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Evaluation of Cerebrospinal Fluid Results in Patients Presenting with a Prediagnosis of Meningitis

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ABSTRACT

Aim: It was aimed to determine the microorganisms and antibiotic susceptibilities obtained from cerebrospinal fluid samples (CSF).

Material and Methods: CSF samples of 138 patients who admitted with a prediagnosis of meningitis to Ondokuz Mayıs University Pediatric Emergency Department between 01/01/2021-01/01/2022 was included. CSF cultures, identification of bacteria and their antibiotic susceptibilities were evaluated by VITEK 2 (bioMeriux, France) automated identification system. If the growth was detected by blood culture system, the specimen was cultivated onto the 5% blood agar, Eosin Methylene Blue agar and chocolate agar. Also, CSF samples were studied for both viral and bacterial agents with the Smart Cycler (Cepheid, USA) device working with Real-Time PCR method. In this system, *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Cytomegalovirus, Herpes simplex type-1, Herpes simplex type-2, Human herpes virus type-6 (HHV-6), Varicella zoster virus and Epstein Barr virus* agents were detected.

Results: Of the 138 CSF samples, 50,88% were male and 49,12% were female. The mean age of the patients was 36 ± 55 months. Bacterial agents were detected in 18 (13,8%) of the CSF samples and viral agents in 12 (9,2%) samples. *Staphylococcus epidermidis* (64.6%), *Staphylococcus aureus* (7.7%) and *Streptococcus pneumonia* (6.9%) took the first three places in bacterial agents. Among the viral agents, *HHV-6* was the most common followed by *Enteroviruses*.

Conclusion: It has been observed that the profiles and resistance status of microorganisms frequently seen in CSF have changed over the years.

Keywords: Meningitis; cerebrospinal fluid; antimicrobial susceptibility; PCR.

Menejit Ön tanısıyla Başvuran Hastalarda Beyin Omirilik Sıvı Sonuçlarının Değerlendirilmesi

ÖZ

Amaç: Beyin omurilik sıvısı örneklerinden (BOS) elde edilen mikroorganizmaların ve antibiyotik duyarlılıklarının belirlenmesi amaçlanmıştır.

Gereç ve Yöntemler: Ondokuz Mayıs Üniversitesi Çocuk Acil Servisi'ne 01/01/2021-01/01/2022 tarihleri arasında menenjit ön tanısı ile başvuran 138 hastanın BOS örnekleri alındı. BOS kültüründe üreyen bakterilerin tanımlanması ve antibiyotik duyarlılıkları VITEK 2 (bioMeriux, Fransa) otomatik tanımlama sistemi ile değerlendirildi. Kan kültürü sisteminde üreme saptandıysa örnek %5 kanlı agar, Eosin Methylene Blue agar ve çikolatalı agarda ekildi. Ayrıca Real-Time PCR yöntemi ile çalışan Smart Cycler (Cepheid, ABD) cihazı ile BOS örnekleri hem viral hem de bakteriyel etkenler açısından incelendi. Bu sistemde *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Sitomegalovirüs, Herpes simpleks tip-1, Herpes simpleks tip-2, Human herpes virüsü tip-6 (HHV-6), Varicella zoster virüsü ve Epstein Barr virüsü* ajanları tespit edildi.

Bulgular: 138 BOS örneğinin, %50,88'i erkek, %49,12'si kadındı. Hastaların yaş ortalaması 36 ± 55 ay idi. BOS örneklerinin 18'inde (%13,8) bakteriyel, 12'sinde (%9,2) viral etkenler saptanmıştır. Bakteriyel etkenlerde *Staphylococcus epidermidis* (%64,6), *Staphylococcus aureus* (%7,7) ve *Streptococcus pneumonia* (%6,9) ilk üç sırayı aldı. Viral ajanlar arasında en yaygın olanı *HHV-6* idi ve onu *Enterovirüsler* izlemiştir.

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Sonuç: BOS'ta sıklıkla görülen mikroorganizmaların profillerinin ve direnç durumlarının yıllar içinde değiştiği gözlenmiştir.
Anahtar Kelimeler: Menenjit; beyin omurilik sıvısı; antimikrobiyal duyarlılık; PZR.

INTRODUCTION

Meningitis is an acute inflammation of the membranes surrounding the brain caused by various microorganisms (1). Bacteria are usually detected as the causative agent in acute purulent meningitis; in acute aseptic meningitis, the causative agent is usually viruses. *Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenza* are responsible for 80-85% of acute bacterial meningitis cases, but the incidence of meningitis agents varies according to age and underlying condition (2). It has been reported that non-polio enteroviruses are responsible for 80-95% of aseptic meningitis cases (3).

In order to make a definitive diagnosis of acute bacterial meningitis, clinical symptoms and findings should be supported by laboratory findings. If there is no contraindication, lumbar puncture should be performed and cerebrospinal fluid (CSF) examination should be performed in every patient with suspected meningitis (4).

Initiation of treatment as soon as CSF examination is performed in meningitis affects morbidity and mortality. Recognizing age groups, service characteristics, microorganisms frequently isolated in that region and their antibiotic resistance; will guide the clinician correctly in empirical treatment until culture results are obtained (5).

For the diagnosis of central nervous system (CNS) infections; many methods such as cell count, Gram stain, bacterial culture and polymerase chain reaction (PCR) are used for nucleic acid detection. The most important advantage of PCR against bacterial culture is its high sensitivity, fast results and no need for live bacteria. For this reason, it has been the most frequently used method in recent years (6,7).

There may be a change in the profile of the agents of meningitis with the new vaccines that have been routinely applied in recent years (8). In this study, it was aimed to determine the microorganisms and antibiotic susceptibilities obtained from CSF samples sent from the pediatric emergency department of our hospital with a preliminary diagnosis of meningitis.

MATERIAL AND METHODS

In current study, between 01/01/2021 and 01/01/2022 CSF samples of 138 patients hospitalized in Ondokuz Mayıs University Faculty of Medicine Pediatric Emergency Service with a preliminary diagnosis of meningitis was evaluated retrospectively. The clinical and laboratory findings of the patients were obtained from the automation system. Ethics committee approval was obtained for the study from the Institutional Review Board (OMU KAEK No: 2022/435, Date: 29/09/2022).

CSF samples were tested with The BioFire® FilmArray®Meningitis/Encephalitis (ME) Panel (Biomeriux, France) for both bacterial and viral agents. In this panel, *Escherichia coli K1, Haemophilus influenzae, Streptococcus pneumoniae, Listeria monocytogenes, Streptococcus agalactiae, Neisseria meningitides, Varicella zoster virus (VZV), Cytomegalovirus (CMV), Enterovirus (EV), Herpes simplex virus 1 (HSV-1), Human herpesvirus 6 (HHV-6), Herpes simplex virus 2 (HSV-2), Human parechovirus (HPeV) and Cryptococcus (C. neoformans/C. gattii)* were tested by nested PCR. CSF samples were tested according to the recommendations of the manufacturer.

CSF samples were also incubated in a BacT/Alert (Biomeriux, France) blood culture system. Cell count and Gram staining were also studied from the samples. If the growth was detected by the blood culture system, the specimen was cultivated onto the 5% blood agar, Eosin Methylene Blue (EMB) agar and chocolate agar and incubated at 36°C for 24 hours. Identification of bacteria was performed by Vitek MS (Biomeriux, France) and their antimicrobial susceptibility was tested by Vitek2 Compakt (Biomeriux, France) automated systems.

RESULTS

CSF samples of 138 patients were included in the study. Of the patients included in our study, 80 (58%) were male and 58 (42%) were female. The mean age of the patients was 36 ± 55 months. Of the patients diagnosed with meningitis, one was admitted with febrile convulsions and the others with fever and/or vomiting and headache. Biochemical (protein, glucose) and Gram stained microscopic examinations of the patients who were found to be positive were compatible with meningitis.

While the agent was not isolated in 108 (78%) of the CSF samples sent to the laboratory, it was isolated in 30 (22%). Of these agents, 18 were bacterial (13.8%) and 12 were viral agents (9.2%). The distribution of bacterial and viral agents detected for growth is summarized in Table 1 and 2.

Table 1. The distribution of isolated bacterial agents.

	n
S.epidermidis	9
S.pnemonia	3
Klebsiella	1
S.aureus	4
E.coli	1

Table 2. The distribution of isolated viral agents.

	n
Escherichia coli DNA	
Haemaphilus influenza DNA	
Listeria monocytogenes DNA	
Neisseria meningitidis DNA	
Streptococcus agalactiae DNA	
Streptococcus pneumoniae DNA	1
Cyto megalo virüs (CMV) DNA	
Human herpes virüs type 6 (HHV6) DNA	7
Human parechovirus DNA	
Herpes simplex 1 (HSV1) DNA	1
Herpes simplex 2 (HSV2) DNA	
Varicella zoster virüs DNA	
Cryptococcus neoformans/gattii DNA	
Enterovirus DNA	4

Table 3. The resistance to antibiotics in gram-positive bacteria isolated from cerebro spinal fluid (%)

	Clindamycin	Oxacillin	Vancomycin	Linezolid	Ceftriaxone
S.epidermidis	50	77	0	0	70
S.aureus	50	25	0	0	50
S.pnemoniae	66	66	0	0	50

Table 4. The resistance to antibiotics in gram-positive bacteria isolated from cerebro spinal fluid (%)

	Amikacin	Ampicillin	Meropenem	Ceftriaxone	Cefepime	Trimethoprim-sulfamethoxazole
Klebsiella	S	R	S	R	R	R
E.coli	S	R	S	R	R	R

R: Resistant, S: Susceptible

Accordingly, *Staphylococcus epidermidis* (64.6%), *Staphylococcus aureus* (7.7%) and *Streptococcus pneumonia* (6.9%) took the first three places in bacterial agents. Among the viral agents, *HHV-6* was the most common followed by *Enteroviruses*.

10 patients had ventriculo-peritoneal shunt. *Staphylococcus epidermidis* was detected in six of them and *Staphylococcus aureus* in four of them.

Antibiotic resistance in gram-negative and gram-positive bacteria is shown in Table 3 and 4.

DISCUSSION

Despite the advances in antimicrobial therapy and vaccines compared to the last century, meningitis continues to be a serious threat to global public health with high morbidity and mortality and can lead to important complications (9). The incidence of bacterial meningitis in developed countries is 5 cases per 100,000, and it is ten times higher in developing countries (10). Various bacteria can cause meningitis, but excluding the neonatal period, more than 80% of meningitis cases are caused by *Haemophilus influenzae type b, Streptococcus pneumoniae* and *Neisseria meningitidis* (8,11).

After the introduction of specific vaccines against *Streptococcus pneumonia* and *Haemophilus influenzae type b* into the routine vaccination program, a decrease in the cases of meningitis caused by these microorganisms was observed (11,12). In this study, growth was detected in 30 samples (22%) and 18 (13%) were bacterial, 12 (8.7%) were viral out of 138 samples. *Neisseria meningitidis* and *Haemophilus influenzae* could not be isolated in any patient, and *S. pneumonia* was isolated in only 3 cases (2.1%). In the study conducted by Özdemir et al. (13) in 2010, the rates of *Streptococcus pneumonia* was 27%, 11% for *Haemophilus influenza* and 11% for *Neisseria meningitidis* reported in patients with meningitis.

Streptococcus pneumoniae is the most common cause of community-acquired bacterial meningitis (8). Its incidence tends to decrease in countries that include vaccination studies in the national immunization program. Conjugated pneumococcal polysaccharide vaccine was introduced into routine vaccination in 2008 in our country (14). In the study of Akkaya et al. (6), *Streptococcus pneumoniae* was found to be the causative agent in 7 (3.5%) patients and ranked first among bacterial meningitis agents. In the current study *Streptococcus pneumoniae* was isolated in 2 female (9 and 15 years old) and 1 male (11 years old) patients (2.1%). They did not have any underlying diseases and all three had fever and headache at presentation. Two of these three samples had penicillin resistance (66%). In the study of Duman et al. (13), *Streptococcus pneumoniae* strains were isolated in a three-year period and penicillin resistance was found at a rate of 18.2%. In various publications in our country, *Streptococcus pneumoniae* penicillin resistance has been reported as 3-38% (15,16).

In the study performed by Sümer et al. (17), no agent could be isolated in 283 (68.5%) of the CSF samples, while *Coagulase-negative staphylococci (CNS)* (64.6%) took the first place in 130 CSF cultures. This was followed by *Acinetobacter spp* (7.7%) and *Enterococcus spp* (6.9%).

In studies where CSF samples were examined bacteriologically, Dadağlıoğlu et al. (18) detected *Neisseria meningitidis*, *Enterobacter* and *Escherichia coli*; Durmaz et al. (19) *E.coli, Klebsiella spp* and *Pseudomonas spp*; Ulusoy et al. (20) identified *Streptococcus pneumoniae, Neisseria meningitidis* and Gram-negative rods in the first three rows. In the study of Yağcı et al. (21), while CNS took the first place, *Acinetobacter spp* took the third place. In the current study CNS took first and *Staphylococcus aureus* was second place. All patients with *Staphylococcus aureus* had ventriculo-peritoneal shunt. Methicillin resistant rate was 25% for *Staphylococcus aureus*. Yanık et al (22) reported 9% rate and 36% methicillin resistance for *Staphylococcus aureus*.

In various studies, CNS is isolated from CSF cultures between 14.9% and 64%, and the rate of methicillin-resistant CNS varies between 30% and 64% from hospital to hospital (17). In the study of Duman et al. (12), 25 (19.7%) CNS strains were isolated and 16 of the strains (64%) were found to be methicillin resistant. In the current study CNS was isolated in 9 patients (7%) and methicillin resistant rate was 77% similar to Duman et al. While Yağcı et al. (21) found methicillin resistance as 64.0%, Kocagöz et al. (23) reported it as 30.0%.

Sümer et al. (17) evaluated antibiotic susceptibilities of the agents and found no resistance to amikacin, vancomycin and netilmicin in CNS. In the current study, there was no resistance to vancomycin, 50% resistance to clindamycin and 70% resistance to ceftriaxone.

In our study, the most common viral agents were *HHV-6*, while *Enteroviruses* were the second most common viral agent. Akkaya et al. (6) reported that *Enteroviruses* were the first meningitis agents with a rate of 25%. In the study conducted by Sarinoglu et al. (24) in 2016, *HSV-1* was the most detected factor in 12 (34.2%) patients, followed by

Enterovirus detected in 8 (22.8%) patients. In the study of Soylar et al. (7), *EBV* had the highest percentage with a rate of 15% among viral agents. In the study of Çaycı et al. (25), the most common virus was *HSV-1* (20%) and the most common bacterial agent was *Streptococcus pneumoniae* (40%). In another study by Çiçek et al. (26), viruses were found to be the causative agent in 33% of patients diagnosed with acute meningitis, of which 22.5% were *Enterovirus*, 9.9% were *Herpes viruses*, and 8.1% were *West Nile virus*.

In conclusion; the profile and resistance of microorganisms isolated from CSF have been changed. It was aimed to investigate the change in meningitis agents isolated from CSF with the effect of vaccination and it was seen that the decrease in the most frequently isolated bacteria was at a significant level.

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