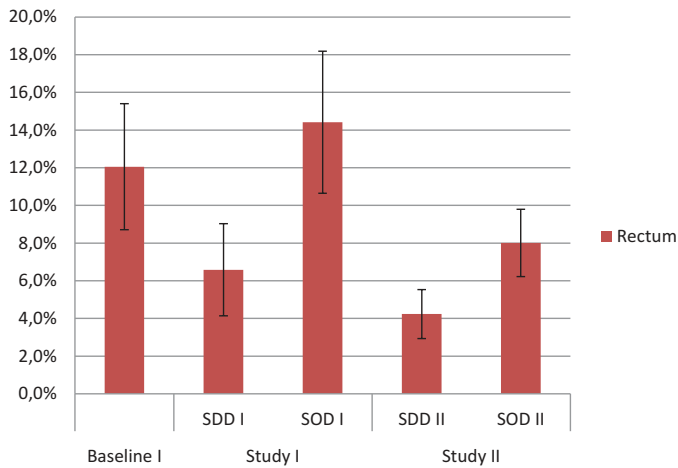


Figure 4. Tobramycin resistance in rectal samples

Prevalence of Gram-negative bacteria with intermediate susceptibility (I) or resistant (R) to tobramycin in rectal samples obtained during study period 1 and 2 respectively.

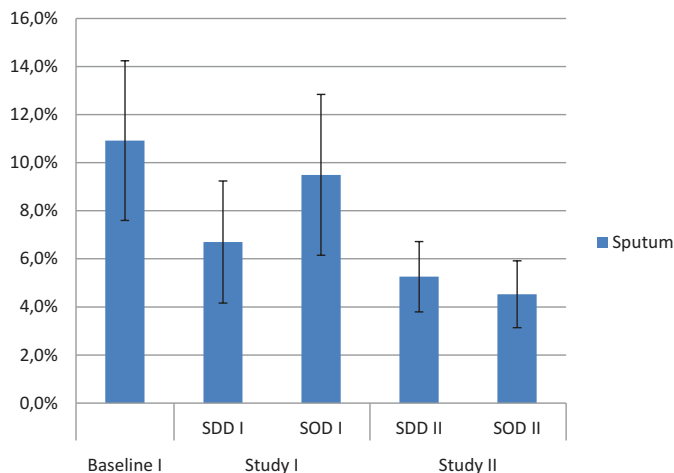


Figure 5. Tobramycin resistance in respiratory samples

Prevalence of Gram-negative bacteria with intermediate susceptibility (I) or resistant (R) to tobramycin in respiratory samples obtained during study period 1 and 2 respectively.

As compared to the standard care period in study period I, the average point prevalence of tobramycin resistance in rectum samples had declined in study period II; from 12.1% in the standard care period of study period I to 4.2% during SDD and 8% during SOD in rectal swabs (RR 0.35 (95%CI 0.23-0.53) and 0.66 (95%CI 0.47-0.95), respectively), and from 10.9% in the standard care period of study period I to 5.3% during SDD (RR 0.48 (95%CI 0.32-0.73) and 4.5% during SOD in respiratory tract samples (RR 0.42 (95%CI 0.27-0.64)) (Table 1).

The identified species and their counts in each study period are available in the supplement, table S2.

Discussion

In this longitudinal ecological study, spanning a period of 7 years, we found no evidence of increasing resistance to colistin in ICUs using SDD and SOD. Moreover, resistance to tobramycin among Gram-negative bacteria was lower after several years of SDD and SOD.

These findings provide further evidence on the ecological effects of SDD and SOD in settings with low levels of antibiotic resistance, which was previously documented during short-term use (3, 6).

The current results support previous findings obtained from two longitudinal studies in Germany and Spain using clinical culture results and surveillance cultures, respectively (7, 8). In a French retrospective study, spanning 6 years, carriage of antibiotic-resistant bacteria based on clinical culture results was compared for individual patients receiving or not receiving SDD, yielding no changes in resistance among Gram-negative bacteria (9). In 17 Dutch ICUs that continuously used SDD or SOD during 4 years of follow-up, there was no increase in resistance against colistin and tobramycin among Gram-negative bacteria, while resistance against third-generation cephalosporins and ciprofloxacin decreased during the follow-up period (10).

The current results are based on point prevalence samples obtained from all patients present in the ICU on a predefined moment, also including patients not directly exposed to SDD or SOD, thus reflecting the ICU ecology. If SDD or SOD would directly cause antibiotic resistance in exposed patients, inclusion of non-exposed patients would dilute this effect, creating a bias towards null. However, in a previous analysis of patients receiving SDD or SOD during study period I antibiotic resistance was lower than during standard care (11).

Results of the second cluster-randomized cross-over study, performed in 16 ICUs yielded a 7% and 4% monthly increase in the prevalence of aminoglycoside-resistant Gram-negative bacteria in rectal samples during 12 months of SDD and 12 months of SOD, respectively (6). In addition, SDD was associated with an increase in aminoglycoside resistance genes in the non-culturable intestinal flora in some patients (12). These findings are in contrast with the current findings, which might be related to differences in the duration of follow-up (7 years versus 24 months), study population (5 ICUs versus 16 ICUs), and detection methods (conventional microbiology versus metagenomics approaches). Careful monitoring of aminoglycoside resistance should, therefore, be performed during SDD or SOD.

Clonal spread of colistin-resistant extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* has been described after introduction of SDD

during an outbreak that could not be controlled with classical infection control measures (13). That situation markedly differed from the non-outbreak study settings. In the Netherlands, the prevalence of multidrug resistance among both Gram-negative and Gram-positive bacteria in general is low (6, 10, 11).

This study has some limitations. Adjustment for secular trends of antibiotic resistance was not possible, since there was no data from ICUs that did not use SDD or SOD. Yet, in an analysis of trends of resistance against aminoglycosides and colistin among Enterobacteriaceae between 2008 and 2012 in 13 Dutch hospitals in which no SDD or SOD was used, a significant change could not be demonstrated (10).

Furthermore, distributions of MIC values were lacking in the current study. Changes in case mix on the ICUs and implementation of other interventions (that is infection control measures) that influence the prevalence of antibiotic resistance could have occurred during the interval between the two studies, although based on reports of the participating hospitals we have no indication that either of these took place.

Analysis of third-generation cephalosporin resistance was not performed as different selective culture media were used for screening in both study periods. Moreover, polymyxin B (5 mg/l) and polymyxin E (4 mg/l) were used for screening in study periods I and II, respectively, but it is unlikely that this had consequences for our findings, since there is complete cross-resistance between colistin and polymyxin B (13).

Conclusion

This study did not find an increase in the prevalence of resistance against colistin and tobramycin among Gram-negative isolates during a mean of 7 years of SDD or SOD use. The effect of SDD and SOD in settings with higher levels of antibiotic resistance than the Netherlands remains to be determined.

Acknowledgements

The authors wish to acknowledge the ICUs and microbiologists for their participation in the trials that contributed data and their assistance in checking the manuscript for any inconsistencies.

References

1. Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ, et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. *American journal of respiratory and critical care medicine*. 2002;166(8):1029-37.
2. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomized controlled trial. *Lancet*. 2003;362(9389):1011-6.
3. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *N Engl J Med*. 2009;360(1):20-31.
4. Bastin AJ, Ryanna KB. Use of selective decontamination of the digestive tract in United Kingdom intensive care units. *Anaesthesia*. 2009;64(1):46-9.
5. Daneman N, Sarwar S, Fowler RA, Cuthbertson BH. Effect of selective decontamination on antimicrobial resistance in intensive care units: a systematic review and meta-analysis. *The Lancet Infectious diseases*. 2013;13(4):328-41.
6. Oostdijk EAN, Kesecioglu J, Schultz MJ, Visser CE, de Jonge E, van Essen EHR, et al. Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. *JAMA*. 2014;312(14):1429-1437.
7. Heininger A, Meyer E, Schwab F, Marschal M, Unertl K, Krueger WA. Effects of long-term routine use of selective digestive decontamination on antimicrobial resistance. *Intensive care medicine*. 2006;32(10):1569-76.
8. Ochoa-Ardila ME, Garcia-Canas A, Gomez-Mediavilla K, Gonzalez-Torralba A, Alia I, Garcia-Hierro P, et al. Long-term use of selective decontamination of the digestive tract does not increase antibiotic resistance: a 5-year prospective cohort study. *Intensive care medicine*. 2011;37(9):1458-65.
9. Leone M, Albanese J, Antonini F, Nguyen-Michel A, Martin C. Long-term (6-year) effect of selective digestive decontamination on antimicrobial resistance in intensive care, multiple-trauma patients. *Crit Care Med*. 2003;31(8):2090-5.
10. Houben AJ, Oostdijk EA, van der Voort PH, Monen JC, Bonten MJ, van der Bij AK. Selective decontamination of the oropharynx and the digestive tract, and antimicrobial resistance: a 4 year ecological study in 38 intensive care units in the Netherlands. *The Journal of antimicrobial chemotherapy*. 2014;69(3):797-804.
11. de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernards AT, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomized, crossover study. *The Lancet Infectious diseases*. 2011;11(5):372-80.
12. Buelow E, Gonzalez TB, Versluis D, Oostdijk EA, Ogilvie LA, van Mourik MS, et al. Effects of selective digestive decontamination (SDD) on the gut resistome. *The Journal of antimicrobial chemotherapy*. 2014;69(8):2215-23.
13. Halaby T, Al Naiemi N, Kluytmans J, van der Palen J, Vandenbroucke-Grauls CM. Emergence of colistin resistance in Enterobacteriaceae after the introduction of selective digestive tract decontamination in an intensive care unit. *Antimicrobial agents and chemotherapy*. 2013;57(7):3224-9.

Supplement material

Table S1. Characteristics of the two studies

	Study I ^(B)	Study II ⁽⁶⁾
Years in which inclusion took place	2004-2006	2009-2013
Participating hospitals (n)	13	16
Patients included (n)	5939	11997
Duration of intervention		
SDD	6 months	12 months
SOD	6 months	12 months
Standard care	6 months	NA
Inclusion criteria to receive SDD/ SOD	Mechanical ventilation > 48 hours and/or Length of stay > 72 hours	Mechanical ventilation > 48 hours
Antibiotics used in SDD/SOD		
Tobramycin	Mouthpaste: 2% concentration Suspension: 80mg*	<i>idem</i>
Colistin	Mouthpaste: 2% concentration Suspension: 100mg*	<i>idem</i>
Amphotericin B	Mouthpaste: 2% concentration Suspension: 500mg*	<i>idem</i>
Systemic prophylaxis	Cefotaxim 4000mg daily*	<i>idem</i>
Methods		
Point prevalence surveys available (n)	18	24
Culture media used for screening in point prevalence surveys	McConkey with - polymyxin B 50iu/ml (5mg/l) - tobramycin 8mg/l - cefotaxim 8mg/l - ciprofloxacin 2mg/l	McConkey with - polymyxin E (colistin) 4mg/l - tobramycin 8mg/l ESBL chromogenic agar VRE chromogenic agar

* only during SDD

Abbreviations: SDD: selective digestive tract decontamination; SOD: selective oropharyngeal decontamination; NA: not applicable; ESBL: extended spectrum beta-lactamase; VRE vancomycin resistant enterococcus.

Table S2. Count of resistant isolates of species during each study period

		Species	Study period				
			Baseline	SDD I	SOD I	SDD II	SOD II
Colistin resistant							
rectum	Enterobacter spp.	0	2	1	3	6	
	Escherichia coli	3	2	2	3	2	
	Klebsiella spp.	5	3	1	10	3	
	Pseudomonas spp.	1	0	0	0	0	
	Proteus spp.*	15	1	6	0	1	
	Serratia spp.*	3	6	6	0	0	
	Stenotrophomonas spp.*	5	2	3	0	0	
	Morganella spp.*	7	9	26	0	0	
	Hafnia spp.*	0	8	0	0	0	
respiratory tract	Enterobacter spp.	0	4	2	1	3	
	Escherichia coli	0	0	0	2	0	
	Klebsiella spp.	0	1	0	8	2	
	Pseudomonas spp.	2	0	0	0	0	
	Proteus spp.*	6	0	4	0	0	
	Serratia spp.*	18	8	12	0	0	
	Stenotrophomonas spp.*	13	2	7	0	0	
	Morganella spp.*	3	2	2	0	0	
	Hafnia spp.*	0	1	1	0	0	
	Gram negative rods NOS	1	1	3	0	0	
Gram-negative rods - non fermenter NOS	0	2	0	0	0		
Tobramycin resistant							
rectum	Enterobacter spp.	8	1	6	4	15	
	Escherichia coli	18	3	18	17	43	
	Klebsiella spp.	12	2	6	20	13	
	Pseudomonas spp.	6	5	3	2	10	
	Proteus spp.	1	0	1	4	5	
	Serratia spp.	0	4	3	1	1	
	Stenotrophomonas spp.	5	3	3	0	0	
	Morganella spp.	0	1	2	1	4	
	Hafnia spp.	0	1	0	0	0	
	Citrobacter spp.	6	1	11	1	7	
	Achromobacter spp.	0	0	3	0	0	
Gram-negative rods NOS	0	2	2	0	0		
respiratory tract	Enterobacter spp.	6	2	3	3	2	
	Escherichia coli	5	2	2	2	6	
	Klebsiella spp.	7	1	2	10	3	
	Pseudomonas spp.	9	3	2	9	8	
	Serratia spp.	4	3	5	6	8	
	Stenotrophomonas spp.	16	9	8	0	0	
	Citrobacter spp.	0	0	0	0	1	
	Achromobacter spp.	0	2	2	0	0	
	Acinetobacter spp.	1	1	0	0	0	
	Burkholderia spp.	1	0	1	0	0	
	Gram-negative rods NOS	0	4	0	0	0	

Abbreviations: spp.: species; NOS: non otherwise specified.

Chapter 9

General discussion

Bastiaan H.J. Wittekamp

Introduction

The aim of this thesis is to compare the effectiveness and ecological safety of three decontamination strategies, SDD, SOD and chlorhexidine (CHX) mouthwash, for ICU patients in European countries with higher endemicity of antibiotic resistance than the Netherlands.

Effectiveness of SDD and SOD

This thesis confirms the hypothesis that Gram-negative colonization of the gut and respiratory tract increases the risk for infections (chapter 2). In the cluster randomized study among 13 ICUs in 6 European countries with high levels of antimicrobial resistance (chapter 5) there was no effect of decontamination strategies CHX mouthwash, SDD and SOD on survival of ICU patients and a statistically non-significant trend towards a reduction of ICU-acquired bacteremia (Cause specific hazard rate (CSHR) 0.79, 95% confidence interval (CI) (0.60-1.05)) during SDD, compared to standard care. This is in contrast with previous cluster-randomized studies performed in the Netherlands, in which SDD and SOD were more effective than standard care in reducing both mortality and the incidence of bacteremia acquired in the ICU and in which SDD was more effective than SOD in reducing both mortality and the incidence of bacteremia acquired in the ICU (1, 2).

Differences between the previous Dutch and current European study have been described in the discussion of chapter 5. A possible explanation for the difference in findings is that less optimal decontamination might have been achieved in the current European study due to differences in the decontamination regimens (Table 1).

In the current cluster study the proportion of positive rectal surveillance cultures was lower during SDD, compared to SOD, CHX and the baseline period (Figure 1), but direct comparison of the efficacy of decontamination between the previous Dutch studies and current study is hampered by differences in culturing methods for respiratory and rectal surveillance samples, including non-selective in the former and selective (ESBL) culture media in the current study.

Table 1. Differences between the European cluster study and previous Dutch studies

	European cluster study (chapter 5)	Previous Dutch studies (1-3)
Intensification of SDD regimen upon persistent colonization	No	Increasing frequency of SDD administration / initiation of antibiotic nebulization
Duration of decontamination treatment	Start of mechanical ventilation until extubation	ICU admission until discharge
Systemic antibiotics as part of SDD	None	Third generation cephalosporin during the first 4 days of ICU admission
Hand hygiene protocol and chlorhexidine 2% bodywash for all patients in ICU during all study periods (including baseline)	Yes	No

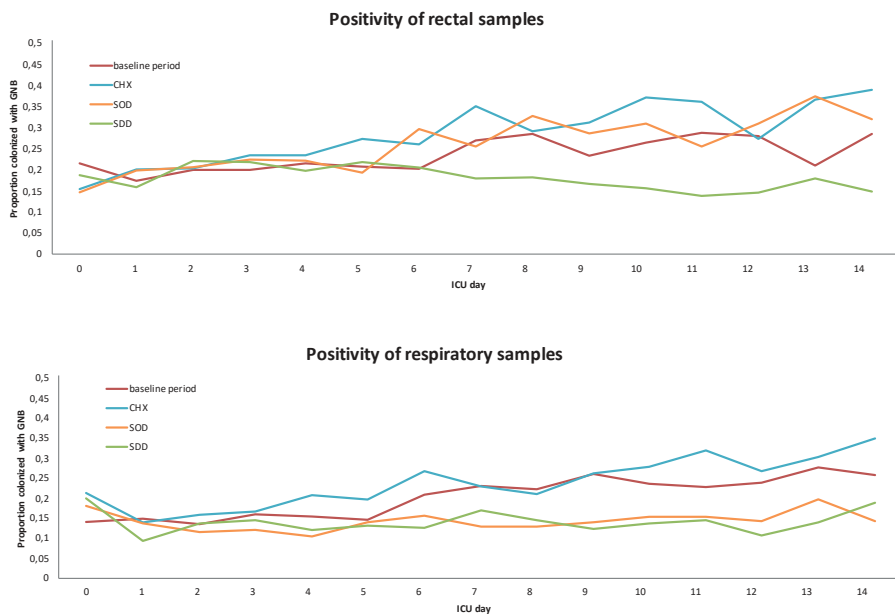
Possibly, intensification of the SDD and SOD regimen upon persistent colonization and treatment until ICU discharge instead of until extubation might have led to more effective decontamination and thus larger preventive effects in the Dutch study, although it is unlikely that treatment with cephalosporins and decontamination until ICU discharge would have led to an improved outcome, as was shown with a sensitivity analysis in chapter 5 (discussion section).

Furthermore, there were differences between the participating ICUs in trends of effectiveness of decontamination strategies in preventing mortality and bacteremia. In some ICUs SDD was associated with a trend towards a protective effect for in-ICU and in-hospital mortality, while in others there was a trend in the opposite direction. This may result from heterogeneity in ICU characteristics, caused by the pragmatic nature of the study and the ICU selection criteria that were based on the (high) prevalence of antimicrobial resistance only. Also, eradication of Gram-negative bacteria from the gut or respiratory tract may have been more effective in some ICUs than others.

In addition, there were differences between the current European and the former Dutch studies in study population, as patient inclusion criteria from the current study were less strict with the aim to include all ICU patients receiving mechanical ventilation for 24 hours or more, whereas previous studies included long-stay ICU patients, regardless of ventilation status. This resulted in a slightly more severely ill study population in the current cluster study (mean APACHE II score 19.8-21.8

in the current, versus 18.6-19.6 in the Dutch cluster study by De Smet et al. (2)). However, more severely ill patients are expected to benefit at least as much from decontamination strategies as patients with a lower disease severity score, as the risk of bacteremia and infection would be expected to be higher in more severely ill patients. Also, the length of stay in ICU in the current study was longer than in previous studies (median 10-11 days in the current study, versus median 9 days in the previous Dutch study), while the median duration of mechanical ventilation was shorter (6-7 days in the current, versus 7-8 days in the previous Dutch study, respectively). As decontamination was stopped at the end of mechanical ventilation in the current study, patients remained unexposed to decontamination for a median of 2 days until ICU discharge which may have increased the risk for recolonization.

Figure 1. Proportion of surveillance samples positive for Gram-negative bacteria on day 1 – day 14 of ICU-admission



The pragmatic nature of the study and the current results suggest that in ICUs with higher levels of antimicrobial resistance than the Netherlands decontamination with SDD and SOD and CHX mouthwash as applied according to the current

protocol are not effective in reducing mortality among ventilated ICU patients. SDD may reduce the occurrence of ICU-acquired bacteremia, compared to standard care without decontamination, although the latter effect was not statistically significant and less pronounced in the current study than in the previous Dutch studies.

Ecological safety of SDD and SOD

During SDD and SOD in the current European study the prevalence of carriage with antimicrobial resistant Gram-negative bacteria remained stable or tended to decrease and the prevalence of VRE and MRSA carriage remained stable, as measured during monthly point prevalence cultures among all patients in the ICU, confirming previous findings from the Dutch setting (2, 4).

A limitation of the current study is the surveillance method for colistin resistance in monthly point prevalence measures. During the study it became apparent that the colistin E-test may lead to 'false-susceptible' test results (i.e., a low sensitivity) (5). As the colistin E-test was used as primary testing method this may have resulted in underreporting of colistin resistance. Nevertheless, in *three*-monthly point prevalence measures a random selection of Gram-negative bacteria were tested with an automated testing method (BD Phoenix, BD) and resistance to colistin remained rare, with the prevalence ranging from 1.1% in the rectum during the baseline period to 2.3% during SDD. The prevalence in respiratory samples was lower ranging from 0.2% (baseline) to 0.9% (SOD) (chapter 5, supplement table S11). Based on these findings, SDD and SOD do not seem to promote antimicrobial resistance in settings with higher levels of antimicrobial resistance than the Netherlands.

The finding that SDD and SOD did not lead to increased resistance to tobramycin and colistin in 5 Dutch ICUs with relatively low levels of antimicrobial resistance, even after 7 years of use (chapter 8), is in line with previous studies (6-9), and requires further confirmation for settings with higher levels of antimicrobial resistance.

Two outbreaks of highly resistant micro organisms (HRMO) occurred during the cluster study in two participating ICUs, both during the SOD period. In one ICU the hospital infection control committee ordered to stop the administration of SOD after an increase in the prevalence of carbapenem resistant Enterobacteriaceae

was noted, which later appeared to reflect a hospital-wide increase, including wards not directly related to the ICU. In the other ICU the outbreak was caused by clonal expansion of a colistin resistant strain of *Klebsiella pneumoniae*. To explore the effects of the decontamination regimens on the occurrence of cross-transmission, whole genome sequencing (WGS) data will become available for selected resistant *Klebsiella* and *E. coli* isolates that were identified in surveillance cultures throughout all study periods in the cluster study. However, it will be impossible to disentangle the effects of the interruption of SOD that was initiated simultaneously with outbreak control measures in clonal expansion (i.e., cross transmission) in the outbreak settings.

SDD and SOD were associated with a higher rate of decontamination of third generation cephalosporin resistant Enterobacteriaceae (3GCR-E) from the respiratory tract during treatment with SDD and SOD and 3GCR-E and Carbapenem resistant Gram-negative bacteria (CR-GNB) from the rectum during treatment with SDD (chapter 7). Eradication of these HRMO during ICU stay with SDD (for the lower gastro-intestinal tract and respiratory tract) and SOD (for the respiratory tract) could protect patients against HRMO infections, related morbidity and prevent cross-transmission of these HRMO. However, this did not translate in a reduction in ICU-acquired bacteremia with HRMO during SDD and SOD (CSHR 0.77 (95%CI 0.38-1.52) and CSHR 0.83 (95%CI 0.46-1.51) for SDD and SOD compared to baseline, respectively) (chapter 5), in contrast to the previous Dutch studies in which a significant effect on bacteremia with HRMO was present (1). Apart from differences in study protocols and the ecological setting mentioned above, the observed eradication of 3GCR-E and CR-GNB may have been insufficient or too late to prevent translocation in some patients or may have been counterbalanced by acquisition of new HRMO species in these patients later during ICU stay. Additional analyses will explore the effects of the three decontamination strategies on acquisition of 3GCR-E and CR-GNB during ICU stay.

Effectiveness and safety of chlorhexidine mouthwash

The position of chlorhexidine as decontamination strategy appears to have changed with two recent meta-analyses reporting that chlorhexidine reduced the incidence of lower respiratory tract infections among cardiac surgery patients, as opposed to other ICU patients, and that CHX mouthwash may be associated with increased mortality (10, 11). The increase in mortality (odds ratio 1.25, 95% CI 1.05

to 1.50) became first apparent in a meta-analysis of 11 studies with a total of 1,288 patients treated with chlorhexidine with concentrations up to 0.2%, although only one of these trials showed a statistically significant increase in mortality and none had mortality as a primary endpoint (10).

In the current study, decontamination with CHX mouthwash, partially performed with chlorhexidine with a concentration of 2% and predominantly with a 1% concentration, did not lead to a statistically significant increase or decrease in mortality. However, it is important to note that 11 of 13 participating ICUs used a lower concentration of CHX mouthwash (0.12% or 0.2%) during the baseline period, as this was part of standard care before the start of the study and it was deemed unethical to abandon this strategy for study purposes. This might have led to an underestimation of the effects of CHX 1% during the intervention period. As a consequence, we cannot rule out that CHX mouthwash with a concentration of 0.12% or higher may have a detrimental effect on the survival of ICU patients, although the mechanism underlying these findings would remain unclear (11). Result from the current study show that, compared to CHX 0.12 % and 0.2%, oropharyngeal decontamination with CHX concentrations up to 2% do not lead to an increase in mortality among ventilated ICU patients.

CHX 1-2% mouthwash did not reduce the incidence of ICU-acquired bacteremia compared to lower concentrations in the current study. In previous studies decontamination of the respiratory tract with CHX reduced the incidence of ventilator associated pneumonia (VAP) (12). Decontamination could prevent translocation of bacteria from the respiratory tract to other body sites (chapter 2). However, the proportion of respiratory surveillance samples yielding growth during ICU stay with CHX mouthwashes was comparable to standard care and was higher than during SDD and SOD (figure 1), suggesting that decontamination of the (upper) respiratory tract was not achieved with CHX mouthwash, or at least suboptimal.

Moreover, during CHX 2% side effects occurred in 10% of patients mainly consisting of oral mucosal lesions which disappeared after cessation of CHX mouthwash (chapter 6) (13). Similar side effects were reported in one other study (14) and these appeared to be related with rubbing of the CHX 2% solution during application. Although rubbing was not part of the current protocol, side effects did occur, suggesting that the CHX 2% concentration leads to mucosal irritation

regardless of the method of application and should therefore not be used in ICU patients for oropharyngeal decontamination.

The future of SDD, SOD and CHX mouthwash

The most recent Dutch cluster-randomized cross-over study revealed that SDD was superior to SOD in preventing bacteremia and improving survival (1). SDD will, therefore, replace SOD as preferred decontaminating strategy, at least in the Netherlands. For other countries, there is no evidence that SDD or SOD improve ICU patients outcome in terms of survival or occurrence of bacteremia.

Beneficial effects on patient level outcomes, such as survival and bacteremia, would be a prerequisite for implementation of SDD and SOD outside the Netherlands, but even if these effects would be shown, there are other relevant aspects such as cost-effectiveness that require further exploration, especially in ICUs where resources are more scarce than in the Netherlands.

During the current study in 13 ICUs outside the Netherlands there was no increase in the prevalence of antimicrobial resistance among Gram-negative bacteria, nor in the prevalence of MRSA and VRE. This leaves the opportunity for new studies, possibly with a different SDD regimen including intensification of the SDD protocol upon persistent colonization. Systemic cephalosporins that are used as part of SDD in Dutch ICUs during the first days of ICU stay are not appropriate in settings with high(er) prevalence of cephalosporin resistance. There was no increase in colistin resistance during SDD or in the current study, although the use of the colistin E-test may have led to underreporting of colistin resistance. Future studies should therefore use broth microdilution methods for colistin susceptibility testing, as this is currently considered the most reliable testing method (5).

Furthermore, the occurrence of outbreaks with micro-organisms resistant to antimicrobial agents and the increasing trends of antimicrobial resistance over time are likely to continue to feed the discussion on ecological safety of SDD. For example, the finding of new resistance mechanisms against colistin, which is considered a last resort antibiotic to treat carbapenem resistant Gram-negative bacteria, may lead to reconsiderations of prophylactic strategies using colistin, such as SDD and SOD, even if these may not be causally related to colistin resistance. In fact, the current study was not allowed in ICUs in France by the French regulatory

authorities because of the prophylactic use of colistin, despite the lack of evidence that SDD or SOD are associated with colistin resistance.

In the current study there was no beneficial effect of CHX 1% mouthwash on survival or the occurrence of bacteremia among mechanically ventilated ICU patients, compared to lower concentrations of CHX. As concentrations higher than 1% were associated with side-effects and meta-analyses suggest an increase in mortality associated with concentrations up to 0.2%, there is no evidence to support the use of CHX mouthwash with a concentration of 1% (or higher) in the general (surgical and medical) ICU population.

The results of this thesis are therefore not likely to lead to further implementation of SDD, SOD or CHX 1% mouthwash in settings with higher antimicrobial resistance rates than the Netherlands.

References

1. Oostdijk EAN, Kesecioglu J, Schultz MJ, Visser CE, de Jonge E, van Essen EHR, et al. Notice of Retraction and Replacement: Oostdijk et al. Effects of Decontamination of the Oropharynx and Intestinal Tract on Antibiotic Resistance in ICUs: A Randomized Clinical Trial. *JAMA*. 2014;312(14):1429-1437. *Jama*. 2017;317(15):1583-4.
2. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *The New England journal of medicine*. 2009;360(1):20-31.
3. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet (London, England)*. 2003;362(9389):1011-6.
4. de Smet AM, Kluytmans JA, Blok HE, Mascini EM, Benus RF, Bernards AT, et al. Selective digestive tract decontamination and selective oropharyngeal decontamination and antibiotic resistance in patients in intensive-care units: an open-label, clustered group-randomised, crossover study. *The Lancet Infectious diseases*. 2011;11(5):372-80.
5. Matuschek E, Ahman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin - evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2017.
6. Heininger A, Meyer E, Schwab F, Marschal M, Unertl K, Krueger WA. Effects of long-term routine use of selective digestive decontamination on antimicrobial resistance. *Intensive care medicine*. 2006;32(10):1569-76.
7. Ochoa-Ardila ME, Garcia-Canas A, Gomez-Mediavilla K, Gonzalez-Torrallba A, Alia I, Garcia-Hierro P, et al. Long-term use of selective decontamination of the digestive tract does not increase antibiotic resistance: a 5-year prospective cohort study. *Intensive care medicine*. 2011;37(9):1458-65.
8. Houben AJ, Oostdijk EA, van der Voort PH, Monen JC, Bonten MJ, van der Bij AK. Selective decontamination of the oropharynx and the digestive tract, and antimicrobial resistance: a 4 year ecological study in 38 intensive care units in the Netherlands. *The Journal of antimicrobial chemotherapy*. 2014;69(3):797-804.
9. Leone M, Albanese J, Antonini F, Nguyen-Michel A, Martin C. Long-term (6-year) effect of selective digestive decontamination on antimicrobial resistance in intensive care, multiple-trauma patients. *Critical care medicine*. 2003;31(8):2090-5.
10. Price R, MacLennan G, Glen J. Selective digestive or oropharyngeal decontamination and topical oropharyngeal chlorhexidine for prevention of death in general intensive care: systematic review and network meta-analysis. *BMJ (Clinical research ed)*. 2014;348:g2197.
11. Klompas M, Speck K, Howell MD, Greene LR, Berenholtz SM. Reappraisal of routine oral care with chlorhexidine gluconate for patients receiving mechanical ventilation: systematic review and meta-analysis. *JAMA internal medicine*. 2014;174(5):751-61.
12. Liberati A, D'Amico R, Pifferi S, Torri V, Brazzi L, Parmelli E. Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care. *The Cochrane database of systematic reviews*. 2009(4):Cd000022.
13. Plantinga NL, Wittekamp BH, Leleu K, Depuydt P, Van den Abeele AM, Brun-Buisson C, et al. Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU. *Intensive care medicine*. 2016;42(4):620-1.
14. Tantipong H, Morkchareonpong C, Jaiyindee S, Thamlikitkul V. Randomized controlled trial and meta-analysis of oral decontamination with 2% chlorhexidine solution for the prevention of ventilator-associated pneumonia. *Infection control and hospital epidemiology*. 2008;29(2):131-6.

Closing pages

Summary

Nederlandse samenvatting

Dankwoord

Curriculum vitae

List of publications

Summary

Patients admitted to the intensive care unit (ICU) have an increased risk to develop infections due to their underlying illness. Also, the ICU environment facilitates transmission of micro-organisms. Potential pathogenic micro-organisms (PPMO) colonizing the oropharynx and lower gastro-intestinal tract are the target of decontamination strategies, such as selective digestive tract decontamination (SDD), selective oropharyngeal decontamination (SOD) and chlorhexidine (CHX) mouthwash.

The introduction of this thesis describes the theory on which decontamination strategies are based and summarizes the evidence that is available from previous studies. SDD and SOD are strategies relying on antibiotic prophylaxis and have been shown to improve survival of ICU patients and prevent the occurrence of ICU-acquired bloodstream infection, although studies with these results were performed in settings with relatively low levels of antimicrobial resistance, mainly the Netherlands. In other countries, SDD and SOD are not often used, mainly because of concerns over increasing antimicrobial resistance that may be accelerated by SDD and SOD and uncertainty about the effectiveness in these settings with higher levels of antimicrobial resistance. The aim of this thesis is, therefore, to compare the effectiveness and ecological safety of three decontamination strategies, SDD, SOD and CHX mouthwash, for ICU patients in other European countries with higher endemicity of antibiotic resistance.

Outline of this thesis

The associations between Gram-negative bacteria (GNB) colonizing the digestive tract and the respiratory tract and the occurrence of GNB infections during ICU admission were determined in **chapter 2**. Colonization status of the rectum and respiratory tract was determined using twice weekly microbiological surveillance cultures in 2,066 mechanically ventilated subjects receiving SDD. 1,157 (56.0%) subjects ever had documented GNB carriage in the rectum during ICU-stay. Rectal colonization was an independent risk factor for both respiratory tract colonization (cause specific hazard ratio (CSHR) 2.93, 95% confidence interval (CI) 2.02-4.23) and new GNB infection in the ICU (CSHR 3.04, 95% CI 1.99-4.65). Both rectal and respiratory tract colonization were associated with bacteremia (CSHR 7.37, 95% CI 3.25-16.68 and CSHR 2.56, 95% CI 1.09-6.03, respectively). The association between

colonization of the rectum and bacteremia was stronger than the association between respiratory tract colonization and bacteremia, which provides evidence that the gut acts as an important reservoir for PPMO and decontamination strategies aimed at the gut have a higher potential to prevent bacteremia than strategies aimed at the respiratory tract.

Chapter 3 describes the comparison of two SDD regimens, one with the antifungal agent amphotericin B and the other with nystatin as the antifungal component. Amphotericin B has most often been used in SDD, but shortage in raw materials have led to supply problems in the past and an increasing price. Nystatin belongs to the same class of antifungal agents as amphotericin B, but is cheaper. In a before-after study, SDD with amphotericin B was replaced by SDD with nystatin in two steps: first replacement of amphotericin B in the gastro-enteral suspension and second the replacement of amphotericin B in the mouth paste. The occurrence of acquisition of *Candida* colonization in the rectum and respiratory tract and decolonization of *Candida* from the rectum was determined with results from surveillance cultures. Nystatin was independently associated with less rectal acquisition of *Candida* (adjusted hazard ratio (HR) 0.52, 95% CI 0.33-0.83) and associated with faster rectal decolonization (adjusted HR 1.70, 95%CI 1.18-2.45), compared to amphotericin B. Acquisition rates in the respiratory tract were not significantly different in the three study periods. Based on these findings nystatin is a suitable alternative for amphotericin B and will improve the cost-effectiveness of SDD and SOD.

Chapter 4 is a comment on the European trial directive 2001 (*European Clinical Trials Directive 2001/20/EC*) on which national legislation regarding clinical trials in European countries has been based. This directive does not contain a provision for waiving informed consent, thereby obstructing cluster randomized studies which may require a waiver for individual informed consent, for example to study the ecological effects of decontamination strategies. Accordingly, a provision in the new European Clinical Trials Regulations allowing the possibility to alter the informed consent procedure when this is not in conflict with ethical standards and essential for the methodological validity, is needed.

To determine the most effective decontamination strategy among SDD, SOD and CHX mouthwash, a cluster randomized study among 13 European ICUs was

initiated, as described in **chapter 5**. Following a baseline period which included universal daily body washings with CHX and implementation of a hand hygiene program, ICUs were cluster randomized to three six-month intervention periods with CHX 1% oral care, SOD or SDD (order randomized per ICU). Patients with an expected length of mechanical ventilation of more than 24 hours were included to determine the occurrence of ICU-acquired bloodstream infection (BSI) with highly resistant micro-organisms (HRMO) (primary endpoint), survival (at three time points) and unit-wide prevalence of HRMO carriage. 8,665 patients were included from 13 ICUs in 6 European countries. Adjusted hazard ratios (95% CI) for ICU-acquired HRMO BSI were 1.06 (0.58-1.99), 0.83 (0.46-1.51) and 0.76 (0.38-1.52) during CHX, SOD and SDD, versus baseline, respectively. Adjusted odds ratios (95% CI) for day 28 mortality were 1.07 (0.86-1.32), 1.05 (0.85-1.29) and 1.03 (0.80-1.32) for CHX, SOD and SDD, versus baseline, respectively. In ICUs with moderate-to-high antibiotic resistance prevalence, use of CHX 1% oral care, SOD or SDD were, compared to standard care not associated with reductions in ICU-acquired BSI rates or mortality. The unit-wide prevalence of HRMO carriage in respiratory tract and rectum remained stable during interventions.

Chapter 6 describes the occurrence of side-effects in 29 of 295 (9.8%) patients treated with CHX 2% mouthwash in the multicenter study. Side-effects consisted mostly of oral mucosal lesions and disappeared after cessation of CHX mouthwash. The occurrence of side-effects was associated length of stay in the ICU and duration of mechanical ventilation, suggesting a dose-relationship. The CHX 2% concentration was therefore replaced with 1% chlorhexidine oral gel for the remainder of the study. CHX 2% mouthwash can therefore not be recommended as decontamination strategy for mechanically ventilated ICU patients.

To quantify the effects of SDD, SOD and CHX mouthwash on rectum and respiratory tract carriage of third generation cephalosporin resistant Enterobacteriaceae (3GCR-E) and carbapenem resistant Gram-negative bacteria (CR-GNB) a post-hoc analysis was performed on data from the multicenter study, as described in **chapter 7**. Carriage with 3GCR-E or CR-GNB in the rectum and respiratory tract was determined at least twice weekly and persistence of carriage was compared to standard care. 3GCR-E and CR-GNB present during initial ICU stay were more frequently eradicated in rectum samples during SDD with respective cause specific hazard ratios (CSHR) of 1.76 (95%CI 1.31-2.36) and 3.17 (95% CI 1.60-6.29) for SDD

versus standard care. For 3GCR-E there was a tendency towards eradication in respiratory samples during SDD (CSHR 1.47 (95% CI 0.98-2.20)) and SOD (CSHR 1.38 (95% CI 0.92-2.06)), compared to standard care, which was not observed during CHX or for CR-GNB. Suppression of 3GCR-E and CR-GNB might prevent the occurrence of ICU-acquired infections and cross-transmission of such bacteria.

In **chapter 8** the long-term effects of SDD and SOD on colistin and tobramycin resistance were determined by comparing results of point prevalence measurements in two Dutch cluster randomized studies. The first study took place between 2004 and 2006 and the second study between 2009 and 2013. Five ICUs participated in both studies and continued to use SDD or SOD in the interval between the studies, leading to an average follow-up of 7 years. Tobramycin resistance decreased during the follow-up period (rectal samples risk ratio RR (95% CI) 0.35 (0.23-0.53); respiratory samples RR 0.48 (0.32-0.73)), compared to baseline, and there was no difference in colistin resistance. Based on these results, long-term use of SDD or SOD does not lead to increased tobramycin or colistin resistance in the Dutch setting with relatively low levels of antimicrobial resistance.

The **discussion** summarizes the most important findings of this thesis and positions these in the context of previous studies. To conclude, the results of this thesis are not expected to lead to an increased use of CHX mouthwash, SDD and SOD in other countries than the Netherlands with higher endemicity of antibiotic resistance, as there were no effects on the survival of ICU patients with these interventions and a statistically non-significant trend towards less bloodstream infections.

Nederlandse samenvatting

Patiënten op de intensive care (IC) zijn ernstig ziek en hebben daardoor een verhoogd risico op infecties. Bacteriën en gisten die de darm en mond-keelholte koloniseren spelen een belangrijke rol in het ontstaan van infecties en vormen het aangrijpingspunt van decontaminatie strategieën, zoals selectieve darm decontaminatie (SDD), selectieve orofaryngeale decontaminatie (SOD) en chloorhexidine (CHX) mondspoeling.

Kenmerken van SDD, SOD en chloorhexidine

SDD is een strategie die bestaat uit antibiotica profylaxe, meestal een combinatie van colistine, tobramycine en een antifungaal middel zoals amfotericine B. SDD richt zich op (aerobe) Gram-negatieve bacteriën, *Staphylococcus aureus* en gisten, zoals *Candida* soorten. Deze micro-organismen koloniseren de tractus digestivus en zijn bekende verwekkers van infecties. Daarom worden ze ook wel potentieel pathogene micro-organismen (PPMO) genoemd. Anaerobe bacteriën koloniseren eveneens de darm, maar worden door SDD juist gespaard omdat zij (her)kolonisatie van PPMO zouden voorkomen (1).

SDD bestaat uit mondpaste en een suspensie die via de neus-maag sonde wordt gegeven. Naast deze lokale middelen wordt een breed spectrum antibioticum intraveneus toegediend gedurende de eerste vier dagen van de IC opname, bijvoorbeeld een derde generatie cefalosporine, om infecties met PPMO tijdens de eerste dagen van een IC opname te voorkomen. SOD bestaat uit alleen de mondpaste.

Meerdere studies, waarvan de grootste en meest recente zijn uitgevoerd in gebieden met een relatief lage prevalentie van antibiotica resistentie, met name Nederland (2-4), laten een overlevingsvoordeel zien voor IC patiënten die SDD krijgen (relatief risico afname van 10% voor sterfte op dag 28 na IC opname, absolute risico afname 3%). SOD heeft ook een gunstig effect op de overleving, maar in minder mate dan SDD. Daarnaast zijn SOD en SDD beiden effectief gebleken in het voorkomen van bacteriëmieën en wordt geen toename in antibiotica resistentie waargenomen tijdens SDD en SOD, maar juist een afname (4). Op basis van deze gegevens worden SDD en SOD in de Nederlandse IC richtlijn uit 2014 aanbevolen voor alle patiënten die langer dan 72 uur op IC verblijven of

die langer dan 48 uur op de IC beademd worden (5). Een recentere Nederlandse studie laat zien dat SDD effectiever is dan SOD in het voorkomen van sterfte en bacteriëmieën bij IC patiënten (6).

CHX mondspoeling wordt veelvuldig gebruikt als onderdeel van standaard mondzorg voor IC patiënten, zowel in Nederland als daarbuiten en is geassocieerd met een afname in het vóórkomen van beademing geassocieerde pneumonie (VAP) bij patiënten die hartchirurgie ondergaan (7). CHX is in tegenstelling tot SDD en SOD niet selectief in het spectrum micro-organismen die het beoogt te elimineren en het is geen antibioticum, maar een antiseptisch middel. Resistentie tegen CHX is zeldzaam en het middel is goedkoper dan SDD en SOD.

Ondanks de gunstige effecten van SDD en SOD op de overleving van IC patiënten in de Nederlandse studies worden SDD en SOD buiten Nederland weinig gebruikt, met name omdat er onvoldoende bewijs is voor effectiviteit in landen met een hogere prevalentie van antibiotica resistentie. Ook wordt er gevreesd dat SDD en SOD zullen leiden tot een verdere toename van antibiotica resistentie in deze landen (8), hoewel dit tot nu toe niet is aangetoond. De effectiviteit van CHX mondspoeling is nog nooit direct vergeleken met SDD en SOD, terwijl het een goedkoper alternatief zou kunnen zijn met minder effecten op antibiotica resistentie. Het doel van dit proefschrift is het vaststellen van de effectiviteit en veiligheid van SDD, SOD en CHX mondspoeling bij IC patiënten in landen met een hogere prevalentie van antibiotica resistentie dan Nederland.

Opbouw van dit proefschrift

In **hoofdstuk 2** wordt dieper ingegaan op de relatie tussen dragerschap van Gram-negatieve bacteriën in de darm en luchtwegen en het vóórkomen van infecties bij IC patiënten. De relatie tussen dragerschap in de darm en dragerschap in de luchtwegen is nog niet eerder onderzocht en de relatieve bijdrage van dragerschap in elke tractus aan het vóórkomen van bacteriëmie is onbekend. Bij 2,066 IC patiënten die met SDD behandeld werden is gekeken naar dragerschap in de darm en luchtwegen aan de hand van surveillance kweken: 1,157 (56.0%) patiënten waren op enig moment tijdens IC opname gekoloniseerd met Gram-negatieve bacteriën in het rectum. Dragerschap in het rectum was een onafhankelijke risicofactor voor infectie met Gram-negatieve bacteriën (*cause specific hazard ratio* (CSHR) 3.04, 95% betrouwbaarheidsinterval (BI) 1.99-4.65) en een risicofactor voor dragerschap in

de luchtwegen ((CSHR) 2.93, 95% BI 2.02-4.23), hoewel de overeenkomst tussen species in de darm en luchtwegen niet groot was. Zowel dragerschap in het rectum als dragerschap in de luchtwegen was geassocieerd met het vóórkomen van Gram-negatieve bacteriëmie (respectievelijke CSHR 7.37, 95% BI 3.25-16.68 en CSHR 2.56, 95% BI 1.09-6.03). De relatie tussen rectaal dragerschap en bacteriëmie was dus sterker dan de relatie tussen respiratoir dragerschap en bacteriëmie. Dit ondersteunt de hypothese dat de darm een reservoir is voor PPMO die infecties kunnen veroorzaken en een belangrijk aangrijpingspunt is voor decontaminatie strategieën.

In **hoofdstuk 3** worden twee antifungale middelen vergeleken, nystatine en amfotericine B. Amfotericine B is van oudsher een onderdeel van SDD en SOD, maar schaarste van grondstoffen hebben het middel duurder gemaakt en in het verleden geleid tot leveringsproblemen. Nystatine behoort tot dezelfde klasse antifungale middelen als amfotericine B en heeft dezelfde eigenschappen, maar het is goedkoper en makkelijker verkrijgbaar. Op een Nederlandse IC afdeling werd SDD met amfotericine B in twee stappen vervangen door SDD met nystatine: eerst in de mond pasta en vervolgens in de gastro-intestinale suspensie. SDD met nystatine bleek effectiever dan SDD met amfotericine B in het verwijderen van *Candida* uit het de darm (Hazard ratio (HR) 1.70, 95% BI 1.18-2.45). Ook raakten minder mensen gekoloniseerd in het rectum met *Candida* tijdens SDD met nystatine (HR 0.52, 95% BI 0.33-0.83), vergeleken met amfotericine B. Er was geen verschil tussen nystatine en amfotericine B in het verwijderen van *Candida* species uit de luchtwegen. Op basis van deze gegevens is nystatine een geschikt alternatief voor amfotericine B en zal nystatine de kosteneffectiviteit van SOD en SDD vergroten.

Hoofdstuk 4 bevat een betoog voor aanpassingen in de EU richtlijn uit 2001 voor het verrichten van klinische studies (*European Clinical Trials Directive 2001/20/EC*). In de richtlijn werd individuele toestemming van de patiënt voor studiedeelname als vereiste gesteld en werd geen voorziening getroffen voor het doen van cluster gerandomiseerd onderzoek zonder individuele toestemming van deelnemers. Deze studieopzet is voor de multicenter studie in hoofdstuk 5 gebruikt, omdat met het randomiseren op cluster niveau (IC niveau) de situatie kan worden nagebootst waarin een decontaminatie strategie gelijktijdig wordt toegepast bij alle patiënten die daarvoor in aanmerking komen, als ware het een standaard behandeling.

Alleen dan kunnen de ecologische effecten van de strategieën op het vóórkomen van antibiotica resistentie op de IC afdeling onderzocht worden. In hoofdstuk 4 wordt daarom gepleit voor een voorziening in de nieuwe Europese richtlijn voor het doen van cluster gerandomiseerd onderzoek zonder individuele toestemming van patiënten, onder bepaalde voorwaarden zoals het minimale risico van de te onderzoeken interventies voor patiënten.

In **hoofdstuk 5** wordt de cluster gerandomiseerde studie beschreven naar de effectiviteit en de ecologische effecten van SDD, SOD en CHX mondspoeling. De studie vond plaats op 13 IC afdelingen in 6 Europese landen, buiten Nederland. De prevalentie van antibiotica resistentie was in deze centra hoger dan in Nederland. Eindpunten van de studie waren de incidentie van bacteriëmie met een bijzonder resistent micro-organisme (BRMO) en overleving op dag 28 na IC opname. Elke IC begon met een baseline periode van minimaal 6 maanden met alleen de standaard behandeling (zonder SDD, SOD of CHX 2% mondspoeling). Hierna volgden 3 interventie perioden: SDD, SOD en CHX mondspoeling in willekeurige volgorde, ieder gedurende 6 maanden. SDD en SOD bestonden uit tobramycine, colistine en nystatine. Systemische antibiotica waren geen onderdeel van de interventies. Er werden 8,665 beademde IC patiënten geïncludeerd. De HR (95% BI) voor bacteriëmie met een BRMO waren 1.06 (0.58-1.99), 0.83 (0.46-1.51) en 0.76 (0.38-1.52) voor respectievelijk CHX, SOD en SDD, vergeleken met de standaard behandeling. Odds ratio's (95% BI) voor sterfte op dag 28 na IC opname waren respectievelijk 1.07 (0.86-1.32), 1.05 (0.85-1.29) en 1.03 (0.80-1.32) voor CHX, SOD and SDD, vergeleken met de standaard behandeling. De prevalenties van antibiotica resistentie op de IC afdelingen en van BRMO dragerschap bleven stabiel. Hoewel in deze setting met meer antibiotica resistentie dan in Nederland geen toename werd gezien in antibiotica resistentie, was er ook geen effect van SDD, SOD of CHX mondspoeling op de overleving van IC patiënten. Er was bovendien een niet-significante trend voor afname van bacteriëmieën tijdens SOD en SDD, in tegenstelling tot eerdere studies die in Nederland werden uitgevoerd, waarbij een statistisch significante afname in bacteriëmieën en sterfte werd gevonden tijdens behandeling met SDD en SOD.

Tijdens de studie traden onverwacht bijwerkingen op door het gebruik van CHX 2% mondspoeling. Deze worden beschreven in **hoofdstuk 6**. De bijwerkingen, met name mucosale laesies in de mond en op de lippen, traden op bij 29 van de

295 (9.8%) patiënten die met CHX 2% behandeld werden. Na het staken van CHX verdwenen de laesies. De duur van mechanische beademing en van het verblijf op IC waren geassocieerd met het vóórkomen van bijwerkingen, wat duidt op een dosis afhankelijke relatie. Dit was aanleiding om voor de resterende duur van de studie CHX 1% orale gel te gebruiken, waarna geen bijwerkingen meer werden waargenomen. CHX 2% mondspoeling is derhalve niet geschikt als orale decontaminatie strategie bij IC patiënten.

Om de effecten van de drie decontaminatie strategieën op resistente micro-organismen te onderzoeken wordt in **hoofdstuk 7** gekeken naar de mate van decontaminatie van *Enterobacteriaceae* resistent voor 3^e generatie cefalosporinen (3GCR-E) en carbapenem resistente Gram-negatieve bacteriën (CR-GNB) in de darm en de luchtwegen. Hiervoor werden gegevens uit multicenter studie gebruikt (hoofdstuk 5). Respectievelijk 643 en 154 patiënten werden geïncludeerd omdat ze tijdens de eerste 4 dagen van IC opname drager waren van 3GCR-E en CR-GNB in het rectum en respectievelijk 291 and 143 patiënten omdat ze drager waren van 3GCR-E en CR-GNB in de luchtwegen. Dekolonisatie van 3GCR-E en CR-GNB in het rectum kwam vaker voor tijdens SDD (3GCR-E: CSHR 1.76 (95%BI 1.31-2.36) en CR-GNB: CSHR 3.17 (1.60-6.29), vergeleken met standaard behandeling). Voor 3GCR-E werd een trend gevonden voor frequentere dekolonisatie van de luchtwegen tijdens SDD (CSHR 1.47 (0.98-2.20)) en SOD (CSHR 1.38 (0.92-2.06)), maar dit werd niet gevonden voor CHX mondspoeling. Op CR-GNB in de luchtwegen hadden de drie decontaminatie strategieën geen effect. Decontaminatie van 3GCR-E en CR-GNB gedurende de IC opname kan de kans op kruistransmissie en infecties met deze bacteriën verkleinen.

Om meer inzicht te krijgen in de lange termijn effecten van SDD en SOD wordt in **hoofdstuk 8** het vóórkomen van colistine en tobramycine resistentie geëvalueerd in 5 Nederlandse IC afdelingen die deelnamen aan 2 cluster gerandomiseerde studies naar het effect van SDD en SOD. De eerste studie vond plaats tussen 2004 en 2006 en de tweede studie tussen 2009 en 2013. De methoden voor het vaststellen van antibiotica resistentie verschilden niet tussen de twee studies. Tijdens beide studies werd maandelijks bij alle patiënten op de IC een rectum en respiratoir sample afgenomen en de prevalentie van colistine en tobramycine resistentie onder Gram-negatieve bacteriën bepaald. De prevalentie van tobramycine resistente Gram-negatieve bacteriën daalde (rectale samples risk ratio RR (95% BI)

0.35 (0.23-0.53); respiratoire samples RR 0.48 (0.32-0.73), vergeleken met standaard behandeling) en de prevalentie van colistine resistente Gram-negatieve bacteriën veranderde niet over een tijdspanne van gemiddeld 7 jaar.

In de **discussie** worden de belangrijkste bevinden van dit proefschrift samengevat en worden de resultaten beschouwd in de context van resultaten uit eerdere studies. Mogelijke verklaringen voor de verschillen in resultaten tussen de huidige multicenter studie (hoofdstuk 5) en voorgaande Nederlandse studies worden besproken. De discussie eindigt met een blik op de toekomst voor SDD, SOD en CHX buiten Nederland, waarbij het waarschijnlijk lijkt dat de resultaten van dit proefschrift niet zullen leiden tot een frequenter gebruik van de drie strategieën buiten Nederland.

Referenties

1. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk L-v. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *The Journal of hygiene*. 1971;69(3):405-11.
2. Krueger WA, Lenhart FP, Neeser G, Ruckdeschel G, Schreckhase H, Eissner HJ, et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. *American journal of respiratory and critical care medicine*. 2002;166(8):1029-37.
3. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet (London, England)*. 2003;362(9389):1011-6.
4. de Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *The New England journal of medicine*. 2009;360(1):20-31.
5. Oostdijk EADJ, E.; Kullberg, B.J.; Natsch, S.; De Smet, A.M.G.A.; Vandenbroucke-Grauls, C.M.J.E.; Van Der Vorm, E.; Bonten, M.J.M. SWAB-Richtlijn: selectieve decontaminatie bij patiënten op de intensive care. Stichting Werkgroep Antibioticabeleid, 2014.
6. Oostdijk EA, Kesecioglu J, Schultz MJ, Visser CE, de Jonge E, van Essen EH, et al. Effects of decontamination of the oropharynx and intestinal tract on antibiotic resistance in ICUs: a randomized clinical trial. *Jama*. 2014;312(14):1429-37.
7. Klompas M, Speck K, Howell MD, Greene LR, Berenholtz SM. Reappraisal of routine oral care with chlorhexidine gluconate for patients receiving mechanical ventilation: systematic review and meta-analysis. *JAMA internal medicine*. 2014;174(5):751-61.
8. Bastin AJ, Ryanna KB. Use of selective decontamination of the digestive tract in United Kingdom intensive care units. *Anaesthesia*. 2009;64(1):46-9.

Curriculum vitae

In 1984, Bastiaan Wittekamp was born in Huizen, the Netherlands. He lived there with his brother Wouter and parents until completing secondary school at the *Erfgooiers College* in Huizen in 2002, after which he moved to Maastricht to study medicine.

During his study he got the opportunity to do an elective in immunology in Linköping (Sweden), an internship in paediatrics in Bloemfontein (South-Africa) and an elective in emergency medicine in Pretoria (South-Africa).

After six years he graduated with distinction, and worked two years as a resident in the department of intensive care and internal medicine in *De Gelderse Vallei* hospital in Ede.

After being admitted to the specialization in internal medicine at the *University Medical Center Utrecht* in 2010, he worked in *Gelre ziekenhuis*, Apeldoorn. After one year and a meeting with prof. Bonten, he joined the R-GNOSIS consortium to work on the 'R-GNOSIS, decolonization strategies in intensive care' study. Combining this with his training in internal medicine and working in collaboration with Nienke Plantinga, resulted in this thesis in 2018.

After finishing this thesis, Bastiaan started his specialization in intensive care medicine at the UMC Utrecht.

List of publications

Associations between enteral colonization with Gram-negative bacteria and ICU-acquired infections and colonization of the respiratory tract.

Frencken JF and Wittekamp B.H., Plantinga N.L., Spitoni C., Groep K. v.d., Cremer O.L., Bonten M.J.M.
Clinical infectious diseases. 2018;66(4):497-503

Oral mucosal adverse events with chlorhexidine 2% mouthwash in ICU.

Plantinga N.L., Wittekamp B.H., Leleu K., Depuydt P., Van den Abeele A.M., Brun-Buisson C., Bonten M.J.M.
Intensive Care Medicine. 2016;42(4):620-621.

Nystatin versus amphotericin B to prevent and eradicate Candida colonization during selective digestive tract decontamination in critically ill patients.

Wittekamp B.H., Ong D.S., Cremer O.L., Bonten M.J.M.
Intensive Care Medicine. 2015;41(12):2235-2236.

Colistin and tobramycin resistance during long-term use of selective decontamination strategies in the intensive care unit: a post hoc analysis.

Wittekamp B.H., Oostdijk E.A.N., de Smet A.M.G.A., Bonten M.J.M.
Critical Care. 2015 Mar;19:113.

Fighting antibiotic resistance in the intensive care unit using antibiotics.

Plantinga N.L., Wittekamp B.H., van Duijn P.J., Bonten M.J.M.
Future Microbiology. 2015;10(3):391-406. (Review)

Regulatory obstacles affecting ecological studies in the ICU.

Wittekamp B.H., Wise M.P., Brun-Buisson C., Bonten M.J.
Lancet Infectious Diseases. 2014;14(10):913-915.

Catheter-related bloodstream infections: a prospective observational study of central venous and arterial catheters.

Wittekamp B.H., Chalabi M., van Mook W.N., Winkens B., Verbon A., Bergmans D.C.
Scandinavian Journal of Infectious Diseases. 2013;45(10):738-745.

Studies of selective decontamination.

Wittekamp B.H., Oostdijk E.A.N., Cooper BS, Brun-Buisson C., Bonten M.J.M.
Lancet Infectious Diseases. 2013;13(9):736-737.

Antibiotic prophylaxis in the era of multidrug-resistant bacteria.

Wittekamp B.H., Bonten M.J.M.
Expert Opinion on Investigational Drugs. 2012;21(6):767-772. (Review)

Selective decontamination in European intensive care patients.

Oostdijk E.A.N., Wittekamp B.H., Brun-Buisson C., Bonten M.J.M.
Intensive Care Medicine. 2012;38(4):533-538.

Clinical review: post-extubation laryngeal edema and extubation failure in critically ill adult patients.

Wittekamp B.H., van Mook W.N., Tjan D.H., Zwaveling J.H., Bergmans D.C.
Critical Care. 2009;13(6):233. (Review)
Critical Care. 2015;19:295. (Updated Review)