

## Full Length Research Paper

## Antibiotic susceptibility pattern of bacterial isolates in children with otitis media in Zamfara, North-Western Nigeria

Bilkisu Ilah Garba<sup>1\*</sup>, Bello Alhaji Mohammed<sup>2</sup>, Fatima Mohammed<sup>3</sup>, Murtala Rabi<sup>4</sup>, Usman Mohammed Sani<sup>1</sup>, Khadija Omene Isezuo<sup>1</sup> and Usman Mohammed Waziri<sup>1</sup>

<sup>1</sup>Department of Paediatrics, Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria.

<sup>2</sup>Department of Obstetrics and Gynaecology, Ahmad Sani Yariman Bakura Specialist Hospital, Gusau, Zamfara State, Nigeria.

<sup>3</sup>Department of Paediatrics, Ahmad Sani Yariman Bakura Specialist Hospital, Gusau, Zamfara State, Nigeria.

<sup>4</sup>Laboratory Department, Ahmad Sani Yariman Bakura Specialist Hospital, Gusau, Zamfara State, Nigeria.

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Otitis media has been reported as a common childhood disease and is associated with multiple microbial pathogens within the middle ear. Though the causative agents of this infection have largely remained the same over the years, current literature reviews show that the antibiotic susceptibility pattern has changed considerably. This study aimed to identify bacterial isolates and determine their antibiotic susceptibility and resistance patterns in children who had otitis media. This is a retrospective analysis of ear swab microscopy, culture and sensitivity (MCS) results of children between the age of 0 and 13 years who presented with clinical episodes of otitis media over a four year period. A total of 53 results were retrieved from the laboratory record book of which 43 were culture positive giving a yield of 84.9%. Males were 33 (62.3%) giving a M:F ratio of 1.7:1 and 71.7% were below the age of 5 years. Majority (68.9%) of the isolates were Gram positive organisms with *Staphylococcus aureus* being the commonest bacteria isolated (53.3%), followed by *Pseudomonas aeruginosa* (20.0%) and then *Streptococcus pyogenes* (13.3%). The isolates were highly susceptible to ciprofloxacin, ofloxacin and gentamicin, while all were found to be resistant to tetracycline, cefixime and levofloxacin. *S. aureus*, *P. aeruginosa* and *S. pyogenes* were the commonest bacterial isolates in the patients. Ciprofloxacin, ofloxacin and gentamicin were the antibiotics with the highest bacterial susceptibility rate. Children with ear discharge were recommended to be investigated and treatment should be based on antimicrobial test to prevent resistance and probably complications.

**Key words:** Otitis media, bacterial isolates, antibiotic, susceptibility, resistance.

### INTRODUCTION

Otitis media which is inflammation of the middle ear cleft has been reported as a common childhood disease (Cripps and Kyd, 2003; Afolabi et al., 2012; Wasihun and Zemene, 2015; Ngo et al., 2016). It is associated with

multiple microbial pathogens within the middle ear (Ngo et al., 2016). It has a high incidence and prevalence in both developed and developing countries (Afolabi et al., 2012). It is classified as acute, chronic or recurrent and

can also be suppurative or non-suppurative. Children tend to have higher predisposition to ear infection than adults due to the anatomy of the Eustachian tube in children which permits easier access of organism through the nasopharynx (Afolabi et al., 2012).

Current literature reviews show that the causative agents of this infection have not changed much over the years but the antibiotics susceptibility pattern has changed (Nnebe-Agumedu et al., 2011). Normal skin flora such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* which can enter through a perforated ear have been implicated as common organisms causing otitis media (Wasihun and Zemene, 2015).

Several studies have been conducted on antibiotic susceptibility and resistant pattern of bacterial pathogens causing otitis media in various parts of Nigeria: Abakaliki (Nnebe-Agumedu et al., 2011); Ilorin (Afolabi et al., 2012); Zaria (Jido et al., 2014); and Awka (Ejiofor et al., 2016). However, there is no published data from Gusau, Zamfara State North-Western Nigeria to guide empiric antibiotic choice and prompt treatment. Hence, the aim of this current study is to fill the existing knowledge gap.

This study aimed to identify bacterial isolates and determine their antibiotic susceptibility and resistance patterns in children who had otitis media.

## MATERIALS AND METHODS

### Study design, site, and data collection

This was a retrospective analysis of ear swabs MCS results of children aged 0 to 13 years with otitis media irrespective of the subtype seen at the Ahmad Sani Yariman Bakura Specialist Hospital (ASYBSH), Gusau, Zamfara State, North-Western Nigeria. The study was conducted over a four year period between May 2013 and April 2017. Patient information registered in the microbiology laboratory unit's registration books were collected which included patient's age, gender, type of organism isolated from the ear discharge as well as pattern of antibiotic susceptibility and resistance.

### Isolation and identification of bacteria in the laboratory of ASYBSH

Although the study is retrospective, methods of isolation and identification of bacteria from ear swab samples in the laboratory is briefly discussed. All collected specimen were subjected to Gram stain and were inoculated on blood, chocolate and MacConkey agar plates. The plates of blood and MacConkey were placed in an aerobic incubator while the chocolate plate was incubated in a carbon-dioxide enriched atmosphere for 18 to 24 h after which the plates were read and isolates identified according to standard bacteriological methods. Zone diameters of inhibition around each disc for susceptibility testing were measured using a calibrated ruler

and interpreted according to Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2014, 2015).

### Antibiotic susceptibility testing

*S. aureus* (ATCC 25922), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC2785) were used as controls for Gram positive, Gram negative, and *P. aeruginosa* isolates, respectively. Disk diffusion assay was performed to assess the antibiotic susceptibility and resistance pattern of bacterial isolates. Selection of these antibiotics was based on availability of the discs and the frequently used antibiotics in the country. Two types of discs were used in the laboratory during the study period which were Abtek Biological Ltd. disc one and Himedia disc. Abtek Biological Ltd. disc one (as written by manufacturer) consisted of ceftriaxone (30 µg), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg), and ciprofloxacin (5 µg). While Himedia disc (as written by manufacturer) consisted of ceftriaxone (30 µg), gentamicin (10 µg), co-trimoxazole (25 µg), levofloxacin (5 µg), neticillin (30 µg), tetracycline (30 µg), amoxycylav (30 µg) and ofloxacin (5 µg). For the purpose of the study, results of augmentin and amoxycylav would be reported as augmentin (both contain amoxycillin clavulanic acid).

Isolated bacteria were reported as susceptible or resistant to the antibiotics according to CLSI (2014, 2015) guidelines. The bacteria was defined as being multidrug resistant if it is resistant to three or more of the antibiotic agents tested and based on the antibiotic categories (Magiorakos et al., 2012).

### Ethical approval

Ethical approval for the study was obtained from the ethical committee of the ASYBSH, Gusau, Zamfara State.

### Statistical analysis

The data obtained were checked, entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 20 (Chicago Illinois). Continuous variables were summarized using mean and standard deviation. Descriptive statistics was used to describe the frequency of categorical variables and are presented in tables. The Fisher's exact test was used to measure the association between qualitative variables. A p value of <0.05 was considered to be statistically significant.

## RESULTS

A total of 53 results were retrieved from the laboratory record book of which 43 were culture positive giving a yield of 84.9%. Males constituted 33 (62.3%) while females were 20 (37.7%) giving a M:F ratio of 1.7:1, however, this was not significant (Fishers exact=0.162). The mean age was 3.05 ± 0.36 years, with a range of 10 days to 13 years. Majority of the children were aged five years and below as shown in Table 1.

\*Corresponding author. E-mail: bgilah@yahoo.com. Tel: +2348036782582.

**Table 1.** Age distribution of study population.

| Age range (years) | Frequency (n) | Percentage |
|-------------------|---------------|------------|
| 0 - <5            | 38            | 71.7       |
| 5.0 - <10.0       | 10            | 18.9       |
| 10.0 - 15.0       | 5             | 9.4        |
| Total             | 53            | 100.0      |

**Table 2.** Distribution of the isolated bacteria according to age range.

| Bacteria                        | 0-5 years [n (%)] | 6-10 years [n (%)] | 10-15 years [n (%)] | Total (%)  |
|---------------------------------|-------------------|--------------------|---------------------|------------|
| <i>Staphylococcus aureus</i>    | 18 (40.0)         | 4 (8.9)            | 2 (4.4)             | 24 (53.3)  |
| <i>Pseudomonas aeruginosa</i>   | 5 (11.1)          | 2 (4.4)            | 2 (4.4)             | 9 (20.0)   |
| <i>Streptococcus pyogenes</i>   | 5 (11.1)          | 1 (2.2)            | 0 (0.0)             | 6 (13.3)   |
| <i>Escherichia coli</i>         | 2 (4.4)           | 1 (2.2)            | 0 (0.0)             | 3 (6.7)    |
| <i>Streptococcus pneumoniae</i> | 1 (2.2)           | 0 (0.0)            | 0 (0.0)             | 1 (2.2)    |
| <i>Proteus vulgaris</i>         | 1 (2.2)           | 0 (0.0)            | 0 (0.0)             | 1 (2.2)    |
| <i>Neisseria meningitides</i>   | 1 (2.2)           | 0 (0.0)            | 0 (0.0)             | 1 (2.2)    |
| Total                           | 33 (73.3)         | 8 (17.8)           | 4 (8.9)             | 45 (100.0) |

Fishers exact p=0.882.

**Table 3.** Distribution of the isolated bacteria according to gender.

| Bacteria                        | Male [n (%)] | Female [n (%)] | Total (%)  |
|---------------------------------|--------------|----------------|------------|
| <i>Staphylococcus aureus</i>    | 16 (35.6)    | 8 (17.8)       | 24 (53.3)  |
| <i>Pseudomonas aeruginosa</i>   | 6 (13.3)     | 3 (6.7)        | 9 (20.0)   |
| <i>Streptococcus pyogenes</i>   | 4 (8.9)      | 2 (4.4)        | 6 (13.3)   |
| <i>Escherichia coli</i>         | 3 (6.7)      | 0 (0.0)        | 3 (6.7)    |
| <i>Streptococcus pneumoniae</i> | 0 (0.0)      | 1 (2.2)        | 1 (2.2)    |
| <i>Proteus vulgaris</i>         | 0 (0.0)      | 1 (2.2)        | 1 (2.2)    |
| <i>Neisseria meningitides</i>   | 0 (0.0)      | 1 (2.2)        | 1 (2.2)    |
| Total                           | 29 (64.5)    | 16 (35.5)      | 45 (100.0) |

Fisher's exact p=0.369.

The predominant isolates were Gram positive which were 31 (68.9%), while Gram negative organisms were 14 (31.1%). *S. aureus* was the commonest bacteria isolated, followed by *P. aeruginosa*, and then *S. pyogenes*. There were no mixed isolates found. Table 2 shows the distribution of the bacterial isolates according to age range with majority of the isolates seen in children of 5 years and below. Table 3 shows the distribution of the bacterial isolates according to gender, however, there was no significance

Table 4 shows the antimicrobial susceptibility pattern of the bacterial isolates. Ciprofloxacin, ofloxacin and gentamicin were the antibiotics with the highest bacterial susceptibility of about 50%. The bacterial isolates were found to be resistant to tetracycline, cefixime and levofloxacin.

*S. aureus* and *P. aeruginosa* were most susceptible to ciprofloxacin, gentamicin and ofloxacin. The highest susceptibility rate of 83.3% was observed with *Streptococcus pyogenes* to ofloxacin. *S. pyogenes*, *E. coli* and *S. pneumoniae* were found to be multidrug resistant, while *Proteus vulgaris* and *Neisseria meningitides* were resistant to all the antibiotics.

## DISCUSSION

This is the first reported study on bacterial isolates from discharging ears of children in Gusau, Zamfara State, North-Western Nigeria. The laboratory record of only 53 samples over a four year period reflects the lack of request by some doctors to identify the bacterial isolate

**Table 4.** Antibiotic susceptibility pattern of the isolated bacteria.

| Antibiotic | Total bacterial isolates [No (%)] |                          |                          |                      |                          |                              |                            | Total (n=45) |
|------------|-----------------------------------|--------------------------|--------------------------|----------------------|--------------------------|------------------------------|----------------------------|--------------|
|            | <i>S. aureus</i> (n=24)           | <i>Pseudomonas</i> (n=9) | <i>S. pyogenes</i> (n=6) | <i>E. coli</i> (n=3) | <i>P. vulgaris</i> (n=1) | <i>N. meningitidis</i> (n=1) | <i>S. pneumoniae</i> (n=1) |              |
| GEN        | 12 (50.0)                         | 6 (66.7)                 | 3 (50.0)                 | 1 (33.3)             | 0 (0.0)                  | 0 (0.0)                      | 1 (100.0)                  | 23 (51.1)    |
| OFL        | 12 (50.0)                         | 5 (55.6)                 | 5 (83.3)                 | 1 (33.3)             | 0 (0.0)                  | 0 (0.0)                      | 1 (100.0)                  | 24 (53.3)    |
| AUG        | 11 (45.8)                         | 2 (22.2)                 | 3 (50.0)                 | 0 (0.0)              | 0 (0.0)                  | 0 (0.0)                      | 1 (100.0)                  | 17 (37.8)    |
| CTZ        | 6 (25.0)                          | 1 (11.1)                 | 2 (33.3)                 | 0 (0.0)              | 0 (0.0)                  | 0 (0.0)                      | 0 (0.0)                    | 9 (20.0)     |
| CIP        | 15 (62.5)                         | 7 (77.8)                 | 1 (16.7)                 | 1 (33.3)             | 0 (0.0)                  | 0 (0.0)                      | 1 (100.0)                  | 25 (55.6)    |
| CRX        | 5 (20.8)                          | 2 (22.2)                 | 2 (33.3)                 | 0 (0.0)              | 0 (0.0)                  | 0 (0.0)                      | 0 (0.0)                    | 9 (20.0)     |
| CEF        | 1 (4.2)                           | 1 (11.1)                 | 0 (0.0)                  | 0 (0.0)              | 0 (0.0)                  | 0 (0.0)                      | 0 (0.0)                    | 2 (4.4)      |
| LEV        | 4 (16.7)                          | 0 (0.0)                  | 0 (0.0)                  | 0 (0.0)              | 0 (0.0)                  | 0 (0.0)                      | 0 (0.0)                    | 4 (8.9)      |
| NET        | 1 (16.7)                          | 1 (11.1)                 | 1 (16.7)                 | 0 (0.0)              | 0 (0.0)                  | 0 (0.0)                      | 0 (0.0)                    | 3 (6.7)      |
| TCN        | 0 (0.0)                           | 0 (0.0)                  | 0 (0.0)                  | 1 (33.3)             | 0 (0.0)                  | 0 (0.0)                      | 0 (0.0)                    | 1 (2.2)      |
| NIT        | 7 (29.2)                          | 7 (77.8)                 | 0 (0.0)                  | 0 (0.0)              | 0 (0.0)                  | 0 (0.0)                      | 1 (0.0)                    | 9 (20.0)     |
| CTX        | 1 (4.2)                           | 1 (11.1)                 | 0 (0.0)                  | 1 (33.3)             | 0 (0.0)                  | 0 (0.0)                      | 0 (0.0)                    | 3 (6.7)      |

No: Number; %: percentage; GEN: gentamicin; OFL: ofloxacin; AUG: augmentin; CTZ: ceftriaxone; CIP: ciprofloxacin; CRX: cefuroxime; CEF: cefixime; LEV: levofloxacin; NET: neticillin; TCN: tetracycline; NIT: nitrofurantoin; CTX: co-trimoxazole.

causing ear discharge. This highlights the empiric treatment of otitis media since ear discharge is a common presentation in children. Another reason for the low number of ear discharge samples could be due to lack of an ear, nose and throat specialist and clinic in our hospital; hence, children with otitis media are more likely to seek medical attention at a tertiary hospital in the state where such services are available.

Majority of the children were below five years of age which is similar to the findings in Abakaliki (Nnebe-Agumede et al., 2011), Zaria (Jido et al., 2014), Rabat (Es-Said et al., 2014), and Awka (Ejiofor et al., 2016). There were more males in the study although not significant, which was similar to the findings in Abakaliki (Nnebe-Agumede et al., 2011), Ilorin (Afolabi et al., 2012), and other studies (El-Manama et al., 2014; Garima et al., 2016). This is in contrast to the findings in Ife (Ako-Nai et al., 2002), Iraq (Al-Marzoqi et al., 2013), and Zaria (Jido et al., 2014) where they found more females than males having otitis media. Reason for the disparity may be due to age differences and location of the studies.

Our culture positivity rate of 84.9% was high similar to 87.7% obtained in Abakaliki (Nnebe-Agumede et al., 2011) and 83.6% in North-Eastern Ethiopia (Argaw-Denboba et al., 2016). It was however lower than 96.4% from Iraq (Al Marzoqi et al., 2013) and 98.2% reported from Northern Ethiopia (Wasihun and Zemene, 2015), but higher than 7.4% reported from Colombia (Trujillo et al., 1989), 62% from a multinational study (Jacobs et al., 1998), 64% from Mexico (Parra et al., 2011), 75% from Awka (Ejiofor et al., 2016), and 79.7% from India (Garima et al., 2016). Reasons for these disparities could be attributed to difference in methodology, sample size and

prior use of antibiotics.

Majority of the isolates were Gram positive organisms similar to the findings in Ile-Ife (Ako-Nai et al., 2002) and Morocco (Es-Said et al., 2014), but contrasting findings from Abakaliki (Nnebe-Agumede et al., 2011), Ilorin (Afolabi et al., 2012), Zaria (Jido et al., 2014), and other studies (Wasihun and Kyd, 2003; Al Marzoqi et al., 2013; Elmanama et al., 2014; Argaw-Danboba et al., 2016). This could be explained by the fact that different pathogens cause otitis media in different geographic localities.

*S. aureus* was the commonest isolate in our study similar to the findings by Iraq (Al-Marzoqi et al., 2013) and Ethiopia (Wasihun and Zemene, 2015). *P. aeruginosa* was the commonest Gram negative isolate similar to findings in many studies (Nnebe-Agumede et al., 2011; Afolabi et al., 2012; Jido et al., 2014; Es-Said et al., 2014; Elmanama et al., 2014; Wasihun and Zemene, 2015; Garima et al., 2016; Ejiofor et al., 2016).

Even though *S. pneumoniae* is a common bacteria isolated as a cause of otitis media in some studies (Jacobs et al., 1998; Commisso et al., 2000; Rosenblut et al., 2001; Kilpi et al., 2001; Aquilar et al., 2009; Pumarole et al., 2013; Al Marzoqi et al., 2013; Wasihun and Zemene., 2015; De-Antonio et al., 2016); this study identified the organism in only 2.2% which is higher than 0.9% obtained in Iraq (Al Marzoqi et al., 2013).

This study did not isolate *Haemophilus influenzae* probably due to the availability of the vaccination incorporated into the immunisation schedule and majority of the children are below the age of five whom may have been vaccinated against the organism. The organism has been shown to be the most prevalent aetiological agent in Colombia (Trujillo et al., 1989), Argentina (Commisso et

al., 2000), and Mexico (Parra et al., 2011).

The antibiotics with the highest bacterial susceptibility rate in this study were ciprofloxacin, ofloxacin and gentamicin; this is in line with some studies conducted both in Nigeria and other countries (Mansoor et al., 2009; Afolabi et al., 2012; Nnebe-Agumedu et al., 2011; Jido et al., 2014; Elmanama et al., 2014; Wasihun and Zemene., 2015). However, the highest susceptibility pattern was seen in ofloxacin against *S. pyogenes*. In the report by Ako-Nai et al. (2002), all their isolates were susceptible to ofloxacin. *P. aeruginosa* was found to be the only isolate with some susceptibility to almost all the antibiotics ranging from 11.1 to 77.8%. The bacteria have been reported as a multidrug resistant strain from Ile-Ife (Ako-Nai et al., 2002) and Gaza (Elmanama et al., 2014). The present study found *P. vulgaris* and *N. meningitides* were resistant to all the tested antibiotics, this has not been reported by other studies, the reason could be due to infection by multidrug resistant strains of the organisms.

Various susceptibility and resistance patterns were observed with other antimicrobials similar to what was found by other authors. Even though augmentin and cefixime are antibiotics commonly prescribed for otitis media, most of our isolates were found to be resistant to them. Hence, it is imperative to investigate children with ear discharge so as to reduce the rate of development of complications, treatment failure or extra expenses of buying various medications by the parents or care givers.

## CONCLUSION AND RECOMMENDATIONS

*S. aureus*, *P. aeruginosa* and *S. pyogenes* were the most common organisms in this study. Ciprofloxacin, ofloxacin and gentamicin were the antibiotics with the highest bacterial susceptibility rate.

It is recommended in this study that children with ear discharge be investigated and treatment should be based on antimicrobial test to prevent resistance and probably complications.

## LIMITATIONS

Due to the retrospective nature of this study, there was limitation of describing the various types of otitis media, clinical characteristics of the patients and prior antibiotic usage.

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