Early Detection and Control of Methicillin resistant Staphylococcus aureus Outbreak in an Intensive Care Unit

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Background: Although Methicillin resistant Staphylococcus aureus (MRSA) is one of the major pathogens of healthcare associated infections, we had only sporadic cases in our intensive care unit (ICU) for years.

Aims: To investigate the sudden increase in the number of MRSA cases in ICU.

Study design: Descriptive study.

Methods: From the 5th December 2016 to 26th January 2017, we detected 11 new MRSA cases in ICU. Screening of 73 ICU healthcare workers (HCWs) and screening of 13 patients was performed for outbreak investigation. Nine clinical isolates available in stocks and eight screening MRSA isolates were included in molecular studies. PFGE, spa-mecA-mecC-PVL in-house multiplex PCR assay and spa typing, SCCmec typing were performed for all isolates. Sequence type of the representative strain was determined by Multi-Locus Sequence typing (MLST).

Results: All strains were mecA positive, PVL negative, and have the same antimicrobial susceptibility pattern except for two strains. All clinical, two patient screening and three nasal isolates of HCWs showed the same pulsotype, named clone A. The spa type of outbreak isolates is t030 and the SCCmec type is SCCmecIII; the MLST type of representative strain is ST239 (PFGE pulsotype A, ST239-SCCmecIII-t030). Unrelated three isolates had PFGE pulsotype B-SCCmec-t030, PFGE pulsotype C-SCCmecIII-t1459, PFGE pulsotype D-SCCmecIII.

Conclusion: Molecular typing techniques are the cornerstones for the investigation of outbreaks. Infection control measures, such as enhancing cleaning procedures, promoting hand hygiene, should be enforced in the ICU unit. All patients, including those who have already been discharged to other departments, must be put on contact isolation. HCWs carrying the MRSA strains could be offered decolonization.

Methicillin-resistant Staphylococcus aureus (MRSA) was described for the first time in 1961, after the use of methicillin in 1959. MRSA is a causative agent of a broad spectrum of infectious diseases and considered as a frequent pathogen of health care-associated infections (1, 2). Methicillin resistance in S aureus is encoded by mecA or mecC genes that are carried on a mobile genetic element known as staphylococcal cassette chromosome (SCC) (1, 3). MRSA outbreaks are serious situations that can result in significant mortality and morbidity (4). Rapid diagnostic tests are needed to detect infections caused by MRSA strains and to limit the spread of these strains (3). Genotyping of outbreak strains to detect the possible source of the outbreak and immediate implementation of control measures are necessary. Frequently used molecular methods for typing purposes are pulsed-field gel electrophoresis (PFGE), spa typing, SCCmec typing, and multilocus sequence typing (MLST).

In this study, we aimed to investigate new MRSA cases in the intensive care unit (ICU). We screened patients and health care workers (HCWs) and performed spa typing, PFGE, SCCmec typing, and MLST on MRSA strains to decide whether any transmission was occurring and measures of infection prevention were mandatory.

MATERIALS AND METHODS

Ethical Considerations

Ethics committee approval was received for this study from the Local Ethics Committe of Şişli Hamidiye Etfal Training and Research Hospital (date: 30.04.2019; number: 2377).
Outbreak description and bacterial isolates

From December 5, 2016, to January 26, 2017, we detected 11 new MRSA cases in the adult ICU. On December 20, 23, and 24, 2016, the Clinical Microbiology Department noticed 3 consecutive MRSA isolates, 2 from the blood and 1 from tracheal aspirate culture, having the same antibiotic susceptibility pattern belonging to 3 different patients in the adult ICU. We searched the Laboratory Information System for MRSA growths starting from October; we found 3 more isolates and 2 more cases who had already been discharged from the ICU to the neurology and general surgery wards. The first isolate was from a blood culture dated December 5 and belonged to a male patient who had a positive blood culture on December 20. We determined him as the index case. The index case was a morbidly obese patient hospitalized in the ICU because of respiratory failure owing to pneumonia and left ventricular dysfunction.

Nasal screening of 74 ICU HCWs and nasal, throat, axilla, groin, and rectum screening of 13 patients were performed for outbreak investigation. Identification and antimicrobial susceptibility test (AST) were done by MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) and Phoenix100 ID/AST system (Becton Dickinson, Sparks, MD, USA), respectively.

A total of 9 clinical isolates available in stocks (7 blood, 1 tracheal aspirate, 1 abscess; 1 isolate per patient) and 8 screening MRSA isolates (5 HCWs and 3 patients) were included in molecular studies.

Evaluation of mecA, mecC, spa, and PVL genes by multiplex polymerase chain reaction

In-house multiplex polymerase chain reaction (PCR) assay was performed as described by Stegger et al. (5). Instagene Matrix (Bio-Rad) was used for DNA extraction. Confirmation of methicillin resistance and identification of isolates were done by amplification of mecA/mecC genes and spa gene, respectively. We also searched for the Panton-Valentine leukocidin (PVL) gene.

spa typing

spa gene amplicons were purified and sequenced. The open-source spa typing program at http://spatyper.fortinbras.us/ was used for spa sequence analysis.

Pulsed-field gel electrophoresis

PFGE typing of S aureus strains was performed following the protocol of Yetkin et al. (6). Briefly, bacterial cells were embedded into low melting agarose including lysostaphin (5 U/mL). Cells in plugs were digested with proteinase K. After washing the plugs, genomic DNA in the plugs was restricted by 30 U of Smal (Promega Corporation, Durham, NC, USA) for 24 hours at 25°C in a water bath. DNA fragments were separated on 1% agarose gels run in 0.5 × Tris-borate-ethylenediaminetetraacetic acid buffer using a CHEF-DR III system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14°C at 6 V/cm² for 20 hours. The initial and final switch times were 5.3 and 34.9 seconds, respectively. The gel was stained with ethidium bromide (5 µg/mL) for 20 minutes and destained with distilled water for 30 minutes. The DNA band profiles were visualized under ultraviolet light, photographed using a Gel Logic 2200 Imaging System (Kodak Co, Rochester, NY, USA), and analyzed by GelCompar software (version 7.0; Applied Maths, Sint-Martens-Latem, Belgium). A 1% band tolerance was used for the comparison of DNA profiles. The clonal relationship among isolates was evaluated using the criteria of Tenover et al. (7).

SCCmec typing

SCCmec typing was performed using in-house multiplex PCR according to the procedure described by McClure-Warnier et al. (8). A total of 9 primer pairs were used for the screening of major SCCmec types and subtypes I to V.

Multilocus sequence typing

The allelic profile of the outbreak MRSA strain was obtained by sequencing internal fragments of 7 house-keeping genes as described at http://saureus.mlst.net/misc/info.asp. (9): carbamate kinase (arc), Shikimate dehydrogenase (aro), Glyceral kinase (glp), Guanylate kinase (gmk), Phosphate acetyltransferase (pta), triose-phosphate isomerase (tpi), and acetyl coenzyme A acetyltransferase (yqi). The DNA sequence of each allele analyzed using data analysis software at the http://saureus.mlst.net/sql/singlelocus.asp website and allele numbers of 7 ampiclons were found out. By entering all allelic numbers of the strain in a program at the http://saureus.mlst.net/sql/allelicprofilechoice.as website, the sequence type (ST) of the outbreak strain was determined.

RESULTS

Patient and HCW demographics, clonal characteristics, and antibiotic susceptibilities of MRSA isolates and molecular typing test results are presented in Table 1.

Screening cultures

Screening was performed in the ICU on December 24. Of the 13 patients, 5 were carrying MRSA strains, in multiple sites of their body; 2 of 5 sharing the same antibiotic susceptibility profile were shown to carry the outbreak strain by molecular typing methods (15%). Of these 2 colonized patients, one carried the outbreak strain at nasal and groin sites, and the other one at the throat, nasal, groin, and rectum sites. Rectum-colonized patient was an 86-year-old man with a Fournier’s gangrene; 18 of 73 HCWs were S aureus positive for their nasal swab cultures (10 methicillin-susceptible Staphylococcus aureus [MSSA]; 8 MRSA), and molecular typing was done for 5 MRSA sharing the same or similar antibiotic susceptibility pattern with outbreak strain (Table 1). Moreover, 3 of HCWs’ nasal isolates were the outbreak strain (4%).

Antibiotic susceptibilities

We performed the AST using the recommended methods and interpretation of the European Committee on Antimicrobial Susceptibility Testing. The outbreak strain was resistant to ciprofloxacin (CIP), levofloxacin (LEV), clindamycin (DA), erythromycin (E), tetracycline (TE), gentamicin (GN), and tobramycin (TOB) and susceptible to trimethoprim-sulfamethoxazole (TMP-SXT), daptomycin (DPC), fusidic acid (FD), linezolid (LZD), and vancomycin (VA) except clinical isolate 8 and clinical isolate 9. Clinical isolate 8 and clinical isolate 9 were susceptible to DA, E, GN, and TOB.

Decolonization of HCWs

All of the HCWs, except 1 pregnant nurse, were decolonized by TMP-SXT tablets (800/160 mg twice a day for 10 days). We checked the colonization status of HCWs 1 week after the completion of treatment. They were all cleared of the MRSA.
<table>
<thead>
<tr>
<th>Isolate identifier</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Isolation site</th>
<th>Therapy</th>
<th>Outcome</th>
<th>PFGE</th>
<th>spa type</th>
<th>SCCmec type</th>
<th>Cefoxitin</th>
<th>Ciprofloxacin</th>
<th>Chloramphenicol</th>
<th>Daptomycin</th>
<th>Erythromycin</th>
<th>Fusidic acid</th>
<th>Gentamicin</th>
<th>Linezolid</th>
<th>Rifampicin</th>
<th>Tetracycline</th>
<th>TMP-SX</th>
<th>Tobramycin</th>
<th>Vancomycin</th>
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<td>CI-1</td>
<td>48</td>
<td>M</td>
<td>Pneumonia</td>
<td>Blood</td>
<td>PIP-TZP 4.5 g IV TID; Daptomycin 500 mg IV daily</td>
<td>Died</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
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<td>Trachea</td>
<td>Linezolid 600 mg IV BID</td>
<td>Discharged</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>74</td>
<td>F</td>
<td>Meninx benign neoplasm</td>
<td>Trachea</td>
<td>Vancomycin 1 g IV daily</td>
<td>Died</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
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<td>CI-4</td>
<td>68</td>
<td>M</td>
<td>Colon cancer</td>
<td>Blood</td>
<td>Daptomycin 350 mg IV daily</td>
<td>Discharged</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<td>46</td>
<td>F</td>
<td>Falling from high</td>
<td>Trachea</td>
<td>Tigecycline 50 mg IV BID; Meropenem 1 g IV TID</td>
<td>Discharged</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
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<td>S</td>
<td>S</td>
<td>R</td>
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<td>Over cancer</td>
<td>Blood</td>
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<td>Discharged</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
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<td>R</td>
<td>S</td>
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<td>Colon cancer</td>
<td>Abscess</td>
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<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>S</td>
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<td>M</td>
<td>Drug user, intoxication</td>
<td>Blood</td>
<td>Tigecycline 50 mg IV BID; Meropenem 1 g IV TID</td>
<td>Discharged</td>
<td>A</td>
<td>t030</td>
<td>Type I</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>CI-9</td>
<td>86</td>
<td>M</td>
<td>Fournier’s gangrene</td>
<td>Blood</td>
<td>Linezolid 600 mg BID</td>
<td>Died</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<td>K</td>
<td>Cerebellar tumor, epilepsy</td>
<td>Throat, nasal</td>
<td>Vancomycin 1 g IV BID</td>
<td>Died</td>
<td>C</td>
<td>t459</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
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<td>Pt-S-2*</td>
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<td>Meninx benign neoplasm</td>
<td>Nasal, groin</td>
<td>Vancomycin 1 g IV daily</td>
<td>Died</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
<td>S</td>
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<tr>
<td>Pt-S-3**</td>
<td>86</td>
<td>M</td>
<td>Fournier’s gangrene</td>
<td>Throat, nasal, groin, rectum</td>
<td>Linezolid 600 mg IV BID</td>
<td>Died</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
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<td>S</td>
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<td>HCW-1</td>
<td>44</td>
<td>E</td>
<td></td>
<td>Nasal</td>
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<td>A</td>
<td>t030</td>
<td>Type III</td>
<td>R</td>
<td>R</td>
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<td>D</td>
<td>ND</td>
<td>Type III</td>
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<td>Type V</td>
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<td>Nasal</td>
<td>Not decolonized</td>
<td>A</td>
<td>t030</td>
<td>Type III</td>
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<td>S</td>
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<td>S</td>
<td>S</td>
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<td>HCW-5</td>
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<td></td>
<td>Nasal</td>
<td>TMP-SX 160/800 mg PO BID</td>
<td>B</td>
<td>t459</td>
<td>Type I</td>
<td>R</td>
<td>S</td>
<td>S</td>
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</table>


*Pt-S-2 and CI-3 belonged to the same patient; **Pt-S-3 and CI-9 belonged to the same patient

**TABLE 1. Patient and healthcare workers demographics, isolation sites, clonal characteristics, and antibiotic susceptibilities of MRSA isolate

**Evaluation of mecA-mecC-SPAPVL multiplex PCR**

All strains were positive for the mecA gene and negative for the PVL gene.

**Molecular typing**

All clinical isolates and 2 patient screening and 3 nasal isolates of HCWs showed the same PFGE pulstype (PT) that was named
as clone A (Figure 1). The spa ST of these isolates was t030, and the SCCmec type was SCCmecIII. The MLST result was ST239. Clinical isolate 8 and HCW-3 have different SCCmec types, SCCmecI and SCCmecV, respectively. Unrelated 3 isolates had PFGE PT B-SCCmecI-t030, PFGE PT C-SCCmecI-III-t459, and PFGE PT D-SCCmecIII (Figure 2).

**DISCUSSION**

In this study, we report our experience about the MRSA outbreak in an adult ICU. The outbreak was noticed in the middle of December 2016; by that time, 4 patients had already been discharged from the ICU to other clinical wards. We assume that our index case was a male patient who stayed in the ICU for more than a month. Few HCWs were colonized with the outbreak strain, and we assume HCWs might have transmitted the outbreak strain to other patients during the medical care as reported in the literature before (10).

To interrupt the MRSA transmission, various infection control measures were established immediately. Nasal screening of HCWs and nasal, throat, axilla, groin, and rectal screening of ICU patients were performed (11). All of HCWs carrying the MRSA strains except 1 pregnant employee were treated with co-trimoxazole as a decolonization procedure because mupirocin ointment for nasal usage is not available in the market in our country (12). We put our infected and colonized patients in contact isolation, including those who had already been discharged to other wards (13). Infection control nurses gave educational sessions to HCWs on promoting hand hygiene and enhancing cleaning procedures. We screened patients a month later and there were still patients colonized with the outbreak strain, but no new MRSA cases occurred. Therefore, we declared the outbreak was over and decided to follow up on the situation. Identification of an outbreak is usually based on molecular typing, which is a significant tool in infection control. Molecular typing methods show the nosocomial transmission of the successful clone and elucidate its transmission routes and sources in an outbreak setting (14). Different molecular methods are available for typing of S aureus isolates, including PFGE, multilocus variable number of tandem repeat analysis, SCCmec typing, spa typing, MLST, and full-genome sequencing (1, 3, 15). PFGE is still accepted as the “gold standard” for molecular typing of MRSA, and spa is the enzyme of the choice for macrorestriction (16). The major drawback of PFGE is the insufficient comparability of results between laboratories, which might be overcome by using standardized protocols nationally (2, 16). In contrast, results of MLST are comparable between laboratories, but its routine use in infection control is restricted by high cost, labor intensity, and availability of DNA sequencer (16). Recently, it has been reported that spa typing and MLST typing have comparable performance in a macroepidemiologic study (15). Moreover, spa typing has a higher discriminatory power over MLST, so 1 ST type can harbor several spa types, but they remain within the same clonal complex (17). In this study, we used several typing methods, PFGE, SCCmec typing, spa typing, and MLST, simultaneously, for the characterization of the outbreak strain. The outbreak isolates showed the same PFGE PT named as clone A and were ST239-SCCmecIII-t030 strains. ST239-SCCmecIII-t030 has previously been reported as the most frequent hospital-associated (HA) MRSA clone in Turkey (18) and named as TR09. Other studies from Turkey also reported the dominance of MLST ST239 and spa type t030 (19-21) characterized MRSA clones. Oksuz et al. (21) found Vienna/Hungarian/Brazilian clone (ST239-MRSA-III) as the most prevalent clone (53.9%) in a university hospital for more than 5 years, and the rest of the MRSA clones were largely diverse. Geographic distribution of major HA MRSA clones is known worldwide, but sometimes shifts of these MRSA clones have occurred over time in countries, in a region, or even in a single hospital (22). MRSA evolved from MSSA in 1961 by the acquisition of SCCmec (1). Since then, a limited number of clones with a certain genetic background and SCCmec type have...
spread worldwide (22). MRSA ST239 lineage is a globally disseminated HA MRSA consisting of more than 5 clades, such as Asia, North America, South America, Europe, and Australia (23). ST239 is a successful clone in terms of infiltration and adaptation in hospital settings (24). Several studies are being conducted to identify the reasons behind this success. Recently, Hong et al. (24) reported that a higher expression of staphylococcal protein A (SpA) in HA MRSA ST239 helps for durable colonization and immune evasion of the clone. Furthermore, they stipulated that SpA may be a significant factor for the adaptation and persistence of the ST239 clone in hospital environments. Moreover, 2 colonization factors, sax protein first described in ST239 clone during an outbreak in London and arginine catabolic mobile element (ACME), were found to be associated with the epidemiologic success of the MRSA. ACME was first described in CA-MRSA USA300 and has spread horizontally to HA sequence types such as ST3 and ST239 (25, 26). Finally, enteric carriage of HA MRSA has been shown for ST228 and was considered responsible for maintaining a long-term outbreak in a tertiary hospital (27). In our patient group, we found out that 1 patient had enteric colonization with the outbreak strain. However, he was not the index case.

The outbreak strain was resistant to CIP, LEV, DA, E, TE, GN, and TOB and susceptible to TMP-SXT, DPC, FD, LZD, and VA. This resistance pattern was very similar to that indicated in previous reports (21, 23, 28). PVL was not detected on the outbreak strain.

In conclusion, we described an MRSA outbreak in the ICU owing to the clonal spread of the specific strain. Infection control measures were put in practice immediately upon the detection of clustering. Through the microbiologic typing methods, outbreak strain was described in detail and the index case was identified. The outbreak was successfully controlled through universal contact precautions and treatment of infected patients and decolonization of HCWs for nasal carriage.

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