

Isolation and identification of aerobic bacteria that causes otitis media and detection of their production of β -lactamases

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ABSTRACT

The dissemination of β - lactamases production by bacteria is worldwide problem, this study aimed to detection of β - lactamase and multidrug resistant patterns in aerobic bacteria that causes otitis media, ear swabs collected from 53 patients attending Al-Haboubi hospital in Al-Nasiriyah city during year 2019. The study found 46 out of 53 ear swabs gave positive culture that distributed to 29 (90.6%) female and 18 (8.7% male). The age group >50 year was the higher (12 (25.5 %)) infected with otitis media, it appeared that patients with a chronic otitis media (COM) were more (32 (68.1%) that than in acute otitis media (AOM) that registered 15 (31.9%). AOM appeared with a higher rate (100%) in age group (1-10) year, while the largest age group affect by COM was the age group >50 year. Fifty-five bacterial isolates distributed to 39 (70.9%) isolated from COM and 16 (29.1%) from AOM, *Pseudomonas aeruginosa* was predominant bacteria (18(32.7%)) among other aerobic bacteria. The most antibiotic which resistant by isolates was erythromycin, with a total resistance 89.1%, while amikacin was the most effective with 98.2% of sensitivity. The total number of isolates that produced β -lactamases enzymes reached 49 (89%), and *Pseudomonas aeruginosa* ranked first among these isolates, as 17(34.6%) isolates were recorded, 43 (78.2%) of isolates had multidrug resistance, the most frequency bacteria with MDR were *Proteus* spp, *E. coli*, *Enterobacter* spp. and *Klebsiella* spp, it registered 100%.

Keywords: Otitis media, β - lactamases, Bacteria, AOM, COM

INTRODUCTION

Otitis media (OM) is infection of middle ear and may appeared as otitis media with effusion (OME), acute otitis media (AOM) and chronic suppurative otitis media (1).

In acute otitis media, the found of middle ear fluid (MEF) is accompanied by earache, otorrhea and fever between other severe and symptoms and signs infection. In OME which can be developed as a supplement to acute otitis media or as a new start afterwards viral infection, MEF is appear in the absence of and symptoms and signs of infection, with the most common change detected is hearing loss (2).

AOM is recognize by the rapid fluid accumulation in the middle ear with signs and symptoms of acute infection, like ear pain otorrhea, vomiting and fever (3). OME is characterized by The a glue-like liquid in the Middle ear behind an intact tympanic membrane without symptoms and signs of acute cases (4), CSOM depends on persistent presence of purulent middle ear effusion with usually ear drainage through the tympanic membrane pore (5). Otitis media often recurs after

acute upper respiratory infections as secondary infection.

It is also caused by sensitivity and changes in the middle ear and

Anatomically or functionally of the Eustachian tube. Many pathogenic bacteria like non-typable *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* *Streptococcus pyogenes*, *Escherichia coli*, *Moraxella catarrhalis* and *Proteus mirabilis* and are the causative gents of otitis media (6).

The main organisms in acute otitis media are *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*. In chronic otitis media, these organisms are common, in addition to *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and anaerobic bacteria.

The causative agents associated with otitis media include viruses, fungi and more frequency, bacteria (7). Highlighting the etiology of ear infection and antibiotic sensitivity pattern will help reduce severe complications of infection and guide experimental antibiotics prescribed by doctors, especially in developing countries (8).

A doctor may advocate treatment with antibiotics regardless of the aetiology. This may cause unwanted economic loss, patient stress if the ear infection is caused by a virus or fungus, and above all antibiotic resistance (9). Beta lactam are group of

antibiotics include penicillins, cephalosporins and other. These drugs are effective against many Gram-negative, Gram-positive and anaerobic bacteria (10), these antibiotics are choice for first line therapy of acute otitis media like amoxicillin – clavulanate, cedinir, cefpodoxime and cefuroxime (11).

The clinical outcomes of patients with acute otitis media have changed since the development of bacterial resistance to the commonly antibiotics, like amoxicillin, treat other forms of otitis such as otitis externa and chronic suppurative otitis media has been hindered because increase of bacteria that resistant antibiotics albeit to lower extent than acute otitis media (12). Beta-lactam resistance in Enterobacteriaceae and other Gram-negative bacteria it is mediated primarily by production beta-lactamases (13). beta-lactamases are the most common cause of resistant to beta lactam antibiotics (14).

Beta-lactamases subsequently hydrolyze the beta-lactam ring. The beta-lactam antibiotic becomes inactive before it reaches PBP targeting (15), due to increase the resistance of bacteria with otitis media to antibiotics especially beta lactams, this research was aimed to isolation of bacteria from otitis media patients and determine the ability of bacteria to produce beta –lactamases.

MATERIALS AND METHODS

Specimens Collections

A total of 53 ear swab were collected from patients suffering from ear infection that attending Al-Haboubi hospital in Al-Nasiriyah city, Thi -Qar province, Iraq, cotton swabs had been inoculated culture media MacConkey agar and blood agar and incubated aerobically at 37 for 24 hr. isolates identification was performed by morphological and biochemical tests according to (16) API 20 E system (BioMerieux , France) .

Testing of antibiotic susceptibility

This test was performed by standard disk diffusion method using Mueller Hinton agar (BD&BBL, USA) according to (17), antibiotics disks tested (symbol /in µg) were amikacin (AK /30), vancomycin (VA /30), ceftriaxone (CRO /30), gentamycin (CN/10) Amoxicillin-clavulanic acid (AMC/20-10), ciprofloxacin (CIP /5 µg), erythromycin (E /15), trimethoprim (TMP /10), AZM: azithromycin (AZM /15), Ampicillin\ cloxacillin (AX /15) (Bioanalyses, Turkey).

Detection of β - lactamase production

β - lactamase production test was performed by using capillary tubes method according to (18).

RESULTS**Table (1):** Distribution of otitis media patients with aerobic bacteria according the sex

Sex	Positive Culture		Negative Culture		Total	
	No.	%	No.	%	No.	%
Male	18	85.7	3	14.2	21	39.62
Female	29	90.7	3	9.37	32	60.38
Total	47	88.6	6	12.7	53	100
CalX ² =1.228 TabX ² = 3.84 DF= 1 p. value 0.268						

Results of ear swab culture (table 1) revealed that positive bacterial culture indicating for otitis media was observed in 47(88.6%) out of 53 patients. it was appeared that 47 cases were distributed to 18 (8.7 %) among males and 29 (90.6%) among females.

The results expressed in table (2) shows that, the age group >50 year was the higher infected with otitis media, it was registered 12(25.5%) among other age groups that included in this study

Table (2): Distribution of patients with otitis media according to age groups

Age groups in years	Male with OM		Female with OM		Total	
	No.	%	No.	%	No.	%
1 - 10	2	11.1	7	24.1	9	19.15
11 - 20	6	33.3	4	13.7	10	21.28
21 - 30	3	16.6	4	13.7	7	14.89
31 - 40	1	55.5	4	13.7	5	10.64
41 - 50	1	55.5	3	10.3	4	8.51
> 50	5	27.8	7	24.1	12	25.53
Total	18	38.3	29	61.7	47	100

CalX²= 41.10 TabX²= 11.07 DF= 5 p. value < 0.001**

It appears that patients with otitis media were more (32(68.1%)) infected with a chronic otitis media (COM) than that in acute otitis media (AOM). AOM appeared with a higher rate (100%) in age group (1-10) year. In contrast, the largest age group affected by COM was the age group >50-year, table (3).

Table (3): Distribution the types of otitis media according to age groups

Age groups in years	Patients with AOM		Patients with COM		Total	
	No.	%	No.	%	No.	%
1 - 10	9	100	0	0.0	9	19.15
11 - 20	4	40.0	6	60.0	10	21.28
21 - 30	2	28.6	5	71.4	7	14.89
31 - 40	0	0.0	5	100	5	10.64
41 - 50	0	0.0	4	100	4	8.51
> 50	0	0.0	12	100	12	25.53
Total	15	31.9	32	68.1	47	100
CalX²= 29.38 TabX²= 11.07 DF= 5 p. value < 0.001**						

Out of 55 bacterial isolates with otitis media patients ,39 (70.9%) isolated from COM and 16 (29.1%) from AOM. *Pseudomonas aeruginosa* was predominant bacteria ((18(32.7 %)) among other aerobic bacteria that causes otitis media and appeared in higher percentage (38.46%) in COM, followed by *Staph. aureus* (14 (25.45%)) that recorded higher rate (43.75%) in AOM, table (4).

Table (4): Types of aerobic bacteria isolated from otitis media

Bacteria I type	Species	Bacteria in AOM		Bacteria in COM		Total		Overall total		p. value
		No.	%	No.	%	No.	%	No.	%	
Gram positive	<i>S. aureus</i>	7	43.75	7	17.9	14	25.45	16	29.1	0.001
	<i>S. pneumonia</i>	0	0.0	2	5.12	2	3.6			
Gram negative	<i>P. aeruginosa</i>	3	18.75	15	38.46	18	32.7	39	70.9	0.015
	<i>Proteous spp.</i>	4	25.0	6	15.38	10	18.18			
	<i>E. coli</i>	1	6.25	4	10.25	5	9.09			

	<i>Enterobacter</i> spp.	1	6.25	3	7.67	4	7.27			
	<i>Klebsiella</i> spp.	0	0.0	2	5.12	2	3.60			
Total		16	29.1	39	70.9	55	100	55	100	
CalX²= 31.01 TabX²= 12.59 DF= 6 p. value < 0.001**										

Eight samples of total positive growth (55) appeared mixing growth of different types of bacteria, *Staphylococcus aureus* and *E. coli* found in 3(5.45%) of positive culture, while 5(9.09 %) of *Pseudomonas aeruginosa* with *Proteus* spp. showed coexistence growth in COM infections, in contrast, the coexistence growth not found in AOM infections.

Table (5): Types of mixing growth with chronic otitis media

Type of mixing bacteria	No. of Mix growth	%
<i>Staph aureus</i> + <i>E. coli</i>	3	5.45
<i>Pseudomonas aeruginosa</i> + <i>Proteus</i> spp.	5	9.09
Total	8	14.54

The results of antibiotics susceptibility test revealed that, the most antibiotic which resistant was erythromycin, with a total resistance 89.1 %, while amikacin was the most effective, as the isolates recorded 98.2% sensitivity to this antibiotic, table (6).

Table (6): Antibiotic susceptibility of isolates

Antibiotic Bacteria		AK	VA	CRO	CN	AMC	CIP	E	TMP	AZM	AX	P. value
<i>P. aeruginosa</i> No. 18	R	1 (6.0)	18 (100)	11 (61.0)	5 (28.0)	17 (94.0)	4 (22.0)	18 (100)	14 (78.0)	10 (56.0)	16 (89.0)	< 0.001
	S	17 (94.0)	0 (0.0)	7 (39.0)	13 (72.0)	1 (6.0)	14 (78.0)	0 (0.0)	4 (22.0)	8 (44.0)	2 (11.0)	
<i>S. aureus</i> No. 14	R	0 (0.0)	0 (0.0)	14 (100)	0 (0.0)	10 (71.4)	2 (14.0)	8 (57.0)	0 (0.0)	2 (14.0)	14 (100)	< 0.001
	S	14 (100)	14 (100)	0 (0.0)	14 (100)	4 (28.6)	12 (86.0)	6 (43.0)	14 (100)	12 (86.0)	0 (0.0)	
<i>Proteous Spp</i> No. 10	R	0 (0/0)	10 (100)	5 (50.0)	9 (90.0)	7 (70.0)	5 (50.0)	10 (100)	10 (100)	3 (30.0)	7 (70.0)	< 0.001
	S	10 (100)	0 (0.0)	5 (50.0)	1 (10.0)	3 (30.0)	5 (50.0)	0 (0.0)	0 (0.0)	7 (70.0)	3 (30.0)	
<i>E. coli</i> No. 5	R	0 (0/0)	5 (100)	2 (40.0)	0 (0/0)	5 (100)	0 (0/0)	5 (100)	0 (0/0)	0 (0/0)	0 (0/0)	< 0.001
	S	5 (100)	0 (0.0)	3 (60.0)	5 (100)	0 (0.0)	5 (100)	0 (0.0)	5 (100)	5 (100)	5 (100)	
<i>Enterobacter</i> Spp No. 4	R	0 (0/0)	4 (100)	3 (75.0)	4 (100)	4 (100)	4 (100)	4 (100)	2 (50.0)	3 (75.0)	0 (0/0)	0.001
	S	4 (100)	0 (0/0)	1 (25.0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	2 (50.0)	1 (25.0)	4 (100)	

<i>Streptococcus</i> Spp No. 2	R	0 (0/0)	1 (50.0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	2 (100)	0 (0/0)	0 (0/0)	0 (0/0)	< 0.001
	S	2 (100)	1 (50.0)	2 (100)	2 (100)	2 (100)	2 (100)	0 (0/0)	2 (100)	2 (100)	2 (100)	
<i>Klebsiella</i> Spp No. 2	R	0 (0/0)	2 (100)	0 (0/0)	0 (0/0)	2 (100)	2 (100)	2 (100)	1 (50.0)	1 (50.0)	2 (100)	< 0.001
	S	2(100)	0 (0/0)	2 (100)	2 (100)	0 (0/0)	0 (0/0)	0 (0/0)	1 (50.0)	1 (50.0)	0 (0/0)	
Total No. 55	R	1 (1.8)	40 (72.7)	35 (63.6)	18 (32.7)	45 (81.8)	17 (30.9)	49 (89.1)	27 (49.1)	19 (34.5)	39 (70.9)	< 0.001
	S	54 (98.2)	15 (27.3)	20 (36.4)	37 (67.3)	10 (18.2)	38 (60.1)	6 (10.9)	28 (50.9)	36 (65.5)	16 (29.1)	

AK: Amikacin; VA: vancomycin; CRO: ceftriaxone; CN: gentamycin; AMC: Amoxicillin/ clavulanic acid; CIP: ciprofloxacin; E: erythromycin; TMP; trimethoprim; AZM: azithromycin; AX: Ampicillin\ cloxacillin

The total number of isolates that produced β -lactamases enzymes reached 49 (89%), and *Pseudomonas aeruginosa* ranked first among these isolates, as 17(34.6%) isolates were recorded. In contrast, none of the two isolates of *Streptococcus* bacteria recorded production of these enzymes, table (7).

Table (7): Numbers and percentages of β -lactamase producers isolates

Types of Bacteria	β - Lactamase producers		Non- β - Lactamase producers	
	No.	%	No.	%

<i>P. aeruginosa</i>	17	34.6	1	16.67
<i>S. aureus</i>	14	28.5	0	0.0
<i>Proteus spp.</i>	7	14.2	3	50.0
<i>E. coli</i>	5	10.2	0	0.0
<i>Enterobacter spp.</i>	4	8.16	0	0.0
<i>Streptococcus spp.</i>	0	0.0	2	33.3
<i>Klebsiella spp.</i>	2	4.08	0	0.0
Total	49	89.0	6	11.0
CalX²= 110.4 TabX²= 12.59 DF= 6 p. value < 0.001^{**}				

Out of 55 aerobic isolates with otitis media, 43 (78.2%) had multidrug resistance, the most frequency bacteria with MDR were *Proteus spp.*, *E. coli*, *Enterobacter spp.* and *Klebsiella spp.*, it registered 100%, table (8).

Table (8): Numbers of multidrug resistance isolates

Type of bacteria	No. & (%) of multi drug resistance bacteria	No. & (%) of non multi drug resistance bacteria	Total
<i>Pseudomonas aeruginosa</i>	14(77.8)	4(22.2)	18

<i>Staphylococcus aureus</i>	8(57)	6(43)	14
<i>Proteus</i> spp.	10(100)	0 (0)	10
<i>E. coli</i>	5(100)	0(00)	5
<i>Enterobacter</i> spp.	4(100)	0(0)	4
<i>Streptococcus</i>	0(0)	2(100)	2
<i>Klebsiella</i> spp.	2(100)	0 (0)	2
Total	43(78.2)	12(21.8)	55

DISCUSSION

Although otitis media may heal spontaneously and not result complications, it can cause hearing loss and other lifelong complications. The origin of otitis media can be bacterial or viral, during the common cold, viruses can ascend from Eustachian tubes into the middle ear and make way for bacterial ear pathogens residing in the nasopharynx (2).

The results showed that 88.6% of middle ear swabs for patients gave positive result for aerobic bacterial culture, women were more likely than men to have otitis media, these results were disagreed with the results of (19) who found 45.91% of patients with OM were male and female registered lower percentage (45.09), these results were similar with results of (20) who found the females were more infected with AOM than males in study period from 2015 to 2018.

The COM was more frequency in this study than AOM , this result found to be similar to the results of (21) at North east Ethiopia , were found the COM recorded 83.3%, AOM were appeared in high percentage in age group (>1-10) year , this may be due to the fact , that this age group is a category of children, the common risk factor can predispose to develop AOM in children is a preceding infections of upper respiratory tract (22), and Eustachian tube in children is also shorter and horizontal more than that in adults (23) . The results showed the chronic otitis media was infected the age group > 50 more than other age groups, these results were agreement with the results of (24) who found that the prevalence of COM increased with age, these may be due to considered the COM often develops from an AOM, and people in this old age group

are less immune than those who are younger and therefore acute otitis media may turn into a chronic infection. Smoke, seasonal allergies and regularly contact with air pollution may be predisposing factors that increase the risk of developing chronic otitis media.

The present study appeared, that, *Pseudomonas aeruginosa* were more frequency in COM than other bacterial isolates, this result in line with the results of study conducted by (25) that revealed *P. aeruginosa* was common among other isolates from chronic otitis media, *Pseudomonas* spp. Can reproduces at room temperature, it can grow in absence of special feeding and are highly resistant to antimicrobials (26).

The study found, the highest resistance of bacteria to antibiotics was to erythromycin. It has been suggested that Gram negative bacteria are lack of susceptible to erythromycin due to the action of efflux pumps (27). Amikacin is antibiotic was more effective against bacterial isolates under study, this antibiotic is particularly effect against bacteria that are possess resistant to other antibiotic of aminoglycosides group, because its chemical structure makes it less sensitive to inactivate enzymes (28).

The results of this study showed a high percentage of bacterial isolates were β -lactamase producing, these finding were similar to results of (29) who found β -lactamase producers of bacterial isolated from 33 patients (69%). The increased prevalence of β -lactamase may be due to the bacteria containing plasmids or the presence of mutations containing the genes encoding these enzymes.

Pseudomonas aeruginosa was more frequency as β -lactamase producer than other isolates, these results was in the same line with the results of (30) who found that, *Pseudomonas aeruginosa* registered a highest percentage of β -lactamase producing.

In addition to innate resistance in *Pseudomonas aeruginosa*, acquired resistance due by plasmids in these bacteria is also a problem. Plasmid-mediated resistance with modification enzymes is specifically associated with indiscriminate use of antibiotics and sites where elevated levels of antimicrobials are achieved (31). High percentages of isolates (*Pseudomonas aeruginosa*, *E.coli*, *Proteus* spp., *Klebsiella* spp. and *Enterobacter* spp.) were appeared as multidrug resistance (MDR) in this study. MDR is the antimicrobial resistance exhibited by a type of microbes to at least one antibiotics in three or more classes of antibiotics (32), these results were in line with a study of (33) that showed 67% of isolates with otitis media was MDR bacteria, the most pathogenic bacteria that possessed this characteristic was *Enterobacter* spp.(100%), *Proteus mirabilis* (90%), *E.coli* (77.8%) and *Staphylococcus aureus* (74.5%).

MDR is widespread among bacteria, resulting in effective antibacterial drugs are often compromised the treatment of clinical infection diseases are particularly difficult if continuous use antibiotics provide a suitable environment for multidrug resistance bacteria which can then acquire R plasmid to expand the range of resistance available mechanisms (34).

The current study concluded that the bacteria that cause otitis media were high levels of β -lactamase, in addition to being multidrug resistance bacteria.

The increased prevalence of beta lactamases may be due to the bacteria containing plasmids or the presence of mutations containing the genes encoding these enzymes.

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Abbreviation	Terms
OM	Otitis media
OME	otitis media with effusion
AOM	acute otitis media
COM	chronic otitis media
CSOM	chronic suppurative otitis media
MEF	middle ear fluid

MDR	Multi – Drug -Resistant tuberculosis
PBP	Penicillin – Binding protein
API 20 E system	Analytical profile index 20 Enterobacteriaceae System
CLSI	Clinical Laboratory standards Institute