Retrospective Study Of Bacterial Isolates And Susceptibility Patterns From Paediatric CSF Samples At Federal Teaching Hospital, Gombe

S Charanchi, A Samaila, Y Alkali

Citation

S Charanchi, A Samaila, Y Alkali. *Retrospective Study Of Bacterial Isolates And Susceptibility Patterns From Paediatric CSF Samples At Federal Teaching Hospital, Gombe*. The Internet Journal of Microbiology. 2015 Volume 13 Number 1.

DOI: 10.5580/IJMB.25763

Abstract

Between the years 2009 and 2014, one thousand two hundred and eighty five (1,285) CSF samples were collected at paediatric wards of federal teaching hospital Gombe. Using standard laboratory procedures the samples were processed for microscopy, culture and sensitivity of bacterial isolates at medical microbiology department of the same institution. The results were retrospectively analyzed. Generally, 54 (4.2%) bacterial pathogens were isolated from the patients in the years under study. Of the seven hundred and sixty seven (767) male samples processed 34 (4.4%) were culture positive while of the five hundred and eighteen (518) female samples processed, 20 (3.9%) were also culture positive. The highest number of positive samples was 29 (53.7%) from age group 0-2 years while the least positive sample was 1(1.9%) from age group 13-15years. Nine bacterial species were isolated, among which Neisseria meningitidis was the most frequent isolate with 31(57.4%) isolates. The least were Salmonella species and Citrobacter species with 1 (1.9%) isolate each. Using Oxoid sensitivity discs for the susceptibility testing, Gentamicin was the most effective drug as 6 of the 9 species isolated were excellently sensitive to it while the least was Cotrimoxazole where 8 of the isolated species were completely resistance to it. The results of this study therefore, re-establish the high vulnerability of children to bacterial meningitis, the importance of laboratory analysis of the CSF and recommends that Gentamicin and Augmentin should be included in syndromic treatment of CSM where laboratory investigations of CSF are not readily available.

INTRODUCTION

Meningitis is the inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges (1).The inflammation may be caused by infection with viruses, bacteria or other microorganisms, and less commonly by certain drugs (2,3).

Meningitis can be life- threatening because of the inflammation's proximity to the brain and spinal cord; therefore, the condition is classified as a medical emergency (1, 4). Meningitis being a medical emergency requires urgent rational antibiotic therapy, especially among the neonates and young infants (5).

Statement of the Problem

Bacterial meningitis is one of the most feared infectious diseases of children and epidemic meningitis can have a devastating impact on entire populations. It can be quite severe and may result in brain damage, loss of hearing, learning disability and death if not treated (6). It accounted for 2.7% of infant deaths among hospitalized children in Ilorin, Nigeria and 3.4% of post-neonatal deaths in Sokoto, Nigeria (7). Bacterial meningitis has a global incidence of about 20 -100 cases per 100,000 live births during the newborn period (8). It is common in the so-called meningitis belt area between 10°-15°N of the equator, in which Nigeria lies.

Bacterial meningitis is characterized by acute onset of fever (usually >38.5°C rectal or >38.0°C axilliary), headache, neck stiffness, altered consciousness, vomiting, and inability to tolerate light (photophobia) or loud noise (phonophobia). Children only exhibit nonspecific symptoms such as irritability and drowsiness. If a rash is present, it may indicate a particular cause of meningitis; for instance, meningitis caused by meningococcal bacteria may be accompanied by a characteristic rash (5, 9).

The types of bacteria that cause meningitis normally vary

with age group. In premature babies and newborns up to three months old, common causes are group B Streptococci (subtypes III which normally inhabit the vagina and are mainly a cause during the first week of life) and those that normally inhabit the digestive tracts such as Escherichia coli (carrying K1 antigen). Listeria monocytogenes (serotype IV b) may affect the newborn and occurs in epidemics. While those under five are affected by Heamophilus influenzae type b (in countries that do not offer vaccination), older children above five years are more commonly affected by Neisseria menigitidis (meningococcus) and Streptococcus pneumoniae (serotypes 6, 9, 14, 18 and 23) (4,7)

Head trauma also, potentially allows nasal cavity bacteria to enter the meningeal space. Similarly, devices in the brain and meninges, such as cerebral shunts, extra ventricular drains or Ommaya reservoirs, carry an increased risk of meningitis. In these cases, the persons are more likely to be infected with Staphylococci, Pseudomonas, and other Gram negative bacteria(4). These bacteria are also associated with meningitis in people with compromised immune system(1). The relative frequency of isolation of various bacterial species as a cause of meningitis varies with age, and among geographical regions (11, 12).

Aims and Objectives

Considering the fact that bacterial meningitis affects people of all ages and predominantly infants and newborns, this study is aimed at

Determine the bacterial species with the highest frequency among male and female paediatric patients.
Identify the most sensitive antibiotics to the isolated organisms.
Determine the age group with the highest risk among paediatric patients attending FTH, Gombe.
Documenting the distribution and profile of the common bacterial etiological agents of meningitis in paediatric patients of FTH Gombe.

MATERIALS AND METHOD

This is a laboratory-based retrospective analysis of One thousand two hundred and eighty five (1,285) Cerebrospinal Fluid specimens for microscopy, culture and antibiogram sensitivity testing. Investigations were carried out at the Bacteriology Laboratory of the Department of Microbiology and Immunology of Federal Teaching Hospital, Gombe in North-East Nigeria within a five year period between January 2009 and December 2013.

Collection and Transportation

The CSF samples were collected as part of the routine clinical management of children admitted in E.P.U and S.C.B.U wards of the hospital. The CSF samples were collected in sterile containers by lumbar puncture using a hypodermic needle after cleansing the site thoroughly with 70% methyl alcohol. This was done by the attending clinician who is wearing a pair of sterile hand gloves. Specimens were allowed to stand on the table for five minutes and inspected for colour change or 'cobweb' formation and subsequently delivered to the laboratory within half an hour after its collection.

Macroscopy

The specimens were further examined in the laboratory for change in appearance or color and for the presence of contaminants or blood. Normal CSF was clear and colorless (13).

Gram stain:

The CSF was spun to sediment cells and bacteria. The films of sediments were stained by Gram's stain technique and examined microscopically (13). None of samples showed 'cobweb' formation which would have necessitated ZN-stain.

Culture:

The samples were processed according to the standard operation procedures by inoculating heavily on to culture media (blood agar, chocolate agar and MacConkey agar) prepared as per the manufacturer's instruction and incubated at 35-370C aerobically .The chocolate agar plates were however incubated by placing them in a candle light jar, which provided 5-10% CO2 concentration microaerophilic condition for the growth of fastidious bacteria. After 18-24 hours of incubation, the plates were observed for bacterial growth; those that showed no growth were further incubated for another 24 hours. Organisms were identified based on standard microbiological procedures which include colony morphology, staining reaction, biochemical and serological tests (8, 9, 14, 15, 16).

Antibiotic susceptibility testing

Antibiotic susceptibility testings were done on pure cultures

isolated according to Kirby Bauer disc diffusion technique (20) against commonly available commercial antibiotics sensitivity discs prepared by Oxoid Ltd and include: Gentamicin (10µg), Cloxacillin (5µg), Tetracycline (10µg), Erythromycin (5µg), Amoxicillin (10µg), Augmentin (30µg) Chloramphenicol (10µg), Cotrimoxazole (25µg) and Streptomycin (10µg).

RESULTS

As shown in Table1: One thousand two hundred and eighty five (1,285) CSF cultures were analyzed. Seven hundred and sixty seven (767) were from male patients with positive cultures of 34 (4.4%) while five hundred and eighteen (518) were from female patients with positive cultures of 20 (3.9%). The highest number of positive cultured samples recorded was 29 from the age group (0-2 years) while the least recorded positive cultured samples was 1 from the age group (13-15years). Generally bacterial pathogens were isolated from 54 (4.2%) patients in the years under study.

Table 1

Distribution of Bacterial Isolates According to Age Groups and Sex

Age group (yrs)	Male		Female		Total	
	NT	NP (%)	NT	NP (%)	NT	NP (%)
0-2	386	18 (4.7%)	248	11(4.4%)	634	29 (4.6%)
3-5	274	9 (3.3%)	186	5 (2.7%)	460	14 (3.0%)
6-8	83	5 (6.0%)	68	3 (4.4%)	151	8 (5.3%)
9-11	15	1 (6.7%)	9	1 (11.1%)	24	2 (8.3%)
12-15	9	1(11.1%)	7	0 (0.0%)	16	1(6.3%)
TOTAL	**767**	**34(4.4%)**	**518**	**20 (3.9%)**	**1285**	**54 (4.2%)**

Key: NT: Number Tested, NP: Number Positive

As presented in Table 2: Among the 9 different types of bacterial isolates observed in the period under review 7 were Gram negative organisms while the remaining 2 were Gram positive. Neisseria meningitidis was found to be the most frequent isolate with 31 (57.4%) followed by Heamophilus influenzae (16.7%) and Streptococcus pneumoniae (9.3%). The least are Salmonella species and Citrobacter species with 1.9% each. Out of the total 54 isolated organisms, 34 (63%) were from the male patients while 20 (37%) from the female patients.

Table 2

Types and Distribution of Isolates according to sex

Isolate	Male (%)	Female (%)	Total (%)
Neisseria meningitidis	20 (58.8%)	11(55%)	31(57.4%)
Hemophilus influenzae	5 (14.7%)	4 (20%)	9 (16.7%)
Sreptococcus pneumoniae	4 (11.8%)	1(5%)	5 (9.3%)
Klebsiella species	1 (2.9%)	1(5%)	2 (3.7%)
Staphylococcus aureus	2 (5.9%)	0 (0%)	2 (3.7%)
Escherichia coli	1 (2.9%)	1 (5%)	2 (3.7%)
Pseudomonas aeruginosa	0 (0%)	1(5%)	1(1.9%)
Citrobacter species	1 (2.9%)	0 (0%)	1(1.9%)
Salmonella species	0 (0%)	1 (5%)	1(1.9%)
TOTAL	**34 (63%)**	**20 (37%)**	**54**

Table 3 presented the antibiogram activity of the available sensitivity discs used. Gentamicin was more effective than the other drugs as 6 of the 9 isolates were almost 100% sensitive to it. The organisms include Neisseria meningitides (96%), Staphylococcus aureus (100%), Escherichia coli (100%), Pseudomonas species (100%), Citrobacter species (100%) and Salmonella species (100%). The drug next in activity was Augmentin with 5 out of the 9 isolates excellently susceptible to it. However, 8 of the isolates were completely resistant to Cotrimoxazole, with the only exception being Neisseria meningitides that was 3.2% and this is equally very low susceptibility. This was followed by Amoxicillin with 7 of the isolates equally completely resistant to it. The remaining 2 isolates; Neisseria meningitides and Haemophilus species were only 32% and 11.1% sensitive to it respectively.

Table 3

Antibiogram Sensitivity Pattern of Isolates (% sensitivity)

ORG (TI)	GEN	CXC	TET	ERY	AMX	AUG	COT	CHL	STR
Neisseria sp (31)	96%	32%	67%	57.6%	32%	80%	3.2%	80%	16%
Heamophillus (9)	66.6%	33.3%	66.6%	44.4%	11.1%	33.3%	0%	55.5%	33.3%
Strep pneumo(5)	40%	60%	60%	100%	0%	100%	0%	40%	0%
Klebsiella sp (2)	0%	50%	0%	0%	0%	100%	0%	0%	0%
Staph aureus (2)	100%	0%	0%	0%	0%	100%	0%	50%	50%
Escherichia coli (2)	100%	0%	0%	0%	0%	0%	0%	0%	0%
Pseudomonas (1)	100%	0%	0%	0%	0%	0%	0%	0%	0%
Citrobacter sp (1)	100%	0%	100%	0%	0%	100%	0%	0%	0%
Salmonella sp (1)	100%	0%	100%	0%	0%	0%	0%	0%	100%

KEY: ORG-Organism, (TI)-Total Isolate, GEN–Gentamicin, CXC–Cloxacillin, TET– Tetracycline, ERY-Erythromycin, AMX–Amoxicillin, AUG-Augmentin, COT-Cotrimoxazole, CHL–Chloramphenicol, STR-Streptomycin.

As presented in Table 4, Infants at 0-2 years are more

susceptible to Neisseria meningitides and other isolates. Distribution of bacterial isolates is almost uniform among other age groups, although 3-5 and 6-8 are fairly more susceptible than others.

Table 4

Distribution of Bacterial species According to Age Group

Age group (yrs)	a	b	c	d	e	f	g	h	i	Total
0-2	19	2	4	1	1	2	0	0	0	29
3-5	5	6	1	1	0	0	0	0	1	14
6-8	6	1	0	0	1	0	0	0	0	8
9-11	1	0	0	0	0	0	0	1	0	2
12-15	0	0	0	0	0	0	1	0	0	1
TOTAL	**31**	**9**	**5**	**2**	**2**	**2**	**1**	**1**	**1**	**54**

Key: **a-***Neisseria meningitides,* **b-***Heamophillus sp,* **c-***Streptococcus pneumonia,* **d-***Klebsiella sp,* **e-***Staphylococcus aureus,* **f-***Escherichia coli,* **g-***Pseudomonas sp,* **h-***Citrobacter sp,* **i-***Salmonella species*

DISCUSSION AND CONCLUSION

It was observed that most of the patients who visited the hospital in the period under review were male and of the age group 0-2 years. This is in line with previous works that equally indicated that male children were more significantly affected than the female (5, 21). In 2004, the US infant mortality rate was 7.4 for boys and 6.1 for girls. Children of both genders get childhood infectious diseases such as mumps and meningitis at the same rate, but boys are more prone to complications(3,4), which from this study, tallies with the high number of male patients admitted to the hospital and the high value of positive cultures (4.3%) isolated.

Also from Table 1, it was observed that the highest positive culture rates were from infants less than 5 years from which the occurrence drastically came down. This result agrees with other studies carried out in Nigeria and Kenya, wherein pediatric patients under 5 years had the highest prevalence of bacterial meningitis compared to the other paediatric age groups (1,7,22). It was equally recorded that over two-thirds of all cases of bacterial meningitis occur in children less than five years old(12). In a district hospital in Kenya, bacterial meningitis was reported among 1.3% of children on admission; 88% of whom were under 5 years of age(23). The prevalence of pathogens in this particular pediatric group is probably due to immature immune system and permeability of the blood brain barriers (29).

The rate of positive culture isolated among children with suspected meningitis in this retrospective study (4.2%) was closely compatible to 3.3% among children in the study done in North-eastern Ethiopia in the year 2005(2). Several studies reported culture negative cases of meningitis or a low CSF culture positivity ranging from 3-5% (14, 15, and 23). The low bacterial isolation rate (4.2%) recorded in this study is in agreement with that reported by Akuhwa, et.al(20). This might have resulted from depletion of delicate organisms such as N. meningitidis, caused by delay in transportation and testing of CSF samples or intake of antibiotics prior to presentation of patients to hospitals (12, 23,24) which are usual in Nigerian hospitals (13,25) .

The predominance of Neisseria meningitidis (31%), Heamophilus influenzae (9%) and Streptococcus pneumoniae (5%), is in concordance with some previous studies, that equally recorded predominance of these three organisms in CSF samples from pediatric patients (3, 16, 23, 26, 27 and 28).

The high isolation of different species of Gram negative enteric bacilli tallies with work done by Andargachew in 2005(29). The presence of E. coli and other members of Enterobactericeae in pediatrics in this study might be as a result of infections acquired during passage through colonized mothers' vaginal vault as suggested by some specialists (5, 30 and 31). Equally, the isolation of some other pathogens may be accelerated by the presence of certain underlying conditions, such as otitis media, pneumonia, diabetes mellitus or immunodeficiency (1, 29).

Bacterial meningitis is a life threatening neurological condition and needs prompt antibiotic treatment compared to viral and aseptic meningitis which carries relatively lower morbidity. Delay in the start of proper therapy may introduce the potential for increased morbidity and mortality, if the patient does indeed have acute bacterial meningitis (9 and 21) . However, in Nigeria multidrug resistance to conventional antibiotics as observed in this study with other less frequent isolates like Pseudomonas aeruginosa, Klebsiella species, Staphylococcus aureus E. coli and Citrobacter species is a serious hurdle which may be connected to factors such as misuse of common drugs like cloxacillin, amoxicillin and Cotrimixazole (2,16).

Conclusion:

In this study Gentamicin was observed to show effective anti-bacterial activity across all the bacterial isolates except Klebsiella species. It is therefore our candid suggestion that in such a life threatening emergency as meningitis and with knowledge of its epidemics, the risk factors associated with

it and prior abuse of common drugs which may bring about resistance to antibiotics, treatment with wide-spectrum antibiotics like Gentamicin should not be delayed while confirmatory tests are being conducted. This is rightly supported by specialists in the field that successful management of patients suffering from bacterial illness depends on the identification of the types of organisms that cause the disease and the immediate selection of an effective antibiotic(23). Most often therapy for bacterial meningitis has to be initiated before the etiology is known and this could only be achieved by the use of known effective wide-spectrum antibiotic (21).

A limitation in this study which might have led to a high culture negative CSF (95.8%) may be due to loss of fastidious bacteria, caused by delay in sample transportation and processing. We would like to suggest that other methods of diagnosis which do not require the growth of live pathogens such as latex agglutination and polymerase chain reaction should be included in the routine medical laboratory practice in Federal Teaching Hospital, Gombe as a tertiary healthcare providing institution.

References

1. Sanya EO,Taiwo SS,Azeez O,Oluyaombo R.Bacterial meningitis:Problems of empirical treatment in a Teaching hospital in the tropics.Internent .J.Infect .Dis.2007;(6(1):1-4.

2. Andargachew Mulu,Afework Kassu,Belay Tessema.Bacterial isolates from cerebrospinal fluids and their antibiotic susceptibility patterns in Gondar University Teaching Hospital, North East Ethiopia.Ethiop J. Health Dev.2005;19(2).

3. Heyderman RS,Lambert HP,O'Sullivan I,Stuart JM,Taylor BL,Wall RA(2003).Early management of suspected bacterial meningitis and meningococcal septicaemia in adult.Journal of Infection 46(2):75-77.

4. Van de Beek D,de Gans J,Tunkel AR,Wijidicks EF.Community-acquired bacterial meningitis in adults.The New England.Journal of Medicine 2006;.354(1):44-53.

5. Rabab F,Marwa K,Waleed F,Taha G,Badawy E,Ayman Y.Role of clinical presentations and routine CSF analysis in the rapid diagnosis of acute bacterial meningitis in cases of negative gram stained smears.Journal of Tropical Medicare.2014,Article 213762.

6. Richard E.B et al. Nelson textbook of pediatric, 16th ed. W.B.Saunders Company. New York,USA.2000.p.707.

7. Segal S,Pollard AJ.Vaccines against bacterial meningitis.British Medical Bulletin.2004;72(1)65-81.

8. World Health Organization. Basic Laboratory procedures in clinical bacteriology.WHO,Geneva,Switzerland. 1991:78-95

9. World Health Organization.Control of epidemic meningococcal diseases.WHO Practical Guidelines.2 ed.1998.Geneva,Switzerland.

10. World Health Organization. Basic Laboratory procedures in clinical bacteriology.WHO,Geneva,Switzerland. 1991:78-95

11. World Health Organization.Detecting meningococcal meningitis in epidemics in highly endemic African countries.Wkly Epidemiol.Recd.2003;78(33):294-296.12. Tang LM,Chen ST,Hsu WC,Lyu RK.Acute bacterial meningitis in adults:A hospital – based epidemiological study.QJM 1999;92:719-25.

13. Chessborough M.Medical Lab.Manual for Tropical Countries II.1987:255-275.

14. Chinchankar N,Mane M,Bhave S,Bapat S,Bavdekar A,Pandit,et al.Diagnosis and outcome of acute bacterial meningitis in early childhood.Indian Pediatr 2002;39:914-21.

15. Kabra SK,Praveen K,Verma IC,Mukherjee,Chowdhary BH,Sengupta S, et al.Bacterial meningitis in India: An IJP Survey.Indian J Peadiatr 1991;58:505-11.

16. Mani R,Pradhan S,Nagarathna S,Wasiulla R,ChandramukinA.Bacterial profile of community acquired acute bacterial meningitis:A ten-year retrospective study in a Tertiary Neurocare Centre in South India.Indian J.Med. Microbiol.2007;25:108-14.

17. Kristos TG,Muhe L.Epidemic meningococcal meningitis in children.A retrospective analysis of cases admitted to ESCH..Ethiop Med J.1993;31 (1):9-14.

18. Maleeha A,Rubeena H,Tahir M.Bacterial meningitis:A diagnostic approach.Biomedica 2006;22:96-98.

19. Mani R,Pradhan S,Nagarathna S,Wasiulla R,ChandramukinA.Bacterial profile of community acquired acute bacterial meningitis:A ten-year retrospective study in a Tertiary Neurocare Centre in South India.Indian J.Med. Microbiol.2007;25:108-14.

20. Akuhwa RT ,Alhaji MA, Bello MA, Okon KO. Susceptibility pattern of meningococcal meningitis outbreak in Nguru, Yobe state Nigeria. Internet J Trop Med 2010;7 (1)1-4.

21. Ray P,.G,Badarou-Acossi,A.Viallon et al.Accuracy of the cerospinal fluid results to differentiate bacterial from non-bacterial meningitis;in case of negative gram stained smear.The American Journal of Emergency Medicine;2007(25):179-184.

22. Shanson DC.Microbiology in Clinical Practice.3rd ed.Butterworth Heinemann:305-306.

23. Jawetz,Melnick,Adelbergis.Medical Microbiology.19th ed.Prentice-Hall International Inc.Antibacterial and Antifungal Chemotherapy.149-179.

24. Robert F. Basic Medical Microbiology.5th ed .Lippincott Williams and Wilkins Country of Publication.252-267.

25. Das BK,Gurubacharya RL,Mohapatra TM,Mishra OP.Bacterial antigen detection test in meningitis.Indian J.Peadiatr 2003;70:799-801.

26. Mattijs C,Brouwer,Allan R,Tunkel,Diederik V.Epidemiology,diagnosis and antimicrobial treatment of acute bacterial meningitis.Clin.Microbiol.Rev.2010;23(3):467-492.

27. Kristos TG,Muhe L.Epidemic meningococcal meningitis in children.A retrospective analysis of cases admitted to ESCH..Ethiop Med J.1993;31 (1):9-14.

28. Hailu M,Muhe L.Childhood meningitis in a tertiary hospital in Addis Ababa.Clinical and epidemiological features.Ethiop.Med J.2001;39(1):29-38.

29. Thomas N.Riaz AS,Qasim S.Cerebrospinal fluid analysis in childhood bacterial meningitis.2007.

30. Mwangi,I,Berkley J,Lowe B,P,Peshu N,Marrh K,Newton CR.Acute bacterial meningitis in children admitted to a rural Kenya hospital:Increasing antibiotic resistance and outcome.Pead.Infect.Dis.J.2002;21(11):1042-1048.

31. Nwadioha SI,Onwuezube I,Egesie JO,Kashibu E,Nwokedi EOP.Bacterial isolates from cerebrospinal fluid of suspected acute meningitis in Nigerian children.International Infectious Dis.2011.Vol, 1:8

32. Ogunlesi TA,Okeniyi JAO,Oyelaemi OA.Pyogenic meningitis in Ilesa,Nigeria.Indian Paed.J.2005;42:1019-1023.

Author Information

S.M. Charanchi
Medical Microbiology Department, FTH Gombe
Gombe, Nigeria
charanchis@yahoo.ca

A.B. Samaila
Department of Biological Sciences, ATBU Bauchi
Bauchi, Nigeria

Y.S. Alkali
Paediatric Department, FTH Gombe
Gombe, Nigeria