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STAPHYLOCOCCUS AUREUS MENINGITIS AMONG PAEDIATRIC EMERGENCIES IN A TERTIARY CENTER IN NIGERIA USING MOLECULAR METHODS

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ABSTRACT

Background: Bacterial meningitis remains a major cause of morbidity and mortality in children, particularly in low- and middle-income countries. While *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b were tradionally the most common pathogens, *Staphylococcus aureus* is increasingly being reported as a cause of paediatric meningitis in sub-Saharan Africa. This study aimed to determine the burden of *S. aureus* meningitis among children presenting with fever and central nervous system (CNS) signs at the University College Hospital, Ibadan, Nigeria, using molecular diagnostic methods.

Methods: A prospective study was conducted among 98 consecutively admitted children aged one week to nine years with fever and CNS features. Cerebrospinal fluid (CSF) samples were analysed using Gram stain, culture, and a two-step molecular biology workflow: broad bacterial detection via 16S rRNA PCR, followed by specific PCR targeting *S. aureus.*

Results: There 98 participants, 57(58.2%) males and 41(41.8%) females, aged one week to 9 years, of the 98 CSF samples tested, bacteria DNA was in 19 (19.4%). Among these, 7(7.1%) were positive for *S. aureus*. All *S. aureus* cases occurred in children older than one year, with a slight female predominance (57.1%). Seizures (84.7%) and lethargy (70.4%) were the most common presenting features. Two of the 7 who had *S. aureus* detected in their CSF were malnourished. Bacteria was not detected by CSF microscopy or culture.

Conclusion: This study highlights a notable incidence of *S. aureus* meningitis among paediatric emergencies in Nigeria, accounting for nearly one-third of confirmed bacterial meningitis cases. The findings underscore the importance of incorporating molecular diagnostics into routine practice to improve detection and guide timely antimicrobial therapy.

KEYWORDS: *Staphylococcus aureus* meningitis, paediatric bacterial meningitis, meningitis by molecular methods.

INTRODUCTION

Paediatric bacterial meningitis is a serious central nervous system infection characterized by the inflammation of the meninges. It is a major global health challenge, causing significant morbidity, mortality, and long-term neurological sequelae in children.[1-3] In 2023, an estimated 2.5 million infections and 259,000 deaths occurred worldwide due to meningitis.[4] Children under five years of age bear a disproportionate share of this burden, accounting for more than one-third of all global deaths (~90,000).[4] The incidence of meningitis is disproportionately higher in low- and middle-income countries (LMICs), with sub-Saharan Africa, particularly countries such as Nigeria lying within the "African meningitis belt" bearing the heaviest impact with a case fatality rate as high as 7%.[5] Prompt diagnosis and identification of the causative organism is crucial in prevention of morbidity or mortality as any delay in initiating appropriate antimicrobial therapy can lead to permanent neurological sequelae or death.[6]

Streptococcus pneumoniae, Neisseria meningitidis, and Haemophilus influenzae type b [7] have been the predominant organisms responsible for the majority of bacterial meningitis cases worldwide.[5, 8, 9] With the establishment of routine childhood immunization programs, the incidence of these organisms has reduced in many regions.[7] Although a less common aetiologic agent in meningitis, Staphylococcus aureus is emerging as a significant cause of community and hospital-acquired meningitis, particularly in neonates, patients with prior neurosurgical procedures, or the immunocompromised, accounting for up to 32.2% of bacterial isolates in some paediatric CSF samples in Nigeria.[10, 11]

The gold standard for the diagnosis of meningitis is cerebrospinal fluid (CSF) culture and Gram staining, however, these phenotypic methods are often slow, require viable organisms, and can yield false negatives, particularly if the child has received prior antibiotic treatment. In some Nigerian settings, only about 4–5% of suspected cases are confirmed by culture, often due to prior antibiotic use.[12] To address these gaps, this study utilizes a two-step

molecular biology workflow. We first employ 16S ribosomal RNA (rRNA) gene detection as a broad screening mechanism to confirm bacterial presence, followed by specific PCR for the specific identification of S. aureus.[13, 14] This approach aims to enhance diagnostic speed and yield, aligning with the WHO "Defeating Meningitis by 2030" global roadmap, which targets a 70% reduction in deaths and a 50% reduction in cases of vaccine-preventable bacterial meningitis.[15]
Therefore, this study aimed to evaluate the burden of these specific pathogens among children presenting to the emergency department of a tertiary hospital in Nigeria using molecular methods, intending to provide data for improved clinical management and infection control strategies.

METHODS

Study design

This was a study of all consecutively admitted patients presenting with fever and CNS signs at the children's emergency ward of the University College Hospital Ibadan, Nigeria by testing for bacteria 16sRNA gene and S.aureus nuc A gene.

Clinical procedure

All children under the age of five years admitted with fever and any acute onset features of central nervous system disturbance such as: seizures, altered consciousness, irritability, lethargy at the Otunba Tunwase Children's Emergency Ward of the University College Hospital, Ibadan were recruited consecutively until the desired sample size of 100 was reached. Those with absolute contraindication to lumbar puncture were excluded. Details of patients' demography, presenting symptoms and physical examination findings were be taken into a proforma.
All eligible subjects had samples taken for cerebrospinal fluid (CSF) analysis, CSF samples were sent to the laboratory in a cold box.

Microscopy and Bacterial Culture

Cerebrospinal fluid samples were processed upon receipt in the microbiology laboratory. On receipt, each CSF specimen was examined microscopically for appearance, including clarity, colour, turbidity, blood staining, clot formation, and presence of xanthochromia. The CSF was centrifuged at approximately 1,500–3,000 × g for 10–15 minutes to concentrate organisms and cells. The supernatant was carefully removed, and the sediment was used to prepare a thin smear on a clean grease-free glass slide. The smear was air-dried, heat-fixed, and Gram stained according to standard procedures. Stained slides were examined under oil immersion for the presence of bacteria, yeast cells, inflammatory cells, and other microscopic features. Bacterial morphology and Gram reaction were recorded where organisms were observed, including Gram-positive cocci, Gram-negative diplococci, Gram-negative bacilli, Gram-positive bacilli, or yeast-like cells. Quality control was maintained by ensuring the use of clean slides, properly prepared stains, calibrated microscopes, and trained laboratory personnel. Positive and negative control slides were used where applicable to confirm staining quality. Using a sterile Pasteur pipette, an aliquot of the CSF sediment was inoculated onto blood agar, chocolate agar, and MacConkey agar plates. The inoculum was streaked using standard quadrant streaking technique to obtain isolated colonies. The inoculated blood agar and chocolate agar plates were incubated at 35–37°C in 5-10% carbon dioxide for 24–48 hours. MacConkey agar plates were incubated aerobically at 35–37°C for 24–48 hours. Plates were examined after 18–24 hours and again at 48 hours for bacterial growth. Where no growth was observed after 48 hours, cultures were reported as no bacterial growth, provided that quality control and incubation conditions were satisfactory.

Molecular detection of Bacteria

DNA extraction

Bacterial DNA was extracted directly from cerebrospinal fluid (CSF) specimens using a commercially available nucleic acid extraction kit from Jena Bioscience (Jena Germany) according to the manufacturer's instructions. Briefly, CSF samples were processed immediately upon receipt or stored at −80°C until analysis. Prior to extraction, specimens were centrifuged to concentrate bacterial cells. The resulting pellet was resuspended in the appropriate lysis buffer, and total nucleic acid was purified using silica membrane-based spin column technology. DNA was eluted in elution buffer and stored at −20°C until PCR analysis.

Bacteria 16sRNA Detection by PCR

Bacterial detection was performed by amplification of the bacterial 16S ribosomal RNA (16S rRNA) gene using primers targeting the V1-V3 region of the gene[16]. Polymerase chain reaction (PCR) was carried out using a ABS 9700 thermal cycler (Applied Biosystems USA). Each reaction contained extracted 5ul of DNA template, 5ul of 5X red load PCR master mix by Jena Biosciences (Jena Germany), 1ul each of 10pM forward and reverse primers, and 13ul of nuclease-free water. Appropriate positive controls containing bacterial genomic DNA and no-template negative controls were included in every amplification run to monitor assay performance and contamination. The cycling profile consisted of an initial denaturation step at 95ºC, followed by repeated cycles of denaturation at 95ºC for 5 minutes, primer annealing at 56ºC for 45 seconds, and extension at 72ºC for 45 seconds, with a final extension step at 72ºC for 5 minutes. PCR products were resolved by agarose gel electrophoresis using an sybr

DNA stain by Jena Bisocience (Jena Germany). Samples producing bands of the expected 485-500bp for the target gene were interpreted as positive for the corresponding organism.

Detection of Staphylococcus aureus by PCR

All the DNA with detetctable bacteria 16sRNA were further tested for S.aureus by amplification of the S.aureus using primers targeting the nuclease gene (nuc) [17]. The PCR amplification was carried out in a 25ul reaction volume containing 5ul of 5X red load PCR master mix by Jena Biosciences (Jena Germany), 1ul each of 10pM forward and reverse primers, 13ul of nuclease-free water, and 5ul of extracted DNA template. Each PCR run included a positive control consisting of DNA from confirmed reference or previously characterized isolates of S. aureus. A no-template control was included to monitor contamination. Amplification was performed in a ABS 9700 thermal cycler (Applied Biosystems USA). The cycling profile consisted of an initial denaturation step at 95ºC, for 5 minutes followed by repeated cycles of denaturation at 95ºC for 30 secounds, primer annealing at 56ºC for 45 seconds, and extension at 72ºC for 45 seconds, with a final extension step at 72ºC for 5 minutes. PCR products were resolved by agarose gel electrophoresis using an sybr DNA stain by Jena Bisocience (Jena Germany). Samples producing bands of the expected 279bp for the target gene were interpreted as positive for the corresponding organism.

Table 1: Details of Primer used

Target Gene	Primer name	Sequences	Amplicon size
V1-V3 of 16sRNA	27F	AGAGTTTGATCMTGGCTCAG	485-500
	519R		
S.aureus nuc gene	Nuc A_F	GCGATTGATGGTGATACGGTI	279bp
	Nuc A_R	-AGCCAAGCCTTGACGAACTAAAGC	

Ethical Approval and Consideration

Ethical approval was obtained from the University of Ibadan/University College Hospital ethical review committee. Efforts were made to ensure patient's confidentiality and also to minimize suffering of patients while collecting samples.

RESULTS

Socio-demographics

There were 98 participants, 57(58.2%) males and 41(41.8%) females, aged one week to 9 years over the study period. The majority, 89(90.8%) of the participants were aged under 5 years (see Table 1). The mean age was 26.6 ± 22.68 months and the median age- 23 (IQR 10 – 35.5 months.

Table 2: Age and sex distribution of study participants

Sex / Age	Male n(%)	Female n(%)	Total n(%)
<29 days	5 (5.1)	3(3.1)	8(8.2)
29 days to <12 months	11(11.2)	8(8.2)	19(19.4)
12 months to <5 years	35(35.7)	27(27.6)	62(63.3)
≥ 5 years	6 (6.1)	3(3.1)	9(9.2)
Total	57(58.2)	41(41.8)	98(100)

Thirty-five (35.7%) of the parents belonged to the upper and middle socio-economic class respectively while 28(28.6%) belonged to the lower class.

Presenting clinical features

The most common CNS features at presentation were seizures 83(84.7%), lethargy 69(70.4%) and coma 17(17.3%). Other signs of systemic illness such as tachypnoea (26[26.5%]), pallor (25[25.5%]), and poor feeding [47(48%)] were commonest among age group 1 to 5 years (Table 2).

Table 3: Presenting clinical features by age

Age / Clinical Feature	<29 days (N=8)	29 days to <1 year (N=19)	1 to <5 years (N=62)	≥5 years (N=9)	Total (N=98)
Fever	8(8.2)	17(17.3)	59(60.2)	7(7.1)	91(92.9)
Seizures	6(6.1)	13(13.3)	58(59.2)	6(6.1)	83(84.7)
Lethargy	8(8.2)	11(11.2)	45(45.9)	5(5.1)	69(70.4)
Coma	0(0.0)	0(0.0)	14(14.3)	3(3.1)	17(17.3)
Tachypnoea	2(2.0)	7(7.1)	17(17.3)	0(0.0)	26(26.5)
Pallor	0(0.0)	6(6.1)	18(18.4)	1(1.0)	25(25.5)
Poor Feeding	6(6.1)	6(6.1)	32(32.7)	3(3.1)	47(48.0)
Bleeding Diathesis	1(1.0)	0(0.0)	4(6.5)	1(1.0)	6(6.1)

Incidence of Bacteria

16s ribosomal RNA –

Nineteen (19.4%) of the patient had 16s rRNA detected in their CSF, of those who tested positive, 15(78.9%) were under five years of age, 11(58%) were males and 8(42%) females.

Staphylococcus Aureus –

Out of the 19(19.4%) who had 16s RNA detected in their CSF, 7(7.1%) had staphylococcus Aureus DNA detected in their CSF. Of these 7(7.1%) who tested positive, 3(42.9%) were males and 4(57.1%) females and all 7 of them were above the age of one year, all but one were under 5 years (Table 3).

Table 4: Incidence of Bacteria by Socio-demographics

Characteristics	16s RNA	Staph.	Negative
Age			
<29 days	1(12.5)	0(0)	7(87.5)
29 days to <12 months	2(10.5)	0(0)	17(89.5)
12 months to <5 years	12(19.4)	6(9.7)	50(80.6)
≥ 5 years	4(44.4)	1(11.1)	5(55.6)
Sex			
Male	11(19.3)	3(5.3)	46(80.7)
Female	8(19.5)	4(9.8)	33(80.5)
Mother's Occupation			
I	2(33.3)	1(0)	4(66.7)
II	4(23.5)	2(11.8)	13(76.5)
III	5(17.2)	0(0.0)	24(82.8)
IV	6(15.8)	4(10.5)	32(84.2)
V	1(14.3)	0(0.0)	6(85.7)
Mother's Education			
I	7(25.0)	2(7.1)	21(75.0)
II	4(30.8)	1(7.7)	9(69.2)
III	6(15.8)	3(7.9)	32(84.2)
IV	1(6.3)	0(0.0)	15(93.8)
Father's Occupation			
I	6(31.6)	2(10.5)	13(68.4)
II	3(18.8)	1(6.3)	13(81.3)
III	6(15.8)	0(0.0)	32(84.2)
IV	4(17.4)	4(17.4)	19(82.6)
Father's Education			
I	9(29.0)	3(9.7)	22(71.0)
II	3(21.1)	0(0.0)	10(76.9)
III	5(12.8)	3(7.7)	34(87.2)
IV	2(16.7)	1(8.3)	10(83.3)

SES			
Upper Class	3(33.3)	0(0.0)	6(66.7)
Middle Class	11(18.0)	3(4.9)	50(82.0)
Lower Class	5(17.9)	4(14.3)	23(82.1)

Presenting features in those with bacterial meningitis

All the patients who had 16s RNA had at least one CNS feature, 16 out of the 19 patients had seizures and 11 had lethargy- Table 4.

There were 18 malnourished patients, and 5 of the malnourished patients had 16s RNA detected in their CSF out of which 2 had *S. aureus*.

None of the patients had any previous or ongoing surgical diagnosis.

Table 5: Presenting clinical features by bacterial infection

Clinical Feature	**16s RNA**	**Staph.**
Fever	16(17.8)	6(6.7)
Seizures	16(19.5)	6(7.3)
Lethargy	11(16.2)	4(5.9)
Poor Feeding	8(17.0)	3(6.4)
Tachypnoea	6(23.1)	2(7.7)
Pallor	5(20.0)	1(4.0)
Coma	2(11.8)	1(5.9)

OTHER RESULTS

There were 9 patients with pleocytosis out of which 3 had 16s RNA in their CSF - 1 was positive for *S. aureus* DNA.

Of the seven patients (7.7%) who had hypoglycorrhachia, 3 had 16s DNA positive in their CSF one of which also had *S. aureus* DNA detected in their CSF.

Thirty-eight (38.8%) patients had raised CSF protein, 7 had 16s DNA positive in their CSF out of which 2 had S. aureus meningitis.

DISCUSSION

In this study a 19.4% overall positivity rate for acute bacterial meningitis, with Staphylococcus aureus accounting for nearly one-third (36.8%) of all positive molecular isolates. This is in support of an epidemiology shift in the aetiology of bacterial meningitis as traditionally, the main organisms in paediatric bacterial meningitis were Streptococcus pneumoniae, haemophilus influenzae and neisseria meningitidis and S. aureus was only known to cause central nervous system (CNS) infections among immunocompromised patients or as post-neurosurgical complications or in patients with anatomical defects. [16]

An overall meningitis positivity rate of 19.4% in this pediatric emergency cohort is much lower than the results of Kwambana-Adams *et. al* [11] in their molecular-based surveillance across the West African sub-region, which reported a much higher percentage positivity of 78.5% among paediatric emergencies presenting with fever and CNS signs from Eastern Nigeria. The reason for this difference may be that Kwambana-Adams et al recruited patients who had fever and late signs of meningitis such as loss of consciousness. The yield from studies that used conventional CSF MCS, are consistently lower than those which used molecular methods.[17] This may suggest as some authors have that logistical issues involving laboratory testing may contribute to poor yield and therefore underestimate the prevalence of culture proven bacterial meningitis reported in studies.[18] This and the fact that meningitis is a serious infection and rapidly fulminant has influenced the use of presumptive clinical diagnosis which may be beneficial in not missing out on any patient requiring antibiotics but may lead to unjudicious use of antibiotics and contribute to the development of drug resistance. While molecular methods are expensive, more studies using molecular methods are required to properly document the prevalence of bacterial meningitis

In this study, S. aureus was 36.8% of isolates detected, this is in keeping with the findings of Iregbu *et. al*[10]*.,* who found that of the 28 bacterial pathogens obtained, 32.2% were Staphylococcus Aureus. Kwambana-Adams *et. al.,* in their review of aetiology of meningitis in 5 countries in sub-Saharan Africa found that staph aureus was reported as in 4 out of the 5 countries representing 17.8%, 6.1%, 21.4% and 33.3% of all pathogens obtained from Ghana, Nigeria, Senegal and Togo respectively.[11] In high income countries (HIC), Streptococcus pneumoniae and

Neisseria meningitidis maintain a near-monopoly in the aetiology of bacterial meningitis in children, with staph aureus rarely implicated except in cases of secondary infections such as in head injury patients. The reason for this difference maybe partly due to successfully reduction of the transmission of traditional vaccine-preventable strains Haemophilus influenzae type b [7] vaccine and Pneumococcal Conjugate Vaccines (PCVs) in Nigeria has unmasked the presence of non-vaccine pathogens like S. aureus. Especially because unlike high-income nations, *S. aureus* remains a cause of serious infections Nigerian children.

Clinically, the high proportion of *S. aureus* isolates identified in this study carries grave therapeutic implications for the management of meningitis as many reports of community acquired methicillin resistant staph aureus across Nigeria. Ceftriaxone is the empirical antibiotic of choice on most standard empirical guidelines for meningitis in most Nigerian centers, if the strain of *S. aureus* causing meningitis is MRSA, using ceftriaxone or any β-lactam antibiotics would be entirely ineffective. This delay in therapy accelerates the disease process and increases the risk of severe neuro-disabilities (such as sensorineural deafness or secondary epilepsy) and mortality.

The increasing reports of *S. aureus* meningitis also has a significant public health implication as there is vaccine against *S. aureus*, therefore reducing its burden cannot rely on immunization campaigns, public health strategies must therefore pivot towards education regarding improved hand and personal hygiene as well as promoting prompt health seeking behaviour. Efforts must also be made towards improving hospital diagnosis as continuing to rely solely on conventional, culture methods in resource-limited settings may lead to delays in diagnosis. Furthermore, a significant portion of pediatric patients receive pre-hospital antibiotics that may partially sterilize the CSF prior to sample collection further reducing the yield of conventional methods. Although culture-independent molecular methods are expensive, transitioning to rapid, molecular methods in the emergency rooms has become a clinical necessity to ensure rapid diagnosis and prompt appropriate treatment.

This study was conducted in a single centre therefore generalisability must be done with caution, also being a referral centre we receive very sick patients potentially overstating the absolute community incidence of staphylococcal meningitis. Furthermore, while molecular detection provided unparalleled diagnostic sensitivity, the lack of widespread real-time quantification (such as PCR cycle threshold values) prevented our team from correlating intrathecal bacterial loads directly with specific clinical severity scores. Finally, the lack of genomic sequencing restricted our ability to determine if these seven S. aureus cases were driven by specific community-acquired clonal complexes (such as CC30 or CC80) or hospital-acquired strains.

In conclusion, an incidence of 19.4% with Staphylococcus aureus accounting for 7 of the 19 isolates demonstrates that staphylococcal meningitis is likely a major player in Nigerian paediatric central nervous system emergencies. This shift demands an immediate re-evaluation of empirical antibiotic protocols and highlights the urgent need to integrate rapid molecular diagnostics into tertiary emergency frameworks to reduce childhood mortality.

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