

## β - Lactamase Producing Bacteria Isolated From Patients Infected With Otitis Media

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### Abstract

This study was carried out to evaluate the relation between the causative agents of otitis media and the parameter related to the patients (e.g., gender, age) the criteria of causative agents include type of bacteria, growth conditions, susceptibility to antibiotics, and the ability to produce β-lactamase enzymes. A total of 50 ear swabs were obtained from outpatients infected with otitis media.

The results showed that *Staphylococcus epidermidis* (30%) was the predominant pathogens followed by *Staphylococcus aureus* (20%), *Pseudomonas aeruginosa* (18%), *Proteus mirabilis* (8%), and *Proteus vulgaris* (4%). The antimicrobial susceptibility pattern showed that all bacteria were resistant to cephalothin and moderate resistant to other antibiotics.

For detection of β-lactamase producing bacteria, results showed that *Ps. aeruginosa* was recorded a high percentage in producing of β-lactamase enzymes (55.6 %) in comparison with the other bacteria.

### الخلاصة

أجريت هذه الدراسة لتقييم العلاقة بين التهاب الإذن الوسطى ومعايير تتعلق بالمريض مثل الجنس والعمر إضافة إلى معايير تتعلق بالبكتريا مثل نوع البكتريا ، ظروف النمو ، حساسيتها للمضادات الحيوية وقابليتها لإنتاج إنزيمات ألبينا لاكتاميز حيث تم جمع 50 مسحة اذنية مأخوذة من المرضى الخارجيين المصابين بالتهاب الإذن الوسطى .

من الممرضات السائدة ثم (30%) تشكل *Staphylococcus epidermidis* أظهرت النتائج إن (18%) ، *Staphylococcus aureus* ، *Pseudomonas aeruginosa* ، (20%) أظهر فحص الحساسية للمضادات *Proteus mirabilis* (8%) و *Proteus vulgaris* (4%) الحياتية إن جميع البكتريا المعزولة من التهابات الإذن الوسطى كانت مقاومة للمضاد سيفالوثين ومتوسطة المقاومة للمضادات الاخرى.

استخدمت طريقة Iodometric لتحديد قابلية البكتريا لإنتاج إنزيم ألبينا لاكتاميز وأظهرت النتائج إن بكتريا *Ps. aeruginosa* قد سجلت أعلى نسبة بلغت (55.6%) في إنتاج إنزيم ألبينا لاكتاميز بالمقارنة مع البكتريا الأخرى.

## Introduction

Otitis media is an infection or inflammation of part or all of the mucous membrane lining the middle ear cleft (1). Which is the most common illness in infant and young children and its complications and sequelae often persist into the adult years<sup>(2,3)</sup>.

$\beta$ -lactamase producing bacteria (BLPB) may have an important role in otitis media infections<sup>(4)</sup>. The resistance of these bacteria against  $\beta$ -lactam antibiotics is increasing at significant rate and has become a common problem in primary care medicine. There are several mechanisms of antimicrobial resistance to  $\beta$ -lactam antibiotics (5,6). One important mechanism is the production of  $\beta$ -lactamases, which are enzymes that cleave the  $\beta$ -lactam ring (7).  $\beta$ -lactamase activity can occur in Gram-positive (e.g., *S. aureus* and *S. epidermidis* and Gram-negative bacteria (e.g., *Pseudomonas*, *Proteus*, and other Enterobacteriaceae).

For these reasons the present study was undertaken to determine the occurrence of  $\beta$ -lactamase producing bacteria isolated from patient infected with otitis media in Al-Najaf city.

## Materials and method

**Subjects:** Subjects were outpatients, attending to ENT department in Al-Hakeem teaching hospital in Al-Najaf from November, to May 2006; they were diagnosed by a physician as having otitis media on the basis of symptoms and signs. Patients were divided into three groups according to age.

**Specimen collection and processing:** Ear swabs were inoculated onto the surface of blood, MacConkey, and mannitol salts agars (Himedia, India). After incubation for 24 and 48 h at 37°C, the isolates were subjected to biochemical test for identification (8).

Antibiotic susceptibility of clinical isolates were studied against the following antibiotics: amikacin, cirpofloxacin, cefotaxim, amoxicillin, rifampin, clarithromycin, cephalothin, oxacillin, trimethoprim, gentamicin, carbencillin, and cephalexin (Bioanalyse, Turkey), by the disk diffusion technique on Muller-Hinton agar, using inhibition zone size criteria recommended by the disk manufacture and based on method of Barry (9). The selection of antibiotic disks was performed according to the recommendation of National Committee for Clinical Laboratory Standards (10).

For detection of  $\beta$ -lactamase producing isolates a loop full of grown culture was transferred into small tube containing 1ml of penicillin G solution and incubated at 37°C for 30 minutes then 0.5 ml of iodine solution was added and mixed for 2-3 minutes. A change the color to colorless, indicates as a positive result (9).

## Results

The age and sex distribution of patients were reported in Table (1). Rate of the otitis media was highest in the first group and mainly occurred in patients aged 1 to 15 years old (22%), mild in patients aged 16 to 30 years old (15%), and lowest in patients aged 30 to 65 years old.

A total of 31 (62%) females and 19 (38%) males had positive ear swab culture.

However, there is a significant variation ( $P < 0.05$ ) between males and females.

Table 1. Susceptibility of different groups of individual infected with otitis media regarding to gender and age

Age group	Male		Female		Total
	NO.	%	NO.	%	
1-15	12	63	10	32	22
16-30	2	10.5	13	41.9	15
30-65	5	26.3	8	25.8	13
Total (%)	19	38	31	62	50

$$X^2 = 6.36, P < 0.05$$

The bacterial isolates obtained as a pure and predominant growth from ear specimens were only considered for the present study. All these bacteria were identified. Among 50 ear swabs, only 40

(80%) clinical isolates were recovered as follows: *S. epidermidis* (30%), *S. aureus* (20%), *Ps. aeruginosa* (18%), *P. mirabilis* (8%), and *P. vulgaris* (4%) (Table 2).

Table 2. distribution of etiological agents in patients infected with otitis media

Type of bacteria	No. of isolates	%
<i>S. epidermidis</i>	15	30
<i>S. aureus</i>	10	20
<i>Ps. aeruginosa</i>	9	18
<i>P. mirabilis</i>	4	8
<i>P. vulgaris</i>	2	4
Non pathogenic bacteria	10	20
Total	50	100

The frequency of antibiotic resistance of the isolates was determined (Table 3). The etiological agents were found to be resistant to a minimum 5 antibiotics to which they were tested. However, all the isolates were resistant to cephalothin. The majority of isolates were highly resistant to cefotaxim, amoxicillin, clarithromycin, oxacillin, trimethoprim, carbencillin, and cephalixin and moderately resistant to amikacin, cirpofloxacin, and gentamicin,.

The β-lactamase-production among bacterial isolates is illustrated in Table (4). Of the total of 40 isolates, only 12 (30%) were recognized as β-lactamase-producing bacteria. These included; *S. epidermidis* (13.3%), *S. aureus* (20%), *Ps. aeruginosa* (15.6%), *P. mirabilis* (50%), and *P. vulgaris* (50%). The results from the present study show that all 12 β-lactamase-producing isolates gave positive reactions after few seconds to three minutes from the additional of the reagent.

Table 3. Antibiotic resistance of etiological agents isolated from patients infected with otitis media

Antibiotic	Percentage of bacteria resistant to antibiotic				
	<i>S. epidermidis</i> n=15	<i>S. aureus</i> n=10	<i>Ps. aeruginosa</i> n=9	<i>P. mirabilis</i> n=4	<i>P. vulgaris</i> n=2
Amikacin	13.3	37.5	55.5	0	0
Ciprofloxacin	13.3	60	33.3	0	0
Cefotaxim	80	100	77.7	50	50
Amoxicillin	80	80	100	100	100
Rifampin	33.3	33.3	100	100	100
Clarithromycin	84.6	83.3	100	100	50
Cephalothin	100	100	100	100	100
Oxacillin	46.6	30	100	100	100
Trimethoprim	90	80	77.7	75	50
Gentamicin	57.1	30	77.7	33.3	33.3
Carbencillin	84.6	100	100	100	100
Cephalexin	73.3	62.5	100	100	100

Table 4. The occurrence of β-lactamase producing bacteria

Type of bacteria	Total numbers	β -lactamase production	
		No.	%
<i>S. epidermidis</i>	15	2	13.3
<i>S. aureus</i>	10	2	20
<i>Ps. aeruginosa</i>	9	5	55.6
<i>P. mirabilis</i>	4	2	50
<i>P. vulgaris</i>	2	1	50
Total	40	12	30

## Discussion

In this investigation, the peak of occurrence of the otitis media was on the first age group (1-15 years old) and decline with the advancement of age. Such finding indicates that the risk of otitis media decreased sharply with age. This may be associated with the development of immune system (17) and may be attributed to the shorter, wider, and horizontal Eustachian tube in children than in adults, offering greater opportunities for

pathogens to ascend from nasopharynx to the sterile middle ear activity (14).

In this study, *S. epidermidis* was the most frequent microorganism among bacterial isolates followed by *S. aureus* and *Ps. aeruginosa*, this may be attributed to these bacteria may have a role as opportunistic pathogens in the presence of weakened local tissue defenses. The results are in agreement with Brook and Yocum(11), who found that *S. epidermidis* strains were predominate and isolated from patients with present clinical finding of otitis media. However, Brook *et al.*(12) reported

that *S. aureus* was the dominant causative agent of this disease, in contrast, Ibekwe *et al.*(13) found that

*Ps. aeruginosa* was the first pathogen. These differences may be associated to the geographical variations and socio-economic factors.

The resistance of all Bacterial isolates to 12 common antimicrobial agents was detected as shown in Table (3), which indicate a remarkable resistance of *Ps. aeruginosa* to the most of antimicrobial agents were used this resistance resulting from many factors such as structural and genetic factors which protect it from the action of antibiotic. these results may be attributed to the widely use of these drug which result in emergence of resistant strains to penicillins, due to production of β-lactamase enzymes which breaks the β-lactam ring of penicillins rendering them into inactive product (15).

The results revealed that all isolates were found to be resistant to at least 5 antibiotics. Hence these isolates were considered to multidrug resistant. Bacterial resistant to antibiotics is now widespread and possesses serious clinical threats. However, the resistance rates in this study were higher than those reported by others(16), who found that the vast majority of the bacterial strains isolated from patients with otitis media were susceptible to common used antibiotics. The high resistance in the present study may be due to antibiotic abuse which to development of resistant isolates. The causal antibiotics usage without antibiotic sensitivity testing is the most important factor promoting the emergence of multidrug resistance which lead to selection and dissemination of antibiotic resistant pathogens in clinical medicine. Therefore, more careful use of antibiotics and control of the spread of these resistant isolates are necessary in our

country where antibiotics are easily available in pharmacies without prescription.

In conclusion, beta-lactamase producing *Ps. aeruginosa* is existed in our hospital (Al-Hakeem Hospital) and it might be existed in other hospitals in Al-Najaf Governorate. The tests for beta-lactamase production should be added to the routine tests performed in hospital microbiology laboratory in order to decrease the mortality and morbidity rates due to infection with these pathogens.

Future studies, may be need to detection of the type of β-lactamase enzymes produced by bacteria causes multidrug resistance in patients with otitis media.

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