

Incidence of *Haemophilus influenzae* type b among children less than 5 years and resistance to antibiotics in Iraq.

Maha F. Almelan* VMBCbB, MSc

Summary:

Back ground: Meningitis is an inflammation of the protective membranes covering the brain and spinal cord that known as meninges. It is the major cause of morbidity and mortality among infants and children less than the age of 5 years.

Objectives: To determine *Haemophilus influenzae* type b meningitis incidence in children aged less than 5 years and choice a drug for treatment

Patients and methods: During period from Febraury 2012 to first March 2013 ,250 Cerebrospinal fluid (CSF) specimens were collected from children less than 5 years ,which clinically diagnosed with meningitis ,in several hospitals of Iraq.Microbiological, biochemical, latex agglutination test and antibiotics sensitivity were used to identify and choice the antibiotic that should be used for treatment

Results: Culturing CSF specimens identified 18 confirmed cases of *Haemophilus influenzae* type b meningitis among 250 CSF specimens depending on biochemical and serotyping. The incidence of *Haemophilus influenzae* type b meningitis among infants less one years of age was 12(66.6%) compared to 6(33.3%) for older children . Incidence among males was 11(61.1%) compared to 7(38.8%) for females. *Haemophilus influenzae* type b meningitis is sensitive to Ampencillin , Ciprofloxacin, Cefotaxime, Cefotriaxone, Chloramphenicol, while resistant to Trimetheprim-sulfamethoxazole and Gentomycin.

Conclusions: *Haemophilus influenzae* type b meningitis was most common in children less than 1 years.

Key words: Meningitis, children, *Haemophilus influenzae* type b, Cerebrospinal Fluid, Antibiotics.

Fac Med Baghdad
2015; Vol.57, No.1
Received: Dec.,2014
Accepted: Jan.,2015

Introduction:

Meningitis is a serious public health problem demanding early diagnosis, effective treatment prevention and control. It is a major cause of morbidity and mortality among infants and children below the age of five years (1,2).

Meningitis might be caused by infection with viruses, bacteria, fungi, parasite and less common by certain drugs. Bacterial meningitis (BM) with 6-8 % was sometimes fatal and frequently associated with sever neurological sequel (3).BM is one of the most potentially serious infections in infants and older children because it is associated with a high rate of acute complications, risk of chronic morbidity and death. Beyond the neonatal period the main agents of BM are *Haemophilus influenzae* type b ,meningococcus and pneumococcus being the reported mortality 7.7%, 3.5% and 30% respectively (4).

Haemophilus influenzae type b is considered to be the most common cause of bacterial meningitis and one the most common cause of invasive infections among young children worldwide (5 ,6).H.influenzae exists in two forms capsulated (a-f) and non capsulated (7). Type b capsular strains are associated with invasive disease which is estimated by world health organization (WHO) in 2005 to cause 386000 deaths world per year in children aged less than 5 years old (8). These

cases primarily in developing countries where the disease was not readily recognized antibiotic treatment was scare and vaccine was not available (9) .Eradication of the infecting organism from CSF is entirely dependent on antibiotics, and bacteriocidal antibiotics should be administered intravenously at the highest clinically validated doses to patients with suspected bacterial meningitis(10).The aim of this study is to determine *Haemophilus influenzae* type b meningitis incidence in child aged less than 5 years and choice a drug for treatment.

Materials and Methods:

A definitive diagnosis of meningitis is dependent on examination and culture of CSF, whenever the physician suspects meningitis a lumber puncture should be under taken. CSF Specimens collection: Two hundred fifty CSF specimens were collected from infants and children aged from one month to five years during period from February 2012 to first March 2013 in different hospitals in Iraq (Central Teaching Hospital of pediatric, Children Hospital of Alilwia and Children welfare Hospital).

1 ml of CSF was collected in one sterile tube that was used for microbiological examinations [Gram stain and culture on selective media (GCmedia)].

The specimens were delivered to the Central Public Health Laboratory in T-I M (Trance –Isolate Medium) for

*Dept. of basic science, College of Dentistry, University of Baghdad.

Email:maha_almelan@yahoo.com

transportation of CSF sample.

Laboratory methods: The present study implemented standard operating laboratory procedures based on routine microbiological procedures and WHO recommendations. A Gram stain was performed on CSF and then routine practice of culture media modified to optimize isolation rates of *H.influenzae*. CSF was cultured for *H.influenzae* on chocolate and Gonococcal GC medium (supplemented with 1% hemoglobin and 2% isovtlex (mast group UK) for X and V factors) then incubated at 37C° for 24-48 hours under 10% CO₂ condition. Oxidase and API –NH system (Bio-Merieux France) used according to manufacture instructions for the diagnosis and serotyping of Haemophilus. Biochemical was on the bases of indol production, urease and Ornithine decarboxylase activity (11). As well as the nutritional requirements of *H.influenzae* to both XV factors were tested on Muller –Hinton agar all isolates were serotyped by latex agglutination test with specific antisera from a-f (BD BBL mast group, UK) according to manufacture instructions for detecting the serotype of capsulated *H.influenzae* isolates. studies on *H.influenzae* with susceptibility or resistance to Antibiotics depend on Disc diffusion method after culturing *H.influenzae* isolate on Chocolate agar, a pure growth of an organism fully sensitive to several drugs tested.

Results:

specimens of cerebrospinal fluid collected from 250 children aged less than five years old that clinically diagnosed to have meningitis were obtained through the period February 2012 to March 2013 from different hospitals we identified 18 (7.2%) confirmed cases of *Haemophilus influenzae* type b meningitis among 250 CSF specimens depending on X V factor, API-NH system and serotype (a-f) (Fig1,2,3),(Table 1).

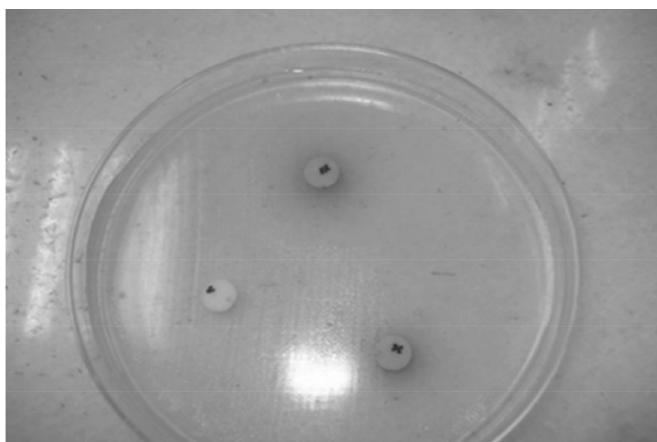


Figure 1: growth of H. influenzae around (XV) factors after inoculated on mueller –hinton agar for 24 hours of inoculation.



Figure 2: API-NH system results of the Haemophilus influenzae

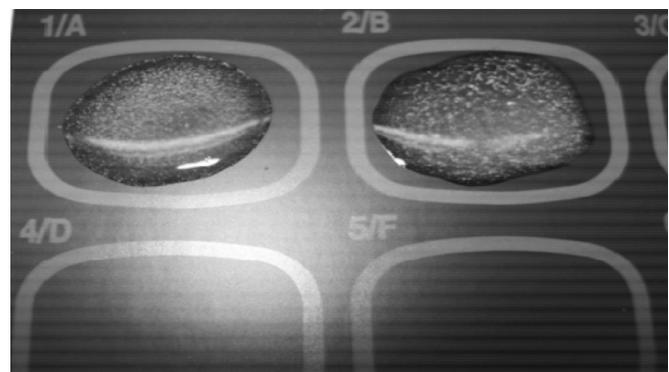


Figure 3: Serotyping of Haemophilus influenzae type b by latex agglutination test with type specific antisera type b.

Table (1): Depended on API-NH and X V factors for identification of Haemophilus influenzae.

Test	Result	
X V factor	+	
Oxidase	+	
API-NH	Glucose	+
	Furctose	V
	Maltose	-
	Sucrose	-
	Ornithine de carboxylase	V
	Urease	V
	Lipase	-
	Alkaline phosphate	+
	B Galactosidase	-
	Praline arylamidase	-
	Gamma glutamyl transferase	-
	Indol	V

+ : Positive, - : Negative, V : variable

The incidence of *Haemophilus influenzae* type b meningitis among infants less 1 year of age was 12(66.6%) compared to 6(33.3%) for older children. Incidence among males was 11(61.1%) compared to 7(38.8%) for females. (Table 2).

Table (2): Demographic characteristics of patients with meningitis:

Age group (year)	Haemophilus influenzae type b Meningitis patients (NO.=18)	%
≤1 years	12	66.6
2	3	16.5
3	1	5.5
4-5	2	11.5
Gender	Number	%
Male	11	61.1
Female	7	38.9

Ill appearance of the child, drowsiness, irritability and poor sucking or refusal of feed, vomiting, seizures, neck stiffness and Kernig's sign.

We depend on antibiotic sensitivity (Ampicillin 10µg, Trimethoprim-sulfamethoxazole 1.25-23.75µg, Gentomycin 10µg, Ciprofloxacin 5µg, Cefotaxime 30 µg, Ceftriaxone 30 µg, Chloramphenicol 30 µg) depending on use Kirby bauer method by use chocolate agar (Table3).

Table (3): The results of antibiotics sensitivity for Haemophilus influenzae type b

Antibiotics		Haemophilus influenzae (18) isolate			
		R	%	S	%
Ampicillin	10µg	0	0	18	100%
SXT (Trimethoprim-sulfamethoxazole)	1.25-23.75 µg	18	100%	0	0
Gentomycin	10µg	18	100%	0	0
Ciprofloxacin	5 µg	0	0	18	100%
Cefotaxime	30 µg	0	0	18	100%
Ceftriaxone	30 µg	0	0	18	100%
Chloramphenicol	30 µg	0	0	18	100%

Discussion

Meningitis caused by *Haemophilus influenzae* serotype b is an important human disease worldwide especially in children less than five years old. Hence its important that early diagnosis improves clinical outcomes (12,13).

This study was carried out in Iraq to report *Haemophilus influenzae* type b meningitis incidence. The happening of *Haemophilus influenzae* type b meningitis among infants less

two years of age was 15(83.2%) this result agree with other study that found *Haemophilus influenzae* type b meningitis in Asia mostly affects children under 2 years of age. Almost 80% of *Haemophilus influenzae* type b disease in the other study was in those aged less than 23 months (14). In 1998 Peltola's report for south east Asia showed 50-60% of *Haemophilus influenzae* type b meningitis cases occurring in the first 5 months of life (15). Europe study was found that *Haemophilus influenzae* type b meningitis was most common in children 1-3 years of age, a finding similar to that seen in Europe before introduction of vaccine. These findings contrast with children from developing countries show higher *Haemophilus influenzae* type b meningitis incidence rates among infants and young children (16).

Haemophilus influenzae type b meningitis is mainly a disease of infancy. Babies in the first year of life have the highest rates most cases are in children aged 3 months to 3 years. The disease is uncommon in infants smaller than 3 months and in children older than 5 years of age. Children naturally develop immunity to *H. influenzae* After the third year of life and concentrations of polyribosylribitol phosphate antibodies reach adult values by 7 years of age (17, 18). Culture methods identified only 18(7.2%) confirmed cases of *H. influenzae* type b of 250 meningitis cases were caused by any microorganism this result is compatible to the result found by Korean study Lee who identified eight confirmed *Haemophilus influenzae* type b meningitis cases. In Sri Lanka found culture methods identified only 12 *Haemophilus influenzae* type b meningitis cases and 26 of any microorganism, giving isolation rate of 2% from 1146 CSF cultures. Factors may contribute to this fastidious nature of *H. influenzae* coupled with suboptimal microbiology laboratory facilities and the use of antibiotics prior to admission to the hospital. Choice of antibiotic treatment entails the selection of agents that are effective against the probable pathogens and are able to attain adequate bacteriocidal activity in CSF. The ability of an antimicrobial agent to penetrate the blood-brain barrier is the most important factor that determines whether efficient bacterial killing happens in the CSF (20). Emergence of antimicrobial-resistance bacteria presents a constant challenge to the development of new bacteriocidal antibiotics for the treatment of bacterial meningitis (21).

Conclusion

Our conclusions are recommending that *Haemophilus influenzae* type b meningitis was most common in children less than 1 year that identified by culturing, biomedical and serotyping as well as the antimicrobial susceptibility of the *Haemophilus influenzae* type b.

References

1- Faraj H., Abedel-Fatah M., and Youssr A.i, *Epidemiological*

- clinical and prognostic profile of acute bacterial meningitis among children in Alexandria Egypt, *Indian Journal of Medical Microbiology*.2005;23(2):95-101(IVSL).
- 2- Corless C.E., Guiver M., Borrow R., Edward-jones V., Fox A.J. and Kaczmarek E.B.i, Simultaneous Detection of Neisseria meningitidis, Haemophilus influenzae and Streptococcus pneumoniae in suspected cases of meningitis and Septicemia using Real Time PCR, *Journal of Clinical Microbiology*. 2001;39(4):1553-1558.
- 3-Dubos F., Lammotte B., Bibi-Tirik B.i, Moulin F., Raymond J., Gendre D.I, Breat B., and Chalumeau M., Clinical Decision Rules to Distinguish between Bacterial Meningitis and Aseptic Meningitis, *Arch.Dis.Chil*.2006; 91: 647-650.
- 4- Cristiana M.,and Otavio M., Etiology of bacterial meningitis among children aged 2-59 months in Salvador , Northeast Brazil before and after routine use of Haemophilus influenzae type b vaccine, *Arq.Neuro-psiquiatr*.2004;62(2A): 250-252(IVSL).
- 5- MacNeil .R.,Cohn A.C.,Farley M.,Mair R.,Baumbach J.,Bennett N.,Gershman K.,Harrison L.,Lynfield R.Petit S.,Reingold A.,Schaffner W.,Thomas A.,CoronadoF., Zell E.,Mayer L.,and Massonnier N.E.Current Epidemiology and trends in invasive Haemophilus influenzae Disease –united states 1989-2008 ,*Clinical Infectious Disease* ,2011;53(12):1230-1236.
- 6- Andreson E.C., Beg N.T.g, Crawshaw S.C., Hargreave R.M.s, Howard A.J., and Slack M.P., Epidemiology of invasive Haemophilus influenzae infections in England and wales in the pre-vaccination era(1990-2), *Epidemiol.infec*.1995;115 : 89-100.
- 7- Schweda E.K., Richard J.C., Hoo D.W.d,and Moxon E.R., Expression and structural diversity of the LPS of Haemophilus influenzae implication in virulence, *International J.Medical Microbiology*. 2007;297:297-306.
- 8- Abdulhasan G.A., Sabal M.A., Jasem K.A., and Hassani H.H., Identification and typing of Haemophilus influenzae in Iraqi children diagnosed with meningitis, *International journal of microbiology research*. 2013; 5(3): 417-423(IVSL).
- 9-Broker M.,Burden of invasive Disease P.G.caused by Haemophilus influenzae type b in Asia,*J.infec.Dis*. 2009;62: 87-92.
- 10- Chaudhuri A., Martinez-Martin P. , Kennedy ,EFNS guideline on the management of community-acquired bacterial meningitis:report of an EFNS task force on acute bacterial meningitis in older children and adults .*Jeur J Neurol*. 2008;15: 649-59.
- 11- Brook G.F.s, Carroll K.C.I,I.S.Bute I.S.I,and Moras S.A. ,Haemophilus in Jawetz,*Medical Microbiology*.24thed. (McGraw Hill 2007):280-238.
- 12- Tzanakaki G.,and Mastrantonio P., Aetiology of bacterial meningitis and resistance to antibiotics of causative pathogens in Europe and in the Mediaterrian region . *International Journal of Antimicrobial Agent*. 2007;29: 621-629.
- 13- Aabdulrab, Salem F.,and Mohammed.Y.A.K., Acute bacterial meningitis in adults a hospital based study in yemen. *jpn.j.infec.Dis*. 2010;63: 128-131.
- 14- Ranjith B., Lalan R.i, Pranitha S., Malka D.,and Naihal A.,incidence of childhood Haemophilus influenzae type b meningitis in SriLanka. *International Journal of Infectious Disease*. 2010;14: 372e-376e.
- 15- Peltola H.,Need for Haemophilus influenzae type b vaccination In Asia as evidenced by epidemiology of bacterial meningitis.*Pediatr Infect .Dis J*. 1998;17(Suppl1): S148-51.
- 16- Peltola H.,World Wide Haemophilus influenzae type b disease at the beginning of the 21st century:global analysis of disease burden 25 years after the use of the polysaccharide vaccine and a decade after the evedent of conjugates.*Clin. Microbiol.*, 2000 ;13(2): 302-17.
- 17- Saiz-liorens X., McCracken jr G.H., Bacterial meningitis in children.*Lancet*. 2003;36:, 2139-48.
- 18-Kim J.S., Jang Y.T., Kim J.D., Park T.H., Park J.M., Kilgore P.E., and Kennedy W.A., Incidence of Haemophilus influenzae type b and other invasive disease in south Korean children.*Vaccine*.2004; 22: 3952-3962.
- 19- Lee H.J., Epidemiology of systemic Haemophilus influenzae in Korean children (miscellaneous). *Pediatric Infectious Disease Journal*.1998;17(9): S185-9.
- 20 Sinner S.W.,Tunkel A.R , Antimicrobial agent in the treatment of bacterial meningitis, *Infect Dis Clin North Am*.2004;18: 581-602.
- 21- Kwang S. K., Acute bacterial meningitis in infants and children., *Lancet infect Dis* .2010;1:, 32-42(IVSL).