ORAL DISEASES

Oral Diseases (2010) 16, 465–468. doi:10.1111/j.1601-0825.2010.01657.x

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ORIGINAL ARTICLE

Methicillin-resistant staphylococci carriage in the oral cavity: a study conducted in Bari (Italy)

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OBJECTIVES: The oral cavity may represent a site of colonization by antibiotic-resistant bacteria, such as methicillin-resistant staphylococci (MRS). To define the prevalence of staphylococci and MRS in the oral cavity, an observational study was carried out in the city of Bari (Italy).

METHODS: Sixty subjects were asked to provide oral samples and a questionnaire about risk factors of colonization by MRS. An enrichment medium specific for staphylococci was used for the isolation.

RESULTS: Swabs and corresponding questionnaires were available from 36 out of 60 patients. Staphylococci were isolated from seven out of 36 samples (prevalence 19.4%). Among the seven staphylococcal isolates, three were *Staphylococcus aureus*, and one strain, belonging to *S. epidermidis* species, was found to be MR (1.7%). No methicillin-resistant *S. aureus* were isolated. Five out of seven staphylococcal isolates exhibited resistance to more than two classes of non-beta-lactams antimicrobials. None of the risk factors analysed correlated with the status of MRS carriers, except the presence of oral disease.

CONCLUSIONS: The results underline the potential role of the oral cavity as a reservoir of staphylococci.
Oral Diseases (2010) **16**, 465–468

Keywords: staphylococci; methicillin-resistance; oral cavity

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCNS) are an important cause of hospital-acquired (HA) infections in humans (Voss and Doebbeling, 1995). Staphylococci become methicillin-resistant (MRS) by the acquisition of the *mec*A gene and the production of an altered penicillin-binding protein (PBP2a) which confers resistance to all beta-lactams and monobactams antimicrobial agents (Chambers, 1997). MRS infections are found world-wide. In Italy, MRSA infections account for 35–41% of *S. aureus* HA infections, and a trend of general increase is reported (Stefani and Varaldo, 2003). The infections by MRCNS such as *S. epidermidis* tend to show a higher level of prevalence (60–70%) (Stefani and Varaldo, 2003). In recent years community-acquired (CA) rather than HA infections have been reported increasingly, demonstrating the ability of staphylococci to spread from the hospital environment to the community and to colonize healthy individuals, regardless the presence of conventional risk factors for MRS colonization (Zetola *et al*, 2005).

Colonized human hosts are responsible for person-to-person transmission of MRCNS and MRSA infection. In particular, their hands and aerosol can spread the bacteria (Coia *et al*, 2006). The anterior nares have been shown to be the main site of colonization of MRS. However, other niches of infection may have been overlooked, such as the throat (Mertz *et al*, 2007) and oral cavity (Smith *et al*, 2003a,b; Small *et al*, 2007). In recent years, the attention of bacteriologists has been focused on the mouth as a reservoir of opportunistic pathogens and on the role played by those bacteria in the onset of oral diseases. To date, few studies have analyzed the prevalence of either staphylococci or MRS carriage on human oral mucosa. Studies about the colonization by MRSA are of particular interest considering the difficulties in eradicating the carriage of such organisms by the oro-pharynx (Smith *et al*, 2003a,b).

Therefore, the aim of the study was to analyse the oral carriage of staphylococci and MRS in volunteers recruited from private dental cabinets and to determine if any risk factors predict MRS carriage.

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Received 23 September 2009; revised 24 November 2009; accepted 29 November 2009

Materials and methods

Experimental design
An observational, cross-sectional study was carried out during the period January–December 2008. A criterion of exclusion was the presence of acute, chronic, systemic and organ-specific diseases, in individuals with the exception of pyogenic granulomas or abscesses. One person was recruited per week among the patients of two private dental cabinets of the city of Bari (Italy). A total of 60 individuals were selected, distributed into two groups: healthy people ($n = 30$, group A); individuals with pyogenic granulomas or abscesses ($n = 30$, group B).

Samples
Amies swabs with transport medium were used for the sample collection. Samples from group A were obtained by streaking the swab on the oral mucosa (Small *et al*, 2007), while pus from the site of lesions in all the patients of group B was collected after disinfection with chlorhexidine. In both cases, swabs were stored at 4°C the bacteriological analysis.

Questionnaire
Patients were asked to complete a questionnaire containing questions about the risk of colonization by HA or CA-MRSA, as indicated in Table 1. A written informed consent was obtained from all patients by participating dentists who adhered to the study protocol according to the Italian law D.lgs. n.196/2003 and according to ethical principles.

Bacteriological analysis
A protocol that allowed the selective culture of staphylococci was used. Briefly, the swabs were cultured in an enrichment broth for staphylococci (Hanselman *et al*, 2008) and incubated for 48 h at 35°C. Subcultures were made onto Mannitol Salt Agar (MSA, Liofilchem, Teramo, Italy) and incubated for 48 h at 35°C. The organisms grown on MSA were identified by means of the biochemical system *api staph* (Biomerieux, Marcy l'Etoile, France).

Identification of MRS
All the strains identified as belonging to the genus *Staphylococcus* (Table 2) were subjected to a PCR for the detection of the *mecA* gene using a primers pair described previously (Murakami *et al*, 1991). For DNA extraction, an overnight culture in Brain Heart Infusion (BHI, Oxoid, Milan, Italy) of the strain was centrifuged and treated with 2 μl of lysostaphin (10 mg ml^{-1}) (Sigma, Milan, Italy) for 1 h at 37°C (Araj *et al*, 1999). Then, DNA was extracted using the QIAamp Tissue kit (QIAGEN GmbH, Hilden, Germany). For amplification, the Accuprime TM Taq DNA polymerase was used (AccuprimeTM SupermixII; Invitrogen, Milan, Italy).

Antimicrobial susceptibility test
All the isolates were tested for susceptibility to antimicrobials (Table 3) by the disk diffusion test (Clinical and Laboratory Standards Institute 2006).

Statistical analysis
The data were analyzed by the software SAS (SAS 1998). Dichotomous variables were evaluated by chi-squared test with Yates correction, considering a *P*-value < 0.05 as statistically significant. Other data were analysed by univariate analysis, with carriage of MRS as dependent variable, and risk factors as independent variables. Odds ratio (OR) values were calculated, with a confidence interval of 95% (Table 1).

Results

Thirty-six out of 60 subjects answered the questionnaire and provided the swab (response rate 60%). The remaining individuals did not provide a response (no questionnaire and/or no sample) and their samples were excluded from the study. Twenty-one individuals belonged to group A and 15 to group B. The median age was 42.5 years (range 21–72 years, first quartile 35.7, third quartile 50.5).

Nineteen participants were females, 17 were males. Seven out of 36 patients were found to be carriers of staphylococci (19.4%). The characteristics of the carriers are reported in Table 1. One of the carriers of staphylococci belonged to group A, and six belonged to

Table 1 presence of Risk factors in staphylococcal carriers: odds ratio values

Risk Factor	*Staphylococcal carriers* (n = 7)	*Non-carriers* (n = 29)	*Odds ratio (IC 95%)*	P-*value*
1. Health worker/Health worker in the family				
Yes	1	1	4.67 (0.25–85.56)	>0.05
No	6	28		
2. Previous hospitalization[a]				
Yes	1	2	2.25 (0.17–29.06)	>0.05
No	6	27		
3. Previous antibiotic treatment[b]				
Yes	1	1	4.67 (0.25–85.56)	>0.05
No	6	28		
4. Use of mouthwash				
Yes	3	10	1.43 (0.27–7.66)	>0.05
No	4	19		

[a] In the last 2 years.
[b] In the last 6 months.

Table 2 Characteristics of the carriers of staphylococci

Sample	*Group*	*Sex*	*Age*	*Staphylococcus* spp.
1/08	A	F	49	*S. chromogenes*
25/08	B	F	47	*S. epidermidis*
35/08	B	M	40	*S. epidermidis*
40/08	B	F	40	*S. aureus*
42/08	B	M	70	*S. aureus*
50/08	B	F	50	*S. epidermidis*
60/08	B	M	47	*S. aureus*

Group A, healthy people; Group B, patients with pyogenic lesions; F, female; M, male.

Table 3 Species and pattern of antimicrobial resistance of staphylococcal isolates. 25/08: methicillin-resistant strain; R, resistant; S, susceptible

	Strains						
Antimicrobials	*S. chromogenes* 1/08	*S. epidermidis* 25/08	*S. epidermidis* 35/08	*S. aureus* 40/08	*S. aureus* 42/08	*S. epidermidis* 50/08	*S. aureus* 60/08
Nalidixic acid	R	R	S	S	R	S	S
Pipemidic acid	R	R	S	S	R	S	S
Ciprofloxacin	S	R	S	S	S	S	S
Clindamicycin	S	S	S	R	S	S	R
Chloramphenicol	S	S	S	S	S	S	S
Co-trimoxazole	R	R	S	S	S	S	S
Doxycline	S	S	S	S	S	S	S
Erytrhomycin	R	R	S	R	R	R	S
Gentamicin	S	R	S	R	S	S	S
Imipenenem	S	S	S	S	S	S	S
Lincomycin	S	S	R	R	R	R	S
Norfloxacin	S	R	S	S	S	S	S
Rifampicin	S	S	S	S	S	S	S
Sulfamethoxazole	R	R	R	R	R	R	S
Teicoplanin	S	S	S	S	S	S	S
Tetracyclin	S	S	R	R	R	R	S
Vancomycin	S	S	S	S	S	S	S

group B (difference statistically significant, $P < 0.05$, chi-squared test). The different staphylococcal isolates are indicated in Table 2. Three out of seven staphylococci were characterized as *S. aureus* none in group A and three in group B respectively ($P > 0.05$, chi-squared test).

MRS

When PCR specific for *mec*A gene was run on the seven staphylococcal isolates, one strain (14.3%), belonging to the *S. epidermidis* species, was found to be positive. The overall rate of MRS carriers was 1.7% (1 out of 36 samples). None of *S. aureus* isolates was found to be MR.

Antimicrobial susceptibility test

The results of antimicrobial susceptibility tests are reported in Table 3. Interestingly, MR *S. epidermidis* (25/08) was found to be multiresistant, i.e. resistant to more than two classes of non-beta-lactam antimicrobials.

Statistical analysis

None of the risk factors was found to be related to the status of carriers (OR < 1).

Discussion

It is well known that staphylococci, either MR or methicillin-sensitive, live in the nasal cavity and body surfaces. More recently, some authors have reported the presence of MRS in the throat and oral cavity (Nilsson and Ripa, 2006; Small *et al*, 2007). Such organisms may reside solely in the oral cavity or can derive from the anterior nares as a result of migration trough the oropharynx. The persistence in the mouth may be favoured by the fitness that antibiotic-resistant strains have over susceptible staphylococci. It has been demonstrated that the status of MRS carriers can persist even for 2 years (Lu *et al*, 2005). Moreover, the mouth may represent a portal of entry for staphylococci causing systemic infections (Rautemaa *et al*, 2007).

In the cross-sectional study reported here, a good response rate to the survey was obtained, despite the fact that volunteers were recruited and personal information was required. Thus, some epidemiological considerations were drawn. The rate of staphylococcal carriers was relatively high, when considering the age of the patients. Data about the prevalence of staphylococcal carriage in children, elderly people and immunocompromised persons are available in literature (Smith *et al*, 2003a,b; Barr *et al*, 2007); however, staphylococcal prevalence in adults has been poorly studied. The differences observed in the rate of isolation in humans might depend on the protocols used for the isolation of staphylococci. In the survey reported here, a selective enrichment broth was used to enhance the growth of such bacteria. Thus, in the screening of oral mucosa samples, if a selective protocol is not used, the predominant flora of the mouth, such as streptococci, might overgrow staphylococci.

The results suggest that persons with oral pathology are more likely to be colonized with staphylococcal species. Thus an aetiological role of such organisms might be hypothesized. Three out of seven staphylococcal isolates belonged to *S. aureus*, whose pathogenic attitude is well known (Smith *et al*, 2001; Robinson and Enright, 2003).

Three strains were characterized as *S. epidermidis*, a species that has been isolated in systemic infections and that may be well adapted to the dental plaque, due to its ability to produce biofilm (Rautemaa *et al*, 2007). The overall rate of MRS isolation was rather low, and in agreement with other surveys performed in adults (Smith *et al*, 2001), although the relative percentage of MRS *vs* staphylococci was higher.

Moreover, the MR isolate was multiresistant, and present in the group of patients with pyogenic infections

(group B). Thus, it cannot to be excluded that a previous use of antibiotics might have selected multiresistant strains (Robinson and Enright, 2003). Staphylococcal isolates exhibit resistance to several classes of antimicrobials, such as fluoroquinolones, macrolides, aminoglicosides and sulphonamide. This is a relevant feature, as staphylococci easily contribute to the diffusion of the determinants of antibiotic resistance by genetic transfer (Livermore, 2000).

Despite the useful data provided, the present study may have some limitations, due to the small number of samples. In fact, when the risk factors were related to the status of staphylococcal carrier, no significant differences were observed except for the presence of oral disease.

In conclusion, this study highlights that staphylococci are a component of oral flora and that the oral cavity can play a role as a reservoir of MRS.

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