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ORIGINAL ARTICLE

Detection of FOX-AmpC-β-lactamase gene and antibiogram of AmpC-beta-lactamase-producing pathogens isolated from chronic suppurative otitis media patients in Nigeria

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ABSTRACT

Background and Objectives: AmpC-producing Gram-negative bacterial (GNB) pathogens are distributed worldwide, especially in clinical settings. This study aimed to determine the antibiogram and the type of AmpC-β-lactamase gene harboured by GNB pathogens implicated in chronic suppurative otitis media (CSOM) cases.

Materials and Methods: Ear swab samples (300) collected from patients with active CSOM were analysed using standard microbiological techniques. Phenotypic and molecular detection of AmpC β-lactamase production was done by cefoxitin/cloxacillin double-disk synergy test and PCR respectively. Antibiogram was determined by disk diffusion technique.

Results: Among the GNB pathogens isolated from CSOM patients, *P. aeruginosa* was the most predominant (36.3%); followed by *K. pneumoniae* (22.3%), and *E. coli* (13.7%). Patients with active CSOM showed increased bacteria isolation rate from bilateral ear discharges than unilateral ear discharges. *E. coli* and *P. aeruginosa* were more prevalent among patients with duration of discharge >2 weeks; recording 9.0% and 20.3% respectively. AmpC β-lactamase producers accounted for 14.0%; they were highly resistant (60%-100%) to cephalosporins, trimethoprim-sulfamethoxazole, ofloxacin, amoxicillin, and tetracycline, but very susceptible (70.4%-100%) to ciprofloxacin, imipenem, and amikacin. Multiple antibiotic resistance indices of isolates ranged from 0.7-0.8. FOX-AmpC-β-lactamase gene was detected in 3.9% of the isolates.

Conclusion: The detection of AmpC β-lactamase-producing multidrug-resistant GNB pathogens harbouring FOX-AmpC-β-lactamase gene among patients with CSOM infections in our study is a serious public health problem which needs urgent intervention.

Keywords: AmpC-β-lactamase; Chronic suppurative otitis media; Gram-negative bacterial pathogens; FOX-AmpC gene; Multidrug resistance

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INTRODUCTION

Chronic suppurative otitis media (CSOM) is a chronic inflammation of the middle ear and the mastoid cavity with perforated tympanic membrane, resulting in ear discharges persisting for at least 14 days (1, 2). If not properly treated, the disease might have long-term effects on language development, early communication, educational process, physiological and cognitive development, and auditory processing (3). By one year of age, at least 60% of children have experienced an episode, and 17% have suffered at least 3 episodes of acute otitis media (AOM) (4) which may progress to CSOM. Worldwide, the disease is distributed and ranks as the fifth global burden of diseases. It is the second cause of hearing loss (5) and one of the commonest diseases responsible for receiving antibiotics among immunocompromised, especially children (5). In developing countries, it has been recognized to cause severe disability and in extreme cases, leads to death if not properly and promptly managed. The etiological agents are either resident or transported to the middle ear by insufflations of the lower respiratory tract system through the Eustachian tube (6). In Nigeria and abroad, *Proteus* species, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* are the commonly reported Gram-negative bacteria isolated from cases of CSOM (1, 6, 7).

Beta-lactams, fluoroquinolones, and aminoglycosides are the most frequently prescribed antibiotics to treat bacterial ear infections; however, the widespread use of these antimicrobials has caused the emergence and spread of antibiotic-resistant bacteria (8). The development of resistance by these GNB pathogens has been attributed to production of β-lactamases, including extended-spectrum-β- lactamases (ESBLs), AmpCs, and carbapenemases (9). AmpC β-lactamases are cephalosporinases that belong to molecular class C β-lactamases (10). The genes encoding AmpC β-lactamases are much more frequently chromosomal than plasmid-mediated such as the CTM, MOX, and FOX gene. The AmpC β-lactamases are clinically important β-lactamases because they confer antimicrobial resistance to the narrow-spectrum, expanded-spectrum, and the broad-spectrum cephalosporins including cefotaxime, ceftazidime, ceftriaxone, aztreonam, and the penicillins. Resistance is also expressed towards β-lactamase inhibitors such as amoxicillin-clavulanic acid (10). Most of the genera of the family Enterobacteriaceae produce AmpC enzymes through an inducible mechanism; in which case, the presence of broad-spectrum antibiotics sparks enzyme production in the organism. Although AmpC β-lactamases are not widely reported when compared to ESBL. AmpC β-lactamase FOX gene are more commonly reported in few literatures than other AmpC β-lactamase genes (11-13). Due to the threat of antibiotic resistance in hospitals across the globe, the spread of antimicrobial resistance and associated genetic determinants; studies on the prevalence of ear infection with β-lactamase-producing bacteria are indispensable. At the same time, baseline information on the magnitude of beta-lactamase-producing bacteria implicated in CSOM cases is limited and attest to reported poor treatment outcome in patients. According to literature, there is paucity of information on the prevalence of AmpC β-lactamase-producing GNB pathogens implicated in CSOM. Previous studies on the etiologies of CSOM did not adequately address the burden of β-lactamase-producing pathogens in ear infections. This study was designed to determine the frequency, antimicrobial resistance profiles, and the presence of AmpC-β-lactamase resistance genes in GNB pathogens implicated in CSOM cases.

MATERIALS AND METHODS

Study area. This study was carried out at Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA). AEFUTHA is in Abakaliki town, the capital city of Ebonyi State. It is situated at an elevation of 117 meters above sea level with latitude 6.32°N latitude and longitude 8.12°E. The hospital provides services such as surgical, medical, pediatric, gynecologic, and obstetrics, ear-nose-and-throat (ENT), and intensive care to the community. The hospital has an accredited laboratory, more than 1200 beds, and provides healthcare referral services for thousands of people from the surrounding zones and nearby regions. The climate of Abakaliki is characterized by a hot dry period which stretches from November-April, while the rainy season is from May-October. The maximum temperature during dry season is 36°C, while the minimum temperature is 27.1°C (14). The major occupations of people in Abakaliki are farming and trading; there are also civil servants and students, and all these people engage in busy activities of life (15).

Sample size determination. Sample size was calculated based on the assumption of 5% expected margin of error and 95% confidence interval, taking the prevalence of 17.8% from a previous study which was conducted by Molla et al. (16) on bacterial profile and antimicrobial susceptibility patterns in Chronic Suppurative Otitis Media (CSOM) using Cochran's formular:

$$n = \frac{Z^2 Pq}{e^2}$$ (Cochran's formular)

The approximate total calculated sample size for this study was 300 study participants. The study participants were enrolled consecutively using a convenience sampling technique until a sample size of 300 study participants was achieved between the periods of February 2018 - September 2019.

Ethical clearance. The ethical approval for this study was granted by the ethical committee of Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA) with reference number: SMOH/ERC/042/21. All methods were carried out in compliance with the ARRIVE guidelines. Every fundamental study was done in line with the World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human and animal subjects, and identifiable human and animal material or data (17).

Sampling method. The study utilized simple random sampling to select CSOM patients. Subjects were recruited into the study as they came to the hospital until the required number was obtained with strict application of the inclusion and exclusion criteria.

Inclusion and exclusion criteria. All study participants with active CSOM i.e., perforated tympanic membranes with active purulent discharge, unilateral or bilateral draining ears resulting from CSOM of two weeks or more were included in the study; whereas patients with discharges less than 2 weeks duration, discharges with intact tympanic membrane (otitis externa), and patients receiving antibiotic therapy (topical or systemic) within 7 days before data collection were excluded.

Clinical demographic and medical history. The detailed information regarding age, sex, duration of discharge, unilateral or bilateral draining ears were collected from each study participant using a structured questionnaire by the attending Ear, Nose, and Throat (ENT) specialist after medical examination of the patient.

Sample collection. Three hundred (300) middle-ear discharge samples were collected by an ENT specialist according to the protocol described by Molla et al. (16). This was done under strict aseptic conditions using single mini-tip culture swabs after cleaning the external auditory canal with a spirit swab. Pus specimens were collected from both ears of patients with bilateral draining. Swabs were immediately transported within 1 hour of collection to the laboratory for microbiological analysis.

Microbiological analysis. Swabs were firstly enriched in nutrient broth (Oxoid, UK) and were aerobically incubated overnight at 37°C for 24 hours. After overnight incubation, a loopful of the turbid broth culture was aseptically streaked on cetrimide agar, MacConkey agar (Oxoid, UK), and Eosin Methylene Blue (EMB) agar (Oxoid, UK), and incubated at 37°C for 24 hours. Isolates were identified morphologically with reference to colour and shape on the selective and differential media. Each atypical colony was purified through successive streaking before physiological and biochemical identification such as Gram staining, catalase, oxidase test, triple sugar iron agar, indole production, citrate utilization, motility test, and carbohydrate utilization were done (18, 19). Isolates were further identified by PCR using specific 16S rRNA primers (20).

Phenotypic detection of AmpC β-lactamase enzymes. Isolates were subjected to the antimicrobial activity of cefoxitin (30 µg, Oxoid, UK) on Mueller-Hinton agar (MHA) plates (Oxoid, UK). Each plate was incubated at 30°C for 18 hours (13). Inhibition zones were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria (21). Cefoxitin-cloxacillin double-disk synergy test (CC-DDST) was performed according to previously described methods (12, 13). Single disks containing 30 µg of cefoxitin were placed 20 mm away from a disk containing 20 µg of cloxacillin on MH agar plates already inoculated with the test bacteria (equivalent to 0.5 McFarland turbidity standards). Each plate was incubated at 30°C for 18 hours. A difference of 4 mm in the cefoxitin-cloxacillin inhibi-

tion zones minus the cefoxitin disk used alone was indicative of AmpC enzyme production phenotypically.

Antimicrobial susceptibility studies. This was performed by the disc diffusion method following the Clinical Laboratory Standards Institute guidelines (21). The following antibiotics (Oxoid, UK) were tested against the isolates: amikacin (10 µg), amoxicillin (30 µg), amoxicillin/clavulanic acid (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), ciprofloxacin (10 µg), imipenem (30 µg), ofloxacin (30 µg), piperacillin (30 ug), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (25 µg). Results were interpreted as susceptible and resistant according to the Clinical Laboratory Standards Institute (CLSI) guidelines (21).

Multiple antibiotic resistance indices (MARI). The MARI of the isolates was calculated as the number of antibiotics to which the isolates exhibit resistance (a) divided by the total number of antimicrobial agents tested against the isolates (b) (22, 23).

Molecular detection of FOX-AmpC-β-lactamase-encoding gene. Genomic DNA extraction of bacterial pathogens was performed using Zr Fungal/Bacterial DNA Miniprep™ (Manufactured by Zymo research cat number: D6005) kit according to the manufacturer's protocol. The PCR mix components was made up of 12.5 µL of Taq 2× Master Mix from New England Biolbs (M0270); 1uL each of 10 µM forward and reverse primer; 2 µl of DNA template and then made up with 8.5 µL nuclease free water. The oligonucleotide primers (12) used and their amplicon size is shown in Table 1. PCR cycling conditions were initial denaturation at 94°C, annealing at 55°C for 5 mins for 36X, followed by denaturation at 94°C for 30 secs, elongation at 72°C for 45 secs, and final elongation step at 72°C for 7 minutes and hold temperature at 10°C. Amplified PCR products were run on 1.5% agarose at 110 volts for 45-60 minutes. The DNA fragments or PCR fragments were then visualized under UV transilluminator.

Table 1. Primer sequences and their amplicon sizes

Primers	Nucleotide sequence	Base pair (bp)
FOX-F	AACATGGGGTATCAGGGAGATG	190
FOX-R	CAAAGCGCGTAACCGGATTGG	

Statistical analysis. This was performed with the SPSS statistical software package 18.0 version using the ANOVA and Independent samples T-test tools for the comparative evaluation of categorical variables. Results were only considered to be statistically significant if the p-value was less than 0.05 ($p < 0.05$).

RESULTS

Frequency of bacterial pathogens recovered from CSOM patients. The proportion of CSOM patients infected with *E. coli* was higher in patients within the age group of 21-30 years with a frequency of 13 (4.3%) while *K. pneumoniae* was predominant in patients aged 41 years and above with a frequency of 29 (9.7%), followed by the most predominant frequency of 42 (13.7%) for *P. aeruginosa* among patients aged 1-10 years (Table 2). Patients with active CSOM showed increased occurrence rate of bacteria on bilateral ear (7.7%, 9.3%, and 26.0%) than unilateral ear discharges (6.0%, 13.0%, and 10.3%) for *E. coli, K. pneumoniae*, and *P. aeruginosa* respectively (Table 2). *E. coli* (9.0%) and *P. aeruginosa* (20.3%) were more prevalent among patients with duration of discharge >2 weeks while *K. pneumoniae* was observed in 17.0% cases of discharge within the period of 2 weeks (Table 2). Gram-negative bacteria were more predominant in male patients with frequencies of 26 (8.7%), 40 (13.3%), and 63 (21.0%) for *E. coli, K. pneumoniae*, and *P. aeruginosa* respectively (Table 3). Out of the three hundred samples collected, *P. aeruginosa* accounted for 109 (36.3%) as the most prevalent bacterial pathogens recovered from CSOM patients. This was closely followed by *K. pneumoniae* and *E. coli* with prevalence frequencies of 67 (22.3%) and 41 (13.7%) respectively (Table 3). There was no statistically significant difference in the occurrence frequency of the bacterial pathogens with respect to age group ($p = 0.132$), gender ($p = 0.088$), discharge type ($p = 0.503$), and duration of discharge ($p = 0.969$) based on one-way ANOVA between subjects and independent samples T-test conducted.

Antimicrobial resistance pattern of GNB pathogens isolated from CSOM patients. The AmpC β-lactamase-producing GNB pathogens (*E. coli, K. pneumoniae*, and *P. aeruginosa*) were highly resistant

Table 2. Frequency distribution of Gram-negative bacterial pathogens causing CSOM regarding demographic and clinical characteristic of patients

Patient Information	Sample Distribution	CSOM Bacteria n (%)		
Age (years)		*E. coli*	*K. pneumoniae*	*P. aeruginosa*
1-10	51	9 (3.0)	15 (5)	42 (13.7)
11-20	79	6 (2.0)	7 (2.3)	19 (6.7)
21-30	91	13 (4.3)	16 (5.3)	15 (5.0)
31-40	45	5 (1.7)	0 (0.0)	8 (2.7)
41years & Above	34	8 (2.7)	29 (9.7)	25 (8.3)
Gender				
Male	131	26 (8.7)	40 (13.3)	63 (21.0)
Female	169	15 (5.0)	27 (9.0)	46 (15.3)
Discharging ear				
Bilateral	215	23 (7.7)	28 (9.3)	78 (26.0)
Unilateral	85	18 (6.0)	39 (13.0)	31 (10.3)
Duration of discharge				
2 weeks	227	14 (4.7)	51 (17.0)	48 (16.0)
>2 weeks	73	27 (9.0)	16 (5.3)	61 (20.3)
Total	300	41 (13.7)	67 (22.3)	109 (36.3)

Table 3. Frequency distribution of bacteria pathogen causing CSOM isolated from ear swabs of patients visiting AEFEUTHA

CSOM Bacteria (n=300)	Occurrence rate (%)
E. coli	41 (13.7)
K. pneumonia	67 (22.3)
P. aeruginosa	109 (36.3)
Total	217 (72.3)

Key: n-number of samples

(60%-100%) to amoxicillin-clavulanic acid, amoxicillin, cefotaxime, cefuroxime, cefoxitin, ceftriaxone, ceftazidime, piperacillin, trimethoprim-sulfamethoxazole, tetracycline, and ofloxacin but very susceptible (80%-100%) to amikacin, ciprofloxacin, and imipenem (Table 4). The average multiple antibiotic resistance indices (MARI) of the AmpC-producing *E. coli, K. pneumoniae,* and *P. aeruginosa* were 0.8, 0.7, and 0.8 respectively (Table 5). No statistically significant difference was observed in the percentage resistance frequency between *E. coli, K. pneumoniae,* and *P. aeruginosa*, based on the one-way ANOVA between subjects' test conducted (p = 0.949).

Frequency of AmpC β-lactamase producers and FOX-AmpC-β-lactamase-harbouring genes among GNB isolates recovered from CSOM patients. *P. aeruginosa* was the most predominant AmpC β-lactamase producer with a frequency of 42 (14%), followed by *E. coli* and *K. pneumoniae* with frequencies of 5 (1.7%), and 1 (0.3%) respectively (Table 6). FOX-AmpC- β-lactamase gene was detected in 1 (2.1%) *E. coli,* 1 (2.1%) *K. pneumoniae,* and 2 (4.2%) *P. aeruginosa* isolates (Table 6).

DISCUSSION

This study investigated the frequency and antibiogram of AmpC-β-lactamase-producing Gram-negative bacterial (GNB) pathogens isolated from patients with chronic suppurative otitis media (CSOM). In this study, AmpC-producing GNB pathogens (*E. coli, K. pneumoniae*, and *P. aeruginosa*) with multidrug-resistant traits and harbouring FOX-AmpC-β-lactamase genes were isolated from bilateral and unilateral ear discharges of CSOM patients, with *P. aeruginosa* being the most predominant.

Among the majority of patients with ear discharges for over two weeks (>2 weeks) before visiting the hospital, high frequency of GNB pathogens [*E. coli* (9%), *K. pneumoniae* (5.3%), and *P. aeruginosa* (20.3%)] were recorded. As evidenced in this study, CSOM was more common with microbial proliferation in age group of 1-10 years (30.9%) and the age group of 41 years and above. Bacterial pathogens

Table 4. Antibiotic susceptibility pattern of AmpC β-lactamase-producing Gram-negative bacteria isolated from ear swab samples of CSOM patients

Antibiotic (µg)	*E. coli*		*K. pneumonia*		*P. aeruginosa*	
	Resistance n (%)	Susceptible n (%)	Resistance n (%)	Susceptible n (%)	Resistance n (%)	Susceptible n (%)
Amikacin	0 (0.0)	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)
Amoxycillin-Clavulanic acid	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Amoxicillin	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Cefotaxime	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Cefuroxime	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Cefoxitin	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Ceftriaxone	4 (80)	1 (20)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Ceftazidime	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Colistin Sulphate	5 (100)	0 (0.0)	1 (100)	0 (0.0)	40 (95.2)	2 (4.8)
Ciprofloxacin	1 (20)	4 (80)	0 (0.0)	1 (100)	3 (7.1)	39 (92.6)
Clindamycin	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Imipenem	0 (0.0)	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)
Ofloxacin	3 (60)	2 (40)	1 (100)	0 (0.0)	31 (73.8)	11 (26.1)
Piperacillin	4 (80)	1 (20)	1 (100)	0 (0.0)	42 (100)	0 (0.0)
Tetracycline	5 (100)	0 (0.0)	0 (0.0)	1 (100)	41 (97.6)	1 (2.3)
Trimethoprim-Sulfamethoxazole	5 (100)	0 (0.0)	1 (100)	0 (0.0)	42 (100)	0 (0.0)

Table 5. Average multiple antibiotic resistance indices (MARI) of AmpC β-lactamase-producing bacterial pathogens isolated from ear swab samples of CSOM patients

CSOM Bacteria	Average MARI Value
E. coli	0.8
K. pneumonia	0.7
P. aeruginosa	0.8

Table 6. Frequency of AmpC β-lactamase-producing Gram-negative bacterial pathogens isolated from ear swab samples of CSOM patients

CSOM Bacteria	AmpC β-lactamases Positive (%)	FOX-AmpC-β-lactamase gene n (%)
E. coli	5 (1.7)	1 (2.1 %)
K. pneumonia	1 (0.3)	1 (2.1 %)
P. aeruginosa	42 (14.0)	2 (4.2 %)
Total	48 (16.0)	

prevalence patterns in CSOM patients within the age group of 1-10 years have been reported in previous studies (4, 24-26); with the burden of disease greatest between 6 months and 18 months of age. In contrast to our study, other studies reported maximum number of CSOM cases in the first (1-10 years) and second decades (11-20 years) of life (24, 27). Poor hygiene and unorthodox treatment approaches by care-givers, such as the use of unconventional ear drops and concoctions (oil and honey) into the middle-ear, especially in Nigeria, may initiate the proliferation of opportunistic pathogens, thereby leading to blockage of eustachian tubes in children.

Gram-negative bacteria in CSOM patients were more frequent in males than females in this study. Males have also been reported to have a higher risk factor than females for the development and acquisition of otitis media (24, 25). The reason for this observation is still unknown. As this study involved a random selection of cases, the predominance of male patients over female may possibly be an incidental finding.

The isolation rate of 72.3% observed for Gram-negative bacterial pathogens in CSOM cases in our study is in agreement with previous studies done in Nigeria (76.3%), Ethiopia (67.6%) and Malawi (72.4%) (16, 28, 29). The observed slight differences in rates of bacterial isolation could be attributed to differences in population characteristics, variation in climate, sample size, culture techniques, duration of ear discharge, indiscriminate use of antibiotics, and cultural differences (16, 29). The most predominant bac-

terial species isolated from CSOM samples in this study was *P. aeruginosa* as it accounted for 36.3%. Although there seems to be geographical differences in the proportions between the species presently identified in other studies; this present observation is in parallel with previous reports (25, 30, 31). In our study, *K. pneumoniae* and *E. coli* were identified in 22.3% and 13.7% cases of CSOM respectively. The findings of our study are in tandem with the work of Prakas and Deepak (27) who reported *K. pneumoniae* (9.42%) and *E. coli* (7.33%) in CSOM cases. Mansoor et al. (32) reported a prevalence frequency of 8% and 4% for *K. pneumoniae* and *E. coli* respectively, whereas Poorey and Lyer (33) reported a higher *K. pneumoniae* prevalence (25.4%) in their study. More frequent isolation of faecal bacteria (*E. coli* and *K. pneumoniae*) and environmental bacteria (*Pseudomonas* spp.) indicated that individuals are at high risk of infection due to poor hygienic conditions.

In consonance with earlier studies, overall proportion of AmpC β-lactamase-producing *P. aeruginosa* was 37.89% (7) when compared with the 20% observed in our study. In Nigeria and abroad, most studies on AmpC β-lactamases and ESBL-producing bacteria focused on isolates from clinical origin, particularly uropathogenic bacteria, and in animals. Meanwhile, this current study has been able to report AmpC β-lactamase-producing Gram-negative bacterial pathogens in CSOM cases.

AmpC β-lactamase-producing bacterial pathogens in this study exhibited high frequency of resistance (100-74%) to cephalosporins (ceftazidime, ceftriaxone, cefoxitin, cefuroxime, and cefotaxime). In contrast to our study, beta-lactamase-producing Enterobacteriaceae isolates with low resistance to ceftazidime (14.8%), cefotaxime (11.9%), and ceftriaxone (20%) in CSOM cases have been reported (34). In the present study, all the bacterial isolates showed low level of resistance to ciprofloxacin (0- 29.6%). This result is in agreement with the study by other authors in Africa where 0-6.9%, 0-33% and 0- 37.5% resistance frequencies were observed with ciprofloxacin (34). All the AmpC β-lactamase-producing *P. aeruginosa* were resistant (100%) to tetracycline. This pattern of tetracycline resistance is common in *P. aeruginosa* and has been reported in other studies with resistance frequencies of 59.1%-100% (35, 36).

AmpC β-lactamase-producing bacteria in CSOM in this study expressed MDR phenotype with multiple antibiotic resistance index (MARI) value ranging from 0.7-0.8. This is in tandem with reports from other studies (7, 37). Our findings collectively showed the rising trend of β-lactamase-producing multidrug-resistant (MDR) which might be linked to the misuse of antibiotics or lack of appropriate antimicrobial resistance diagnosis for accurate treatment in our study area.

Interestingly, all the AmpC β-lactamase-producing bacterial strains in this study were completely susceptible (100%) to amikacin and imipenem. The effectiveness of these antimicrobial agents against CSOM bacterial pathogens substantiates reports in many literatures (5, 7, 16, 25, 30, 31).

PCR results showed that 1 (2.1%) *E. coli*, 1 (2.1%) *K. pneumoniae*, and 2 (4.2%) *P. aeruginosa* isolates harboured the FOX-AmpC- β-lactamase gene. Other reports have reported higher frequencies of FOX-AmpC- β-lactamase gene when compared to the present study. In Abakaliki, Nigeria, 6.3% *E. coli* isolates harbouring the FOX-1 plasmid-mediated AmpC gene was reported in faecal samples of farm animals (12). In India, the prevalence of AmpC-producing *K. pneumoniae* and *E. coli* isolates was 10.1% and 12.5% respectively (37). A study from Egypt detected the FOX-1 gene in 33.3% *E. coli* isolates (11). Another study from Egypt reported AmpC-producing strains in 88.46% isolates among cefoxitin-resistant *E. coli* and *Klebsiella* species (38). A limitation in our study was our inability to sequence the isolates habouring the FOX-AmpC-β-lactamase genes due to some constraints. As higher incidence of disease was seen among children, there is need for educating parents and guardians or care-givers on possible risk-factors of CSOM disease which may be a preventive strategy that might reduce the disease burden and occurrences.

CONCLUSION

To the best of our knowledge, this is the first report of AmpC β-lactamase-producing multidrug-resistant GNB pathogens (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) harbouring FOX-AmpC-β-lactamase gene in bilateral and unilateral ear discharges of chronic suppurative otitis media (CSOM) patients in Nigeria. This study advocates for good personal hygiene among patients. However, continuous and periodic evaluation of antimicrobial resistance profiles, and molecular studies of bacterial pathogens

implicated in CSOM infections are needed to comprehensively understand the etiology, pathogenesis, and clonal spread of bacterial pathogens implicated in CSOM cases, so as to help in its effective management.

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