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REGULAR ARTICLE

AETIOLOGIC AGENTS OF ACUTE OTITIS MEDIA (AOM): PREVALENCE, ANTIBIOTIC SUSCEPTIBILITY, β-LACTAMASE (βL) AND EXTENDED SPECTRUM β-LACTAMASE (ESBL) PRODUCTION

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ABSTRACT

Two hundred and seventy-two samples from patients with acute otitis media attending Ear, Nose and Throat clinics in Uyo and Ikot Ekpene were collected using sterile swab sticks between January 2009 and December, 2010. *In vitro* antibiotic susceptibilities of the isolates were evaluated using Kirby-Bauer technique. Beta-lactamase and extended spectrum beta-lactamase producers were determined using starch paper test, chromogenic cephalosporin test and double disc synergy test, respectively. The highest prevalence of AOM was observed in age group ≤ 10 years with 84 (30.9%) cases and lowest prevalence observed in age group ≤ 61 having 12 (4.4%) cases with significant difference in the prevalence of AOM between age groups ≤ 10 years and other age groups at ($P<0.5$). One hundred and sixty-five samples showed growth of single isolates, 69 (25.4%) showed growth of two isolates and polybacterial growth was present in 29 (10.7%). Bacteria isolated were *Staphylococcus aureus*, *Pseudomonas aureginosa*, *Proteus mirabilis, Streptococcus pneumoniae,* Coagulase negative *Staphylococcus* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Bacteriodes fragilis*. The isolates were highly sensitive to

moxifloxacin and levofloxacin. The starch paper test identified 152 (38.2%) β-lactamase producers, while β-lactamase enzyme was detected in 163 (41.0%) of isolates through chromogenic cephalosporin test, with no statistical difference at P>0.5 between the results obtained using the two methods. Thirty-three (33) of the 81 Gram negative bacilli were ESBL producers. Consequently, this study has updated data on the incidence of the AOM and also revealed the actual therapy.

Keywords: Otitis Media, Prevalence, Susceptibility, Betalactamase, EsBL

INTRODUCTION

Over two billion dollars of the annual cost of health care in the United States of America is attributable directly or indirectly to otitis media **(Bondy *et al*., 2000; Marcy *et al*., 2001).** where as in Africa, particularly in Nigeria the importance of otitis media has not been fully established despite the reports of its higher prevalence in the developing countries compared to advanced countries **(Acuin, 2007; Lasisi, 2008).** Otitis media is the infection associated with the malfunctioning or inflammation of the middle ear due to pathogenic micro-organisms that are resident in the middle ear **(Ekpo *et al*., 2009).** Sources of infection in otitis media is solely dependent on the route by which infection reaches the middle ear and the chief route by which this occurs is through the eustachian tube **(Daly, 1997; Akinjogunla and Eghafona, 2011).** In acute otitis media, the bacteria found in the middle ear include *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae* and *Moraxella catarrhalis***.** These are respiratory pathogens that may have been insufflated from the nasopharynx into the middle ear through the eustachian tube during bouts of upper respiratory infections **(Faden *et al*., 1994; Akinjogunla and Enabulele, 2010)**. Symptoms of upper respiratory infections are often associated with otitis media in 94% cases **(Arola *et al*., 1990)**

Recent development in the treatment of patients with acute otitis media include the evidence of the efficacy of antibiotics especially cephalosporins and newer topical flouroquinolones **(Bearden and Danziger, 2001; Loy *et al*., 2002).** The introduction of the fluoroquinolones about three decades ago provided clinicians with a class of broad spectrum agents applicable to a range of bacterial infections **(Hooper, 1995).** Flouroquinolone antibiotics target DNA gyrase and topoisomerase IV by disrupting DNA synthesis and

causing lethal double-strand DNA breaks during DNA replication **(Akinjogunla and Eghafona, 2011)**. The growing resistance to antimicrobial agents of all respiratory tract pathogens has made the management of acute otitis media more difficult (**Itzhak and Alan, 2005)**. The problems of antimicrobial resistance for medically important pathogens was first recognized in the 1950s with the emergence of multidrug resistant strains of *S. pneumoniae*, *S. aureus* and *E. faecalis* **(Atlas, 1997)**. Multidrug resistant bacteria in both the hospitals and community environments are important concern to the clinician, as it is the major cause of failure in the treatment of infectious diseases, increased morbidity, mortality and the evolution of pathogens **(Hacker *et al*., 1997; Jones and Phaller, 1998**). Microorganisms mechanisms of overcoming the activities of antimicrobial agents include the production of structure-altering or inactivating enzymes (e.g. beta-lactamase or amino glycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell-wall target sites, alteration of DNA gyrase targets, permeability mutations, active efflux and ribosomal modification **(Aaterson, 2001; Levy, 2002)**. The most common causes of bacterial resistance to β-lactam antibiotics are the production of β-lactamases, the presence of plasmid and mutation **(Bronson and Barret, 2001)**. Extended spectrum betalactamases (ESBLs) are often plasmid mediated and derived from mutations in the classic TEM (Temoria) and SHV (Sulphydyl Variable) genes by one or more amino acid substitution around the active sites **(Paterson *et al*., 2001).**

In view of the fact that acute otitis media pose a great challenge to the medical personnels and the patients, and also the non-existence of sufficient data in this environment, there are needs for providing and updating data on the incidence of the disease/infection. Thus, this research was carried out to determine the bacterial isolates associated with acute otitis media, its antibiotic susceptibility, beta-lactamase and ESBL production.

MATERIAL AND METHODS

Study Population

The study was carried out prospectively from January 2009 to December 2010. Information on age and sex were obtained during the medical examinations from the patients and also from their medical records. The study groups consist of 272 patients with acute otitis media and 80 individuals without the middle ear infections as the control group.

Collection and Transportation of Samples

Middle-ear samples from patients (aged ≤1 year to ≥ 60 years) attending Ear, Nose and Throat (ENT) clinics in Uyo and Ikot Ekpene in Akwa Ibom State , who had not received antibiotic therapy (topical or systemic) for the previous three days were collected aseptically using sterile swab sticks and transported using peptone water to maintain the swabs moist until being taken to the Microbiology Laboratory for bacteriological analyses.

Isolation, Characterization and Identification of Bacterial Isolates

The middle ear swabbed samples were streak inoculated onto plates of MacConkey Agar (MCA), Blood Agar (BA), Eosin Methylene Blue (EMB) Agar, Mannitol Salt Agar (MSA) and incubated aerobically at 37^{o}C for 24hours. Plates of Blood Agar and Chocolate Agar were incubated at 37^{o}C for 48hours using anaerobic jar with a lit candle for anaerobic bacteria. After incubation, the colonies were sub-cultured onto Petri dishes containing nutrient agar and incubated as described above. Routine conventional laboratory techniques including Gram staining, motility, coagulase, catalase, oxidase , indole, urease production, citrate utilization, methyl red, Vogues Proskauer, and carbohydrate fermentation tests such as mannitol, sucrose, glucose and lactose were all carried out using the methods described by **Fawole and Oso (1988); Holt *et al* (1994); Cowan (1999); Cheesbrough (2006).**

Antibiotic Susceptibility Testing

In vitro susceptibility of the pure bacterial species to twelve different antibiotics was determined using Kirby-Bauer disk-diffusion technique **(Bauer *et al*., 1996; NCCLS, 2004).** One (1) ml of each bacterial isolates prepared directly from an overnight agar plate adjusted to 0.5 McFarland Standard was inoculated using sterile pipette into each of the Petri dishes containing Mueller-Hinton agar (MHA) and were allowed to stand for 30 minutes for pre-diffusion of the inoculated organisms. The commercially available discs containing the following antibiotics: Penicillin (Pen,10µg/disk), streptomycin (Stp,10µg/disk), amoxicillin (Amy,10µg/disk), imipenem (Imi,10µg/disk), ceftriaxone, (Cef,30µg/disk), cephalothin (Cep,30µg/disk) ,ceftazidime, (Cfp ,30µg/disk), cefotaxime (Cfo, 30µg/disk), ofloxacin (Ofl,5µg/disk), ciprofloxacin (Cip, 5µg/disk), levofloxacin (Lev, 5µg/disk) and moxifloxacin (Mox,5µg/disk) (Oxoid, UK) were aseptically placed onto the surfaces of the sensitivity agar plates with a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37^{o}C for 18 hrs for aerobic bacteria and anaerobically using anaerobic jar with a lit candle at 37^{o}C for 24-48 hrs for the anaerobic bacteria**.** Zones of

inhibition after incubation were observed and the diameters of inhibitory zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative manual of CLSI (2005). Percentage resistance was calculated using the formula *PR=a/b×100, where 'PR'* was percentage resistance, '*a'* was the number of resistant isolates and '*b'* was the number of isolates tested with the antibiotic. The percentage sensitivity was calculated using the formula *PS=c/d×100, where 'PS'* was percentage sensitivity, '*c'* was the number of sensitive isolates and '*d'* was the number of isolates tested with the antibiotic **(Akinjogunla and Enabulele, 2010).**

Phenotypic Detection of Extended Spectrum Beta-lactamase (ESBL) Producing Bacteria

Double Disc Synergy test (DDST) was used for presumptive detection of the presence of extended spectrum β-lactamase (ESBL) producing bacteria. Suspension of test organisms were inoculated onto the surface of the Mueller-Hinton agar plate using sterile pipettes. A disc of co-amoxiclav (amoxicillin, 20 µg / clavulanic acid 10 µg) was placed at the centre of the Petri-dish on the agar surface and ceftriaxone (30 µg/disk), ceftazidime (30 µg/disk) and cefotaxime (30 µg/disk) were placed 20 mm apart center to center on the plates. This was incubated at 37°C for 18 hrs. An enhanced zone of inhibition between any one of the β-lactam discs and the amoxicillin-clavulanic acid disc was interpreted as presumptive evidence for the presence of ESBL.

Phenotypic Detection of Beta-lactamase (Starch Paper Method)

Beta-lactamase test was carried out using the method of **Odugbemi *et al.* (1977).** Strips of starch paper were sterilized using 70% ethanol and soaked for 10 min in benzyl penicillin solution containing 100, 000 µg/ml in phosphate-buffered saline (PBS) Oxoid, pH 7.3. The strips of starch paper were spread evenly on Petri dishes and 24-48 hrs old cultures grown on nutrient agar were inoculated on the surface of starch paper, spread over an area of 2-3 mm and incubated at 37^{o}C for 30 minutes. Gram's iodine solution was used to flood the plate and drained off immediately. The starch paper turned uniformly blue-black within 30 seconds of application while colonies with decolourized zones were positive for beta-lactamase and colonies with black background showed beta-lactamase negative.

Phenotypic Detection of Beta-lactamase (Chromogenic Cephalosporin Method)

The phenotypic production of β-Lactamase enzymes was also determined by Chromogenic Cephalosporin Method using Nitrocephin (Oxoid, UK). The colonies of the test bacteria were picked from overnight nutrient agar plates and inoculated into sterile nutrient broth, incubated both aerobically for 24 hours at 37°C for aerobic bacteria and anaerobically as described above for anaerobic bacteria. Two drops of nitrocephin solution were added to each broth culture for colour change within 30 minutes. The β-lactamase production was inferred when the broth turned red within 30 minutes of addition of the nitrocephin solution.

Statistical Analysis of Results

Frequencies and percentages were calculated for study variables. The data were analyzed using statistical packages for the social sciences (SPSS) 15.0 statistical softwares. Chi-square ($\chi 2$) test was used to calculate probabilities and determine significance. A p-value of less than or equal to 0.5 was considered to be statistically significant ($P \leq 0.5$), while p-value more than or equal to 0.5 was considered to be statistically not significant (NS).

RESULTS AND DISCUSSION

Out of 272 patiently clinically diagnosed of AOM, 124 (45.6%) were males and 148 (54.4%) were females (Table 1). Although, numerous studies have reported that males are at higher risk of otitis media than females, but several studies found no male-female disparity **(Bluestone *et al.*, 1992)**. **Ahmed *et al.* (1999), Li *et al.* (2005)** and **Variya *et al.* (2002)** reported that occurrence of otitis media was more in male than female and this is contrary to the results obtained in these studies as 124 (45.6%) males and 148 (54.4%) females had acute otitis media. From these results, there was higher prevalence of acute otitis media in female than male patients and this is also supported by the study carried out in Singapore **(Loy *et al.*, 2002)**. The highest prevalence of AOM was observed in age group ≤ 10 years with 84 (30.9%) cases and lowest prevalence of AOM was observed in age group ≤ 61 having 12 (4.4%) cases. The results showed that there was significant difference in the prevalence of AOM between age groups ≤ 10 years and other age groups at $P<0.5$ (Table 1). The highest prevalence of AOM in age group ≤ 10 years could be ascribed to physiological, anatomical and socio-cultural reasons. Based on physiological and anatomical structures, the immune systems of the children within this age group are not well developed compared to those in the higher age groups **(Li *et al.*, 2005).** Also the eustachian tube of younger children is shorter

and more horizontal than in older children, therefore, the organisms from the nasopharynx can reach the middle ear more readily in younger children as a consequence **(Li *et al.*, 2005; Akinjogunla and Eghafona, 2011)**.The socio-cultural aspect involves inserting contaminated objects into the ears, swimming in dirty and stagnant river **(Li *et al.*, 2005)**.

Table 1 Age-wise and Gender-wise Distribution of Acute Otitis Media and Uninfected (Control)

Age Range (Years)	Acute Otitis Media			Uninfected (Control)		
	Male	Female	Total	Male	Female	Total
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
≤ 10	36 (29.0)	48 (32.4)	84 (30.9)	7 (17.5)	8 (20.0)	15 (18.8)
11-20	19 (15.3)	27 (18.2)	46 (16.9)	8 (20.0)	7 (17.5)	15 (18.8)
21-30	15 (12.1)	17 (11.5)	32 (11.8)	5 (12.5)	5 (12.5)	10 (12.5)
31-40	9 (7.3)	20 (13.5)	29 (10.7)	5 (12.5)	5 (12.5)	10 (12.5)
41-50	18 (14.5)	13 (8.8))	31 (11.4)	5 (12.5)	5 (12.5)	10 (12.5)
51-60	9 (7.3)	8 (5.4)	17 (6.3)	5 (12.5)	5 (12.5)	10 (12.5)
≥ 61	5 (4.0)	7 (4.7)	12 (4.4)	5 (12.5)	5 (12.5)	10 (12.5)
USP	13 (10.5)	8 (5.4)	21 (7.7)	-	-	-
Total	124 (45.6)	148 (54.4)	272 (100)	40 (50.0)	40 (50.0)	80 (100.0)

Legend: USP: Unspecified; Values in parenthesis are percentages ($P< 0.5$; X^2: 8.28; df: 7)

Of the 272 middle ear samples, 263 (96.7%) showed positive growth, while 9 (3.3%) samples showed no growth in all the culture media used. Among the 263 samples with positive growth, 165 (60.7%) samples showed growth of single isolates, 69 (25.4%) showed growth of two isolates, while poly-bacterial growth was present in 29 (10.7%) samples (Table 2). These results corroborated similar studies carried out by **Loy *et al*. (2002) and Asif *et al*. (2007).**

Table 2 Number and Percentage of Bacterial spp. obtained from Patients with Acute Otitis Media and Uninfected (Control) in relation to Sex

Number of Isolates	Acute Otitis Media (AOM)			Control (Uninfected)		
	Male No. (%)	Female No. (%)	Total No. (%)	Male No. (%)	Female No. (%)	Total No. (%)
0	6 (4.8)	3 (2.0)	9 (3.3)	25 (62.5)	28 (70.0)	53 (66.3)
1	82 (66.1)	83 (56.1)	165 (60.7)	13 (35.5)	11 (27.5)	24 (30.0)
2	25 (20.2)	44 (29.7)	69 (25.4)	2 (5.0)	1 (2.5)	3 (7.5)
3	8 (6.5)	13 (8.8)	21 (7.7)	-	-	-
4	3 (2.4)	5 (3.4)	8 (2.9)	-	-	-
Total	124 (100)	148 (100)	272 (100)	40 (100)	40 (100)	80 (100)

USP: Unspecified; Values in parenthesis are percentages

The pattern of bacterial isolates showed that 50.5% of the isolates were Gram positive, 49.5% were Gram negative while 98.5% of the isolates were aerobes while 1.5% were anaerobes. The frequency of occurrence of the bacterial isolates showed that *Staphylococcus aureus* had the highest prevalence of 84 (21.1%), while *Enterococcus faecalis* had the lowest prevalence of 5 (1.3%). Other bacterial frequencies were: *Moraxella catarrhalis* 51 (12.8%), *Haemophilus influenzae* 58 (14.6%), *Streptococcus pneumoniae* 47 (11.8%), *Streptococcus pyogenes* 23 (5.8%), Coagulase negative *Staphylococcus* spp. 42 (10.6%), *Pseudomonas aeruginosa* 27 (6.8%), *E. coli* 21 (5.3%), *Proteus mirabilis* 15 (3.8%) , *Proteus vulgaris* 9 (2.3%) , *Klebsiella pneumoniae* 10 (2.5%) and *Bacteriodes fragilis* 6 (1.5%) (Table 3). The isolation of *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. from acute otitis media is in conformity with the reports of **Ekpo *et al*. (2009)**. The occurrence of *H. influenzae, S. pneumoniae* and *M. catarrhalis* as the commonly encountered bacteria in this study is in agreement with **Canter (1997), Koksal and Reisli (2002); Siegel and Bien (2004), Rovers *et al*. (2004).** The

higher percentage of occurrence of *S. aureus* in females than males observed in these studies is in agreement with the earlier report of **Oyeleke (2009).**

Table 3 Frequency of Distribution of Bacterial spp. Associated with Acute Otitis Media (AOM)

Bacterial spp. Isolated	Number of Occurrences	Percentage of Occurrences
Gram Positive / Aerobes		
Streptococcus pneumoniae	47	11.8
Streptococcus pyogenes	23	5.8
Staphylococcus aureus	84	21.1
CoN *Staphylococcus* spp.	42	10.6
Enterococcus faecalis	5	1.3
Gram Negative / Aerobes		
Moraxella catarrhalis	51	12.8
Haemophilus influenzae	58	14.6
Pseudomonas aeruginosa	27	6.8
Escherichia coli	21	5.3
Proteus mirabilis	15	3.8
Proteus vulgaris	9	2.3
Klebsiella pneumoniae	10	2.5
Gram Negative / Anaerobe		
Bacteriodes fragilis	6	1.5
TOTAL	398	100

CoN : Coagulase negative

S. aureus was the most frequent bacterial species in both sexes with 30 (17.9%) and 54 (23.5%) frequencies in male and female patients, respectively. *M. catarrhalis* 20 (11.9%), *H. influenzae* 24 (14.3%), *S. pneumoniae* 19 (11.3%), *P. aeruginosa* 12 (7.1%), *E. coli* 9 (5.4%), *P. mirabilis* 6 (3.6%), *K. pneumoniae* 7 (4.2%), and *B. fragilis* 2 (1.2%) were isolated from the males. For females, the corresponding values were *M. catarrhalis* 31(13.5%), *H. influenzae* 34(14.8%), *S. pneumoniae* 28 (12.2%), *P. aeruginosa* 15 (6.5%), *E. coli* 12 (5.2%), *P. mirabilis* 9 (3.9%), *K. pneumoniae* 3 (1.3%), and *B. fragilis* 4 (1.7%) (Table 4).

Table 4 Age and Gender Specific Distribution of Bacterial spp. among Patients with Acute Otitis Media

Bacterial spp. Isolated	Gender		Age Group								Total
	Male No. (%)	Female No. (%)	≤10 No. (%)	11-20 No. (%)	21-30 No. (%)	31-40 No. (%)	41-50 No. (%)	51-60 No. (%)	≥ 61 No. (%)	USP No. (%)	No. (%)
M. catarrhalis	20 (11.9)	31(13.5)	14 (27.5)	9 (17.6)	6 (11.8)	4 (7.8)	6 (11.8)	5 (9.8)	2 (3.9)	5 (9.8)	51 (12.8)
H. influenzae	24 (14.3)	34(14.8)	16 (27.6)	9 (15.5)	7 (12.1)	5 (8.6)	8 (13.8)	5 (8.6)	4 (6.9)	4 (6.9)	58 (14.6)
S. pneumoniae	19 (11.3)	28 (12.2)	12 (25.5)	12(25.5)	6 (12.8)	5 (10.6)	3 (6.4)	4 (8.5)	1 (2.1)	4 (8.5)	47 (11.8)
S. pyogenes	13 (7.7)	10 (4.3)	5 (21.7)	3 (13.0)	2 (8.7)	2 (8.7)	3 (13.0)	6 (26.1)	-	2 (8.7)	23 (5.8)
S. aureus	30 (17.9)	54 (23.5)	25 (29.8)	17(20.2)	10 (11.9)	7 (8.3)	7 (8.3)	8 (9.5)	4 (4.8)	6 (7.1)	84 (21.1)
CoN*S* spp	18 (10.7)	24 (10.4)	12 (28.6)	8 (19.0)	7 (16.7)	5 (11.9)	2 (4.8)	2 (4.8)	2 (4.8)	4 (9.5)	42 (10.6)
P. aeruginosa	12(7.1)	15 (6.5)	9 (33.3)	3 (11.1)	3 (11.1)	1 (3.7)	5 (18.5)	3 (11.1)	1 (3.7)	2 (7.4)	27 (6.8)
E. coli	9 (5.4)	12 (5.2)	2 (9.5)	5(23.8)	1(4.8)	4 (19.0)	4(19.0)	5(23.8)	-	-	21(5.3)
P. mirabilis	6 (3.6)	9 (3.9)	3(20.0)	1(6.7)	3(20.0)	1(6.7)	4(26.7)	1(6.7)	1(6.7)	1(6.7)	15(3.8)
P. vulgaris	6 (3.6)	3 (1.3)	5 (55.6)	-	-	2(22.2)	1(11.1)	1(11.1)	-	-	9(2.3)
K. pneumoniae	7 (4.2)	3 (1.3)	2 (20.0)	1 (10.0)	1 (10.0)	1 (10.0)	2(20.0)	1 (10.0)	1 (10.0)	1 (10.0)	10 (2.5)
E. faecalis	2 (1.2)	3 (1.3)	-	-	3 (60.0)	-	2(40.0)	-	-	-	5 (1.3)
B. fragilis	2 (1.2)	4 (1.7)	1 (16.7)	-	-	-	1 (16.7	-	3(50.0)	1(16.7)	6 (1.5)
Total	168 (100)	230 (100)	106 (26.6)	68(17.1)	49 (12.3)	37 (9.3)	48(12.1)	41(10.3)	19(4.8)	30 (7.5)	398 (100)

Key: USP: Unspecified; CoN*S:* Coagulase negative *Staphylococcus*; Values in parenthesis are percentages

The *in-vitro* antibiotic susceptibility profiles of the bacteria isolated showed varied percentages of sensitivity (Tables 5a and 5b). Of the 398 isolates, 60.1% were sensitive to penicillin, 63.8% were sensitive to streptomycin, 55.8% were sensitive to amoxycillin, 71.1% were sensitive to imipenem, 67.8% were sensitive to both ceftriaxone and cefotaxime each, 73.9% were sensitive to cephalothin, 76.9% were sensitive to ceftazidime, 71.6% were sensitive to ofloxacin, 73.4% were sensitive to ciprofloxacin, 80.2% were sensitive to levofloxacin and 81.7% were sensitive to moxifloxacin. The high sensitivity of *S. pneumoniae* to both levofloxacin and moxifloxacin is in agreement with **Ken *et al.* (2008).** Although, **Force *et al.* (1995)** reported ciprofloxacin to be an effective and safe therapy for acute otitis media but widely varying percentages of ciprofloxacin resistance have been reported with a global trend of increasing resistance. Resistance of *Pseudomonas aeruginosa* to cephalosporins as well as to other antibiotics has been reported by **Carsenti-Etesse *et al.* (2001).** The ranges of values for the sensitivity of each of the bacteria were as follows: *M. catarrhalis* [ceftriaxone 30 (58.8%), amoxycillin 30 (58.8%) to moxifloxacin 45 (88.2%) and levofloxacin 45 (88.2%)]; *H. influenzae* [penicillin 33 (56.9%) to moxifloxacin 47 (81.0%)]; *S. pneumoniae* [penicillin 23 (48.9%) to ceftazidime 39 (83.0%)]; *S. aureus* [amoxycillin 48 (57.1%) to moxifloxacin 71 (84.5%)]; Coagulase negative *Staphylococcus* spp. [streptomycin 19 (45.2%) to moxifloxacin 31 (73.8%)]. The varied values and percentages obtained for the resistance are also shown in Tables 5a and 5b.

Table 5a *In-vitro* Antibiotic Susceptibility Spectrum of Bacterial spp. Isolated from Acute Otitis Media

Bacterial spp.	No. of Isolates	Pen^s No.(%)	Pen^r No.(%)	Stp^s No.(%)	Stp^r No.(%)	Amy^s No.(%)	Amy^r No.(%)	Imi^s No.(%)	Imi^r No.(%)	Cef^s No.(%)	Cef^r No.(%)	Cfo^s No.(%)	Cfo^r No.(%)
M. catarrhalis	51	34(66.7)	17(33.3)	37(72.5)	14(27.4)	30(58.8)	21(41.2)	42(82.4)	9(17.6)	30(58.8)	21(41.2)	34(66.7)	17(33.3)
H. influenzae	58	33(56.9)	25(43.1)	39(67.2)	19(32.8)	34(58.6)	24(41.4)	42(72.4)	16(27.6)	34(58.6)	24(41.4)	40(69.0)	18(31.0)
S. pneumoniae	47	23(48.9)	24(51.1)	27(57.4)	20(42.6)	31(66.0)	16(34.0)	30(63.8)	17(36.2)	29(61.7)	18(38.3)	29(61.7)	18(38.3)
S. pyogenes	23	15(65.2)	8(34.8)	15(65.2)	8(34.8)	14(60.9)	9(39.1)	16(69.6)	7(30.4)	19(82.6)	4(17.4)	18(78.3)	5(21.7)
S. aureus	84	62(73.8)	22(26.2)	65(77.4)	19(22.6)	48(57.1)	36(42.9)	67(79.8)	17(20.2)	55(65.5)	29(34.5)	53(63.1)	31(36.9)
CoN *Staphylococcus* spp.	42	25(59.5)	17(40.5)	19(45.2)	23(54.8)	20(47.7)	22(52.3)	20(47.7)	22(52.3)	27(64.3)	15(35.7)	24(57.1)	18(42.9)
P. aeruginosa	27	14(51.9)	13(48.1)	14(51.9)	13(48.1)	16(59.3)	11(40.7)	21(77.8)	6(22.2)	23(85.2)	4(14.8)	21(77.8)	6(22.2)
E. coli	21	11(52.4)	10(47.6)	10(47.6)	11(52.4)	10(47.6)	11(52.4)	14(66.7)	7(33.3)	16(76.2)	5(23.8)	16(76.2)	5(23.8)
P. mirabilis	15	7(46.7)	8(53.3)	9(60.0)	6(40.0)	8(53.3)	7(47.7)	9(60.0)	6(40.0)	12(80.0)	3(20.0)	12(80.0)	3(20.0)
P. vulgaris	9	6(66.7)	3(33.3)	6(66.7)	3(33.3)	4(44.4)	5(55.6)	6(66.7)	3(33.3)	7(77.8)	2(22.2)	7(77.8)	2(22.2)
K. pneumoniae	10	5(50.0)	5(50.0)	7(70.0)	3(30.0)	3(30.0)	7(70.0)	7(70.0)	3(30.0)	9(90.0)	1(10.0)	8(80.0)	2(20.0)
E. faecalis	5	1(20.0)	4(80.0)	3(60.0)	2(40.0)	2(40.0)	3(60.0)	4(80.0)	1(20.0)	4(80.0)	1(20.0)	4(80.0)	1(20.0)
B. fragilis	6	3(50.0)	3(50.0)	3(50.0)	3(50.0)	2(33.3)	4(66.7)	5(83.3)	1(16.7)	5(83.3)	1(16.7)	4(66.7)	2(33.3)
Total	398	239(60.1)	159(39.9)	254(63.8)	144(36.2)	222(55.8)	176(44.2)	283(71.1)	115(28.9)	270(67.8)	128(32.2)	270(67.8)	128(32.2)

CoN: Coagulase negative*;* Values in parenthesis are percentages; s: sensitive; r: resistant; Pen: Penicillin; Stp: Streptomycin; Amy: Amoxicillin; Imi: Imipenem; Cef: Ceftriaxone; Cfo: Cefotaxime

Table 5b *In-vitro* Antibiotic Suscesptibility Spectrum of Bacterial spp. Isolated from Acute Otitis Media (Cont'd)

Bacterial spp.	No. of Isolates	Cep^s No.(%)	Cep^r No.(%)	Cfp^s No.(%)	Cfp^r No.(%)	Ofl^s No.(%)	Ofl^r No.(%)	Cip^s No.(%)	Cip^r No.(%)	Lev^s No.(%)	Lev^r No.(%)	Mox^s No.(%)	Mox^r No.(%)
M. catarrhalis	51	37(72.5)	14(27.5)	42(82.3)	9(17.6)	38(74.5)	13(25.5)	44(86.3)	7(13.7)	45(88.2)	6(11.8)	45(88.2)	6(11.8)
H. influenzae	58	40(69.0)	18(31.0)	38(65.5)	20(34.5)	42(72.4)	16(27.6)	42(72.4)	16(27.6)	45(77.6)	13(22.4)	47(81.0)	11(19.0)
S. pneumoniae	47	37(78.7)	10(21.3)	39(83.0)	8(17.0)	32(68.1)	15(31.9)	29(61.7)	18(38.3)	36(76.6)	11(23.4)	36(76.6)	11(23.4)
S. pyogenes	23	20(87.0)	3(13.0)	19(82.6)	4(17.4)	17(73.9)	6(26.1)	17(73.9)	6(26.1)	19(82.6)	4(17.4)	20(87.0)	3(13.0)
S. aureus	84	59(70.2)	25(29.8)	61(72.6)	23(27.4)	61(72.6)	23(27.4)	64(76.2)	20(23.8)	69(82.1)	15(17.9)	71(84.5)	13(15.5)
CoN *Staphylococcus* spp	.42	29(69.0)	13(31.0)	27(64.3)	15(35.7)	24(57.1)	18(42.8)	29(69.0)	13(31.0)	30(71.4)	12(28.6)	31(73.8)	11(26.2)
P. aeruginosa	27	21(77.8)	6(22.2)	24(88.9)	3(11.1)	20(74.1)	7(25.9)	18(66.7)	9(33.3)	22(81.5)	5(18.5)	22(81.5)	5(18.5)
E. coli	21	18(85.7)	3(14.3)	19(90.5)	2(9.5)	16(76.2)	5(23.8)	16(76.2)	5(23.3)	18(85.7)	3(14.3)	17(81.0)	4(19.0)
P. mirabilis	15	11(73.3)	4(26.7)	13(86.7)	2(13.3)	12(80.0)	3(20.0)	12(80.0)	3(20.0)	12(80.0)	3(20.0)	11(73.3)	4(26.7)
P. vulgaris	9	7(77.8)	2(22.2)	8(88.9)	1(11.1)	6(66.7)	3(33.3)	6(66.7)	3(33.3)	7(77.8)	2(22.2)	7(77.8)	2(22.2)
K. pneumoniae	10	7(70.0)	3(30.0)	8(80.0)	2(20.0)	8(80.0)	2(20.0)	9(90.0)	1(10.0)	7(70.0)	3(30.0)	9(90.0)	1(10.0)
E. faecalis	5	3(60.0)	2(40.0)	4(80.0)	1(20.0)	4(80.0)	1(20.0)	3(60.0)	2(40.0)	5(100.0)	0(0.0)	4(80.0)	1(20.0)
B. fragilis	6	5(83.3)	1(16.7)	4(66.7)	2(33.3)	5(83.3)	1(16.7)	3(50.0)	3(50.0)	4(66.7)	2(33.3)	5(83.3)	1(16.7)
Total	398	294(73.9)	104(21.1)	306(76.9)	92(23.1)	285(71.6)	113(28.4)	292(73.4)	106(26.6)	319(80.2)	79(19.8)	325(81.7)	73(18.3)

CoN: Coagulase negative*;* Values in parenthesis are percentages; s: sensitive; r: resistant; Cep: Cephalothin;Cfp:Ceftazidime;Ofl:Ofloxacin;Cip:Ciprofloxacin; Lev: Levofloxacin; Mox; Moxifloxacin

The results showed that Starch Paper Test (SPT) identified 152 (38.2%) β-lactamase producing isolates, while β-lactamase enzyme was detected in 163 (41.0%) of isolates through Chromogenic Cephalosporin Test (CCT). Concordance between SPT and CCT was observed in 142 (35.7%) of the analysis. There was no statistical difference between the results obtained using the two methods at (P >0.5). The results obtained using Starch Paper Test (SPT) showed that 23 (39.7%) *H. influenzae,* 19 (40.4%) *S. pneumoniae,* 22 (52.4%) CoN *Staphylococcus* spp, 7 (33.3%) *E. coli,* 8 (53.3%) *P. mirabilis*, 4 (44.4%) *P. vulgaris,* 5 (50.0%) *K. pneumoniae*, 2 (40.0%) *E. faecalis* and 2 (33.3%) *B. fragilis* were β-lactamase producers, while Chromogenic Cephalosporin Test detected 26 (44.8%) *H. influenzae,* 19 (40.4%) *S. pneumoniae,* 20 (47.6%) Coagulase negative *Staphylococcus* spp, 10 (47.6%) *E. coli,* 7 (46.7%) *P. mirabilis*, 4 (44.4%) *P. vulgaris,* 5 (40.0%) *K. pneumoniae,* 2 (40.0%) *E. faecalis* and 3 (50.0%) *B. fragilis* β-lactamase producers (Table 6a). β-lactamase positivity was detected in 19 (37.3%) of the *M. catarrhalis* strains using chromogenic cephalosporin method in this study and the occurrence of β-lactamase producing *M. catarrhalis* has been reported by **Doern *et al*. (1996)**.

The results showed that 33 of 82 (40.2%) of the isolates were ESBL producers, while 49 of 82 (59.8%) were non ESBL producers. The prevalence of ESBL-producing bacteria among the Gram negative bacilli is shown in Table 6b. ESBLs were detected in *P. aeruginosa* 12/27 (44.4%), *E. coli* 8/21 (38.1%), *P. mirabilis* 6/15 (40.0%), *P. vulgaris* 3/9 (33.3%) and *K. pneumoniae* 4/10 (40.0%). The high isolation frequency of ESBL producing *K. pneumoniae* and *E. coli* has been reported in the Latin America (45.4%), the Western Pacific (24.6%), Europe (22.6%) and Nigeria **(Thomson *et al*., 1999; Akinjogunla *et al.*, 2010)**. The occurrence of ESBL producing *E. coli* and *K. pneumoniae* in acute otitis media in this study is similar to the reports of **Quinn *et al*., (1989) and Livermore, (1995)**.

Table 6a The Prevalence of Beta-Lactamase (βL) Producing Bacteria Isolated from Acute Otitis Media

Bacterial spp	Number of Occurrence	Starch Paper Test (SPT)		Chromogenic Cephalosporin Test (CCT)	
		No / (%) of βL Producers	No / (%) of βL Non Producers	No / (%) of βL Producers	No / (%) of βL Non Producers
Moraxella catarrhalis	51	15 (29.4)	36 (70.6)	19 (37.3)	32 (62.7)
Haemophilus influenzae	58	23 (39.7)	35 (60.3)	26 (44.8)	32 (55.2)
Streptococcus pneumoniae	47	19 (40.4)	28 (59.6)	19 (40.4)	28 (59.6)
Streptococcus pyogenes	23	8 (34.8)	15 (47.8)	8 (34.8)	15 (65.2)
Staphylococcus aureus	84	26 (31.0)	58 (69.0)	29 (34.5)	55 (65.5)
CoN *Staphylococcus* spp	42	22 (52.4)	20 (47.6)	20 (47.6)	22 (52.4)
Pseudomonas aeruginosa	27	11 (40.7)	16 (59.3)	12 (44.4)	15 (55.6)
Escherichia coli	21	7 (33.3)	14 (66.7)	10 (47.6)	11 (52.4)
Proteus mirabilis	15	8 (53.3)	7 (46.7)	7 (46.7)	8 (53.3)
Proteus vulgaris	9	4 (44.4)	5 (55.6)	4 (44.4)	5 (55.6)
Klebsiella pneumoniae	10	5 (50.0)	5 (50.0)	4 (40.0)	6 (60.0)
Enterococcus faecalis	5	2 (40.0)	3 (60.0)	2 (40.0)	3 (60.0)
Bacteriodes fragilis	6	2 (33.3)	4 (66.7)	3 (50.0)	3 (50.0)
TOTAL	398	152 (38.2)	246 (61.8)	163 (41.0)	235 (59.0)

CoN: Coagulase negative; Values in parenthesis are percentages

Table 6b The Prevalence of Extended Spectrum Beta-Lactamase (ESβL) Producing Bacteria Isolated from Acute Otitis Media (AOM)

Source	Bacterial spp.	Number of Occurrence	No / (%) of ESβL Producers	No / (%) of ESβL Non Producers
AOM	*Pseudomonas aeruginosa*	27	12 (44.4)	15 (55.6)
	Escherichia coli	21	8 (38.1)	13 (61.9)
	Proteus mirabilis	15	6 (40.0)	9 (60.0)
	Proteus vulgaris	9	3 (33.3)	6 (66.7)
	Klebsiella pneumoniae	10	4 (40.0)	6 (60.0)
	TOTAL	82	33(40.2)	49 (59.8)

Values in parenthesis are percentages

CONCLUSION

This study has provided and updated data on the incidence of the middle ear infection; the aerobic and anaerobic bacteria associated with acute otitis media and also revealed the actual therapy for acute otitis media. However, routine tests for both β-lactamase (βL) and extended spectrum β-lactamase (ESBL) productions among resistant bacteria should be incorporated into diagnostic laboratory.

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