

Characterisation of *Staphylococcus aureus* nasal and skin carriage among patients undergoing haemodialysis treatment

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SUMMARY

The aim of the study was to investigate the rate of *Staphylococcus aureus* nasal and skin carriage in patients undergoing haemodialysis. The cultured staphylococcal isolates were subsequently characterized by molecular methods. The study group comprised 43 haemodialysed patients from whom nasal and skin swabs from the vascular access sites were collected. The identification of staphylococcal isolates and antibiotic susceptibility testing were performed on the basis of conventional diagnostic procedures. The staphylococci were further characterized using Pulsed-Field Gel Electrophoresis (PFGE). *S. aureus* was cultured from 12 (27.9%) patients. Only one (8.3%) patient was colonized with the microorganism both in the anterior nares and the vascular access site representing a single strain, as evidenced by PFGE analysis. Antibiotic susceptibility testing identified one (7.6%) methicillin-resistant *S. aureus* (MRSA) strain. PFGE typing identified several *S. aureus* genotypes with the lack of one specific strain responsible for colonization. However, it should be noted that among two (A and D) PFGE patterns genetically indistinguishable and closely related isolates (two isolates for each pattern) were identified. The obtained results revealed a relatively low rate of *S. aureus* carriage accompanied by low methicillin resistance rate and a significant genetic diversity of cultured isolates with the lack of one predominant strain responsible for colonization.

KEY WORDS: *Staphylococcus aureus*, nasal and skin carriage, haemodialysis

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INTRODUCTION

Staphylococcus aureus is a pathogen isolated from haemodialysed patients most frequently (Kluytmans *et al.*, 1997; Chow & Yu, 1989) and it has been reported that a large majority of infections in this clinical setting are of endogenous origin (Kluytmans *et al.*, 1997; Ena *et al.*, 1994;

Nouwen *et al.*, 2001). Factors responsible for the increased risk of invasion by infectious agents among renal unit patients include decreased immunity, defective mucocutaneous barriers, multiple needle punctures over the vascular access site required for dialysis, and the presence of prosthetic devices that disrupt the normal host barriers and give direct access to normally sterile body sites, but also provide a site of colonization to which staphylococci are well adapted (Peacock *et al.*, 2002; Chow & Yu, 1989; Goldblum & Reed, 1980; Kluytmans *et al.*, 1997; Nouwen *et al.*, 2001). The infections caused by *S. aureus* have been considered to originate from areas of impaired skin as a result of bacterial

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spread from anterior nares since colonization of this ecological niche appears to play a key role in the epidemiology and pathogenesis of invasive infections (Kluytmans *et al.*, 1997; Nouwen *et al.*, 2001; von Eiff *et al.*, 2001; Costa *et al.*, 2004). It has also been suggested that *S. aureus* may cause clinically significant bacteremia in certain groups of patients as a result of translocation from colonized mucosa directly to the bloodstream (Costa *et al.*, 2004).

Compounding the problem of diseases caused by this microorganism is the emergence and global spread of methicillin-resistant *S. aureus* (MRSA) (Crisóstomo *et al.*, 2001) and the striking ability of MRSA to acquire resistance against other classes of antimicrobial agents including vancomycin (Hiramatsu *et al.*, 2002; Lowy 2003; Hiramatsu *et al.*, 2001). Hence, screening of haemodialysed patients for *S. aureus* nasal carriage supported by molecular typing methods characterized by high discriminatory power and the ability to differentiate among strains cultured from multiple sources in one patient (von Eiff *et al.*, 2001) is essential to prevent the development of infectious complications but also to investigate the origin of invasive clones. The aim of the present study was to evaluate *S. aureus* nasal colonization rate among patients on haemodialysis followed by characterization of these isolates by molecular methods.

MATERIALS AND METHODS

Patients

Nasal and vascular site access swabs were collected from 43 haemodialysed patients in February 2004. The patients ranged in age between 30 and 79 years (mean age: 53.3 ± 11.82 years). Duration of dialysis treatment at the time of swab collection ranged from 5 months to 27 years (mean: 136.76 ± 104.99 months). Nineteen (44.1%) patients had a history of previous immunosuppression due to kidney transplantation (9 patients) or glomerulonephritis (10 patients). Eight (18.6%) patients suffered from diabetes mellitus, one patient (2.3%) had malignancy (true polycythaemia). Moreover, 25 (58.1%) patients were infected with the hepatitis C virus (HCV), whereas 7 patients in this group were additionally co-infected with hepatitis B

virus (HBV). All patients had an endogenous arteriovenous fistula as a vascular access site.

Nasal and skin swabs

All patients included in the study were screened for *S. aureus* colonization in their anterior nares. Additionally, cultures were also taken from the skin overlying the insertion site. The specimens were immediately streaked on blood agar and mannitol/salt agar (MSA) and incubated at 37°C for up to 48 hours. Preliminary identification of isolates resembling staphylococci was performed on the basis of standard microbiological procedures such as colonial morphology, cultural characteristics on blood agar and MSA, Gram-reaction, catalase and coagulase tests. Staphylococcal isolates were further identified to the species level using the API STAPH system according to manufacturer's instructions (bioMérieux, France).

Antibiotic sensitivity test

Susceptibility testing was performed on cultured isolates using the agar disc diffusion method according to recommendations given by the National Reference Centre for Antimicrobial Susceptibility Testing in Poland (NRCST) (Hryniewicz *et al.*, 2004).

The antibiotics used in the disc diffusion method included oxacillin (1 µg), penicillin (10 U), tetracycline (30 µg), erythromycin (15 µg), lincomycin (15 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), mupirocin (200 µg), mupirocin (5 µg), fusidic acid (10 µg), vancomycin (30 µg), and teicoplanin (30 µg).

Pulsed-field gel electrophoresis (PFGE)

The PFGE typing of SmaI digested DNA was performed by a modification of a previously described method (Bannerman *et al.*, 1995; Leonard *et al.*, 1995). Briefly, each tube containing 250 µl of NET Buffer (10 mM Tris, 1 mM EDTA, 10 mM NaCl) was inoculated with the relevant bacterial culture. The suspension was then mixed with 200 units of achromopeptidase (Sigma, UK) and an equal volume of 2% SeaPlaque Agarose (Flowgen, UK) cooled to 50°C. Agarose/cell mixture was immediately loaded into block molds (Bio-Rad Laboratories Ltd, UK) and allowed to solidify at 4°C. Solidified blocks were subse-

quently dispensed into appropriate test tubes containing lysis buffer (6mM Trizma base, 100mM EDTA, 1M NaCl, 0.5% Brij 58, 0.2% sodium deoxycholate, 0.5% lauroyl sarcosine) and incubated in a waterbath at 50°C for 60 min. After incubation the lysis buffer was removed from the tubes and the blocks were washed three times for 10 min at 50°C each in TE buffer (10 mM Trizma base, 1 mM EDTA). One quarter of each agarose block was then digested with SmaI (10 units/ μ l) at 30°C for 3 hours and loaded into the wells of 1% PFGE certified agarose gel [Bio-Rad Laboratories Ltd, UK]. Electrophoresis was performed in 0.5 x TBE buffer (44.5 mM Trizma base, 44.5 mM boric acid, 1 mM EDTA) [Biowhittaker, UK] by the contour-clamped homogenous electric field method with a CHEF system [Bio-Rad Laboratories Ltd, UK]. Fragments were separated with a linear ramped pulse time of 6.8-63.8 s for 23 hours at 14°C. Gels were subsequently stained with ethidium bromide solution (1 μ g/ml) for 30 min, visualized under UV and photographed. The PFGE results were interpreted according to Tenover *et al.* (1995) criteria.

Data analysis

Data were described as mean value \pm standard deviation (SD). The statistical significance of the difference between the duration of the haemodialysis treatment between the *S. aureus* carriers and non-carriers was evaluated using the t-test. The analysis was performed using statistical software (SigmaStat, version 2.03). $P < 0.05$ was regarded as significant.

RESULTS

S. aureus was cultured from the nares of 12 (27.9%) of the 43 haemodialysis patients. Only one patient (8.3%) was colonized with *S. aureus* both in the anterior nares and at the vascular access site.

The mean age of *S. aureus* nasal carriers was 48.08 ± 8.07 , whereas the mean age of patients, who were not found to be colonized with this microorganism was 55.35 ± 12.51 . There was not a statistically significant difference in the age between the carriers and non-carriers ($P = 0.7$). Among *S. aureus* nasal carriers, seven (58.3%)



FIGURE 1 - PFGE analysis of *S. aureus* isolates cultured from the anterior nares and the vascular access sites of haemodialysed patients. Lanes 1, 6, 13 - Standard *S. aureus* NCTC 8325; Lanes 2, 16 - type A; Lane 3 - type B; Lanes 4, 10 - type D; Lane 5 - type C; Lane 7 - type I; Lanes 8, 9 - type E; Lane 11 - type J; Lane 12 - type H; Lane 14 - type F (MRSA); Lane 15 - type G.

patients had a history of previous immunosuppression, three (25%) persons suffered from diabetes mellitus, and one (8.3%) patient had malignancy.

The mean time on dialysis treatment among *S. aureus* carriers at the time of swab collection was 100 ± 87.03 months (8.3 years), whereas five (41.6%) carriers were on haemodialysis for more than 10 years (mean 189.6 ± 49.03 months). The mean time of the haemodialysis treatment for non-carriers was 151 ± 109.10 months (12.5 years). There was not a statistically significant difference in the duration of haemodialysis treatment between the *S. aureus* nasal carriers and non-carriers ($P = 0.15$).

Antibiotic susceptibility testing identified only one (7.6%) MRSA strain. Methicillin-susceptible *S. aureus* (MSSA) isolates demonstrated high rates of resistance to penicillin (76.9%) and tetracycline (69.2%). One isolate was additionally resistant to trimethoprim/sulfamethoxazole. Moreover, both *S. aureus* isolates which were found to colonize the anterior nares and the vas-

cular access site of the patient had the identical antibiotic resistance pattern evidenced by their resistance to tetracycline.

S. aureus isolates were subsequently tested by PFGE (Figure 1). Since the aim of the study was to evaluate genetic relatedness between *S. aureus* isolates cultured from patients in the same hospital unit within a short (a month) time period any staphylococcal strain was assumed to be endemic if there were three or less band differences between isolates representing the strain. Any isolate with four or more band differences was classified as a different PFGE type. Hence, The PFGE analysis grouped the 13 *S. aureus* isolates into ten types. PFGE type A and D were each isolated from 2 patients. Type E was isolated from the nares and the vascular access site of another patient which indicated that the patient was colonized with a single *S. aureus* strain at both sites. All other types, including the MRSA, were isolated from single patients.

DISCUSSION

In the present study, 12 (27.9%) out of 43 studied patients carried *S. aureus* in their anterior nares. The carriage rate in this patient population was below rates reported by Kluytmans *et al.* (1997) (30.1-84.4%) and Wanten *et al.* (1998) (37%). Furthermore, we did not find a statistically significant association regarding the *S. aureus* carriage status and the duration of the haemodialysis treatment between the carriers and non-carriers ($P > 0.05$).

The susceptibility testing revealed a low methicillin resistance rate as only one (7.6%) isolate demonstrated this type of resistance. It must be emphasized, however, that in spite of reported higher rates of autoinfections following carriage of methicillin-resistant strains in comparison with methicillin-susceptible isolates (Kluytmans *et al.* 1997), the risk of invasive infections associated with the previous carrier state concerns both methicillin-resistant and susceptible microorganisms (Archer & Climo, 2001). Therefore, screening of patients prone to infections of staphylococcal etiology should not be focused only on identifying methicillin-resistant isolates but also on the clarification of their pathogenic potential and clinical significance.

Interestingly, only one patient (female, 49 years old), who was colonized with *S. aureus* in the anterior nares, carried the microorganism at the vascular access site as well. The reason for that might be the reported less susceptibility of endogenous arteriovenous fistulae (AVF), through which all patients included in the present study were dialysed, to infection than prosthetic shunts and intravenous catheters (Peacock *et al.* 2002). According to Saxena *et al.* (2003), patients, in whom AVFs were placed for haemodialysis, carried statistically insignificant risk of developing *S. aureus* - associated infection, even if they carried the bacterium in their nares.

The PFGE analysis of two *S. aureus* isolates cultured from the nasal swab and from the skin of the patient in this study revealed that they were genetically identical, which indicated that the *S. aureus* strain might have disseminated from the nasal reservoir to the surface of the skin. Although the patient did not develop infection during the study period, staphylococcal colonization of the area of impaired skin, as in this case, should be regarded as a preliminary step predisposing to bacterial invasion.

The results of our study also showed that together with a relatively low nasal colonization and methicillin resistance rates, there was no specific *S. aureus* PFGE type responsible for colonization. We observed significant genetic diversity among analyzed *S. aureus* isolates with only two PFGE patterns (A and D), which were represented by two isolates each and between which either genetic identity (A pattern) or close relatedness (D pattern) were revealed. Other genetic backgrounds were represented by single isolates only. It is worth noting that both carriers of the A PFGE subtype were dialysed at close time intervals, similarly to another two patients harbouring the MSSA isolates representing the D PFGE type. Moreover, the patients received their treatment in the same room.

These data suggest that there was a possibility of a limited extent of bacterial transmission among the haemodialysed patients. No environmental or staff samples were included hence it was impossible to elucidate whether members of the hospital staff and the haemodialysis equipment may have contributed

to spread of these strains. Our study had several limitations. Namely, the patients included in the study were screened for *S. aureus* nasal carriage only once, which could only reveal colonization with this microorganism at the time of swab collection, without distinguishing between the intermittent and persistent nasal carriage status. Moreover, the study was focused on the evaluation of the frequency of mucosal colonization; no infections of staphylococcal etiology were observed among patients during the study period.

It should be mentioned that the rational administration of antibiotics among patients at the presented ward as well as high awareness of the hospital staff about the threat of MRSA transmission through hands and, as a consequence, routine wearing of protective gloves during contacts with patients and frequent hand washing played a key role in the low rate of strain transmission between patients and also the low rate of MRSA detection among cultured isolates. Generally, the results of the study concerning the rate of resistance against methicillin among *S. aureus* isolates reflected relatively low frequency of MRSA isolation from clinical specimens in the University Hospital (12% in 2004).

The results obtained revealed a relatively low rate of *S. aureus* carriage accompanied by low methicillin resistance rate and a significant genetic diversity of cultured isolates with the lack of one predominant strain responsible for colonization. Furthermore, the study demonstrated that only a limited degree of staphylococcal transmission among patients treated in the unit might have occurred.

Screening of predisposed patients for mucosal colonization with *S. aureus* should be performed routinely in order to better understand the epidemiology of this process but also to subsequently prevent the development of infectious complications. Particular attention should be focused not only on isolation of MRSA but also on the ability of invasion by methicillin-sensitive isolates.

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