Supplementary Online Content

Wittekamp BH, Plantinga NL, Cooper BS, et al. Decontamination strategies and bloodstream infections with antibiotic-resistant microorganisms in ventilated patients: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2018.13765

eTable 15. Results for Competing End Points for Primary and Secondary Outcomes

This supplementary material has been provided by the authors to give readers additional information about their work.

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**, **MsC,** 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, BsC,** 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.

eAppendix 1. eMicrobiology 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 (Supplementary eTable 2). Five centers used automated antibiotic susceptibility testing (AST), five relied on disk diffusion and three used both methods (Supplementary eTable 1). 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 (Supplementary eTable 11).

Clinical cultures

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

eAppendix 2. Sample Size Calculation

The sample size calculation in the study protocol states that “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), including a margin of 600 patients per study arm to include cluster-effects and differences in baseline characteristics. The required sample size to detect a 50% reduction of ICU-acquired BSI due to MDRGNB for each intervention (from a baseline incidence of 1.4%) was 6,700.”

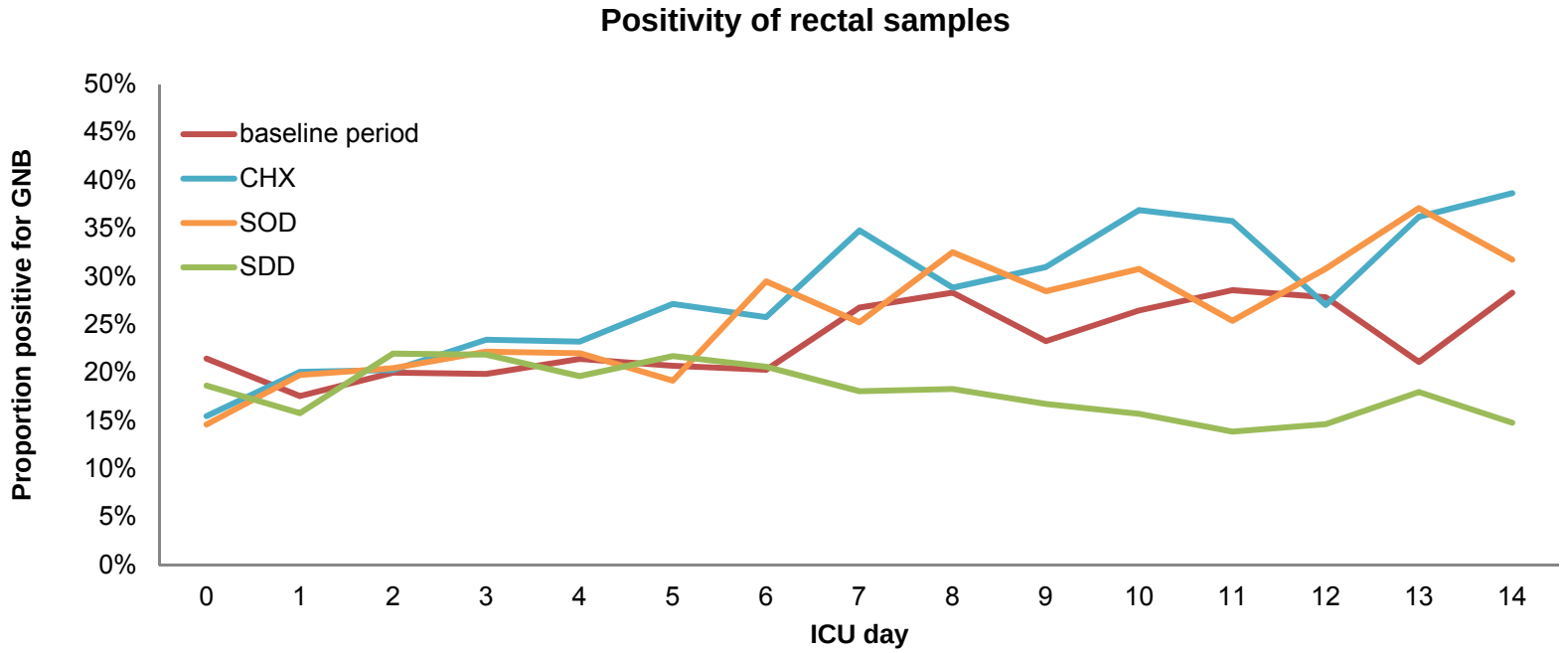
In fact, a power of 80% instead of a beta of 0.80 was used. .

It was discovered – after study completion - that an error had occurred in the calculation of variance between study groups in the formula which led to lower patient numbers than required. This mistake was partly attenuated by the higher mortality rate observed in the baseline period.

The ICC was determined with the final data with the R-package ICCbin (rm method) and yielded a value of 0.001.

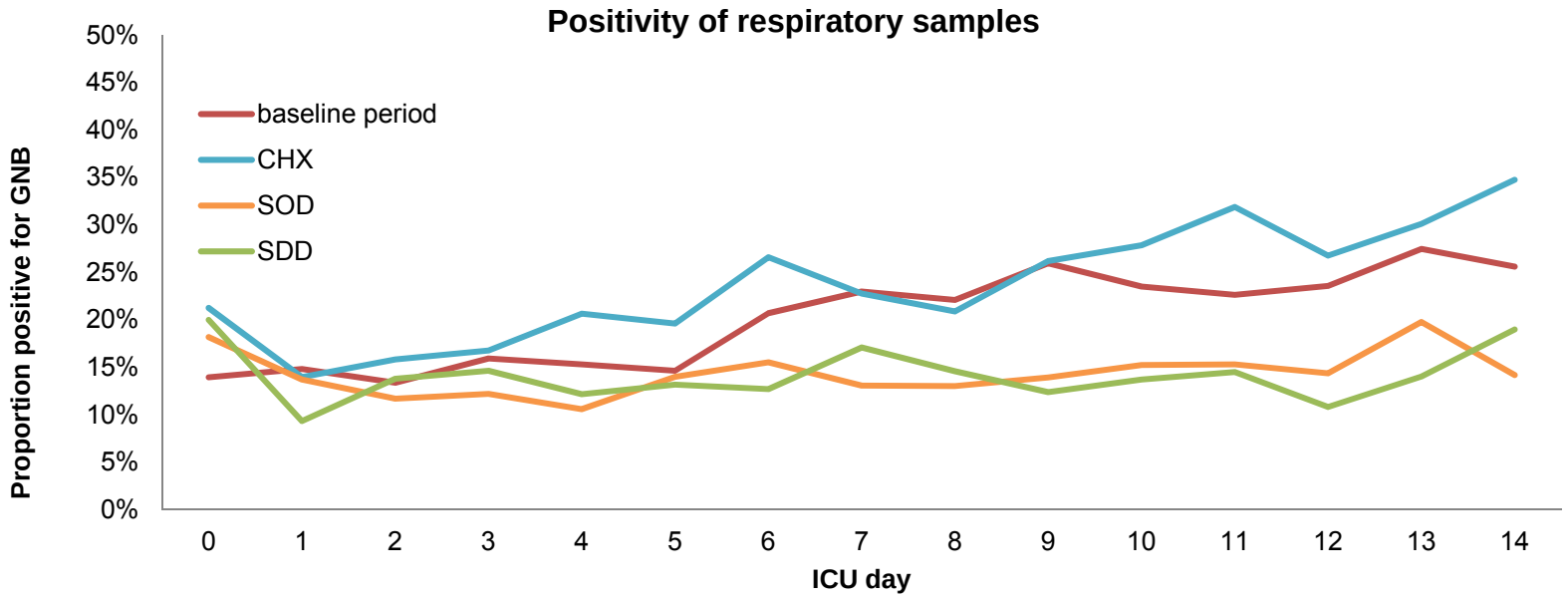
The funding for this study was obtained from a call text that specifically asked for evaluation of interventions in ICU that could reduce the incidence of ICU-acquired BSI. We, therefore, designed our study with this primary endpoint, but decided to power the study on mortality, as this is the more clinically relevant endpoint.

eFigure. Proportion of Surveillance Samples Positive for Antibiotic-Resistant Gram-Negative Bacteria on Day 1-14 of ICU Admission (ESBL-Selective Media)



No. 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

eFigure (continued). Proportion of Surveillance Samples Positive for Antibiotic-Resistant Gram-Negative Bacteria on Day 1-14 of ICU Admission (ESBL-Selective Media)



No. 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

eTable 1. Characteristics of Participating Centers

ICU	Coun-try	no. of beds	Type of ICU (medical /surgical /mixed)	CHX oral care in standard care	Standard care (study period 1)		Randomization sequence (order of study periods)			No. of patients included (% of screened)	prevalence of colonization with 3GC-R Enterobacteriaceae in rectum (standard care)	susceptibility testing GNB surveillance cultures	
					Start date	Duration (months) [d]	2	3	4			disk diffu-sion	automated
1 [a]	BE	36	mixed	0.12%	01-Dec-2013	6	CHX (5.6)	SOD (6)	SDD (6)	900 (14.9%)	19.0%	x	
2 [a]	BE	24	mixed	0.20%	01-Jan-2014	6	CHX (4.6)	SDD (6)	SOD (6)	317 (12.8%)	13.6%		phoenix
3	SL	12	medical	*none*	06-Jan-2014	6	SOD (6)	SDD (6)	CHX (6)	168 (22.0%)	7.5%	x	
4	BE	42	mixed	0.20%	01-Mar-2014	6	SDD (7)	CHX (6)	SOD(6)	1231 (19.4%)	7.2%	x	
5	SP	30	mixed	0.12%	14-Apr-2014	6	SOD (8.5)	CHX (6)	SDD (6)	1043 (32.7%)	30.0%	x	sensititre
6	IT	12	mixed	0.20%	15-May-2014	6	SDD (6)	SOD (3.5+2.5)	CHX (6)	598 (76.4%)	23.2%	x	vitek / phoenix
7	PT	10	mixed	0.12%	01-Jun-2014	14.5	SDD (6)	CHX (6)	SOD (6)	639 [b]	5.7%		vitek/mi-croscan
8	BE	15	mixed	0.20%	01-Jul-2014	6	SOD (6)	SDD (6)	CHX (6)	337 (20.9%)	15.2%	x	vitek
9	BE	42	mixed	0.20%	01-Sep-2014	6	CHX (6)	SOD (5)	SDD (6)	1297 (28.0%)	18.8%		vitek
10	SP	24	medical	0.12%	01-Nov-2014	8	SDD (6)	SOD (6)	CHX (6)	375 (18.3%)	35.2%	x	
11	SP	8	mixed	0.12%	03-Nov-2014	9	CHX (6)	SDD (5)	SOD (6)	237 (23.1%)	11.3%		phoenix
12	UK	22	mixed	*none*	11-Jan-2015	8	SOD (6)	CHX (6)	SDD (6)	1109 (44.1%)	8.9%	x	
13	PT	9	mixed	0.20%	19-Jan-2015	7	SDD (6)	SOD (6)	CHX (6)	414 (53.1%)	0.0% [c]		micro-scan

eTable 1. Characteristics of Participating Centers

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

a) Oral mucosal lesions occurred during the CHX study period with CHX 2% concentration, which was replaced by CHX 1% for the remainder of the study.
b) Only excluded patients with point prevalence data were registered as part of the screened population.
c) 31/31 rectal samples were negative for 3GC-R; in the respiratory tract the prevalence of 3GC-R Enterobacteriaceae was 2/31 (6.5%).
d) In some ICUs, the baseline period was extended pending authorization to conduct the trial.

eTable 2. Micro-organisms in Positive Blood Cultures That are Not Included in the Study Definition of ICU-Acquired Bloodstream Infection

Study period **Number of patients**	**Baseline** **n=2251**		**CHX** **n=2108**		**SOD** **n=2224**		**SDD** **n=2082**	
	No. of episodes [a]	Column%	No. of episodes[a]	Column%	No. of episodes[a]	Column%	No. of episodes[a]	Column%
Other ICU-acquired positive blood cultures [b]	**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; ICU, intensive care unit; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination; spp., species.

a) First occurrence of unique species on day 2 of ICU admission onwards.
b) Excluding species considered contaminants (Bacillus, Atopobium, Corynebacterium and Propionibacterium species).

eTable 3. Antibiotic Susceptibility Testing and Definition of Highly Resistant Micro-organism

Minimum required antibiotic susceptibility testing list	HRMO criteria [a]
Multidrug resistant Gram-negative bacteria (primary outcome)	
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**
MDRGNB regardless of susceptibility testing result	
Stenotrophomonas spp.	
Achromobacter spp.	
Burkholderia spp.	
Gram-positive HRMO	
Methicillin-resistant *S. aureus* (MRSA)	
Vancomycin resistant *E.faecium/E.faecalis* (VRE)	

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

a) 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.

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 resistant HRMO.

**** Resistance to third-generation cephalosporins directly qualified for HRMO and was counted as one of three antibiotic classes for criterium B.

eTable 4. 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)	43.3 (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 (days); SD, standard deviation; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

* 137 missing values.
** 2306 missing values (within these selected hospitals).

eTable 5. All Baseline Characteristics (Study Population)

	baseline	CHX	SOD	SDD
	n = 2251	n = 2108	n = 2224	n = 2082
ICU admission in prior 30 days *with inclusion*	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 from 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%)

continued	baseline	CHX	SOD	SDD
	n = 2251	n = 2108	n = 2224	n = 2082
If admitted from a hospital or long-term care facility, prior location *(continued)*				
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%)

continued	baseline	CHX	SOD	SDD
	n = 2251	n = 2108	n = 2224	n = 2082
Comorbidities				
Any malignancy (incl. 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 / immunesupression)	190 (8.4%)	223 (10.6%)	193 (8.7%)	206 (9.9%)
Periferal 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; incl., including; SD, standard deviation; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

eTable 6. Compliance Measures

	CHX	SOD	SDD
Protocol compliance (monthly unit-wide point prevalence screening)			
Patients admitted	1667	1693	1588
Ventilated (% of admitted) - *representing % eligible*	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)	**n = 2108**	**n = 2224**	**n = 2082**
Treatment compliance (% of included)			
Intervention interrupted for more than > 24h (4 gifts 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			
Allergy	0 (0.0%)	1 (0.0%)	0 (0.0%)
Intolerance	15 (0.7%)	2 (0.1%)	1 (0.0%)
Colonization with HRMO, only sensitive to colistin	0 (0.0%)	1 (0.0%)	0 (0.0%)
Colonization with HRMO, that is resistant to carbapenems *and* tobramycin (or if tobramycin was not tested, to gentamicin)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gastrointestinal 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%)

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

eTable 7. Average Hand Hygiene Compliance per Study Period

Center	Baseline period	CHX	SOD	SDD
ICU1	45.3%	59.1%	60.3%	54.7%
ICU2	66.0%	74.8%	66.2%	66.3%
ICU3	76.2%	80.7%	71.9%	78.8%
ICU4	67.0%	71.2%	74.1%	76.1%
ICU5	61.4%	70.3%	75.0%	73.7%
ICU6	74.1%	73.3%	64.2%	75.2%
ICU7	64.9%	86.5%	93.6%	65.0%
ICU8	66.3%	70.4%	70.3%	74.6%
ICU9	61.1%	70.3%	75.5%	64.3%
ICU10	58.4%	72.7%	70.6%	65.0%
ICU11	61.7%	67.3%	74.0%	76.6%
ICU12	32.6%	61.7%	48.7%	78.0%
ICU13	86.5%	86.4%	91.1%	87.7%
Overall	64.1%	72.4%	72.5%	72.2%

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

eTable 8. Incidence of ICU-Acquired Bloodstream Infection With Multidrug Resistant Gram-Negative Bacteria (Primary Outcome) per ICU [a]

	baseline		CHX		SOD		SDD		Total	
Centre	**n**	**incidence n (%)**	**n**	**incidence n (%)**	**n**	**incidence n (%)**	**n**	**incidence n (%)**	**n**	**incidence n (%)**
ICU1	212	4 (1.9%)	214	7 (3.3%)	245	2 (0.8%)	2 (229)	0.9%	900	15 (1.7%)
ICU2	77	1 (1.3%)	59	0 (0.0%)	101	3 (3.0%)	2 (80)	2.5%	317	6 (1.9%)
ICU3	45	0 (0.0%)	46	0 (0.0%)	36	0 (0.0%)	0 (41)	0.0%	168	0 (0.0%)
ICU4	266	0 (0.0%)	285	0 (0.0%)	334	0 (0.0%)	0 (346)	0.0%	1231	0 (0.0%)
ICU5	169	4 (2.4%)	277	4 (1.4%)	349	3 (0.9%)	2 (248)	0.8%	1043	13 (1.3%)
ICU6	122	9 (7.4%)	177	7 (4.0%)	155	8 (5.2%)	3 (144)	2.1%	598	27 (4.5%)
ICU7	297	0 (0.0%)	109	1 (0.9%)	104	1 (1.0%)	1 (129)	0.8%	639	3 (0.5%)
ICU8	85	2 (2.4%)	92	2 (2.2%)	85	0 (0.0%)	0 (75)	0.0%	337	4 (1.2%)
ICU9	333	9 (2.7%)	338	11 (3.3%)	309	8 (2.6%)	8 (317)	2.5%	1297	36 (2.8%)
ICU10	113	8 (7.1%)	85	1 (1.2%)	85	7 (8.2%)	6 (92)	6.5%	375	22 (5.9%)
ICU11	63	1 (1.6%)	50	2 (4.0%)	70	0 (0.0%)	1 (54)	1.9%	237	4 (1.7%)
ICU12	352	6 (1.7%)	272	2 (0.7%)	248	0 (0.0%)	1 (237)	0.4%	1109	9 (0.8%)
ICU13	117	3 (2.6%)	104	1 (1.0%)	103	1 (1.0%)	0 (90)	0.0%	414	5 (1.2%)
Total	**2251**	**47 (2.1%)**	**2108**	**38 (1.8%)**	**2224**	**33 (1.5%)**	**2082**	**26 (1.2%)**	**8665**	**144 (1.7%)**

Abbreviations: CHX, chlorhexidine; n, number of inclusions; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

a) Incidence refers to the number of patients with an ICU-acquired bloodstream infection with multidrug resistant Gram-negative bacteria (n=144); there were 154 unique episodes

eTable 9. Systemic Antibiotics Used[a]

Study period	Baseline	CHX	SOD	SDD
% Patient days included	54%	56%	54%	52%
Antibiotic type (DDD per 1,000 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
Total	**1,058**	**1,000**	**1,030**	**1,115**

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

a) Secondary exploratory analysis

eTable 10. Prevalence of Unit-Wide Carriage of Antibiotic Resistant Microorganisms in Rectum and Respiratory Tract (Complete Results)

	baseline	**CHX**		**SOD**		**SDD**	
Rectum	prev.	prev.	aRR [a]	prev.	aRR [a]	prev.	aRR [a]
HRMO Enterobacteriaceae	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)
third-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%	0.80 (0.64-1.01)
resistance to ≥3 antibiotics (or classes)	10.8%	15.5%	1.07 (0.97-1.19)	14.2%	1.06 (0.96-1.17)	10.0%	1.10 (0.97-1.24)
colistin resistance [b]	0.5%	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%	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 [c]	2.9%	2.9%	0.75 (0.60-0.93)	2.7%	0.95 (0.76-1.18)	1.8%	0.80 (0.60-1.06)
colistin resistance	0.0%	0.1%		0.1%		0.3%	
resistance to ≥3 antibiotics (or classes)	2.4%	2.0%	0.66 (0.50-0.89)	2.7%	0.82 (0.65-1.05)	1.7%	0.71 (0.52-0.98)
MDRGNB, regardless of antibiotic susceptibility	1.0%	1.5%	0.80 (0.50-1.27)	1.1%	0.80 (0.49-1.30)	1.6%	1.01 (0.64-1.58)
Any MDRGNB (aggregate)	**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)**
VRE	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)
Enterobacteriaceae or GNF-GNB with gentamicin resistance [d]	*8.3%*	*9.0%*	*0.95 (0.84-1.08)*	*10.4%*	*0.99 (0.89-1.10)*	*7.2%*	*1.00 (0.87-1.15)*

eTable 10. Prevalence of Unit-Wide Carriage of Antibiotic Resistant Microorganisms in Rectum and Respiratory Tract (Complete Results) (continued)

	baseline	**CHX**		**SOD**		**SDD**	
Respiratory tract	prev.	prev.	aRR [a]	prev.	aRR [a]	prev.	aRR [a]
HRMO Enterobacteriaceae	6.6%	7.6%	0.94 (0.81-1.09)	4.2%	0.93 (0.80-1.09)	4.7%	0.94 (0.78-1.13)
third-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)
resistance to ≥3 antibiotics (or classes)	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 [b]	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)
HRMO Glucose non-fermenting GNB	3.4%	2.9%	0.80 (0.64-1.00)	3.8%	0.84 (0.70-1.00)	2.7%	0.75 (0.58-0.96)
carbapenem resistance [c]	3.1%	2.8%	0.80 (0.63-1.00)	3.4%	0.83 (0.69-1.00)	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)
MDRGNB, 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 MDRGNB (aggregate)	**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)**
MRSA	1.7%	1.1%	0.95 (0.66-1.36)	1.3%	0.77 (0.59-1.00)	1.7%	0.73 (0.54-0.97)
Enterobacteriaceae or GNF-GNB with gentamicin resistance [d]	*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: aRR; adjusted relative risk per month; CHX, chlorhexidine; GNB, Gram-negative bacteria; HRMO, highly resistant micro-organism; MDRGNB, multidrug resistant Gram-negative bacteria (resistance to 3 or more antibiotics/classes of antibiotics [(Supplementary eTable 2]);MRSA, methicillin resistant *S. aureus*; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination; VRE, vancomycin-resistant *E. faecium/E. faecalis*.
a) all models were corrected for underlying time trends per center.
b) excluding Enterobacteriaceae with intrinsinc colistin resistance (Proteus spp., Morganella spp., Serratia spp., Providencia spp., *Hafnia alvei*).
c) for Pseudomonas spp., resistance to at least one other antibiotic was required to be reported as carbapenem-R.
d) these were not part of the definition of HRMO.

eTable 11. Compliance With Antibiotic Susceptibility Testing in Point Prevalence Samples

	Rectum		Respiratory tract	
	EB	**GNF-GNB**	**EB**	**GNF-GNB**
Number of unique micro-organisms	1,153	467	559	399
Antibiotics for which AST was requested				
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; EB Enterobacteriaceae; GNF-GNB Glucose non-fermenting Gram-negative bacteria (including Pseudomonas spp.); no., number.

eTable 12. Prevalence of Colistin-Resistant Gram-Negative Bacteria 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 [a])				
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 *intrinsically* colistin-R GNB [a]				
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; no., number; SDD, selective digestive tract decontamination; SOD, selective oropharyngeal decontamination.

Methods: The use of the colistin E-test in eleven hospitals (see supplementary microbiological methods) might have underestimated the prevalence of colistin resistance during monthly point prevalence surveys. Therefore, 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.

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

eTable 13. Results of Sensitivity Analyses

A) Post-hoc sensitivity analysis on the effects of SDD on mortality [a]

	Results from adjusted analysis [b]
	SDD vs. baseline
ICU mortality (aHR, 95% CI)	0.93 (0.79-1.08)
Hospital mortality (aHR, 95% CI)	0.93 (0.79-1.10)
28-day mortality (aOR, 95% CI)	1.01 (0.78-1.29)

Abbreviations: aHR, adjusted hazard ratio; aOR, adjusted odds ratio; CI, confidence interval; ICU, intensive care unit; SDD, selective digestive decontamination.

a) In this analysis 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 alive at all mortality endpoints.
b) 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) A prior sensitivity analysis: Effects of interventions on mortality, excluding patients with ICU-LOS <3 days.

	Results from adjusted analysis [a]		
	CHX vs. baseline	**SOD vs. baseline**	**SDD vs. baseline**
ICU mortality (aHR, 95% CI)	1.09 (0.96-1.24)	1.04 (0.92-1.19)	0.99 (0.84-1.15)
Hospital mortality (aHR, 95% CI)	0.99 (0.86-1.15)	1.02 (0.89-1.17)	0.98 (0.84-1.15)
28-day mortality (aOR, 95% CI)	1.08 (0.87-1.35)	1.06 (0.86-1.32)	1.04 (0.81-1.34)

Abbreviations: aHR, adjusted hazard ratio; aOR, adjusted odds ratio; CHX, chlorhexidine; CI, confidence interval; HR, hazard ratio; ICU, intensive care unit; OR, odds ratio; 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).

eTable 14. Head-to-Head Comparisons Between Study Interventions; Primary and Secondary Outcomes

	SDD vs. CHX	SOD vs. CHX	SDD vs. SOD
Primary outcome [(a)]			
ICU-acquired BSI with MDRGNB (aHR, 95% CI) [(b)]	0.62 (0.39-0.98)	0.79 (0.53-1.17)	0.79 (0.52-1.19)
Secondary outcomes [(a)]			
ICU-acquired BSI with HRMO (aHR, 95% CI)	0.72 (0.43-1.19)	0.78 (0.55-1.10)	0.92 (0.57-1.48)
ICU-acquired BSI with any pathogen (aHR, 95% CI)	0.73 (0.57-0.93)	0.87 (0.75-1.00)	0.84 (0.67-1.05)
Mortality in ICU (aHR, 95% CI)	0.92 (0.82-1.02)	0.97 (0.90-1.05)	0.94 (0.84-1.06)
Mortality in hospital (aHR, 95% CI)	0.99 (0.90-1.08)	1.03 (0.95-1.12)	0.96 (0.87-1.06)
Mortality at 28th day from ICU admission (aOR, 95% CI)	0.96 (0.82-1.14)	0.98 (0.86-1.12)	0.98 (0.83-1.16)

Abbreviations: aHR, adjusted hazard ratio; aOR adjusted odds ratio; BSI, bloodstream infection; CHX, chlorhexidine; CI, confidence interval; MDRGNB, multidrug resistant Gram-negative bacteria; 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) multidrug resistant Gram-negative bacteria are defined in Supplementary eTable 2.

eTable 15. Results for Competing End Points for Primary and Secondary Outcomes

Study period (comparisons with baseline period)	CHX	SOD	SDD
Competing events	CSHR (95% CI)	CSHR (95% CI)	CSHR (95% CI)
ICU-acquired BSI with multidrug resistant Gram-negative bacteria			
ICU discharge	0.97 (0.87-1.07)	1.00 (0.90-1.12)	0.99 (0.88-1.11)
Death in ICU	1.02 (0.90-1.15)	0.99 (0.87-1.12)	0.94 (0.80-1.09)
ICU-acquired BSI with highly-resistant micro-organisms			
ICU discharge	0.97 (0.87-1.07)	1.00 (0.90-1.12)	0.99 (0.88-1.11)
Death in ICU	1.01 (0.89-1.15)	0.98 (0.86-1.12)	0.92 (0.79-1.08)
ICU-acquired BSI with any pathogen			
ICU discharge	0.96 (0.87-1.06)	1.01 (0.91-1.12)	0.98 (0.87-1.09)
Death in ICU	1.00 (0.88-1.14)	0.97 (0.85-1.11)	0.93 (0.79-1.09)
Mortality in ICU			
ICU discharge	0.97 (0.88-1.08)	1.01 (0.91-1.12)	1.00 (0.90-1.11)
Mortality in hospital			
Hospital discharge	1.03 (0.93-1.14)	1.10 (1.02-1.18)	1.03 (0.96-1.11)

Abbreviations: CSHR: cause specific hazard ratio; CHX, chlorhexidine; CI: confidence interval; ICU: intensive care unit; SDD, selective digestive decontamination; SOD, selective oropharyngeal decontamination.