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Survey of community-associated-methicillin-Resistant *Staphylococcus aureus* in Slovenia: Identification of community-associated and livestock-associated clones

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ABSTRACT

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Slovenia is poorly documented. The aim of this study was to investigate susceptibility patterns, virulence gene profile and clonality among MRSA isolates with positive screened resistance phenotype for CA-MRSA collected from patients in Slovenia, from January 2010 to December 2010.

We included only MRSA isolates that were resistant to cefoxitin and oxacillin, and susceptible to at least two of the following four antibiotics: ciprofloxacin, erythromycin, clindamycin or gentamicin (presumptive CA-MRSA). Altogether 151 isolates fulfilled our screening phenotypic definition, 126 MRSA isolates were classified as CA-MRSA and 25 as HA-MRSA. Thirty-six per cent of them were resistant to ciprofloxacin, 24% to clindamycin, 33% to erythromycin and 13% to gentamicin. The *mec*A gene was detected in 150 isolates, while the *mec*C gene only in 1 isolate. The MRSA isolates were classified to 19 different clones. The most prevalent sequence types were ST5 (26.4%), ST45 (25.2%), ST22 (10.6%), ST398 (9.9%), ST8 (5.9%), ST7 (4.6%), ST1 (3.9%), ST152/377 (3.3%), ST228 (2.6%) and ST2883 (1.3%). The ST6, ST9, ST30, ST72, ST88, ST111, ST130, ST225 and ST772 were identified sporadically. The Panton–Valentine leukocidin (PVL) gene was detected in 13 (8.6%) isolates that belonged to ST5, ST7, ST8, ST22, ST72, ST88, ST 152/377 and ST772.

Our results show high variability of CA-MRSA circulating in Slovenia and also the presence of LA-MRSA clones.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens responsible for hospital-associated (HA-MRSA), community-associated (CA-MRSA) and livestock-associated (LA-MRSA) infections (Grundmann et al., 2006; Deurenberg and Stobberingh, 2008). Global epidemiology studies of HA-MRSA and CA-MRSA have shown the spread of several heterogeneous clones all over the world (Deurenberg and Stobberingh, 2008; Monecke et al., 2010; Chua et al., 2011; Mediavilla et al., 2012). HA-MRSA clones circulating in hospitals are Archaic (sequence type (ST) 250, staphylococcal cassette chromosome (SCC*mec*) I, Iberian (ST247, SCC*mec*I), Southern Germany (ST228, SCC*mec*I), New York–Japan (ST5, SCC*mec*II), Paediatric (ST5, SCC*mec*IV), Brazilian/Hungarian (ST239, SCC*mec*III), UK EMRSA-15 (ST22, SCC*mec*IV) and UK EMRSA-16 (ST36, SCC*mec*II) clones Deurenberg and Stobberingh, 2008). CA-MRSA belongs mainly to five predominant lineages, USA400 or WA-1 (ST1, SCC*mec*IV), USA300 (ST8, SCC*mec*IV), South West Pacific (ST30, SCC*mec*IV), Taiwan (ST59, SCC*mec*V) and European (ST80, SCC*mec*IV) clones (Chua et al., 2011; Mediavilla et al., 2012). Among LA-MRSA, pig-associated (ST398, SCC*mec*IV/V) and *mec*C-positive (clonal complex (CC) 130, SCC*mec*XI) clones are predominant (Chua et al., 2011; Fluit, 2012; Paterson et al., 2014).

MRSA is well-controlled in Slovenian hospitals. Data on the antimicrobial resistance of *S. aureus* and MRSA have been reported, but unfortunately, the data on virulence gene profiles and on clonality remain incomplete in our country. According to Deurenberg

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and Stobberingh (2008) the Southern German, the UK-EMRSA-3 (ST5, SCC*mec*I), the Iberian and the Brazilian/Hungarian clone was predominating among HA-MRSA in previous years in Slovenia. CA-MRSA infections are less frequent in Europe than in the United States (Deurenberg and Stobberingh, 2008; Chua et al., 2011; Mediavilla et al., 2012). In Slovenia, some documented outbreaks have been published. Mueller-Premru et al. (2005) described an outbreak among members of a football players caused by PVL positive CA-MRSA strains (*spa* type t002, ST5 and *spa* type t454, ST152). Dermota et al. (2011) documented outbreaks of four cases of skin and soft tissue infections due to a CA-MRSA strain obtained from one Slovenian hospital in 2011 (*spa* type t044, ST80).

In Slovenia, a complete overview of CA-MRSA is lacking. HA-MRSA strains are widely distributed in our country, and are typically resistant to gentamicin, ciprofloxacin, erythromycin and clindamycin. CA-MRSA are less frequent, therefore, the aim of this study was to determine which CA-MRSA clones are circulating in Slovenia. For this purpose, we reviewed presumptive CA-MRSA isolates in the strain collection database of the Medical microbiology department of the National Laboratory for Health, Environment and Food based on phenotypic characteristics retrospectively during a 12-month period in the year 2010. Only MRSA isolates resistant to oxacillin and cefoxitin, and susceptible to at least two of the following four antibiotics: ciprofloxacin, erythromycin, clindamycin and gentamicin, were included in further analyses (Grmek-Košnik et al., 2009). With the present study we also tried to confirm our phenotypic rules/definition based on antimicrobial resistance pattern as useful tools for suspecting CA-MRSA strains in Slovenia, and to assess the accordance between phenotypic, genotypic characteristics and epidemiological data among presumptive CA-MRSA isolates.

Materials and methods

Setting

Slovenia has an area of 20,273 km^2 and a population of 2 million. Department of Clinical Microbiology of the National Laboratory for Health, Environment and Food (located in Kranj, Nova Gorica, Koper, Celje, Novo mesto, Murska Sobota and Maribor) and the Institute of Microbiology and Immunology of Medical Faculty University of Ljubljana (located in Ljubljana) serve 13 hospitals, primary care and long-term care facilities in Slovenia. During a 12-month period in 2010, 8 laboratories participating in the study were asked to include all patients harbouring a MRSA strain that was resistant to oxacillin and cefoxitin, and susceptible to at least two of the following four antibiotics: ciprofloxacin, erythromycin, clindamycin and gentamicin. MRSA isolates with a positive screening phenotypic pattern and with a clinical data form were sent to the National Laboratory for Health, Environment and Food Kranj for molecular analyses. A CA-MRSA strain was defined as strains isolated from ambulatory patients or during the first 48 h after admission to the hospital in a patient with no risks factors for nosocomial acquisition in the previous year, such as colonization or infection with MRSA and hospitalization history. All other isolates were considered as HA-MRSA. Information was extracted from the laboratory information system (MBL, Src Infonet, Kranj).

MRSA isolates

From January 2010 to December 2010, 1675 MRSA isolates were collected in 8 microbiological laboratories in Slovenia and 151 MRSA isolates had a positive phenotypic pattern. Among 151 MRSA isolates 126 MRSA isolates fulfilled the criteria for classification as CA-MRSA, and 25 were classified as HA-MRSA. One isolate per patient was included in the study. Most MRSA isolates were isolated from asymptomatic carriers, during screening for colonization in health care settings. All isolates were identified by mass spectrometry (MALDI-TOF MS, Biotyper, Bruker Daltonic GmBH, Bremen, Germany).

Antimicrobial susceptibility testing

The susceptibility patterns of the MRSA isolates were determined using a standardized agar disk diffusion method according to the guidelines of the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2010). The antibiotics tested were penicillin, cefoxitin, vancomycin, gentamicin, tobramycin, kanamycin, erythromycin, clindamycin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, rifampin, linezolid, mupirocin and fusidic acid (BD, Maryland). Minimal inhibitory concentration (MIC) determination of oxacillin and vancomycin was performed using the *E*-test (bioMerieux, France).

Molecular characterization

Methicillin resistance was confirmed by *mec*A PCR (Kondo et al., 2007). We used PCR targeting the *mec*C gene for those isolates which were *mec*A negative, but showed phenotypic methicillin resistance (30 μg cefoxitin disk on Mueller-Hinton II agar; BD, Maryland) (Stegger et al., 2012). Genes encoding staphylococcal enterotoxin (*sea* to *see, seg, seh, sei, sek, sel, sen, seo, seu, seq*), toxic shock syndrome toxin (*tst*), staphylococcal exfoliative toxins (*eta, etb, etd*), leukocidin M (*luk*M) and Panton–Valentine leukocidin (*luk*S-*luk*F) were detected by multiplex PCR (Jarraud et al., 2002; Monday and Bohach, 1999). SCC*mec* typing was performed by a multiplex PCR strategy previously described by Kondo et al. (Kondo et al., 2007). Amplification, sequencing and analysis of the polymorphic region of the protein A (*spa* typing) and MLST were performed according to a method described previously (Harmsen et al., 2003; Enright et al., 2000).

Results and discussion

This is the first systematic study analyzing the phenotypic and genotypic characteristics and clonality among presumptive CA-MRSA isolates in Slovenia. In the year 2010, 151 MRSA isolates with the positive screened resistance phenotype for presumptive CA-MRSA were found. One hundred twenty four MRSA isolates were collected as surveillance screening specimens upon admission and 27 from clinical specimens (8 from blood-cultures, 8 from wound swabs, 4 from abscess, 2 from skin swabs, 2 from ear swabs, 1 from tissue, 1 from urine and 1 from aspirate tracheae). Of those, 126 (83.4%) MRSA strains were classified as CA-MRSA and 25 (16.6%) as HA-MRSA. The median age of patients was 64.1 years (range 1-96), 105 of them were male (69.5%) and 46 female (30.5%). The patients' characteristics and epidemiology are shown in Table 1.

Overall, 19 sequence types (STs) were identified, one of which is a novel ST, ST2883. 26.4% of the strains were classified as ST5, 25.2% as ST45, 10.6% as ST22, 9.9% as ST398, 5.9% as ST8, 4.6% as ST7, 3.9% as ST1, 3.3 as ST152/377, 2.6% as ST228 and 1.3% as ST2883. The ST6, ST9, ST30, ST72, ST88, ST111, ST130, ST225 and ST772 were identified sporadically. Characteristics of presumptive CA-MRSA strains are shown in Table 2. Fig. 1 shows the distribution of presumptive CA-MRSA clones in Slovenia.

The first major clone (32 isolates, 21.2%) was related to the Berlin clone, ST45, *agr* type I, SCC*mec* type IV, *spa* type t015 ($n = 18$), t026 ($n = 3$), t230 ($n = 1$), t728 ($n = 6$), t737 ($n = 1$), t1179 ($n = 1$), t1231 ($n = 1$) and t13070 ($n = 1$). We detected this clone in six of