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Bacteriological examination of children diagnosed of meningitis in Lagos, Nigeria

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Abstract— Bacterial meningitis results from an inflammation of the meninges of the brain by bacteria and bacterial products which is characterized by high prevalence in neonates and children. Patients at risk for bacterial meningitis include newborns, people in developing countries and those already infected with Gram-negative bacilli. This study was carried out to isolate, identify and characterize bacterial pathogens associated with cerebrospinal meningitis in children as well assess their antibiotic resistant patterns. A total of 318 samples were collected for bacteriological analysis at the children emergency wards of two hospitals for 24 months. All children diagnosed of meningitis were enrolled after obtaining informed consent from their parents. Aliquots of cerebrospinal fluids were collected by attending physician after lumbar puncture. Microscopy and culture, CSF protein and CSF glucose analyses were done. Of 318 samples analyzed, eighty two Gram stains indicated the presence of bacterial cells, 36 only of which had positive culture. Relationship between glucose and protein levels with Gram staining of CSF was observed. Bacteria were identified with standard microbiological methods included: *Streptococcus* species (12), Staphylococci (8), *Acinetobacter iwoffi* (6), *Escherichia coli* (4), *Klebsiella pneumoniae* (4) and *Serratia rubidala* (2). Most previously dominant organisms in bacterial meningitis were not isolated. Male patients were more predisposed to bacterial invasion with a higher number of cases in those less than one year. The routine use of polymerase chain reaction (PCR) and nucleic acid probes will surely provide rapid and definitive diagnosis of meningitis.

Keywords—Bacterial meningitis, children, Gram-negative bacilli, cerebrospinal fluid, meninges.

I. INTRODUCTION

Meningitis is an acute inflammation of the meninges which covers the brain and spinal cord. Meningitis can result from many causes which are either infectious or non-infectious. The etiology of the inflammation may be caused by infection with viruses, bacteria or other microbial pathogens, but less commonly by certain drugs [1].

Meningitis is often life threatening because the inflammation is proximal to the brain and spinal cord, so therefore it is classified as a medical emergency [2].

Over 1.2 million cases of bacterial meningitis occurs globally every year [3]. The prevalence and case fatality rates (CFR) 70%, with at least one of the survivors left with permanent sequelae which include disability or a limb loss [4].

The commonest bacterial causes of meningitis include group B. *Streptococcus, Escherichia coli, Listeria monocytogenes, Haemophillua influenzae* typhi b (Hib), *Streptococcus pneumonia* and *Neisseria meningitides* [5], [6].

Age-related incidence of Hib and *N. meningitidis* is inversely related the prevalence of serum bacterial activity and lack of type specific antibody as a major risk factor in neonatal group B Streptococcus disease [7], [8].

Bacterial etiology of meningitis have been known to cross the blood brain barrier either trans-cellularly, para-cellularly or by infected phagocytes [9].

Meningitis can result in serious long term consequences such as deafness, epilepsy, hydrocephalus and cognitive deficit, especially if not treated quickly and effectively [5].

Most human bacterial pathogens have the potential to cause meningitis, but a relatively small number of pathogens such as group B *streptococcus , Escherichia coli Listeria monocytogenes, Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae* and *Neissena meningitis* account for most of acute bacterial meningitis in neonates and children, although the reason for this association incompletely understood [6].

Age-related incidence of Hib and *N. meningitidis* disease is inversely related to prevalence of serum bacterial activity [7] and lack of type-specific antibody is a major risk factor neonatal group B Streptococcal disease [8].

Because bacterial meningitis as a medical energy that must be treated promptly, it is important to the cause of the symptoms as quickly as possible prompt laboratory diagnosis for successful treatment [10], [11].

This study was a descriptive longitudinal study carried out to assess the incidence of bacterial agents, isolate and identify such bacteria and assess microbial resistance pattern of such bacterial pathogens.

II. RELATED WORKS

Many studies about Cerebrospinal meningitis in children have been carried in Bida [12], Gusau [13], Abuja [14] and in Ghana [15]. So to the best of our knowledge studies on Cerebrospinal meningitis in children have not been adequately carried out in recent time in the South Western Nigeria, especially Lagos. This research work focused on isolating, identifying and characterizing bacterial species associated with Cerebrospinal meningitis in children in Lagos, Nigeria.

III. MATERIALS AND METHODS

Study Area
This CSF samples were collected by Physicians study at the children emergency ward, Lagos State University Teaching Hospital, Ikeja and General Hospital, Ifako-Ijaye, both of which serve as referral centers of both urban and rural setting in Lagos, Nigeria. Lagos, which falls within Latitude and Longitude coordinates of 6.457171 and 3.3277709 respectively, is the capital of Lagos State with a population of over 21 million, and is the second largest city in Africa and the third largest in the world.

2.2 Study Population
Children whose age ranged from 0-15 years who were admitted and diagnosed clinically as having bacterial meningitis in the two study sites that were eligible patients based on the clinical features of meningitis, were recruited consecutively for two years. Three hundred and eighteen (318) samples were collected from the subjects who fell within inclusion criteria. These were patients who were presented with one or more of the following symptoms: Decreased liquid intake, vomiting, rash, stiff neck, bulging fontanel, seizure activity, lethargy, fever, loss of consciousness, diarrhea, altered sleep pattern, jaundice, trachypnea, or after having obtained informed consent.

Exclusion criteria included:
i. Patients who did not sign the informed consent form.
ii. Patients not diagnosed of meningitis and
iii. Patients who declined to participate in the study.

Sample Collection
Experienced physicians collected the CSF after lumbar puncture from the patients under the aseptic conditions
i. 2 milliliters of CSF was collected into a sterile universal bottle for microscopy and culture.
ii. 1 milliliters of CSF into a sterile chemistry bottle for CSF proteins.
iii. 0.5 milliliters of CSF into a fluoride oxalate bottle for CSF glucose and 2 milliliters [16}.

Sterilization of Materials
All glass wares and laboratory equipment used for the study were washed with detergents with clean water and sterilized in hot air oven at 170ºC for 1 hour. Inoculating loops and spatula were sterilized by flaming red hot in Bunsen burner prior to use.

Media Preparation
All culture media which included blood agar, chocolate agar, Mueller-Hinton agar and media for biochemical tests were prepared according to manufacturer's instructions. Five percent human blood was added to the sterile basal medium at about 45ºC for blood agar while blood was added when the basal medium was very hot for chocolate agar.

Sample Analysis
The cerebrospinal fluid was observed visually for color and clarity. A direct Gram stain was done to determine the presence of bacterial agents before plating the samples out on blood agar, chocolate agar and MacConkey agar which were incubated aerobically at 37^0C for 24-48 hours.

CSF Studies
CSF was observed visually for color and clarity. Glucose and protein levels as well as white blood cells count were determined manually with a modified Neubauer counting chamber.

Isolation of Bacteria
A direct Gram's stain was done to presumptively determine the presence of bacterial agents. The samples were plated out on blood agar, chocolate agar which were incubated under aerobic conditions at 37ºc for 24-48 hours.

Identification of Bacteria
Primary plate cultures were examined for bacterial growth. The isolates were further identified on the basis of their colonial morphology, which were further sub-cultured for microscopy and biochemical identification using standard microbiological techniques.

Isolates were identified on the basis of cultural morphological and biochemical characteristics [16]. The bacterial isolates were subjected to relevant biochemical tests on the basis of their Gram's reactions on the basis of which the isolates were identified and characterized according to Buchanan and Gibbson [17].

Antibiotic Susceptibility Testing
Antimicrobial susceptibility testing was done for all bacterial isolates using Kirby-Bauer disc diffusion method. The antibiotics used and their concentrations in micrograms were ceftriazone 30µg, meropenem 10µg, clindamycin 30 µg, levofloxacin 10µg, erythromycin

15µg, amoxicillin 10µg, cefotaxime 30µg, amikacin 30µg, cefuroxime 30µg, ceftazidine 10µg and cefixime 5µg. Cultures were inoculated directly into 0.85% saline water which was adjusted to a turbidity of 0.5 McFarland's standard using densitometer. A sterile swab stick was then used to spread inoculum evenly on the Mueller-Hinton agar after which the antibiotic discs were placed evenly on the surface. Plates were read between 18-24 h and diameters of zones of inhibition were interpreted according to zones recommended from NCCLS [16], [18].

3.0 **Statistical Analysis.**

The data were analyzed using statistical package for social sciences version 14.0 (SPSS,Inc. 2001, Chicago iii), a statistical computer program. Continuous variables were presented as means and standard deviations while categorical variables were presented as proportions, those with P-value less than 0.005 was considered statistically significant (Confidence level= 95%)

IV. RESULTS

Of the 318 CSF samples analyzed, 82 confirmed cases of meningitis with both Gram positive and Gram negative bacteria while only 36 were cultured on the selective media. The most prevalent bacteria were *Streptococcus pneumonia* (33.3%), *Acinetobacter iwoffi* (16.7%), *Staphylococcus aureus* (11.1%), *Klebsiella pneumoniae* (11.1%), *E. coli* (11.1%) and *Serratia rubidalae* (5.5%) had the lowest occurrence (Table 1)

The relationship between CSF, Gram's stain, glucose level and number of associated pathogens is shown in Table 2. The highest occurrence of positive Gram's stain was found between glucose levels of 45-80mg/dL, with 24 positive culture plates. However, glucose levels of between 81-120 mg/ dL, yielded more positive cultures of 18 isolates but 20 positive Gram's stain. The relationship between protein levels and number of positive Gram's stain reaction is as shown in Table 3. The highest number of positive Gram's stain (40) was observed between protein levels of 81-20 mg/dL, followed by 40-80 mg/ dL which had only 14 positive Gram's stain reactions while those with 121-160 mg/dL protein level had the least positive Gram's stain of 6 (t>0.05).

The distribution of bacterial cells among sex indicated that males were more susceptible than females with 48 males as against 34 females having positive Gram's stain (Table 4). The relationship between age, frequency, Gram's stain reaction and microbial culture among the study population are shown in Table 5. Children under one year of age had the highest incidence of bacterial meningitis from both Gram's stain and microbial culture.

Antimicrobial resistance pattern of the isolates to various antimicrobial agents are shown in Table 6. Bacterial resistance was highest to the macrolide, erythromycin, in which all strains except *E. coli* exhibited 100% resistance. Some antibiotics such as amikacin, cefuroxime and ceftriazone had low levels of resistance but all isolates showed high susceptibility to carbapenems, meropenem, levofloxacin and clindamycin [16].

Table 1: Occurence of bacteria in CSF of children.

Organism	Frequency	Percentage
Streptococcus spp	12	33.3
Staphylococcus aureus	4	11.1
Staphylococcus spp	4	11.1
Acinetobacter iwofi	6	16.7
E coli	4	11.1
Klebsiella pneumonia	4	11.1
Serratia rubidalae	2	5.5

Table 2: Relationship between, Gram's stain glucose and culture.

Glucose levels (mg/ml)	Bacterial cells frequency by Gram's stain	No. of pathogen isolated (culture)
45-80	34	12
81-120	20	18
121-160	6	10
161-200	10	0
201-250	10	0

Table 3: Correlation of CSF protein Gram's stain and culture.

Protein levels (mg/ml)	**Bacterial frequency by Gram's Stain**	**Culture**
0-40	8	0
41-80	14	0
81-120	40	4
121-160	6	0
161-201	12	2

Table 4: Sex distribution of bacteria in CSF of children.

Sex		**Bacteria by Gram's stain**	**Bacteria cultured**
Male	194	48	24
Female	124	34	12
Total	318	82	36

Table 5:.Relationship between Age group, bacterial frequency, Gram's reaction on positive culture.

Age group	**No. examined**	**Gram positive**	**Gram negative**	**NBC**	**Culture**
<1	188	34	2	152	20
1-3	78	16	10	52	8
4-5	10	4	0	6	4
6-7	20	4	0	16	2
8-9	8	0	2	6	2
10-11	6	0	0	6	0
12-13	8	0	0	8	0
Total	318	58	14	246	36

Table 6: Antibiotics Susceptibility pattern.

Antibiotic	*S.aureus(4)*	*Staph.spp(4)*	*A.Iwofi(6)*	*E.coli (4)*	*Klebsiella. pneumoniae(4)*	*S.rubidalae(2)*	*Strep spp(12)*
CAZ	100	66.7	66.7	100	100	0	66.7
CRO	75	66.7	66.7	100	100	0	66.7
ERY	25	0	0	0	100	0	0
CXM	50	75	33.6	100	100	0	50
CTX	100	75	100	100	100	0	50
CL	100	100	66'7	100	50	100	100
MEM	100	100	100	100	100	100	100
AK	100	50	66.7	0	100	100	100
LEV	100	100	100	100	100	100	100
AMC	50	100	100	100	50	0	100

Legend: CAZ- Ceftazidine, CRO-Ceftrizone, ERY-Erythromycin, CXM-Cefuroxime, CTX-Cefotaxime, CL-Clindamycin, MEM-Meropwnem, AK-Amikacin, LEV-Levofloxacin, AMC-Amoxicillin.

V. DISCUSSION

Over the years, the studies have been done and are still ongoing concerning the causative agents, effects of antibacterial agents and prevalence of bacterial meningitis worldwide which has high mortality and morbidity rates [19].

This study shows that six different bacterial species were cultured which included *Streptococcus pneumoniae, Acinetobacter iwoffi, S. aureus, Klebsiella pneumoniae, E. coli* and *Serratia rubidalae* which had been rarely implicated in meningitis contrary to the report by Tunkel et al {5}. The prevalence rate of bacteria is low in this study compared to previous studies and no known outbreak was recorded. The low prevalence rate of bacterial isolates could be attributed to the possible use of antibiotics before hospitalization at the reference centers where all cases of suspected meningitis are referred. It is quite remarkable that no case of *Haemophilus influenzae* and *Neisseria meningitidis* were recorded all through the study period. This could be explained by the introduction of MenA vaccine with routine conjugate vaccine in Nigeria since late 2013 after initial infant trials as the impact is quite high in south west Nigeria. It is likely that the conjugate vaccine has indeed helped to reduce the incidence of the formerly predominant organisms. Certain elements of the patients' history such as predisposing factors, medical conditions, epidemiology, occupation and immune state can also suggest specific bacterial agents of meningitis. The bacteria isolated from the children have been implicated in several human infections, thus may be as a result of complication of such infections.

Male patients were more predisposed to bacterial invasion of the central nervous system than females. In addition, a higher number of cases are reported n patients less 1 year from this study. This brings to fore the question of personal hygiene, environment, mother's immunity level and where the baby was delivered as predisposing factors surrounding birth of such children {20}. The findings of this study correlates previous studies done in Africa and other zones that fall on the meningitis belt and that of national surveillance studies that both the etiological agents and mortality rates of 0-34% of bacterial meningitis depend on the season of the year and the age, sex ethnic background and geographical location [5],[19].

Of the 318 samples, 82 had positive Gram's stains cases in this study .Gram's stain examination of CSF permits a rapid, accurate and prompt identification of the causative bacteria in 60-90% especially in patients with community acquired bacterial meningitis, and it has been reported to have a specificity of 97% [20]. The likelihood of visualizing bacteria on Gram's stain, however correlates with the CSF concentrations of 10^3 cfu/ mL are associated with a positive Gram's positive stain results 25% of the time; 10^3 to 10^5 cfu/mL yields a positive Gram's stain results in 60% of patients and CSF concentrations of 10^5 cfu/mL leads to positive microscopy results in 97% of cases {1}. According to Scarborough and Thwaites [21], there is the likelihood of having positive Gram's stain result which often depend on the specific bacterial pathogens causing meningitis. Although, false positive CSF Gram's stain results may arise from misinterpretation, contaminated reagents or use of an occluded needles for lumbar puncture, the test is rapid, inexpensive and highly specific for the diagnosis of bacterial meningitis [22].

It was observed that 33.3% of the children examined had CSF glucose level that fell within the normal range, also had positive cultures and the highest incidence of positive Gram's stain of 41.5%. Similarly, 44.4% of those whose protein levels fell within the normal range had about 95% actually falling within higher. This shows that CSF protein is a reliable tool in prognostics of bacterial meningitis as against CSF glucose. In addition to culture, both CSF glucose and protein analyses would definitely make diagnosis definitive thus enhance prompt and effective diagnosis and treatment [10].

VI. Conclusion

Several pathogens are associated with meningitis. Some unusual bacterial species were recovered from the CSF examined and that CSF glucose was not as predictive of bacterial meningitis as against CSF protein. Males are more predisposed to meningitis as against females. Gram's stains was found to be a more effective diagnostic method than the culture method , even when the latter method appeared to be more specific and reliable .A high susceptibility of bacteria recovered to antibiotics tested was observed. More bacteria were recovered from children less or equal to eight years of age. Extensive hospital based surveillance are required to confirm the trends of new organisms which a source of worry or are opportunistic infections due to other illnesses mimicking bacterial meningitis, in addition to developing new vaccines against such organisms. Further studies on etiology of meningitis especially in developing countries would definitely be of tremendous health benefits. Use of molecular methods in the diagnosis of meningitis is strongly recommended.

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