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RESEARCH PAPER

Prevalence of common bacterial etiology and antimicrobial susceptibility pattern in patients with otitis media in Duhok Province –Iraq

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A B S T R A C T:

Otitis media is the most frequent type of ear infection. It is a major health problem for both children and adults but the proportion is different in different countries. The current study was designed to determine the frequency of bacterial isolates causing otitis media and their antimicrobial resistance profile to assess the incidence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan drug resistance (PDR) among the isolated pathogens. Also, to determine the various risk factors (age, gender, type, and site of infection) associated with ear infection. A total of 200 ear discharge samples were collected from patients aged 1-77 years attending the Ear, Nose, and Throat (ENT) unit in Azadi Teaching Hospital in Duhok city from November 2018 to September 2019. The clinical samples were cultured and bacterial isolates were identified by standard microbiological methods then confirmed by VITEK® 2 Compact automated system. All gram-negative bacterial isolates were studied phenotypically for Extended Spectrum Beta Lactamase production. Out of 200 ear swabs, 95% confirmed positive culture; 85.8% were bacterial isolates. From the total bacterial isolates, 57% were gram-positive bacteria, while gram-negative bacteria were 43%; with predominant of *Pseudomonas aeruginosa*, (22%) followed by *Staphylococcus aureus* (19%) and Coagulase negative *Staphylococci* (18%). The prevalence of otitis media was not significantly affected by gender. The majority of patients belonged to the age group (1-10) years old. 64.1% of the isolates were characterized as MDR, 31.7% were XDR, with one (0.7%) as PDR. Among these, 60.7% isolates were ESBL producers, *Pseudomonas aeruginosa* was the most frequent ESBL- producing isolates (45.9%), followed by *Proteus spp* (18.9%). The *in-vitro* sensitivity results indicated that Gentamycin and Ciprofloxacin were effective antibiotics in the treatment of otitis media. Additionally, Meropenem, Imipenem, Cefepime, and Azetronam were also the most effective drug against *Pseudomonas aeruginosa*. We concluded that the drug resistant isolates were common, worryingly high, leaving only limited drugs as a treatment choice.

KEY WORDS: *Ear infection, Otitis Media, Bacterial etiology, MDR, XDR, ESBL.*
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1.INTRODUCTION :

Otitis media is an inflammatory process of the mucosa of the inner ear with exudate production. Its consequences are hearing weakness; or even loss in adults and problems in a speech in children (Chamberlain, 2009). The most common causes of disease of the middle ear are respiratory infections producing acute or chronic otitis media. The middle ear, being connected to the respiratory tract (nasopharynx) through the Eustachian tube, is subjected to the same infections as the nose and sinuses and is frequently involved when they become inflamed (Alberti, 2001). Acute otitis media (AOM) is considered to be the initial three weeks of inflammation usually presents with ear pain. Chronic suppurative otitis media (CSOM) is characterized by chronic ear discharge through a perforated tympanic membrane for more than 6 weeks to 3 months (Lieberthal *et al*., 2013). Worldwide, around 1.23 billion people are

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affected by otitis media; thus it is ranked as the fifth global burden of disease and the second cause of hearing loss (Tesfa *et al.,* 2020; Morris and Leach, 2009). Ear infection is caused by bacteria, viruses, and fungi. Bacteria being the most common cause (Bello *et al.,* 2011). *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus mirabilis* and *E.coli* are the common bacteria isolated from the cases of otitis media (Al-Mosawi, 2018; Seid *et al*, 2013). The emergence of the resistance to antimicrobial agents constantly develops seriously, affecting the assessment and treatment of infections in the community and hospitals. ESBLs are enzymes that are capable of hydrolyzing the Beta lactam ring of penicillin, broad spectrum Cephalosporins, and Monobactams (Fernando *et al.,* 2017). ESBLs production is most commonly seen among gram-negative bacteria including *Klebsiella pneumonia*, *E.coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* (Ogefere *et al.,* 2015). In addition, ESBL-producing bacteria exhibit co-resistance to other classes of antibiotics, which may limit treatment options available. Therefore, this study was carried out to determine the frequency of bacterial isolates associated with otitis media and to investigate their antimicrobial resistance profile. Also, to assess the demographic distribution of bacterial ear infection in Duhok City and to assess the prevalence of ESBL-producing bacteria.

2. Materials and Methods

2.1. Study design:

This study was carried out at Ear, Nose and Throat (ENT) unit in Azadi Teaching Hospital in Duhok city from November 2018 to September 2019. Two hundred ear discharge samples were obtained from patients, of both genders, clinically diagnosed with ear infection, their ages ranged between 1-77 years old. Only patients who didn't receive topical or systemic antibiotic treatment for two weeks were included in this study.

2.2. Sample collection

The ear discharge samples were collected immediately after clinical examination by an Otolaryngologist with caution and were taken to avoid surface contamination. The swabs were transported to the microbiology laboratory using Amies transport media (cultiplast tampon swab, Italy) for culture and antibiotics sensitivity investigation. The samples were labeled with the patient's name, identification number, age, gender, site, and type of infection, date, and time of collection. In addition, complete information was obtained directly from the patients, and a questionnaire form was used for each patient.

2.3. Culture and identification

All ear discharge samples were inoculated on the following culture media: Blood, MacConkey, Chocolate, and selective agar plates for bacterial isolation. Then, the samples were incubated aerobically and anaerobically at 37°C for 24 hours; whereas the Sabouraud dextrose agar was used as a selective media for fungi. The plates were incubated under aerobic condition for 24-48 hours at 35°C. All bacterial isolates were identified according to standard microbiological methods: cultural characteristic, gram stain and conventional biochemical tests (MacFaddin, 2000). The bacterial species were identified by VITEK® 2 Compact *(*BioMérieux, USA*)* microbiological automated system, using ID-GN(REF21341) cards specific for Gram-negative bacteria and ID-GP(REF 21342) cards for Gram-positive bacteria (Pincus, 2010).

2.4. Antimicrobial susceptibility testing

All gram-negative isolates were tested against seventeen antibiotic discs (Bioanalyse/Turkey), which include: Amikacin, Gentamycin, Netilmicin, Ertapenem, Imipenem, Meropenem, Cefuroxime, Ceftriaxone, Ceftazidime, Cefepime, Azetronam, Ampicillin, Piperacillin, Amoxicillin clavulanate, Pip-Tazobactum, Trimethoprim, and Ciprofloxacin. While twenty antibiotics were used for gram-positive bacteria which include: Gentamycin, Ampicillin, Ciprofloxacin, Tobramycin, Cefoxitin, Penicillin G, Oxacillin, Amoxicillin clavulanate, Daptomycin, Teicoplanin, Vancomycin, Clindamycin, Erythromycin, Fusidic acid, Linezolid, Fosfomycin, Levofloxacin, Rifampin, Tetracyclin, and Trimethoprim sulphamethoxazole. An antimicrobial sensitivity test was performed by the Kirby Bauer disc diffusion method on Mueller-Hinton agar (Himedia, India). The results were detected after overnight incubation at 37 ºC, by measuring the zone of inhibition according to Clinical and Laboratory Standards Institute guidelines (CLSI.,2016).

MDR, XDR, and PDR strains of pathogenic bacteria were determined according to European Center for Disease Prevention and Control (ECDC), and the Centers for Disease Control and Prevention (CDC). MDR was defined as the isolate resistant to at least one agent in three or more antimicrobial classes. Any bacterial isolate that remains sensitive to only one or two classes of antibiotics is characterized as XDR. While PDR refers to non-susceptibility to all agents in all antimicrobial classes (Magiorakos *et al*., 2012)

2.5. Extended Spectrum β-Lactamases detection methods:

2.5.1. Double Disc Synergy Test:

Double Disc Synergy Test (DDST) was performed using 3rd generation cephalosporin discs (Cefotaxime (30µg), Ceftazidime (30µg) and Ceftriaxone (30µg)), which were placed with a distance of 20mm from an Augmentin (20 µg Amoxicillin /10 µg Clavulanic acid) disc on a cultured Muller-Hinton agar then incubated overnight at 37 ºC. Extension of the inhibition zone of any type of 3rd generation cephalosporin toward the Augmentin disc was considered as a positive to the ESBL production.

2.5.2. ESBL CHROMagar™:

For the phenotypic detection of gram-negative ESBL producing bacteria, the isolates were cultured on CHROMagar™ ESBL (Conda pronadisa, Spain) after adding ESBL Supplement (CAT:6042). They then incubated at 37 ºC for 24 hours. *Klebsiella pneumoniae*, *Enterobacter* and *Serratia* produce green-blue to browny-green colonies, while *E. coli* and *Klebsiella oxytoca* produce pink to burgundy coloration colonies, *Proteus* produce dark to light brown colonies; the colorless colonies considered as ESBL producing *Pseudomonas* and *Acinetobacter*.

2.6. Statistical Analysis

All data was analyzed by SPSS version 24 and Microsoft Excel (2013). Chi-square tests ($\chi 2$) were used to test for variable categories; and probability value (P-value) of less than 0.01 was taken as being statistically highly significant.

3. Results:

Two hundred ear discharge samples were collected from patients attending ENT unit in Azadi Teaching Hospital with ear infection. Of these, 190 (95%) cases were confirmed as positive culture, while 10/200(5%) samples showed culture negative. Among the positive culture, 163/190(85.8%) were positive for bacterial isolates, and 27(14.2%) showed the growth of candida species. 160 clinical samples had single bacterial growth and 3 samples were mixed along with candida. No anaerobic bacteria were detected.

3.1. Isolated pathogens:

The current study relied on bacterial ear infection. The overall prevalence of bacterial isolates was 163/190 (85.8%) and demonstrated that the most frequent bacterial infection diagnosed was otitis media 142/163(87.1%). Out of 142 otitis media cases, 55(38.7%) were acute otitis media and 87(61.3%) were chronic suppurative otitis media. From the total bacterial isolates (142), 81(57%) were gram-positive bacteria, while gram-negative bacteria gave 61(43%). The results shown in figure (1) indicated that the most common bacteria isolated from patients with acute otitis media were: 15(10.6%) *S. aureus*; 12 (8.5%) CoNS; 11(7.7%) *P. aeruginosa*; 4(2.8%) *Kocuria;* 2(1.4%) for each *Proteus*, *Streptococcus*, *M.luteu*, *Enterobacter*, and *G.adiacense*; while *Klebsiella*, *E. faecalis*, and *S. marcescens* were 1(0.7%).

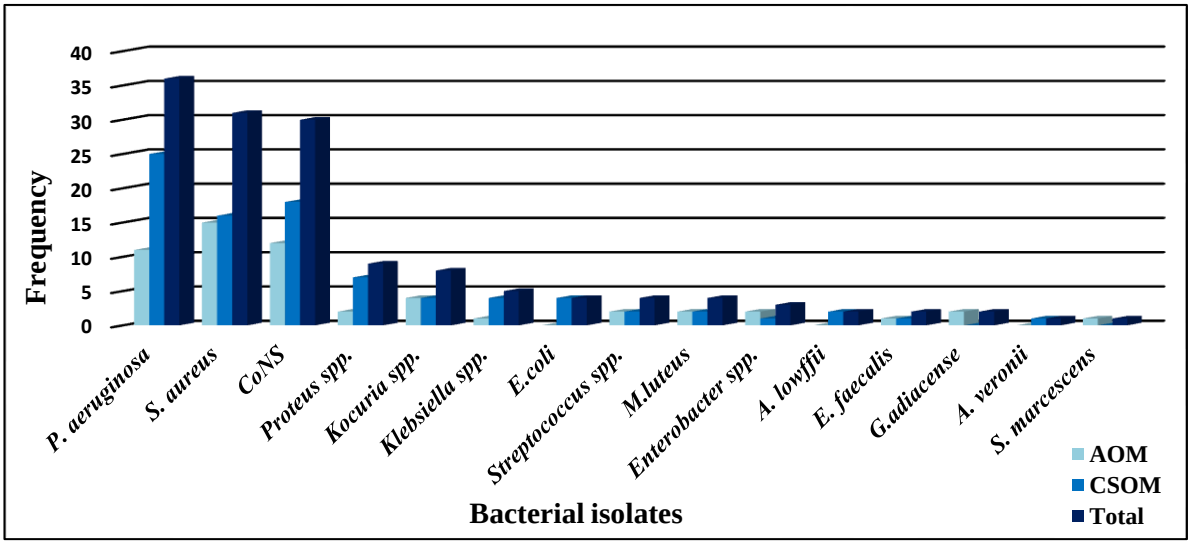

Figure (1): Frequency of bacterial isolates from patients with otitis media.

On the other hand, the same figure showed that the predominant bacteria isolated from patients with chronic suppurative otitis media, were: *P. aeruginosa* 25(17.6%); CoNS 18(12.7%); *S. aureus* 16(11.3%); *Proteus spp*. 7(4.9%); 4(2.8%) for each *Kocuria*, *Klebsiella* and *E.coli;* followed by *Streptococcus spp.*, *M.luteus* and *A. lowffii* were 2(1.4%); while the least prevalent were *Enterobacter spp.*, *E. faecalis*, and *A. veronii* 1(0.7%). The results illustrated that the distribution of various bacterial isolates was not significantly (P>0.01) associated with the types of otitis media.

3.2. Age and gender distribution:

A total of 142 patients with otitis media were enrolled in this study. The participants ages ranged from 1-77 years old, and the mean age was 27.6 (27.6 ± 19.4). Based on the results of table (1), the majority (39: 27.5%) of patients belonged to the age group of (1-10) years old. Acute otitis media was more common in this age group. Whereas chronic suppurative otitis media (19: 21.8%) was more common in the age group (45-55) years old. The chi-square test illustrated that the age groups had a statistically significant (P< 0.01) association with various types of otitis media. On the other hand, the same table showed that the males (74: 52.1%) were more infected with bacterial ear infection than female (68: 47.9%), but was statistically not significant (P>0.01).

Table (1): Distribution of otitis media according to gender and age groups of cases.

Variables		Types of otitis media			Chi-square and p- value
		AOM n(%)	CSOM n(%)	Total n(%)	
Age groups in year	1-10	25(17.6%)	14(9.9%)	39(27.5%)	Chi-square=22.554 p- value < 0.01
	11-21	8(5.6%)	17(12%)	25(17.6%)	
	22-32	8(5.6%)	13(9.2%)	21(14.8%)	
	33-44	10(7%)	12(8.5%)	22(15.5%)	
	45-55	2(1.4%)	19(13.4%)	21(14.8%)	

	>55	2(1.4%)	12(8.5%)	14(9.9%)	
Gender	Male	32(22.5%)	42(29.6%)	74(52.1%)	Chi-square=1325
	Female	23(16.2%)	45(31.7%)	68(47.9%)	p- value >0.01

3.3. Antimicrobial susceptibility testing:

3.3.1. Gram-positive bacteria:

The results in table (2) revealed that all gram-positive isolates were 100% resistant to Penicillin G, while 90% (27/30) and 75% (6/8) for CoNS and *Kocuria spp.* respectively. Regarding, Fusidic acid, all gram-positive bacteria showed high resistance (100%); and 96.8% (30/31), 93.3% (28/30) and 87.5% (7/8) for each (*S. aureus*, CoNS, and *Kocuria spp*). As for Rifampin, most isolates were found to be 100% resistant, while 86.7% (26/30) for CoNS and 75% (6/8) for *Kocuria spp.* On the other hand, the same table indicated variable sensitivity patterns for the isolates against other antibiotics.

Table (3): Pattern of susceptibility of gram-negative bacteria to various antibiotics.

Isolated bacteria		Types of Antibiotics (%)																
		AK	CN	NET	ETP	IPM	MEM	CXM	CRO	CAZ	FEP	ATM	AM	PRL	AMC	TPZ	TMP	CIP
P.aeroginosa	R	61.1%	44.4%	27.8%	86.1%	5.6%	8.3%	100%	100%	63.9%	33.3%	8.3%	100%	47.2%	100%	19.4%	100%	16.7%
	S	38.9%	55.6%	72.2%	13.9%	94.4%	91.7%	0	0	36.1%	66.7%	91.7%	0	52.8%	0	80.6%	0	83.3%
Proteus spp.	R	33.3%	44.4%	33.3%	22.2%	33.3%	11.1%	55.6%	55.6%	44.4%	33.3%	55.6%	100%	66.7%	44.4%	33.3%	77.8%	33.3%
	S	66.7%	55.6%	66.7%	77.8%	66.7%	88.9%	44.4%	44.4%	55.6%	66.7%	44.4%	0	33.3%	55.6%	66.7%	22.2%	66.7%
Klebsiella spp.	R	60%	0	0	40%	40%	20%	20%	40%	40%	0	0	100%	60%	20%	40%	40%	0
	S	40%	100%	100%	60%	60%	80%	80%	60%	60%	100%	100%	0	40%	80%	60%	60%	100%
E.coli	R	50%	25%	25%	50%	0	0	75%	75%	75%	50%	25%	100%	50%	100%	75%	50%	25%
	S	50%	75%	75%	50%	100%	100%	25%	25%	25%	50%	75%	0	50%	0	25%	50%	75%
Enterobacter spp.	R	33.3%	33.3%	0	33.3%	33.3%	0	33.3%	66.7%	33.3%	0	0	100%	0	100%	33.3%	33.3%	33.3%
	S	66.7%	66.7%	100%	66.7%	66.7%	100%	66.7%	33.3%	66.7%	100%	100%	0	100%	0	66.7%	66.7%	66.7%
A. Lowffii	R	50%	0	0	100%	0	0	50%	100%	50%	0%	100%	100%	50%	100%	0	50%	0
	S	50%	100%	100%	0	100%	100%	50%	0	50%	100%	0	0	50%	0	100%	50%	100%
A. veronii	R	100%	0	0	100%	100%	100%	0	0	0	0	0	100%	100%	100%	100%	0	0
	S	0	100%	100%	0	0	0	100%	100%	100%	100%	100%	0	0	0	0	100%	100%
S. marceses	R	0	0	0	0	0	0	100%	0	0	0	0	100%	0	100%	100%	0	0
	S	100%	100%	100%	100%	100%	100%	0	100%	100%	100%	100%	0	100%	0	0	100%	100%

*R: Resistance *S: Sensitive

3.4. Incidence of drug resistance patterns of all bacterial isolates studied:

The resistance profiles were used to recognize MDR, XDR, and PDR amongst all bacterial isolates studied which determined, out of total 142 bacterial isolates, 91 (64.1%) bacterial isolates were MDR and 45(31.7%) isolates were XDR; with one (0.7%) isolate of *Pseudomonas* was PDR. Amongst 81 gram positive pathogen isolated, 69(85.2%) and 12(14.8%) were MDR and XDR, respectively; with no PDR was detected. Out of 61 gram negative isolates, 22(36.1%) isolates were MDR, 33(54.1%) were XDR, and one isolate (1.6%) was PDR. Out of 31 *S. aureus* isolated, 26(83.9%) were MDR, and 5(16.1%) were XDR. Thirty CoNS were isolated and 27(90%) were MDR, whereas 3(10%) were XDR. *Kocuria* isolates have shown high (100%) MDR rate. Three (75%) and 1 (25%) out of 4 *Streptococcus* isolates were MDR and XDR respectively. Additionally, all four isolates of *Micrococcus* were MDR (100%). While 2 (100%) *Enterococcus* isolates were detected as XDR. Finally, of two *Granulicatella* isolates, 1(50%) was MDR, and 1 (50%) was XDR, as shown in table (4).

Table (4): Frequency of MDR, XDR, and PDR of gram-positive bacterial isolates for selected antimicrobial classes.

Isolated Bacteria (No.)	Types of Resistance		
	MDR	XDR	PDR
S .aureus (31)	26(83.9%)	5(16.1%)	-
CoNS (30)	27(90%)	3(10%)	-
Kocuria. spp (8)	8(100%)	-	-
Streptococcus .spp (4)	3(75%)	1(25%)	-
M .luteus (4)	4(100%)	-	-
E .faecalis (2)	-	2(100%)	-
G .adiacense (2)	1(50%)	1(50%)	-
Total (81)	69(85.2%)	12(14.8%)	-

Table (5) shows incidence of MDR, XDR, and PDR pathogens isolated from each species of gram negative bacterial isolates. Thirteen (36.1%), 22 (61.1%) and 1(2.8%) out of 36 *Pseudomonas* isolates were MDR, XDR, and PDR respectively. Among 9 isolates of *Proteus*, 4 (44.4%) and 5 (55.6%) were MDR and XDR respectively. Five *Klebsiella* were isolated, 1 (20%) were MDR, whereas 1 (20%) were XDR. Of four isolates *E.coli* 1 (25%) were MDR and 2 (50%) were XDR. Furthermore, out of 3 isolates of *Enterobacter* 1 (33.3%) were MDR and 1(33.3%) were XDR. Two (100%) *A. Lowffii* isolates were XDR. The overall rate of MDR among all isolates *A. veronii* and *S. marcescens* were 100%.

Table (5): Frequency of MDR, XDR, and PDR of gram-negative bacterial isolates against the antibiotics used.

Isolated Bacteria (No.)	Types of Resistance		
	MDR no.(%)	XDR no.(%)	PDR no.(%)
P. aeruginosa (36)	13 (36.1%)	22(61.1%)	1(2.8%)
Proteus.spp (9)	4(44.4%)	5(55.6%)	-
Klebsiella. spp (5)	1(20%)	1(20%)	-
E.coli (4)	1(25%)	2(50%)	-
Enterobacter. spp (3)	1(33.3%)	1(33.3%)	-
A .lowffii (2)	-	2(100%)	-
A .veronii (1)	1(100%)	-	-
S. marcescens (1)	1(100%)	-	-
Total (61)	22(36.1%)	33(54.1%)	1(1.6%)

3.5. Screening for ESBL producers:

Sixty-one gram-negative bacterial isolates obtained from patients with otitis media were studied phenotypically for ESBL production; the results indicated that 37(60.7%) isolates were ESBL producers and 24 (39.3%) isolates were non-ESBL producers. A significant correlation was observed between ESBL and non-ESBL producers and the prevalence of otitis media cases. The predominant ESBL producing isolates were *P. aeruginosa* 17/37 (45.9%), followed by *Proteus spp.* 7/37 (18.9%), *Klebsiella spp.* 5/37 (13.5%), *E.coli* 4/37 (10.8%), *Enterobacter* 3/37 (8.1%) and *S. marcescens* 1/37 (2.7%). Phenotypically, ESBL production was demonstrated by both Double Disk Synergy Test and ESBL CHROM agar methods as shown in (Fig.2 and Fig.3) respectively. Among 37 ESBL producers, 31(83.8%) isolates were detected by Double Disk Synergy Test, while ESBL CHROM agar detected 37(100%) isolates.

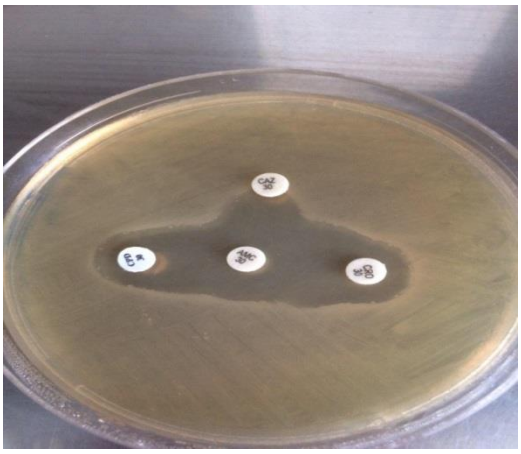

Figure (2): Double Disk Synergy Test showing positive ESBL production, when swabbed on Muller Hinton agar and incubated over night at 37°*C.*
with Cefotaxime, Ceftazidime and Ceftriaxone discs; which were placed with distance of 20mm from an Amoxicillin-clavulanic acid disc.

Figure (3): ESBL producers: colonies on CHROM agar
1- *P. aeruginosa*, 2-*Kl. pneumonia*, 3- *Kl. oxytoca* , 4-*E.coli*, 5- *Enterobacter spp.* , and 6- *S. marcescens.*

3.6. Antimicrobial susceptibility patterns of ESBL producing isolates:

The resistance profile of the ESBL and non-ESBL producing isolates was shown in table (6). It was found that all ESBL producing isolates resistant 100% to Ampicillin; other resistance rates were 78.4% Amoxicillin-clavulanic acid, 73% Ceftriaxone and Trimethoprim, and 70% Cefuroxime. Additionally, ESBL producers demonstrated high sensitivity 91.9%, 86.5%, and 83.8% for Meropenem, Azetronam and Impenem respectively. A significant difference ($p < 0.01$) in resistance pattern was observed with Gentamicin, Ertapenem, Cefuroxime, Ceftriaxone, Amoxicillin-clavulanic acid and Trimethoprim between ESBL and non ESBL isolates.

Table (6): Antibiotic susceptibility pattern of ESBL and non- ESBL producing isolates

Antibiotic	ESBL positive		ESBL negative		P-value
	Resistance	Sensitive	Resistance	Sensitive	
Amikacin	19 (51.4%)	18 (48.6%)	14 (58.3%)	10 (41.7%)	P>0.01
Gentamicin	15 (40.5%)	22 (59.5%)	7 (29.2%)	17 (70.8%)	p<0.01
Netilmicin	10 (27%)	27 (73%)	4 (16.7%)	20 (83.3%)	P>0.01
Ertapenem	20 (54.1%)	17 (45.9%)	21 (87.5%)	3 (12.5%)	p<0.01
Impenem	6 (16.2%)	31 (83.8%)	3 (12.5%)	21 (87.5%)	P>0.01
Meropenem	3 (8.1%)	34 (91.9%)	3 (12.5%)	21 (87.5%)	P>0.01
Cefuroxime	26 (70.3%)	11 (29.7%)	22 (91.7%)	2 (8.3%)	p<0.01
Ceftriaxone	27 (73%)	10 (27%)	23 (95.8%)	1 (4.2%)	p<0.01
Ceftazidim	20 (54.1%)	17 (45.9%)	14 (58.3%)	10 (41.7%)	P>0.01
Cefepime	10 (27%)	27 (73%)	7 (29.2%)	17 (70.8%)	P>0.01
Azetronam	5 (13.5%)	32 (86.5%)	6 (25%)	18 (75%)	P>0.01
Ampicillin	37 (100%)	0	24 (100%)	0	P>0.01
Pipracillin	18 (48.6%)	19 (51.4%)	12 (50%)	12 (50%)	P>0.01
Amoxicillin-clavulanic acid	29 (78.4%)	8 (21.6%)	23 (95.8%)	1 (4.2%)	p<0.01
Pip-Tazobactum	13 (35.1%)	24 (64.9%)	5 (20.8%)	19 (79.2%)	P>0.01
Trimethoprime	27 (73%)	10 (27%)	22 (91.7%)	2 (8.3%)	p<0.01
Ciprofloxacin	8 (21.6%)	29 (78.4%)	3 (12.5%)	21 (87.5%)	P>0.01

4. Discussion

Multi microbial infections are commonly reported in otitis media with mixed bacteria-bacteria, viral-bacterial and fungal-bacterial infections. Based on the present study results, the single infection was more frequent than mixed infection. The incidence of single infection was 98.2%, while it was 1.8% for mixed infection. This finding was consistent with work published by several studies (Haider, 2002; Karim, 2008; Chaudhary and Shukla, 2014**;** Balan and Viswanatha, 2017**;** Gorems *et al*., 2018). No growth was observed in 5% ear swabs, similar results were reported by (Karim, 2008; Al-Marzoqi *et al*., 2013; Gorems *et al*., 2018). This may be attributed to the possibility of viruses

(respiratory syncytial virus, influenza virus and adenovirus), chlamydia or mycoplasma as a pathogen of the otitis media (Chonmaitree, 2000; Prakash *et al*., 2013).

It was found in this study that males 52.1% more infected than females and prevalence of otitis media was not significantly affected by gender. Which was in line with other studies conducted in Iraq (Aldhaher *et al*., 2018), Nigeria (Nwokoye *et al.*, 2012), Pakistan (Javed *et al*. 2020), Ethiopia (Worku *et al.* 2014; Gorems *et al*. 2018), Uganda (Justin *et al.*, 2018), and India (Chaudhary and Shukla, 2014). But several studies showed that the infection in females were predominant (Abera *et al.*,2011; Al-Marzoqi *et al*.,2013; Akter *et al*.,2015; Jik *et al*., 2015; Basnet *et al*., 2017). Male predominance could be because life style choices, environmental effect; Kvestad *et al*., (2004) confirms that there is sufficient evidence for the presence of sex-based differences due to relative genetic effects.

The results of this study indicated that the children patients belonged age group 1-10 years had the high prevalence of otitis media. Similar to previous studies carried out by (Nwabuisi and Ologe, 2002; Nwokoye *et al.*, 2012; Saleh *et al.*, 2014; Basnet *et al*., 2017; Hassooni *et al*., 2018) and dissertation conducted by (Karim, 2008) also found similar findings. However, high children´s susceptibility to otitis media is could be attributed to their immune system; it was also shown that the Eustachian tubes were shorter, wider and more horizontal than in the adults. This position allows opportunistic organisms from noes, adenoids and sinuses pass from nasopharynx in to the sterile middle ear along the Eustachian tube "particularly during coughing, sneezing, vomiting and forced feeding commonly practiced in our environment with child´s nose blocked" (Nwabuisi and Ologe, 2002; Basnet *et al*., 2017).

Etiological agents of otitis media and their antimicrobial susceptibility varied with geographical location, age, type of infection whether acute or chronic and time (Hassan and Adeyemi, 2007). The observation in present study indicated that gram positive bacteria were the predominant isolates (57%) from patients with otitis media when compared to gram negative bacteria (43%) which was in agreement to earlier studies performed by (Al-Marzoqi *et al.* , 2013; Vaidya *et al*., 2015; Jik *et al*., 2015; Basnet *et al*., 2017; Gorems *et al*., 2018; Sah *et al.*, 2020). This, however, differs with the studies conducted by (Ogbogu *et al.*, 2013; Hassooni *et al*., 2018; Aldhaher *et al*., 2018) where gram negative bacteria were predominant. The difference might be attributed to the variation in the bacterial profile of otitis media in accordance to climate and geographical variation of the study regions. Out of 142 cases studied, 55(38.7%) were acute type of otitis media and 87 (61.3%) of chronic type in this study. Similar results were obtained from other studies (Seid *et al.*, 2013; Wasihun and Zemene, 2015**;** Vijayvargiya *et al*., 2016; Basnet *et al*., 2017; Hassooni *et al*., 2018). Low socioeconomic status, crowded living conditions, and malnutrition related to poor hygiene which is considered as risk factors for the development of chronic otitis media (Verhoeff *et al.*, 2006). In this study, patient with chronic otitis media were frequently between 45-55 years old. In contrast, Loy *et al*., (2002) showed the increased prevalence of chronic otitis media in 30-40 years old in their study. Vijayvargiya *et al*. reported high incidence in 6-68 years of age; Seid *et al.*, (2013) reported highest among 11-20 years age group. Whereas Shyamala and reddy, (2012), Nazir and Kadri (2014), and Hailu *et al*. (2016) reported that chronic otitis media was seen in first and second decade of life.

The predominant bacterial etiology of chronic otitis media was *P. aeruginosa* followed by CoNS, *S. aureus*, *Proteus* and other enteric bacteria, previous studies also found similar results (Alsaimary *et al*. 2010; Shyamala and reddy, 2012; Ogbogu *et al.*, 2013**;** Al-Marzoqi *et al*., 2013; Nazir and Kadri, 2014; Vijayvargiya *et al*., 2016; Juyal *et al*.,2017; Hassooni *et al*., 2018; Javed *et al*., 2020). While, various studies from different countries have reported that *S. aureus* is the most predominant pathogen that causes chronic otitis media (Ettehad *et al.*, 2007; Ahmad, 2013; Jakribettu *et al.* 2014; Wasihun and Zemene, 2015 ; Basnet *et al*., 2017). The dominance isolation rate of *P. aeruginosa* in this study could be related to its biofilm phenotype property which allows entry to the middle ear through the external canal. It can grow well in the environment of the ear and is difficult to eliminate. It has been suggested to damage tissues, interfere with the host defenses and inhibit antibiotic activity by enzymes and toxins (Seid *et al.*, 2013; Gellatly and Hancock 2013). Moreover, the isolation of fecal pathogens such as

K.pneumoniae and *E.coli* may indicate that individuals were at risk of infection due to low socioeconomic status related to poor hygiene. On the other hand, the most prevalent bacteria responsible for acute otitis media was *S. aureus* (10.6%), and this result was similar with previous studies (Ako-Nai *et al.* 2002; Seid *et al.*, 2013; Gorems *et al*., 2018), but differs from other investigators that indicated *Strep. pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenza* predominance (Sierra *et al.*, 2011; Qureishi *et al.* 2014;). The reason for this might be due to several factors like immunological, genetical, infectious, and environmental conditions leading to an individual´s ear infection.

Table (1) indicates that acute otitis media was more common at younger ages and the rate of acute otitis media appears to be decreasing with advancing age. The reason for this may be due to antimicrobial and immunological factors, which indicates that upper respiratory tract colonized abundantly with pathogens and the short and straight of Eustachian tube allows organisms to penetrate the middle ear. Additionally, high incidence of URTI induces middle ear bacterial infection, could be due to immature immune system (Marom *et al.*, 2012). The frequency of *S. aureus* in middle ear infections can be due to its biofilm forming organism, ubiquitous nature, and carrying of resistant strains in the external auditory canal and upper respiratory tract.

The antimicrobial susceptibility patterns of all bacterial isolates in the current study were completely variable. Gentamycin and Ciprofloxacin were effective antibiotics against more than 86% of studied isolate. These results were closely associated with many previous studies (Seid *et al.*, 2013; Gorems *et al*., 2018). Meanwhile the majority (85%-100%) of gram negative bacterial isolates showed high levels of resistance to Ampicillin and Amoxicillin-clavulanic acid in this study. Different studies reported different sensitivity patterns, in a similar study by (Ettehad *et al.*, 2007), it was found that Ciprofloxacin was an effective antibiotic for treatment of otitis media. While Alsaimary noted that Ciprofloxacin, Amoxicillin-clavulanic acid and Gentamycin were an effective for gram positive and gram negative bacteria in otitis media. Gehanno and French Study Group, 1997 found that Ciprofloxacin and Amoxicillin-clavulanic acid was more bacterial agents for many gram positive and gram negative bacteria in AOM and CSOM. These variations depend on the type of infection, type of isolate and type of antibiotic. Additionally, Meropenem, Imipenem, Cefepime and Azetronam were also found to be most effective drug against *P. aeruginosa* with less sensitive to Ceftazidime. While a study conducted by Mansoor *et al.*, (2009) showed less sensitivity to Azetronam but there was good sensitivity to Ceftazidime which contrast to our findings. On the other hand, most gram positive bacterial isolates showed highest rate of resistance to Tetracyclin, Rifampin, Erythromycin, Fusidic acid and Penicillin G. *S.aureus* was found to be highly susceptible to Fosfomycin, Gentamycin followed by Tobramycin, Levofloxacin, Trimethoprim-Sulphamethoxazole, Ciprofloxacin and Oxacillin. Sah *et al.*, (2020) showed that *S. aureus* was high susceptible to Ofloxacin followed by Ciprofloxacin, Tobramycin and Gentamycin. The reason for this variation could be due to antimicrobial susceptibility profile of isolated bacteria which differ among population according to geographical locations and prevalence of resistant bacterial strains. The drug resistant isolates in this study were common, worryingly high. Overall, 64.1% of the bacterial isolates in this study characterized as MDR pathogenic bacteria, 31.7% isolates were XDR, with one isolate (0.7%) of *P. aeruginosa* was PDR; and this detection of drug resistant isolates may limit treatment options. Hence, the prudent use of appropriate antimicrobial agent is recommended. In addition, the current results indicated high level of MDR isolates which were consistent with other studies (Seid *et al*., 2013; Muluye *et al*., 2013 Hailu *et al*., 2016; Gorems *et al*., 2018). The reason for this high drug resistance might be associated with misuse of antibiotics, inappropriate prescribing habits, and biofilm bacterial properties of common isolates (Seid *et al*., 2013).

The current study designed to detect the prevalence of ESBL production and their effect on antimicrobial susceptibility patterns using phenotypic methods (DDST and ESBL CHROM ager) in gram negative isolates from patients with otitis media. There was a significant geographical difference in the occurance of ESBL worldwide (Paterson and Bonomo, 2005; Leylabadlo *et al.*,

2017). Overall, the rate of ESBL producing isolates was 60.7% in ear study. It was lower compared to a study conducted by Sahu and Swain, (2019) 42.2%, while (Khatoon *et al.*, 2015; Mushi *et al.*, 2016; Kashyap *et al.*, 2017; Endaylalu *et al.*, 2020) reported a lower ear infection prevalence of 18.3%, 16.3%, 8.9% and 7.4% respectively. However, a study in Nigeria reported the absence of ESBL producing bacteria in ear infection (Chika *et al.*, 2013). This may attributed to variation in the ESBL detection methods, the study participants and extent of antibiotic use. Based on our results, ESBL CHROM agar method was found to be better than DDST in detection of ESBL producing bacteria. Among the total 37 ESBL producing isolates found in 83.3% by DDST and 100% by ESBL CHROM agar. This explains that this media is the most reliable for the detection of ESBL in high accuracy and rapid identification with very low false positive rates (Filius *et al.*, 2003; Uyanga *et al*., 2019)

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