British Journal of Medicine & Medical Research
4(29): 4812-4823, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antimicrobial Susceptibility Pattern of *Haemophilus influenzae* among Under-five Children Presenting at the Emergency Paediatric Unit (EPU) of Two Teaching Hospitals in Jos, Nigeria

Yunusa Thairu[1*], Idris Abdullahi Nasiru[2] and O. H. Kolade-Yunusa[3]

[1]Department of Microbiology Microbiology, University of Abuja Teaching Hospital, P.M.B. 228, Gwagwalada, Abuja, Nigeria.
[2]Department of Medical Microbiology, University of Abuja Teaching Hospital, P.M.B. 228, Gwagwalada, Abuja, Nigeria.
[3]Department of Radiology, Jos University Teaching Hospital Jos, Plateau State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author YT conceived the idea, participated in data collection and preparation of draft manuscript. Author OHKY reviewed the work and manuscript for intellectual content. Author IAN performed data collection, preparation of the draft manuscript and in the statistical analysis of results. All authors read and approved the final manuscript.

Original Research Article

Received 10th April 2014
Accepted 5th June 2014
Published 27th June 2014

ABSTRACT

Background: *Haemophilus influenzae* meningitis *is* a leading cause of endemic bacterial meningitis in infants and under-five children globally. *H. influenza* infection is severe where vaccine is not routinely used and one-third to half of the children either dies or suffers permanent disability such as deafness, paralysis or mental retardation when prompt and appropriate treatment is not instituted.
Aim: This research sets to study and document the antibiotic susceptibility pattern of *H. influenzae* isolates from cerebrospinal fluids of under-five children presenting at the Emergency Paediatric Units of two Teaching Hospitals in Jos, Nigeria.
Methodology: This was a descriptive cross–sectional prospective study conducted from

**Corresponding author: Email: samhaamal200@gmail.com;*

October 2009 to March 2010. One hundred and sixty consecutive under-five children who presented with signs and symptoms consistent with *H. influenzae* meningitis were recruited. Socio-demography data was obtained with structured questionnaire. Specimens were aseptically collected and carefully processed for isolation and identification of *H. influenza* and subsequently the antimicrobial susceptibility pattern of the isolates.
Results: The prevalence of *H. influenzae* meningitiswas low in Jos with prevalence of 6.3% among 160 under-five children studied, with mean age of 34 months and M: F ratio of 1:1. About 60% of these isolates were obtained from patients with acute pyogenic bacterial meningitis. Majority of the isolates were ampicillin resistant, β-lactamases producers and were all sensitive to ceftriaxone and azithromycin.
Conclusion: The low prevalence of *H. influenzae* meningitis suggests substantial but not complete coverage of vaccine activity in this region while the susceptibility pattern of the isolate reveals and supports the vital role ceftriax one plays in the management of invasive *H. influenzae* infections to avoid pathologic complications.

Keywords: Haemophilus influenza; antimicrobial susceptibility pattern; under-five and Jos.

1. INTRODUCTION

Haemophilus influenzae is a fastidious Gram negative bacillus which was initially recognized as the viral agent causing influenza [1]. The type species (*H. influenzae*) is responsible for a variety of diseases in humans. It is a common aetiological agent of diseases such as pneumonia, meningitis, otitis media, and conjunctivitis [2]. *Haemophilus influenzae* was first isolated in 1892 by Robert Pfeiffer from the sputum of patients with pandemic influenza infection [3]. It was named 'influenzae' because it was isolated from the lungs of individuals who died during an epidemic of influenza virus infection [3].

H. influenza has encapsulated (serotypes a through f) and nonencapsulated forms (nontypeable). The most important serotype is *H. influenzae* serotype b (Hib), which was a frequent cause of bacteremia, meningitis, and other invasive infections prior to the routine use of Hib conjugate vaccines in children [4]. Other capsular serotypes and unencapsulated *H. influenza* strains can also cause disease, mainly mucosal infections (sinusitis, otitis, bronchitis, andpneumonia), but occasionally cause more invasive infections.

H. influenza are small, pleomorphic gram-negative rods that are oxidase-positive, facultatively anaerobic, and nonmotile. In clinical specimens obtained from patients who have received beta-lactam antibiotics, *H. influenzae* can appear as filamentous rods. In vitro growth requires a CO-enriched atmosphere, hemin (factor X), and nicotinamide adenine dinucleotide (NAD, factor V); therefore, isolation from clinical specimens on solid medium requires the use of chocolate agar or other X and V factor supplemented media. *H. influenzae* appear as transparent or slightly opaque colonies on solid media [4].

The presence or absence of a polysaccharide capsule is an important distinguishing characteristic of *H. influenzae* species. The polysaccharide capsule can be serologically classified into six serotypes (a through f), while *H. influenzae* lacking a polysaccharide capsule are considered to be nontypeable [5]. The type b capsule consists of aribosyl and ribitol phosphate polymer and is the primary antigenic constituent of polysaccharide and polysaccharide conjugate Hib vaccines [5]. Nontypeable strains are genetically heterogeneous; some carry genetic elements for capsule production that are not expressed,

while other isolates do not have any of these genes and are more distantly related [5]. Even among identically classified nontypeable strains, electrophoresis of outer membrane proteins or enzymatic analysis demonstrates great heterogeneity of nonencapsulated strains. Infections of the upper and lower respiratory tract are largely caused by nontypeable strains. Nontypeable strains have been classified into eight biotypes based upon the presence or absence of indole, urease, and ornithine decarboxylase. Some biotypes have been associated with specific clinical syndromes; *H. influenza* biotype 3 isolates (also known as biotype aegyptius) are associated with the syndrome of Brazilian purpuric fever [6], and biotype 4 isolates are associated with maternal genitourinary tract infections and neonatal sepsis [7,8].

H. influenzae can survive within respiratory epithelial cells; this intracellular sequestration mayexplain the ability of these organisms to colonize the respiratory epithelium for extended periods [9]. Furthermore, *H. influenza*e can gain access to the subepithelial space, either by transmural migration across epithelial cells or by anindependent intercellular mechanism. This provides access to the vascular system, which can lead to bacteremia andmetastatic infection, including meningitis [9].

The clinical spectrum of *H. Influenza* infections are: Invasive infection; Neonatal and maternal infection; Non-invasive respiratory infection. Invasive infection occurs in children less than four years (90%), and caused by mainly *H. influenzae* type b (90%) and non-capsulated strain (10%). Neonatal and maternal infection is caused mostly by non-capsulated strains, meningitis in children older than one year presents with neck pain, headache, nausea vomiting, and neck stiffness. In infants, symptoms are difficult to pinpoint and they can present with irritability, refusal to feed, inconsolable crying, and bulged fontanelle [10].

By 18 months of age, one-third of children have had *H. Influenza* asopharyngeal colonization with both typeable and nontypeable *H. Influenza* [11]. Carriage rates in households or daycare centers with an index casecan exceed 60 percent [12]. Colonization can persist for months, and intercurrent upper respiratory infection may promote invasive disease as well as enhance spread among close contacts [13]. Simultaneous colonization by multiple strains is common, and longitudinal monitoring demonstrates that colonization is a dynamic process with a turnover of individual strains over time [14]. Since the introduction of Hib conjugate vaccines in 1985, the incidence of invasive disease cause by *H. influenzae* in the United States has decreased dramatically. Between 1989 and 2008, 7559 cases of *H. Influenza* disease were reported in the United States; the estimated mean annual incidence was 1.62 per 100,000 population, and 15 percent of cases were fatal [15]. A considerable burden of non-Hib disease is still present in the oldest and youngest age groups. The largest burden of disease among children occurred in infants <1 year; many of these cases occurred during the first month of life in preterm or low birth weight infants [15].

Prior to routine vaccination, the incidence of invasive Hib disease varied from 67 to 130 cases per 100,000 children under five years per year, and the incidence of Hib meningitis was 40 to 69 cases per 100,000 children per year. This incidence was equivalent to approximately 25,000 cases of acquired invasive Hib annually in the United States, orinvasive infection in 1 per 200 children in the first five years of life [16]. Some populations at increased risk had incidence up to four times this rate. Before widespread immunization, the secondary attack rate among children who were household contacts of an index case was 0.3 percent, which is 500-fold higher than the age-adjusted risk in the general population [16]. This risk of secondary infection increased inversely with age; children under

four years of age were at greatest risk, and clinical disease was most likely in the first 30 days after exposure to the index case. However, the widespread use of conjugate Hib vaccines in infancy has resulted in a dramatic decline in invasive Hib disease in children to one case or less per 100,000 [16]. The incidence of invasive disease among individuals over five years of age has been stable at approximately 0.5 per 100,000 population. Hib immunization has reduced carriage (and presumably transmission) and facilitated herd immunity. This reduction in carriage seems to be influenced by number and type of Hib immunizations as well as time post immunization. Consequently, the differences in immunization schedules internationally may result in differing carriage and disease patterns [17].

The global burden of Hib disease is substantial; worldwide Hib caused about 8.13 million serious illnesses worldwide in 2000, with 371,000 deaths [18]. This is almost entirely vaccine preventable; expanded use of the Hib vaccine could reduce pneumonia, meningitis, and mortality among children. Most cases of invasive *H. influenza* infection since introduction of the Hib vaccination have been attributable to non-type B strains [19].

In general, beta-lactam agents (eg, amoxicillin or a second-or third-generation cephalosporin) are the preferred antimicrobial agents if the organism is susceptible. Alternative agents with activity against *H. influenzae* include fluoroquinolones, macrolides, tetracyclines, and aminoglycosides. Among the macrolides, azithromycin is more active *In vitro* against most strains of *H. influenzae* and has more rapid killing and a longer post-antibiotic effect than clarithromycin [20,21].

Beta-lactamase–negative, ampicillin-resistant (BLNAR) *H. influenzae* is an emerging pathogen 73. Depending on local susceptibility findings, ceftriaxone may be an appropriate choice for treatment of clinical infections due to BLNAR *H. influenzae* pending further study of clinical infections with this pathogen. There have also been reports of increased prevalence of nontypeable *H. influenza* strains with resistance toampicillin and/or other beta-lactams. In a retrospective study that evaluated 465 *H. influenza* isolates from the blood or cerebrospinal fluid from patients in Sweden between 1997 and 2010, a significant increase in beta-lactamase–negative, beta-lactam–resistant isolates was observed over the course of the study period. Ninety-one isolates (20 percent) were beta-lactam resistant (defined as resistance to one or more beta-lactam, including penicillin, ampicillin, a cephalosporin, or a carbapenem), of which 43 (10 percent) were beta-lactamase negative and beta-lactam resistant [22].

Currently, paucity of data in the tropics regarding the bacteriology and antibiogram of *H. influenzae* has contributed to poor understanding of its possible resistance pattern which could make treatment difficult. Consideringthis, in mind we set out to study and document the antibiotic susceptibility pattern of *H. influenzae* isolates from cerebrospinal fluids of under-five children presenting at Emergency Paediatric Units of two teaching hospitals in Jos, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Background

This study was carried out at the Emergency Paediatric Unit (EPU) of Jos University Teaching Hospital (JUTH) and Bingham University Teaching Hospital (BUTH), Jos. JUTH is

a 600-bed capacity and BUTH a 350-bed capacity tertiary health institution serving Plateau state and majority of the states in the north central geopolitical zone of Nigeria. The temperature in the state is generally low. The harmattan period encourages clustering and crowding of family units. The housing units, constructed mostly from clay mostly are closely packed together and poorly ventilated.

2.2 Study Population

Consecutive children between six months and five years who presented in the Emergency Paediatric Units of Jos University Teaching Hospital and Bingham University Teaching Hospital, with symptoms and signs of meningitis, otitis media, conjunctivitis, sinusitis, pneumonia, and epiglottitis, and whose parents or guardians consented to participate in the study were enrolled and were informed about the need for this work.

2.3 Inclusion Criteria

i. Children of both sexes aged six months to five years with symptoms consistent with *H. influenzae* meningitis.

2.4 Exclusion Criteria

i. Children with known history of bleeding disorder, skin disease extending to the lumbar area and raised intracranial pressure.
ii. Children with history of lumbar puncture.
iii. Children below six months or above five years.
iv. Children with no history of antibiotics medication two weeks prior to sample collection.

2.5 Study Design

This was a descriptive cross–sectional prospective study conducted from October 2009 to March 2010. Using the prevalence rate of 28% documented by (Babalola and Coker, 1982), the minimum sample size was set at 160.

2.6 Sampling Methods

Diagnosis was achieved in collaboration with the Paediatrician. Children diagnosed of *H. influenzae* infection were recruited based on signs and symptoms of; meningitis (fever, neck pain, headache, nausea, vomiting, and neck stiffness), otitis media (fever irritability, difficulty in sleeping, fever, ear discharges and ear pain), conjunctivitis (red eyes and eye discharge), sinusitis (sneezing fever and breathing difficulty), pneumonia (cough fever flaring of alar nasal and tachpnoea), epiglottitis (presenting with drooling of saliva, inability to talk and mouth agape).

Interviewer-administered, structured questionnaires were used as the study tool. The questions outlined in the data forms were explained to the parent or guardian and then completed with the required information which included the child's bio-demographic data (such as child's age, sex, school, type of house and number of people sleeping in a room), provisional diagnosis and laboratory processes, such that the eventual result was noted in the data forms and communicated to the physicians and the parents.

The data obtained was entered and analysed using Epi Info version 3.5.1 package. Confidence interval was 95% and the p value was 0.05.

2.7 Specimen Collection, Transportation and Processing

Cerebrospinal fluid (CSF) samples were collected in universal sterile container; Blood was collected into two aerobic blood broth; Throat swabs were collected with sterile Stuart swabs and returned into the containers. Ear and eye swab samples were collected using pen torch to illuminate the ear. Eyes and Ears were examined for evidence of exudates. A moistened sterile swab stick was used to swab the conjunctiva and the middle ear to obtain the specimen, the swab was carefully removed and returned back to the container.

The specimens were macroscopically and microscopically examined, Gram staining was done. Using a sterile wireloop, the specimens were streaked onto a sheep chocolate agar, sheep blood agar, sheep chocolate agar plate with vancomycin and clarithromycin and Haemophilus testing media with supplement.

The inoculated plates were placed in a canister and incubated at 37℃ in a moist atmosphere supplemented with 5–10% CO_2 for 18 to 24hrs. The colonies were observed and those that were mucoid, shinny, convex and greyish were recorded. The colonies were Gram stained [11] for evidence of Gram negative bacilli or coccobacilli. Pure colonies were identified by satellitism and X+V factor requirement [23].

2.8 Antibiotics Susceptibility Testing

Antibiotics susceptibility pattern was determined using the *Haemophilus* test mediaby the disk diffusion method and MIC by antibiotic gradient strip (Etest) testing methods.

Materials used were; *Haemophilus* test medium, Petri dish, antibiotics disks (Oxoid)^{R}, McFarland standard, sterile swab sticks, control strain (*Haemophilus influenzae* ATCC 49247).

Three well-seperated colonies of similar appearance to the control (*Haemophilus influenzae* ATCC 49247) were emulsified in two separate test tubes, containing 4ml of sterile physiological saline each labelled ‘test’ and ‘control’ respectively. The turbidity of the suspensions (both test and control) was compared with 0.5 McFarland standards. Using sterile swab, *Haemophilus* testing media plates were swabbed with test suspension and control suspension respectively and labelled appropriately. After 5mins, sterile forceps were used to place the antibiotic discs, evenly distributed on both plates (similar antibiotics were tested in both the test and the control). Within 30 minutes of applying the discs, the plates were incubated at 35ºC for 18 to 24 hours with CO_2 supplementation. After overnight incubation, the test and the control plates were examined. Using a ruler on the underside of the plates the diameter of each zone of inhibition was measured in millimetres [23].

To Interpret the result of susceptibility tests; The zone diameter of each antibiotics in the control experiment was compared with the CLSI standards, if within the CLSI acceptable limits for Quality control strains, then the zone diameter of each antibiotics of the test were compared with CLSI zone diameter breakpoints and was recorded sensitive, intermediate or resistance.

2.9 Minimum Inhibitiory Concentration Determination (M.I.C)

Materials used were; Haemophilus test medium (HTM BASE CM0898+HTM SUPPLEMENT SR0158) and MIC evaluator Etest (Biomerieux, USA)^{R} strips for Ceftriaxone, forceps.

2.10 Procedure for Etest

TwoHaemophilus testing media plates swabbed with broth culture made from the test organisms (*Haemophilus influenzae* isolates) and control organism (*Haemophilus influenzae* ATCC 49247) were used. The MIC evaluator strips of ceftriaxone was carefully placed on the HTM of test plate and the control plate, the underside with graded concentration making contact with the media while the surface with the concentration scale faces upwards. These plates were incubated at 35ºC for 18 to 24 hours with Co_2 supplementation. Where the ellipse intersects the scale of the strip, was observed to be the M.I.C of the antibiotics.

2.11 Interpretation

The value at the intersects of ceftriaxone in the control plate was compared with the CLSI acceptable limits and if within the limits of the CLSI standards, the value at the intersects of ceftriaxone in the plate of test organism were read for each isolates and compared with the CLSI MIC interpretive standard.

2.12 Determination of β Lactamases

Materials used include; Petri dishes, Nitrocefin chromogenic discs (R211667) and isolates. The procedure involves the use of Nitrocefin discs, loopful colony of the isolate was smeared on the discs and this was placed in a closed petri dish and after 15min colour change was observed. Colour change of the discs from yellow to red colour signified that the isolate produced β-lactamases and the isolate was non-β-lactamases producers when the discs colour remained yellow.

3. RESULTS

This study was carried out among 160 children between the ages of six months and five years. Samples were collected from these children and examined between November 2009 and March 2010, and no parent withdrew their child after consented to the study. There were 76 males (47.5%) and 84 females (52.5%) and the male to female ratio (M:F) was 1:1. The mean age of children studied was 34 months, with the highest proportion within the age range of 31-40 months accounting for 22.0% of the children and the lowest proportion being 0-10 months accounting for 8.2%. However, this distribution was not statistically significant (P >0.05) (Table 1).

From the 160 children, *H. influenzae* was isolated from 10 children, three from Jos University Teaching Hospital (JUTH) and seven from Bingham University Teaching Hospital (BUTH) representing 6.3% of the study population (JUTH and BUTH represents 1.9% and 4.4% respectively) (Fig.1). The interpretive zone diameter breakpoints showed that all the 10 (100.0%) isolates obtained from the specimens in the study were sensitive to ceftriaxone and Azithromycin, while 8 (80.0%) each of the isolates were sensitive to cefuroxime and co-amoxiclav, 3 (30.0%) of the isolates were sensitive to chloramphenicol, 2 (20.0%) to ampicillin (Table 2). The MIC of ceftriaxone to *H. influenzae* ATCC 49247 was 0.12µg/mL,

which was within CLSI MIC acceptable limits (12µg/mL) of 0.06-0.25 and the MIC of each of the isolates were tabulated (0.008-0.12µg/mL) (Table 3). Six *H. influenzae* isolates (60%) were β-lactamase producers.

From the study, 13 (8.1%) of the isolates obtained from the children examined were *S. aureus* and 5 (3.1%) of the isolates obtained from the children examined were *S. pneumoniae* while 2 (1.3%) each of the isolates were *S. pyogenes* and *P. mirabilis*. Other bacterial pathogens isolated from the children examined were *P. aeruginosa* and *N. meningitidis* with frequencies of 4 (2.5%) and 1 (0.6%) respectively. Therefore, other bacteria isolates represents 11.7% of the study population while samples without isolates were however one hundred and twenty three representing 82.0% of the study population (Table 4).

Table 1. Distribution of *Haemophilus influenzae* isolates among under-five children in Jos

Age group (months)	Total	Percentage %	*H. influnzae isolates*	Percentage %
0-10	13	8.1	0	0.0
11-20	22	13.1	3	13.6
21-30	33	20.6	2	6.1
31-40	35	21.9	1	2.1
41–50	32	20.6	3	9.4
51-60	25	15.6	1	4.0
Total	160	100.0	10	6.3

$\chi^2=3.24;df=5;P>0.05$

Table 2. Antibiotics susceptibility pattern of *Haemophilus influenzae* isolated from under-five children in Jos

Antibiotics (n=6)	Sensitivity	Percentage
Ampicillin	2	20.0
Azithromycin	10	100.0
Chloramphenicol	3	30.0
Cefuroxime	8	80.0
Co-amoxyclav	8	80.0
Ceftriaxone	10	100.0

Table 3. Results of Etest determination of minimum inhibitory concentration of ceftriaxone for *Haemophilus influenza* isolates among under-five children in Jos

Isolates N=10	MIC of *H. influenza* ATCC 49247 (µg/mL)	MIC of isolates (µg/mL)	Interpretation (≤2=susceptible=[S])
1	0.12 (0.06-0.25)	0.02	S
2	0.12 (0.06-0.25)	0.06	S
3	0.12 (0.06-0.25)	0,03	S
4	0.12 (0.06-0.25)	0.01	S
5	0.12 (0.06-0.25)	0.12	S
6	0.12 (0.06-0.25)	0.02	S
7	0.12 (0.06-0.25)	0.06	S
8	0.12 (0.06-0.25)	0.01	S
9	0.12 (0.06-0.25)	0.03	S
10	0.12 (0.06-0.25)	0.03	S

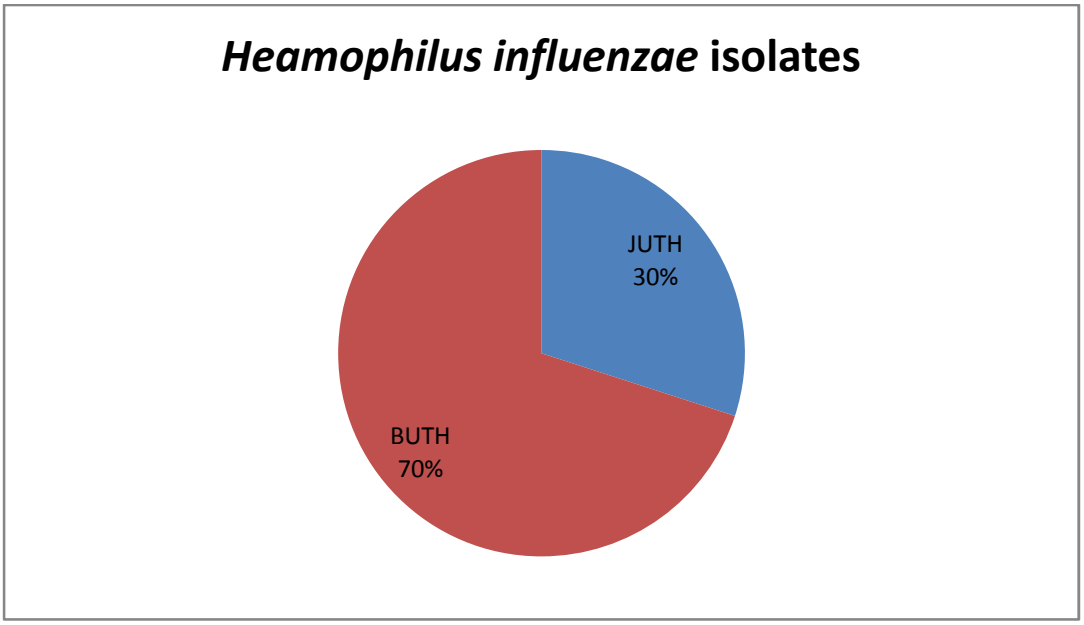

Fig. 1. Distributions of isolates in JUTH and BUTH in Jos

Table 4. Other bacterial isolates in under-five children in Jos

Other bacteria isolates	Total	Percentage %
N. meningitidis	1	0.7
S. pneumoniae	5	3.3
S. pyogenes	2	1,3
S. aureus	13	8.7
P. aeruginosa	4	2,7
P. mirabilis	2	1.3
Culture negative	123	82.0

4. DISCUSSION

Data obtained in this study revealed that the prevalence of *H. Influenza* meningitis among under-five children presenting at the emergency unit of JUTH and BUTH was 6.3%. This figure varies with findings in Nigeria and other parts of the world [24].

The finding of 6.3% prevalence in the present study was higher than values reported from studies in UK [24]. In Nigeria the value obtained from this study is lower than a study done in south-west Nigeria, where prevalence value of 28% was obtained by Babalola and Coker at Lagos [25]. This suggests a successful decline in *H. influenzae* meningitis in the country which could be attributed to active vaccination or successful antimicrobial therapy. Similarly, this value was lower than studies in East Africa. A value of 26% was documented in Kilifi district of Kenya [26]. The differences observed may be due to differences in the geographic and climatic conditions of the countries [25,26]. Also, in this study the age limit was restricted to under-five, whereas in the other studies, children aged five years and above were examined [26].

From this study, more than 60% of the isolates were obtained from older children within the age groups of 21-30, 31-40, and 41-50 months. No isolate was obtained from children within the 0-10 months age group. These findings were consistent with established that acquired maternal immunity wanes as the children grows older [2,27] and this was statistically significant.

From the study the pattern of antibiotics sensitivity of isolates to antibiotics tested was consistent with other findings [23,27]. The susceptibility of all the isolates to ceftriaxone and high resistance to ampicillin agrees with study by Enting and colleagues in the Netherlands [11]. Most of the isolate were β-lactamases producers, a direct test for ampicillin resistance. The low MIC of ceftriaxone to the isolate was consistent with other findings and significantly shows that use of ceftriaxone against *Haemophilus influenza* was appropriate and should be encouraged in clinical practice.

5. CONCLUSION

Haemophilus influenzae causes largely invasive life-threating meningitis and pneumonia in under-five children and this condition has largely been caused by the encapsulated form. There has been ongoing *Haemophilus influenzae* type-b vaccination of children in the country however, there is a strong association betweenunder-five children and *H. influenzae* meningitis. This suggests the need to strengthen Hib vaccination in children. More so majority of the *Haemophilus influenzae* isolates were ampicillin resistance, β-lactamases producers and ceftriaxone susceptible. The outcome of this study reveals and supports the vital role ceftriaxone in the management of invasive *H. influenzae* infections to avoid pathologic complications.

CONSENT

The purpose of this work was explained to the parents before their consent to participate was sought. The consent form was filled by the investigator and the parent or guardian of each child signed the form.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the ethical committee of the Jos University Teaching Hospital and permission was sought from Bingham University Teaching Hospital.

ACKNOWLEDGMENT

The secretariat staff of Jos University Teaching Hospital, Aladelokun Bonuola and Folake Olabode for their technical services.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kayser FH. Haemophilus and Pasteurella. In: H. Fritz. M.D. Kayser, A. Kurt, J. Eckert, D.V.M. Kayser (eds). Medical Microbiology. 10th ed. Stuttgart Georg Thieme Verlag. 2005;300-301.

2. Mogens K. *Haemophilus*. In: E.H. Lennette, A. Ballows, WJ. Hausler., HJ. Shadomy (eds) Manual of clinical microbiology. 4th ed. Washington DC U.S.A.: American society for microbiology. 1985;387-392.
3. Daniel M.M. Bacteriology- *Haemophilus* species. Baron medical microbiology, 8th ed. London, Wright's books. 2000;724-739.
4. Eskola J, Käyhty H, Takala AK. A randomized, prospective field trial of a conjugate vaccine in the protection of infants and young children against invasive Haemophilus influenzae type b disease. N Engl J Med. 1990;323:1381.
5. St Geme JW, Takala A, Esko E, Falkow S. Evidence for capsule gene sequences among pharyngeal isolates of nontypeable *Haemophilus influenzae*. J Infect Dis. 1994;169:337.
6. Segada LM, Carlone GM, Gheesling LL, Lesse AJ. Characterization of P1-deficient isogenic mutant of *Haemophilus influenzae* biogroup aegyptius associated with Brazilian purpuric fever. Microb Pathog. 2000;28:145.
7. Quentin R, Musser JM, Mellouet M. Typing of urogenital, maternal, and neonatal isolates of Haemophilus influenzae and *Haemophilus parainfluenzae* in correlation with clinical source of isolation and evidence for a genital specificity of *H. influenzae* biotype IV. J Clin Microbiol. 1989;27:2286.
8. Wallace RJ Jr, Baker CJ, Quinones FJ. Nontypable *Haemophilus influenzae* (biotype 4) as a neonatal, maternal, and genital pathogen. Rev Infect Dis. 1983;5:123.
9. Moxon ER. Molecular basis of invasive *Haemophilus influenzae* type b disease. J Infect Dis. 1992;165(Suppl1):S77.
10. Daum R. *Haemophilus influenzae* infection. In: RE. Behrman, RM. Kliegman., HB. Jenson (eds). Nelson Textbook of Paediatrics. 17th ed. Philadelphia, W.B. Saunders. 2004;904-908.
11. Rao VK, Krasan GP, Hendrixson DR. Molecular determinants of the pathogenesis of disease due to non-typable Haemophilus influenzae. FEMS Microbiol Rev. 1999;23:99.
12. Krasan GP, Cutter D, Block SL, St Geme JW 3rd. Adhesin expression in matched nasopharyngeal and middle ear isolates of nontypeable *Haemophilus influenzae* from children with acute otitis media. Infect Immun. 1999;67:449.
13. Kubiet M, Ramphal R, Weber A, Smith A. Pilus-mediated adherence of *Haemophilus influenzae* to humanrespiratory mucins. Infect Immun. 2000;68:3362.
14. Mäkelä PH, Takala AK, Peltola H, Eskola J. Epidemiology of invasive *Haemophilus influenzae* type b disease. J Infect Dis. 1992;165(Suppl 1):S2.
15. MacNeil JR, Cohn AC, Farley M. Current epidemiology and trends in invasive *Haemophilus influenzae* disease--United States, 1989-2008. Clin Infect Dis. 2011;53:1230.
16. Cochi SL, Broome CV. Vaccine prevention of *Haemophilus influenzae* type b disease: past, present and future. Pediatr Infect Dis. 1986;5:12.
17. Ward JI, Fraser DW, Baraff LJ, Plikaytis BD. *Haemophilus influenzae* meningitis. A national study of secondary spread in household contacts. N Engl J Med. 1979;301:122.
18. Watt JP, Wolfson LJ, O'Brien KL. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. Lancet. 2009;374:903.
19. Adam HJ, Richardson SE, Jamieson FB. Changing epidemiology of invasive *Haemophilus influenzae* in Ontario, Canada: Evidence for herd effects and strain replacement due to Hib vaccination. Vaccine. 2010;28:4073.
20. Zuckerman JM. Macrolides and ketolides: Azithromycin, clarithromycin, telithromycin. Infect Dis Clin North Am. 2004;18:621.72.

21. Resman F, Ristovski M, Forsgren A. Increase of"-lactam-resistant invasive Haemophilus influenzae in Sweden, 1997 to 2010. Antimicrob Agents Chemother. 2012;56:4408.
22. Kenji G, Liang Q, Kiwao W. Prevalence of *Haemophilus influenzae* with resistance genes isolated from young culture with acute lower respiratory tract infection. Journal of infection and chemotherapy. 2008;14:349-353.
23. Enting RH, Spanjaard L, van de Beek D, Hensen EF, de Gans J, Dankert J. Antimicrobial susceptibility of *Haemophilus influenzae*, *Neisseria meningitidis and Streptococcus pneumoniae* isolates causing meningitis in The Netherlands. Journal of Antimicrobial Chemotherapy. 1996;38(5):777-786.
24. Ladhani S, Slack MP, Heath PT, ME. Ramsay. Changes in ascertainment of Hib and its influence on the estimation of disease incidence in the United Kingdom: Epidemiol. Infect. 2007;135:861–867.
25. Babalola AA, Coker AO. Pyogenic meningitis among Lagos children: Causative organisms, age, sex and seasonal incidence. Central Africa Journal of Medicine. 1982;28(1):14-18.
26. Abdullahi O, Nyiro J, Lewa P, Slack M. The descriptive epidemiology of *Streptococcus pneumoniae* and *Haemophilus influenzae* nasopharyngeal carriage in children and adults in Kilifi District, Kenya. Paediatric Infectious Disease Journal. 2008;27(1):59–64.
27. WHO. *Haemophilus influenzae* type b in Africa.
Available: http://www. Hibaction.org/resources/HibinAfrica. Nov 2006. Last updated Feb 2009. Accessed 5th May 2010.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=581&id=12&aid=5086