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Isolation and Identification of Some Pathogenic Bacteria from Otitis Media in Babylon Governorate

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Abstract

This study was conducted for the period from February to November 2016 in Babylon Teaching Hospital, during this period, 200 swab ear of people with otitis media in the city of Babylon were collected. 120 sample was diagnosed with bacterial isolates depending on morphology on the different culture media and some biochemical tests. The highest rate of infection in the age group (21-40)years, and the least in the age group (41-60)years as it was (46 %,15%) respectively. In this study were isolate five types of a bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumonia* and *Klebsiella pneumonia*) and the highest rate of infection has reached (35 %, *Pseudomonas aeruginosa*), (26%, *Staphylococcus aureus*). As for the results of examination of the sensitivity of the bacterial isolates to antibiotics was the highest rate of antibiotic resistance was (94%Tetracyclin, 92%Ampicilin and 91% Chloramphenicol), while the resistance decreased in the case of antibiotics (42%Amikacin, 42%Gentamicin and 31%Tobramycin). *Streptococcus pneumonia* were detected by PCR, 16 cases were PCR positive for *Streptococcus pneumonia*, there was no samples displayed culture positive and PCR negative for *Streptococcus pneumonia*.

Keywords: *Pathogenic bacteria, Otitis media, Sensitivity test, PCR.*

Introduction

Otitis media is the most common health problems among patients in health centers and hospitals [1]. The early diagnosis of pathological condition will prevent the complications associated with an infection similar occurrence such as brain abscesses, meningitis and thrombus side pockets [2].

There exist many pathogens that cause inflammation of the middle ear which causes bacterial, viral and fungal, either bacterial infection can be attributed to several bacterial genera such as (*Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) [3]. Health education to the community and the environment play an important role in the spread of pathogens and for the case of inflammation as the cleaning of the ear canal is incorrect and does not follow the conditions of hygiene and swimming in contaminated water all lead to get infection and the spread of pathogens [4].

Many studies have indicated that the source of Gram positive bacteria that causes otitis

media is nasopharyngeal cavity, and the source of Gram negative bacteria is not the nasal cavity and pharyngeal but auditory canal contamination of these bacteria may be the cause of the infection status [5]. Middle ear lining with a layer of ciliated pseudo stratified epithelium cells containing goblet cells and glands separator shall mucous substance [6]. The connection of middle ear with the upper respiratory system led to the contamination with pathogen which infects this system such as bacteria, viruses and other, and the opening of the ear on the external environment through the tympanic membrane also led to the exposure of many pathogens opportunism that cause middle ear infection [7].

The changes occurring in the airway pressure caused pay next aetiology of liquid nasal and throat upwards through the Eustachian tube [8]. Many studies show that most children who suffer from cases of otitis media who are suffering from infections earlier in the respiratory tract and the bacteria involved in this inflammation occurs; there is a normal

flora in the respiratory tract [9]. One of the ways of incidence of this infection is the occurrence of a hole in the tympanic membrane, which will provide a pathway for normal flora which found in external auditory canal to reach to the middle ear [10]. This study aims to isolate and diagnose the bacteria that cause inflammation of the middle ear with the study of their sensitivity to antibiotics act in order to identify the most appropriate antibiotic use.

Materials and Methods

Samples Collection

This study was conducted on 200 patients who suffer from otitis media and by taking a swab from an area inflammation and we record information about each patient in terms of sex, age and make sure that patients do not take any antibiotic before taking the swab for a period of not less than a week.

Bacterial Isolation

Samples were planted directly on the culture media including (Blood Agar, MacConkey's Agar and Manitol Salt Agar), Petri dishes were incubated aerobically for 24 hours and at a temperature of 37 °C, colonies were diagnosed initially depending on the phenotypic and culture characteristics, then colored by Gram stain after that promotional tests were conducted which (IMViC, Coagulase, Catalase and Oxidase) tests [11].

DNA Extraction

DNA was extracted from samples according to the manufacture company (Geneaid, China) by participation of bacteria by 7000rpm/min, and the product was stored to the PCR [12].

Nested - PCR

First PCR was performed by adding 12.5 µl of (Accu Power® PCR Pre Mix) which supplied from Bioneer company, 7 µl of DNA extracts, 1 µl of each primer RW01 and DG74, 3.5 µl of sterile distilled water and PCR was done with conditions denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, this is was repeated for 34times, followed by a final extension at 72°C for 10 min. And the PCR product separated on 1.5% agarose gel stained with ethidium bromide, the gels then visualized on a UV-light transilluminator and detect 370 bp. These PCR were used as temp let to second PCR with specific primer for *Streptococcus pneumonia*STR1 and DG74, and PCR performed with the similar conditions that used in first amplifications and then detect 105bpby 1.5% agarose gel.

Measurement of Bacterial Sensitivity to Antibiotics

To estimate the sensitivity of bacteria which isolated from cases of inflammation of the middle ear to antibiotics, we follow the method that is used by [13], which used Muller-Hinton Agar for this purpose, with the exception of *S. pneumonia* that was added to their culture media 5% blood, dishes were incubated at a temperature 37 °C for 24 hours and measured diameters of inhibition zone surrounding the disk antibiotic by listed ruler and compared this particular record schedules.

Results and Discussion

Bacteria were detected in (120) of 200 samples isolates for the duration of the study, and the number of infected males 76 (63%) while we found bacteria in 44 (37%) female as in the Table (1).

Table 1: Distribution of pathogenic bacteria according to age group and genders

Age group	Male		Female		Positive No. Total	%
	Positive No.	%	Positive No.	%		
1 - 20	31	41 %	16	36 %	47	39 %
21 - 40	36	47 %	19	43 %	55	46 %
41 - 60	9	12 %	9	21 %	18	15 %
total	76(63%)	100 %	44(37%)	100 %	120	100 %

It is clear from this table that the rate of infection among males is much higher than in females, and this may be due to the opportunity for males to practice swimming, whether in swimming pools or in other rivers and ponds, this is in an agreement with the results of several studies which indicate that the number of infected males was higher than

that in females [14, 15], these results are not consistent with those indicated by the [16].

In the same table we notice the rate of infection according to the age groups, it became clear that the age group (21-40) years. It is the most susceptible to infection and reached (47%) this result may be due to the

fact that age into this category represents the younger age groups that engage in business by larger and frequent exposure to external factors [17,18]. Evident from the Table 2 that *Ps. aeruginosa* constitute the highest

percentage of the events of the disease than the rest of other species and then followed by *Staph. aureus* (26 %), while the lowest rate was in *Klebsiella pneumonia* (6 %).

Table 2: Species of bacteria that isolated from middle ear infection and their percentage

Bacteria species	No.	%
<i>Pseudomonas aeruginosa</i>	43	36
<i>Staphylococcus aureus</i>	31	26
<i>Escherichia coli</i>	23	19
<i>Streptococcus pneumonia</i>	16	13
<i>Klebsiella pneumonia</i>	7	6
Total	120	100 %

Some studies showed that *Ps. aeruginosa* are the most common cases of otitis media, followed by *S. aureus*, and the infection rates for the first three types are the highest among the total cases of infection by other bacterial species [19].

Some researchers showed that the source of infection in otitis media with enteric bacteria (Gram negative) and *Ps. aeruginosa* is not necessary to be by the nasopharyngeal stream but may be through fecal contamination of the auditory stream. Another way that helps to transfer pathogens to the middle ear is to use incorrect methods for cleaning the external ear [21], and swimming in rivers and swamps itself one of the important ways to the occurrence of otitis media with *Ps. aeruginosa* and enteric bacteria, where these water sources are vulnerable to contamination with waste of human and animal, which is an important source of environmental pollution sources [22].

As for the sensitivity assay, it was observed that there is a considerable variation in the sensitivity of the isolates to antibiotics (Tetracyclin and Ampicilin) and reached the highest rate (94%, 92 %) respectively, these percentage measured according to [23], the reason for this resistance may be due to the production of bacteria in a certain enzymes that coded by plasmids, which will be able to break down aggregates of β -lactam antibiotics [24].

As well as the widespread and indiscriminate, the use of many antibiotics in the treatment of middle ear inflammatory which led to increased resistance to the commonly used antibiotics [25], and it has been observed that *Ps.aeruginosa* are the most common types of bacteria resistant to all antibiotics, because

these bacteria possess a variety of defense mechanisms, including the production of β -Lactamases enzymes which crash β -lactam ring in β -lactam antibiotics, and the resistance of bacteria to antibiotics is considered as one of the medical problems and one of the reasons for the failure of most cases treatment of otitis media [26].

Perhaps this resistance can be attributed to the modulating in one or more Penicillin Binding Proteins (PBPs) which represent the target site in antibiotics, and this type of resistance due to chromosomal mutations leading to non-arrival of antibiotics to the target site in bacterial cell [27]. As well as *Ps. aeruginosa* have many efflux pumps which pull the antibiotics and pushed them out of the bacterial cell [28].

Some studies have pointed that some strains of *Ps. aeruginosa* in cases of chronic inflammation produce mucous material surrounding the bacterial cell that consists of Mucoïd Exopoly Saccharide which is called Alginate, which suppresses the arrival of the bactericidal of some antibiotics into the bacteria [29]. It is also worth noting that the multiple resistance to antibiotic a portable on a moving plasmid among pathogenic bacteria, especially for new generations of antibiotics [30].

Streptococcus pneumonia were detected by PCR, 16 cases were PCR positive for *Streptococcus pneumonia*. There was no samples displayed culture positive and PCR negative for *Streptococcus pneumonia*. These results have been matched with Gok [31], some studies show that the *S. Pneumonia* and *M.catarrhalis* were the most common isolated organisms from otitis media.

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