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Analysis of Antibiotic Resistance Profiles of Gram-Positive Bacteria Isolated from Blood Culture Samples in Patients with Catheter Infection

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ABSTRACT

Bloodstream enfections, which is common and often lethal, is a serious public health problem. There is consensus that the incidence is increased in patients with sepsis due to an aging population, increased use of immunosuppressive therapy, and high-risk interventions.

In this study, 750 patients with suspected sepsis hospitalized in intensive care units in the Health Education University Training and Research Hospital were evaluated. 750 patients were classified according to their age and sex. Bacteria were isolated from their blood cultures. Biochemical tests such as catalase test, oxidase test, Gram staining and Coagulaz test were performed. Vitek 2 Compact (Biomerieux, USA) device was used for identification of bacteria and evaluation of *the* antibiogram test. The *mecA* genes were analyzed by polymerase chain reaction (PCR).

Two hundred and four Gram-positive bacteria from 750 (375 male and 375 female) were isolated in the blood culture samples taken from the intensive care units. 101 (26.9%) and 103 (27.5%) bacteria were isolated from 375 each male and female patients, respectively. Slime factor was positive in 47 of the coagulase negative bacteria. Twelve methicillin resistant *Staphylococcus aureus* (MRSA) isolates were positive for *mecA* gene carriage.

Identification of the bacteria causing sepsis in our hospital and determining the antibiotic resistance rates were found quite important. Among the causative agents of sepsis, effect of the presence of methicillin and multidrug resistance bacteria on humman health were found to be very important.

Keywords: Bloodstream enfections, *mecA*, *S. aureus*, CNS

Introduction

Circulatory system diseases are serious public health problems that can be prevalent and often lethal (1). The incidence studies conducted worldwide show that the disease has an incidence rate of about 300% in a population of 100.000 (2, 3). However, there is a consensus that the incidence is increasing because of an aging population with multiple comorbid infections, increasing the use of immunosuppressive therapy, and high-risk interventions (4, 5). Antimicrobial susceptibility of pathogenic microorganisms and rapid and accurate identification of them play a critical role in reducing the incidence of circulatory system diseases (6, 7). Gram-positive bacteria such as in particular coagulase-negative *Staphylococcus* (CNS) and *Staphylococcus aureus* cause serious circulatory system diseases. The increase in antibiotic resistance rates of these microorganisms is a matter of concern in terms of public health (8, 9).

Drug-resistant gram-positive bacteria can affect people of all ages in the population annually either directly or indirectly (10, 11). Staphylococci are evaluated in two parts according to their ability to produce coagulase. *S. aureus* and *Staphylococcus intermedius* are the most common infectious agents among the coagulase-positive species. Methicillin-resistant *S. aureus* (MRSA), defined among these bacteria in the world, poses a great risk in hospitals. In some studies, the prevalence of MRSA has been reported to be 18% in Europe, 44% in the USA, and 16% in Canada (12, 13, 14). Also, it was observed that the MRSA rate started to decrease to 28% in South Africa after the improvements in antibiotic administration (13, 15). It has been reported that the MRSA prevalence in the Southern Sahara region of Africa, India, Latin America, and Australia continued to rise (13). Coagulase-negative species

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(CNS) are identified as *Staphylococcus epidermidis*, *S. hominis*, *S. haemolyticus*, *S. cohnii*, *S. capitis* (16). CNS naturally colonize skins in humans and animals. They can cause circulatory system diseases in newborns without any interventional applications as well as sepsis after catheter and implant applications. In recent years, it has been observed that methicillin resistance levels in CNSs increased as well as *S.* epidermidis (9, 16).

This study aimed to evaluate the Gram-positive bacteria species isolated from the blood cultures of the patients, resistance rates that were found to be significant, and the presence of virulence factor (for CNS). It was considered important to determine how often, in which sex, and at what age the bacteria were observed. It is thought that the determination of antibiotic resistance and subsequent identification of resistance gene characteristics of bacterial species whose reproduction is found to be significant will contribute to surveillance studies. Also, the study aimed to contribute to the understanding of the flora that colonized the hospital, revealing the risk that the patients are exposed to, and organizing the measures to be taken in light of the information obtained in this study.

Material and Methods

Isolation and Identification of Bacteria, and Antibiogram Test: Seven hundred and fifty patients hospitalized on suspicion of sepsis in the intensive care units of Van Training and Research Hospital of the University of Health Sciences were evaluated. The 750 patients were classified by age and sex. The blood culture bottles were monitored in a Bactec/Alert 3D (Biomerieux, USA) device for 5 days. The blood culture bottles were inoculated into 5% sheep-blooded agar (Acumedia, USA), McConkey Agar (Oxoid, UK), and Eosin Methylene Blue agar (EMB, Oxoid, UK). The cultures were left for incubation at 37℃ for 48 hours. The colony morphology of the cultures was evaluated. Their biochemical tests such as the Catalase test, Oxidase test, and Gram staining were carried out. Vitek 2 Compact (Biomerieux, USA) system was used for the identification of the bacteria and antibiogram test. The bacteria that were identified to be CNS and *Staphylococcus aureus* were stored at -20℃.

Genomic DNA Extraction and Amplification: Bacterial DNA extraction was performed at the Pharmaceutical Microbiology Laboratory of the Faculty of Pharmacy. The bacteria stored at -20℃ were thawed at room temperature. They were inoculated into Trypton Soy Agar (Acumedia, USA) and were incubated at 37℃ for 24 hours. Then, the DNAs of the strains with multiple drug resistance were using G-SpinTM Total DNA Extraction kit (IntronBio, Korea) protocol. The bacterial DNA samples were stored at -20℃.

Bacterial DNA amplification was carried out by taking Sahebnasagh et al. (17) study as a reference. 5 µl of template DNA, 200 µM of deoxynucleotide triphosphate (Life Technologies) each, 1,5 U Taq DNA polymerase (ABM, Canada), buffer (20 mMTris-HCL, 50 mM KCL), and 3 mM MgCL2 (Biotools) were adjusted to be a 50 µl of final solution for PCR (Polymerase Chain Reaction). The PCR conditions for the gene *mecA* were arranged to be 2 min at 94℃, 20 sec at 94℃, 45 sec at 54℃, 60 sec at 72°C, 5 min at 72°C with 40 cycles. Amplicon products of the bacteria were run at Thermo EC300XL2 electrophoresis device at 100 Volts for 1,5 hours on 1.5% agarose gel. Amplicons were visualized using Bio-Print- ST4 (Vilber Lourmant, France) system. DNA amplification of the isolated and identified bacteria was performed using the reference primer *mecA* F: 5'-CCAATTCCACATTGTTTCGGTCATA-3' and R:5'-GTAGAAATGACTGAACGTCCGAT AA-3'. Antibiotic resistance identifications of each bacterium were approved.

Slime Analysis: An evaluation was performed with Congo red agar method to determine the slime production (18, 19). Congo red agar was obtained by stirring of 5% sucrose-supplemented (50 gr/L) Brain heart infusion broth (BHI, 37 gr/L) and Congo red stain (0.8 gr/L). The bacteria were inoculated into the Congo red agars and left for incubation at 37℃ for 48 hours. The cultures in which black colony formation was observed were considered to be positive. The CNS colonies that produced weak slime were observed to be pink or in the form of a black center and pink periphery. The experiments of these colonies were reiterated three times and the result was considered positive or negative.

Ethics: The Ethics Committee of Van Training and Research Hospital of the University of Health Sciences approved this study (No: 2018/ 02).

Results

Isolation and Identification of Bacteria, and Antibiogram Results: Two hundred four Gram-positive bacteria from 750 (375 male and 375 female) were isolated in the blood culture samples

Table 1. Gender and Age Distribution of Gram Positive Bacteria Isolated From Blood Culture Samples

	Female/ Age(%)			Male/ Age (%)		
Bacteria sp.	0-18	18-50	50+	0-18	18-50	50+
S. aureus	5 (13,5)	3 (16,6)	11 (23,9)	11 (35,5)	8 (40)	15 (28,8)
S. epidermidis	17 (46)	13 (72,2)	21 (45,7)	6 (19,4)	5 (25)	18 (34,6)
S. hominis	6 (16,2)	1 (5,6)	9 (19,6)	11 (35,5)	5 (25)	14 (26,9)
S. haemolyticus	8 (21,6)	1 (5,6)	4 (8,7)	1 (3,2)	2 (10)	4 (7,7)
S. capitis	1 (2,7)	-	1 (2,1)	2 (6,4)	-	1 (2)
Sum	46 (100)	51 (100)	83 (100)	40 (100)	30 (100)	70 (100)

taken from the intensive care units. 101 (26,9%) and 103 (27,5%) bacteria were isolated from 375 each male and female patients, respectively. In the identification of the Gram-positive bacteria, 53 (26%), 80 (39.2%), 46 (22.5%), 20 (9.8%), and 5 (2.5%) were found to be *Staphylococcus aureus*, *S. epidermidis*, *S. hominis*, *S. haemolyticus*, and *S. capitis*, respectively. The distributions of the bacteria by sex and age were given in Table 1. The percentages of the distributions of bacteria were determined by evaluating them in the age groups.

In this study, those who have multiple drug resistance among our bacteria were evaluated in terms of antibiotic resistance. Only twelve isolates out of 53 *S. aureus* strains were observed to be methicillin-resistant. It was detected that there were no bacteria with both multiple drug resistance and methicillin resistance among the CNS isolates. Percentage distributions of antibiotic resistance rates of Gram-positive bacteria isolated from patients are given in Table 2.

Genomic DNA Extraction and Amplification Results: 12 of the 203 bacteria we isolated and identified were analyzed as they were phenotypically multiple-drug-resistant. After the phenotypic evaluation, 12 MRSA of the gram-positive bacteria were included in the study and they were evaluated in terms of being a *mecA* carrier. 12 MRSA isolates were found to be positive in terms of being a gene *mecA* carrier (Figure 1).

Slime Analysis Results: The slime positivity of 151 CNS strains was analyzed using Congo red agar. Slime positivity was found in 25 (31.25%), 17 (37%), 4 (20%), and 1 (20%) of 80 *S. epidermidis* isolates, 46 *S. hominis* isolates, 20 *S. haemolyticus* isolates, and 5 *S. capitis* isolates, respectively.

Discussion

S. aureus is one of the pathogens most commonly seen in circulatory system diseases worldwide (20, 21). Methicillin-resistant *S. aureus* (MRSA) causes more resistant circulatory system diseases when compared with the susceptible strains and their mortality rates are higher (22, 23). In some studies, the mortality rates of methicillin-susceptible *S. aureus* (MSSA) circulatory system diseases were reported to be higher (24, 25). It was reported that *S. aureus* was the most common agent in the circulatory system diseases reported annually by the hospitals in Ireland (2006-2016) at a rate of approximately 19-32%. It was reported that while the incidence of MRSA among the *S. aureus* strains that cause circulatory system diseases was 41.9% in 2006, it decreased to 14.7% in 2016 (20). The positivity of methicillin resistance of the *Staphylococcus* species was investigated in a blood culture study on 80 patients in İzmir province of Turkey. The researchers reported that 1 (2%) *S. aureus* and 1 (2%) CNS were methicillin-resistant (26). In our study, the activity of gram-positive bacteria in sepsis patients was found to be quite higher. The rate of *S. aureus* isolated from our male and female patients was found to be 26% (n=53). The incidence of *S. aureus*-induced sepsis was found to be higher in male patients than it was in female patients. The highest isolation rate was found to be in male patients over 50 years of age. The lowest isolation rate was found to be in female patients under 18 years of age. In our region, it was detected that *S. aureus*-induced sepsis activity could be seen in all age groups. However, it was revealed that our male patients were more prone to the sepsis induced by this agent. In the antibiotic resistance evaluation conducted by the Vitek 2 system, it was observed that 12 MRSA isolates had a high resistance profile. The *mecA* gene carrying of the MRSA isolates was found to be positive by a PCR. It was found to be important that treatment protocol to be used in sepsis patients be performed by revealing MRSA risk factors.

Table 2. Rates of Antibiotic Resistance of Gram-Positive Bacteria Isolated From Patients

	S. aureus (n=53)			S. epidermidis (n=80)			S. hominis (n=46)			S. haemolyticus (n=20)			S. capitis (n=5)		
Cefoxitin	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Benzylpenicillin	40 (75,5%)	-	13 (24.5%)	64 (80%)	-	16 (20%)	34(73.9%)	-	12(26.1%)	16(80%)	-	4 (20%)	4(80%)	-	1 (20%)
Oxacillin	12 (22.6%)	-	41 (77.4%)	-	-	80 (100%)	-	-	46 (100%)	-	-	20 (100%)	-	-	5 (100%)
Gentamicin	36 (67.9%)	-	17 (32.1%)	57 (71.25%)	-	23 (28.75%)	27 (58.7%)	-	19 (41.3%)	17 (85%)	-	3 (15%)	3 (60%)	-	2 (40%)
Ciprofloxacin	27 (50.9%)	-	26 (49.1%)	54 (67.5%)	-	26 (32.5%)	24 (52.2%)	-	22 (47.8%)	6 (30%)	-	14 (70%)	2 (40%)	-	3 (60%)
Levofloxacin	14 (26.4%)	-	39 (73.6%)	49 (61.25)	-	31 (38.75%)	15 (32.6%)	-	31 (67.4%)	7 (35%)	-	13 (65%)	1 (20%)	-	4 (80%)
Moxifloxacin	17 (32%)	-	36 (68%)	45 (56.25%)	-	35 (43.75%)	7 (15.2%)	-	39 (84.8%)	9 (45%)	-	11 (55%)	2 (40%)	-	3 (60%)
Inducible Clindamycin Resistance	17 (32%)	-	36 (68%)	6 (7.5%)	-	74 (92.5%)	3 (6.5%)	-	43(93.5%)	2 (10%)	-	18(90%)	1 (20%)	-	4 (80%)
Erythromycin	34 (64.1)	-	19 (35.9%)	63 (78.75%)	-	17 (21.25%)	27 (58.7%)	-	19 (41.3%)	14 (70%)	-	6 (30%)	4 (80%)	-	1 (20%)
Clindamycin	38 (71.7%)	-	15 (28.3%)	36 (45%)	-	44 (55%)	4 (8.7%)	-	42 (91.3%)	3 (15%)	-	17 (85%)	3 (60%)	-	2 (40%)
Linezolid	42 (79.2%)	-	11 (20.8%)	57 (71.25%)	-	23 (28.25%)	12 (%26)	-	34 (74%)	7 (35%)	-	13 (65%)	1 (20%)	-	4 (80%)
Dalfopristin	26 (49%)	-	27 (51%)	17 (21.25%)	-	63 (78.75%)	17 (37%)	-	29 (63%)	8 (40%)	-	12 (60%)	2 (40%)	-	3 (60%)
Vancomycin	-	-	53 (100%)	-	-	80 (100%)	-	-	46 (100%)	-	-	20 (100%)	-	-	5 (100%)
Tetracycline	38 (71.7%)	-	15 (28.3%)	43 (53.75%)	-	37 (46.25%)	33 (71.7%)	-	13 (28.3%)	11 (55%)	-	9 (45%)	4 (80%)	-	1 (20%)
Tigecycline	5 (9.4%)	-	48 (90.6%)	9 (11.25%)	-	71 (88.75%)	4 (8.7%)	-	42 (91.3%)	6 (30%)	-	14 (70%)	2 (40%)	-	3 (60%)
Nitrofurantoin	12 (22.6%)	-	41 (77.4%)	13 (16.25%)	-	67 (83.75%)	6 (13%)	-	40 (87%)	5 (25%)	-	15 (75%)	2 (40%)	-	3 (60%)
Trimethoprim/ Sulfamethoxazole	7 (13.2%)	-	46 (86.8%)	21 (26.25%)	-	59 (73.75%)	18 (39.1%)	-	28 (60.9%)	12 (60%)	-	8 (40%)	1 (20%)	-	4 (80%)

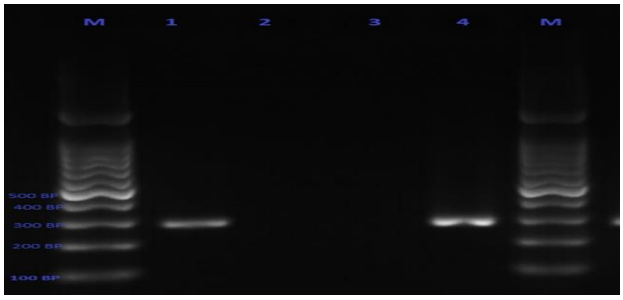

Fig. 1. 1. *mecA* positive *S. aureus* in patients with bacteremia; **2, 3:** *mecA* negative *S. aureus* in patients with bacteremia; **4:** mecA positive S. aureus 43300 (reference isolate); **M:** Marker (100 bp), BP: Baz pair

For years, CNS has been considered as contaminants and not true clinical pathogens (27). Since the last 10 years, CNS has taken its place among the agents of hospital infections (28). Especially CNS was identified as an important cause of infections in patients with immunodeficiency (29) and premature babies (9). It was revealed that CNSs were more common in individuals with prosthetic heart valves, catheter-applied patients, and patients receiving hemodialysis and corticosteroid therapy (30). In a study conducted by May et al. (27) between 1999 and 2012, they reported that they isolated over 540,000 CNS and 80,000 of them were *S. epidermidis*. In a study in Brazil, 25 staphylococci were isolated from the blood cultures. It was revealed that *S. haemolyticus* had the highest rate (31). In this study, the bacteria that were most highly isolated from the patients with sepsis (39.2%, 80) were found to be *S. epidermidis*. The highest incidence of *S. epidermidis*-induced septicemia was found to be in the female patients over 50 years of age. *S. epidermidis* was found to cause infection at the lowest in the female patients between 18-50 years of age. It was revealed that *S. haemolyticus* was the third cause between the CNS group of bacteria in our patients. The highest incidence of sepsis induced by this bacterium was found to be in female patients with 0-18 years of age. *S. capitis* was found to have the lowest rate among the gram-positive bacteria inducing sepsis. In our region, it was detected that the sepsis induced by this agent was seen in the groups of 0-18 and over 50 years of age.

Glycopeptides are the most commonly used antibiotics in CNS infections. However, in recent times, the increase in resistance rates to these antibiotics is alarming (32). The resistance rates of hospital-associated *S. epidermidis* strains to methicillin, aminoglycosides, and macrolides were reported to increase over 90% (27). The isolation rates of methicillin-resistant CNSs (MR-CNS) from patients are increasing. It was observed that the most frequently isolated agent in MR-CNS was *S. epidermidis* (9, 16). In a study conducted in Istanbul in 2009, methicillin-resistant and methicillin-susceptible 200 CNS isolates were found to show 90% and 17% resistance to gentamicin, 80% and 37% resistance to erythromycin, 72% and 18% resistance to clindamycin, 68% and 38% resistance to trimethoprim-sulfamethoxazole, 67% and 23% resistance to ciprofloxacin, 60% and 45% resistance to tetracycline, 56% and 13% resistance to chloramphenicol, and 25% and 15% resistance to fusidic acid. It was revealed that all of the 200 isolates were susceptible to vancomycin and teicoplanin (28). In a study conducted by Pereira et al. (31) in Brazil, they found that 15 CNS isolated from blood culture were oxacillin-resistant as well as carried *mecA* gene. In a study conducted at Ohud hospital, 24.1% of the CNS isolates were reported to be oxacillin-resistant. It was revealed that they were resistant to other antibiotics penicillin (99.24%), ampicillin (98.87%), erythromycin (90.9%), co-trimoxazole (84.2%), cephalothin and gentamicin (79.7%), amoxicillin-clavulanic acid (72.2%), and clindamycin (59.4%). All the isolates were reported to be vancomycin-resistant (33). May et al. (27) reported that the ciprofloxacin and clindamycin resistances of *S. epidermidis* isolates they isolated from blood cultures between 1999 and 2012 increased from 58% to 68.4% and 43.4% to 48.5%, respectively. Also, they detected that levofloxacin resistance between 1999 and 2005 increased rapidly from 57.1% to 78.6%. They reported that the rate of this resistance decreased to 68.1% in 2012. In a study in Italy, 166 CNS were isolated from blood culture samples. Antibiotic susceptibility rates of them were reported to be 15.7% (n=26), 98.8% (n=164), and 90.4% (n=150) for oxacillin, vancomycin, and teicoplanin, respectively. The susceptibility rates according to the number of bacteria tested in the same study were found to be 99.3% (155/156) for linezolid and 98.9% (97/98) for daptomycin (34). In our study, antibiotic resistance rates of the CNS strains isolated from the patients with sepsis were very low. It was also observed that the CNS strains isolated from the female and male patients with sepsis did not have methicillin, vancomycin, and multiple drug resistance profiles. It was seen that the prognosis of the patients could be treated by a treatment protocol that would be set with an antibiotic to be chosen correctly.

CNS species can adhere to surfaces with medical instruments through slime with mucopolysaccharide structure and found on the cell surface (19, 28). Slime-positive bacteria can be protected from antibiotics, phagocytosis, and chemotaxis (35, 36). Slime production is thought to play an important role in gaining resistance to antibiotics (19). In a study in Turkey, 86 out of 200 CNS isolates were found to be slime-positive. Slime-positivity by species were reported to be 71% for *S. epidermidis*, 35% for *S. haemolyticus*, 26% for *S. hominis*, 22% for *S. lugdunensis*, 10% for *S. xylosus*, and 7% for *S.* capitis (28). Deighton et al. (37) reported that the slime production of *S. saprophyticus* isolates was 50%. The studies revealed that there was a correlation between antibiotic resistance and slime production, but there was no clear information on its mechanism (28, 38, 39). The slime-positivity of the 151 CNS strains was investigated in this study. Slime positivity was detected in 25 (31.25%) of 80 *S. epidermidis* isolates, 17 (37%) of 46 *S. hominis* isolates, 4 (20%) of 20 *S. haemolyticus* isolates, and 1 (20%) of 5 *S. capitis* isolates. Slime positivity was found to be higher in *S. epidermidis* and *S. hominis* isolates. However, the antibiotic resistance rates of the slime-positive strains were not found to be high.

In conclusion, it was observed that the identification of Gram-positive bacteria causing sepsis in our hospital and revealing their antibiotic resistance rates were highly important. It was revealed that the presence of methicillin- and multiple drug-resistant bacteria among the agents causing sepsis had a highly significant effect on human health. It was found that the risk factors in patients with sepsis might vary by age and sex. It was determined that it was important that the surveillance reports to be kept be arranged accordingly. Annual follow-up of the analysis of gene regions that cause antibiotic resistance of isolated and identified bacteria was determined to be important in terms of MRSA prevalence.

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