Prevalence of Etiological Agents and Antimicrobial Resistance Patterns of Bacterial Meningitis in Nemazee Hospital, Shiraz, Iran

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Background: Meningitis is a serious inflammation of the meninges, mainly caused by bacteria. Treatment of this life threatening disease should not be delayed; therefore, rapid empirical antibiotic therapy should be immediately started. Antimicrobial therapy based on early Cerebrospinal Fluid (CSF) examination has an important role in limitation of bacterial meningitis.
Objectives: The current study aimed to identify the prevalence of etiological agents of bacterial meningitis and their susceptibility patterns against commonly used antibiotics.
Patients and Methods: This cross-sectional study was performed within a two-year period 2011-2013 on all patients with suspected meningitis referring to Nemazee Hospital, Shiraz, Iran. Cerebrospinal fluid specimens were collected aseptically, and conventional methods were employed to isolate and identify the bacteria. Antimicrobial susceptibility tests were performed using disk diffusion method according clinical and laboratory standards institute (CLSI) recommendation.
Results: Out of 2229 suspected meningitis cases, 255 (11.4%) were detected as positive culture. Most of the isolates were Gram-positive cocci, which 116 (45.5%) were *Staphylococcus epidermidis,* and 20 (7.8%) identified as *Streptococcus* spp. Among Gram-negative isolates, *Escherichia coli* and *Acinetobacter* spp., each with 19 isolates (5.9%), were predominant. Overall, the most effective antibiotics against Gram-positive cocci were vancomycin (91.5%), chloramphenicol (75.2%), and gentamicin (59.3%), respectively. Gram-negative isolates were mostly susceptible to gentamicin (65.1%), chloramphenicol (42.9%), and ciprofloxacin (39.7%), respectively.
Conclusions: Gram-positive cocci, such as (Coagulase-Negative Staphylococci) CoNS and aerobic Gram-negative bacilli, were identified as the common etiologic agents of bacterial meningitis. Since the majority of these isolates was part of the normal flora and considered as opportunistic nosocomial infections, it is required to consider the importance of infection control programs in the healthcare centers.

Keywords: Bacterial Meningitis; Cerebrospinal Fluid; Nosocomial Infections

1. Background

The management of bacterial meningitis is still a priority of public health because of its rapid onset and high level of morbidity and mortality (1). Bacterial meningitis approximately causes more than one hundred thousand deaths per year worldwide (2). Meningitis continues to remain among the leading causes of childhood death, particularly in the developing countries (3). Nowadays in spite of the great improvement in modern antibiotics and healthcare services, the life-threatening problem of bacterial meningitis is not eliminated (4). Prolonged persistence of infection in the Cerebrospinal Fluid (CSF) may result in quite severe neurological dam-ages as well as mortality, if rapid measures are not taken (4). Rapid identification and immediate treatment of meningitis causative agent is necessary because of its potential devastating effects (5). Gram staining is widely available and as an inexpensive and rapid procedure is routinely performed for primary diagnosis of bacterial meningitis (6). However, gram staining sensitivity is not totally reliable, so CSF culture is mandatory to diagnose bacterial meningitis (5, 6). Implementation of rational antimicrobial therapy based on the findings of early CSF examination has an important role in limitation of bacterial meningitis and also its future outbreaks (5). Bac-

terial meningitis often presents nonspecific signs and symptoms, especially in neonates; therefore, empirical antimicrobial therapy should be initiated based on low clinical suspicion until CSF culture results would be prepared (1). Empirical antibiotic treatment is advised in cases of suspected meningococcal disease, depends on local resistance patterns and the medical environment (4). But, because of the selective pressure, extensive empirical uses of broad-spectrum antibiotics often lead to the emergence of resistant isolates against many antimicrobial agents (7). New information of antimicrobial susceptibility testing for routinely used antibiotics could guide clinicians to better choices of antibiotics for prophylaxis purposes (2, 8). Antibiotic susceptibility patterns may also vary among hospitals; therefore, this information is particularly vital for the effectiveness of empirical therapy in acute cases (7, 9). *Neisseria meningitides*, *Streptococcus pneumonia,* and *Haemophilus influenzae* type b are most commonly associated with bacterial meningitis (5). However, the relative prevalence of the reported bacterial meningitis isolates vary with age and geographical regions (10). To the authors' best knowledge there is a lack of published reports on the analysis of bacterial meningitis in suspected patients and determination of antibacterial susceptibility patterns, especially in all the patients ranging neonates to adults the studied region. Such studies could indicate appropriate therapy of bacterial meningitis, reduce the length of hospitalization and decrease the relative morbidity and mortality rate. Besides, information on such a regional study might be extended to the rest of the world.

2. Objectives

The current study aimed to determine the prevalence of etiologic bacteria and their susceptibility patterns against commonly used antibiotics in Nemazee Teaching Hospital, Shiraz, Iran.

3. Patients and Methods

3.1. Study Area and Subjects

This descriptive cross-sectional study was conducted within two years from March 2011 to February 2013. All patients with suspected meningitis who referred to Nemazee Teaching Hospital, affiliated to Shiraz University of Medical Sciences, were included. Nemazee Hospital is a major tertiary care hospital in South-West of Iran with one thousand beds. Totally, 2229 CSF samples were collected during the study period. The patients were categorized into three groups: neonatal and children up to two years old, children aged 2-14 years, and another group including all the patients older than 14 years. There was no age limit in the inclusion criteria and just

suspecting to meningitis based on compatible clinical manifestations (fever, headache, and neck stiffness) by the physician was examined. Bacterial meningitis was confirmed by CSF positive cultures. Exclusion criteria were taking antibiotic 24 hours before admission to the hospital.

3.2. Specimens and Bacterial Identification

Cerebrospinal fluid specimens were collected aseptically by lumbar puncture at the discretion of the attending physicians and transferred into sterile tubes. Clinical specimens were sent to microbiology laboratory within 20 minutes. Conventional bacteriological methods were employed to isolate and identify the bacteria. Cerebrospinal fluid specimens were seeded on to cultures media, blood agar, chocolate agar and thioglycolate enrichment broth (Merck, Germany), for 48 hours incubation in humid air plus 5 - 10% CO_2.

3.3. Antimicrobial Susceptibility Testing

Susceptibility profiles were determined by following locally available antibiotics using disk diffusion method according to clinical and laboratory standards institute (CLSI) recommendation (11). Antimicrobial disks (Mast, UK) used for Gram-positive isolates included ampicillin (10 μg), vancomycin (30 μg), erythromycin (15 μg), cotrimoxazole (25 μg), chloramphenicol (30 μg), gentamicin (10 μg), lincomycin (2 μg), ciprofloxacin (5 μg), cephalexin (30 μg), cloxacillin (1 μg), ofloxacin (5 μg), and azithromycin (15 μg). In addition, amikacin (30 μg), ceftizoxime (30 μg), and cefixime (5 μg) disks (Mast, UK) were also considered for the Gram-negative isolates. The test was performed on Mueller-Hinton agar (Merck, Germany). Statistical analysis of the infectious agents and antimicrobial susceptibility frequency were performed using SPSS software version 19. The results were presented as descriptive statistics in terms of relative frequency.

4. Results

From a total of 2229 CSF specimens obtained from suspected meningitis cases, 255 (11.4%) samples were detected as bacterial culture positive. Out of the 255 positive cases, 137 (53.7%) belonged to males and the remaining 118 (46.3%) belonged to females. The mean age of the patients was 34 ± 26 years and the age range was from 1 month to 93 years. The majority of positive cases (179 = 70.2%) belonged to patients older than 14 years. The common bacterial isolates were Gram-positive cocci, which *Staphylococcus epidermidis* with 116 (45.5%) and *Streptococcus* spp. with 20 (7.8%) isolates were on the top, respectively. Among Gram-negative isolates, *Escherichia coli* and *Acinetobacter* spp., each with the frequency of 19 (5.9%) species, were predominant. *S. epider-*

midis (n = 18) and *Klebsiella* spp. (n = 2) were the most prevalent Gram-positive and Gram-negative isolates from neonates and children aged less than two years. For the children with an age range of 2-14 years, *S. epidermidis* (n = 20) and *Pseudomonas* spp. (n = 4) were the most prevalent ones. *S. epidermidis* (n = 78) and *E. coli* (n = 12) were mostly isolated from the adult patients. The age and isolate distribution patterns of the studied subjects are summarized in Table 1. Antibacterial susceptibility tests revealed that the highest resistance rates in Gram-positive isolates were observed against cloxacillin (81.3%), erythromycin (70.1%), and cephalexin (62.1%), respectively. On the other hand, the most effective antibiotics against Gram-positive cocci were vancomycin (91.5%), chloramphenicol (75.2%), and gentamicin (59.3%), respectively. *Enterococcus* spp., as the most resistant isolates among Gram-positive cocci, showed the highest sensitivity to vancomycin. Among the Gram-negative isolates, the highest resistance rates were observed against tetracycline (98.4%), cefixime (95.2%), and cephalexin (92.1%), respectively. The most susceptibility rates in Gram-negative isolates belonged to gentamicin (65.1%), chloramphenicol (42.9%), and ciprofloxacin (39.7%), respectively. Few isolates of *Acinetobacter* spp., as the most resistant Gram-negative iso- lates, were only sensitive to gentamicin, amikacin, and chloramphenicol. The results of antibiotic susceptibility patterns for Gram-positive and -negative isolates are presented in Tables 2 and 3, respectively.

5. Discussion

Since an exact etiological diagnosis of the pathogen is not immediately available in the infectious diseases, the primary choice of antibiotics is usually empirical (4). Meningitis without appropriate therapy is usually fatal (1). Previously, in a seven-year study by Mosavi-Jarrahi et al. meningitis fatality rate was reported 4.5% for Iran (12). Microbiological identification and susceptibility testing of the causative agents are the key to select effective antibiotic therapy (1). Identifying the most prevalent organisms causing bacterial meningitis and their susceptibility patterns are usually studied in children, but rarely in adults. The current study included patients aged one month to 93 years old and categorized them in three groups. Bacterial isolation rate from CSF samples was 11.4% in the present study; previous isolation rates in Iran were reported with various ranges. Yousefi Mashouf et al. reported a rate of 25.1% in children aged less than 10 years old from Hamadan (13).

Table 1. The Distribution of Recovered Bacterial Isolates Based on the Patients' Age [a]

Isolates	Patients' Age			
	≤ 2	2-14	≥ 14	Total, No. (%)
Staphylococcus epidermidis	18	20	78	116 (45.5)
Streptococcus **spp.**	3	3	14	20 (7.8)
CoNS	1	1	15	17 (6.7)
Staphylococcus aureus	1	1	13	15 (5.9)
Escherichia coli	1	2	12	15 (5.9)
Acinetobacter **spp.**	1	3	11	15 (5.9)
Klebsiella **spp.**	2	1	11	14 (5.5)
Enterococcus **spp.**	2	2	6	10 (3.9)
Pseudomonas **spp.**	1	4	5	10 (3.9)
Enterobacter **spp.**	0	3	6	9 (3.5)
Diphtheroids	1	4	4	9 (3.5)
Bacillus **spp.**	1	0	3	4 (1.6)
Proteus **spp.**	0	0	1	1 (0.4)
Total, No. (%)	32 (12.5)	44 (17.3)	179 (70.2)	255 (100)

[a] Abbreviation: CoNS, Coagulase-negative Staphylococcus (CoNS).

Table 2. Antibiotic Susceptibility Pattern of Gram-Positive Species Isolated From Cerebrospinal Fluid Cultures [a,b]

Antibiotics	*Staphylococcus aureus* (**n = 15**)	*Enterococcus* **spp.** (**n = 10**)	*Streptococcus* **spp.** (**n = 20**)	**CoNS** (**n = 17**)	*Staphylococcus epidermidis* (**n = 116**)
Cloxacillin					
R	11 (73.3)	10 (100)	15 (75)	13 (76.5)	91 (78.5)
I	0	0	0	0	4 (3.4)
S	4 (26.7)	0	5 (25)	4 (23.5)	21 (18.1)
Lincomycin					
R	8 (53.3)	10 (100)	11 (55)	11 (64.7)	60 (51.7)
I	0	0	1 (5)	0	3 (2.6)
S	7 (46.7)	0	8 (40)	6 (35.3)	53 (45.7)
Chloramphenicol					
R	0	2 (20)	6 (30)	3 (17.6)	15 (12.9)
I	2 (13.3)	6 (60)	5 (25)	0	5 (4.3)
S	13 (86.7)	2 (20)	9 (45)	14 (82.3)	96 (82.8)
Vancomycin					
R	0	6 (60)	8 (40)	0	0
I	0	1 (10)	0	0	0
S	15 (100)	3 (30)	12 (60)	17 (100)	116 (100)
Co-trimoxazole					
R	1 (6.7)	10 (100)	18 (90)	12 (70.6)	51 (44)
I	2 (13.3)	0	0	0	5 (4.3)
S	12 (80)	0	2 (10)	5 (29.4)	60 (51.7)
Ciprofloxacin					
R	9 (60)	8 (80)	10 (50)	12 (70.6)	43 (37)
I	1 (6.7)	2 (20)	2 (10)	0	2 (1.8)
S	5 (33.3)	0	8 (40)	5 (29.4)	71 (61.2)
Erythromycin					
R	10 (66.7)	9 (90)	14 (70)	13 (76.5)	77 (66.4)
I	0	1 (10)	0	0	1 (0.9)
S	5 (33.3)	0	6 (30)	4 (23.5)	38 (32.7)
Gentamicin					
R	6 (40)	9 (90)	12 (60)	5 (29.4)	30 (25.9)
I	1 (6.7)	1 (10)	2 (10)	2 (11.8)	4 (3.4)
S	8 (53.3)	0	6 (30)	10 (58.8)	82 (70.7)
Cephalexin					
R	8 (53.3)	10 (100)	15 (75)	13 (76.5)	57 (49.1)
I	1 (6.7)	0	0	0	6 (5.2)
S	6 (40)	0	5 (25)	4 (23.5)	53 (45.7)
Ofloxacin					
R	-	8 (80)	12 (60)	-	-
I	-	2 (20)	2 (10)	-	-
S	-	0	6 (30)	-	-
Azithromycin					
R	-	10 (100)	15 (75)	-	-
I	-	0	0	-	-
S	-	0	5 (25)	-	-
Ampicillin					
R	-	10 (100)	10 (100)	-	-
I	-	0	0	-	-
S	-	0	0	-	-

[a] Abbreviations: CoNS, Coagulase-negative Staphylococcus; R, Resistant; I, Intermediate; S, Sensitive.
[b] Data are presented as No. (%).

Table 3. Antibiotic Susceptibility Patterns of Gram-Negative Species Isolated From Cerebrospinal Fluid Cultures [a,b]

Antibiotics	*Acinetobacter* **spp.** (n = 15)	*E. coli* (n = 15)	*Enterobacter* **spp.** (n = 9)	*Klebsiella* **spp.** (n = 14)	*Pseudomonas* **spp.** (n = 10)
Ceftizoxime					
R	15 (100)	13 (86.7)	7 (77.8)	12 (85.7)	10 (100)
I	0	0	0	0	0
S	0	2 (13.3)	2 (22.2)	2 (14.3)	0
Tetracycline					
R	15 (100)	12 (80)	8 (88.9)	14 (100)	10 (100)
I	0	2 (13.3)	1 (11.1)	0	0
S	0	1 (6.7)	0	0	0
Amikacin					
R	14 (93.3)	2 (13.3)	2 (22.2)	5 (35.7)	5 (50)
I	0	7 (46.7)	1 (11.1)	1 (7.1)	2 (20)
S	1 (6.7)	6 (40)	6 (66.7)	8 (57.2)	3 (30)
Chloramphenicol					
R	14 (93.3)	0	3 (33.3)	7 (50)	10 (100)
I	0	0	1 (11.1)	1 (7.1)	0
S	1 (6.7)	15 (100)	5 (55.6)	6 (42.9)	0
Ciprofloxacin					
R	12 (80)	6 (40)	5 (55.6)	10 (71.5)	4 (40)
I	0	0	0	1 (7.1)	0
S	3 (20)	9 (60)	4 (44.4)	3 (21.4)	6 (60)
Gentamicin					
R	1 (6.7)	4 (26.7)	2 (22.2)	10 (71.5)	2 (20)
I	0	0	0	1 (7.1)	2 (20)
S	14 (93.3)	11 (73.3)	7 (77.8)	3 (21.4)	6 (60)
Cefixime					
R	15 (100)	15 (100)	7 (77.8)	12 (85.7)	10 (100)
I	0	0	1 (11.1)	0	0
S	0	0	1 (11.1)	2 (14.3)	0
Cephalexin					
R	15 (100)	13 (86.7)	8 (88.9)	12 (85.7)	10 (100)
I	0	0	0	0	0
S	0	2 (13.3)	1 (11.1)	2 (14.3)	0
Co-trimoxazole					
R	15 (100)	4 (26.7)	5 (55.6)	9 (64.3)	10 (100)
I	0	0	1 (11.1)	0	0
S	0	11 (73.3)	3 (33.3)	5 (35.7)	0

[a] Abbreviations: R, Resistant; I, Intermediate; S, Sensitive.
[b] Data are presented as No. (%).

Also in a ten-year retrospective study by Rezaeizadeh et al. (14) the rate was reported 2.9% in Tehran; and Ghotaslou et al. (15) reported culture positive rate of 3.9% in Children Hospital of Tabriz. Thomas et al. determined this rate as 10.6% in children suspected to meningitis in Royal Hospital of Oman (16). In similar studies from two African countries, Namibia and Ethiopia, reported frequencies are 9.6% and 5.6%, respectively (17, 18). This may be a reflection of the different infection control policies and other factors involved in the mentioned regions or countries (19). In the current study, the most common bacteria causing meningitis was *S. epidermidis*

(45.5%). CoNS, especially *S. epidermidis*, are considered as the main cause of nosocomial infections in the pediatric age groups; while they are commonly considered as the contamination in adult samples. However, ventriculoperitoneal shunt infections by these bacteria are a common complication in patients with these devices even in adults (20). It is in agreement with the results of a previous study from Iran by Shayanfar et al. (21). Also the results from repeated cultures and the similar isolation rates in other studies would reject the possibility of contamination of specimens. However, final approval for bacterial meningitis caused by CoNS was accompanied with the results of CSF examination (glucose and protein level) to differentiate from asymptomatic meningitis (22). Although *N. meningitides*, *S. pneumonia* and *H. influenza* type b are the most common causes of bacterial meningitis, based on the previous reports from the Iranian studies, patterns seem to be different from other parts of the world. While Yousefi Mashouf et al. form Hamadan, Iran, introduced *S. pneumonia* as the most common isolate (13), Rezaeizadeh et al. (14) in Tehran and Japoni et al. (7) in Shiraz introduced coagulase-negative Staphylococcus (CoNS) species as the most prevalent causative agents of bacterial meningitis with a frequency of 40.1% and 36.3%, respectively; as the closest rates to the current study findings. Also, Bahador et al. (23) in Kerman and Ghotaslou et al. (15) in Tabriz reported *N. meningitides*, *S. pneumonia* and *H. influenza* as the most common bacteria isolated from the Iranian patients with meningitis. Gram-positive cocci as the causative agent of bacterial meningitis was almost confirmed in all the previous studies from Iran, which was in agreement with the findings of the current study; but Aletayeb et al. (24) reported *Klebsiella pneumoniae* and *Enterobacter* spp. as the two main causative agents of neonatal bacterial meningitis form Emam Khomeini Hospital in Ahvaz, Iran. Although *Klebsiella* spp. was the most frequent Gram-negative isolate among children younger than two years, in the current study it was not the most prevalent causative agent of meningitis in these patients. Abdinia et al. (25) and Yousefi Mashouf et al. (13) reported *S. pneumonia* as the most common causative agent of bacterial meningitis among the Iranian pediatric patients in Tabriz and Hamadan. It is in not in agreement with the findings of the current study in which *S. epidermidis* was the predominant isolate. Similar finding reported from Oman and Yemen specified *S. pneumonia* as the most common pathogen among neonates (16, 26). In Ethiopia, *N. meningitides* was reported as the most common isolate among neonates and young children, and *S. pneumonia,* as the leading causes of bacterial meningitis in the adults groups, in a one -year hospital survey (17). All these reports highlight the variable nature of etiological agents of bacterial meningitis. According to the current study, with increasing age in the studied groups, bacterial isolation rates also increased, which was similar to the findings of Domingo et al. from Spain indicating that the

elderly patients are at a much higher risk of developing bacterial meningitis than younger adults (27). Overall in the present study, gentamicin, as an aminoglycoside, was one of the best choices for Gram-positive and -negative isolates with 59.3% and 65.1% sensitivity, respectively, which was in agreement with the report of Shayanfar et al. in Tehran (21). However in a previous study by Rezaeizadeh et al. (14), Gram-negative isolates showed more sensitivity to amikacin in comparison with gentamicin. Although they reported Gram-positive susceptibility patterns almost same as those of the current study, and their *Enterococcus* spp. isolates were more sensitive to the tested antibacterial agents than the isolates of the current study. Although the results of the study by Japoni et al. (7) were not specific for CSF samples, a previous study in Shiraz provided good information on the susceptibility patterns of species isolated from CSF cultures. Overall, it seems that the Gram-positive isolates of the current study were more sensitive to co-trimoxazole and ciprofloxacin than the isolates in that of the Japoni et al. (7). Vancomycin resistance in Gram-positive cocci of the current study was more than that of Japoni et al.'s with less than 10%. They evaluated the susceptibility of the isolates by determining the minimum inhibitory concentration (MIC) by E-test (7) as a more precise test than disk diffusion method, which was a limitation of the current study. Finally, based on the current study results, nosocomial meningitis was prevalent in the studied hospital. Opportunistic organisms, such as CoNS and aerobic Gram-negative bacilli, were considered as the common causes of nosocomial meningitis in the hospitalized patients. The prevention and control of nosocomial bacterial meningitis need serious attention, since the majority of diseases are caused by multidrug-resistant pathogens (28). The results of the current study provide useful information to prescribe more effective empirical therapy and optimize institutional infection control policies. Therefore, the variable nature of etiological agents and antimicrobial susceptibility patterns of bacterial meningitis made it reasonable to provide continuous future updates on local resistance patterns of the most frequent bacterial pathogens associated with meningitis.

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