Methicillin-resistant *Staphylococcus aureus* (MRSA) in the community – laboratory based study

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Received: 25. 02. 2007.
Accepted: 24. 05. 2007.

Objective To determine the occurrence and antibiotic resistance of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. **Methods used** In 2003-2005, consecutive samples of nasal, throat, eye, ear and genitourinary tract swabs, swabs of wound infections and soft and skin tissue infections and samples of sputum obtained from outpatients submitted to the Laboratory with clinical indications were analyzed for the presence of *Staphylococcus aureus*. The disc diffusion method using Mueller-Hinton agar (Oxoid, Besingstoke, UK) was used to test against nine antimicrobials. Oxacillin-resistance was confirmed by E-test (AB Biodisc, Solna, Sweden). **Results** A total of 1583 (11.3%) nonduplicate *S. aureus* isolated from 13 937 samples. MRSA was detected in 63 (4.1%) of S. *aureus* isolates. MRSA isolates more frequently from infected genitourinary tract and wounds than other sites ($p<0.0001$). The patients in both age groups ≥65 and 0-6 years of age were more frequently infected with MRSA than patients of other age groups ($p=0.02$). Statistically significant differences in susceptibility rates between MSSA and MRSA isolates were found for all antibiotic tested ($p=0.0053$ to $p<0.000$). MRSA isolates were more frequently multidrug resistant (MDR) than MSSA isolates ($p=0.0009$). SCC*mec* type IV or V phenotype was detected in 30 (47.6%) of MRSA isolates. **Conclusion** Although low MRSA prevalence was noted, the presence of SCC*mec* type IV/V phenotypes in the community is of particular concern. Effective control of dissemination of MRSA throughout the community will likely require effective control and monitoring of nosocomial MRSA transmission.

Key words: *S. aureus*, MRSA, MSSA, SCC*mec*, Resistance, Multidrug resistance.

Introduction

Methicillin-resistant *S. aureus* (MRSA) has traditionally been considered a hospital-acquired pathogen (HA-MRSA) in patients with established risk factors (recent hospitalization or surgery, dialysis, residence in a long-term care facility, and presence of a permanent indwelling catheter or percutaneous medical device) at the time of culture) (1, 2). But more recently MRSA has emerged as a highly virulent organism in the community of patients without established risk factors for the acquisition of MRSA (3-5). Moreover, the spread of community-acquired methicillin resistant *S. aureus* (CA-MRSA) into hospitals has been reported, causing nosocomial infections (6, 7).

Most studies have been based on hospitalized patients, or patients upon admission to hospital, which has probably resulted in an overestimation of the true prevalence of CA-MRSA (8, 9). Accordingly, epidemiological definitions of CA-MRSA have commonly been based on the timing of isolation of MRSA in relation to the time of admission to hospital, so that MRSA isolates were classified as community-acquired if they were isolated within the first 48-72 h of hospitalization, or if they were isolated in a community setting (10).

Reported prevalence rates of CA-MRSA vary widely among studies, in part because of the use of different definitions used to distinguish between CA-MRSA and HA-MRSA, but also because of the different settings in which studies have been performed. Only a limited number of studies has been performed in outpatient settings and among randomly selected healthy community members (4, 5, 11, 12).

A combination of molecular typing techniques with good resolving power provides a reliable means of analysing isolates of MRSA to determine their genetic relatedness (13, 14). Recent studies have indicated that well-defined CA-MRSA strains carry SCC*mec* type IV or V (14), whereas the majority of HA-MRSA strains carry SCC*mec* type I, II or III (13).

Recently two MRSA strains isolated from the noses and hands of food handlers prompted a retrospective review of Laboratory outpatient records identifying patients from whom *S. aureus* was isolated from any site in the period 2003-2005. The objective of this study was to report the frequency of *S. aureus* isolation in outpatients from the Zenica-Doboj Canton, Bosnia and Herzegovina, according to methicillin resistance, origin of isolates, age and gender of patients, and to determine the antibiotic susceptibility patterns. For comparison, *S. aureus* isolates obtained from food handlers and food products (routinely analysed in the Laboratory during 2003-2004) were also included in the study.

Methods

The Laboratory for Sanitary and Clinical Microbiology of the Cantonal Public Health Institution in Zenica covers a population of 331,229 in the Zenica-Doboj Canton (112,471 males and 218,758 females). In the 2003-2005 period, 13,937 consecutive samples of nasal, throat, eye, ear and genitourinary tract swabs, swabs of wound infections and soft and skin tissue infections (SSTIs) and sputum obtained from outpatients submitted to the Laboratory with clinical indication, were analyzed for the presence of *S. aureus*.

Sterile cotton swabs were used. Swabs were streaked onto sheep blood agar (5% columbia agar base) for detection of gram-positive bacteria, and incubated overnight at 37°C. Morphologically distinct colonies were tested for the production of bound coagulase (Staphylase Test, Oxoid, Basingstoke, UK) and identified as *S. aureus*.

The disc diffusion method using Mueller-Hinton agar (Oxoid, Besingstoke, UK) was used to test against nine antimicrobials (Oxoid, UK). Clinical and Laboratory Standards Institute (CLSI) criteria were used for the interpretation of antibiotic sensitivity testing results (15). Oxacillin-resistant strains were further tested by the E-test (AB Biodisc, Solna, Sweden). Isolates were considered resistant to oxacillin if the MIC exceeded 4 mg/L. The isolates characterized as intermediate by both disk diffusion and E-test were considered susceptible. *Staphylococcus aureus* ATCC 25923 control strains were used. Isolates resistant to oxacillin and susceptible to gentamicin, clindamycin, and trimethoprim-sulfamethoxasole were designated as having a SCC*mec* type IV or V phenotype.

The name, surname, ID, address, gender and age of the patient (0-6, 7-14, 20-64, >64 years), date of isolation, specimen number, source of isolates and susceptibility results of *Staphylococcus aureus* isolates were recorded, as well as the number of specimens submitted during the study.

For comparison, *S. aureus* strains isolated from 4439 successive nasal swabs of food-handlers and 6517 samples of food collected during routine mandatory examination in the Laboratory during 2003-2004 were also included in this study. Microbiological analysis of food products was performed according to the standards and legal regulations of the Republic/Federation of Bosnia and Herzegovina. Routine antimicrobial susceptibility testing of *S. aureus* isolates from these samples was terminated at the end of 2004, and for that reason the data for 2005 were not available.

The significance of differences in resistance rates was determined by means of the χ^2 test and Fisher exact test for independence. A statistically significant difference was defined as a p value of <0.05 and 95% confidence interval.

Results

A total of 1583 (11.3%) nonduplicate *S. aureus* isolates from 13 937 consecutive outpatients presented to the Laboratory because of different clinical symptoms were collected during 2003-2005. MRSA was detected in 63 (4.1%) of S. *aureus* isolates and in 0.6% of submitted samples. *S. aureus* was identified in 322 out of 4439 (7.3%) nasal swabs of food handlers, five of which were MRSA (1.6%). MRSA was isolated in 0.1% of submitted food handler samples. Thirty five *S. aureus* strains were isolated from 6517 (0.5%) food samples, and two of them (5.7%) were MRSA. All *S. aureus* isolated from ice cream samples obtained from local patisseries and fast food restaurants.

Table 1 shows the distribution of methicillin susceptible *S. aureus* (MSSA) and MRSA isolates according to the origin of isolates.

MRSA isolates were more frequently isolated from genitourinary tract and wounds than from other sites (p<0.0001).

The patients in age groups ≥65 and 0-6 years of age were more frequently infected with MRSA than patients of other age groups (p=0.02) (Table 2). Female patients were significantly more often infected with MRSA than male patients (p=0.003) (data not shown). The median age of patients infected with MRSA and MSSA was 30.09 and 20.88, respectively.

Statistically significant differences in susceptibility rates between MSSA and MRSA clinical isolates were found for all antibiotic tested (p=0.0053 to p<0.0001) (Table 3). No resistance to vancomycin or ciprofloxacin was detected in MRSA isolates. MRSA isolates were more frequently multidrug resistant (MDR) than MSSA isolates (p=0.0009). According to origin, MDR was more often detected in wound infection isolates, 28.6%, than in isolates from GU tract and nose, 12.5% and 0.6%, respectively, but with no statistically significant difference (data not

Table 1 Distribution of MRSA and MSSA clinical isolates of different origin in the 2003-2005 period

Origin of isolates	Site of isolation	No of samples submitted	No of MSSA	No of MRSA (% of SA)	No of MRSA with SCCmec IV or V phenotype	Total S. aureus (% of submitted samples)
Clinical	Nos	7978	1146	34 (2.9)	21 (61.2)	1180 (14.8)
	Throat	12.032	10	1 (9.1)	0	11 (0.09)
	Sputum	14	2	0	0	2 (14.3)
	Wound	444	168	14 (7.7)	5 (35.7)	182 (41.0)
	SSTI	217	4	0	0	4 (1.8)
	Eye	1808	106	5 (4.5)	3 (60)	111 (6.1)
	Ear	379	45	1 (2.2)	0	46 (12.1)
	Genito-urinary tract	1065	39	8 (17.0)	1 (12.5)	47 (4.4)
	Total clinical	13937	1520	63 (4.0)	30 (47.6%)	1583 (11.3%)
Food handlers	Nose	4439	317	5 (1.6)	5 (100)	322 (7.3)
Food	Food samples	6517	33	2 (5.7)	2 (100)	35 (0.5)

Table 2 Distribution of MRSA and MSSA clinical isolates according to age groups

	Age groups				
	0-6	7-14	15-19	20-64	≥ 65
	Number (%) of patients				
MRSA	11 (20%)	10 (18.2%)	4 (7.3%)	23 (41.8%)	7 (12.7%)
MSSA	441 (32.4%)	331 (24.3%)	107 (7.9%)	415 (30.5)	68 (5.0%)
Total	452 (31.9%)	341 (24.1%)	111 (7.8%)	438 (30.9%)	75 (5.3%)

Table 3 Antimicrobial resistance patterns of MSSA and MRSA isolates in the 2003-2005 of different origin

	Origin of isolates	S	R	MDR	Percentage of resistance to antimicrobial agents*								
					VAN	GEN	KAN	ERY	TET	CIP	CLI	SXT	CHL
MSSA													
	clinical (1520)	1091 (71.8%)	429 (28.2)	23 (1.5)	0	5.3	8.7	7.1	17.1	0.6	1.6	4.4	2.7
	food (33)		8 (24.2)	0	0	3.6	6.1	6.1	28.1	0	0	0	0
	food handlers (317)		64 (20.2)	0	0	0.7	3.3	5.9	14.6	0.7	0	2.6	2.6
MRSA													
	clinical (63)	16 (25.4%)	47 (74.6)	10 (15.9)	0	17.9	36.8	37.1	31.7	0	23.0	31.7	9.8
	food (2)		2	0	0	0	0	100.0	50.0	0	0	0	0
	food handlers (5)		3	0	0	0	25.0	40.0	40.0	0	0	0	0

MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; S, susceptible; R, resistance to one or more antimicrobials; MDR (multidrug resistance), resistance to three or more antimicrobials
*Antimicrobial agents tested: vancomycin (VAN), gentamicin (GEN), kanamycin (KAN), erythromycin (ERY), tetracycline (TET), ciprofloxacin (CIP), clindamycin (CLI), trimethoprim-sulfamethoxasole (SXT), chloramphenicol (CHL)

shown). No MDR was detected in MSSA and MRSA isolated from food handlers or food products.

SCC*mec* type IV or V phenotype (isolates resistant to oxacillin and susceptible to gentamicin, clindamycin, and trimethoprim-sulfamethoxasole) was detected in 30 (47.6%) of MRSA isolates. These MRSA phenotypes were significantly more often isolated from GU tract, wounds and nose than from eyes (p=0.0005), but they were not isolated from throat, sputum or ear (Table 1).

Discussion

The finding of 30 MRSA isolates showing good sensitivity to antibiotics other than beta-lactams and the low prevalence of multidrug resistance (MDR) in MRSA suggests the presence of true CA-MRSA in our population (2-4, 16) Multidrug resistance characterizes nosocomially acquired MRSA strains isolated from patients with identified risk (2,4).

Nasal carriage of *S. aureus* is an important risk factor for infections by this organism in both community and hospital settings (16). Health-care exposure is significantly associated with MRSA carriage (10, 18). In our study MRSA was detected in 0.6% of clinical samples submitted to our Laboratory, which is in agreement with colonization reported among community members without healthcare contacts in the USA (0.2%) and Europe (0.7%) (10, 19).

It has been documented that CA-MRSA infections have been increasing among adults and children (4, 20). The results of the present study have also shown that MRSA more often infected the oldest (≥65) and youngest (0-6) age groups of patients than other age groups. Therefore, microbiologic culture and antimicrobial susceptibility testing would be recommended to guide treatment.

The prevalence of colonization of both *S. aureus* and MRSA in food handlers and their appearance in food products was low and in agreement with the prevalence of *S. aureus* and MRSA infections in our region. Reportedly, MRSA-contaminated food can be a vehicle of outbreaks affecting low-risk persons within the community and the food was contaminated by an asymptomatic carrier (21). There were no *S. aureus* foodborne outbreaks noted in this period.

The spectrum of illness is similar for MRSA and MSSA infections in our community, but we found that MRSA were more often isolated from the GU tract and wound infections than from other sites.

Susceptibility results for MRSA demonstrated that the prevalence of resistance to ciprofloxacin and erythromycin was as high as 80% and 90%, respectively (22, 23). Fluoroquinolone resistance emerged very rapidly in HA-MRSA in the years after widespread utilization of these agents (23-25). No resistance to fluoroquinolones was noted in this study in MRSA isolates of any origin investigated, but interestingly, it was detected in MSSA isolated from clinical samples and food products.

We found 47.6% MRSA isolates having the SCC*mec* type IV / V phenotype, which is typical for CA-MRSA isolates (7). All MRSA isolated from food handlers and food products (ice cream) were SCC*mec* type IV or V phenotype. SCC*mec* type IV/V type has increased mobility and therefore greater potential for horizontal spread to diverse *S. aureus* genetic backgrounds, compared with other SCC*mec* types (13, 14). We did not perform genotype confirmation of SCCmec type IV or V phenotype, but according to the high correlation between the genotype and phenotype we could assume that at least some of these MRSA strains are generated in the community.

Our investigation has some limitations. This is a retrospective study with a relatively small sample size and accordingly, a small number of MRSA were analysed. Additionally, molecular analysis was not perfomed and a risk factors involved in acquisition of MRSA infections wre not investigated. Also, data on the prevalence of HA-MRSA in this region are missing. But, since we found that 25.4% (16/63) MRSA isolates were fully susceptible to all antibiotic tested and 30 (47.6%) MRSA isolates had SCC*mec* IV/V phenotype we could estimate that MRSA generated in the communitya might be present in this region.

The origin of CA-MRSA strains is still the subject of debate**.** Only studies based on appropriate molecular analysis would be able

to determine these newly identified community-acquired strains. Further population-based studies in outpatient settings are warranted in order to define fully the extent of MRSA infections without identified risk.

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