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Phenotypic and Genotypic Analysis of the Antibiotic Resistance Profiles of Gram Negative Bacteria Isolated from the Blood Culture Samples

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ABSTRACT

Sepsis, 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, which leads to an increase in the use of immunosuppressive therapy, and high-risk interventions.

Seven hundred fifty patients with suspected sepsis hospitalized in intensive care units in the Health Education University Van Training and Research Hospital were evaluated. These patients were classified according to their age and sex. Bacteria were isolated from their blood cultures. Biochemical tests such as catalase, oxidase tests and Gram staining were performed. Vitek 2 Compact (Biomerieux, USA) device was used for identification of bacteria and evaluation of the antibiogram test. The bla_{OXA-48} and bla_{IMP} genes were analyzed by polymerase chain reaction (PCR) for those which display multidrug resistance to certain antibiotics.

One hundred and sixteen Gram negative bacteria were isolated. Among them, 44 *Klebsiella pneumoniae* (37.9%), 40 *Acinetobacter baumannii* (34.5%), 27 *Escherichia coli* (23.3%) and 5 *Pseudomonas aeruginosa* (4.3%) were identified. Eleven multidrug-resistant bacteria were assessed by Vitek 2 Compact device. Among them, two *K. pneumoniae* isolates were found to be bla_{OXA-48} carriers, whereas bla_{IMP} gene was not found in any of eleven isolates.

The presence of the bacteria with carbapenem, extended β-lactamase and multidrug resistance among the infectious bacteria may create a risk for human health. The risk factors may vary depending on age and gender in patients with sepsis and bloodstream infections. It was concluded that surveillance reports should be regulated according to this fact.

Keywords: Sepsis, bla_{OXA-48}, bla_{IMP}, PCR

Introduction

Sepsis is a common serious public health problem that may be fatal (1). Worldwide incidence studies have revealed an incidence of 300,000 cases per 100,000 population for the disease (2, 3). On the other hand, there is a consensus on the fact that its incidence increases due to the aging population with multiple infectious comorbidities, increasing use of immunosuppressive treatment, and high-risk interventions (4, 5). Assessment of antimicrobial susceptibility of pathogenic microorganisms as well as their rapid and accurate identification play a critical role in reducing the incidence of bloodstream infections (6, 7). Bacteria, such as Enterobacteriaceae family, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, cause serious bloodstream infections. Increased antibiotic resistance rates of these microorganisms create concerns with respect to public health (8, 9).

Carbapenem resistance bacteria has emerged as an important public health problem due to high mortality, length of hospital stay and high hospital cost (10). Because it is seen that carbapenems are ineffective used in severe bacteremic infections caused by multi-resistant Gram-negative bacteria (11). The reason for the ineffectiveness of carbapenems is the hydrolysis enzymes (carbapenemase) of Gram-negative bacteria (12). The most widely known carbapenemase are classified as KPC (Ambler class A), NDM and VIM (Ambler class B; metallo-β-lactamases, MBLs) and OXA-48 (Ambler class D) (12). OXA-48, was first described in a *K. pneumoniae* in Turkey (13). OXA-48 producing bacteria were reported from different parts of the world (14, 15). In addition, the imipenemase (IMP) enzyme encoded by the blaIMP gene was first described in the MBLs class in Japan (16). Later, it was detected less frequently in Enterobactericeae in England and Europe (17).

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However, information on bloodstream infections caused by IMP-producing bacteria is limited (12).

Carbapenem-resistant Enterobacteriaceae (CRE) pose a critical threat to human health by developing resistance to carbapenems. The reports published in 2013 in the five countries of Europe (19) have presented at least a rate of 10%. Carbapenem-resistant *K. pneumoniae* and *E. coli* were reported at the rates of 11% and 2% in the hospitals of the USA in 2012, respectively (20). In India, the rate of carbapenem-resistant *E. coli* was 13% whereas this rate was reported as 57% for carbapenem-resistant *K. pneumoniae* (21). Multidrug-resistant *P. aeruginosa* causes serious clinical infections in hospitals (22). It has been reported in a study that mortality rate was over 20% in the bacteremia cases associated with *P. aeruginosa* (23). *A. baumannii* is one of the major cause of the hospital-acquired infections with high mortality and morbidity rates (24). *A. baumannii* has the capability to develop resistance to various antimicrobial drugs. In recent years, it is known as one of the most common pathogens among the agents with multidrug resistance (25).

There are hundreds of biomarkers that can be used for diagnosis and prognosis in patients with septic and bloodstream infections (26). It is known that characteristics of the biomarkers such as specificity, sensitivity, availability for patient monitoring, and financial affordability are essentially effective in diagnostic success. It is obvious that the use of indicators such as C-reactive protein, procalcitonin, cytokines, lipopolysaccharide-binding protein (LBP), surface markers of circulating leukocytes (CD64), D-dimer, TREM-1, and CD73 provides critical data in diagnosis of sepsis. In microbiological approach, identification of the agents causing sepsis and bloodstream infections with rapid and accurate diagnosis of their antibiotic susceptibility are given high importance. It is known that antibiotic susceptibility testing plays a very effective role in treatment of the common bacteria such as *A. baumannii*, *P. aeruginosa* and members of Enterobactericeae family (27). In recent years, Vitek-2 has been widely used for the antibiotic susceptibility of bacteria isolated from blood cultures in sepsis patients (10, 11). Also, it is obviously seen that use of this method such as PCR (Polymerase Chain Reaction) technique for diagnosis of sepsis and bloodstream infections has increased (27).

In the literature research, there are some studies in different populations regarding the antibiotic resistance of different bacterial species (11, 18), however, we could not find a study performed in Van province regarding patients with sepsis having multi-drug resistance bacteria. Therefore, the study aims to identify bacteria species isolated from blood culture samples taken from patients with sepsis. It was expected that the determination of antibiotic resistance of bacterial species with significant growth and clarification of the characteristic of the resistance genes will contribute to the surveillance studies. It is also targeted to contribute to the procedures with respect to identification of the bacterial flora colonized in the hospital and regulation of the precautions by clarifying the risk that the patients are exposed to.

Materials and Methods

Isolation, Identification and Antibiotic Susceptibility of Bacteria: Seven hundred fifty patients with suspected sepsis admitted in the intensive care unit of TR Ministry of Health H.S.U., Van Training and Research Hospital were evaluated. The patients were classified based on age and gender. Blood culture bottles were monitored for 5 days in the BacTec/Alert 3D (Biomerieux, USA) instrument. Blood culture fluids from the bottles were inoculated onto 5% sheep blood agar base (Acumedia, USA), MacConkey Agar (Oxoid, UK) and Eosin Methylene Blue (EMB, Oxoid, UK) Agar. The plates were incubated at 37 °C for 48 hours. The colony morphologies of the cultures were evaluated. The biochemical tests including catalase test, oxidase test and Gram staining were performed. Vitek 2 Compact (Biomerieux, USA) instrument was used for bacterial identification and antibiogram test.

Extraction and Amplification of Genomic DNA: DNA extraction of the bacteria was performed in Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy of Van 100th Year University. The bacteria stored as bacterial stocks at -20 ℃ were thawed at room temperature. The bacteria were inoculated onto Tryptone Soy Agar (Acumedia, USA) and incubated at 37 ℃ for 24 hours. Following, DNAs of multidrug resistance bacteria were extracted using the protocol of G-SpinTM Total DNA Extraction kit (IntronBio, Korea). DNA samples of the bacteria were stored as bacterial stocks at -20℃.

DNA amplification of the bacteria was performed by taking reference the study of Poirel et al. (30). The concentrations of the components in the PCR mixture were calculated as 5 µl DNA template, 200 µM for each deoxynucleotide triphosphate (Life Technologies), 1.5 U Taq DNA polymerase (abm, Canada), buffer (20 mMTris-HCL, 50 mM KCL) and 3 mM $MgCL_2$ (Biotools) to get a 50 µl final solution. PCR for bla_{OXA-48} and bla_{IMP} was carried out under the conditions adjusted as 94℃ for 10 min, 94℃ for 30

Table 1. Reference oligonucleotide series used in the study

Genes	Primer Sequence	Base Size	Reference
blaOXA-48	F: 5'- GCGTGGTTAAGGATGAACAC-3'	438bp	30
	R: 5'- CATCAAGTTCAACCCAACCG -3'		
blaIMP	F: 5'- GGAATAGAGTGGCTTAATTCTC-3'	232bp	30
	R: 5'- GGTTTAATAAAACAACCACC-3'		

Table 2. The distribution of the bacteria isolated from the blood cultures based on gender and age

	FEMALE/ AGE (%)			MALE/ AGE (%)		
Bacteria spp.	0-18	18-50	50+	0-18	18-50	50+
K. pneumoniae	3 (33.3)	10 (30.3)	20 (54.1)	3 (33.3)	6 (60)	2 (11.1)
A. baumannii	2 (22.2)	20 (60.6)	10 (27)	4 (44.4)	1 (10)	3 (16.7)
E. coli	3 (33.3)	3 (9.1)	6 (16.2)	1 (11.1)	3 (30)	11 (61.1)
P. aeruginosa	1 (11.1)	-	1 (2.7)	1 (11.1)	-	2 (11.1)
Sum	9 (100)	33 (100)	37 (100)	9 (100)	10 (100)	18 (100)
		79 (21.1)			37 (9.9)	

sec, 52℃ for 40 sec, 72℃ for 50 sec and 40 cycles of 72℃ for 5 min. Bacterial amplicon products were run on the 1.5% agarose gel at 100 Volts for one hour using the Thermo EC300XL2 electrophoresis system. The bacterial amplicons were displayed using Bio-Print-ST4 (Vilber Lourmat, France). DNA amplification of the isolated and identified bacteria were performed using the reference primers given at Table 1. Identification of antibiotic resistance for each bacterium was confirmed by PCR.

Results

Isolation, Identification and Antibiogram Test Results of Bacteria: One hundred and sixteen Gram-negative bacteria were isolated from the blood culture samples taken from 750 patients (375 females and 375 males) in the intensive care units. Seventy nine (21.1%) and 44 (37.9%) bacteria were isolated from 375 female and 375 male patients, respectively. The distribution of the isolated Gram-negative bacteria were found as follows: *K. pneumoniae* 44 (37.9%), *A. baumannii* 40 (34.5%), *E. coli* 27 (23.3%) and *P. aeruginosa* 5 (%4,3). The distribution of the bacteria based on gender and age is presented in Table 2. The percentages of bacterial distribution were determined by an intragroup evaluation of age. The highest rate of *K. pneumoniae* strains were isolated from the female patients aged over 50 years old whereas the lowest rate of those were isolated from the male patients aged over 50 years old. *A. baumannii* strains were found to be isolated in higher rates from the female patients aged between 18 and 50 years old. The highest rate of *E. coli* strains were isolated from the male patients aged over 50 years old whereas the lowest rate of those were isolated from the male patients aged between 0-18 years old. *P. aeruginosa* strains were species isolated in the lowest rates from both female and male patients.

The bacterial species with mutlidrug resistance evaluated in terms of antibiotic resistance is given in Table 3. The number of the isolates with multidrug-resistance was 5 (11.4%) among the isolated *K. pneumoniae* (44; 37.9%) strains. These isolates were found to have both carbapenem and extended β-lactam resistance. The number of the isolates with multidrug resistance was 3 (7.5%) among the isolated *A. baumannii* (40; 34.5%) strains. These isolates were detected to have both carbapenem and extended β-lactam resistance. Multidrug resistance phenotype was detected in 2 (7.4%) *E. coli* isolates among *E. coli* (27; 23.3%) isolates. These isolates were found to have both carbapenem and extended β-lactam resistance. According to the result of antibiotic resistance analysis of *P. aeruginosa* (5; 4.3%) strains isolated from the patients with sepsis; only 1 (20%) *P. aeruginosa* strain demonstrated multidrug antibiotic resistance. This isolate demonstrated both carbapenem and extended β-lactam resistance. It was determined that sepsis due to *K. pneumoniae, A. baumannii* and *E. coli* with multidrug-resistance can be treated most effectively with colistin. It was encountered that colistin, imipenem, amikacin, and gentamicin can be used in the treatment of *P. aeruginosa* strain with multidrug resistance isolated from a female patient with sepsis.

Extraction and Amplification Results of Genomic DNA: Since 11 of the 116 bacterial strains isolated

Table 3. Antibiotic resistance rates of the bacteria with multidrug resistance isolated from the patients with sepsis

	K. pneumoniae (n=5)			A. baumannii (n=3)			E. coli (n=1)			P. aeruginosa (n=1)		
Antibiotics	R	I	S	R	I	S	R	I	S	R	I	S
Ampicillin	5	-	-	3	-	-	1	-	-	1	-	-
Amoxicillin/ Clavulanic Acid	5	-	-	3	-	-	1	-	-	1	-	-
Piperacillin/ Tazobactam	5	-	-	3	-		-	-	1	1	-	-
Cefazolin	5	-	-	3	-	-	1	-	-	1	-	-
Cefuroxime	5	-	-	3	-	-	1	-	-	1	-	-
Cefuroxime Aksetil	5	-	-	3	-	-	1	-	-	1	-	-
Cefoxitin	5	-	-	3	-	-	-	-	1	1	-	-
Ceftazidime	5	-	-	3	-	-	1	-	-	1	-	-
Ceftriaxone	5	-	-	3	-	-	1	-	-	1	-	-
Sefepim	5	-	-	3	-	-	1	-	-	1	-	-
Ertapenem	5	-	-	3	-	-	1	-	-	1	-	-
Imipenem	4	1	-	3	-	-	1	-	-	-	-	1
Meropenem	4	1	-	3	-	-	1	-	-	1	-	-
Amikacin	4	1	-	2	-	1	1	-	-	-	-	1
Gentamycin	3	2	-	3	-	-	1	-	-	-	-	1
Ciprofloxacin	4	1	-	3	-	-	1	-	-	1	-	-
Tigecycline	3	2	-	1	1	1	-	-	1	1	-	-
Colistin	-	-	5	-	-	3	-	-	1	-	-	1
Nitrofurantoin	1	-	4	3	-	-	1	-	-	1	-	-
Netilmicin	1	-	4	3	-	-	1	-	-	1	-	-
Aztreonam	1	-	4	3	-	-	1	-	-	1	-	-
Levofloxacin	2	-	3	3	-	-	1	-	-	1	-	-
Tobramycin	1	-	4	3	-	-	1	-	-	1	-	-
SXT*	1	-	4	3	-	-	1	-	-	1	-	-

*SXT: Sulfamethoxazole/ trimethoprim; R: Resistance; I: Intermadiate; S: Susceptible

and identified displayed multidrug-resistance phenotypically, these bacterial strains were analyzed molecularly, as well. According to phenotypic antibiotic resistance of Gram negative bacteria; 5 *K. pneumoniae*, 2 *A. baumannii*, 1 *E. coli* and 1 *P. aeruginosa* strains that showed carbapenem and extended β-lactam resistance were included in the study and they were evaluated with respect to carriage of bla_{OXA-48} and bla_{IMP} genes. Only 2 *K. pneumoniae* isolates of the Gram-negative bacteria were found to be carrier of bla_{OXA-48} gene (Fig. 1). None of the Gram negative bacteria that caused sepsis was found to include bla_{IMP} gene.

Discussion

No regression was encountered in the mortality rates in patients with sepsis despite improvements in medical practice. This fact causes both human loss and high-cost health expenses (31). Bloodstream infections are defined as serious infections progressing with high mortality and morbidity rates (32). However, it has been reported that identification of the patient and infectious agent, microbiological evaluation, and treatment process improves prognosis very effectively in the intensive care units (33). In patients with bloodstream infections, rapid selection, and administration of the antibiotic is very crucial for the prevention of the potential epidemics (34).

K. pneumoniae is a very important Gram-negative bacterium that is related to hospital epidemics and have hypervirulent strains in community-borne infections (35). *K. pneumoniae* plays an important role as the sepsis agent in the hospitals (especially in the babies) (36). It may cause pneumonia, wound, soft tissue, or urinary tract infections in the hospitalized

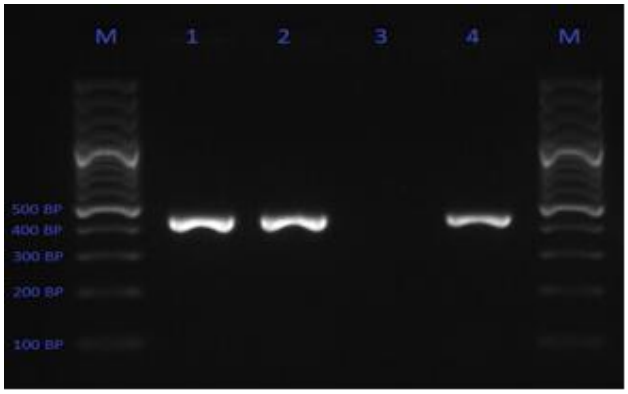

Figure 1. 1, 2: bla$_{oxa-48}$ positive *K. pneumoniae*; 3: Negative Control; 4: Positive Control: bla$_{OXA-48}$ positive *Escherichia coli* BAA-2523; M: Marker (100 bp); BP: Base pair

patients. *K. pneumoniae* may also lead to community-borne infections such as liver abscesses, pneumonia, and meningitis (37). The localization of *K. pneumoniae* isolates with multidrug resistance around the hospital and the clinic increases concerns (38). In recent years, carbapenem-resistant *K. pneumoniae* has created an important problem in bloodstream system infections (11;39). In Spain, 38 *K. pneumoniae* strains isolated from patients (>18 years of age) with bacteremia were reported to be positive for the *bla$_{OXA-48}$* gene. Additionally, all of these isolates were reported to be resistant to ertapeneme, co-trimoxazole, and ciprofloxacine (11). In another study, it was reported that 210 *K. pneumoniae* were isolated from blood cultures of patients (older than 16 years of age). In addition, 78% of colistin resistant *K. pneumoniae* isolates were found to carry the *bla$_{OXA-48}$* gene. Researchers also found that *K. pneumoniae* strains were most susceptible to aminoglycosides (39). It was shown in the present study that sepsis due to *K. pneumoniae* was found more commonly in the female patients aged 50 years old and over. Five *K. pneumoniae* strains isolated from the patients with sepsis were detected to have carbapenem, extended β-lactam and multidrug resistance. *K. pneumoniae* strains isolated from 2 patients were *bla$_{OXA-48}$* gene positive. It was concluded that serious complications may emerge due to the colonization of *K. pneumoniae* strains with multidrug resistance in the hospital.

A. baumannii may change to multidrug-resistant, extreme-drug resistance and pan-drug resistant *A. baumannii* strains in the regions in which predisposition to use of broad spectrum antibiotics is present (40, 41). Serious complications may emerge in the clinical practice since the number of treatment options against high-resistant *A. baumannii* strains is limited (42). It was reported that in a study carried out in Isparta 34 *A. baumannii* strains were isolated from the blood samples. Antibiotic susceptibility test suggested that these isolates showed the highest and the lowest sensitivity to tobramycin (122, 94.6%) and ciprofloxacin (9, 7%), respectively. Their sensitivities to imipenem and meropenem were found 66.7% and 50.4%, respectively (43). Gözütok et al. (44) reported that they isolated 161 (100%) *A. baumannii* strains from clinical samples including 63 (39%) blood samples. They suggested that 161 *A. baumannii* isolates showed a high rate of resistance 90% to many antibiotics (cephalosporins, carbapenems, β-lactamase inhibitors and kinolons). Colistin has been reported as the best antibiotic option to be recommended for the treatment of the isolates. The antibiotic resistance characteristics of 50 clinical *A. baumannii* isolates (12, 24% from blood samples) with multidrug resistance were shown using the techniques such as disc diffusion, liquid microdilution and agar dilution in a study conducted between 2011 and 2012 in Adana. The isolates showed 92% and 96% resistance to imipenem and meropenem, respectively. Amikacin susceptibility was stated to be 14% (45). Another study carried out in 2014 in Izmir reported that 65 *A. baumannii* isolates were isolated from the clinical samples (including 14 *A. baumannii* isolates from blood samples). Antibiotic susceptibilities of the isolates were identified by Vitek 2 instrument. It was shown that all of the isolates were resistant to ciprofloxacin, imipenem, cefepime, ampicillin-sulbactam and trimetoprim-sulfametoxazol. Sixty five *A. baumannii* isolates demonstrated the lowest resistance to colistin (46). In the present study, we have detected more *A. baumannii* strains from the female patients aged between 18 and 50 years old. Antibiotic resistance analysis performed using Vitek 2 instrument revealed that 3 *A. baumannii* strains had carbapenem, extended β-lactam and multidrug resistance profiles. The *A. baumannii* strains isolated from the patients were found not to carry *bla$_{OXA-48}$* and *bla$_{IMP}$* genes. It was encountered that *A. baumannii* strains with multidrug resistance showed the highest sensitivity to colistin. It was concluded that increasing inservice trainings is crucial to prevent contamination and infection in the hospital.

Hospital-borne infections are accepted as an important problem worldwide and the rates of the hospital-borne infections related to *P. aeruginosa* were stated to range between 10- 25 % (47). The patients may reveal pyogenic abscesses, wound infections (especially burn wounds), septicemia, pneumonia, ecthyma gangrenosum, otitis, meningitis, septic arthritis, urinary tract infections, and osteomyelitis associated with this bacterium (48). It has been reported in a study carried out in the USA that 596 *P. aeruginosa* strains were isolated from the blood cultures of the patients who had bloodstream infections, and 305 of these patients were included in that study. Of

the 241 survivor patients; 134 (55.6%) and 107 (44.4%) were male and female, respectively. Non-survivor 64 patients were composed of 39 (60.9%) and 25 (30.1%), respectively (49). It was noted that 120 *P. aeroginosa* strains isolated from blood cultures between 2009 and 2011 consisted of 76 (63.3%) male and 44 (36.7%) female patients according to the distribution based on age and gender. The mean age of the patients was reported as 51 (50). In the present study, a lower rate of *P. aeruginosa* (5, 4.3%) was detected than the other Gram-negative bacteria in the patients with sepsis in Van Training and Research Hospital. *P. aeruginosa* was the bacterial species with the lowest rate isolated from both female and male patients aged between 0-18 and older than 50 years. A study carried out in 2007 in Turkey has reported that 10 *P. aeruginosa* isolates were positive for bla_{IMP-1} and bla_{VIM-1} genes by the rates of 90% and 10%, respectively (51). A study conducted in Brasilia has investigated the presence of metallo-β-lactamase genes (bla_{SPM-1} and bla_{VIM-1}) in the carbapenem-resistant *P. aeruginosa* strains. The authors have reported that 45.8% (46) of 120 *P. aeruginosa* strains isolated from the patients with bacteremia were carbapenem-resistant. These strains were found positive for the bla_{SPM-1} and bla_{VIM-1} genes by respectively by 57% and 43% according to the MBL gene analysis performed by PC (50). In our study, only one *P. aeruginosa* isolate was carbapenem, extended β-lactam and multidrug resistant. This agent was found not to carry bla_{OXA-48} and bla_{IMP} genes according to the PCR analysis. It was concluded that such low impact of *P. aeruginosa* strains as the infectious agents would be associated with geographical conditions, predisposition of the population, colonization rate in the hospital and carriage in healthcare staff.

In recent years, antibiotic resistance reached alarming rates worldwide and the burden on the national economy due to especially *E. coli* associated with bacteremia has increased (52). It has been additionally reported that the mortality rate may exceed 40% if an effective antibiotic is not administered (53, 54). Lehmann et al. (34) have investigated the efficacy of diagnosis with PCR in the patients with sepsis. They reported to have isolated 271 microorganisms from 467 patients, composed of 154 and 117 isolates from PCR and blood samples, respectively. They suggested that 6, 5 and 2 *E. coli* isolates were obtained from using direct PCR, using PCR after blood culture and only from blood culture, respectively. The surveillance studies carried out between 2004 and 2008 indicates that incidence of *E. coli* has increased by rate of approximately 33%. It was suggested in 2008 that *E. coli* was identified in one third of the patients aged over 70 years old and more than 20% of all bacteremia cases (55). It has been noted in a diagnostic study performed in a one-thousand bed hospital in Switzerland that 20 various microorganisms were isolated from the blood cultures. According to the results of the identification studies by MALDI-TOF-MS methodology, *E. coli* showed the highest rate by 28.6% among the infectious agents that cause sepsis (32). In our study, *E. coli* (27; 23.3%) ranked in the third position of all. We have observed that the highest rate of *E. coli* isolates were from the male patients aged over 50 years old whereas the lowest rate of *E. coli* isolates were from the male patients aged between 0-18 years old. The incidence of multidrug-resistant *E. coli* reached alarming levels in Europe. It has been determined that resistance of *E. coli* strains against third-generation cephalosporins, aminoglycosides, and fluoroquinolones increased. Especially invasive *E. coli* reports demonstrated an increase of 70% (56). In the study conducted by Ma et al. (57), 5223 blood cultures were collected from the patient group with bacteremia at a mean age of 39 years old and including females by 51%. They reported to have isolated important microorganisms from 553 of those and *E. coli* caused the highest rate of sepsis. It was noted that 70 (61.9) of 113 *E. coli* isolates produced extended β-lactam resistance. Carbapenem resistance was encountered at a rate of 6%. Amikacin and piperacillin-tazobactam susceptibilities were presented as 92% and 80.6%, respectively. Among *E. coli* strains, SXT-resistance was 80.6% whereas ciprofloxacin resistance was detected as 74.1%. Resistance to ampicillin and ampicillin-sulbactam were 91.6% and 79.5%, respectively. A study conducted in China investigated the changes in the antibiotic resistance rates of *E. coli* strains leading to neonatal sepsis in two periods between 2002 to 2008 and 2012 to 2018. The antibiotic resistance rate of the *E. coli* isolates to third-generation cephalosporins was found to increase from 14.3% to 46.7%. In parallel to this rate; extended β-lactam resistance were reported to increase from 13.3% to 46.2%. Antibiotic resistance rates to ampicillin and ciprofloxacin were shown to increase from 50% and 9.5% to 73.1% and 38.5%, respectively. It was also stated that no change emerged in the rate of gentamicin resistance among *E. coli* isolates (23.8% in 2002-2008; 26.9% in 2012-2018) during these time intervals (58). In our study, 7.4% (2) *E. coli* among the *E. coli* strains (27; 23.3%) isolated from the sepsis patients showed multidrug resistance phenotypically. These strains were also shown to have both carpapenem and extended β-lactam resistance. However, *E. coli* strains were found not to carry bla_{OXA-48} and bla_{IMP} genes. The risk that our patients with sepsis may carry due to *E. coli* is

exactly related with their compliance to hygiene rules. It is concluded that training hospital personnel and patients about personal hygiene will contribute to the solution and prevention of hospital-borne complications.

As a conclusion, it was observed that identification of Gram negative bacteria isolated from the blood cultures in our hospital and determination of antibiotic resistance rates are very critical. The presence of the bacteria with carbapenem, extended β-lactamase and multidrug resistance among the infectious bacteria may create a risk for human health. Risk factors may vary depending on age and gender in the patients with sepsis and bloodstream infections with respect to the determination of those examinations. Surveillance reports should be regulated according to this fact. Analysis of the gene regions responsible for antibiotic resistance of the the bacteria is very crucial in the prediction of the risk in the hospital.

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