Supplementary Appendix

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

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Preventing surgical site infections in *Staphylococcus aureus* nasal carriers

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Methods

Enrolment

PCR and the application of the first dose of nasal ointment were done within 24 hours of admission. PCR results were reported to the researchers immediately after becoming available in the afternoon, and the first dose of nasal ointment was administered within one hour by the researcher. The subsequent doses of nasal ointment were applied by the patient himself if possible, or by nursing staff if the patient was unable to do so. The patient was instructed to use the soap with the next bathing; this could be either the same day or the next morning. Patients bathed themselves if possible, if they were unable to do so they were washed by nursing staff.

At time of enrolment, patient characteristics were collected, as listed in Table 1 (Baseline characteristics of the 917 randomized patients). A patient was considered immunocompromised if one or more of the following conditions were present: solid organ transplant; daily use of steroids; absolute neutrophil count of <500; HIV positive or AIDS patient; immunodeficiency syndrome.

Microbiology

For screening of *S. aureus* carriage, a dry sterile swab (Rayon Swab, Becton Dickinson) was rotated four times in both nares. The swab was placed in 100 microliters of saline and centrifuged. Part of the sample was processed for DNA extraction with the S.E.T.S. II kit (Roche Diagnostics, Almere, The Netherlands). DNA amplification and detection were performed with the LightCycler Staphylococcus kit® (For research use only, Roche Diagnostics, Almere, The Netherlands) as recommended by the manufacturer. A peak-height cut-off of the *S. aureus* meltingcurve was used to avoid false-positive

results. High numbers of *S. aureus* (>100 CFU in the sample) are detected reliably with this kit with a sensitivity of approximately 97%.

Ten microliters of the remaining sample was inoculated on a blood agar plate and incubated for 48 hours. After processing, the swab itself was placed in phenol-red mannitol salt broth (PHMB) and incubated for three days. If the directly inoculated blood agar plate remained negative, the PHMB was subcultured on a second culture plate. Identification of *S. aureus* was performed by a latex agglutination test (Slidex, Biomérieux, France).

Clinical specimens were processed according to standard microbiological methods. Strains were compared by pulsed-field gel electrophoresis analysis and evaluated according to standard criteria[1].

Statistical analysis

A group sequential analysis was conducted as a double triangular test[2] on the cumulative dataset by sequentially entering data in the statistical program PEST4 of each consecutively enrolled group of 100 patients[3]. Assumptions regarding the a priori incidence of healthcare-associated *S. aureus* infections, the expected effect size, the type I error rate, and power were identical to the original design of the study. The assumptions determine the boundaries of the triangular test, as depicted in Figure 2 of the manuscript. If the upper red boundary is crossed, the null hypothesis can be rejected and the trial stopped: there is a beneficial effect of the intervention therapy. If the lower red boundary is crossed, the placebo therapy is significantly more beneficial. If one of the purple interrupted lines is crossed, there is no significant difference between the intervention and the placebo therapy. Note that the inner green serrated boundaries act as a continuity correction, the so-called Christmas tree correction. These boundaries are the real stopping boundaries.

The relative risk for healthcare-associated *S. aureus* infections was calculated using PEST4, adjusted for multiple analyses on the same data and for immunocompromised state.

Differences in patient characteristics and outcomes between the two trial groups were analyzed by a Chi square, Mann Whitney U, *t* test, log-rank test or regression analysis with the statistical program SPSS 15.0.

Contributions

This study was designed by HAV, MCV, HFLW, JAJWK, CMJEV-G, AT, and AV. LGMB, AvB, DB, RR, and ATAB collected the data. All authors were involved in the analysis of the data, they all vouche for the data and the analysis and decided to publish the manuscript. The manuscript was written by LGMB with repeated reviews by all other authors.

Results

Outcomes

Figure 1 of the original article shows the study flow chart. Of those patients who did not meet the inclusion criteria, 85 were not able to start the intervention within 24 hours and 55 were expected to be admitted for less than four days. Other reasons for exclusion were an inability to speak Dutch (10), randomization in previous admission period (4), lack of cooperation of nursing personnel (4), uncertainty about source of the swab (2), uncertainty about the effect of medication a patient with porphyria (1), and unknown reasons (26).

Table 1 shows the baseline characteristics for the 917 patients analyzed in the intention-to-treat analysis. The following data are missing: Admission during month before current admission: No data available for 3 patients (1 in the mupirocin-chlorhexidine group, 2 in the placebo group) Diabetes mellitus type I or II: No data available for 2 patients (1 in the mupirocin-chlorhexidine group, 1 in the placebo group). Malignant condition: No data available for 1 patient (in the placebo group). Skin disease: No data available for 8 patients (3 in the mupirocin-chlorhexidine group, 5 in the placebo group). Antibiotic therapy during month before admission: No data available for 9 patients (4 in the mupirocin-chlorhexidine group, 5 in the placebo group).

Table A of this Appendix shows the primary and secondary outcomes in all patients, and in surgical and non-surgical patients separately. Table B shows the primary outcome for different types of surgery and the numbers of perioperative antibiotic prophylaxis used.

Table C shows the adverse events reported in this trial.

	Mupirocin and Chlorhexidine	Placebo treatment	RR (95% CI)	p-value
All patients (n=917)	**n=504**	**n=413**		
Healthcare-associated *S. aureus* infections – no (%)	17 (3.4)	32 (7.7)	0.42 (0.23-0.75)	
All-cause mortality – no (%)	13 (2.6)	13 (3.1)	0.82 (0.37-1.78)	
Mortality among patients with *S. aureus* infection – no (%)	1 (5.9)	3 (9.4)	0.60 (0.06-6.30)	
Duration of hospitalization – median (IQR)	9 (7-12)	10 (7-15)		0.08
Duration of hospitalization – mean	12.2	14.0		0.04
Surgical patients (n=808)	**n=441**	**n=367**		
Healthcare-associated *S. aureus* infections – no (%)	16 (3.6)	31 (8.4)	0.41 (0.22-0.76)	
All-cause mortality – no (%)	7 (1.6)	12 (3.3)	0.48 (0.19-1.23)	
Mortality among patients with *S. aureus* infection – no (%)	1 (6.3)	3 (9.7)	0.62 (0.06-6.51)	
Duration of hospitalization – median (IQR)	9 (7.5-12)	10 (7-14)		0.04
Duration of hospitalization – mean	11.8	14.0		0.01
Non-surgical patients (n=109)	**n=63**	**n=46**		
Healthcare-associated *S. aureus* infections – no (%)	1 (1.6)	1 (2.2)	0.73 (0.04-11.92)	
All-cause mortality – no (%)	6 (9.5)	1 (2.2)	4.74 (0.55-40.78)	
Mortality among patients with *S. aureus* infection – no (%)	0 (0.0)	0 (0.0)		
Duration of hospitalization – median (IQR)	11 (6-20)	10.5 (5.75-19.25)		0.58
Duration of hospitalization – mean	15.2	14.0		0.63

Table A. Outcomes of the intention-to-treat analysis for the use of mupirocin and chlorhexidine versus placebo for healthcare-associated *S. aureus* infections, all cause mortality and duration of hospitalization.

Outcomes are shown for all patients included as well as for surgical and non-surgical patients separately. IQR: Interquartile range

	Mupirocin and Chlorhexidine		Placebo		RR (95% CI)
All surgical patients (n=808) (No. of *S. aureus* infections / No. of patients)	16/441	(3.6%)	31/367	(8.4%)	0.41 (0.22-0.76)
Cardiothoracic surgery (n=391)	3/220	(1.4%)	15/171	(8.8%)	0.14 (0.04-0.51)
Cephalosporin	3/172		10/117		
Cephalosporin + Aminoglycoside	0/40		5/48		
Cephalosporin + Penicillin + clavulanate acid	0/1		0/1		
Cephalosporin + Penicillin + clavulanate acid + Aminoglycoside	0/0		0/1		
Cephalosporin + Vancomycin	0/1		0/0		
Cephalosporin + Aminoglycoside + Vancomycin	0/0		0/2		
Vancomycin	0/1		0/1		
Aminoglycoside	0/1		0/0		
Aminoglycoside + Vancomycin	0/2		0/1		
No prophylaxis	0/2		0/0		
Orthopedics (n=172)	1/85	(1.2%)	4/87	(4.6%)	0.25 (0.03-2.26)
Cephalosporin	1/83		3/79		
No prophylaxis	0/2		1/8		
Vascular surgery (n=95)*	7/53	(13.2%)	6/42	(14.3%)	0.91 (0.28-2.96)
Cephalosporin	4/34		5/24		
Cephalosporin + Metronidazole	0/1		0/1		
Cephalosporin + Metronidazole + Penicillin + clavulanate acid	0/1		0/0		
Cephalosporin + Vancomycin	0/1		0/0		
Cephalosporin + Rifampicin	0/0		0/1		
Penicillin + clavulanate acid	0/1		0/0		
Aminoglycoside	0/1		0/0		
Rifampicin	0/1		0/0		
Quinolone	0/1		0/0		
No prophylaxis	2/11		1/14		
Gastrointestinal surgery (n=43)†	2/22	(9.1%)	3/21	(14.3%)	0.60 (0.09-4.01)
Cephalosporin	0/2		1/2		
Cephalosporin + Metronidazole	1/13		2/15		

Cephalosporin + Metronidazole + Penicillin	0/1		0/0		
Cephalosporin + Metronidazole + Penicillin + clavulanate acid	0/0		0/1		
Penicillin + clavulanate acid + Aminoglycoside	0/1		0/0		
No prophylaxis	1/4		0/2		
General surgery (n=107)‡	3/61	(4.9%)	3/46	(6.5%)	0.74 (0.14-3.85)
Cephalosporin	0/19		2/19		
Cephalosporin + Penicillin	0/1		0/0		
Cephalosporin + Penicillin + clavulanate acid	0/1		0/0		
Cephalosporin + Penicillin + Aminoglycoside	0/1		0/0		
Cephalosporin + Metronidazole	0/8		1/7		
Cephalosporin + Penicillin + Metronidazole	1/1		0/0		
Penicillin + clavulanate acid	0/2		0/2		
Cephalosporin + Sulphonamide + Trimethoprim	0/0		0/2		
Cephalosporin + Sulphonamide + Trimethoprim + Macrolide	0/0		0/1		
No prophylaxis	2/28		0/13		

Table B. Healthcare-associated *S. aureus* infections in patients undergoing surgery, per type of surgery and perioperative antibiotic prophylaxis used

* No data available for 2 patients who underwent vascular surgery and received placebo

† No data available for 1 patient who underwent gastrointestinal surgery and received placebo, and for 1 patient who received mupirocin/chlorhexidine

‡‡ No data available for 2 patients who underwent general surgery and received placebo

Microbiology

The infection rate due to microorganisms other than *S. aureus* among patients treated with mupirocin and chlorhexidine was 11%, versus 12% in patients receiving placebo (p=0.59).

All colonizing and infecting strains were compared using pulsed-field gel electrophoresis (PFGE). No nosocomial outbreaks were identified. However, serial cross-infection from a common source, e.g. a persistent *S. aureus* carrier among health care workers, or contamination of the environment, affecting a few patients over a period of several months may occasionally have occurred.

Adverse events

	Mupirocin	Chlorhexidine	Placebo nasal ointment	Placebo soap
	n=504		n=413	
Adverse event				
Itching of the skin – no (%)		4 (0.8)		4 (1.0)
Itching of the nose – no (%)	2 (0.4)		2 (0.5)	
Epistaxis – no (%)	1 (0.2)			
Nausea – no (%)			1 (0.2)	
Irritation of the skin – no (%)		1 (0.2)		1 (0.2)
Irritation of the nose – no (%)	1 (0.2)			
Total	4 (0.8)	5 (1.0)	3 (0.7)	5 (1.2)

Table C. Adverse events from the use of study medication.

No serious adverse events were reported. All events resolved after discontinuation of treatment.

References

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