

Bacteria Isolated From the Cerebro-Spinal Fluid (Csf) of Suspected Cases of Meningitis In Enugu State, Nigeria

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Abstract: 742 cerebro-spinal fluid (CSF) samples were taken from suspected cases of bacterial meningitis at University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu state, Nigeria. The patients were of various ages and sexes. The samples were analyzed bacteriologically to determine the various causative bacteria associated with these cases of suspected meningitis. Eleven (1.5%) were positive cases. The main etiological agents detected were *Escherichia coli* (36.4%), *Neisseria meningitides* (18.2%), *Streptococcus pneumoniae* (18.2%), *Staphylococcus aureus* (18.2%) and *Pseudomonas aeruginosa* (9.1%). 64% of these cases of bacterial meningitis were children under 2years of age. The susceptibility of the isolates to antibiotics was investigated. All isolates were found to be sensitive to cephalosporins.

I. Introduction

Nigeria has the highest incidence, morbidity and mortality of meningitis in the West African sub-region (Ojide et al., 2012). Epidemics of meningitis occur annually in the country and in many sub-saharan African countries.

In view of the recorded high rates of morbidity and mortality, this study was embarked upon to determine the bacteria associated with meningitis cases found in Enugu state, Nigeria.

Enugu state is located in the South East of Nigeria and though it does not lie within the “Meningitis belt”, the meningitis epidemic often spreads to many parts of Nigeria. *Neisseria meningitidis* is not the only etiologic agent responsible for these epidemics. There are other bacteria which are associated with meningitis and which have not been implicated in epidemic outbreaks in Nigeria.

Meningitis is an inflammatory disease of the brain and spinal cord (Rosenstein, 2001). It is convenient to classify meningitis topographically as a triad of blindness, deafness and paralysis as well as other serious complications in their wake.

Many people who carry bacteria associated with meningitis will never develop the disease. In some people, however, for reasons not fully understood, the bacteria will migrate through the body's outer immune defences (for example, through nasal passages) and into the bloodstream.

Bacterial meningitis is not a disease to be taken lightly. It is a degenerative, rapidly progressing disease that can result in death or permanent disability. Acute bacterial meningitis is dangerous and needs to be diagnosed and treated with appropriate antibiotics as quickly as possible. In the past, it was fatal in more than 50 percent of cases. However, with better and early treatment, fatality has dropped between 10 to 14 percent. Nevertheless, about 15 percent of survivors have longterm disabilities, including hearing loss and brain damage.

The most common strains of bacteria that cause meningitis are:

- *Streptococcus pneumoniae* (in about 50 percent of bacterial meningitis cases).
- *Neisseria meningitides* (in about 25 percent of bacterial meningitis cases and up to 60 percent in cases that involve children).
- *Listeria monocytogenes* is a Gram positive bacillus that is spread mostly by contaminated foods. Listerial meningitis occurs mostly among the new born, immuno-compromised patients and elderly people (Brouwer et al., 2006).

In recent years, common causes of bacterial meningitis have changed because of vaccines that target *Haemophilus influenzae* and, to a lesser extent, *N. meningitides*. Previously, these two bacteria were responsible for most bacterial meningitis infections. *Haemophilus influenzae* is also an important cause of bacterial meningitis. Its incidence rate was very high for children in the 80's but has declined since the introduction of Hib conjugate vaccines (Pranatharthi, 2013). *H. influenzae* type b is the most common bacterial cause of bacterial meningitis between the neonatal period and the age of 6. The number of children in the United States who get meningitis from this organism has decreased by about 90 percent (Todar, 2012). Today, *S. pneumoniae* accounts for about half of all bacterial cases.

Other organisms of the maternal birth canal like *E. coli*, *Proteus* sp., *Klebsiella* sp., and infectious flora of the hospital nurseries e.g. *Pseudomonas* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis* dominate

the invading bacteria linked with neonatal meningitis. Spirocheatal and tuberculous meningitis may occur at any age as they do not have definite patient – age relationship.

Patients suspected of having meningitis should have a specimen of CSF examined in the laboratory as soon as possible. Such patients have best chances of recovery if the diagnosis is made promptly and therapy instituted early.

The flagship of the fight against bacterial meningitis in developing countries is the use of conjugate vaccines. However, in the rural areas where the incidence of bacterial meningitis is highest, early treatment with antibiotics is imperative.

In the wake of this, emerging antibiotic resistance has become an increasing problem especially to β -lactam antibiotics. In many developing countries, there is increasing antibiotic resistance by *S. pneumoniae*, *H. Influenzae* and *N. meningitides*.

It is now necessary for doctors to make wise choices of appropriate antibiotics especially those backed up by laboratory antibiotic sensitivity testing of specimens.

II. Methods

742 cerebrospinal fluid samples were taken from suspected cases of bacterial meningitis in University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Nigeria in 2013.

The samples which were obtained aseptically from suspected cases of meningitis by lumbar puncture were quickly taken to the laboratory for immediate analysis. No samples were stored in the refrigerator for more than one hour. Each sample was split into two with one half used for cell counts and the other half centrifuged. The deposits were cultured in blood agar and chocolate agar. These cultures were incubated aerobically and anaerobically. Gram stained smears were examined microscopically.

Antimicrobial sensitivity tests were carried out using disc diffusion method (CLSI, 2008).

III. Results

Table 1 shows that of the 742 samples of CSF analyzed, 11 (1.5%) were positive.

Table 2 shows high incidence (54%) of meningitis among patients of 2 years and below. This confirms that cases of meningitis are high amongst children and infants (Duke et al., 2003).

Table 3 shows that more males than females were infected.

Table 4(A) shows that the two isolates of *N. meningitides* were sensitive to Sulphonamide, Chloramphenicol and Cephalosporin.

Table 4(B) shows that the four isolates of *E. coli* were sensitive to Gentamicin, Cloxacillin and Cephalosporin.

Table 4(C) shows that the two isolates of *S. aureus* were sensitive to Septrin, Erythromycin and Cephalosporin while *P. aeruginosa* was sensitive to Gentamicin, Cloxacillin and Cephalosporin.

Table 4(D) shows that the two isolates of *S. pneumoniae* were sensitive to Penicillin, Ampicillin, Erythromycin, Gentamicin and Cephalosporin.

These results show that all the isolates were sensitive to Cephalosporin

Table 5 shows the white blood cell counts from all positive cases.

Table 1: Organisms isolated from 742 samples of CSF analyzed bacteriologically

Organisms	No of Isolates	Percentage (%)
<i>N. meningitides</i>	2	18.2%
<i>S. pneumoniae</i>	2	18.2%
<i>P. aeruginosa</i>	1	9.1%
<i>E. coli</i>	4	36.4%
<i>S. aureus</i>	2	18.2%
Total	11 (1.5%)	100%

Table 2: Organisms isolated according to age distribution

Age groups	Organisms					Total
	<i>S. aureus</i>	<i>N. meningitides</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. pneumoniae</i>	
Under 1 year	-	2	1	2	-	5
1-2 years	-	-	-	2	-	2
3-12 years	-	-	-	-	-	-
13-30 years	-	-	-	-	-	-
31-70 years	-	-	-	-	-	-
above 70 years	2	-	-	-	2	4
	-	-	-	-	-	-
Total	2	2	1	4	2	11

Table 3: Organisms isolated according to sex distribution

Organism	Male%	Female%
N. meningitides	1	1
S. pneumoniae	1	1
P. aeruginosa	-	1
E. coli	3	1
S. aureus	2	-
Total	7 (64%)	4 (36%)

Table 4 (A): Antibiotic Sensitivity Patterns of the two isolates of N. meningitides

Antibiotics	1 st isolate	2 nd isolate
Ampicillin	S	R
Penicillin	R	R
Tetracycline	S	R
Sulphonamide	S	S
Erythromycin	R	S
Gentamicin	R	S
Streptomycin	R	R
Chloramphenicol	S	S
Cloxacillin	R	R
Cephalosporin	S	S

Key:

R = Resistant
S = Sensitive

Table 4 (B): Antibiotic Sensitivity Patterns of the four isolates of E. coli

Antibiotics	1 st isolate	2 nd isolate	3 rd isolate	4 th isolate
Ampicillin	R	R	R	R
Penicillin	R	R	R	R
Tetracycline	R	S	S	R
Septrin	R	S	R	R
Erythromycin	R	R	R	R
Gentamicin	S	S	S	S
Streptomycin	S	S	S	S
Chloramphenicol	R	R	R	R
Cloxacillin	S	S	S	S
Cephalosporin	S	S	S	S

Table 4 (C): Antibiotic Sensitivity Patterns of the isolates of S. aureus and P. aeruginosa

Antibiotics	S. aureus		P. aeruginosa
	1 st isolate	2 nd isolate	
Ampicillin	R	S	R
Penicillin	R	S	R
Tetracycline	R	S	R
Septrin	S	S	R
Erythromycin	S	S	R
Gentamicin	S	R	S
Streptomycin	R	R	R
Chloramphenicol	R	R	R
Cloxacillin	R	R	S
Cephalosporin	S	S	S

Table 4 (D): Antibiotic Sensitivity Patterns of the isolates of *S. pneumoniae*

Antibiotics	1 st isolate	2 nd isolate
Penicillin	S	S
Ampicillin	S	S
Tetracycline	R	S
Seprtin	R	R
Erythromycin	S	S
Gentamicin	S	S
Streptomycin	R	R
Chloramphenicol	R	S
Cephalosporin	S	S

Table 5: White Cell Count (Total and Differential) CSF of Positive Cases

Organisms	Total/ Count	Polymorphs(%)	Lymphocytes(%)
E. coli			
1 st isolate	20WBC	75	25
2 nd isolate	15 WBC	70	30
3 rd isolate	36 WBC	66	36
4 th isolate	25 WBC	60	40
S. aureus			
1 st isolate	200 WBC	55	45
2 nd isolate	90 WBC	60	40
P. aeruginosa	10 WBC	60	40
N. meningitides			
1 st isolate	1920 WBC	80	20
2 nd isolate	720 WBC	75	25
S. pneumoniae			
1 st isolate	15 WBC	60	40
2 nd isolate	28 WBC	75	25

IV. Discussion

Although *N. meningitidis* is the most common cause of meningitis in Africa, it is not the only causative organism. *E. coli* was the most frequently isolated bacterium in this study, followed by *S. pneumoniae*, *S. aureus*, *N. meningitidis* and lastly, *P. aeruginosa* (Table 1). This shows that *E. coli* is a common cause of meningitis in Enugu, Nigeria.

Table 2 shows that age influences the incidence rates as well as the causal organisms. Acute bacterial meningitis is most common in children aged 1 month to 2 years. 7 of the isolates (64%) came from children below 2 years of age while the remaining 4 isolates (36%) were from adults (31 – 70 years). In line with other studies, this research showed that infants and children 2 years and below are more susceptible to organisms from the birth canal like *E. coli*, *Pseudomonas* species, and *S. aureus*. (Rosenstein, 2001). Also, neonates are at greater risk from meningitis than other age groups because of deficiencies in humoral and cellular immunity and because their meninges are more susceptible to invasion whenever bacteremia occurs (Dredge, 2013)

Defence mechanisms play an important role in limiting infections in adults. *H. influenzae* has been found to be rare among adults because antibodies against this organism have been demonstrated but the level of this antibody in children is low. Nonetheless, in this study, 4 (36%) cases of bacterial meningitis were observed in adults (31 – 70 years).

The two isolates of *N. meningitidis* came from CSF samples that had high WBC counts. This development could be as a result of the patients being on drugs before CSF samples were collected. Self-medication is prevalent in Nigeria. This condition is referred to as cryptogenic meningitis. These could be patients who have been treated previously and came for review. Such were not indicated in the request forms of these patients.

Acid-fast staining and culture for tubercule bacilli were not carried out in this study. However, it would have been useful to do so because of the increasing incidence of antibiotic resistant *Mycobacterium tuberculosis* and emergence of this pathogen as an opportunistic pathogen of HIV/AIDS.

The emergence of antibiotic resistant strains or organisms is usually a major drawback in healthcare delivery, especially if the drugs the organisms are sensitive to are no longer available or the available ones are very expensive. Cephalosporins are usually the drug of choice in meningitis especially if the causative agents are *S. pneumoniae*, *H. Influenzae* and *N. meningitidis* (Brouwer et. al, 2010). All the isolates were sensitive to Cephalosporin making it a drug of first choice. Other drugs followed no definite pattern.

The low incidence bacterial meningitis seen in this study could be viewed from many perspectives. It could be attributable to the period of study which coincided with the period of the onset of rains. Epidemics and high incidence of meningitis usually occur in the dry season (October – February) in the savannah areas of Nigeria. Respiratory infection rates are highest in this area at this time. Enugu is not regarded as being in the meningitis belt of Nigeria.

Moreover, increased level of education, with its consequent increase in personal hygiene and health awareness are possible factors contributing to the low incidence of meningitis observed in this study

V. Conclusion

It is concluded that *N. meningitidis* is not the only etiologic bacterial agent of meningitis in Nigeria. A study of viral aetiologies of meningitis is advisable.

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