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Panton–Valentine leucocidin gene carriage among *Staphylococcus aureus* strains recovered from skin and soft tissue infections in Turkey

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Objectives: Regardless of methicillin resistance, Panton–Valentine leucocidin (PVL)-positive *Staphylococcus aureus* isolates are associated with various types of infections and outbreaks. Limited data exist about the PVL content of *S. aureus* strains in Turkey. In this multicentre study, we aimed to assess the PVL positivity and antimicrobial susceptibilities of *S. aureus* isolates recovered from skin and soft tissue samples of both community and nosocomial origin in the study period, 2007–08.

Methods: Two hundred and forty-two [92 community-acquired (CA) and 150 hospital-acquired (HA)] isolates were included in the study. Analysis of *mecA* and PVL was carried out using PCR. All isolates underwent susceptibility testing according to the CLSI.

Results: Out of 242 isolates, 77 were *mecA* positive. PVL was not found among methicillin-resistant *S. aureus* (MRSA) isolates, but 8 (5.3%) HA methicillin-susceptible *S. aureus* (MSSA) and 14 (15.2%) CA-MSSA, mostly isolated from furuncles (71.4%), were positive for PVL. Among PVL-positive strains, the penicillin resistance rate was 90.9%. Low resistance rates, <10%, were detected for erythromycin, fusidic acid and co-trimoxazole. PVL-positive strains showed higher rates of susceptibility to erythromycin, gentamicin and rifampicin than negative isolates.

Conclusions: Based on the findings of this study, infection related to PVL-carrying CA-MRSA is not at an alarmingly high level, but population-based surveillance studies should be done to determine the real status.

Keywords: *mecA*, CA-MRSA, PVL, skin infections, antimicrobial resistance

Introduction

The rapidly increasing prevalence of infection and outbreaks due to methicillin-resistant *Staphylococcus aureus* (MRSA) carrying Panton–Valentine leucocidin (PVL), a pore-forming cytotoxin commonly associated with skin and soft tissue infections, has been reported worldwide.[1] Similar to in Europe, PVL-associated infections are not common in Turkey, which is in contrast to the situation in the USA, where PVL-MRSA strains are a leading cause of skin and soft tissue infections.[2,3] The aim of this study was to determine using PCR the frequency of PVL among community-acquired (CA) and hospital-acquired (HA) *S. aureus* isolates recovered from skin and soft tissue infections, and also to evaluate the antimicrobial susceptibility profiles.

Materials and methods

Study group and strains

Clinicians from multiple clinics were asked to collect wound swab samples and complete a data form (including sex, age, lesion type, chronic diseases, personal or family history of recurrent skin infections, history of hospitalization, surgery in the past year and hospital attendance of close relatives) for any patient with skin and soft tissue infection admitted to the outpatient clinics between November 2007 and April

2008. All samples ($n=150$) were sent to the National Reference Hygiene Center, Ankara. Swabs were inoculated onto 5% sheep blood agar. Among 150 swab samples, 6 (4.0%) showed no growth, 92 (61.3%) *S. aureus*, 34 (22.6%) coagulase-negative *Staphylococcus* spp., 17 (11.3%) Gram-negative bacilli and 1 (0.6%) *Micrococcus* sp. A set of *S. aureus* isolates ($n=150$) from skin and soft tissue infections of hospitalized patients admitted to the hospital during the study period were also included.

Methicillin resistance detection and antimicrobial susceptibility

Methicillin resistance was determined using the Kirby–Bauer disc diffusion method with 1 μg of oxacillin and 30 μg of cefoxitin discs (Oxoid, UK). The results were confirmed using oxacillin screen agar supplemented with 4% NaCl and 6 mg/L oxacillin (Sigma–Aldrich, USA), according to the CLSI guidelines.[4] Susceptibilities to penicillin, gentamicin, tetracycline, erythromycin, rifampicin, mupirocin, clindamycin, co-trimoxazole and teicoplanin (Oxoid, UK) were determined based on CLSI guidelines.[4] For fusidic acid, Comité de l'antibiogramme de la Société Française de Microbiologie criteria were used.[5] *S. aureus* ATCC 25923 and ATCC 43300 were used as susceptible and resistant control strains, respectively.

Detection of mecA and PVL

mecA PCR was performed as described by Murakami *et al*.[6] *S. aureus* ATCC 43300 and ATCC 25923 were used as positive and negative controls for this PCR assay, respectively. *lukS-PV/lukF-PV* PCR was performed as described by Lina *et al*.[1] *S. aureus* HT20041200 and HT20041212 were used as positive and negative controls for this PCR assay, respectively.

Statistical analysis

Statistical comparisons were performed using SPSS software 15.0 (SPSS, Inc., Chicago, IL, USA), using the χ^2 test or Fisher's exact test. All hypotheses were two-tailed and were considered significant at the $P<0.05$ level.

Results

Among 242 *S. aureus* isolates, 77 [66 (87.5%) inpatient and 11 (14.2%) outpatient; OR=5.79, 95% CI=2.85–11.73; $P=0.001$] were *mecA* positive. Oxacillin and cefoxitin disc diffusion tests and oxacillin screen agar exhibited sensitivities of 98.7%, 98.7% and 100%, and specificities of 96.9%, 97.5% and 96.9%, respectively.

The most prevalent lesions among patients were abscess ($n=43$), furuncle ($n=31$) and pyoderma ($n=31$). Evaluation of the risk factors showed that the only statistically significant risk factor was hospital attendance (OR=17.8; $P=0.037$). According to *mecA* PCR, 242 *S. aureus* isolates were classified as CA methicillin-susceptible *S. aureus* (MSSA) [$n=81$ (33.5%)], HA-MSSA [$n=84$ (34.7%)], CA-MRSA [$n=11$ (4.5%)] and HA-MRSA [$n=66$ (27.3%)]. After excluding outpatients with risk factors, five isolates were identified as true CA-MRSA.

Out of 242 *S. aureus* isolates, 22 (9.1%) were PVL positive, including 14 (15.2%) CA ($n=92$) and 8 (5.3%) HA ($n=150$), none of which was methicillin resistant. CA isolates showed higher PVL positivity than HA isolates: 15.2% versus 5.3% (OR=3.19; $P<0.009$). Among CA samples, PVL-positive strains were isolated from furuncle [10 (71.4%)], abscess [3 (21.4%)] and carbuncle [1 (7.1%)], and a statistically significant relationship was observed between PVL carriage and furunculosis (OR=12.50; $P<0.001$).

All isolates were susceptible to teicoplanin. mecA-positive strains showed higher resistance rates to all antimicrobials except mupirocin, compared with *mecA*-negative isolates, and a statistically significant relationship was detected between *mecA* carriage and resistance to erythromycin, clindamycin, tetracycline, gentamicin and rifampicin. The antimicrobial resistance rates of the strains are shown in Table 1 and Figure 1.

Among PVL-positive CA-MSSA ($n=14$), two were susceptible to all antibiotics and penicillin resistance (42.9%) was the most common resistance phenotype. Resistance rates to antimicrobials were: penicillin, 85.7%; tetracycline, 28.5%; erythromycin, 14.2%; and fusidic acid, 7.1%. Among PVL-positive HA-MSSA, four (50%) were susceptible to all antimicrobials except penicillin and showed resistance to penicillin of 100%, tetracycline of 37.5% and co-trimoxazole of 12.5%. Higher rates of susceptibility to erythromycin, gentamicin and rifampicin were observed among PVL-positive strains compared with among negative isolates ($P=0.001$).

Discussion

In this study, the PVL content of 242 *S. aureus* isolated from skin and soft tissue infections was evaluated. A total of 77 (11 outpatient and 66 inpatient) MRSA were identified by PCR. Among the outpatient group, hospital attendance was defined as an MRSA risk factor. Excluding patients with risk factors, five (5.5%) true CA-MRSA were defined. Including patients with risk factors showed that out of 242 *S. aureus*, 11 (4.5%) were CA-MRSA, representing 14.3% of all MRSA isolates.

Although PVL is believed to be a stable marker of CA-MRSA, it was also detected among MSSA and HA isolates.[2,3,7] Variation in its frequency due to geographical area, patient, infection localization and isolate type has been reported. Limited data exist on the PVL content of *S. aureus* strains in Turkey, with the reported rates being <10% for MSSA and <3% for MRSA.[8] In the present study, PVL was not detected among MRSA isolates, but 9% of all *S. aureus* and 13.3% of MSSA were positive. The low prevalence in Europe and Turkey compared with in the USA could be explained by differences in the distribution of clonal lineages. Dominant

Table 1. Distribution of antimicrobial resistance among *S. aureus* isolates according to *mecA* analysis

	Resistance (%)			
Antimicrobial	CA-MSSA ($n=81$)	HA-MSSA ($n=84$)	CA-MRSA ($n=11$)	HA-MRSA ($n=66$)
Erythromycin	8.6	20.2	27.3	69.7
Clindamycin	1.2	6.0	18.2	30.3
Penicillin	90.1	91.7	100.0	100.0
Mupirocin	3.7	1.2	—	1.5
Co-trimoxazole	—	1.2	—	1.5
Fusidic acid	2.5	—	—	6.1
Tetracycline	21.0	31.0	81.8	92.4
Rifampicin	7.4	17.9	72.7	97.0
Gentamicin	4.9	14.3	63.6	81.8

The MRSA status of the strains was determined by detection of *mecA*.

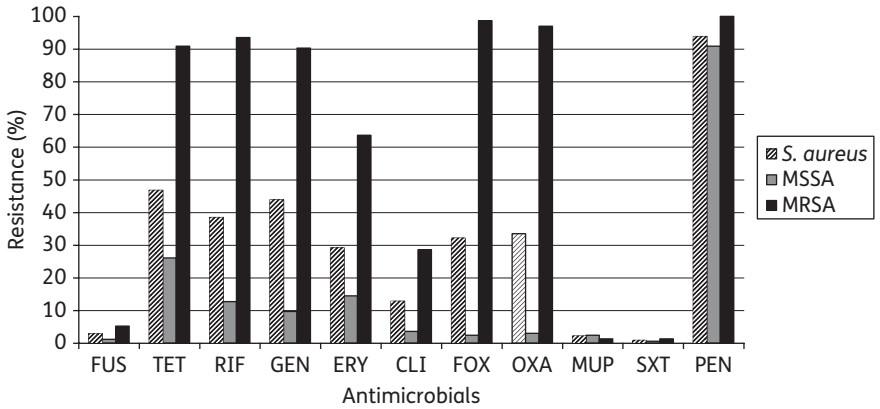

Figure 1. Distribution of antimicrobial resistance of the strains by isolate type ($n=242$). FUS, fusidic acid; TET, tetracycline; RIF, rifampicin; GEN, gentamicin; ERY, erythromycin; CLI, clindamycin; FOX, cefoxitin; OXA, oxacillin; MUP, mupirocin; SXT, co-trimoxazole; PEN, penicillin.

clones frequently isolated in the USA, ST1 and ST8 (where ST stands for sequence type), have the ability to disseminate rapidly, compared with the common ST80 found in Europe, which has a low adaptation feature to the hospitals.[9] We found that 5.3% of the HA isolates have PVL, possibly the result of community acquisition by the inpatient group. It is clear that CA-MRSA infections seem not to be a serious threat in our region yet, but it is essential to conduct prevalence studies on a large scale in the different populations in the community.

Clindamycin and co-trimoxazole have been recommended for the treatment of cutaneous infections related to *S. aureus*. In this study, all isolates except two HA *S. aureus* were susceptible to co-trimoxazole. Although resistance to erythromycin and clindamycin is mediated by a similar mechanism, the erythromycin resistance rate was higher than that for clindamycin among MRSA (63% and 28%, respectively), indicating the fact that the empirical use of macrolides should be monitored closely.

Resistance rates of >60% to tetracycline, rifampicin and aminoglycosides among HA-MRSA strains showed that empirical therapy with these drugs should be avoided, especially in hospitalized patients. Additionally, the higher antimicrobial resistance rates observed among CA-MRSA compared with CA-MSSA strains, except for mupirocin, co-trimoxazole and fusidic acid, could be explained by the extensive use of those antibiotics in our study region. The high resistance rates among CA-MRSA with risk factors for hospital acquisition could be due to the misclassification of HA-MRSA isolates as CA-MRSA. We found lower resistance percentages both in methicillin-resistant and -susceptible strains for mupirocin (1.3% versus 2.4%, respectively) and for fusidic acid (5.2% versus 1.2%, respectively), indicating these drugs can be used, especially in patients with mild skin and soft tissue infections.

Limited data exist on the antimicrobial susceptibility of PVL-carrying CA-MRSA strains, and strains have generally been found to be susceptible to co-trimoxazole, rifampicin, fusidic acid and tetracycline.[10] Low resistance rates were obtained except for tetracycline in this study, but the results have to be evaluated carefully due to the low number of PVL-positive isolates.

Infection control has become increasingly difficult because HA-MRSA and CA-MRSA have been isolated in both locations, i.e. in hospitals and in the community. Moreover, HA-MRSA strains carrying PVL and the SCC*mec* type IV element have also been detected in the community.[3] Recently, genetic relatedness between PVL-positive MSSA lineages, epidemic-associated CA-MSSA and CA-MRSA lineages was observed.[7] In light of this, PVL-carrying MSSA isolates should be considered with caution in order to prevent the spread of PVL to methicillin-resistant isolates.

In conclusion, this study showed that only a small proportion of our isolates harbour PVL. Although we could not detect any PVL-positive CA-MRSA strain in our study group, routine surveillance and population-based studies should be carried out to determine the real status in Turkey, and appropriate infection control measures should be implemented to control the dissemination of PVL to methicillin-resistant isolates.

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Transparency declarations

None to declare.

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