International Journal of Molecular and Clinical Microbiology 6(1) (2016) 608-612

International Journal of
Molecular and Clinical Microbiology

Frequency of neonataland children bacterial meningitis and determining their antibiotic sensitivity patterns in Hospitals of Iran

Neda Moafi[1], Khosro Issazadeh[*2]

1,2Department of Microbiology, College of Basic Sciences, Lahijan Branch, Islamic Azad University, Lahijan, Iran

ARTICLE INFO

Article history:
Received 31 October 2015
Accepted 20 March 2016
Available online 1 June 2016

Keywords:
Meningitis,
Cerebrospinal Fluid,
Antibiotic Sensitivity,
Neonates,
Hospital

ABSTRACT

Meningitis is one of the most important causes of infant mortality and its early and proper diagnosis is of great importance, but there are currently no laboratory facilities optimized and fast in detection of meningitis pathogens. The current standard of bacterial meningitis diagnosis is microscopic examination and cerebrospinal fluid (CSF) culture. This research aimed at isolating the most common bacteria infecting cerebrospinal fluid and determining their antibiotic sensitivity patterns for cerebrospinal fluid of newborns with meningitis admitted in Hospitals of Iran. The study was carried out on 680 cerebrospinal samples from newborns and children during 2013-2014. From among the 680 newborns and children of interest who were LP according to the doctor's order, 35 had positive cultures. In the present research, the resistance pattern and antibiotic sensitivity of the bacteria isolated were also examined. *H.influenzae* was the most prevalent isolate from newborns and *E.coli* took second place. The most isolated sample was *Haemophilus* and a large number of this bacterium was sensitive to kanamycin, gentamicin and chloramphenicol. These antibiotics can be used for early empiric treatment of meningitis. Many of these bacteria were resistant to sulfamethoxazole, ampicillin, and amoxicillin and their prescription is not recommended.

1. Introduction

Central nervous system infections are so dangerous and lethal (Amirmoghadami et al., 2005). Meningitis is a very serious infection of the meninges that surround the brain and the spinal cord. It is usually caused by viral, bacterial or fungal pathogens. Bacterial meningitis is defined by an infection of the arachnoids mater as the middle part of meninges and cerebrospinal fluid (CSF) in both the subarachnoid space and the cerebral ventricles (Zelalem et al., 2013). Most cases of bacterial meningitis occur in childhood and its pathogens are varied in different age groups. *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b are among the prevalent bacterial pathogens of this disease. In recent years, two main changes have been observed in the epidemiology of acute bacterial meningitis. The first is a decrease in the incidence of *H. influenzae* type b and *S. pneumoniae* meningitis in countries where vaccination plan is generally performed against the two bacteria. The second is an increase in resistant strains of pneumococcus across the world. Therefore, early diagnosis and appropriate antibiotic therapy is necessary to avoid further complications. However, the occurrence and etiologies of bacterial meningitis

*Corresponding author: Dr. Khosro Issazadeh
Tel: +:+989112422730,Fax:+9822422605
E-mail address: Issa_kaam@yahoo.com

vary in different geographic regions. Moreover, effectiveness of treatment is limited due to antibiotic resistant bacterial strains. The epidemiology of bacterial meningitis has not been well characterized in our country so far. Furthermore, to make decision regarding children's vaccination against causative bacteria in our country, it is required to know the outbreak of the bacterial meningitis in the area. On the other hand, to make proper decision concerning the treatment of bacterial meningitis, we need to recognize antibiotic resistance patterns of prevalent bacteria in the area. (Abdinia et al., 2014).

Bacterial meningitis has for a long time been treated with a combination of penicillin/ampicillin and chloramphenicol, and this combination is still the widely recommended first choice in most of Africa. However the increasing frequency of reports of bacterial resistance in-vitro to these drugs has raised concern that this choice may no longer be appropriate despite advances in vaccine development and chemoprophylaxis, bacterial meningitis remains a common disease worldwide. The disease is more common in developing countries. (Birehanemeskel et al., 2015).The management of bacterial meningitis is still a priority of public health because of its rapid onset and high level of morbidity and mortality. Bacterial meningitis approximately causes more than one hundred thousand deaths per year worldwide. Meningitis continues to remain among the leading causes of childhood death, particularly in the developing countries. Nowadays in spite of the great improvement in modern antibiotics and healthcare services, the life-threatening problem of bacterial meningitis is not eliminated (Motamedifar et al. 2015). So the aim of this study was to retrospectively assess the bacterial etiologies of meningitis and their antimicrobial susceptibility pattern in Qods Hospital, Qazvin city, Iran.

2. Materials and Methods

The study was carried out during a 20-month period from March 2013 to October 2014. In this regard, 680 samples of cerebrospinal fluid of infants and children in Qods hospital, Qazvin, Iran, that suspected of meningitis were collected. In this study, 500 µl samples containing anticoagulant heparin was distributed in three sterile test tubes in order to do macroscopic, microscopic, biochemical, serological and microbial experiments. Cultivation and isolation of organisms were performed according to the methods outlined in the Bailey & Scott's Diagnostic Microbiology (Forbes 2002). The sediment of spinal fluid was prepared for providing the smear and cultures. Specimens were then cultured on appropriated culture media including Thayer-Martine agar, EosinMethylen Blue (EMB) agar and Blood agar (Merck, Germany). The cultures were incubated in 37^{o}C for 24-48 hours and then the colonies were removed for further studying. Biochemical and antigenic properties of isolates were verified for identification purposes. In essential cases, specific antiserum against bacteria was used for precise identification of bacteria type (Koneman et al., 1997). For diagnosis of *Haemophilus influenzae*, Centrifuged sediment of the cerebrospinal fluid were cultured on a supplemented chocolate agar (blood agar base with 2% hemoglobin and 1% isovitalexcontaining X and V factors) plate. The suspected bacterial colonies were identified by typical Gram stain morphology, catalase, oxidase, Carbohydrate fermentation reactions, ONPG test. The *H.influenzae* isolates were serotyped by slide agglutination using Hib-specific antiserum (Denka-Seiken Co. Ltd, Tokyo, Japan). Susceptibility profiles were determined by following locally available antibiotics using disk diffusion method according to clinical and laboratory standards institute (CLSI) recommendation (Clinical and Laboratory Standards Institute 2011). Antimicrobial disks used for isolates included Amoxicillin, Ampicillin, Chloramphenicol, Sulfamethoxazole, Cephalexin, Ceftizoxime, Gentamicin and kanamycin.

The test was performed on Mueller-Hinton agar (Merck, Germany). Antimicrobial susceptibility of *H.influenzae* was determined by the disc-diffusion method using Mueller-Hinton agar II supplemented with 1%hemoglobin, X and V factors, and commercial antibiotic discs. 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.

3. Results

Out of 680 children less than 10 years suspected to meningitis, only 35 children (5.1%) had positive bacterial culture that 42.9% of isolates were Gram-positive cocci and 57.1% were also Gram-negative bacilli. The most common isolates of Gram-positive cocci were as follow: *Streptococcus pneumoniae* (17.10%), *Enterococcus faecalis* (14.30%), *Streptococcus agalactiae* (11.40%) and *Enterococcus faecalis* (14.30%). The most common isolates of Gram-negative bacilli were: *Haemophilus influenzae* (22.90%), *E.coli* (20.0%), *Kelebsiella pneumoniae* (8.60%) *and* unknown isolates (5.70%). Frequency of Neonatal and children bacterial meningitis are shown in table 1. The most common clinical manifestations of meningitis in children under 10 years of age with bacterial meningitis were Fever (100.0%), Leukocytosis (84.5%), and nausea and vomiting (77.2%), sleepiness (76.4%) and headache (66.5%). Of 35 patients with bacterial meningitis, 23 cases (65.7%) were males and 12 cases (34.3%) were females. The frequencies of age groups of patients with bacterial meningitis are shown intable 2, the most frequent patients were belonged to the 0-2 years old (28.7%) and the least frequent patients were belonged to the 8-10 years old (11.4%). Our results showed that the most frequent bacterial meningitis (38.7%) was occurred during fall and then winter with frequency of 29.6%, and summer 12.8%. Antibiotic resistance patterns of isolated bacteria from patients with bacterial meningitis are shown in table 3.

In the present research, sensitivity and resistance patterns of the isolated bacteria to different antibiotics were also studied (Table 3). Maximum resistance of *H.influenzae* was to the antibiotics sulfamethoxazole, ampicillin, andamoxicillin, that is, 50% to each. Moreover, resistance of this bacterium to the antibiotics ceftizoxime and cephalexin was 25% for each and its minimum resistance was to the antibiotics kanamycin, gentamicin and chloramphenicol (12.5% each). Additionally, the highest resistance of *E.coli* was to ceftizoxime, i.e. 52.14%, and its lowest resistance was to gentamicin with the amount of 14.28%. *S.agalatia* was also studied in terms of antibiotic resistance so that its highest and lowest resistance was to chloramphenicol and kanamycin, that is, 75% and 0%, respectively. Also, maximum and minimum resistance of *S.pneumoniae* was to amoxicillin (50%) and ceftizoxime (0%), respectively. *E.faecalis* was also investigated in terms of antibiotic sensitivity and resistance patterns so that its highest resistance was to the antibiotics gentamicin and ceftizoxime (80% each) and lowest resistance was to the antibiotics chloramphenicol and amoxicillin (40% each). *K.pneumoniae* was another isolated bacterium studied for antibiotic sensitivity and resistance patterns and findings showed that its maximum resistance was to the antibiotic ceftizoxime, i.e. 100%, and minimum resistance was to kanamycin, i.e. 0%. Asmentioned, white blood cells, red blood cells, and resulting spinal fluid glucose and protein were measured in this study. Results showed that average WBC in people with bacterial infections was 304.5 mm^3 and average RBC was 126.42 mm^3, and glucose and protein averages were 29.57 mg/dl and 180.18 mg/dl, respectively.

Table 1. Frequency of Neonatal and children bacterial meningitis in Hospitals of Iran during 2013-2014

Microorganism	Number	Percentage
H.influenzae	8	22.90
E.coli	7	20.0
S.agalactia	4	11.40
S.pneumoniae	6	17.10
E. faecalis	5	14.30
K. pneumoniae	3	8.60
Unknown (Gram -negative bacilli)	2	5.70
Total	35	100.0

Table 2. The frequencies of the age groups of patients with bacterial meningitis

Age groups (Years)	Frequency	Percentage
0-2	10	28.70
2-4	8	22.80
4-6	7	20.0
6-8	6	17.10
8-10	4	11.40
Total	35	100.00

Table 3. Antibiotic sensitivity patterns of bacteria isolated from Hospitals of Iran during 2013 and 2014

Antibiotic / Isolate	AM	CH	CF	CT	GM	KA	SXT	AMX
H.influenzae	4 (50)	1 (12.5)	2 (25)	2 (25)	1 (12.5)	1 (12.5)	4 (50)	4 (50)
E.coli	2 (55.40)	3 (42.85)	4 (57.14)	5 (52.14)	1 (14.28)	2 (28.58)	4 (57.14)	2 (28.58)
S.agalactia	2 (50)	3 (75)	1 (25)	2 (50)	2 (50)	0 (0)	2 (50)	1 (25)
S.pneumoniae	2 (36.33)	2 (33.33)	2 (33.33)	0 (0)	5 (83.33)	1 (16.62)	2 (36.67)	3 (50)
E.faecalis	3 (60)	2 (40)	3 (60)	4 (80)	4 (80)	3 (60)	3 (60)	2 (40)
K.pneumoniae	1 (33.33)	1 (33.33)	2 (66.66)	3 (100)	2 (66.66)	0 (0)	2 (66.67)	2 (66.67)
Unknown	1 (50)	2 (100)	2 (100)	1(50)	1 (50)	2 (100)	2 (100)	0 (0)

AMX=Amoxicillin, AM=Ampicillin, CH=Chloramphenicol, SXT= Sulfamethoxazole, CF=Cephalexin, CT= Ceftizoxime, GM= Gentamicin, KA= kanamycin

4. Discussion

Bacterial meningitis is still a major problem in children and infants. In recent decades, significant advances have been made in quick diagnosis of the disease, such as serological methods and antigens searching. Etiologic factors of bacterial meningitis are relatively diverse and most researchers introduced *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* as main causes of bacterial meningitis, especially in children 27. Given the studies done, these factors can vary due to time, geographical location, and patient's age 31. According to studies on children infected with meningitis by Sirus Bakht and Reza Khaniha (Sirusbakht and Khaniha, 2007) during 2001-2006 in Shohadaye Tajrish and Imam Hossein hospitals in the city of Tehran, 20 patients had pneumococcal meningitis with positive cerebrospinal fluid culture. The average age of children with pneumococcal meningitis was 30months. In this study, resistance to penicillin was 35% in pneumococcal infections 25% of which is the relative resistance and 10% had complete resistance. We found the resistance to chloramphenicol in 10% of pneumococcal infections as well as resistance to penicillin and chloramphenicol in 5% of the patients. Pneumococcal resistance to second-generation cephalosporins was observedin15% of cases and it was of relative type. Resistance to vancomycin was not seen in any cases. Resistance to rifampin was seen in 5% of pneumococcal infection. However, the highest resistance of *H. influenzae* was to sulfamethoxazole, ampicillin, andamoxicillin (50%) and its resistance to the antibiotics kanamycin, gentamicin and chloramphenicol was 12.5% each. Also, maximum and minimum resistance of *S.pneumoniae* was to amoxicillin (50%) and ceftizoxime (0%), respectively. *E.faecalis* had its highest resistance to the antibiotics gentamicin and ceftizoxime (80% each) and its lowest resistance was to the antibiotics chloramphenicol and amoxicillin (40% each). Furthermore, the highest resistance of *E.coli* was to ceftizoxime, i.e. 52.14%, and its lowest resistance was to gentamicin with the amount of 14.28%. In the study of Rasul Yosemite al.(Yusefi Mashuf et al., 1998) during two years from May 1998 to April 2000 on children below 10 years old being hospitalized with the diagnosis of meningitis in Ghaem children hospital and Sina infectious hospital in Hamedan city, 582 children suspected of meningitis were studied in terms of cerebrospinal fluid culture and antibiogram test. Results showed that from the total of 582 children below the age of 10 and suspected of meningitis, only 46 children (7.9%) has positive bacterial culture from which 26 Gram-positive bacteria (58.9%) and 20 Gram-negative bacteria (41.1%) were isolated. The most prevalent isolated Gram-positive bacteria included: 11 *Streptococcus pneumoniae* (23.9%), 6 *Staphylococcus aureus* (13.1%), and alpha hemolytic *Streptococcus* and *Staphylococcus epidermidis*each4 cases (8.7%). Also, the most prevalent isolated Gram-positive bacteria included: 5 *Escherichia coli* (10.9%), 3 *Neisseria meningitidis* and 3 *Pseudomonas aeruginosa* (6.5%), and 2 *Haemophilus influenza* (4.4%). Additionally, 2 Gram-negative bacteria (4.4%) were isolated but not known. Age distribution of the children below the age of 10 infected with bacterial meningitis (28.3%) was in children in the age group of 2 and less. Also, the lowest percentage of bacterial infection (31.1%) was in 8- to 10-year-old children. They

had a very good sensitivity to antibiotics from cephalosporin family such as cephalexin and ceftizoxime but showed relatively high resistance to antibiotics from β-lactam family such as amoxicillin and ampicillin. These bacteria also had moderate drug resistance to chloramphenicol and sulfamethoxazole. At the conclusion of our study, penicillin is not a proper drug for treatment of meningitis. In studies by Vero et al.in the U.S., the most prevalent bacteria isolated from newborns were in turn *Haemophilus influenza* type B (45%), *Streptococcus pneumoniae* (18%), and *Neisseria meningitidis* (14%) whereas in a similar research by Ronald Gold et al. (1992), (Amirmoghadami, Pedram 2005) pathogens in 70% of 1- to 5-year-old children were announced *Escherichia coli*, alpha hemolytic *Streptococcus*, *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. In the present study, however, the highest frequency percentage belonged to *Haemophilus influenzae*. Also, in another similar study done by (Borjian, 2008) in Valiasr hospital in Borujen, the most frequent causes of meningitis in children were in order *Haemophilus influenzae* type (63.8%), *Streptococcus pneumoniae* (26.3%), *Neisseria meningitidis* and *Salmonella para typhi A* (10.5% each). As can be seen, in most studies done around the world as well as Iran, the strain *Haemophilus influenzae* has been identified as the most prevalent bacterial meningitis cause in newborns and children. Considering the results obtained in this study, we can conclude that Gram-negative bacteria, especially *Haemophilus influenzae* and *Escherichia coli*, which have relatively high resistance to broad-spectrum antibiotics like sulfamethoxazole, ampicillin, and ceftizoxime, are among the major factors causing bacterial meningitis in children of the study area.

Acknowledgement

We are grateful to Islamic Azad University, Lahijan Branch Authorities, for their useful collaboration.

Refereces

Abdinia, B., Ahangarzadeh Rezaee, M., Abdoli Oskouie, S. 2014. Etiology and Antimicrobial Resistance Patterns of Acute Bacterial Meningitis in Children: A 10-Year Referral Hospital-Based Study in Northwest Iran. Iran Red Crescent Med J. 16(7): 1-4.

Achamyeleh, A., Aseffa, A., Gedlu, E. 1995. Bacterial isolates from CSF over a five year period in Gondar with a special reference to pediatric admissions and outcome. Proceeding of an Annual Research conference, Gondar College of Medical Sciences: 51–58.

Amirmoghadami, H., Pedram, N. 2005. Laboratory methods for meningitis diagnosis. Ministry of Health and Medical Education, Tehran, Seda Publishing Centre. 37-25.

Birehanemeskel, T., Solomon, G., Nigus, F. 2015. Bacterial Meningitis: a five-year retrospective study among patients who had attended at University of Gondar Teaching Hospital, Northwest Ethiopia. Biomedical Research and Therapy 2015, 2(5): 270-278.

Borjian, S. 2008. The most common causes of bacterial meningitis in children in the city of Borujen.Journalof Medical Sciences, Shahrekord, 1(2):52-58.

Clinical and Laboratory Standards Institute. 2011. Performance Standards for Antimicrobial Susceptibility Testing; 21th Informational Supplement M100-S21.Wayne, PA: CLSI;

Eslami, NP., Kazemi, A. 2007. The methods used for routine tests on body fluids, TehranPress, pp. 6-23.

Forbes, A., Sahm, DF, Weissfeld AS. Bailey & Scott's Diagnostic microbiology 11th ed. Philadelphia: Mosby. 2002: 133-148 and 907-912.

Koneman, E.W., Allen D.A., Janda W.M., Schreckenberger, P.C., Winn-Jr, WC., 1997. Color Atlas and text book of diagnostic microbiology 15th ed. Philadelphia: Lippincott William and Wilkins: 148-51.

Motamedifar, M., Sedigh Ebrahim-Saraie, H., Mansury, D., Nikokar, I., Hashemizadeh, Z. 2015. Prevalence of Etiological Agents and Antimicrobial Resistance Patterns of Bacterial Meningitis in Nemazee Hospital, Shiraz, Iran. Arch Clin Infect Dis., 10(2):1-7.

Sirusbakht, S., Khaniha, R. 2007. Antibiogram study Children with pneumococcal meningitis in hospital Shohada and emam hossein during 2001-2006. Annals of military and health sciences research. 2(5):1216-1213.

Yusefi mashuf, R., et al. 1998-2000. A study of bacterial factors of meningitis in children and determining antibiotic results, Hamedan. Journal of the University of Medical Sciences. 17 31-37.

Zelalemm, A. et al. 2013. Frequency and susceptibility pattern bacterial isolated from CSF in university of Gondar hospital, north west ETHIOPIA. International Journal of Pharmacy and Industrial Research.41-46.