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Methicillin resistance of *S. aureus* bloodstream infections: The data of 15 years

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Abstract
Aim: The aim of this study is to determine the annual changes in the frequency of methicillin resistance in the *S. aureus* strains isolated from blood cultures in our hospital and to find the resistance rate of *S. aureus* strains against other antimicrobial agents.
Material and Methods: *S. aureus* strains isolated from blood cultures of the hospitalized children between 2004 and 2018 were retrospectively analyzed. Patients' age, gender, complex chronic conditions (CCC) and the number of positive *S. aureus* blood cultures were investigated. The study period, 15 years, was classified in 3 periods of 5 years.
Results: Four hundred eleven blood cultures of 337 patients (157 girls, 180 boys) were positive for *S. aureus*. There were 100 MRSA (100/411, 24%) bacteremia in total. Twenty-eight of them were community-acquired (CA-MRSA). One hundred thirty-five patients (%40) had CCC. MRSA bacteremia was significantly higher in patients with CCC compared to patients without CCC (35% to 11%). The highest MRSA rate was detected in the period 2014-2018 among all positive *S. aureus* blood cultures. Clindamycin, erythromycin, gentamicin, trimethoprim/sulfamethoxazole and ciprofloxacin resistance were higher in the MRSA group. Hospital-acquired MRSA strains have a statistically higher rate of clindamycin, erythromycin, gentamicin, and ciprofloxacin resistance than CA-MRSA strains.
Discussion: The frequency of MRSA bacteremia has been increasing in our center in recent years due to the spread in the number of patients with chronic conditions. More attention should be paid to rational antibiotic use in children with CCC.

Keywords
Bacteremia; Complex Chronic Condition; Methicillin- Resistant *S. aureus*

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Introduction

Bloodstream infections are clinical conditions associated with high mortality and morbidity. Its mortality rates are reduced when it is diagnosed and treated early. *S. aureus*, which is one of the most common agents of Gram-positive bacteremia, causes numerous serious infections such as skin and soft tissue infections, surgical site infections, pneumonia, empyema, osteomyelitis, septic arthritis, and endocarditis [1-3].

While *S. aureus* related infections were successfully treated with penicillin G in the early 1940s, *S. aureus* developed resistance to penicillin, especially in hospital-acquired (HA) infections. Resistance of *S. aureus* to erythromycin and tetracycline was observed in the 1950s, then methicillin- resistant *S. aureus* (MRSA) was emerged in 1961 [4]. MRSA strains cause serious problems with treatment, thus increase treatment costs. The multidrug resistance of these strains is the most important treatment problem. A significant part of MRSA strains has a decreased susceptibility to macrolides, clindamycin, chloramphenicol, aminoglycosides and antiseptics [5]. In studies from our country, the rate of MRSA strains among all *S. aureus* strains was found to be 32-61% [1, 6, 7].

The frequency of methicillin-resistant strains has been increasing all over the world until recent years, but it has been reported that the frequency of MRSA has decreased in some centers [8, 9]. A study of a pediatric cohort from our country that evaluated microorganisms isolated from blood cultures between 2000 and 2011 found MRSA strains decreased over the years, and MRSA rate was found to be 0% in 2011 [8].

The antimicrobial resistance status of *S. aureus* strains is extremely important both to determine the treatment protocol and to make epidemiological evaluations. The aims of this study are to assess the prevalence of MRSA, as well as determining the annual frequency of methicillin resistance in the *S. aureus* strains isolated from blood cultures in our hospital over the 15 years, and to evaluate antimicrobial susceptibility patterns of *S. aureus* strains to other common antibiotics.

Material and Methods

S. aureus strains isolated from blood cultures of hospitalized children at Dr. Sami Ulus Obstetrics and Gynecology and Pediatrics Training and Research Hospital, between 2004 and 2018 were retrospectively analyzed. Cultures from patients older than 18 years of age and cultures with more than one strain were excluded from the study. Patients' age, gender, complex chronic conditions (CCC) and the number of positive *S. aureus* blood cultures were investigated. A complex chronic condition was defined as 'any medical condition that are expected to last for at least 12 months (unless death intervenes), and that affects more than one organ system, or that severely affects a system, requiring specialized paediatric care and possibly requiring hospitalization at a tertiary care centre' [10]. Patients were divided into two groups according to the unit where they were hospitalized: patients who were hospitalized in intensive care (pediatric, neonatal and cardiovascular) units and in other services. The rate of MRSA infections was compared with the rate of methicillin-sensitive *S. aureus* (MSSA) infections according to patients' place of hospitalization, CCC and their age. The length of hospital stay after *S. aureus* bacteremia and the mortality status of the patients were examined. The study period, 15 years, was classified in 3 periods of 5 years (2004-2008, 2009-2013 and 2014-2018) to determine the change in MRSA epidemiology obviously.

S. aureus blood cultures obtained within the first 3 days of admission were considered to originate from Community-Acquired (CA) infections [11]; those obtained after the 3rd calendar day of admission were considered to originate from Hospital-Acquired (HA) infections. Blood cultures were performed in BACTEC Vacutainer Culture Systems. To diagnose *S. aureus* strains, presumptive isolates were inoculated onto Blood agar base with a 5% sheep blood medium (Oxoid, England). Microorganisms were evaluated according to colony morphologies, Gram staining properties, the status of beta hemolysis on blood agar and Catalase and Coagulase tests. Both tube and slide coagulase tests were performed. Strains that were positive for beta hemolysis on blood agar Catalase and Coagulase tests (both tube and slide) were identified as *S. aureus*. Methicillin resistance was determined by Cefoxitin disc diffusion test considering the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (Wayne PA. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing 2011;100-21). Antimicrobial susceptibility analyzes were conducted with the first positive *S. aureus* strains of recurrent positive *S. aureus* blood cultures.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The institutional review board of Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital approved the study (Decision No: 2019-12-18). Statistical analysis was performed in SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were given as median and interquartile range for continuous variables and frequency, the percentage for categorical variables. Comparisons were performed using the Mann-Whitney u test and Chi-square test, where $p< 0.05$ was considered statistically significant.

Results

Four hundred-eleven positive blood cultures for *S. aureus* were detected in 337 patients (157 girls, 180 boys). Demographic variables of the patients are shown in Table 1. Forty percent (n=135) of the patients had an underlying CCC. The most common CCC was cardiovascular diseases (n = 32). This was followed by renal (n=24), neurological (n=19), and metabolic (n=17) conditions. Forty-five percent (n=151) of patients had CA *S. aureus* bacteremia.

During the years 2004-2018, there were 100 MRSA (100/411, 24%) and 311 MSSA bacteremia in total. Table 2 shows important variables about *S. aureus* positive blood cultures. The MRSA rates of intensive care units and other services were comparable. There was more CA bacteremia in the MSSA group. MRSA bacteremia was significantly higher in patients with CCC compared to patients without CCC. Patients who were older than 12 months of age had significantly higher rates of MRSA bacteremia (Table 2). The number of positive blood cultures for *S. aureus* in each year is demonstrated in Figure 1. Most of the *S. aureus* bacteremia took place between 2015 and 2018. When all *S. aureus* positive blood cultures were examined in 3 periods,

Table 1. Demographic variables of children who had S. aureus bacteremia between 2004 and 2018

Patients	
Sex (female/male)	157/180
Age (median month, IQR)	3 (35)
Number of one positive blood culture for *S. aureus* n (%)	265 (79%)
≥2 positive blood cultures for *S. aureus* n (%)	72 (21%)
Number of patients with CCC n (%)	135 (40%)
Patients with community acquired *S. aureus* bacteremia n (%)	151 (45%)

Abbreviations: CCC: Complex chronic condition, IQR: Interquartile range

Table 2. Important clinical variables about *S. aureus* positive blood cultures

Blood cultures	MRSA (n=100) (%)	MSSA (n=311) (%)	Total (n=411) (%)	P value
CA *S. aureus* bacteremia	28 (28%)	159 (51%)	187 (45%)	<0.01
Where was the blood culture taken?				
• In intensive care units	33 (20%)	134 (80%)	167 (41%)	0.12
• In other services	67 (27%)	177 (73%)	244 (59%)	
Patients				
• With CCC	70 (40%)	105 (60%)	175 (43%)	<0.01
• Without CCC	30(13%)	206 (87%)	236 (57%)	
Patients who were				
• ≤ 12 months of age	27 (18%)	124 (82%)	151 (37%)	0.02
•> 13 months of age	73 (28%)	187 (72%)	260 (63%)	

Abbreviations: CCC: complex chronic condition, CA: community acquired, MRSA: methicillin resistant S. aureus, MSSA: methicillin susceptible S. aureus

Table 3. The antibiotic resistance of MRSA and MSSA strains*

Antibiotics	MSSA		MRSA		Total		P value
	n	%	n	%	n	%	
Penicillin G	240	91.3	74	100	314	93.2	0.008
Clindamycin	18	7.0	34	46.6	52	15.7	<0.001
Erythromycin	32	15.6	41	70.7	73	27.8	<0.001
Gentamycin	3	1.2	25	35.2	28	8.5	<0.001
Daptomycin	1	1.5	0	0	1	1.1	0.988
Trimethoprim/ sulfamethoxazole	10	3.9	13	17.6	23	6.9	0.001
Ciprofloxacin	15	6.3	24	35.8	39	12.7	<0.01
Vancomycin	0/285	0	0/95	0	0/380	0	-
Teicoplanin	0/274	0	0/89	0	0/363	0	-
Linezolid	0/140	0	0/56	0	0/196	0	-

Abbreviations: MRSA: methicillin resistant S. aureus, MSSA: methicillin susceptible S. aureus
*In cases of ≥2 positive blood cultures for S. aureus only the first positive S. aureus strains were included in the analyses.

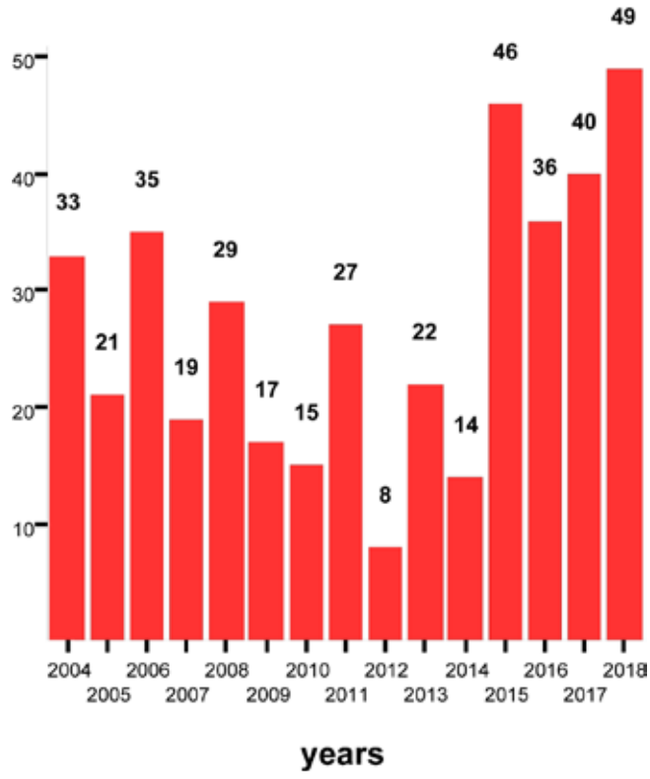

Figure 1. The number of positive blood cultures for S. aureus in each year. Most of the S. aureus bacteremia took place between 2015 and 2018.

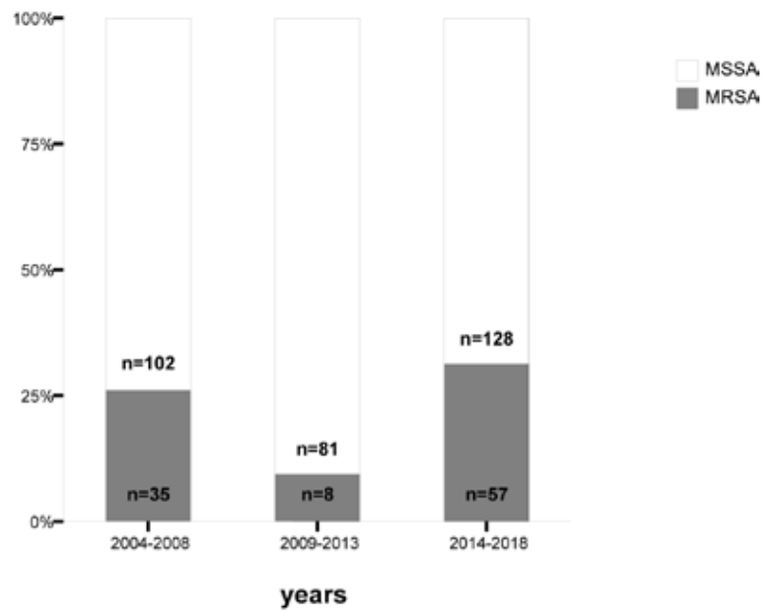

Figure 2. MRSA rates in 3 periods of 5 years. The highest MRSA rate was detected in the period 2014-2018 (p <0.01).

the highest MRSA rate was detected in the period 2014-2018. In the 2004-2008 years, 26%, of all positive *S. aureus* infections were MRSA, in the 2009-2013 years- 9% and 31% in 2014-2018 years (p <0.01) (Figure 2). Among patients with *S. aureus* bacteremia, there were significantly more patients with a CCC in the period 2014-2018 years. The rates of patients with a CCC were 31% in the period 2004-2008, 31% in the period 2009-2013, and 50% in the period 2014-2018 (p <0.01).

The antibiotic resistance of all first positive *S. aureus* strains as well as MRSA and MSSA strains is shown in Table 3. Resistance to penicillin G, clindamycin, erythromycin, gentamicin, trimethoprim/sulfamethoxazole and ciprofloxacin was higher in the MRSA group than in the MSSA group (Table 3). There were no vancomycin, teicoplanin and linezolid resistant strains in both groups. Compared to CA-MRSA strains, HA-MRSA strains had statistically significantly higher rates of resistance to clindamycin (55.1% to 21.1%; p=0.01), erythromycin (81.6% to 37.5%; p<0.01), gentamicin (45.7% to 15.0%; p=0.02), and ciprofloxacin (46.5% to 10.5%; p<0.01).

The median length of hospital stay (LOS) after *S. aureus*

bacteremia was 18 (IQR: 16) days in the MRSA group, while it was 9.5 (IQR: 8) days in the MSSA group (p <0.01). In the 15-year period, 13 patients (4%) died. Six of the 13 patients had MRSA and seven MSSA bacteremia. All 13 patients had CCC.

Discussion

S. aureus bacteremia is associated with severe morbidity and mortality. In this study, we determined that the methicillin resistance of *S. aureus* strains isolated from blood cultures decreased in the period 2008-2012, but increased in the period 2013-2018. In addition, MRSA strains isolated from blood cultures had higher resistance to clindamycin, erythromycin, gentamicin, trimethoprim/sulfamethoxazole and ciprofloxacin compared to MSSA strains. The HA-MRSA strains had higher rates of resistance to clindamycin, erythromycin, gentamicin and ciprofloxacin compared to CA-MRSAs.

According to EARS-Net data, which is a surveillance study involving European countries, the methicillin resistance rates of *S. aureus* strains reduced in Europe [13]. In Norway, the frequency of MRSA was 0.2% in 2002, and 0.8% in 2014, while in EU countries, this rate was 25.6% in 2007 and 18% in 2013 [14]. Low MRSA rates in Scandinavian countries are considered to be associated with low and rational antibiotic use in these countries [14]. In a study from our country, MRSA rates gradually decreased to 0 in 2011 [8]. We observed a decrease in the total number of *S. aureus* bacteremia in the 2009-2013 years in our hospital. Nevertheless, the total number of *S. aureus* bacteremia increased significantly after 2015. A possible explanation of this fact could be an increase in the number of hospital beds, including intensive care beds, after 2015 in our hospital. However, regardless of the increase in the number of hospital beds, MRSA rates were increased to 31% in the 2014-2018 years. The spread in the number of hospitalized patients with CCC and their increased antibiotic use might be the reason for this challenge in recent years. The higher rate of MRSA bacteremia in patients with CCC in this study supports this explanation as well.

Resistance to antibiotics is directly associated with the overuse of antibiotics [15]. Turkey has the highest rate of total antibiotic consumption among European countries [16]. Therefore, the ministry of health in Turkey carefully follows rational antibiotic use policies [17]. In studies conducted in adults in our country, MRSA rates were reported as 50.2% [7]. Although the frequency of MRSA infections among all *S. aureus* bacteremia was 24% in our hospital, the increase of MRSA rates in recent years suggested that infection control measures and restriction programs in antibiotic consumption should be adhered carefully [18].

In our study, no resistance to vancomycin, teicoplanin and linezolid was detected. However, clindamycin resistance was found by 7% in MSSA strains and 40% in MRSA strains. In an Afghan study, the clindamycin resistance rate was found 0% in MSSA and 8.5% in MRSA strains [19]. In a large cohort from US, the clindamycin resistance rate was reported approximately 15% in MSSA and 10% in MRSA strains [9]. Clindamycin is a common antibiotic that could be used for *S. aureus* infections in non-toxic children whose clinic is stable. However, the high clindamycin resistance in MRSA strains in our study made the use of clindamycin in *S. aureus* infections questionable.

The epidemiology of MRSA infections changed significantly. From 1961 to the 1990s MRSA infections were assumed as hospital- acquired infections [20]. Today MRSA infections are increasingly common in the community. Defined risk factors for CA-MRSA infections are intravenous drug use, prior antibiotic use, and prior hospitalizations [21]. Since patients with CA-MRSA bacteremia do not have classical risk factors such as dialysis catheters or central lines, it is difficult to predict whether these patients are at risk of having an MRSA infection. The choice of empirical therapy for serious infections where *S. aureus* is a possible agent could be a great challenge with the evidence that inappropriate initial antibiotic treatment leads to higher mortality [22]. On the other hand, increased consumption of anti-MRSA antibiotics such as vancomycin could cause further resistant strains such as vancomycin-resistant *S. aureus* [20]. In our study, the rate of CA MRSA bacteremia was 28%. In some centers, CA-MRSA reached 86% among MRSA bacteremia [23]. Consistent with the literature, CA-MRSA strains have lower rates of clindamycin, erythromycin, gentamicin and ciprofloxacin resistance compared to HA-MRSA bacteremia in this study [24, 25]. Due to the fact that HA-MRSA strains have higher rates of resistance against antibiotics, more caution is needed in the choice of antibiotics in HA-MRSA infections.

In conclusion, the frequency of MRSA bacteremia has been increasing in our center in recent years due to the spread of patients with CCC and their possibly increased consumption of antibiotics. Multicenter studies are needed to examine this increase. More attention should be paid to the rational antibiotic use in children with CCC.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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