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Short Article

A Study on Bacterial Agents of Patients with Meningitis Referred to Afzalipour Hospital in Kerman, South East Iran

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Background and Aims: Identifying meningitis-causing bacteria play an important role in selecting the appropriate antibiotic vaccine and reducing the complications of meningitis. The present study aimed to identify the most common bacteria causing meningitis in patients referred to Afzalipour Hospital in Kerman.

Materials and Methods: In this cross-sectional study, 60 patients have participated. A 3-5 ml of cerebrospinal fluid specimens were obtained from each patient. Traditional and molecular methods identified bacterial agents.

Results and Conclusions: Twenty-eight females and thirty-two males participated in the study. Bacterial agents were recovered from 33.3% of the samples. *Streptococcus pneumonia* (65%), *Staphylococcus epidermidis* (10%), *Klebsiella* (10%), *Enterococcus* (5%), *Escherichia coli* (5%) and *cocci gram-positive* (5%) were the microbial agents identified in this study. In the present study, the detection rate of bacteria was low. *Streptococcus pneumonia*, especially the 18CFBA serotype, was the most common bacteria.

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Introduction

Meningitis is an infection and swelling of the meninges that requires early diagnosis and treatment. Although most cases of meningitis improve, it can cause serious complications such as brain damage and deafness, and even death if left untreated [1]. Most meningitis has common symptoms such as fever, seizures, nausea, vomiting, headache, stiff neck, sensitivity to light, irritability, chills, and lethargy [1, 2]. Various microorganisms such as bacteria, viruses, fungi, and parasites can play a role in causing meningitis [3]. Bacterial meningitis is very important due to its high morbidity and mortality even with newer antimicrobial agents' introduction and supportive care improvement [1]. Numerous bacteria are involved in causing meningitis. Identifying meningitis-causing bacteria plays an important role in selecting the appropriate antibiotic and reducing the complications of meningitis [2]. Numerous studies have been performed in Iran and other countries to identify meningitis-causing bacteria [3-7]. Depending on age, geographic location, immune system function, and vaccine implementation, the incidence rates and causative organisms of bacterial meningitis can be different [2, 6]. Kerman city is located in the south of Iran, and so far, no comprehensive study has been conducted on identifying meningitis-causing bacteria in this city. Therefore, this study was performed to identify the causative agents of bacterial meningitis in Kerman city. Various serotypes of *Streptococcus pneumonia*, *Haemophilus influenzae*, and *Neisseria meningitides* have been mentioned as the most important bacterial agents in the development of meningitis [2, 6]. So, in this study, *Streptococcus pneumonia*, *Haemophilus influenzae*, and *Neisseria meningitides* serotypes were examined to select the appropriate vaccine.

Materials and Methods

This cross-sectional was conducted in the department of pediatrics at the Kerman University of Medical Sciences, Between February 2017 and June 2019. The ethical code of this study was IR.KMU.AH.REC. 1398.060. Sixty suspected cases of acute bacterial meningitis with signs included: positive kerning sign, neck stiffness, upper and lower Brudzinski sign, and bulging fontanelle-hyperreflexia who were admitted to Afzalipour Hospital in Kerman city, were included in this study. A 3-5 ml of cerebrospinal fluid (CSF) specimens were immediately obtained and transported to the laboratory. In order to identify the type of bacteria in the CSF, conventional methods such as Gram staining, culture in differential media (Chocolate agar, Blood agar, McConkey agar, Tayer Martin) and the morphology of the colonies and standard biochemical tests including catalase, sugar utilization, oxidase, optochin test and bile solubility, Bacitracin test, triple sugar iron (TSI), indole-methyl red, Voges-Proskauer, citrate (IMVIC), sulfide-indole-motility (SIM), and urea was used. Culture media and other

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chemicals were prepared from Merck Co. (Darmstadt, Germany). Real-time polymerase chain reaction (PCR) was used to identify *Neisseria Meningitidis*, *Streptococcus pneumonia*, *Haemophilus influenzae*, and their serotypes, as described by Attarpour-Yazdi et al. [5]. The positive control was *Streptococcus pneumoniae* ATCC 49619. In this study, eleven genes including: *wzy*, *wzx*, *wzg*, *wcrH*, *wcwV*, *wciL*, *wciP*, *wcrG*, *wcwL*, *wciNbeta*, and *galU* were studied.

Data analysis

The data were analyzed by SPSS version 23 software, using descriptive statistics and calculation of ratios and percentages.

Results and Discussion

Sixty patients (28 females and 32 males) suspected of meningitis were studied in this study. Twenty-one (35%) patients were neonates and 39 (65%) patients were aged one month to 70 years (Mean+SD = 18.5 ± 20.8 years). In the present study, conventional and molecular methods proved the presence of bacteria in 33.3% of the samples (Table 1). In the study of Yazdi et al. [5] in Tehran, 62.6% of CSF samples were positive for bacteria. In a study by Tegene et al. [2] in Ethiopia, 3.76% of CSF samples were positive for bacteria. In the Borjian study [7], which was conducted in the city of Borujan, 7.25% of CSF samples were positive for bacteria. In a study by Jayaraman et al. [4] in India, 27.27% of CSF samples were positive for bacteria. In another study in Hamadan, 7.9% of CSF samples were positive for bacteria [6]. Differences in the results of various studies can be attributed to differences in the geographical area, identification methods, and technician's skill in identifying bacteria. In the present study, smear preparation from CSF and gram staining showed bacterial meningitis in 60% of cases. In Tegene et al. [2], and Modi et al. [8] studies, gram staining showed the presence of bacteria in 82.8% and 64.3% of cases, respectively. The difference in the results of the studies can be attributed to the amount of bacteria in the CSF sample. However, due to the time-consuming nature of other methods, smear preparation and gram staining can help in the immediate diagnosis of bacterial meningitis in many cases. In our study, bacterial meningitis was approximately the same in males and females (11 males and 9 females).

Table 1. Absolute and relative abundance of microorganisms isolated from cerebrospinal fluid of meningitis patients

Microorganism	Number	Percent
Streptococcus pneumoniae	13	65%
Staphylococcus epidermidis	2	10%
Klebsiella	2	10%
Enterococcus	1	5%
Escherichia coli	1	5%
Gram-positive cocci	1	5%
Total	20	100%

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This finding contradicts the results of a study by Attarpour-Yazdi et al. [5] in Tehran and Tegene et al. [2] in Ethiopia. The number of participants in the studies may be the cause of differences in the results. *Streptococcus pneumonia, Staphylococcus epidermidis, Klebsiella, Enterococcus, Escherichia coli*, and *gram-positive cocci* were the microbial agents identified in this study. These microorganisms were different from the results of studies conducted by Yazdi et al. [5] In Tehran, Modi et al. [8], in India, and Tegene et al. [2] in Ethiopia. The reason for the difference in the results of the studies can be related to the genetics and methods of detecting microbial agents. *Streptococcus pneumonia* was the most identified microorganism (65%). This finding was similar to the results of studies conducted by Yazdi et al. [5], Tegene et al. [2], and Modi et al. [8], who reported the prevalence of pneumococci as 36%, 31.6%, and 60%, respectively. Although *Streptococcus pneumonia* was predominant in all of these studies, differences in the frequency of the bacteria could be related to lifestyle, test method, and vaccination program. Today, more than 94 serotypes of pneumococci have been reported [2]. In the present study, 18CFBA was the predominant (23.1%) serotype of *Streptococcus pneumoniae* 6ABCD (7.7%), 19a (7.7%), 16F (7.7%), and 1 (7.7%) were the other identified serotypes of the bacteria. These serotypes were different from the serotypes identified in the studies of Yazdi et al. [5] and Tegene et al. [2]. The reason for this difference can be related to the number of samples. 18CFBA was found in age groups: one month to 1 year, 10.1 to 30 years, and 30.1 to 70 years. 6ABCD, 1, 19a, and 16f were found in age groups: 1.1 to 10 years, one month to 1 year, 30.1 to 70 years one month to 1 year, respectively. In the present study, 46.2% of serotypes of *Streptococcus pneumoniae* were not identified, which could be related to the low level of bacteria in the samples. Contrary to the results of studies by Yazdi et al. [5] and Tegene et al. [2], *Neisseria meningitides* and *Haemophilus influenzae* were not found in the present study any of the samples. The reason for this difference can be related to the characteristics of the subjects and previous treatments. Studies have shown that *Escherichia coli* and enterococci can cause meningitis in newborns [9, 10]. In our research, these bacteria were isolated only from newborns.

Conclusion

In the present study, the detection rate of bacteria was low. This may be due to the problematic growth nature of some microbial agents, the low number of bacteria in the samples, or the use of antibiotics before sampling. Because the clinical signs of meningitis are not always reliable, laboratory methods are essential for early diagnosis. Simple, quick and inexpensive tests such as gram staining and adequate knowledge of the bacteriological epidemiology of meningitis can be a good guide for early treatment and reduction of complications. *Streptococcus pneumoniae* was the most common bacteria. Although many serotypes of this bacteria have not been identified, they can significantly help its vaccination program. *Neisseria meningitides* and *Haemophilus*

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influenzae had no role in causing meningitis.

Conflict of interest

The authors declare that they have no competing interests.

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