Antibiotic surgical prophylaxis increases nasal carriage of antibiotic-resistant staphylococci

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Staphylococci are a significant cause of hospital-acquired infection. Nasal carriage of Staphylococcus aureus is an important risk factor for infection in surgical patients and coagulase-negative staphylococci (CNS) are a major cause of prosthetic joint infections. The impact that antibiotic surgical prophylaxis has on the nasal carriage of staphylococci has not been studied. Daily nasal swabs were taken from 63 patients who received antibiotic surgical prophylaxis and 16 patients who received no antibiotics. Total aerobic bacterial count, S. aureus and CNS were enumerated by culture from nasal swabs. Representative isolates were typed by staphylococcal interspersed repeat units (SIRU) typing and PFGE, and MICs to nine antibiotics were determined. After antibiotic administration, there was a reduction in S. aureus counts (median $-2.3 \log_{10} \text{c.f.u. ml}^{-1}$) in 64.0% of S. aureus carriers, compared with only a 0.89 $\log_{10} \text{c.f.u. ml}^{-1}$ reduction in 75.0% of S. aureus carriers who did not receive antibiotics. A greater increase in the nasal carriage rate of meticillin-resistant CNS was observed after antibiotic surgical prophylaxis compared with hospitalization alone, with increases of 16.4 and 4.6%, respectively. Antibiotic-resistant S. epidermidis carriage rate increased by 16.6% after antibiotic administration compared with 7.5% with hospitalization alone. Antibiotic surgical prophylaxis impacts the nasal carriage of both S. aureus and CNS.

INTRODUCTION

Staphylococci are important pathogens that cause healthcare-associated infections and were responsible for 21.3% of all healthcare-associated infections in a UK survey in 2011 (Health Protection Agency, 2012). Endogenous spread of staphylococci is a potential source of infection in surgical patients, with antibiotic surgical prophylaxis being administered to reduce the risk of postoperative surgical-site infection (SSI).

Antibiotic administration is known to cause changes to the resident microbial communities of an individual (Sullivan et al., 2001). However, only two studies have investigated the impact of antibiotics on the microbial community of the nose, both using extended courses of antibiotics (White et al., 1959; Aly et al., 1970). White et al. (1959) treated 10 female long-term hospitalized patients with tetracycline for 14 days and observed a 2.8 log decrease in nasal counts of staphylococci. Aly et al. (1970) observed a similar response when 17 healthy male volunteers received the first-generation cephalosporin, cephalaxin, for 12 days.

Due to increasing antibiotic resistance and changing practice, the use and type of antibiotic agents used for antibiotic surgical prophylaxis have changed. Most guidance now advocates the use of either a single dose or continuation for $<24$ h (Bratzler et al., 2013). The antibiotic agent used for surgical prophylaxis varies between surgical specialties and procedures. There have been no studies specifically investigating the impact of antibiotic surgical prophylaxis...
prophylaxis on staphylococci of the nose. Only one study, >25 years ago, investigated the effects of antibiotics on nasal staphylococci during hospitalization and demonstrated that the use of antibiotics increases antibiotic-resistant coagulase-negative staphylococci (CNS) in the nose (Powell & Sanderson, 1987). In addition, the emergence of antibiotic-resistant CNS on the skin of patients undergoing cardiac (Archer & Tenenbaum, 1980; Archer & Armstrong, 1983) and vascular surgery (Terpstra et al., 1999) has been observed after receiving antibiotic surgical prophylaxis.

The aim of this descriptive microbiological study was to establish the impact of a number of modern surgical prophylaxis regimens on the nasal carriage of staphylococci and their resistance to antibiotics and hence to understand the potential infection risk to patients.

METHODS

Study design, hospital setting and population. A longitudinal observational study was carried out to determine the effect of surgical prophylaxis on the nasal staphylococci of hospital inpatients. Patients over the age of 18 were recruited at random via written consent within 24 h of hospitalization to one of the seven study wards at the Heart of England NHS Foundation Trust. Of the seven wards, six were surgical and one cardiac. The surgical wards covered four surgical specialties: gastroenterology, thoracic, urology, and trauma and orthopaedic. Ethical approval for the study was obtained from the National Research Ethics Service Committee West Midlands (08/H1206/133).

Patient sampling and culture-dependent enumeration. Daily nasal samples were taken using a flocked swab with Liquid Amies transport medium (Sterilin). Swabs were vortexed for 10 s in 1 ml of transport medium and 100 µl neat sample was plated onto Columbia Blood Agar (Oxoid). A 10-fold serial was dilution (10⁻², 10⁻⁴) of the nasal sample was performed and 100 µl of each serial dilution was plated out on to a series of plates that were incubated at 37 °C and read at 48 h. The c.f.u. ml⁻¹ values were calculated for total aerobic bacterial count (TBC) from Columbia Blood agar plates (Oxoid), Staphylococcus aureus from Baird Parker agar (Oxoid), meticillin-resistant (MR) S. aureus from Brilliance MRSA agar plates (Oxoid) and CNS from Staph/Strep agar (Oxoid). Multiple representative CNS isolates, based on enumerated number of CNS colonies (five for > 50 colonies, three for 10–50 and one for > 10 colonies) were stored at −80 °C.

Identification of CNS species and meCA carriage. DNA was extracted from CNS isolates as described previously (Kumari et al., 1997) and used as template in two multiplex PCRs to identify six common staphylococcus species. The first PCR used three primer pairs targeting the meCA gene to identify Staphylococcus epidermidis (124 bp) (Martineau et al., 1996) and Staphylococcus haemolyticus (270 bp) (Schuenck et al., 2008) using previously published primers, and Staphylococcus hominis (230 bp) using Shom_F 5'–ATGGGGAAGCTTGGACAGGC–3' and Shom_R 5'-TGCTCGGCTATTAGGTTGTTG-3' primers designed for this study. The second PCR used three previously described primer pairs to identify Staphylococcus capitis (525 bp), Staphylococcus lugdunensis (695 bp) and Staphylococcus saprophyticus (843 bp) (Hirotaki et al., 2011) by amplifying different size products of the nuc gene. These CNS isolates not specified by PCR were identified using a MALDI Biotyper system (Bruker Dal-}

tonics). All CNS isolates were screened by PCR for carriage of the meCA gene (Pérez-Roth et al., 2001).

Molecular typing and antibiotic sensitivity of staphylococci. A single representative isolate of S. aureus and S. epidermidis from each patient on day 1 and day 3 were selected at random for antibiotic sensitivity testing and molecular typing. If a representative isolate was not present on day 3, an isolate from day 4 or 5 was selected. A single representative isolate of other CNS species isolated from patients on day 1 and day 3 underwent antibiotic sensitivity testing.

The MICs for nine antimicrobial agents (ciprofloxacin, clindamycin, erythromycin, gentamicin, linezolid, mupirocin, teicoplanin, ticacycline and vancomycin) were determined by an agar dilution method (Andrews, 2001) using British Society of Antimicrobial Chemotherapy (2014) MIC breakpoints.

Staphylococcal interspersed repeat unit (SIRU) polymorphisms were used to type S. aureus isolates (Hardy et al., 2006). S. epidermidis isolates were typed by PFGE (Cookson et al., 2007). A dendrogram was created in BioNumerics 7.1 (Applied Maths) using Dice coefficient and UPGMA (unweighted pair group method with arithmetic mean), with 0.8 % optimization and 1.3 % tolerance applied. Using a 79 % similarity cut-off, groups of two or more isolates were assigned to a PFGE cluster (Miragaia et al., 2008).

Statistical analysis. Hierarchical linear regression modelling of the logarithm of TBC and the independent variables, antibiotic and the day since hospital admission, were used to establish whether there was an association between antibiotic surgical prophylaxis and TBC. Linear regression was used to assess whether there was significant change in the CNS counts between day 1 and day 3, between patients who received antibiotics and those that did not. The significances of both statistical tests were assessed by the likelihood ratio test, with a significance level of 5%. The χ²-test was used to establish whether the increase in antibiotic resistance in staphylococci from day 1 and day 3 was statistically significant. All statistical analysis was performed in Stata SEM 13.1 (StataCorp).

RESULTS

Study population and antibiotic regimens received

Of the 99 patients recruited to the study, 79 patients who had three consecutive nasal samples taken from day 1 were included in the analysis. The 79 patients were comprised 39 males and 40 females, with a median age of 68 years (range 37–91 years). The median length of stay was 4 days (range 3–21 days). Sixty-three patients received antibiotic surgical prophylaxis and 16 patients received no antibiotics. Six different antibiotic surgical prophylaxis regimens were administered over four surgical specialities. Five regimens were administered as a single dose intravenously on induction: co-amoxiclav (1.2 g) (n=25), co-amoxiclav (1.2 g) and gentamicin (160 mg) (n=12), teicoplanin (400 mg) (n=6), teicoplanin (400 mg) and gentamicin (160 mg) (n=3), and gentamicin (160 mg) and metronidazole (500 mg) (n=5). Flucloxacinil (1 g) and gentamicin (160 mg) intravenously was administered in three doses: a single dose intravenously on induction and two further
doses of flucloxacillin (1 g) intravenously postoperatively at 6 h intervals (n=12).

**Effect of antibiotic surgical prophylaxis on TBC**

Nasal TBC varied on admission from $1.6 \times 10^2$ to $8.7 \times 10^3$ c.f.u. ml$^{-1}$ (median $2.4 \times 10^4$ c.f.u. ml$^{-1}$). In patients who received no antibiotics, there was patient-to-patient variation in TBC counts. However, within a patient, the TBC remained stable during their hospital stay for the majority of patients (12/16, 75%), varying only by $\pm 0.3 \log_{10}$c.f.u. ml$^{-1}$ in an individual’s nose between day 1 and day 3. In patients that received antibiotics, there was substantial patient-to-patient variation in the change in TBC between day 1 and day 3 (range $-3.13$ to $+2.25 \log_{10}$c.f.u. ml$^{-1}$), with half of patients having an increase in TBC and half having a decrease in TBC. The majority of patients (40/59, 67.8%) had a TBC change of $\pm 1.0 \log_{10}$c.f.u. ml$^{-1}$, with 20.3% (12/59) changing between $\pm 1.0$ and $2.0 \log_{10}$c.f.u. ml$^{-1}$, and 10.2% (6/59) between $\pm 2.0$ and $3.0 \log_{10}$c.f.u. ml$^{-1}$. Multilevel modelling of TBC showed that the pattern of the $\log_{10}$c.f.u. ml$^{-1}$ changes across consecutive days of hospital admission differed significantly between antibiotic regimens ($P=0.02$).

**Effect of antibiotic surgical prophylaxis on S. aureus nasal carriage**

Thirty-six patients carried *S. aureus* (33 positive on admission), with counts ranging from $6.0 \times 10^1$ to $5.53 \times 10^3$ c.f.u. ml$^{-1}$ (median $1.00 \times 10^4$ c.f.u. ml$^{-1}$). In both *S. aureus* carriers who received antibiotics and those that did not, some individual’s *S. aureus* counts increased, whereas others decreased (Fig. 1). A reduction in the *S. aureus* $\log_{10}$c.f.u. ml$^{-1}$ between day 1 and day 3 was observed in 64.0% (16/25) of patients who received antibiotics and 75.0% (6/8) who did not receive antibiotics. In those patients where a reduction in *S. aureus* was observed, a median reduction of $-2.3 \log_{10}$c.f.u. ml$^{-1}$ occurred after antibiotics, as opposed to only a median of $-0.89 \log_{10}$c.f.u. ml$^{-1}$ decrease in those who did not receive antibiotics. In 10 patients (38.5%), after receiving antibiotics, *S. aureus* was undetected by culture for ≥1 day during their hospitalization.

All *S. aureus* strains were meticillin-sensitive. Of the *S. aureus* carriers’ positive on admission, 90.9% (30/33) carried fully sensitive strains and three patients carried strains resistant to only erythromycin. Three patients acquired fully sensitive *S. aureus* strains during hospitalization.

The *S. aureus* strains colonizing the 33 patients on admission were diverse with 32 different SIRU profiles identified; only two patients were colonized with indistinguishable strains (Fig. S1 and Table S1, available in the online Supplementary Material). Twenty-nine patients had *S. aureus* isolated on day 1 and day 3, with 28 patients being colonized with an indistinguishable strain on both days. The antibiotic sensitivity profile and MIC values to all nine antibiotics in any of the *S. aureus* strains did not change between day 1 and day 3. Only one patient who received no antibiotics carried a different strain on day 1 (2 3 5 × 8 5 2) to day 3 (1 2 2 × 9 3 2). The three *S. aureus* strains acquired during hospitalization had different SIRU profiles from each other and from profiles observed on admission.

**Effect of antibiotic surgical prophylaxis on CNS nasal carriage**

**CNS counts.** On admission, 96.2% (76/79) of patients carried CNS, with counts ranging from $1.0 \times 10^1$ to $8.4 \times 10^5$ c.f.u. ml$^{-1}$ (median $4.0 \times 10^5$ c.f.u. ml$^{-1}$). CNS counts altered between day 1 and day 3 in patients who received antibiotics and those that did not, with CNS increasing in some patients and reducing in others (Fig. 2). Even though the magnitude of the change in CNS in patients who received antibiotics (range $-3.02$ to $+3.64$, median $+0.38$) was larger than in patients who received no antibiotics (range $-0.94$ to $+2.60$, median $-0.06$), the difference was not statistically significant ($P=0.8$).

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**Fig. 1.** Change in *S. aureus* $\log_{10}$c.f.u. ml$^{-1}$ counts between day 1 and day 3 following hospital admission for individual patients who received antibiotics (black bars) and patients who did not receive antibiotics (shaded bars). Antibiotic regimens are indicated by a letter: (A) co-amoxiclav, (B) co-amoxiclav and gentamicin, (C) flucloxacillin and gentamicin, (D) teicoplanin, (E) teicoplanin and gentamicin, and (F) gentamicin and metronidazole.
CNS species diversity. Ten CNS species were identified from study patients. On admission, 60% of all patients carried only a single CNS species, 36.7% carried two species and a minority carried three (9.3%) or no (4%) CNS species. CNS diversity remained stable between day 1 and day 3 in 59.3% (35/59) of patients who received antibiotics and 56.3% (9/16) of patients who did not. After antibiotic administration, 22.0% (13/59) of patients decreased to a single species and 18.6% (11/59) of patients increased by a single CNS species. S. epidermidis was the dominant CNS species carried by all patients, and was present on admission and persisted throughout hospitalization. After the administration of antibiotics, there was no clear trend for either acquisition or loss of different CNS species in patients (Table 1). Continued carriage of all CNS species except S. epidermidis was low after receiving antibiotics. Acquisition of S. haemolyticus and S. hominis occurred in patients who had received antibiotics (n=2 and n=3, respectively) and those who had not (n=3 and n=1, respectively). Carriage of Staphylococcus warneri (n=2) and Staphylococcus caprae (n=1) was lost after the administration of antibiotics.

Antibiotic resistance in CNS. S. epidermidis isolates carried in the nose on day 1 were predominantly fully sensitive to antibiotics in both patients who received antibiotics (73.1%) and those patients who did not (61.5%). On day 3, the percentage of isolates resistant to antibiotics increased by 16.6% after the administration of antibiotics as compared with only a 7.5% increase with no antibiotic administration. Cefoxitin resistance in S. epidermidis isolates increased from 6/60 isolates to 23/60 isolates after antibiotic administration, an increase of 28.4% which was statistically significant ($P>0.01$).

Increases in ciprofloxacin, gentamicin and mupirocin resistance (10, 3.3 and 6.7% of isolates, respectively) were also observed after the administration of antibiotics, but were not statistically significant. The number of isolates resistant to three or more antibiotics increased from 3.3% (2/60) on admission to 11.7% (7/60) on day 3 after the administration of antibiotics, but this was not statistically significant ($P=0.08$). In contrast, in patients who received no antibiotics, antibiotic resistance remained largely unchanged.

The antibiotic sensitivity of 54 isolates from nine other CNS species present in patients on day 1 and day 3 were determined. Thirty-four isolates (62.9%) were fully sensitive to all antibiotics tested, and included isolates from seven out of the nine CNS species. All isolates from two CNS species (Staphylococcus pettenkoferi and S. caprae) were fully sensitive. The most frequently observed antibiotic resistance was to erythromycin, present in six CNS species (S. capitis, S. lugdunensis, S. hominis, S. haemolyticus, Staphylococcus cohnii and Staphylococcus simulans). Cefoxitin resistance was observed in three other CNS species (S. hominis, S. haemolyticus and S. simulans). Resistance to three or more antibiotics was observed in two S. haemolyticus isolates and one S. hominis isolate, all three of which were acquired on day 3. One S. haemolyticus isolate, which was resistant to four antibiotics (cefoxitin, ciprofloxacin, erythromycin and gentamicin), and one S. hominis isolate, which was resistant to five antibiotics (cefoxitin, ciprofloxacin, erythromycin, gentamicin and mupirocin), were acquired by different patients after receiving antibiotics. A further patient who received no antibiotics acquired the other S. haemolyticus isolate, which was resistant to seven antibiotics (cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, mupirocin and teicoplanin).

The increase in the percentage of patients carrying MRCNS was greater after the administration of antibiotics than after receiving no antibiotics, with increases of 16.4 and 4.6%, respectively. The increase in MRCNS after administration of antibiotics was not statistically significant ($P=0.07$). The largest increase in the percentage of patients carrying MRCNS was after the administration of flucloxacillin and gentamicin, with an increase of 37.8% (from 16.7 % on day 1 to 54.5 % on day 3). By contrast, after the administration of co-amoxiclav alone only a 6.1% increase in the percentage of patients carrying MRCNS from 41.7% on day 1 was observed. By day 7, MRCNS carriage rate had increased by 30.4% after antibiotic administration. Meticillin resistance was principally present in S. epidermidis. The percentage of patients carrying MR S. epidermidis increased from 27.9 % on admission to 44.8% on day 3 after antibiotic administration, and the increase was statistically significant ($P=0.05$).

S. epidermidis nasal carriage. A total of 146 S. epidermidis isolates from day 1 and day 3 from 73 patients (60 received antibiotics, 13 no antibiotics) were
analysed by PFGE. Thirty-two PFGE clusters and 30 singletons were identified (Fig. S2). Five out of 13 patients (38.5%) who received no antibiotics carried the same PFGE type on day 1 and day 3, compared with only 18.3% of patients (11/60) after receiving antibiotics. Fourteen of the 16 (87.5%) patients that carried the same PFGE type on day 1 and day 3 had the same antibiotic sensitivity profile with no change in MIC values. In two patients who received antibiotics, an increase in resistance to one antibiotic was observed: one became resistant to cefoxitin and the other resistant to mupirocin. Of the 57 patients that changed PFGE type between day 1 and day 3, the antibiotic resistance observed from that of day 1 changed in 31/57 (54.4%), with 22/31 (71%) increasing in resistance (Fig. 3). One patient who received no antibiotics acquired a mecA-positive isolate on day 3, in contrast to 17 who received antibiotics. mecA-positive isolates acquired from patients who received antibiotics consisted of strains from 10 different PFGE clusters.

**Table 1.** Distribution of CNS species in the nose on day 1 and day 3 for patients who received antibiotics and those patients that received no antibiotics. $n=$ number of patients

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<thead>
<tr>
<th>Antibiotics received</th>
<th>No antibiotics received</th>
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<td><strong>CNS species</strong></td>
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<td><em>Staphylococcus epidermidis</em></td>
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<td><em>S. simulans</em></td>
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**DISCUSSION**

This study is the first to demonstrate that hospitalization alone has minimal impact in the staphylococci of the nose. The nasal TBC remained unchanged, and there was only a minimal change in CNS diversity and increase in the antibiotic resistance in patients. This contrasts with the findings of a previous study in which a decrease in CNS species diversity and increased antibiotic resistance in CNS in the nose was reported after 7 days on a surgical intensive care unit (Thurn et al., 1992); however, these
patients will have received antibiotics and this was not taken into account. We found administration of antibiotic surgical prophylaxis was associated with a small decrease in a patient's nasal TBC. A larger 3 log reduction in TBC in a previous study was reported, but this was after administration of 12 days of cephalexin as opposed to a single dose as in the current study (Aly et al., 1970).

*S. aureus* counts were substantially reduced by antibiotic administration; however, despite these reductions complete eradication of *S. aureus* was not achieved in the majority of patients. Therefore, although the risk of postoperative infection is reduced due to a lower number of organisms being present, without eradication there is still a potential source of infection and risk of SSI in surgical patients. These findings may explain the observations of Bode et al. (2010), who reported an *S. aureus* SSI rate of 8.4 % in surgical patients who received surgical prophylaxis. However, when surgical prophylaxis was used in combination with *S. aureus* decolonization with mupirocin and chlorhexidine prior to surgery the *S. aureus* SSI rate reduced to 3.6 %.

The carriage of MRCNs increased after the administration of antibiotic surgical prophylaxis – an increase that was far greater than that seen in patients who received no antibiotics. An Italian study reported a significant increase in MR *S. epidermidis* in patients receiving surgical prophylaxis (for a mean of 4.5 days) from 3.8 % carriage on admission, increasing to 7.5 % by day 7 and 15.1 % by day 14 (Esposito et al., 2003). Our study shows a higher MR *S. epidermidis* rate on admission, and an increase between day 1 and 3, which was statistically significant, that was greater than that reported previously by Esposito et al. (2003) by day 14. The increase in carriage of antibiotic-resistant *S. epidermidis* was greater in patients who received antibiotics than in patients who received no antibiotics. Powell & Sanderson (1987) reported that after at least 1 week in hospital and receiving antibiotic treatment, carriage of antibiotic-resistant CNS in the nose was observed in 73 % of patients as compared with only 32 % of patients who received no antibiotics.

The rapid emergence of resistant CNS on the skin after receiving 72 h of cephalosporin surgical prophylaxis has been reported (Terpstra et al., 1999). Although *S. epidermidis* is less pathogenic than *S. aureus*, there is still a potential risk of SSI, with infections caused by antibiotic-resistant strains being more difficult to treat. With the increase in nasal carriage of antibiotic-resistant *S. epidermidis* the reservoir of antibiotic resistance genes that could potentially transfer into other species, *S. aureus* in particular, is increased. Horizontal transfer of meticillin resistance from *S. epidermidis* to *S. aureus* in a neonatal patient who received antibiotics has been reported (Wielders et al., 2001; Bloemendaal et al., 2010). CNS, including *S. epidermidis*, are the most common cause of prosthetic joint infections; as well as the skin, the nose is a potential source of postoperative infection (Tande & Patel, 2014). Also with the acquisition of *S. haemolyticus* and *S. hominis* there is potential for opportunistic infection such as endocarditis, with multi-antibiotic-resistant strains being more difficult to treat (Becker et al., 2014).

To the best of our knowledge, this is the first study of the impact of antibiotic surgical prophylaxis regimens on nasal carriage of staphylococci. Due to studying multiple antibiotic surgical prophylaxis regimens, a limitation of this study is its small sample size and therefore further study into specific antibiotic regimens used for specific surgical procedures is needed.

In conclusion, this is the first study to demonstrate that current surgical prophylaxis regimens alter the staphylococci of the nose. Surgical prophylaxis reduces *S. aureus* counts in the nose and increases the nasal carriage of *S. epidermidis* strains with increased antibiotic resistance.

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**REFERENCES**


