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Antibacterial susceptibility spectrum of some gram negative bacteria from suspected Otitis media patients

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Otitis media (OM) is a multifactorial disease characterized by high rate of recurrence in young children and considered to cause hearing impairment in children. This study was undertaken to evaluate the antibacterial sensitivity of Gram-negative bacteria of OM to some antibiotics. A total of Fifty four (54) samples were obtained from patients with suspected cases of otitis media coming into the National Ear Care Centre for the first time. Fifty four (54) patients (29 male, 25 female), with mean ages of 3.86 and 3.29 respectively, tested positive to OM pathogens with a total of 84 bacterial isolates while no culturable pathogen was observed in 4 patients (7.4%). The study reveals highest frequency of *Pseudomonas aeruginosa* 60 (71.43 %) followed by *Proteus mirabilis* 14 (16.67), *Klebsiella pnuemoniae* 6 (7.14) and *Escherichia coli* 4 (4.76). *P. aeruginosa* had the highest prevalence among patients < 30 years, whereas *K. pnuemoniae* and *E. coli* had the least isolated across all age groups. Antimicrobial susceptibility test showed highest frequency of resistance among all isolates to amoxicillin, cotrimoxazole, nitrofurantoin and nalidixic acid. However, gentamicin, ofloxacin, augmentin and tetracycline were effective against *Pseudomonas aeruginosa* but ineffective against other isolates. Although antibiotics are the most preferred and prescribed drugs in incidents of OM, it is clear from this study that antimicrobial resistance still remains a persistent among bacterial pathogens of otitis media.

Key words: Otitis media, antibiotic susceptibility, bacteria isolates.

INTRODUCTION

The incidence and chronicity of otitis media is increasing and likewise the effort in the design and development of both topical and systemic antibiotics due to the emerging resistance of bacteria to antimicrobial agents. Otitis media is associated with the inflammatory conditions of the mucosal lining of the middle ear resulting from middle ear microbial infection. The distinguishing features in the structure and anatomy of the ears, the length of the Eustachian tube made up of more flaccid cartilage and its anterior positioning, as well as compromised immunity;

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accounts for the higher prevalence of otitis media in children than in adults (Alsaimary et al., 2010; Weiner and Collison, 2003).

Factors that promote greater adhesion of bacteria to epithelial cells in children than adults includes; ignorance, poverty, poor environmental and personal hygiene. Similarly, socioeconomic burden, anthropogenic activities, as well as poor crowd control, non-compliance to treatment and inadequate health care diagnostics all represent possible predisposing and a perpetuating risk factors for the higher prevalence of otitis media in some developing and less developed countries (Kumar and Seth, 2011; Li et al., 2001; Pereira et al., 2004). Other burden of individual risk factors have been highlighted to relate to the interplay between the microbial load in the middle ear fluid and host factor immune response as well as the health-related behaviour of parents in the care of their young ones (Haggard, 2008; Rovers, 2008).

Researches have revealed OM to constitute an infection of the middle ear with manifestation of complex infective and inflammatory conditions. Based on differences in their presentation and treatment, OM are grouped as acute OM (AOM), OM with effusion (OME), chronic suppurative OM (CSOM) and cholesteatoma with associated complications as mastoiditis, meningitis, brain abscess formation, and sigmoid sinus thrombosis (Qureishi et al., 2014).

Dysfunction of the Eustachian tubes and bacterial infection are major relevant factors for chronic OM with gram-negative bacteria infecting the ear canal. However, fungi and viruses are persistent in some cases (Osazuwa et al., 2011). The normal flora of the skin also causes OM infection, with microbial pathogens gaining entry through acute perforation of the middle ear cleft via the Eustachian tube. Most frequently isolated pathogens associated with chronic and mixed infections in OM patients includes; *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, Staphylococcus pseudintermedius, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae*, *Escherichia coli*, *Pasturella* spp., *Citrobacter* spp., *Enterobacter* spp and *Chlamydia pneumoniae* (Abera and Kibret, 2011; Block 1997). Among the causative pathogens, *E. coli, Klebsiella* spp. and *P. aeruginosa,* are the most resistant and prevalent. However, different antibiotics have shown to have an effective spectrum of activity towards both Gram-negative and Gram-positive pathogens (Sharma et al., 2004). Increasing frequency of bacterial resistance to antibiotics due to indiscriminate administration and use of antibiotics, poor surveillance of patients, inadequate clinical assessment and evaluation and resource-limited settings with inadequate infection control measures have resulted in creating concerns in the management strategy of OM infections.

The rural or grassroots communities in Nigeria have recorded large tales of OM infection.

The *in vitro* antibiotic activity against these causative pathogens and the antibiotic regimen are important in the choice of antibiotics for treatment of OM (Block, 1997).

Dearth of health personnel, inadequate primary health stations and poor diagnostic laboratories are factors militating against efficient care for OM patients in the rural and urban areas in Nigeria. Most cases in northern Nigeria involve prescription of antibiotics empirically to patients with OM in outpatient clinics or patent medicine stores without microbiologic evaluation (Musa et al., Bemu et al., 2015). The National Ear Care Centre Kaduna established to treat cases of OM patients in both rural and urban areas.

This study was undertaken to evaluate the antimicrobial sensitivity of some bacterial pathogens of otitis media among some patients without any record of treatment possible at the National Ear Care Centre, Kaduna, Nigeria.

MATERIALS AND METHODS

Specimen collection and sample size

The study population consisted of 54 patients (29 males and 25 females) between the ages of 0 and 69 years, visiting the clinic for the first time with cases of middle ear infection/or acute otitis media or any complaints of ear problems from June, 2014 to November, 2014. Randomized sampling design was employed. Total of 54 samples of aural discharge were collected with sterilized cotton swabs with the help of the attending physician. All clinical samples were transported in a Stuart transport medium to the microbiology laboratory of the Department of Microbiology Kaduna State University Nigeria within 1 h of collection. Ethical clearance was obtained from the National Ear care centre and with the informed consent of the patients and approval from the clinic management.

Isolation and identification of bacteria

Collected swabs were plated on MacConkey agar, blood agar and chocolate agar plates. MacConkey agar and blood agar were incubated aerobically, whereas chocolate plate was incubated anaerobically at 37°C for 24 h. Isolates were identified by colony morphology, gram staining reaction, catalase test, coagulase test, oxidase test, triple Sugar Iron agar (TSI), citrate utilization test, urease test, motility indole lysine (MIL) and methyl-Red Vogues Proskauer test (MR-VP) with glucose fermentation test (Cheesbrough 2006).

Antimicrobial susceptibility testing

Disk diffusion assay and agar plate dilution was performed according to NCCLS to assess theantibiotic resistance/susceptibility pattern of bacterial isolates. Bacterial suspension was prepared based on the McFarland standard solution 0.5 and inoculated onto Muller Hinton agar (Oxoid). For the disc diffusion technique, dics containing appropriate antibiotics (BBL^{TM} UK), selected among the most commonly used, both with and without prescription - (amoxicillin (30µg), cotrimoxazole (30 µg), nitrofurantoin (100 µg), nalidixic acid (30 µg), gentamicin (10 µ)ofloxacin (1 µg), augmentin (30 µg) and tetracycline (30 µg)) - were tested against each isolate.

Table 1. Distribution of positive suspected cases of OM among patients visiting a clinic, June to November 2014.

Ages (years)	Male	Female	Total
0-9	18	10	28
10-19	5	8	13
20-29	2	3	5
30-39	1	0	1
40-49	0	1	1
50-59	1	0	1
60-69	0	1	1
Total	**27 (54%)**	**23 (46%)**	**50**

Results were reported as susceptible, intermediate or resistant, according to Clinical Laboratory Standards Institute's (CLSI 20) guidelines.

Data analysis

SPSS version 22 software was used for ANOVA Analysis of data and p value of <0.05 was considered statistically significant.

RESULTS

A total of 54 patients, representing 30 males and 24 females with age group of 0 to 69 years, and mean age (35.28, 3.85±6.4 SD and 3.28±4.0 male and female respectively) were investigated in this study. Based on the cultured swab, a total of 50 (92.59%) patients were suspected to have positive cases of OM while 4 (7.4 %) were negative (Table 1). 84 total isolates were obtained and *P. aeruginosa* was the most prevalent, representing 71.43%, *P. mirabilis* 14 (16.67%), *K. pneumoniae* 6 (7.14%) and *E. coli* 4 (4.76%) (Table 2). No significant difference between the genders in terms of cases recorded for OM among those attending the clinic, although there was a male predominance (27 (54%)) over females (23 (46 %)) in terms of positive cases based on the isolation pattern of bacterial isolates. Patients under the age of 20 years had higher identifiable cases recorded (Table 3). Lower incidence of positive cases was recorded for patients above 30 to 70 years.

Antimicrobial susceptibility

The susceptibility pattern of different bacterial isolates tested against eight antibiotics, gentamicin, augmentin, tetracycline, ofloxacin, nitrofurantoin, amoxicillin, nalidixic acid and cotrimozaxole (Table 4) revealed *P. mirabilis, E. coli and K. pneumoniae* have the highest resistance profile (64.24, 125 and 99%, respectively) as compared to other bacterial isolates. About 100% of all isolates were resistant to amoxicillin and cotrimoxazole, while isolates showed sensitivity towards nitrofurantoin, nalidixic and gentamicin (64.3, 32.14 and 104.7, respectively). Only *P. aeruginosa* showed sensitivity to augmentin and tetracycline. *P. mirabilis* isolates showed higher frequency of resistance (100%) to amoxicillin, cotrimoxazole, augmentin and tetracycline. With regards to *E. coli* all isolates showed 100% resistance to amoxacillin, cotrimoxazole, augmentin and tetracycline.

Table 2. Prevalence of bacterial agents of otitis media. among patients visiting a clinic, June to November 2014.

Organisms isolated	Number of isolates (%)
Pseudomonas aeruginosa	60 (71.43)
Proteus mirabilis	14 (16.67)
Klebsiella pneumoniae	6 (7.14)
Escherichia coli	4 (4.76)
Total	**84**

DISCUSSION

The care of the ear is sacrosanct for the overall well-being of every individual. Hearing impairment is imminent in almost all age groups in some parts of Nigeria where lack of medical outreach, poor nutrition and awareness have hampered positive reactions from infected individuals.

In this study, confirmed cases of OM were observed among patients under the age of 30 years. Although children under the age of 10 are mostly vulnerable to OM, studies have revealed that the majority of affected age groups with OM are below 30 years.

Fifty four (54) cases investigated in the study against the 100 cases in the study by Kumar and Seth (2011) suggest a rational observation that adults are also as vulnerable to OM infections as children because all are confronted with similar predisposing factors. Higher number of bacterial isolates were from children under the age of 20 years in contrast to that recorded by Wasihun and Zemene (2015) where higher isolates came from children under age 0 to 5 years; although the study took into account the various stages of infection, not considered in this study.

Pseudomonas aeruginosa was the most isolated pathogen in almost all samples. As often found in all cases of chronic otitis media, *P. aeruginosa* is the most commonly isolated infective pathogen (Adoga et al., 2011; Nwabuisi and Ologe, 2002). The preponderance of *P. aeruginosa* among patients of all sexes and almost all age groups, suggests the diversity of its aetiology across geographical region as a predominant causative agent of otitis media as established in other reported findings across Nigeria and other parts of the world (Kumar et al.,

Table 3. Distribution of bacterial isolates among different age groups of patients visiting a clinic, June to November 2014.

Ages (years)	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Escherichia coli*	*Klebsiella pneumoniae*
0-9	30	9	4	3
10-19	11	3	2	1
20-29	8	2	0	0
30-39	6	0	0	0
40-49	3	0	0	0
50-59	2	0	0	0
60-69	0	0	0	0
Total	**60**	**14**	**6**	**4**

Table 4. Antibiotic sensitivity pattern of bacteria isolates among patients visiting clinic June to November 2014.

Antibiotics	*P. aeruginosa* (%) (%)	*K. pneumonia e* (%)	*E. coli* (%)	*P. mirabilis* (%)
Amoxicillin	0	0	0	0
Cotrimoxazole	1.6	0	0	0
Nitrofurantoin	0	50	0	14.3
Nalidixic acid	0	0	25	7.14
Gentamicin	25	33.3	25	21.4
Ofloxacin	26.6	16.6	75	21.4
Augmentin	3.3	0	0	0
Tetracycline	22	0	0	0

2013; Madana et al., 2011; Orji and Dike, 2015). Although not isolated in this study, infective pathogens such as *S. aureus* have also been isolated as predominant next to *P. aeruginosa* bacterial agent of OM (Prakash et al., 2013; Sharma et al., 2004).

The higher incidence of OM recorded in the study correlates with other reported incidence whereby male predominance is quite appalling than in female (Abubakar et al., 2014; Kumar et al., 2013). No rational explanation have been given for the variation in such prevalence, but it is asserted that over 80% of male experience at least one episode of AOM with concomitant hearing impairment at some stage, which impedes performance (Sambo et al., 2015).

Proteus mirabilis was higher in samples collected from patients between the age of 0 and 9 years and the most prevalent following *P. aeruginosa*, which is in tandem with previous findings by Kumar et al. (2013). Coliforms such as *K. pneumoniae* and *E. coli* were isolated from patients under age 20 with 11.9% occurrence. These pathogens have ubiquitous presence especially in faecal contaminated environment. Their widespread presence in almost all cases reported for OM suggests that they may have a greater pathogenic potential and it is a reflection of their relative high numbers due to poor environmental hygiene. Although the frequency of the isolated coliforms is lower than that reported in other findings, it has also been reported to be the second most commonly isolated pathogenic agent among patients with chronic Otitis media (Afolabi *et al.,* 2012).

Similar study from the National Ear Care Centre revealed the predominance of *K. pneumoniae* and *E. . coli* as the most isolated among patients within the period under review (Bakari et al., 2011). This variation in its prevalence is attributable to the large number of patients investigated in contrast to that reported for this study. *Pseudomonas* sp and *Klebsiella* sp have also been isolated among patients with otitis externa in a recent study from the same clinic (Musa et al., 2015).

The leading role of the gram-negative bacteria such as *Pseudomonas* sp. *Klebsiella* sp. *E. coli*, and *P. mirabilis* in chronic otitis media, makes the choice of the correct antibiotics essential for treatment crucial (Adoga et al., 2011; Wasihun and Zemene, 2015). In most incidences, self-medication and unregulated dosage of antibiotics without laboratory counsel creates problems for treatments since prior attempt by patients to initiate antibiotics therapy can cause irreversible modifications to the pathogenic flora leading to changing patterns in their susceptibility to antibiotics (Park et al., 2008).

In-vitro drug sensitivity pattern of all isolates shows a very disturbing trend of resistance against tested antibiotics. Antimicrobial sensitivity test (AST) based on some selected antibiotics showed gentamicin, ofloxacin

and tetracycline to some extent effective against *P. aeruginosa*. ,Whereas Alsaimary et al. (2010) reported the effectiveness of amoxicillin to *P.aeruginosa*, which contrasts with the observation made in this study. Similarly, only gentamicin and ofloxacin were effective against *P. mirabilis*, *K. pneumoniae* and *E. coli*. Sensitivity pattern for other groups of antibiotics used in this study revealed higher resistance with *K. pneumoniae* and *E. coli* being the least sensitive in contrast to findings by Orji and Dike (2015).

Arbitrary selection of antibiotics in this study was based on the emerging concern on the diversity of microbial profile and AST pattern of most pathogens implicated in COM. Among the commonly available ones used as topical ear drops and investigated in this study, gentamicin was the only effective antibiotics against all isolated pathogens, which is reported to be effective against some commonly isolated organisms in the treatment of COM (Prakash et al., 2013). Arguably, it is proposed with good reasons why gentamicin could be toxic to patients with renal impairment (Ogbogu et al., 2013).

In most, cases, single or multiple antibiotic therapies are administered and the pattern of administration could be oral, parenteral and topical.

Amoxicillin, tetracycline and cotrimoxazole are the most commonly prescribed categories of antibacteria done singly or in combination. Other classes of prescribed antibiotics constitute the quinolones, penicillins and the nitroimidazoles with oral and topical quinolones administered to children (Abubakar *et al.,* 2014; Ain *et al.,* 2010).

In this study, these antibiotics were not effective against all isolates except for tetracycline with a descriptive pattern of sensitivity against 22% of *P. aeruginosa*. The high frequency of resistance displayed by *P. aeruginosa* and other isolates against tested antibiotics can indeed be ultimately linked to the notoriety of some pathogens in developing resistance when prescribed standards for administration of antibiotics are not adhered to (Okeke et al., 1999).

Similar study conducted recently, showed 78.3% of all gram-negative isolates were multidrug resistant and hence not sensitive to all antibiotics tested (Wasihun and Zemene, 2015). This report is also in tandem with this study findings where, although no MDR test was conducted, isolates showed resistance to almost all tested antibiotics. The variation in resistance for all isolates as reported in these findings underscores the task of selecting appropriate antibiotics for treatment since antibiotics seem to be most beneficial in acute otitis media (Rovers et al*.,* 2006).

Consequently, properties that confer resistance to most gram-negative bacterial pathogens such as membrane impermeability and the apparent persistence of pathogens in middle ear fluid causes higher rates of clinical OM that are mostly difficult to be eradicated by initial antibiotic treatment (Leibovitz, 2008). The half-life of these antibiotics found in the environment where these pathogenic bacteria are transmitted via contamination, lingers for a long time after treatment thereby altering the microbial ecology in terms of resistance and bacterial susceptibility (Levy, 2002).

Conclusion

This study reports the antibacterial prevalence of OM infection cases among patients without previous records with the Ear Care Centre. The findings provide evidence that indeed OM persists among most children and adults below 30 years. Despite the high frequency of resistance based on the susceptibility profile of isolates, antibiotics such as gentamicin, ofloxacin and tetracycline remains first line of treatment as recorded in this study. These antibiotics remain beneficial in reducing the risks of hearing impairment and bacteriological eradication in the prevention of subsequent OM episodes. However, our study have also shown that resistance to some antimicrobial drugs by pathogens may develop. Reduction in the indiscriminate use of such antimicrobial agents by creating awareness among patients and preparation of variable antibiotic concentrations may limit the development of resistant bacteria.

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Conflict of interests

The authors have not declared any conflict of interests.

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