Original Article

Methicillin-resistant *Staphylococcus aureus* in a Turkish hospital: characterization of clonal types and antibiotic susceptibility

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Abstract

Introduction: The assessment of the clonal spread of methicillin resistant *Staphylococcus aureus* (MRSA) in nosocomial and community-acquired infections through characterization of the isolates is critical for tracking the evolution of the epidemics, implementing effective control measures, and preventing future outbreaks of MRSA. In this context, it is aimed with this study to determine the clonal relationships between the *S. aureus* isolates obtained from the patients receiving treatment in the intensive-care units of a state hospital in Turkey.

Methodology: A total of 80 MRSA isolates obtained from the patients receiving treatment in three different intensive-care units were analyzed for their antibiotic susceptibilities, pulsed-field gel electrophoresis profiles, and multilocus sequence types.

Results: The dendrogram of the pulsed-field gel electrophoresis profiles revealed two major pulsed-field gel electrophoresis pulsotypes: A and B, which were further divided into two (A1 and A2) and four (B1-B4) subgroups, respectively. Multilocus sequence typeanalysis indicated that all isolates belonged to a single MRSA clone, sequence type 239. No significant difference was found between the antibiotic sensitivity profiles of strains isolated from different intensive-care units. All of the strains were sensitive to linezolid and vancomycin.

Conclusions: It was concluded that the MRSA strains isolated from the patients receiving treatment at the intensive-care units of the hospital constituted two major pulse-field types and belonged to the ST239 lineage, one of the most extensively distributed MRSA lineages throughout the world.

Key words: Clonal typing; methicillin-resistant Staphylococcus aureus; pulsed-field gel electrophoresis.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant to various antibiotics and present a significant challenge in both healthcare (hospital-associated -HA-MRSA- infections) and community (community-associated MRSA -CA-MRSA- infections) settings [1]. Since their first identification in the United Kingdom in 1961, that is, only a year after the introduction of methicillin, MRSA strains caused significant outbreaks globally including developed countries throughout the 1980s and 1990s [1,2]. The incidence and prevalence of MRSA infections have been increasing worldwide, as new strains continue to emerge, spread from country to country and to other organisms, and the existing strains are replaced by newer strains that are resistant to other antibiotics [1,3]. In recent reports, MRSA was stated to account for more than half of *S. aureus* infections in Europe and Eastern Mediterranean and more than three quarters of the cases in Africa, the Americas, and Western Pacific, giving rise to therapeutic difficulties that require the use ofantibiotics other than β-lactams [4].

In MRSA strains, methicillin resistance is encoded by mec genes, which encode a variant of penicillin-binding protein (2a) that does not bind to, and is not affected by, β-lactams and hence confer resistance to these antibiotics [1,5,6]. Various MRSA strains have emerged, became prevalent, and replaced by other strains over a period of more than half a century [7-9]. It has been demonstrated that the MRSA strains that emerged over time formed phylogenetically distinct lineages and that most MRSA outbreaks involved only a few successful pandemic clones [8]. Thus, assessment of the clonal spreading of nosocomial and community-based MRSA infections through the characterization of isolates is critical for tracking their constantly changing epidemiology, implementing effective control

measures, and preventing future outbreaks of MRSA [1,10]. In view of the foregoing, it is aimed in this study to determine the clonal relationships between the *S. aureus* isolates obtained from the patients receiving treatment in the intensive-care units of a state hospital in Turkey with a view to understand the epidemiology of HA-MRSA infections and provide guidance to the future infection control measures.

Methodology

Patient selection

A total of 80 MRSA strains isolated from 80 inpatients who have been receiving treatment in three different (general, internal medicine, and thoracic medicine) intensive-care units (ICUs) of Çankırı State Hospital between February 1st, 2019 and February 1st, 2020 were included in the study. First, patients' blood cultures were taken and analyzed. In the case of patients, who were observed not to have growth in their blood cultures, the MRSA growth observed in the samples taken from other body parts was recorded. Nasal swab samples for MRSA and rectal swab samples for vancomycin-resistant enterococcus (VRE) were obtained from the patients during their admission to the ICU. No growth was observed in these cultures. Hence, the possibility of colonization of the patients was excluded. Nosocomial infection surveillance is actively carried out in the ICUs of the hospital, where this study was conducted. From among the patients who did not have any signs and symptoms for any infection at the time of admission, those with cultures taken at least 72 hours after hospitalization were considered cases with "hospital-acquired infection". As a matter of fact, all patients included in this study were cases with hospital-acquired infections. Of the different clinical specimens of the same patient or strains grown at different times, only the first one was tested. Strains grown from the indwelling catheter were not processed. Samples obtained from the patients were spread on 5% sheep blood agar, Eosin-methylene Blue (EMB) agar, and chocolate (heated blood) agar plates. Blood cultures were evaluated in BacT/Alert 3D automated blood culture system (bioMérieux, Craponne, France). Diagnosis of MRSA isolates was made based on the results of the Gram staining, catalase test, coagulase test, and the Kirby-Bauer disk diffusion method with 30 μg cefoxitin disk (Bioanalyse, Ankara, Turkey) interpreted in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

In order to determine the antibiotic susceptibility, MRSA isolates stored at -20 °C in the stock medium

were spread on 5% sheep blood agar (RT agar, Ankara, Turkey). First, the minimum inhibitory concentration (MIC) of vancomycin and linezolid were determined using the gradient strip diffusion method (E-test, bioMérieux, Craponne, France) as per the manufacturer's recommendations. EUCAST recommends the use of the broth dilution method for the determination of MIC values in glycopeptides. Nevertheless, the laboratory where this study was conducted was not equipped to run the broth dilution method; thus, the gradient test was used instead to determine the MIC values of vancomycin. Quality control testing was performed using the ATCC S. aureus 29213 strain. Similarly, the susceptibility to other antibiotics was investigated using antibiotic disks. The S. aureus ATTC 25923 standard strain was used as the control. The antibiotic susceptibility of the strains was evaluated in accordance with the EUCAST criteria and assessed as susceptible to standard (S) or increased (I) dose (which were considered susceptible - S) and resistant (R).

Pulsed-field gel electrophoresis (PFGE)

Chromosomal DNA was isolated as described by Goering *et al*. [11] and subjected to restriction digestion with *Sma*I restriction endonuclease (Takara, Shiga, Japan). The PFGE was carried out as described by De Lencastre *et al*. [12]. The PFGE band profiles were analyzed using the GelCompar II software (Version 5.0, Applied Maths, Sint-Martens-Latem, Belgium). The dendrogram was constructed based on the Dice similarity coefficients using the unweighted pair group method with arithmetic mean (UPGMA). The resulting dendrogram was verified based on the visual inspection of the banding patterns. The collected research data were interpreted in accordance with the established guidelines [13,14]. The isolates with an 80% band profile similarity were categorized under the same cluster and designated capital letters. The isolates in the same cluster were further categorized into a sub-cluster, were they to be 80% to 100% similar, and designated with the lowercase letter of the name of their cluster followed by a number. Identical (100% similarity) isolates were categorized into the same sub-cluster.

Multilocus sequence typing (MLST)

All isolates were subjected to multilocus sequence typing. Housekeeping genes of Staphylococcus aureus (arcC, aroE, glpF, gmk, pta, tpi, and yqil) were amplified using a BigDye Terminator V3.1 Loop Sequencing Kit (Applied Biosystems, Foster City, CA, US), after which the products were purified.

Sequencing was performed using ABI Prism 3700 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, US). The resulting sequences were compared with the known alleles in the MLST database [15], and clonal complexes (CCs) were determined using eBURST.

Statistical analysis

Descriptive statistics were presented as mean ± standard deviation (SD) values depending on the distribution for continuous variables. Categorical variables were presented as numbers and percentage values. The conformity of numerical variables to normal distribution was checked with the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA)

Figure 1. The origin, antibiotic sensitivity, and dendrogram of the PFGE profiles of 80 *S. aureus* strains isolated from the patients at the intensive care units (ICUs).
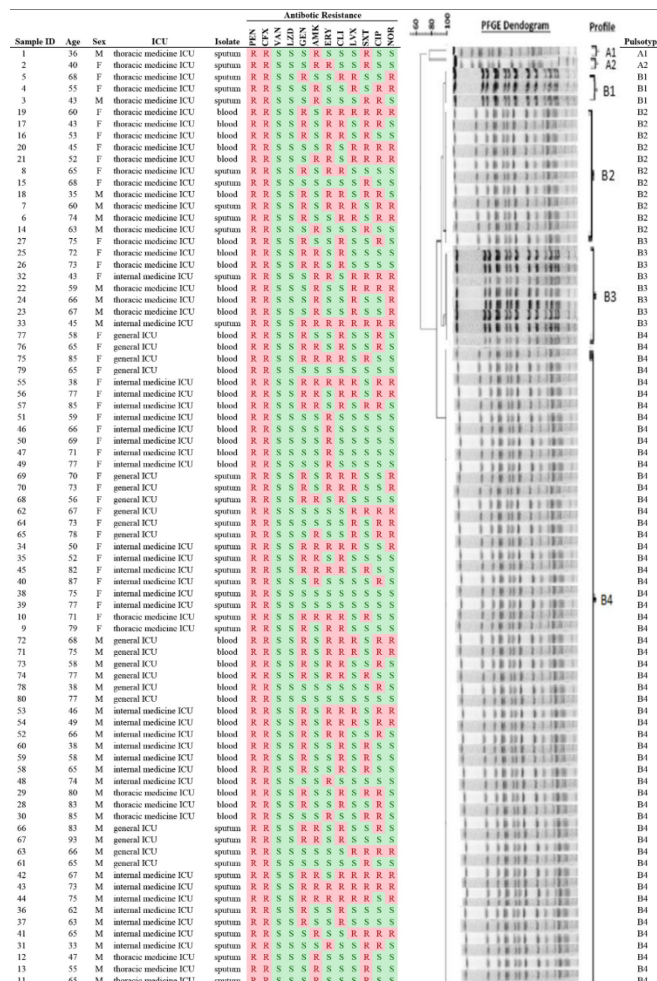
AMK: amikacin, CFX: cefoxitin, CIP: ciprofloxacin, CLI: clindamycin, ERY: erythromycin, GEN: gentamicin, LVX: levofloxacin, LZD: linezolid, NOR: norfloxacin, PEN: penicillin, SXT: trimethoprim-sulfamethoxazole, VAN: vancomycin, ICU: intensive care unit, R: resistant, S: sensitive, PFGE: pulsed-field gel electrophoresis.

Figure 2. Antibiotic sensitivities and pulsotypes of the isolated samples.
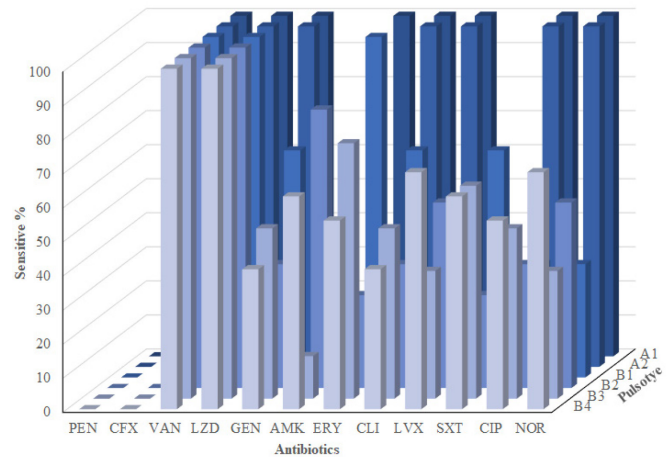
AMK: amikacin, CFX: cefoxitin, CIP: ciprofloxacin, CLI: clindamycin, ERY: erythromycin, GEN: gentamicin, LVX: levofloxacin, LZD: linezolid, NOR: norfloxacin, PEN: penicillin, SXT: trimethoprim-sulfamethoxazole, VAN: vancomycin, ICU: intensive care unit, R: resistant, S: sensitive, PFGE: pulsed-field gel electrophoresis.

was used to compare more than two independent groups involving numerical variables that were determined to conform to normal distribution. In the comparison of the groups involving categorical variables, Pearson's chi-squared test was used in tables with expected cells of 5 and above, whereas the Fisher-Freeman Halton test was used in RxC tables with expected cells below 5. The statistical analyses were performed using Jamovi (version 1.2.24) and JASP (version 0.13.1). The significance level (*p*-value) was deemed as 0.05.

Ethical statement

The study involved no human subjects; only clinical isolates were used. As patient data were anonymized, informed consents and ethics committee approval were not required. This study was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

Results

A total of 80 samples were processed. Of these samples, 40 (50%) were sputum, and the remaining 40 (50%) samples were blood samples. Half of the samples were isolated from female patients. The samples were isolated from the patients receiving treatment in three different ICUs: internal medicine ICU (n = 30, 37.5%), thoracic medicine ICU (n = 30, 37.5%), and general medicine ICU (n = 20, 25%).

The dendrogram of the PFGE profiles of 80 *S. aureus* strains isolated from the patients at the ICU unit indicated two major PFGE pulsotypes: A and B, which

were further divided into two (A1 and A2) and four (B1-B4) subgroups, respectively (Figure 1). The 78 strains with the B pulsotype had a 92% similarity in terms of their PFGE profiles. MLST analysis indicated that all isolates belonged to a single MRSA clone, sequence type (ST) 239 [8,16]. No significant difference was found between the strains isolated from different ICUs in terms of their antibiotic sensitivity profiles ($p > 0.05$).

Antibiotic sensitivities of the isolated samples were plotted versus their pulsotypes, as shown in Figure 2. All strains were resistant to β-lactams (cefazolin, cefoxitin, and penicillin) and linezolid and vancomycin. Sample characteristics are summarized in Table 1. No significant difference was found between the samples obtained from the patients receiving treatment in different ICUs, except for trimethoprim-

sulfamethoxazole resistance, which was found to be significantly more common in the samples obtained from the patients receiving treatment in the thoracic medicine ICU (Table 1).

Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common and increasingly prevalent nosocomial infection in all parts of the world [4]. Epidemiological knowledge about the spatial and temporal distribution of MRSA, which requires typing the MRSA strains isolated at various sites, would enable the development of more effective measures to prevent or control future outbreaks. Pulsed-field gel electrophoresis (PFGE) has been used more commonly than other techniques to investigate and type isolated MRSA strains [17]. PFGE was considered a gold

Table 1. The comparison of patients, samples, and antibiotic resistances at the ICUs.

		All (n = 80)	General ICU (n = 20)	Internal Medicine ICU (n = 30)	Thoracic Medicine ICU (n = 30)	*p*
Age, mean ± SD		63.9 ± 14.1	69.5 ± 12.0	62.9 ± 14.8	61.2 ± 14.1	0.078[a]
Sex, n (%[c])	Female	40 (50.0)	10 (50.0)	15 (50.0)	15 (50.0)	0.999[b]
	Male	40 (50.0)	10 (50.0)	15 (50.0)	15 (50.0)	
Isolate, n (%[c])	Blood	40 (50.0)	10 (50.0)	15 (50.0)	15 (50.0)	0.999[b]
	Sputum	40 (50.0)	10 (50.0)	15 (50.0)	15 (50.0)	
Pulsotype, n (%[c])	A1	1 (1.3)	-	-	1 (3.3)	N/A
	A2	1 (1.3)	-	-	1 (3.3)	
	B1	3 (3.8)	-	-	3 (10.0)	
	B2	11 (13.8)	-	-	11 (36.7)	
	B3	8 (10.0)	-	2 (6.7)	6 (20.0)	
	B4	56 (70.0)	20 (100.0)	28 (93.3)	8 (26.7)	
Antibiotic resistance, n (%[c])						
Penicillin	R	80 (100)	20 (100.0)	30 (100.0)	30 (100.0)	N/A
	S	-	-	-	-	
Cefoxitin	R	80 (100)	20 (100.0)	30 (100.0)	30 (100.0)	N/A
	S	-	-	-	-	
Vancomycin	R	-	-	-	-	N/A
	S	80 (100.0)	80 (100.0)	80 (100.0)	80 (100.0)	
Linezolid	R	-	-	-	-	N/A
	S	80 (100.0)	80 (100.0)	80 (100.0)	80 (100.0)	
Gentamicin	R	45 (56.2)	12 (60.0)	18 (60.0)	15 (50.0)	0.683[b]
	S	35 (43.8)	8 (40.0)	12 (40.0)	15 (50.0)	
Amikacin	R	34 (42.5)	6 (30.0)	13 (43.3)	15 (50.0)	0.372[b]
	S	46 (57.5)	14 (70.0)	17 (56.7)	15 (50.0)	
Erythromycin	R	36 (45.0)	7 (35.0)	17 (56.7)	12 (40.0)	0.251[b]
	S	44 (55.0)	13 (65.0)	13 (43.3)	18 (60.0)	
Clindamycin	R	45 (56.2)	12 (60.0)	18 (60.0)	15 (50.0)	0.683[b]
	S	35 (43.8)	8 (40.0)	12 (40.0)	15 (50.0)	
Levofloxacin	R	29 (36.2)	8 (40.0)	11 (36.7)	10 (33.3)	0.889[b]
	S	51 (63.8)	12 (60.0)	19 (63.3)	20 (66.7)	
Trimethoprim-sulfamethoxazole	R	35 (43.8)	5 (25.0)	12 (40.0)	18 (60.0)	**0.044[b]**
	S	45 (56.2)	15 (75.0)	18 (60.0)	12 (40.0)	
Ciprofloxacin	R	38 (47.5)	11 (55.0)	13 (43.3)	14 (46.7)	0.716[b]
	S	42 (52.5)	9 (45.0)	17 (56.7)	16 (53.3)	
Norfloxacin	R	29 (36.2)	8 (40.0)	11 (36.7)	10 (33.3)	0.889[b]
	S	51 (63.8)	12 (60.0)	19 (63.3)	20 (66.7)	

[a] Student's *t*-test; [b] Pearson chi-squared test; [c] Percentages are for columns; ICU: intensive care unit, SD: standard deviation, R: resistant, S: sensitive, N/A: not applicable.

standard for MRSA typing, in local outbreaks in particular, due to its ability to distinguish MRSA strains better than several other methods, including antibiograms, bacteriophage typing, random amplification of polymorphic DNA (RAPD), ribotyping, and zymotyping [17–21].

In this study, the clonal relationships between the *S. aureus* isolates obtained from the patients receiving treatment in various ICUs of a state hospital in Turkey were investigated. The results of the PFGE analysis revealed that the 80 MRSA strains isolated from this particular hospital belonged to two major PFGE pulse-field types, called A and B, and to a single MRSA clone, multilocus sequence type (ST)239, which is one of the most extensively distributed MRSA lineages throughout the world [8,22,23].

It was reported in a study involving 553 methicillin-sensitive *Staphylococcus aureus* (MSSA) and 359 MRSA strains isolated from several countries through a period of 40 years that the MRSA strains belonged to five ancestral genotypes, called clonal complexes (CCs), which were further categorized into 38 sequence types (STs) based on multilocus sequence typing (MLST) [8]. In the same study, the ST239 lineage was proposed to derive from CC8 with type-III staphylococcal cassette chromosome *mec* (SCCmec) (CC8-MRSA-III) through recombination at the *arc*C locus after the acquisition of methicillin-resistance [8].

The ST239 lineage is representative of HA-MRSA and includes isolates that have distinguishing features concerning their SCCmec-III structure, protein A gene (*spa*) type, and PFGE pattern [22–24]. The ST239 variants constitute epidemic MRSAs (EMRSA) that have been previously reported as Brazilian clone, Portuguese clone, Hungarian clone, Viennese clone, and British EMRSA-1, -4, and -11 clones [16,22,24–27]. The ST239 lineage is resistant to several antibiotics and has been found to account for almost all HA-MRSA infections in China and other parts of mainland Asia [22,28,29]. The ST239 lineage has also been detected in South America and Eastern Europe [22,30–32]. Strains with PFGE profiles similar to strains from ST239 lineage have also been detected in some states in the United States [33].

The ST239 lineage has been previously found to be the dominant strain involved in HA-MRSA infections at various parts of Turkey as well [30]. More recently, ST239 strains were detected in a tertiary care hospital over a period of 2002 to 2012 [34]. The MRSA variants with *spa* type t030, which is associated with the ST239 lineage, were also detected in various units of another tertiary care hospital in Turkey [35]. The strains that

have been analyzed in these previous studies were essentially sensitive to vancomycin, teicoplanin, tigecycline, trimethoprim-sulfamethoxazole, quinupristin-dalfopristin, and linezolid [34–36]. Of these antibiotics, vancomycin, trimethoprim-sulfamethoxazole, and linezolid were tested in our study. A significant portion of the isolates investigated in this study was found to be resistant to trimethoprim-sulfamethoxazole, whereas all were found to be sensitive to linezolid and vancomycin. Trimethoprim-sulfamethoxazole is widely used in Turkey, particularly for the treatment of urinary tract infections. This shift in antibiotic susceptibility profiles may indicate mounting resistance to certain antibiotics yet may also reflect the differences between the sites sampled in those studies.

The PFGE profiles have proven to be highly discriminatory, enabling the comparison of the isolates in a study or a local outbreak. Yet, interpretation of results generated at slightly different running conditions can still be problematic [17]. Therefore, inter-center or inter-study comparison of PFGE results may not be reliable, and a genetic analysis based on MLST and *spa* typing might be more appropriate for making a comparison between the strains investigated in different studies. Limitations of this study include the lack of a comprehensive genetic analysis that covers *spa* types, accessory gene regulator (*agr* locus), and virulence factor profiles (i.e., Panton-Valentine leukocidin (PVL) and enterotoxin A).

Conclusions

In conclusion, the results of the PFGE and MLST analyses of the MRSA strains isolated from various ICUs of the same hospital indicated that these MRSA strains constituted two major pulse-field types and belonged to the ST239 lineage which is one of the most extensively distributed MRSA lineages throughout the world. The results of this study indicate that attention should be paid by the infection control committee to active surveillance cultures in risky areas such as intensive care units, careful implementation of isolation measures, screening and training of personnel at certain periods, and compliance with handwashing and hygiene rules. In summary, strict regulations would allow the early detection of MRSA colonization, and knowledge of the antibiotic resistance profile would be beneficial in preventing hospital-acquired infections.

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