Supplementary Appendix

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

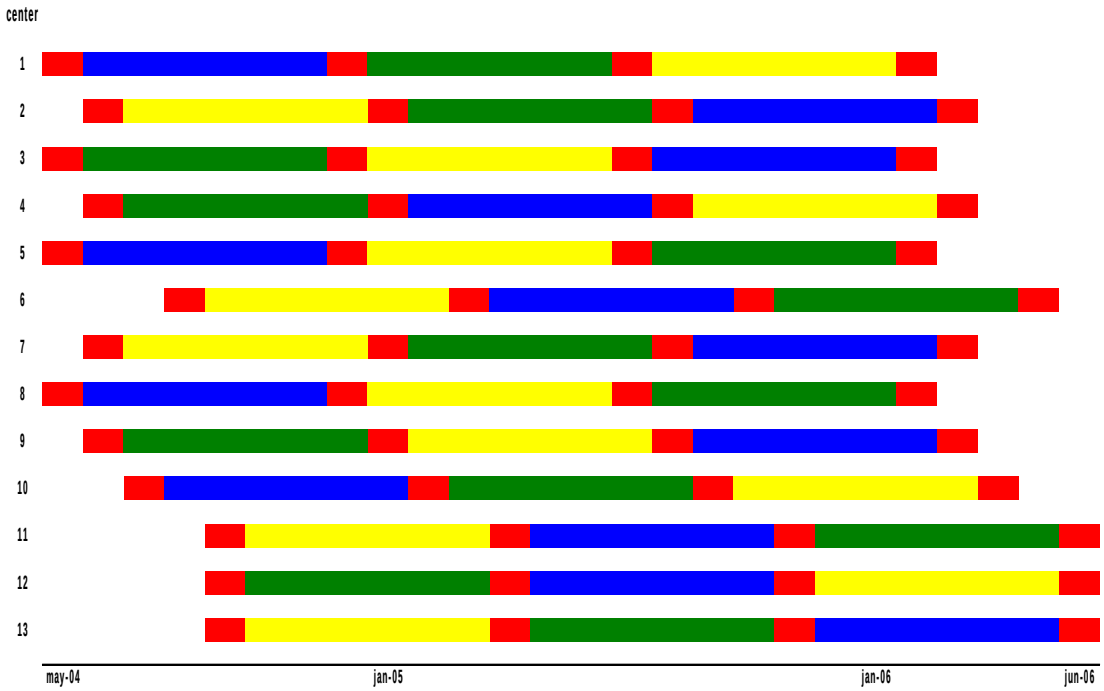
Supplement to: de Smet AMGA, Kluytmans JAJW, Cooper BS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med 2009;360:20-31.

Supplement to manuscript 08-00394

Methods

The first study period was preceded by a one month wash-in period. In this month, newly admitted patients were already treated according to the center-specific allocation treatment for the first period, but actual inclusion started after this wash-in period. Successive study periods were separated by one month wash-out/wash-in periods. In the first two weeks patients included during the previous study period remained to be treated according to that period, but newly admitted patients already received the treatment of the forthcoming period. After two weeks, patients still receiving treatment according to the previous period were switched to treatment of the forthcoming period. Actual inclusion for the new period only started at the first day of the new period (figure study scheme)

Study scheme in each of the 13 participating centers



Red is wash-in/out month (during which no patients were included in the study)

Blue is standard care, yellow is SDD and green is SOD.

SDD regimen

The SDD regimen was identical to that used by de Jonge et al (2), and consisted of oropharyngeal application (every 6 h) of a paste containing polymyxin E, tobramycin and amphotericin B each in a 2% concentration and administration (every 6 h) of a 10 ml suspension containing 100 mg polymyxin E, 80 mg tobramycin and 500 mg amphotericin B via the nasogastric tube. Topical antibiotics were applied until ICU-discharge. The costs of these topical antibiotics was $12 per day. In addition, cefotaxime (1000 mg, every 6 h) was administered intravenously during the first four days of study. Cefotaxime was replaced by ciprofloxacin (twice daily 400 mg) in case of documented cephalosporin allergy. Patients with a clinical suspicion or documented infection when admitted to ICU were treated according to standard clinical practice. In these patients cefotaxime was not added to carbapenems, fluoroquinolones, ceftazidime or piperacillin/tazobactam. Cefotaxime was replaced by ciprofloxacin (twice daily 400 mg) in case of documented cephalosporin allergy.

Protocol modifications for patients with tracheostomy, jejunostomy or colostomy, as well as for those with persistent respiratory tract colonization with yeasts or Gram negative bacteria. In patients with tracheostomy the paste was applied around the tracheostomy. In patients with a duodenal tube or jejunostomy, 5 ml of the suspension was given via the gastric tube and the remaining 5 ml via the duodenal tube or jejunostomy. Patients with colostoma or ileostoma received SDD-suppositories (containing 100 mg polymyxin E, 40 mg tobramycin and 500 mg amphotericin B) twice daily in the distal part of the gut. Surveillance cultures of endotracheal aspirates, oropharyngeal and rectal swabs were performed on admission and twice weekly. Based on these surveillance cultures, several adaptations of the SDD regimen were possible: (a) application of oropharyngeal paste was increased to 8 times daily, if the first surveillance culture of the throat yielded yeasts, until two surveillance cultures were negative; (b) 5 ml (5 mg) amphotericin B was nebulized 4 times daily if a sputum surveillance

culture (not admission culture) yielded yeasts, until two sputum cultures became negative; (c) 5 ml (80 mg) polymyxin E was nebulized 4 times daily if a sputum surveillance culture (not admission culture) yielded Gram negative bacteria, until two sputum cultures were negative.

SOD regimen

Protocol modifications for patients with tracheostomy and persistent respiratory tract colonization with yeasts. In patients with tracheostomy the paste was applied around the tracheostomy. Surveillance cultures of endotracheal aspirates and oropharyngeal swabs were performed on admission and twice weekly. Based on these surveillance cultures, adaptation of the SOD regimen was possible: application of oropharyngeal paste was increased to 8 times daily, if the first surveillance culture of the throat yielded yeasts, until two surveillance cultures were negative. The costs of these topical antibiotics were $1 per day.

Microbiology

Surveillance cultures of throat, rectum and sputum were inoculated on McConkey-agar (with and without tobramycin or cefotaxime), blood agar plate, Sabouraud agar plate and a Chocolate agar plate. Surveillance cultures were not obtained from patients during the control period. Microbiological cultures obtained for clinical reasons were processed according to current clinical practice.

Surveillance cultures obtained during the 1-day point prevalence studies were inoculated on selective media: McConkey-agar plates with cefotaxime (8 mg/L) or ciprofloxacin (2mg/L) or polymyxin E (50 IU/ml) or tobramycin 8 mg/L. Cultures were analyzed qualitatively for the presence of Gram-negative bacteria and minimum inhibitory concentrations (MIC) were determined for all Gram negative bacteria growing on the selective media. We analyzed the proportions of pathogens not susceptible to either gentamicin or tobramycin (breakpoint for non-susceptibility 4 mg/L), ciprofloxacin

(breakpoint for non-susceptibility 2 mg/L) and ceftazidime (breakpoint for non-susceptibility 16 mg/L) (Clinical and Laboratory Standards Institution. Performance standards for antimicrobial susceptibility testing. Fifteenth information supplement (M100-S15). Wayne, PA, USA: CLSI. 2005).

Microbiological cultures, and fecal samples submitted for *Clostridium difficile* toxin determination, obtained for clinical reasons were processed according to current clinical practice. Occurrence of ICU-acquired bacteremia (i.e., documented $\geq$48 hours after ICU-admission) was analyzed for *S. aureus*, Glucose Non-Fermenting Gram-negative Rods (GNF-GNR) (i.e., *Pseudomonas aeruginosa*, *Stenothrophomonas maltophilia* and Acinetobacter species), Enterobacteriaceae, *S. pneumoniae* and *Enterococcus spp*.

Oropharyngeal care

Oropharyngeal care consisted of oral washings with sterile water (3-4 times daily). In case of a visually contaminated oropharyngeal cavity, this was cleaned with a swab moistened in 1.5% hydrogen peroxide. Teeth were brushed twice daily. Chlorhexidine was not used for oral care.

Endpoints and statistical analysis

With an estimated ICU-mortality rate of 20% for eligible patients in the participating ICUs and considering a 20% relative reduction of ICU-mortality to be clinically relevant, it was calculated that 1,150 patients would need to be included in each study arm in the absence of any intracluster correlation ($\beta = 0.8$ and $\alpha = 0.05$). No estimates of the intra-cluster correlation coefficient were available, but assuming an intra-cluster coefficient of 0.05 would have increased the required sample size to 1,840 per study arm.

Time to cessation of ventilation, ICU-discharge and hospital-discharge were

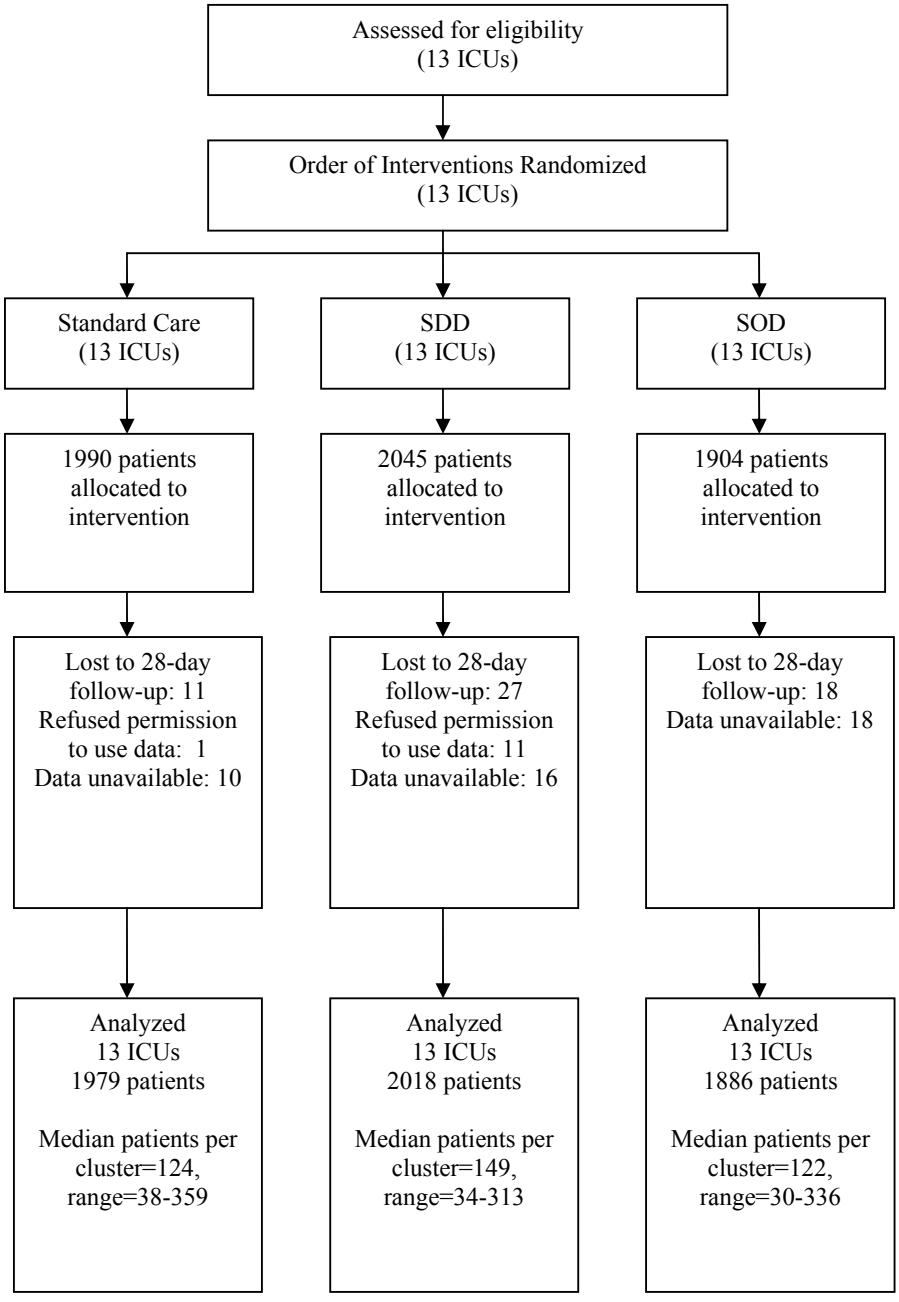
analysed using Cox regression models, with all observations censored at day 28. Since deaths will lead to informative censoring and act in the opposite direction to any positive effect of the interventions on these outcomes, patients who died were considered to have infinite times to cessation of ventilation or discharge. Analysis of mortality and time-to-event data was performed using STATA 8.0 (STATA Corporation, College Station, Texas). All other statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS,Chicago, Illinois) version 12.0.2.

Results

Patients with cross-over to subsequent study period:

- standard care to SOD: 18
- standard care to SDD: 5
- SOD to standard care: 13
- SOD to SDD : 4
- SDD to standard care: 6
- SDD to SOD : 2

Flowchart


Assessed for eligibility
(13 ICUs)
Order of Interventions Randomized
(13 ICUs)
Standard Care
(13 ICUs)
SDD
(13 ICUs)
SOD
(13 ICUs)
1990 patients allocated to intervention
2045 patients allocated to intervention
1904 patients allocated to intervention
Lost to 28-day follow-up: 11
Refused permission to use data: 1
Data unavailable: 10
Lost to 28-day follow-up: 27
Refused permission to use data: 11
Data unavailable: 16
Lost to 28-day follow-up: 18
Data unavailable: 18
Analyzed
13 ICUs
1979 patients
Median patients per cluster=124, range=38-359
Analyzed
13 ICUs
2018 patients
Median patients per cluster=149, range=34-313
Analyzed
13 ICUs
1886 patients
Median patients per cluster=122, range=30-336

Characteristics of the participating ICUs					
Center	Hospital-type	ICU-beds	Number of included patients	Inclusion Rate † (%)	Study order‡
1	university	26	626	92	SC-SOD-SDD
2	teaching	31	681	84	SDD-SOD-SC
3	non-teaching	4	119	71	SOD-SDD-SC
4	teaching	10	200	52	SOD-SC-SDD
5	teaching	10	340	94	SC-SDD-SOD
6	teaching	8	197	90	SDD-SC-SOD
7	teaching	6	147	87	SDD-SOD-SC
8	teaching	12	369	74	SC-SDD-SOD

9	teaching	22	410	57	SOD-SDD-SC
10	university	43	1,013	89	SC-SOD-SDD
11	university	31	777	98	SDD-SC-SOD
12	university	19	646	99	SOD-SC-SDD
13	teaching	23	414	100	SDD-SOD-SC
Totals		197	5,939		

† The number of included patients divided by the number of eligible patients represents the inclusion rate

‡Standard Care (SC), Selective Oropharyngeal Decontamination (SOD) and Selective Decontamination of the Digestive tract (SDD)

Mortality rates (%) on day 28 in patients with APACHE II-score < or ≥ 20 per participating center and per studygroup

Center	APACHE II < 20			APACHE II ≥ 20		
	Study group			Study group		
	Standard Care No. of patients (%)	SOD No. of patients (%)	SDD No. of patients (%)	Standard Care No. of patients (%)	SOD No. of patients (%)	SDD No. of patients (%)
1	94 (17.0)	75 (12.0)	76 (6.6)	126 (41.3)	128 (32.8)	127 (31.5)
2	219 (16.9)	150 (20.7)	137 (19.0)	51 (43.1)	52 (40.4)	72 (37.5)
3	36 (13.9)	22 (13.6)	23 (26.1)	18 (38.9)	8 (62.5)	12 (16.7)
4	22 (13.6)	29 (31.0)	39 (17.9)	16 (31.3)	35 (51.4)	59 (44.1)
5	74 (23.0)	57 (26.3)	76 (22.4)	32 (56.3)	61 (32.8)	40 (45.0)

6	13 (23.0)	5 (0.0)	15 (6.7)	54 (31.5)	65 (29.2)	45 (26.7)
7	21 (19.0)	22 (22.7)	20 (15.0)	26 (38.5)	26 (53.8)	32 (56.3)
8	41 (14.6)	49 (18.4)	39 (12.8)	84 (36.9)	74 (32.4)	82 (40.2)
9	60 (25)	45 (24.4)	82 (20.7)	51 (43.1)	57 (54.4)	115 (41.7)
10	248 (16.9)	242 (13.2)	221 (17.2)	112 (37.5)	97 (41.2)	93 (30.1)
11	128 (13.3)	125 (16.8)	162 (12.3)	119 (32.8)	137 (28.5)	106 (34.9)
12	119 (12.6)	108 (14.8)	110 (13.6)	90 (47.8)	106 (31.1)	113 (43.4)
13	78 (29.5)	78 (25.6)	76 (26.3)	58 (56.9)	51 (29.4)	73 (38.4)

Stepwise random effects logistic regression model accounting for ICU-level clustering.

	SDD	SOD	APACHE>=20	AGE>65	POST-OP /SURGICAL	VENTILATED	MALE	z-score of added covariate
1	0.939 (0.816, 1.081) P=0.384	0.950 (0.823, 1.096) P=0.481	-	-	-	-	-	-
2	0.884 (0.753, 1.037) P=0.131	0.900 (0.770, 1.052) P=0.186	3.022 (2.642, 3.456)	-	-	-	-	16.14
3	0.861 (0.744, 0.996) P=0.044	0.891 (0.768, 1.033) P=0.125	2.820 (2.491, 3.193)	1.770 (1.565, 2.001)	-	-	-	9.09
4	0.851 (0.734, 0.986)	0.884 (0.762, 1.026)	2.618 (2.304, 2.975)	1.868 (1.648, 2.118)	0.624 (0.550, 0.709)	-	-	-7.22

	P=0.031	P=0.104						
5	0.835 (0.720, 0.968) P=0.017	0.859 (0.740, 0.998) P=0.047	2.563 (2.255, 2.914)	1.869 (1.649, 2.119)	0.608 (0.535, 0.691)	1.679 (1.302, 2.165)	-	3.99
6	0.835 (0.720, 0.968) P=0.016	0.858 (0.739, 0.996) P=0.045	2.565 (2.256, 2.916)	1.870 (1.650, 2.120)	0.607 (0.534, 0.690)	1.670 (1.295, 2.154)	1.093 (0.964, 1.238)	1.39

Odds ratios (95% CIs) from random effects logistic regression models accounting for ICU-level clustering (using the xtlogit command in STATA).