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Isolation and Identification of Some Bacterial Species from Otitis Media Patients in Kerbala City

Laith Yahya Taama Khader
University of Karbala College of Applied Medical Sciences Department of Pathological Analysis

Remah nouri fahad ali
University of Fallujah College applied Sciences Department of pathological Analysis

Omal Baneen Aqeel Hamid Jabbar
University of Kufa College of Science Department of Pathological Analysis

Taif Allawi Jiad Gazi
University of Al-Fallujah Collage Applied Sciences Department of Pathological analysis

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Abstract: This investigation began in last January of this year at the Applied Medical Sciences College's Department of Clinical Laboratories. The study's aims were to identify and diagnose the most frequent bacterial pathogens responsible for otitis media, as well as to explore the antibiotic sensitivity of bacterial isolates and the role of different factors on illness spread. (30) samples of purulent material in the middle ear were obtained in the form of ear swabs from patients diagnosed with otitis media by otolaryngologists within private clinics. It was for different age groups, ranging from 18 to 62 years, for both sexes. The age distribution of otitis media patients showes age groups (21-30 years) and (41-50 years) had the highest percentage incidence (both categories were 26.9%). In the Public Health Laboratory of Karbala City, the samples were grown on various culture medium and diagnosed in the laboratory based on cultural

characteristics, Gram stain findings, and a variety of biochemical tests such as Oxidase, Coagulase, Catalase, and others. The results of the bacterial isolation showed a predominance of Pseudomonas aeroginosae bacteria by 34.61%, followed by Proteus mirabilis bacteria by 26.92%, Staphylococcus aureus by 19.23%, and Escherichia. coli by 11.53%. In addition, Serratia marcescens and Providencia were isolated by 3.84%each separately. While no growth occurred in the remaining four sample. The most effective antibiotics were, ciprofloxacin, Levofloxacin, Amikacin, meropenem, and Piperacillin-tazobactam. The least effective antibiotics were found to be tetracycline, Impicilin, Cefotaxima and Vancomycin. on the other hand, were considerably resistant to commonly used antibiotic penicillin. Among the 26 cases, the number of males with OM was 58% higher than number of women (42%). Cold or upper respiratory infection, smoking or passive smoking, seasonal allergy symptoms, other pathological variables such as gastroesophageal reflux disease, hereditary factors and bad behaviors are examples of etiology and risk factors for otitis media infection.

INTRODUCTION

Contact between the middle ear and the upper respiratory system has contaminated it with upper respiratory pathogens such as bacteria, viruses, and others. Furthermore, the tympanic membrane's accessibility to the external environment has exposed it to a variety of opportunistic microorganisms that cause middle ear infections. [1]. Otitis media (OM) is defined as inflammation of the middle ear and mastoid space resultant from the colonization of pathogenic microorganisms. [2]. It is the second most common reason for visits to otorhinolaryngology and pediatrics departments, following upper respiratory tract illness [3]. Otitis media is classified into three types: acute purulent otitis media, otitis media with effusion, and chronic suppurative otitis media [4,5]. acute otitis media (AOM) exhibits rapid-onset middle ear effusion and signs or symptoms of middle ear inflammation, including fever, otalgia, otorrhoea, or irritability in a short duration. [6] *Streptococcus pneumoniae*,

Haemophilus influenzae, and *Moraxella catarrhalis* are the most prevalent causal agents of AOM [7,8] . Whereas OME is middle ear effusion which is often asymptomatic and characterized by accumulation of fluid in the middle ear. [9] Despite antibiotic therapy, AOM can develop to (chronic suppurative otitis media) CSOM, which is distinguished by chronic infection of the middle ear and mastoid air cells that lasts for more than three months. This condition is characterized by a perforation of the tympanic membrane, as well as intermittent or chronic otorrhea [10]. As chronic otomastoiditis and Eustachian tube dysfunction worsen, the tympanic membrane weakens, increasing the chance of an atelectatic ear or the formation of a cholesteatoma. And according to the findings of this study, the most prevalent aerobic organisms in CSOM were *Pseudomonas aeroginosae, Proteus mirabilis*, *Escherichia. coli*, and *Staphylococcus aureus*. These findings are consistent with previous researches. [11, 12]. *Pseudomonas aeroginosae* was also the most common organism, followed by *Staphylococcus aureus*, which was isolated from CSOM patients in multiple investigations. [13, 14]. Many etiologies are thought to have a part in the pathophysiology of OM, including Eustachian tube dysfunction, allergies, viral and bacterial invasion, decreased ciliary function of both the middle ear and the Eustachian tube mucosa, smoking and gastro-esophageal reflux [15]. However, eustachian tube dysfunction is the cause of the majority of cases. Which is a canal that connects the middle ear to the throat is known as the eustachin tube. This tube aids in the balancing of pressure between the outer and inner ears. A cold or allergy might irritate the tube and create edema around it. This can cause fluid outflow from the middle ear to become obstructed. Fluid builds up behind the eardrum. This fluid can promote the development of bacteria and viruses that cause middle ear infections. Furthermore, otitis media, in all of its forms, is a health issue prevalent condition that affects a huge number of individuals in many areas of the world, particularly in early childhood. According to the World Health Organization, CSOM is a primary cause of hearing loss in children. Adults with recurrent episodes of CSOM have a higher risk of developing permanent conductive and sensorineural hearing loss. [16]. Every year, 28,000 individuals die as a consequence of OM complications, the most common of which are meningitis and brain abscesses. [17,18].

Methodology: Sample Collection, Preparation of culture media and Culturing samples

Sample Collection

From the 11th to the 25th of last January, (30) samples were collected from patients diagnosed with otitis media by otolaryngologists. The majority of them had chronic otitis media and were men and females ranging in age from 18 to 62 years old. As information was recorded about each patient in terms of gender, age, and health issues that led to his referral to a specialized doctor and to ensure that the patient did not take any antibiotics before taking the swab for a period of not less than a week. The sample was collected by A sterile cotton swab was inserted into the auditory canal carrying the remaining pus, slightly rotated to make contact with the pus. The swab was then directly put in the transport medium. A day following collection, each group's primary cultures were grown individually.

Preparation of culture media

Culture media were prepared in accordance with the manufacturer's instructions by dissolving an appropriate amount of medium in distilled water and completing the dissolution process with a water bath, after which it was sterilized in an autoclave at 121°C and around 15 pounds of pressure for 10-20 minutes at a time. In the form of blood agar medium, around 5% of defibrinated mammalian blood (human, sheep, or horse) is added aseptically and well mixed after chilling at 40-45°C.the media is then poured into sterile Petri plates under sterile conditions.

The culture media that was used
Blood agar: is an excellent medium for the growth of fastidious bacteria that require special nutrients and do not grow abundantly on standard media (has one or more protein sources, salt, and beef extract for vitamins and minerals, and approximately 5 percent defibrinated blood). It also assists in viewing the hemolytic responses of various microorganisms.
MacConkey agar: is a selective and differential media used to identify and distinguish non-fastidious gram-negative rods, particularly those from *the Enterobacteriaceae* family and the genus *Pseudomonas.* preventing the growth of gram-positive bacteria sensitive to crystil violet dye and bile salt It also differentiates between lactose fermenting bacteria and non-lactose fermenting bacteria.
Mannitol Salt Agar (MSA): is a selective and differential media. The high salt content (7.5 percent) favors members of the genus *Staphylococcus*, which can withstand high saline levels.
Mueller Hinton Agar (MHA): It's a non-differential, non-selective medium. This indicates that virtually all organisms that are grown on this medium will grow. Mueller Hinton Agar is mostly used to test antimicrobial susceptibility. It has become the standard medium for the Bauer Kirby technique, and the NCCLS specifies its performance. Sulfonamide, trimethoprim, and tetracycline inhibitors are present in low concentrations in MHA.

Culture the samples:

The streaking method was used to inoculate blood agar and MacConkey agar with a cotton swab of ear fluid or ear wax, which was then incubated at 37°C for 18-24 hours. Initially, the growing colonies were identified based on their cultural features. This was performed out in the College of Applied Medical Sciences' microbiology laboratory.

IDENTIFICATION OF BACTERIA

In the public health laboratory of Karbala, all growing bacterial isolates were reactivated on blood agar and macConkey agar under sterile conditions. Then the colonies of isolates were initially diagnosed based on their cultural characteristics: morphological, color, Gram stain was then applied to it, as well as some biochemical tests, such as Catalase, Oxidase, Urease, Indole, Coagulase and the possibility of glucose and lactose fermentation. As seen in the table 3.

Microscopic Examination (Gram Stain)
It gives relatively quick results if bacteria is present. Any bacteria that may be present are categorized by color and shape during the microscopic evaluation: ➢ Color — typically bacteria may be either ―Gram positive‖ (purple) or ―Gram negative‖ (pink) ➢ Shape — the most common shapes include round (cocci) or rod-shaped (bacilli) Additional information may be obtained by observing the groupings of the bacteria on the slide, such as cocci that are present singly, in pairs, in groups of four, in clusters or in chains, or bacilli that are thick, thin, short, long, or have enlarged spores on one end. Any bacteria that are present within white blood cells (intracellular) are also noted.

Biochemical Tests
Oxidase production test

The oxidase test is used to detect whether or not an organism has the cytochrome oxidase enzyme. a filter paper is soaked with a little made 1% reagent (p-phenylenediamine dihydrochloride) and then pick the colony to be tested with a wooden stick and smear in the filter paper. macroorganisim is oxidase positive when appearing blue color within 15 to 30 seconds.
Catalase production test
To detect the ability of organisms to produce the catalase enzyme (an enzyme that breaks down the hydrogen peroxide into water and oxygen). place a small amount of a bacterial colony (18 to 24 hours old) on a clean glass slide. Add one to two drops of 3% hydrogen peroxide. when rapid bubble formation on the slide, the microorganism is catalase positive**.**

Indole production test
To determine the ability of the organism to convert tryptophan into indole. Inoculate the tryptophan broth with the test organism and incubate it for 18-24 hours at 37C. After that, add 15 drops of Kovac's reagent to the inner wall of the tube. The development of a bright red color ring within seconds is indicative of the presence of indole.
TSI test and H2S formation
It is used to differentiate bacteria based on the ability to reduce sulfur and ferment carbohydrates. Prepare the medium of the TSI according to the manufacturer's directions in the form slant agar in test tubes. Inoculate the slant with a pure culture by streaking over the entire surface of the slant (zig-zag to cover surface) and then stabbing deep into the butt. incubate at 37C for 24 hours.
Citrate consumption test
is used to detect the organism’s ability to utilize citrate as a sole source of energy. The Simmons citrate medium is prepared according to the manufacturer's directions in the form of slant agar. Inoculate on the slant by touching a colony that is 18-24 hrs old with a straight wire. and incubate at 35oC-37oC for up to 7 days. observe the blue coloration of the media refer to a positive result.
Coagulation test by coagulase enzyme
to determine if an organism generates the exoenzyme coagulase, which causes blood plasma fibrin to clot. and, in particular, to distinguish *S. aureus* from other *Staphylococcal species*. Some colonies from a new culture are taken and emulsified in water using an inoculating loop. The slide is then treated with a drop of rabbit or human plasma, and the clumping is detected within 10 seconds.
Urease test
The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. prepare a Urea broth according to the manufacturer's directions and autoclaving the media to prevent urea from initial breakdown. Inoculate the given sample of organism aseptically using a wire loop. and incubate the tubes at 37°C for 24 hours. examine for the development of a pink color.
API 20E protocol (Analytical profile index for Enterobacteriaceae)

Use the API 20E protocol to confirm identification. Local isolates identified by biochemical testing were further described using the API 20 E Strip (Biomerieux,) system, which is a standardized characterization approach for *Enterobacteriaceae* and other non- fastidious Gram-negative rods. The system was made up of 20 microtubes packed together with dehydrated substrates. These microtubes were inoculated with a single microbe suspension and incubated for 24 hours at 37 °C. During incubation, metabolism caused color changes that were either spontaneous or revealed by reagent addition. The test results were read, and the organism's identification was determined. The positive and negative test results were utilized to produce a seven-digit code number. This number is then used by the company's database to determine the identity of the microbe according to CLSI standards used in the Central Public Health Laboratory. This confirmatory examination was conducted for only four isolates.

ANTIMICROBIAL SUSCEPTIBILITY SENSITIVE

The Kirby-Bauer disk diffusion protocol was used to estimate the sensitivity of bacteria isolated from cases of otitis media to antibiotics. Touching four to five colonies of bacteria with a loop and emulsified with distilled water. gently mix by vortex until the turbidity was equivalent to a 0.5 McFarland standard. The plates of Hinton Muller agar medium were then inoculated with bacterial suspension using sterile cotton swabs over the whole surface of each plate. Allow the dishes to dry. Following that, antibiotic tablets were placed on the plates with a 2 mm space between each disc, and the plates were incubated at 37°C for 18- 24 hours to establish zones of inhibition. The following antibiotics have been used: AK Amikacin, IMP Ampicilin, CTX Cefotaxim, GM Gentamicin TE Tetracycline, TOB Tobramicin, P Penicillin, SXT Co-Trimoxazol, MEM Meropenem, DO Dxycyclin, CIP Ciprofloxacin, LEV Levofloxacin, FEP Cefepim, PRL Piperacillin, CLR Clarithromycin, FOX cefotaxime. And AZM Azithromycin, VA vancomycin, OFX Ofloxacin, ERY Erythromycin, Clindamycin and Chloramphenicol.

RESULT AND DISCUSSION

As previously stated, (30) samples were obtained from individuals diagnosed with otitis media by otolaryngologists. After culture, 26 (86.66) of the samples showed positive growth on blood agar, whereas 4 (13.335) samples showed no growth on this agar.

Following bacteriological identification, *Pseudomonas aerginosa* was found to be the most frequent among isolates, representing for (34.61%) of the total, followed by *proteus mirabilis* bacteria by (26.92%), followed by *staphylococcus aureus* by (19.23%) and *E. coli* by (11.53%). In addition, *serratia marcescens* and *providencia* were isolated by (3.84%) each separately. (As shown on the table 1).

Table 1: List of Bacteria isolated from cultures

Bacterial Isolates	NO. of Isolates	Relative frequency (%)
Pseudomonas aerginosa	9	34.61%
Proteus mirabilis	7	26.92%
Staphylococcus. aureus	5	19.23%
Escherichia. Coli	3	11.53%
Providencia	1	3.84%
Serratia marcescens	1	3.84%
Total	26	100%

In the present study, the results showed that the *Pseudomonas aerginosa* was predominant organism isolated from middle ear, which reached (9/26) (34.61%), were identified by produce some greenish pigmentation on macConkey agar and metallic sheen colonies on blood agar medium and also gave fishy odour. Strongly oxidase positive was confirmed *Pseudomonas aeroginosae.* followed by *proteus mirabilis,* which was observed in (7/26) (26.92%) cases, this result was similar to studies [38,58], but was dis-agreed with studies [37,52,53,56,57] which they found that the most common

isolates are *Staphylococcus aureus*On the other hand, our results were disagreement with previous reports[52,53,54], that identified 35 isolate of *Staphylococcus aureus,* and our results were similar with results by Ch.Al-Yas,[55] that found *Staphylococcus aureus* in only 6 isolates.

Fig. 1. *Pseudomonas aeruginosa* on blood agar

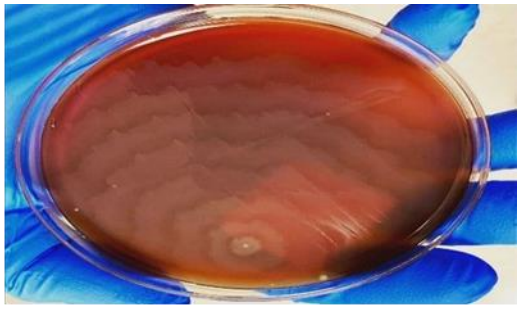

Fig. 2. *Proteus mirabilis* on blood agar

Fig. 3. *Staphylococcus aureus* on blood agar

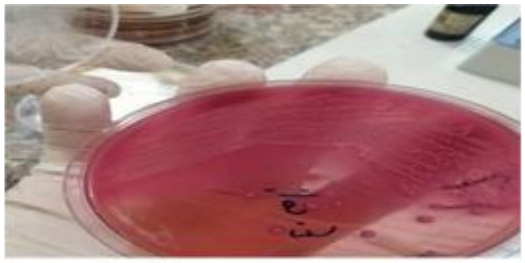

Fig. 4. *E. coli* on macConkey agar

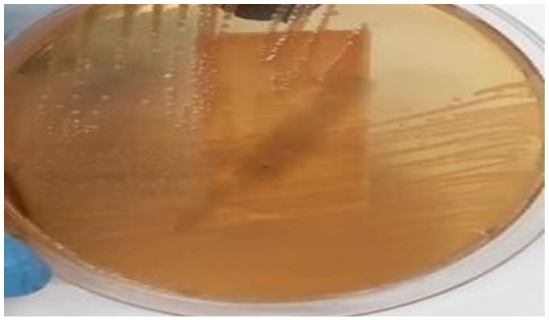

Fig. 5. *Serratia marcescens* on macConkey agar

Fig. 6. *Providencia* on macConkey agar

Pseudomonas aerginosa was initially diagnosed based on the morphology of the colonies in Blood Agar, which appear as typical metallic sheen coloneis. And flat, smooth, and non- lactose fermenting (Colorless) on macConkey agar. Then, using gram stain, identify it as gram negative bacilli. As for the results of its chemical tests, they were positive in Oxidase, Catalase, Urease and Citrate while they exhibited negative results in Indole and Voges- Proskauer (VP), results of the TSI (Triple sugar iron agar) test reveal a K/A, G pattern (Glucose fermentation only, Gas produced, and no H2S formed). *Proteus mirabilis* was the second microbe isolated. With a proportion of (26.92%). These bacteria were identified based on their swarming motility on blood agar. Colonies that are pale or colorless (Non lactos fermenting) on macConkey agar. Gram's stain confirmed the diagnosis; the bacteria showed as gram-negative bacilli in morphology. the outcome of its chemical test were positive for catalase, urease, citrate, and motility. While oxidase, indole, and Voges- Proskauer (VP) exhibited negative findings. The TSI (Triple sugar iron agar) test results show a K/A, H2S pattern (Glucose fermentation only, Gas produced, and H2S formed). The remaining isolates discovered in this study were *Staphylococcus. aureus* (19.23%) and *Escherichia. coli* (11.53%). *Serrctia. marcescens* and *Provenencia* both have a (3.84%). Among them, Staphylococcus is of greater clinical importance. Because of its autochthonic qualities, *Staphylococcus aureus* is the leading cause of nosocomial and community- acquired infections [19]. The relevance of *Staphylococcus aureus* as a causal pathogen of OM stems from the bacteria's growing antibiotic resistance and strong colonization capacity [20,21]. *Staphylococcus aureus* was diagnosed based on zones of clear beta-hemolysis on blood agar. Then, using gram stain, identify it as gram positive cocci. The results

of its chemical testing were positive for Catalase, Urease, and Citrate. Coagulase production and lactose fermentation on Mannitol Salt Agar. As shown on the table (2)

Table 2: Identification of bacteria: Gram stain/shape/Biochemical test

Bacteria	G. S	Shape	Catalase	Oxidase	Indole	V-P	Urease	Citrate	Glucose ferment	lactose ferment	Motility	Coagulase
Pseudomonas aerginosa	-ve	Rod	+	+	–	–	+	+	+	–		
Proteus mirabilis	-ve	Rod	+	–	–	–	+	+	+	–	+ve (swarming)	
Escherichia. coli	-ve	Rod	+	–	+	–	–	–	+	+	+	
Staphylococcus . aurreus	+ve	Cocci	+ve	–	–	+	+	+	+	+		+
Serratia marcescens	-ve	Rod	+	–	–	+	–	+	+	–	+	
Providencia	-ve	Rod	+	–	+	–	+	+	+	–		

As shown in the table (3) The most effective drugs against *Pseudomonas aeroginosae* isolated from the ear samples were Ciprofloxacin , Levofloxacin and Piperacillin- tazobactam (100%), followed by Amikacin and Gentamicin (75%), Chloramphenicol and Cefepim (50%), The least effective drug was Cefotaxima (25%). The most effective antibiotic against *Staphylococcus aureus* isolated was Amikacin (100%), followed by Chloramphenicol and Ciprofloxacin (75%), Clarithromycin, Clindamycin and Tetracycline (50%), The least effective antibiotics were found to be Azithromycin and Vancomycin (25%). Whereas the bacterial isolates showed significantly resistant to the routinely used antibiotic Penicillin (100%) (Table 4). As for the of *Enterobacteriaceae*, the most effective antibiotic was Meropenem with a percentage of 66.66% for *Proteus mirabilis* and 100% for *Escherichia. coli* and *Serratia marcescens*. Followed by the Levofloxacin with a percentage of 66.66% for the *proteus* and 100% for the *Ecoli*. , Impicilin by 100% for proteus and 50% for *E coli*. *E coli* and *Serratia* isolates showed resistance to tetracycline and IMP at 50% and 100%, respectively. As for the *Proteus* isolates, they showed resistance to meropenem, levofloxacin and ciprofloxacin with a percentage of 33.33, respectively. (Tables 5, 6, and 7). The majority of the time, OM is treated clinically, particularly in low-income nation's health institutions. This will result in bacterial antibiotic resistance and avoidable OM problems like as deafness and meningitis [22]. These observations demand for the public to be made aware of this critical health issue through the media.

Table 3 Antibiotic susceptibility pattern of *Pseudomonas aeroginosae*

Antibiotics used	Cons. Mg	Susceptible(%) NO.		Intermediate(%)	Resistant(%)	Total
Amikacin	AK 30 µg	75% (3)		----------	25% (1)	4
Ciprofloxacin	CIP 5µg	100% (4)		----------	----------	4
Gentamicin	GM 10 µg	75%	(3)	----------	25% (1)	4

Cefotaxima	CTX 30 µg	25%	(1)	25% (1)		50% (2)		4
Chloramphenicol	C 30 µg	50% (2)		25%	(1)	25%	(1)	4
Levofloxacin	LEX 5 µg	100% (4)		----------		----------		4
Cefepim	FEP 30 µg	50% (2)		----------		50% (2)		4
Piperacillin-tazobactam	PRL 100/10	100% (2)		----------		----------		2

Taple 4. Antibiotic susceptibility pattern of *Staphylococcus aureus*						
Antibiotics used	Cons. Mg	Susceptible(%)	NO.	Intermediate(%)	Resistant(%)	Total
Amikacin	AK 30 µg	100% (4)		----------	----------	4
Chloramphenicol	C 30 µg	75%	(3)	----------	25% (1)	4
Penicillin	P 10 Units	----------		----------	100% (4)	4
Ciprofloxacin	CIP 5µg	75%	(3)	25% (1)	----------	4
Azithromycin	AZM 15 µg	25% (1)		----------	75% (3)	4
Clarithromycin	CLR 15 µg	50% (2)		----------	50% (2)	4
Clindamycin	CD 2 µg	50% (2)		25% (1)	25% (1)	4
Vancomycin	VA Mg	25% (1)		50% (2)	25% (1)	4
Tetracycline	TE 30 µg	50%(1)			50%(1)	2
Erythromycin	ERY 15 µg	50% (2)		----------	50% (2)	4

Taple 5. Antibiotic susceptibility pattern of *Proteus mirabilis*					
Antibiotics used	Cons. µg	Susceptible(%) NO.	Intermediate(%)	Resistant(%)	Total
Amikacin	AK 30 µg	100% (3)	----------	----------	3
Meropenem	MEM 10 g	66.66(2)	----------	33.33% (1)	3
Impicilin	IMP 10 µg	100% (3)	----------	----------	3
Levofloxacin	LEV 5 µg	66.66(2)	----------	33.33% (1)	3
Gentamicin	GM 10 µg	66.66(2)	33.33% (1)	----------	3
Ciprofloxacin	CIP 5µg	66.66(2)	----------	33.33% (1)	3
Cefepim	FEP 30 µg	66.66(2)	33.33% (1)		3
Piperacillin-tazobactam	PRL 100/10	100% (3)	----------	----------	3

Taple 6. Antibiotic susceptibility pattern of *Escherichia. coli*					
Antibiotics used	Cons. Mg	Susceptible(%) NO.	Intermediate(%)	Resistant(%)	Total
Impicilin	IMP 10 µg	50% (1)	----------	50% (1)	2
Meropenem	MEM 10 g	100%(2)	----------	----------	2
Gentamicin	GM 10 µg	100%(2)	----------	----------	2
Ciprofloxacin	CIP 5µg	100%(2)	----------	----------	2
Levofloxacin	LEV 5 µg	100%(2)	----------	----------	2
Cefotaxima	CTX 30 µg	50% (1)	50% (1)	----------	2

Co- rimoxazol	SXT .25/23.75	100%(2)	----------	----------	2
Tetracycline	TE 30 µg	----------	50% (1)	50% (1)	2

Taple 7. Antibiotic susceptibility pattern of *Serratia marcescens*					
Antibiotics used	Cons. Mg	Susceptible(%) NO.	Intermediate(%)	Resistant(%)	Total
Impicilin	IMP 10 µg	----------	----------	100%(1)	1
Meropenem	MEM 10 g	100%(1)	----------	----------	1
Gentamicin	GM 10 µg	----------	100%(1)	----------	1
Ciprofloxacin	CIP 5µg	----------	----------	100%(1)	1
Dxycyclin	DO 30 µg	100%(1)	----------	----------	1
Cefotaxima	CTX 30 µg	100%(1)	----------	----------	1
Tetracycline	TE 30 µg	----------	----------	100%(1)	1

As shown in the table (7) The age distribution of the patients with otitis media for patients aged 18 to 62 , revealed that the age groups (21-30 years) and (41-50 years) had the highest percentage occurrence (both categories were 26.9%), followed by (31-40 years) 19.2% was registered.

Table (8): Distribution of OM patients according to Age groups and gender						
Age Group	**Male**		**Female**		**Positive No. Total**	**%**
	Positive No.	**%**	**Positive No.**	**%**		
≤ 20	1	6.6%	1	9%	2	7.6%
21-30	5	33.3%	2	18.1%	7	26.9%
31-40	1	6.6%	4	36.3%	5	19.2%
41-50	4	26.6%	3	27.2%	7	26.9%
51-60	3	20%	1	9%	4	15.3%
>60	1	6.6%	0	0%	1	3.8%
Total	15(58%)	100%	11(42%)	100%	26	100%

In this study, the male was seen more susceptible to infection with otitis media than the female (15/26: 58% male) (11/26: 42% female), this result was disagreeing with Ekpo et al.

29. who found the male to female ratio was 1:16, but our results were agreement with some reports [30,31,32,33,34] and other study in South Africa found that the male was more susceptible (64%) than the female (36%) to infect with otitis media[35]. On the other hand, our study were disagreement with study in Pakistan [36] revealed that OM is more common among females.

The most common age group affected was (21-30) years and (41-50) years (26.9%) followed by (≤ 20) years (7.6%), (31-40) years (19.2%), (51-60) years (15.3%), and (> 60) years (3.8%). This result similar to Patigaroo et al. [37] who found the most our patients were young and middleaged, and our result similar to Gul et al., [38] who refer to the most common age group more susceptible to infection with otitis media was (21-30) (22%).

Otitis media, particularly OME, may be related with the following factors: Upper respiratory tract infections, year-round seasonal allergy symptoms, [39] inadequate ciliary clearance, and eustachian tube drainage are the chief causes of OME. Recent study has also revealed that gastroesophageal reflux disease may be linked to eustachian tube dysfunction and otitis media. Reflux of stomach contents into the nasopharynx can result in nasopharyngeal and eustachian tube irritation. It may cause upper respiratory tract problems, including OME in adults and pediatric age group [40]. The study [41] also confirms the relationship between gastric content reflux and the occurrence of otitis media in infants. Because of the angle of the young eustachian tube, these fluids can enter the middle ear and lay the framework for bacterial infections. Multiple studies have looked at the impact of smoking, environmental tobacco smoke (passive smoking) [42] and the incidence of otitis media infections. In addition, maternal smoking during pregnancy has been associated with increased

adverse neonatal events and recurrent otitis media. Childhood morbidity. [43]. NO2 or benzene exposure during prenatal and postnatal exposure in children during their first 12–18 months. The researchers found that exposure to NO2 during the entire prenatal period, 1st, 2nd, 3rd trimester and first year of life to be associated with increased risk of OM Similar associations were found with benzene exposure [44]. Evidence from epidemiological studies supports a link between air pollution and OM; NO2 and benzene exposure in children throughout their first 12–18 months of life, both prenatally and postnatally. The researchers discovered that NO2 exposure over the whole perinatal period, including the first, second, and third trimesters, as well as the first year of life, was connected with an elevated risk of OM. Similar relationships have been discovered with benzene exposure [45]. otitis media (OM) has a substantial genetic component, with innate immunity gene variants likely to contribute to risk. OM candidate genes investigations have mostly involved genes related with innate immunity and inflammation [46]; these are acceptable candidates for study, as the early onset of OM is expected to include a failure in the early steps of pathogen clearance. [47,48]. Other risk factors that might contribute to middle ear infections: incorrect methods for cleaning the outer ear [49], crowded living conditions, pacifier use, and bottle feeding in a supine position, Lack of hygiene, and swimming in polluted waters. [50, 51].

Conclusions and Recommendations

1. Otitis media predominantly occurs as coincident to bacterial upper respiratory tract infections and/or viral infections.
2. *Pseudomonas aeroginosae* was the most predominant organism isolated from the pus swab followed by *Proteus mirabilis* and *Staphylococcus aureus*. Most bacterial isolates prevailing in this study have shown sensitive to Ciprofloxacin and Levofloxacin. Whereas the all *Staphylococcus aureus* isolates showed significantly resistant Penicillin.
3. Otitis media linked with high levels of multiple antibiotic resistant bacteria is a major health concern in all age groups of the study population. So there is a need for culture and susceptibility test facilities for appropriate antimicrobial therapy of otitis media and antimicrobial resistant infections.
4. Increasing social and cultural awareness of these injuries and how to prevent them and exploiting social media, seminars and conferences for that.

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