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Drug resistance profiling, antimicrobial susceptibility and demographic characteristics of children with acute bacterial meningitis in a Southeastern tertiary health facility

Chuks G. Nwala[1]*, Oluchi M. Izuka[2], Ifeyinwa Roseann Chidomere[2], Ikechukwu Frank Ogbonna[2], Ichie Eziyi Kalu[2], Ihuoma Kathleen Ukpabi[2]

[1]Department of Pediatric Neurology, Limi Children's Hospital Wuse 2, Abjua and Federal Medical Centre Umuahia, Abia State, Nigeria
[2]Department of Pediatric, Federal Medical Centre Umuahia, Abia State, Nigeria

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***Correspondence:**
Dr. Chuks G. Nwala,
E-mail: g.nwala@limihospital.org

ABSTRACT

Background: Over the years, varying patterns of bacterial susceptibility and multidrug resistance (MDR) rates have been reported in different settings. Detailed evaluation of the drug profile of the bacterial pathogens implicated in children withinvasive bacterial infection helps to reduce the heightened risk of adverse events that couldfollow poorly managed or complicated cases in resource limited environment like ours. This study therefore, aimed to highlight the antibiotic susceptibility and MDR rate, and establish the relationship if any, between demographic characteristics and positive CSF- bacterial isolates of post neonatal children with suspected acute bacterial meningitis (ABM). The findings would guide practitioners on the empirical antimicrobials to consider in the event of clinical suspicion of ABM pending the availability of CSF isolates' antibiogram.
Methods: A prospective review of 100 children with clinical suspicion of ABM from January 2016-December 2020. Descriptive statistics, chi square and regression analysis were used to establish MDR rates, Isolates' susceptibility pattern and the relationship between demographic variables and positiveisolates respectively. P<0.05 was accepted as significant.
Results: Fluroquinolones, cephalosporins, imipenem and aminoglycosides were susceptible anti-microgram in children with ABM. Sixty-four (85.5%) of the isolates showed MDR pattern,and young children (infants and toddlers) were significantly associated with positive CSF bacterial isolates.
Conclusions: ABM should be treated with combination of CNS penetrating empirical antibiotics due to rising rate of MDR pathogens. Young children with febrile illnesses should be thoroughly evaluated for possibility of CNSinfection.

Keywords: Drug-profiling, Susceptibility, Demographic, Bacterial-meningitis, Southeastern Nigeria

INTRODUCTION

Drug resistance profiling refers to the process of identifying and characterizing the resistance of microorganisms, such as bacteria, viruses, or fungi, to specific drugs or antibiotics. This involves testing the microorganism's ability to grow or survive in the presence of various concentrationsof a drug or antibiotic.

The goal of drug resistance profiling is to; identify the most effective treatment options; monitor the emergence of drug-resistant strains; Develop new drugs or treatment strategies;and improve public health and infection control measures. Drug resistance profiling is essential in various fields, including: Infectious disease management, antimicrobial stewardship; pharmaceutical development; epidemiology and public health.

Techniques used in drug resistance profiling include: minimum inhibitory concentration (MIC) testing; disk diffusion testing; PCR (polymerase chain reaction) and DNA sequencing; and next-generation sequencing (NGS).

By understanding drug resistance patterns, healthcare professionals and researchers can make informed decisions to combat the growing threat of antimicrobial resistance and develop effective treatment strategies.

Bacterial infections among the pediatric population is the most commonly studied condition in resource constrained settings due to its potentially injurious nature, especially when present in the central nervous system of affected individuals.[1-3] Morbidity and mortality from bacterial meningitis have continued to surge in resource limited settings.[4-6] Emerging trend of MDR bacterial infections may have also contributed to this, escalating the current difficulties encountered by healthcare workers in the area of disease containment, ABM inclusive.[7-9] Current brain drain narrative among clinicians in Nigeria and its consequent toll on number of available skilled personnel against background of dearth of medical infrastructure, may have further worsened this trend, thereby portending more adverse physical and neurological outcomes of CNS infections among children.[10]

Despite the introduction of vaccines to cover most common pathogenic bacteria against Meningitis in West Africa, recent surveillance suggests that the incidence of meningitis by *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b remains worrisomely high. This towering disease burden, may not be truly representative of the actual level in the Pediatric population as high dependence on classical features suggestive of diagnosis of clinical syndromes, deterring potential candidates for confirmatory testing, may detract from the actual burden of these conditions like bacterial infection.[11] This calls for use of predictive variables that rouse clinical suspicion for early identification, prompt and adequate containment, and by extension, reduction of disease burden among the Pediatric population. In addition, knowledge of common isolates, susceptibility patterns and MDR profile of pathogens are important steps towards unraveling proficient measures that enhance appropriate infection mitigation and management.

A high susceptibility rate of bacterial isolates for cephalosporins was reported by Nwadioha and co-investigators in Kano.[12] Conversely, Asseid et al in Namibia, also reported an emerging trend of cephalosporin resistance in extended spectrum beta lactamase (ESBL) producing bacteria and *Klebsiella* spp.[13] Few years later Shemse et al in Ethiopia, reported an interesting profile and susceptibility pattern of isolates from inanimate materials within a tertiary hospital, most of which were resistant to cephalosporins.[14]

The hospital environment plays a key role in infection control and spectrum of pathogenic isolates in hospitalized children, with varying resistance patterns to antimicrobials in resource constrained settings. Contacts of these children, including health personnel, convey these pathogens to their communities with a consequent emergence of varying susceptibility patterns to antimicrobials. The researchers in Tikur Anbessa specialist teaching hospital, documented gram positive isolates' resistance to penicillin, while gram negative bacteria were resistant to penicillins, ceftazidime and ceftriaxone. These antimicrobials are often the empirical medications administered to children with suspected ABM on presentation to facilities in resource constrained settings. Very young children with bacterial infections, mostly linked with gram negative bacterial isolates, account for most mortalities in developing countries. These pathogens that had good susceptibility to cephalosporins have reportedly developed resistance to these antibiotics over the years.[15] There is no doubt therefore, that the spectrum of isolates and high sensitivity to cephalosporin reported earlier among the pediatric population, may have been overtaken by more novel resistance pattern.[12-15]

Consequently, this study aimed to identify the common bacterial isolates, their susceptibility pattern and the presence or otherwise of MDR isolates in children with suspected bacterial meningitis seen at a tertiary Southeastern facility in Nigeria. The findings where-from, will add to the available data on childhood infectious diseases and guide stakeholders on the best approach to contain the burden of ABM among the Pediatric population in similar settings and elsewhere.

METHODS

A prospective review of 100 children aged 2 months and 18 years, whose CSF samples were obtained following clinical suspicion of ABM. Participants were enrolled using the consecutive non probability sampling technique (Snowball sampling) due to the sensitivity of the study (lumbar puncture for CSF analysis being a requirement) and small sample size needed.

Initially, sample size was calculated using Cochran's formula, that approximated the subjects to 384 participants.[16] This was not feasible, given the difficulties encountered with caregivers regarding informed consent for CSF sample collection. As a result, a sampling frame of only post neonatal children seen in the emergency room was considered. A total of 4000 children were seen in the emergency room between 2016 and 2020. Out of these patients, a quarter (1000) of them made up the post neonatal age group who represented the sampling frame. Using the Yamane's formula for sample size determination when a population size is known, approximately 100 subjects out of the total pediatric population (4,000) seen within this period was derived and adopted as the sample size.

Inclusion criteria

Children aged 2 months and above, to less than 18 years, whose parental consent and/or assent obtained for study.

Exclusion criteria

Children below 2 months and individuals who refused or parental consent was declined.

The average number of children admitted into the emergency room of the federal medical centre Umuahia per year is about 1,000, so the total number admitted within the period of study was about 4,000. Of this number, the post neonatal children seen over this period of study was 1,000 and a hundred of them had clinical features in keeping with ABM and were enrolled for this study after duly written and signed informed consent was obtained from parents or guardians for their biodata, clinical findings and CSF sample collection.

For each identified isolate, the susceptibility pattern was determined by the Kirby-Bauer disk diffusion method. Descriptive statistics were applied to ascertain demographic characteristics of participants, the rate of pathogen isolation, pattern of susceptibility and resistance to less than 5 or more impregnated antimicrobials.

Descriptive statistics was also used to estimate the rate of antimicrobial susceptibility and MDR patterns. Chi square was used to test for level of significance with categorical variables. Regression analysis established the relationship between demographic characteristics and positive isolates in subjects.

Results were presented in prose and tables. P<0.05 were accepted as significant.

RESULTS

A total of 4000 children were seen at the emergency room of the federal medical centre Umuahia from January 2016 to December 2020. A hundred of these 4000 children had clinical features of ABM during this period and met the inclusion criteria for the study. Of these subjects 59 were males while 41 were female children. Thirty- five of these enrollees were infants, 43 were toddlers, 11 were preschool children. Six school children and 5 adolescents made up the remaining participants. Of the 100 CSF samples collected from the subjects, 69 samples grew pathogenic bacteria.

Multiple isolates obtained in the same samples were *Streptococcus pneumonia* and *Listeria* spp in 5 subjects, and *Listeria cytogenes* and *Klebsiella* in 1 of the samples.

Seventy-five different isolates were cultured from 69 participants' CSF samples, six of which yielded two pathogens in each. Common isolates were *Streptococcus pneumonia* 35 (46.1%), Listeria monocytogenes 12 (16%), *Staphylococcus aureus* 11 (14.7%), *Enterobacter* spp 10 (13.3%), *Streptococcus agalactiae* 2 (2.7), *Klebsiella pneumonia* 2 (2.7%), *Haemophilus influenzae* 1 (1.3%), *Salmonella* 1 (1.3%) and *Escherichia coli* 1 (1.3%).

Table 1 shows the spectrum of bacterial isolates, frequency of culture yield of pathogens and the antibiotic susceptibility of isolates.

Table 1: Bacterial isolate frequency and susceptibility pattern.

Bacterial isolates	N	Percentage (%)	Sensitivity pattern
Streptococcus pneumoniae	35	46.7	Ciprofloxacin, genticin, amoxiclav, erythromycin, cefixime, cefuroxime, imipenem, chloramphenicol, ceftriaxone-sulbactam, cefuroxime-clavulanic acid
Listeria monocytogenes	12	16	Ciprofloxacin, levofloxacin, erythromycin, genticin, ceftriaxone, cefixime, imipenem, chloramphenicol
Staphylococcus aureus	11	14.7	Ciprofloxacin, erythromycin, azithromycin, gentamycin, amoxiclav,ceftriaxone, imipenem, cotrimoxazole, cefuroxime
Enterobacteriaceae	10	13.3	Ceftriaxone, imipenem,ceftriaxone-sulbactam, cefuroxime-clavulanic acid
Streptococcus agalactiae	2	2.7	Ciprofloxacin, cefuroxime-clavulanicacid, gentamycin, chloramphenicol
Klebsiella pneumoniae	2	2.7	Augmentin, streptomycin, septrin,amoxicillin, erythromycin, cefuroxime-clavulanic acid, gentamycin
Haemophilus influenzae	1	1.3	Azithromycin, genticin,ceftriaxone, erythromycin, cefuroxine, chloramphenicol
Salmonella	1	1.3	Genticin, cefixime,imipenem
Escherichia coli	1	1.3	Ciprofloxacin, erythromycin, genticin,ceftriaxone-tazobacta M, Imipenem, levofloxacin
Total	75	100	

Out of the 75 Isolates that grew in the CSF samples, 64 were MDR (resistant to 5 or more antibiotics). The MDR rate was 85.5% in these pathogenic isolates documented.

Age and gender were not significantly associated with positive isolates but there was a strong likelihood that infants and toddlers would have positive CSF culture isolates. Table 3 shows the gender associatedlikelihood of a positive CSF isolate.

Table 2: MDR pattern of bacterial isolates.

Observed susceptibility	Predicted susceptibility		MDR (%)
	Positive	Negative	
Positive	5	7	41.7
Negative	4	60	93.8
Overall percentage			85.5

Table 3: Gender based susceptibility of CSF isolates' cross-tabulation.

Gender	CSF isolate		Total	Pearson chi square	Df	Asymp. sig (2 sided)	Likelihood ratio
	Positive	Negative					
F		35	41	0.422	2	0.810	0.677
M	7	52	59		2	0.713	
Total valid	13	87	100				

Table 4: Age based susceptibility of CSF isolates' cross-tabulation.

Age groups	CSF isolate		Total	Pearsonchi-square	Likelihood ratio	Linear by linear association	Df
	Positive	Negative					
Infants	6	29	35	2.508	3.864	1.468	4
Toddlers	5	38	43				
Preschool	2	9	11				4
School age	0	6	6				
Adolescents	0	5	5				1
Total	13	87	100				

DISCUSSION

The present study applied a longitudinal methodology unlike other studies on the subject.[3,6] Subjects that require repeat or follow up sampling to ascertain adequacy of therapeutic clearance of CNS infection usually benefit from a prospective review of their case management. Antimicrobial resistance evidenced by non-remittance of presenting symptoms is easily identified by this methodological approach which was used in present study.

Streptococcus pneumoniae, *Listeria monocytogenes*, *Staphylococcus aureus* and *Enterobacteriaceae* were the common isolates obtained from present study. This was at variance with studies done 4 years ago in the same study environment (an epidemiological survey).[6]

The change of the spectrum of bacterial isolates from *Neisseria monocytogenes*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type B, to the pattern documented in present study may be reflective of the gains derived from the introduction of pentavalent vaccine (Covering *H. influenzae* as a component), meningococcal vaccines and improved infection control protocol. However, the persistently high rate of isolation of *Streptococcus pneumonia* even in present study despite the introduction of pneumococcal vaccine to the national immunization programme is a cause of concern. Different factors have been reported for the varying patterns of pathogenic isolates.[17-20]

The type of vaccine used vis-à-vis the pathogenic strain it covers may be partly responsible. Bari and colleagues reported a 50% reduction in the prevalence of pneumococcal meningitis after theintroduction of PCV 10 vaccines in Pakistan.[21] It is important however, to note that areas receiving PCV 13 and PCV 23 have a different documentation of efficacy. For instance, individuals that received PCV 13 had 75% and 45% protection against Invasive Pneumococcal Disease and Pneumococcal Pneumonia respectively. The documented efficacy for PCV 13 in such a setting was however better than what was reported with PCV 23 with a lower protection of 45% and 18% against IPD and PP respectively.[22,23] This clearly shows that even with the latter vaccines, the efficacy of PCV in reducing pneumococcal infections is not as good as seen with *Haemophilus influenzae* type b and *Neisseria meningitidis* vaccines. A number of factors including methylation and other adaptive capabilities of this bacteria play important roles in the efficacy of PCV vaccines in containing infections. Therefore, additional measures detracting from the routine, like environmental sanitation, adequate ventilation, early identification of infections from this-pathogens and tailored empirical antibiogram the germ is susceptible to, may help surmount the challenges of pathogen containment among the paediatric population.

The rising rate of multidrug resistant isolates from bacterial infection reported by Nwokedi and colleagues in 2013 may have worsened over the years as present study

showed that the MDR pattern was present in 85.5% of the isolates.[12] This calls for more stringent infection control measures that forestall the exposure of this pathogen to the pediatric population as those with the infection may be faced with poor response to antimicrobials due to this rising level of resistance to more than 5 medications as shown in present study. This deposition agrees with the findings from Ethiopia, Namibia and China and could guide stakeholders in the mitigation measures applied in infection control in similar settings.[8,13]

Stakeholders need to intensify ongoing efforts in areas of childhood immunization, especially the "zero vaccine" children through heightened health education, exclusive and optimal breastfeeding campaigns, environmental sanitation, proper housing, adequate health coverage and other proven preventive measures that could help curtail meningitis burden. This is necessary because the available health facilities are grossly understaffed and do not have the requisite capacity to match the surging burden of MDR infections in the Pediatric population.

There was no association between participants' gender and positive bacterial isolates. Although there have been reports of higher female preponderance to viral and lower respiratory tract bacterial infections, further recent reports also suggest that respiratory infections such as tonsillitis were found frequently in females while lower respiratory tract infections were more common in males.[20,24,25,27] Contrary to this, some researchers have reported male preponderance to bacterial respiratory and skin infections.[27,28] Although the underlying reason may be unclear, attempts at explaining the underlying pathophysiology ascribed this to inherent factors linked to association between X chromosomes' loci (responsible for the production of antibodies) and infection containment. This explanation however, does not support the documentation of more females being at risk of some viral and upper respiratory tract infections.[24] Contrary to aforementioned depositions, present study observed no significant difference in participants' proclivity to bacterial meningitis based on gender. This is a bit ambiguous and detracts from earlier reports on childhood meningitis.

Findings from this study therefore suggest an emerging trend of no-significant-association between gender and invasive childhood bacterial infection like meningitis. This may be due to MDR pattern of pathogenic isolates to body defense mechanisms, emergence of IPD due to non-vaccine serotypes and antimicrobial susceptibility profile different from what has been prevalent over time.[17]

Participants from young age groups at presentation like infants and toddlers, were more likely to have positive bacterial isolates in present study. This finding agrees with reports from developing countries by Zeng, NwadIoha and Agrawal respectively.[8,12,17] The invasive nature of bacterial infection among children with suboptimal immunity, encourages the establishment of life-threatening conditions like meningitis.[15,17] Therefore, a high index of suspicion is required when evaluating children within this category that present with an acute illness. The classical symptoms of bacterial meningitis, may not be evident in this category of children, since the myelination of neurons may have not been completed within this period.[20]

In summary, most of the children with clinical suspicion of ABM had positive isolates of *Streptococcal* species sensitive to fluoroquinolone, 2nd and 3rd generation cephalosporins, aminoglycosides and macrolides. There is an emerging trend of MDR in bacterial isolates. Children below 3 years of age with clinical suspicion of meningitis were likely to have positive pathogenic cultures from their CSF samples.

The hospital-based nature of this study, limits the extrapolation of its findings to the general pediatric population in Umuahia. More so, the invasive nature of CSF collection limited the number of parents and guardians willing to give informed consent for enrollment for index study. Higher numbers of children may be drug resistant but the limited sample population constrained the investigators from determining this position in the study.

CONCLUSION

Fluroquinolone, cephalosporins, carbapenem, chloramphenicol, and macrolide, were susceptible antimicrobials against isolated pathogens and should be considered in children with suspicion of ABM. MDR was exhibited by 85.5% of bacterial isolates and young age at presentation was strongly associated with positive pathogen isolation in the pediatric population studied.

Recommendations

Children with clinical suspicion of ABM should be commenced on empirical antibiogram guided by this susceptibility guide possibly with a combination of CNS penetrating antibiotics and a high index of suspicion is required in infants and toddlers presenting with acute febrile illness to the emergency rooms.

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