Epidemiological and Drug-Resistance Types of Methicillin-Resistant *Staphylococcus Aureus* Strains Isolated From Surgical and Transplantation Ward Patients During 2010 to 2011

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ABSTRACT

Background. The increasing prevalence of multi-drug–resistant methicillin-resistant *Staphylococcus aureus* (MRSA) is a substantial problem in hospitals worldwide, especially in wards with immunocompromised patients undergoing organ transplant. Epidemiological characteristics and antibiotic susceptibility profiles of hospital-acquired (HA) MRSA strains isolated from surgical/transplantation ward patients were studied.

Methods. We analyzed 26 HA-MRSA strains isolated from 22 patients hospitalized at 3 different surgical and transplantation wards at a Warsaw clinical hospital during 2010 to 2011. Eleven patients were MRSA-asymptomatic carriers. Strain relatedness was evaluated through the use of multi-locus sequence typing (MLST), multi-locus variable-number tandem repeat analysis (MLVA), and random amplified polymorphic DNA/arbitrarily primed PCR (RAPD) methods. Antibiotic susceptibility was assessed the use of routine diagnostic methods.

Results. The evaluated strains belonged to 4 clonal complexes (CCs) and 4 sequence types (STs): CC30/ST36 (65.4%), CC8/ST8 (15.4%), CC5/ST1827 (11.5%), and CC1/ST1 (7.7%). Six MLVA types and 6 RAPD types were isolated. A ciprofloxacin-, erythromycin-, and clindamycin-resistant CC30/ST36 clone (MLVA type 1, RAPD type 1A) was isolated in all wards. The isolated HA-MRSA strains were most often resistant to ciprofloxacin (100%), erythromycin (96.2%), clindamycin (84.6%), and gentamycin (30.8%).

Conclusions. A ciprofloxacin-, erythromycin-, and clindamycin-resistant HA-MRSA ST36 CC30 clone, which prevailed on transplantation wards in the years 2010 to 2011, is probably one of the international epidemic clones named UK EMRSA-16 or USA200.

INCREASING prevalence of hospital-acquired, multi-drug–resistant clones of methicillin-resistant *Staphylococcus aureus* (HA-MRSA) constitutes a considerable problem in many hospital wards [1], including the wards with immunocompromised patients who are being qualified for, or have recently undergone organ transplantation. The reason behind the MRSA resistance to a majority of beta-lactam antibiotics is the synthesis of an acquired transpeptidase (PBP2A) encoded by *mec*A or *mec*C genes. These genes can be found in genomic islands, known as staphylococcal chromosome cassettes mec (SCCmec). Apart

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from the *mec* gene, some of these SCCmec types contain genes of resistance to macrolides, lincosamides, or aminoglycosides. Epidemiological patterns of MRSA strains help detect an epidemic and identify epidemic clones [2–4]. The primary aim of the study was to determine antibiotic resistance profiles of methicillin-resistant *S aureus* strains isolated from hospitalized patients, to perform their phylogenetic analysis, and to detect clones belonging to international sequence types (ST) and clonal complexes (CC).

METHODS

Bacterial Strains

This study evaluated 26 MRSA strains isolated from surgical and transplantation ward inpatients in the period between October 1, 2010, and September 30, 2011. The analyzed strains were isolated from asymptomatic bacterial colonizations (n = 10), infected wounds (n = 6), blood samples (n = 5), tracheal aspirate (n = 1), endotracheal tube (n = 3), and tracheostomy tube (n = 1).

Susceptibility testing was conducted with the disk diffusion method in accordance with EUCAST guidelines [5].

Multi-Locus Variable-Number Tandem Repeat Analysis

Multi-locus variable-number tandem repeat (VNTR) polymorphism was analyzed with the polymerase chain reaction (PCR) technique for 8 *S aureus* resistance-encoding genes or virulence factors: *mec*A, *clf*A/*clf*B, *spa*A, *sdr*C/*sdr*D/*sdr*E, and *ssp*A [6,7]. Strains differing in more than 4 aspects of genetic profile were classified as phylogenetically distant.

Random Amplified of Polymorphic DNA/Arbitrarily Primed PCR

The PCR and M13 oligonucleotide techniques were used according to Lee [8]. Strains that demonstrated more than 4 differences in their genetic profiles were classified into separate genotypes.

Multi-Locus Sequence Typing

Multi-locus sequence typing (MLST) was used to determine single-nucleotide polymorphisms (SNPs) in 7 *S aureus* housekeeping genes (*arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi*, and *yqi*L), according to Enright et al [9]. The analyzed strains were classified into international sequence types (ST) and clonal complexes (CC) according to the database available at www.saueus.mlst.net.

RESULTS

In the present study, 26 HA-MRSA strains, isolated from 22 surgical/transplantation ward patients were investigated in detail. Twelve patients were hospitalized for kidney/liver transplantation or post-transplant complications. In 2 patients, more than 1 MRSA isolate was incorporated for the study because of significant interval between strain isolations and additionally different nature of colonization (asymptomatic carrier state or infection).

In the study group, 50% of patients (11 subjects) were MRSA-asymptomatic carriers in the nose, throat, and/or gastrointestinal tract, 3 of which had history of infection caused by MRSA strain. All MRSA carriers were subjected for contact isolation for at least 2 weeks, without antibiotics administration. In 7 patients, control swabs from mucous membranes of the nose and/or throat revealed no MRSA strains. One patient developed endogenous MRSA bloodstream infection and died despite negative swab sampling. There were no available data concerning clinical outcomes of contact separation on other patients with MRSA carriage.

Eleven patients developed symptomatic MRSA infections involving mainly skin, soft tissues, and blood. Patients with infection were treated most commonly with vancomycin or linezolid. The treatment of 7 patients resulted in the desired therapeutic effect; in 3 cases, healthcare improvement was achieved but MRSA eradication failed (infection turned into asymptomatic colonization). In 1 patient, the treatment failed and the patient died.

One patient had primary bacteremia caused by endogenous *S aureus* MSSA strain (methicillin-sensitive) and afterward secondary infection and then asymptomatic colonization with MRSA strain, which had a similar drug susceptibility profile (with the exception of resistance to beta-lactams) and belonged to the same sequence type and clonal complex ST8/CC8 as a previous causal *S aureus* strain.

The isolated 26 HA-MRSA strains demonstrated resistance to ciprofloxacin (100%), erythromycin (96.2%), clindamycin (84.6%), gentamycin (30.8%), tetracycline (15.4%), fusidic acid (11.5%), chloramphenicol (7.7%), and mupirocin (3.9%). Table 1 presents antibiotic resistance patterns. All evaluated strains were susceptible to vancomycin, linezolid, and tigecycline and daptomycin.

Table 1 shows the results molecular typing conducted with the use of 3 methods. The evaluated strains belonged to 4 CCs and 4 STs: CC30/ST36 (65.4%), CC8/ST8 (15.4%), CC5/ST1827 (11.5%), and CC1/ST1 (7.7%). Six MLVA types and 6 random amplified polymorphic DNA (RAPD) types were identified. ST36 strains were classified into 1 MLVA type and 2 RAPD types; ST8 strains were classified into 2 MLVA types and 3 RAPD types; ST1827 strains were classified into 1 MLVA and 1 RAPD type; and ST1 strains were classified into 2 MLVA types and 1 RAPD type. Clone CC30/ST36 (MLVA type 1, RAPD type 1A), which was resistant to ciprofloxacin, erythromycin, and clindamycin, was isolated from patients in the evaluated wards and was decidedly the most common one among the analyzed HA-MRSA strains.

DISCUSSION

The epidemiological success of MRSA in hospital wards is based on several mechanisms: (1) strong adaptive properties, (2) high genomic flexibility, (3) ability to acquire new mechanisms of resistance to antibacterial agents, (4) ability to accumulate nucleotide point mutations and their clonal spread (the spread allows propagation of clones closely related to each other), and (5) ability to colonize asymptomatically different human body sites and