

## Original Article

### Early Detection and Control of Methicillin Resistant *Staphylococcus Aureus* (MRSA) Outbreak in an Intensive Care Unit

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**Received:** 27 February 2020

**Accepted:** 08 September 2020

**DOI:** [10.4274/balkanmedj.galenos.2020.2020.2.153](https://doi.org/10.4274/balkanmedj.galenos.2020.2020.2.153)

**Cite this article as:** Bayraktar B, Gündüz A, Oryaşın E, Erdemir D, Teke L, Aktaş E, Durmaz R, Şen S, Uzun N, Bozdoğan B. Early Detection and Control of Methicillin Resistant *Staphylococcus Aureus* (MRSA) Outbreak in an Intensive Care Unit. *Balkan Med J*

**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:** This study aims to investigate the sudden increase in the number of MRSA cases in ICU.

**Study design:** A descriptive study to investigate MRSA outbreak in an ICU and signify following standard precautions.

**Methods:** From the 5th of 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. ST (Sequence type) clone type of representative strain was determined by Multi-Locus Sequence typing.

**Results:** All strains were positive for *mecA* gene, and have the same AST (**Antimicrobial susceptibility testing**) pattern. None of the strains were carrying *pvl* gene. All clinical, two patient screening and three nasal isolates of HCWs showed the same pulsotype (PT), named as **clone A**. Sequence type of these isolates is t030 and SCCmec type is SCCmecIII. Unrelated three isolates were having PFGE PT B-SCCmecI-t030, PFGE PT C-SCCmecIII-t459, **PFGE PT D-SCCmecIII**. MLST result showed that the outbreak strain belonged to ST239 **clone**.

**Conclusion:** The outbreak clone (PFGE pulsotype A, ST239-SCCmecIII-t030) was determined by using various molecular techniques. Infection control measures were started in ICU unit. Patients who had already been discharged to other services were put on contact isolation. No further cases occurred after 26th January and the outbreak declared over.

**Keywords:** MLST, MRSA, Outbreak

Methicillin resistant *Staphylococcus aureus* (MRSA) has been described for the first time in 1961, following 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 healthcare associated infections [1,2]. Methicillin resistance in *S.aureus* is encoded by *mecA* or *mecC* genes which are carried on 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 those 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, SCC*mec* typing, multilocus sequence typing (MLST). In this study, we aimed to investigate new MRSA cases in the ICU. We screened patients and healthcare workers, and performed *spa* typing, PFGE, SCC*mec* typing and MLST on MRSA strains in order to decide whether any transmission was occurring and infection preventive measures are mandatory.

## **METHODS**

**Outbreak description and bacterial isolates:** From 5<sup>th</sup> of December 2016 to 26<sup>th</sup> January 2017, we detected 11 new MRSA cases in adult ICU. On 20<sup>th</sup>, 23<sup>rd</sup>, 24<sup>th</sup> of December 2016, Clinical Microbiology Department noticed three consecutive MRSA isolates, two from blood and one from tracheal aspirate culture, having the same antibiotic susceptibility pattern belonging to three different patients in the adult ICU. We searched the Laboratory Information System for MRSA growths starting from October; we found three more isolates and two more cases who have already been discharged from ICU to neurology and general surgery wards. The first isolate is from a blood culture dated 5<sup>th</sup> of December and belongs to a male patient who had a positive blood culture on the 20<sup>th</sup> of December. We determine him as the index case. The index case was a morbidly obese patient hospitalized in ICU because of the respiratory failure due to pneumonia and left ventricular dysfunction.

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

Nine clinical isolates available in stocks (7 blood, 1 tracheal aspirate, 1 abscess; one isolate per patient) and eight screening MRSA isolates (5 HCWs and 3 patients) were included in molecular studies.

**Evaluation of *mecA*, *mecC*, *spa* and *PVL* Genes by Multiplex PCR:** In house multiplex PCR assay was performed as described by Stegger et al. [5]. Instagene Matrix (Biorad) was used for DNA extraction. Confirmation of methicillin resistance and identification of isolates was done by amplification of *mecA*/*mecC* genes and *spa* gene respectively. We also searched for Panton Valentin Leukocidin (*PVL*) gene.

***spa* typing:** *spa* gene amplicons were purified and sequenced. The open-source *spa* typing programme at <http://spatyper.fortinbras.us/> is used for *spa* sequence analysis.

**Pulse Field Gel Electrophoresis (PFGE):** PFGE typing of *Staphylococcus aureus* strains was carried out 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 *Sma*I (Promega Corporation, USA) for 24 h at 25 °C in a water bath. DNA fragments were separated on 1% agarose gels run in 0.5× Tris-borate-EDTA buffer using a CHEF-DR III system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14 °C at 6 V/cm<sup>2</sup> for 20 h. The initial and final switch times were 5.3 sec and 34.9 sec, respectively. The gel was stained with ethidium bromide (5 µg/mL) for 20 min and destained with distilled water for 30 min. The

DNA band profiles were visualized under UV 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 comparison of DNA profiles. The clonal relationship among isolates was evaluated by using the criteria of Tenover et al. [7].

**SCCmec typing:** SCCmec typing was carried out by using in-house multiplex PCR according to the procedure described by McClure-Warnier et al. [8]. Nine primer pairs were used for the screening of major SCCmec types and subtypes I to V.

**Multi-Locus Sequence typing (MLST):** The allelic profile of the outbreak MRSA strain was obtained by sequencing internal fragments of seven house-keeping genes as described at <http://saureus.mlst.net/misc/info.asp>. [9]. Carbamate kinase (**arc**), Shikimate dehydrogenase (**aro**), Glycerol kinase (**glp**), Guanylate kinase (**gmk**), Phosphate acetyltransferase (**pta**), Triosephosphate isomerase (**tpi**), Acetyl coenzyme A acetyltransferase (**yqi**). The DNA sequence of each **allele** analyzed by using data analysis software at the <http://saureus.mlst.net/sql/singlelocus.asp> web site, and allele numbers of seven amplicons were found out. By entering all allelic numbers of the strain in a program at the <http://saureus.mlst.net/sql/allelicprofilechoice.as> web site sequence type of the outbreak strain was determined

## Results

**Patient and HCWs demographics; clonal characteristics and antibiotic susceptibilities of MRSA isolates and molecular typing test results were summarized in Table.1.**

**Screening Cultures:** Screening was performed in ICU on 24<sup>th</sup> of December. Five out of 13 patients were carrying MRSA strains, in multiple sites of their body; two out of five sharing the same antibiotic **susceptibility** profile was shown to be the outbreak strain by molecular typing methods (15 %). Of those two colonized patients, the one carried the outbreak strain at nasal and groin sites, the other one at the throat, nasal, groin and rectum sites. Rectum colonized patient was 86 years old man with a Fournier's gangrene. 18 HCWs out of 73 were *S.aureus* positive for their nasal swab cultures (10 MSSA; 8 MRSA) and molecular typing was done for **five** MRSA sharing the same or **similar** antibiotic susceptibility pattern with outbreak strain (**Table.1**). Three of HCW's nasal isolates were the outbreak strain (4%).

**Antibiotic Susceptibilities:** We performed the AST using EUCAST recommended methods and interpretation. 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 CI-8 and CI-9. CI-8 and CI-9 were susceptible to DA, E, GN and TOB.

**Decolonization of HCWs:** All of the HCWs, except one pregnant nurse, were decolonized by TMP-SXT tablets (800/160mg BID for 10 days). We checked the colonization status of HCWs one week after the completion of treatment. They all cleared from the MRSA.

**Evaluation of mecA-mecC-SPAPVL Multiplex PCR:** All strains were positive for *mecA* gene and negative for PVL gene.

**Molecular typing:** All clinical isolates, and two patient screening and three nasal isolates of HCW's showed the same PFGE pulsotype (PT) and named as **clone A (Fig. 1)**. The *spa* sequence type of these isolates is t030, the SCCmec type is SCCmecIII. MLST result is ST239. Clinical isolate 8 (CI-8) and HCW-3 have different SCCmec types, SCCmecI and SCCmecV respectively. Unrelated three isolates were having PFGE PT B-SCCmecI-t030, PFGE PT C-SCCmecIII-t459, PFGE PT D-SCCmecIII (**Fig. 2**).

## Discussion

In this study, we report our experience about the MRSA outbreak in an adult ICU. The outbreak was noticed at the middle of December 2016, by that time four patients have already been discharged from ICU to other clinical wards. We assume that our index case is a male patient who stayed in ICU for over a month. Few healthcare workers 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 one pregnant employee were treated with co-trimoxazole as decolonization procedure since **mupirocin** ointment for nasal usage is not available on the market in our country [12]. We put our infected and colonized patients in contact isolation, including those who have already been discharged to other wards [13]. Infection control nurses gave education sessions to HCWs 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. So, 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 *Staphylococcus aureus* isolates, including PFGE, multilocus variable number of tandem repeat analysis (MLVA), SCCmec typing, *spa* typing, MLST and full-genome sequencing [1,3,15]. PFGE is still excepted as the “gold standard” for molecular typing of MRSA and *SmaI* is the enzyme of the choice for macrorestriction [16]. The major drawback of the PFGE is the insufficient **comparability** of results between laboratories which might be overcome by using standardized protocols nationally [2,16]. On the other hand, results of MLST are comparable between laboratories but its routine use in infection control is restricted by high cost, labour 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 higher discriminatory power over MLST, so one ST type can harbour 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 pulsotype named as cloneA and were ST239-SCCmecIII-t030 strains. ST239-SCCmecIII-t030 has previously been reported 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 [19,20,21] characterized MRSA clones. **"Oksuz et al. found Vienna/Hungarian/Brazilian clone (ST239-MRSA-III) as the most prevalent clone (53.9%) in a university hospital over 5 years, and rest of the MRSA clones were largely diverse [21].** Geographic distribution of major HA-MRSA clones are known worldwide, but sometimes shifts of these MRSA clones have been occurred over time in countries, in a region or even in a single hospital [22]. MRSA was evolved from MSSA in 1961 by the acquisition of a staphylococcal cassette chromosome *mec*(SCCmec) [1]. 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 consisted of more than five 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 looking for the reasons behind this success. Recently, Hong et al. [24] reported that higher expression of staphylococcal protein A (SpA) in HA-MRSA ST239 helps to 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, two colonization factors *sasX* protein, first described in ST239 clone during an outbreak in London and arginine catabolic mobile element (ACME) **are found to be associated with the epidemiological success of the MRSA**. ACME is first described in CA-MRSA USA300 and has spread horizontally to HA sequence types such as ST5 and ST239 [25,26]. Lastly, enteric carriage of HA-MRSA has been shown for ST228 and was taken as **responsible for maintaining a long-term** outbreak in a tertiary hospital [27]. In our patient group, we found out that one patient has enteric colonization with the outbreak strain. But 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 with the previous reports [21,23,28]. PVL was not detected on the outbreak strain. In conclusion, we described an MRSA outbreak in the ICU due to the clonal spread of the specific strain. Infection control measures put in practice immediately upon the detection of clustering. Through the microbiologic typing methods, outbreak strain was described in detail and index case was identified. Universal contact precautions and treatment of infected patients and decolonization of HCW for nasal carriage controlled the outbreak.

#### **Disclosures**

**Ethics Committee Approval:** Local Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital (date: 30.04.2019; number: 2377).

**Financial Disclosure:** No relevant financial relationship exists.

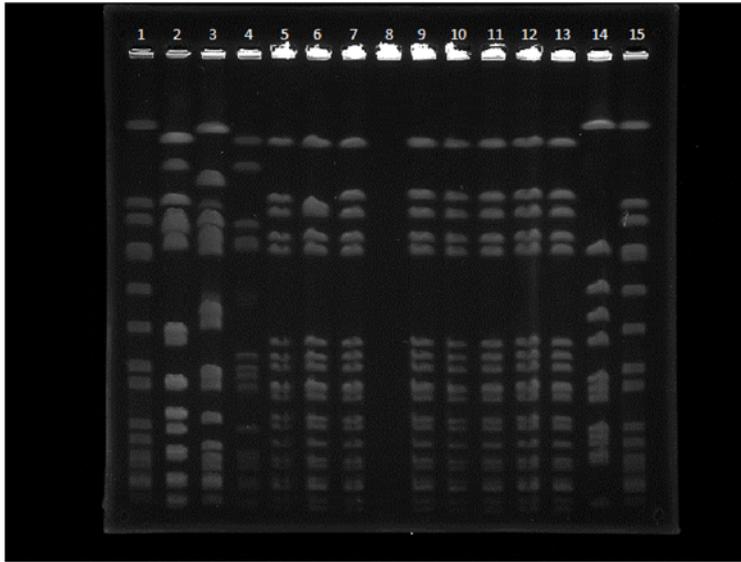
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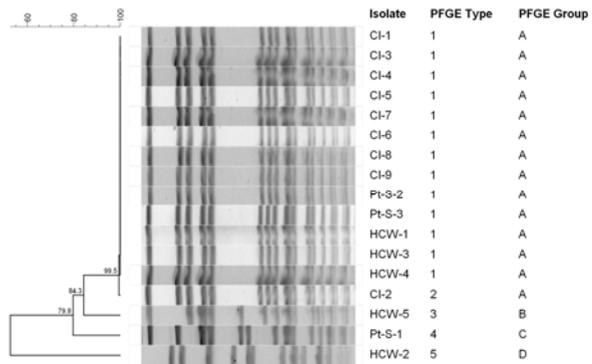
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**FIG. 1** PFGE *smAI* restriction patterns of MRSA clinical isolates(CI) clone A. Lanes 1, 15, Salmonella Braenderup molecular size marker; lanes 4 to 7 CI-1, CI-2, CI-3, CI-4 and lanes 9 to13 CI-5, CI-6, CI-8, CI-7, CI-9 respectively; lanes 2 to 4 and 14 clinical isolates that were unrelated to the outbreak; Lane 8 empty.



**FIG. 2** PFGE dendrogram showing different PTs seen over the duration of outbreak (A to D). PT A belongs to outbreak strain.

Table 1. Patient and HCWs demographics; clonal characteristics and antibiotic susceptibilities of MRSA isolates

Isolate identifier	Age	Gender	Diagnosis	Isolation site	Therapy	Outcome	PFGE	Spa type	SCCmec type	Cefoxitin	Ciprofloxacin	Clindamycin	Daptomycin	Erythromycin	Fusidic acid	Gentamicin	Levofloxacin	Linezolid	Rifampicin	Tetracycline	TMP-SXT	Tobramycin	Trimethoprim	
			<b>Patients' isolates</b>																					
I-1	48	M	Pneumonia	Blood	PIP-TZP 4,5gr IV TID Daptomycin 500mg IV daily	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
I-2	79	F	Cerebrovascular disease	Trachea	Linezolid 600mg IV BID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
I-3	74	F	Meninx benign neoplasm	Trachea	Vancomycin 1gr IV daily	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
I-4	68	M	Colon cancer	Blood	Daptomycin 350mg IV daily	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
I-5	46	F	Falling from high	Trachea	Tigecycline 50mg IV BID; Meropenem 1g IV TID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
I-6	49	F	Over cancer	Blood	Vancomycin 1gr IV BID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
I-7	88	M	Colon cancer	Abcess	Tigecycline 50mg IV BID; Meropenem 1g IV TID	Discharged	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
I-8	23	M	Drug user, toxication	Blood	Tigecycline 50mg IV BID; Meropenem 1g IV TID	Discharged	A	t030	Type I	R	R	S	S	S	S	S	R	S	R	R	S	S	S	S
I-9	86	M	Fournier's gangrene	Blood	Linezolid 600mg BID	Died	A	t030	Type III	R	R	S	S	S	S	S	R	S	R	R	S	S	S	S
			<b>Screening cultures isolates</b>																					
t-S-1	63	K	Crebellar tumor, epilepsy	Throat, nasal	Vancomycin 1gr IV BID	Died	C	t459	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
t-S-2*	74	F	Meninx benign neoplasm	Nasal, groin	Vancomycin 1gr IV daily	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
t-S-3**	86	M	Fournier's gangrene	Throat, nasal, groin, rectum	Linezolid 600mg IV BID	Died	A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R
CW-1	44	E		Nasal	TMP-SXT 160/800 mg PO BID		A	t030	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S	R

CW-2	23	E		Nasal	TMP-SXT 160/800 mg PO BID		D	ND	Type III	R	R	R	S	R	S	R	R	S	R	R	S	R	S
CW-3	28	E		Nasal	TMP-SXT 160/800 mg PO BID		A	t030	Type V	R	R	R	S	R	S	R	R	S	R	R	S	R	S
CW-4	30	K		Nasal	Not decolonized		A	t030	Type III	R	R	S	S	S	S	S	R	S	R	R	S	S	S
CW-5	24	K		Nasal	TMP-SXT 160/800 mg PO BID		B	t030	Type I	R	R	S	S	S	S	S	R	S	R	R	S	S	S

I: clinical isolate; Pt-S: Patient screening; HCW: healthcare worker; F: female; M: male; IV: intravenous; PO: peroral; BID: two times a day; TID: Three times a day; S: susceptible; R: resistant; PIP-TZP: Piperacillin/Tazobactam; TMP-SXT: Trimethoprim/sulfamethoxazole \*Pt-S-2and CI-3 are belonged to same patient, \*\*Pt-S-3 and CI-9 are belonged to same patient.

Uncorrected Proof