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The Agents Isolated from Cerebrospinal Fluid and Antibiotic Susceptibility of Adult Patients in a Training and Research Hospital

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Introduction: This study aims to investigate the antibiotic resistance of the agents isolated from cerebrospinal fluid (CSF) samples of adult patients in our hospital for five years to contribute to surveillance data.

Materials and Methods: 121 CSF samples between January 2015 and January 2020 were included in the study. Identification of isolates and determination of antibiotic susceptibility profile was performed according to EUCAST criteria with VITEK 2 (bioMérieux, France) automated system.

Results: Microorganisms (n=39) were isolated in 37 (30.6%) 121 CSF cultures. The most frequently isolated agent is *coagulase-negative staphylococci* (CoNS) by 30.8%. It is followed by *Micrococcus spp* (15.4%), *Streptococcus pneumoniae* (*S. pneumoniae*) (10.3%), *Klebsiella pneumoniae* (*K. pneumoniae*) (10.3%), *Acinetobacter baumannii* (*A. baumannii*) (10.3%), *S. aureus* (5.1%) and *Corynebacterium spp* (5.1%). 29 (74.4%) of the agents were isolated from hospitalized patients and 10 (25.6%) outpatients. Oxacillin resistance was found at 83.3% in CoNS, and penicillin resistance was found at 25% in *S. pneumoniae*. Antibiotics to which *K. pneumoniae* and *A. baumannii* isolates are most sensitive are tigecycline and amikacin, respectively.

Conclusion: Different regions and patient populations might cause differences in isolated agents and antimicrobial susceptibility. Retrospective analysis of regional data will assist the clinician in treating patients diagnosed with meningitis, where empirical and agent-oriented therapy is vital.

Keywords: Cerebrospinal fluid, antibiotic resistance, bacterial meningitis

Introduction

Bacterial meningitis, an acute infection of the meninges, subarachnoid space, and brain parenchyma is a clinical picture characterized by rapidly developing headache, nuchal rigidity, and neurological findings. Although successful treatment is possible in most cases with early diagnosis and treatment, the morbidity and mortality of bacterial meningitis are still high (1). In bacterial meningitis, a medical emergency,

the therapeutic goal is to initiate antibiotic therapy within 60 minutes of the patient's arrival in the emergency department. Cerebrospinal fluid (CSF) should be obtained for cultures in patients suspected of having bacterial meningitis, and empiric antimicrobial therapy should be initiated without delay. CSF culture is the gold standard in diagnosis and treatment for the agent (2, 3).

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Although *Haemophiles influenza* (*H. influenza*), *Neisseria meningitidis* (*N. meningitidis*), and *Streptococcus pneumoniae* (*S. pneumoniae*) are responsible for most acute bacterial meningitis worldwide, it has been observed that microorganisms isolated from CSF and their antimicrobial susceptibility may differ in populations. In addition, the agent profile has changed after the use of new vaccines (4).

Detection of the causative agents of meningitis and antibiotic resistance guides clinicians in choosing empirical treatment. This can only be achieved by following the resistance profiles of the bacteria over the years and following the treatment of the patients closely (5).

Our study evaluated bacteria isolated within five years from CSF samples sent to the laboratory with a preliminary diagnosis of meningitis and antibiotic susceptibility results.

Materials and Methods

Ethical Statement

This study is retrospective and was conducted in a local medical microbiology laboratory. The study was approved by local ethics committee and was carried out following the Declaration of Helsinki Principles (21.07.2020, 08/16).

Study Design

The study included one hundred and twenty-one CSF samples taken from adult patients and sent to the laboratory between 01.01.2015 and 01.01.2020. Demographic data and culture results were analyzed retrospectively. Samples coming to the laboratory in an automated blood culture media bottle (Biomérieux, France) were loaded into BacT/Alert 3D (Biomérieux, France) device without waiting and left to incubate for five days. Bottles with positive signals were inoculated into 5% sheep blood, Eosin Methylene Blue (EMB), and chocolate medium. Sheep blood and EMB media were incubated at 37°C in an aerobic environment; chocolate media were incubated at 37°C in an environment with 5-10 % CO₂ for 24-48 hours. The automated Vitek 2 Compact (Biomérieux,

France) system identified the growing colonies and antibiotic susceptibility studies. The isolates with carbapenem resistance were confirmed with the E-test method. Reporting of sensitivity results is based on EUCAST guidelines (6).

Statistical analysis

Statistical analysis was performed using the IBM SPSS v. 19 package program (IBM Corp., Armonk, USA). The significance level of for all tests was considered to be 0.05. The normality of distribution for continuous variables was confirmed with the Shapiro-Wilk test. The homogeneity of variances was confirmed with Levene's test. In order to compare samples, an independent sample t-test and Mann-Whitney U test were used for quantitative data, Pearson chi-square test and Fisher's exact test was used for qualitative data.

Results

When the relationship between gender and age distribution of the patients from the outpatient and inpatient was examined, it was similar in the samples ($p=0,522$, $p=0,143$, respectively). The distribution of samples taken from outpatients and inpatients by the year was similar ($p>0.05$) (Table-1).

During the five-year study period, 84 (69.4%) of the 121 CSF samples sent to our laboratory did not reproduce, while 39 agents were grown in 37 (30.6%) samples. *Klebsiella pneumoniae* (*K. pneumoniae*) and *Alcaligenes faecalis* were grown in a female patient, and two types of *coagulase-negative staphylococci* (CoNS) were grown in a male patient. The youngest of the patients with reproduction are 22, the oldest is 89, and the average age is 48.9 years. 19 (51.4%) of the patients with reproduction were male, and the mean age was 46.1 years. Eighteen (48.6%) of the patients with reproduction were female, and the mean age was 49,7. Of the 39 reproducing agents, twelve were CoNS, six were *Micrococcus spp.*, 4 were *S. pneumoniae*, 4 were *K. pneumoniae*, 4 were *A. baumannii*,

2 were *Coryne-bacterium spp.* and 2 were *Staphylococcus aureus* (*S. aureus*). A small number of other factors reproduced. Of the factors, 29 were isolated from inpatients who underwent shunt, trauma, and central nervous system operations, and ten were isolated from outpatients who applied to the outpatient clinic. While CoNS was the most common reproducing agent in inpatient samples (n=11), *S. pneumoniae* (n=4) was the most common in outpatients.

Table 1. Descriptive statistics

Gender	Outpatient	Inpatient	<i>P value</i>
▪ Male	6	13	0,522*
▪ Female	4	14	
Age	54,6±16,5	46,1±14,8	0,143†
Years, <i>agents</i>			
▪ 2015	0	4	>0,05‡
▪ 2016	1	2	
▪ 2017	2	6	
▪ 2018	4	11	
▪ 2019	3	6	
Bacterium		<i>Total</i>	
▪ CoNS	1	11	12 (30.8)
▪ <i>Micrococcus spp.</i>	2	4	6 (15.4)
▪ <i>Strep. pneumoniae</i>	4	0	4 (10.3)
▪ <i>Klebsiella pneumoniae</i>	1	3	4 (10.3)
▪ <i>Acinetobacter baumannii</i>	0	4	4 (10.3)
▪ <i>Corynebacterium</i>	0	2	2 (5.1)
▪ <i>S. aureus</i>	1	1	2 (5.1)
▪ <i>Neisseria meningitidis</i>	1	0	1 (2.6)
▪ <i>Alcaligenes faecalis</i>	0	1	1 (2.6)
▪ <i>Sphingomonas mobilis</i>	0	1	1 (2.6)
▪ <i>Enterococcus gallinarum</i>	0	1	1 (2.6)
▪ <i>Streptococcus. anginosus</i>	0	1	1 (2.6)
▪ Total	10	29	39 (100)

*Pearson chi-square test, †Independent sample t-test, ‡Fisher's exact test (2x2 tables were created, then compared).

The distribution of agents isolated from inpatient and outpatient clinic patients is given in Table 1. Of the isolates, 14 were isolated from Intensive Care, ten from Neurosurgery, ten from Infection, four from Neurology, and one from patients from the Emergency. Considering the distribution of the factors by years, the most reproduction was in 2018 (n=15), and the lowest was in 2016 (n=3). The distribution of the agents by year is given in Table-1.

Oxacillin resistance in CoNS was 83.3% (10/12x100). Penicillin resistance was 25% in *S. pneumoniae* isolates (n=4). Two isolated *S. aureus* strains were sensitive to oxacillin. The susceptibility of CoNS and *S. pneumoniae* isolates are shown in Table-2. *Micrococcus* and *Corynebacterium bacteria*, two types of CoNS growing in one sample, those antibiotic susceptibility studies were not performed because there was no demand from the clinics. 2 (50%) of *K. pneumoniae* isolates were positive for extended-spectrum β -lactamase (ESBL), and one (25%) was positive for carbapenemase - carbapenem resistance confirmed by the E-test method. Antibiotics to which *K. pneumoniae* and *A. baumannii* isolates are most sensitive are tigecycline and amikacin, respectively (Table-2).

Discussion

In bacterial meningitis, an inflammation of the meninges of the brain, clinical findings such as altered consciousness, headache, fever, and neck stiffness are observed. Bacterial meningitis requires immediate attention (2). The age of the patients, the most common factors in age groups, regional differences of the agents, and antibiotic susceptibility should be considered in the selection of empirical treatment. These data contribute to the increase in the success of the treatment and the earlier and sequela-free recovery of the disease (7).

In our study, 39 bacteria were detected in 37 (30.6%) of 121 CSF sent to our laboratory. 12 of them were CoNS, 6 were *Micrococcus spp.*, 4 were *S. pneumoniae*, 4 were *K. pneumoniae*, 4

were *A. baumannii*, 2 were *Coryne-bacterium spp.* and 2 are *S. aureus*. Yanık et al., in their study conducted between 2006-2011, determined the reproduction rate as 7% in 10,632 CSF samples. The most common reproducing agents were CoNS (60.4%), *Acinetobacter spp.* (10.6%), and *Enterobacter spp.* (9.8%)(3). CoNS, which are found as a flora element in many parts of our body, cause meningitis, especially after surgical applications for the central nervous system, trauma, and shunt applications. In studies, CoNS is generally in the top three, and oxacillin resistance rates are up to 82% (8, 9). Consistent with the literature in our study, CoNS was the most frequently isolated factor, with a rate of 30.8%, and oxacillin resistance was 83.3%.

Micrococci, which can be found as a skin flora element, may rarely cause meningitis in the presence of immunosuppression, catheter, or shunt in the body (10). *Micrococci*, detected in our study with a rate of 15.4%, were considered skin contamination, and no antibiotic sensitivity was performed. The lack of clinical feedback for these bacteria has increased the likelihood of skin contamination. *K. pneumoniae* meningitis can spontaneously cause meningitis after head trauma, and after neurosurgical procedures, with the distant spread in patients with liver abscesses. It has been determined that 50-68 % of intensive care patients with diabetes mellitus may be the cause of meningitis (11). Among the agents of postoperative meningitis, the frequency of *A. baumannii* is relatively high, at

Table 2. Antibiotic resistance rates of Gram-positive bacteria (n), *K. pneumoniae*, and *A. baumannii* strains (n)

Antibiotics	Bacteria (n)			
	<i>CoNS</i>	<i>S.pneumoniae</i>	<i>K.pneumoniae</i>	<i>A.baumannii</i>
	(n=12)	(n=4)	(n=4)	(n=4)
Oxacillin	10	*-	-	-
Ampicillin	-	-	4	-
Fusidic acid	7	-	-	-
Ceftazidime	-	-	2	4
Tetracycline	6	1	-	-
Imipenem	-	-	1	4
Ciprofloxacin	6	-	1	4
Meropenem	-	-	1	4
Clindamycin	5	1	-	-
Trimethoprim-sulfamethoxazole	3	0	1	4
Gentamicin	3	-	1	4
Rifampicin	-	0	-	-
Erythromycin	-	1	-	-
Piperacillin-Tazobactam	-	-	1	-
Penicillin	-	1	-	-
Amikacin	-	-	1	0
Daptomycin	3	-	-	-
Cefepime	-	-	2	-
Vancomycin	0	0	-	-
Linezolid	0	0	-	-
Ceftriaxone	-	0	2	-
Ertapenem	-	-	1	-
Tigecycline	0	-	0	1

CoNS: Coagulase-negative staphylococci, * -: Not tested

34.8% (12). In our study, *K. pneumoniae* and *A. baumannii*, in the first place among Gram-negative agents, were detected at a lower rate (10.3%) than in the literature. Ceftriaxone, cefepime, and meropenem treat meningitis cases caused by enteric bacteria (13). In our study, two of *K. pneumoniae* isolates (n:4) were ESBL(+), one was resistant to carbapenems, and tigecycline resistance was not found in any isolate by the literature (11). In 2011, Çıkman et al. found tigecycline resistance to be 14 % in *Acinetobacter* isolates. In our study, tigecycline resistance in *A. baumannii* isolates was 25%, and it was higher than in the literature (14).

In our study, in which we did not find *H. influenzae*, *S. pneumoniae* was isolated in four outpatients, and *N. meningitidis* was isolated in one patient. It has been reported that the most common bacterial meningitis agents in Western countries are *S. pneumoniae* in adults, and In newborns and children, *S. pneumoniae*, *H. influenzae* and *N. meningitidis* (15). Our rates are lower than the literature may be due to the decrease in the rates of meningitis in childhood and over 65 years of age with the use of the vaccine and the that our youngest patient with reproduction was 22 years (7). *N. meningitidis* is seen at a rate of 2.5% in adults, which was 2.6% in our study (16). The most commonly used antibiotics in *S. pneumoniae* meningitis are penicillin and other β -lactam antibiotics. However, increasing resistance worldwide reduces the effect of these antibiotics and increases mortality. There is a linear relationship between the proportion of *S. pneumoniae* isolates that are not penicillin-susceptible and the use of penicillin in outpatients, and this ratio ranges from 1 to 40% (7, 17). The present study observed penicillin resistance in 25% of *S. pneumoniae* strains.

Conclusion

Rapid diagnosis and treatment of bacterial meningitis are life-saving. When deciding on early treatment, the differences in regional

factors according to age and time and knowing their antibiotic susceptibility are the agents that increase the success of the treatment. As a result, we think the data obtained will contribute to selecting empirical/causative antibiotics and surveillance data.

Conflict of Interest

The authors declare no conflict of interest for the present study.

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