Staphylococcus aureus nasal and hand carriage among students from a Portuguese Health School

J. MARQUES, J. BARBOSA, I. ALVES and L. MOREIRA
Escola Superior de Saúde Jean Piaget, Vila Nova Gaia, Portugal

Accepted: 18 September 2009

ABSTRACT

This study aims to compare the frequency of *Staphylococcus aureus* nasal carriage among students from a Portuguese higher health school. Antimicrobial susceptibility testing was also assayed in order to detect methicillin-resistant *S. aureus* (MRSA) strains among the isolates. Nasal swabs and fingerprints from 60 healthy nursing and pharmacy students were collected, followed by inoculation and incubation at 37°C for 24 h. All suspected *S. aureus* isolates were identified by routine laboratory procedures. The susceptibility to antimicrobial agents (tetracycline, gentamicin, chloramphenicol, amoxicillin/clavulanic acid, trimethoprim/sulphamethoxazole, oxacillin and vancomycin) of confirmed isolates was determined by a disc-diffusion method. Results showed 41.7% *S. aureus* colonisation among participants, and that the difference between nursing and pharmacy students was statistically significant. Antibiotic susceptibility testing demonstrated that *S. aureus* isolates showed variable sensitivity to antibiotics but, most importantly, were resistant to oxacillin and vancomycin. Although the frequency and prevalence of colonisation found is within the range previously described in healthy populations, increased resistance to antimicrobials and higher prevalence of MRSA among the student community was found.

KEY WORDS: Antibiotics.
Oxacillin.
Staphylococcus aureus.
Vancomycin.

Introduction

Staphylococcus aureus is a non-motile Gram-positive coccus closely associated with the human body[2] and an important cause of human disease.[1] Although *S. aureus* is an important pathogen,[2,3] many healthy people carry it as part of the normal flora of the nose, throat, perineum or skin.[3] Carrier rate varies among different populations but nasal passages are reported to harbour *S. aureus* in 10–50% of the healthy population.[1,4]

Staphylococccal infection occurs most frequently among persons in hospitals and healthcare facilities who have weakened immune systems and/or prolonged antibiotic exposure, especially to β-lactam antibiotics.[3,5–7] *S. aureus* was always a challenge to chemotherapy, and prior to antibiotics the mortality rate exceeded 80%.[1,8] The introduction of penicillin (β-lactam antibiotics) significantly improved this prognosis but bacterial resistance quickly developed as *S. aureus* acquired the plasmid-borne β-lactamase.[8] Second-generation penicillins (i.e., methicillin) were developed to reverse this problem but the acquisition of a penicillin-binding protein (PBP) with reduced affinity for β-lactam antibiotics[8] increased the number of methicillin-resistant *S. aureus* (MRSA) isolated.[9]

In the early 1960s, the first MRSA strains were described in a hospital environment.[10–12] However, the spread of multi-drug-resistant strains, especially MRSA, in the healthcare setting allowed the isolation of MRSA from community-acquired infections.[1,6,10,12]

Due to the increase of MRSA strains isolated, vancomycin became the main therapy used to treat infected patients. The emergence of MRSA clinical infection with decreased susceptibility to vancomycin is a recent and worrying phenomenon. Since 1996, vancomycin-intermediate *S. aureus* (VISA) strains have been identified increasingly in Europe, Asia and USA.[13] These strains tend to be resistant to a large number of currently available antibiotics, limiting treatment options and increasing morbidity and mortality associated with serious infections such as bacteraemia, endocarditis or osteomyelitis.[1] Hence, establishment of staphylococcal nasal carrier status and characterisation of isolates by antibiotic susceptibility testing is crucial to avoid the spread of multidrug-resistant bacteria in the community.[5,9,14]

The aim of this study is to compare *S. aureus* nasal and fingerprint carrier frequency among students (i.e., nursing, clinical analysis and pharmacy) from a Portuguese higher health school during practical training. Additionally, antimicrobial susceptibility testing is used to detect MRSA strains among the isolates.

Materials and methods

This study was conducted at Escola Superior de Saúde Jean Piaget, a higher health school in Vila Nova de Gaia, Portugal, between March and May 2008. The nasal swabs and fingerprints (n=120 samples) were collected from 60 students on two health degree courses (pharmacy [n=30] and nursing [n=30]). Students were divide into group A (30 students from the second year of both the nursing and pharmacy courses;

Correspondence to: Joana Margarida Moutinho Barbosa
Escola Superior de Saúde Jean Piaget, VN Gaia
Alameda Jean Piaget – Gulpilhares, 4405-678 Vila Nova De Gaia, Portugal
Email: jmbarbosa@gaia.ipiaget.org

Table 1. Colonisation results for *Staphylococcus aureus* isolates obtained from groups A (second year) and B (third year) on the nursing and pharmacy degrees. Binomial test for two proportions of colonisation results are presented.

	Group A			Group B		
	Nursing	Pharmacy	Total	Nursing	Pharmacy	Total
S. aureus carriers	10 (66.7%)	4 (26.7%)	14 (46%)	4 (26.7%)	7 (46.7%)	11 (36%)

Binomial test for two proportions (Groups A and B)	*P* value
Nursing A versus Pharmacy A	0.008
Nursing B versus Pharmacy B	0.123
Nursing A versus Nursing B	0.008
Pharmacy A versus Pharmacy B	0.123

15 from each degree) and group B (30 students from the third year who already had undertaken hospital training in the hospital pharmacy department [n=15] and in medical services [n=15]). Mean age of participants was 23 years (range: 19–47; 75% female, 25% male).

Nasal swabs for each student were rubbed over the anterior nares of both nostrils and streaked on mannitol salt agar (MSA). At the same time, the participants placed their fingerprints, from the right hand, on another MSA plate. All plates were incubated at 37°C for 24 h.

All suspected *S. aureus* isolates were identified by routine laboratory procedures. Identification was performed on the basis of colonial morphology (yellow, large and concave colonies), cultural characteristics on MSA media (reduced pH turned medium colour from red to yellow), catalase (release of O_2 after mixture of suspected colonies with H_2O_2) and coagulase (Staphytec plus, Oxoid Diagnostic Reagents) tests.

Susceptibility of all *S. aureus* isolates to antimicrobial agents was determined by a disc-diffusion method on Mueller-Hinton agar (MHA), performed according to CLSI recommendations.[15] Isolates were tested against tetracycline (TE, 30 μg), gentamicin (CN, 30 μg), chloramphenicol (C, 30 μg), amoxicillin/clavulanic acid (AMC, 30 μg), trimethoprim/sulphamethoxazole (SXT, 30 μg), oxacillin (OX, 1 μg) and vancomycin (V, 30 μg) discs with an inoculum yielding confluent growth (10^7 colony forming units [cfu]/mL).

Statistical analysis was based on descriptive statistics procedures and differences between the two groups were evaluated using the Binomial Test for Two Proportions.

Results

The study showed that 25 (41.7%) of the 60 participants were colonised with *S. aureus*. The isolates were cultured from specimens obtained from groups A and B. Sixteen (26.7%) of the participants were positive for *S. aureus* in their anterior nares and two (3.3%) presented with *S. aureus* on their fingertips. In seven (11.7%) participants, *S. aureus* was cultured from nasal and fingerprint samples. Results are presented in Table 1.

Antibiotic susceptibility testing demonstrated that *S. aureus* isolates were sensitive mostly to gentamicin (100%), followed by chloramphenicol (84.4%), amoxicillin/clavulanic acid (81.3%), trimethoprim/sulphamethoxazole (68.7%) and tetracycline (37.5%). Also, 18/25 (72%) *S. aureus* isolates showed resistance to oxacillin.

Isolates resistant to oxacillin (1 μg disc-diffusion test, confluent growth and interpretation of zone diameter as ≤10 mm resistant [R], 11–12 mm intermediate [I] and ≤13 mm susceptible [S]), according to CLSI (2005) guidelines and classified as MRSA, were submitted to the disc-diffusion susceptibility test to vancomycin (30 μg disc, confluent growth and interpretation of zone diameter as R <11 mm, S ≥11 mm). Fourteen (77.8%) of the 18 MRSA showed resistance to vancomycin. Six (75%) MRSA isolates from students on the second year of the nursing degree and three (60%) isolates from students on the third year were resistant to vancomycin. All MRSA isolates from students on the second year of the pharmacy degree presented as sensitive to vancomycin, but all MRSA samples from students on the third year of the pharmacy degree showed resistance to vancomycin.

Discussion

Methicillin-resistant *S. aureus* has become an important issue in public health, mostly because of community-associated MRSA (CO-MRSA) isolates. Strains that are oxacillin- and methicillin-resistant are also resistant to all β-lactam agents, including cephalosporins and carbapenems. Hospital-associated MRSA isolates often show multiple resistance to other commonly used antimicrobial agents, including erythromycin, clindamycin and tetracycline, while CO-MRSA isolates are often resistant only to β-lactam agents and erythromycin.

In this study, 41.7% of the students presented with *S. aureus* nasal colonisation, which is within the range reported in other cross-sectional surveys.[16–18] This high level of colonisation could be explained by the fact that the anterior nares are the major reservoir of this microorganism.[1,3,14] Statistically significant differences (Table 1) were found between the nursing and pharmacy students in group A (P=0.008) but not in group B (P=0.123). These results could be explained by previous exposure of the second-year nursing students to a hospital environment, as some of them already worked as volunteers or nursing auxiliaries in hospitals and other healthcare facilities, which could also explain the difference between the second- and third-year nursing students.

Koziol-Montewka and colleagues[14] have demonstrated

Table 2. Antibiotic susceptibility results for all *Staphylococcus aureus* isolates from nasal swabs and fingerprints using the disc-diffusion method.

		Group A		Group B		*n*	(%)
		Nursing	Pharmacy	Nursing	Pharmacy		
AMC	R	2	0	1	3	6	18.7
	S	10	5	4	7	26	81.3
SXT	R	2	4	0	4	10	31.3
	S	10	1	5	6	22	68.7
TE	R	6	3	3	8	20	62.5
	S	6	2	2	2	12	37.5
C	R	0	5	0	0	5	15.6
	S	12	0	5	10	27	84.4
CN	R	0	0	0	0	0	0
	S	12	5	5	10	32	100
OX	R	8	5	0	5	18	56.3
	S	4	0	5	5	14	43.7

OX; oxacillin, TE: tetracycline, CN: gentamicin C: chloramphenicol, AMC: amoxicillin/clavulanic acid, SXT: trimethoprim/sulphamethoxazole.
R: resistant, S: sensitive.

that an important factor contributing to *S. aureus* acquisition is contact with the hospital environment. During the hospital training course, in the third year, most of the students begin to work as nurses with distinct types of patient contact.

Many *S. aureus* strains, while resistant to penicillin, remain susceptible to penicillinase-stable penicillins (e.g., oxacillin and methicillin). This study found higher sensitivity to amoxicillin/clavulanic acid so some of those strains might have been sensitive to penicillin, too. All isolates were sensitive to gentamicin, and highly susceptible to chloramphenicol (84.4%) and trimethoprim/sulphamethoxazole (68.7%) (Table 2), as previously documented.[19,20] This very high gentamicin susceptibility may be explained by the need to administer this drug intravenously, and thus a less-commonly used antibiotic that is more difficult to abuse. Studies performed with *S. aureus* community strains and tetracycline found a higher prevalence of isolates with tetracycline resistance – conferring a penicillinase plasmid and a drug resistance gene cluster.[21]

Oxacillin disc diffusion has been the traditional method for routine methicillin resistance screening. In this study very high resistance to oxacillin (75%) was observed, compared to other studies.[14,22] However, this could be due to the fact that a lower antibiotic concentration (1 μg) was used in the present study, in line with CLSI guidelines from 2005, as the students did not report previous *S. aureus* infections.[22] Only in the 2006 CLSI guidelines is the cefoxitin (a potent inducer of the *mecA* regulatory system) disc screening test recommend, or a plate containing 6 μg/mL oxacillin in Mueller-Hinton agar supplemented with NaCl (4% [w/v], 0.68 mol/L) as an alternative methods for the detection and differentiation of CA-MRSA from HA-MRSA strains.

In the past few years, MRSA strains with decreased susceptibility to vancomycin (minimum inhibitory concentration [MIC]: 8–16 μg/mL) and strains fully resistant to vancomycin (MIC: ≥32 μg/mL) have been reported. Now, VISA strains represent an important public health threat and have been implicated in nosocomial infections and outbreaks of infection and colonisation in the healthcare environment. In this study, 56% of *S. aureus* isolates showed resistance to vancomycin, and were also MRSA. *S. aureus* clinical isolates with reduced susceptibility to vancomycin have also been reported using various testing methods. However, only a few cases of VRSA or VISA infections have been documented to date.[23] Thus, accuracy and promptness in the detection of methicillin resistance is of key importance to ensure the correct use of antibiotic treatment, as well as the control of MRSA in the hospital environment.

The present study had several limitations. First, the students were screened only once for staphylococcal colonisation of the nasal mucosa and fingers; thus, it was not possible to comment on the possibility of intermittent or persistent carriage. Second, antimicrobial testing did not include the cefoxitin disc screening test and MIC determination for oxacillin and vancomycin. Third, it was not possible to perform molecular studies on resistant strains to complement the bacterial screening.

These findings indicate the usefulness of investigating staphylococcal colonisation of the nasal mucosa in order to understand the epidemiology of *S. aureus* infection and thus to develop prevention measures and treatment strategies. □

References

1 Kuehnert MJ, Kruszon-Moran D, Hill HA *et al.* Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001–2002. *J Infect Dis* 2006; **193**: 172–9.

2 Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. *Infect Genet Evol* 2008; **8**: 747–63.

3 Hamdad F, Donda F, Lefebvre JF *et al.* Detection of methicillin/oxacillin resistance and typing in aminoglycoside-susceptible, methicillin-resistant and kanamycin/tobramycin-resistant, methicillin-susceptible *Staphylococcus aureus*. *Microb Drug Resist* 2006; **12**: 177–85.

4 Bremer PJ, Fletcher GC, Osborne C. *Staphylococcus aureus*. Lincoln: New Zealand Institute for Crop & Food Research, 2004 (www.crop.cri.nz).

5 Bogut A, Koziol-Montewka M, Baranowicz I *et al*. Characterisation of *Staphylococcus aureus* nasal and skin carriage among patients undergoing haemodialysis treatment. *New Microbiol* 2007; **30**: 149–54.

6 Campanile F, Bongiorno D, Borbone S, Stefani S. Hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in Italy. *Ann Clin Microbiol Antimicrob* 2009; **8**: 22.

7 Rao N, Cannella B, Crossett LS, Yates AJ Jr, McGough R 3rd. A preoperative decolonization protocol for *Staphylococcus aureus* prevents orthopaedic infections. *Clin Orthop Relat Res* 2008; **466**: 1343–8.

8 Gardete S. Towards the understanding of the mechanism of methicillin resistance in *Staphylococcus aureus*. Universidade Nova de Lisboa, Instituto de Tecnologia Química e Biológica, 2006.

9 Velasco D, Tomas MM, Cartelle M *et al.* Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus. J Antimicrob Chemother* 2005; **55**: 379–82.

10 Palavecino E. Clinical, epidemiological and laboratory aspects of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. In: Youndo J ed. *Methicillin-resistant* Staphylococcus aureus *(MRSA) protocols*. Humana Press. 2007: 1–19.

11 Pan A, Lorenzotti S, Zoncada A. Registered and investigational drugs for the treatment of methicillin-resistant *Staphylococcus aureus* infection. *Recent Pat Antiinfect Drug Discov* 2008; **3**: 10–33.

12 Witte W, Pasemann B, Cunny C. Detection of low-level oxacillin resistance in *mecA*-positive *Staphylococcus aureus. Clin Microbiol Infect* 2007; **13**: 408–12.

13 Appelbaum PC. Reduced glycopeptides susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J Antimicrob Agents* 2007; **30**: 398–408.

14 Koziol-Montewka M, Szczepanik A, Baranowicz I, Jóêwiak L, Ksiazek A, Kaczor D. The investigation of *Staphylococcus aureus* and coagulase-negative staphylococcal nasal carriage among patients undergoing haemodialysis. *Microbiol Res* 2006; **161**: 281–7.

15 Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard M100-S15. Fifteenth Informational Supplement. Wayne, PA: CLSI, 2005: Vol 25 (1).

16 Oguzkaya-Artan M, Baykan Z, Artan C. Nasal carriage of *Staphylococcus aureus* in healthy preschool children. *Jpn J Infect Dis* 2008; **61**: 70–2.

17 Price CV, Williams A, Philips G, Dayton M, Smith W, Morgan S. *Staphylococcus aureus* nasal colonization in preoperative orthopaedic outpatients. *Clin Orthop Relat Res* 2008; **466**: 2842–7.

18 Villacian JS, Barkham T, Earnest A, Paton NI. Prevalence of and risk factors for nasal colonization with *Staphylococcus aureus* among human immunodeficiency virus-positive outpatients in Singapore. *Infect Control Hosp Epidemiol* 2004; **25**: 438–40.

19 Obiazi HAK, Nmorsi OPG, Ekundayo AO, Ukwandu NCD. Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* from clinical isolates grown at 37°C and 44°C from Irrua, Nigeria. *Afr J Microbiol Res* 2007;: 57–60.

20 Tillotson GS, Draghi DC, Sahm DF, Tomfohrde KM, Del Fabro T, Critchley IA. Susceptibility of *Staphylococcus aureus* isolated from skin and wound infections in the United States 2005–07: laboratory-based surveillance study. *J Antimicrob Chemother* 2008; **62**: 109–15.

21 Takano T, Higuchi W, Otsuka T *et al.* Novel characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* strains belonging to multilocus sequence type 59 in Taiwan. *Antimicrob Agents Chemother* 2008; **52**: 837–45.

22 Mimica MJ, Berezin EN, Carvalho RLB *et al.* Detection of methicillin resistance in *Staphylococcus aureus* isolated from pediatric patients: is the cefoxitin disk diffusion test accurate enough? *Braz J Infect Dis* 2007; **11**: 415–7.

23 Wang G, Hindler JF, Ward KW, Bruckner DA. Increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital during a 5-year period. *J Clin Microbiol* 2006; **44** (11): 3883–6.