The Relationship Between Bacterial Species Isolated from otitis Media and Bacterial Species Isolated from The Environment of the Infected Patients

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Abstract
Otitis media (OM) is a widespread disease of the middle ear cavity associated with bacterial or viral pathogens. The current study included a pathological list and diagnosis of some types of bacteria that cause otitis media from the extended period 1/11/2021 to 30/4/2022. This study included 115 swabs of patients for the medical clinic in AL- Tarmiyah General Hospital and of different ages. Bacterial isolates were diagnosed morphologically and culturally by using biochemical tests, using these bacteria to produce enzymes and being positive and negative for gram stain, tests have also been confirmed using Vitek. The Staphylococcus aureus bacteria were the most common among the bacterial species that cause infection at 28 (30.7%). Whereas Burkholdera cepacia group bacteria represented the lowest percentage that was at 1 (1.1%). As for the environmental samples, isolates were obtained from the water used for the areas affected by otitis media, Staph. aureus and E. coli were the most common at 8 (30.8%), While *p.aeruginosa, Salmonella enterica* represented the lowest rate at 1(3.8%). As for the soil, *E.coli* and *p.aeruginos* were in the first place at 5 (20%), were *Proteus vulgaris, Proteus mirabilis* represented the lowest at 1(4.0%).

1. Introduction

Otitis media (OM) is a widespread disease of the middle ear cavity associated with bacterial or viral pathogens that affects 80% of children in the United States (Schilder *et al.*, 2016). Where the most common source of earache in children with low socioeconomic background, on the rate of infection in the younger age group, the reason is due to the incomplete immune system, especially in children and weak physical structure (DeAntonio *et al.*, 2016). It also affects adults and often It accompanies colds and influenza or any other type of respiratory system where the middle ear is connected to the upper respiratory system through a small channel called the Eustachian tube (Brennan Jones *et al.* 2020).

One of the most important factors causing otitis media is water and soil, as the world in general and the third world countries in particular face one of the most serious problems faced by humanity, the first being the provision of safe water for drinking and use. (Ahmed Ismail and others, 2009). Therefore, direct use of river water is a reason for the spread of many diseases caused by the presence of microorganisms such as bacteria, parasites and pathogenic fungi (Ayandiran *et al.*, 2018). This is known as biological pollution as well as chemical pollution as well as natural pollution (Fahd and Rabie, 2010). What man sees of the things found in the soil represents only a very simple part of that hidden world that lives in it and of which it is composed. They live in the soil, and these organisms also differ among themselves in their numbers and sizes, as well as their need for light, oxygen and organic matter, and differ among themselves in the ways of coexistence, as well as in the extent of their tolerance to heat and water and the degree of interaction (PH) (Altai, 2020).

2. Material and Method

Sample collection

1- Otitis media

(115) swabs were collected from patients with otitis media who attended the consultative clinic of AL-Tarmiyah General Hospital in from the extended period 1/11/2021 to 30/4/2022. The number of samples with growth was (91) samples and (24) samples without growth. The samples were taken using Sterile cotton swabs. Information about each patient was recorded according to a form prepared for this purpose and to ensure that the patient did not take any antibiotics before taking the swab for a period of no less than three days, then the swabs were transferred directly to the laboratory.

il and water samples of the infected environment

soil and water samples of infected enviroment were collected from the extended period 1/11/2021 to 30/4/2022. The method of soil samples collection was by scraping the surface layer of the soil and taken with a depth ranging between (5-10 cm) from

doi.org/10.31838/hiv22.02.492 Received: 12.05.22, Revised: 25.06.22, Accepted: 12.08.22

the areas of the affected persons from different locations in the Tarmiyah area. The amount of sample taken was (100 gm) from each site were placed in a sterile bag, and the information was recorded (model number, soil type, planted, site) and brought to the laboratory. A series of decimal dilutions (0.1 ml) was prepared from the decimal dilution by spreading (2-10 ml) to the solid medium surface of nutrient agar and incubated at (15-45) C° for (24-48) hours, (Muthanna Hassan, 2008). water samples were collected in sterile glass containers and transferred directly to the laboratory (Asia et al., 2019). It was also prepared through a decimal dilution series as the method used with soil samples (Muthanna Hassan, 2008).

Culture of otitis media swabs

After the sample was taken by swab from the patient, where it was transferred directly to the laboratory and then cultured on different media such as blood, Maconkey, chocolate and nutrient agar, the cultured media then incubated at 37 C° for (18-24) hours. (Betty *et al.*, 2007).

3. Diagnosis

After bacterial isolates microscopic examination were done by stained with Gram stain, bacterial isolates were also diagnosed by based on their cultural, morphological characteristics, and biochemical tests like Oxidase, Catalase and Coagulase tests, in addition to the IMViC tests, which include (indole production test, methyl red test). (Shadman, 2015). To confirm the isolated bacterial species, the Vitec diagnostic device was also used. The (Vitek 2) system was used to confirm the result of the manual biochemical test, and recently this system was used to identify microorganisms (Winn et al., 2006). This system was performed according to the manufacturer's instructions (Biomerieux-France).

4. Results and Discussion

Otitis media Inflammation

1- Distribution according to bacterial growth

Table (1) Distribution according to bacterial growth		
type of bacterial growth	%	Number of isolates
Bacterial growth	79.1	91
Without growth	20.9	24
Total	100	115

115 bacterial isolates were collected throughout the period from 1/11/2021 to 30/4/2022. The results were (91) bacterial growth samples and (24) samples without growth, as shown in (Table 2).

2 – Distribution of Otitis media according to gender

Table (2) Distribution of Otitis media according gender:					
The number of female samples	%	The number of male samples	%	Total	%
49	42.6	66	57.4	115	100

The number of males with otitis media was 66 (57.4%) it was recorded highest rate compared with the number of females that was 49 (42.6%). this may be due to the opportunity available to males to practice swimming, whether in swimming pools or in other rivers and ponds, this is consistent with the results of many studies such as (Al-Attraqchi et al., 2019, Kadhim et al., 2018 and Kumar et al., 2018). This study also agreed with another study of Jabber (2019) who showed that an increase in the number of males with otitis media compared to females. The exposure of males to different working conditions such as dust and humidity, as well as may be more involved in outdoor activities, and thus be more exposed to a polluted environment, and that wearing the veil for females can be considered an important factor to reduce infection (Al-Attraqchi et al., 2019).

3- bacterial isolates distribution according to the gram stain

Table (3) bacterial isolates distribution according to the gram stain.					
The number of positive bacteria	%	The number of negative bacteria	%	Total	%
39	42.9	52	57.1	91	100

The results of the isolated bacteria were divided into two parts, the Gram-negative bacteria group, and the largest percentage was at 52 (57.1%) compared with Gram-positive bacteria which was at 39 (42.9%), this shows that Gram-negative bacteria are the main and most common cause of otitis media, and these results agree with what they found (Abdullah, 2006) (Hussain and Nizam, 2012). Also, this results were agreed with a study conducted by (Shadman, 2015).

4- Number of patients with otitis media according age groups

Table (4) Number of patients with otitis media according age groups.		
ages	%	patients
Less than one year	2.6	3
(1-9)	21.7	25
(10-19)	18.3	21
(20-29)	28.7	33
(30-39)	11.3	13
(40-49)	7.0	8
(50-59)	5.2	6
(60-69)	3.5	4
More than 70	1.7	2
Total	100	115

From the results that showed in table (4) that clarify

number of patients with otitis media according age groups, the age groups (20-29) were represented most rate to infection, reaching (21.7%). One of the causes of this infection is due to the frequent exposure to external factors. (Mahmood et al., 2019). The study conducted by the researcher (Mofatteh et al., 2018) also agreed that the younger age groups are more affected by the disease. The current study also agreed with a study in Saudi Arabia in (Al-Hammar et al., 2018).

5-Distribution of Otitis media according to number of bacterial isolates

Table (5) Distribution of Otitis media according to number of bacterial isolates.

Types of bacteria	Number of bacterial isolates	%
Staph. aureus	28	30.7
Ps.aeruginosa	14	15.4
Acinetobacter baumanni comlex	9	9.9
Klebsiella pneumoniae	9	9.9
E. coli	8	8.8
Proteus mirabilis	6	6.6
Staph.epidermidis	5	5.5
Streptococcus pneumoniae	3	3.3
Enterobacter faecalis	2	2.2
Enterobacter cloca comples	2	2.2
Kocuria kristinae	1	1.1
Gemella bacter	1	1.1
Shigilla group	1	1.1
Morgenal morgan ssp morganii	1	1.1
Burkholder cepacia group	1	1.1
Total	91	100

The results in table (5) clarified Staph.aureus was the first main type of the bacteria isolated from otitis media at 28 (30.7%), this frequency may be due first to its entry into the middle ear from the external canal as normal flora. In a study in Iran conducted on 61 patients with chronic otitis media, the researcher concluded that Staph. aureus is the most common cause of this disease with 19 (31.15%), followed by Ps. aeruginosa by 16 (26.23%). (Samah Hashem, 2012).

Pseudomonas aeruginosa was the second major type of bacteria isolated from otitis media, its number was 14 (15.4%). Pseudomonas aeruginosa is the most common invader of the middle ear when resistance is low and Pseudomonas aeruginosa does not have to be via the nasopharyngeal stream but may be through fecal contamination of the auditory stream. Pseudomonas aeruginosa has many reasons for affecting the middle ear, it spreads easily to vulnerable patients through the external canal from healthy carriers or environmental sites, as swimming in the same rivers and swamps is one of the most important ways for otitis media with Pseudomonas aeruginosa and intestinal bacteria, where sources are This water is subject to contamination with human and animal waste, which is an important source of environmental pollution sources (Kadhim et al., 2018).

As for Acinetobacter baumanni complex and Klebsiella pneumoniae, it ranked third, as the number of isolates was 9, and by (9.9) the sequence of Klebsiella pneumoniae is the fourth among the bacterial genera isolated from middle ear infections, and the source of these bacteria is mostly from the pharynx through the Eustachian tube. The results of the research (Ali, 2011) ranked fourth, as the percentage amounted to (7%) of the total isolated bacterial species. E.coli bacteria ranked fifth in causing otitis media, as coliform bacteria was the cause of 8 patients, with a rate of 8.8%. The main source of this bacteria is swimming in water contaminated with this bacteria, as well as using incorrect methods to clean the outer ear, which contributes to introducing These bacteria enter the middle ear by physical means or by entering the water (Brooks et al, 2007). (Pajor et al, 2006) indicated that Proteus spp.

It is prevalent when it is secondary to the middle ear through the external auditory tube. Also (Rama Rao and Jayakar, 1979) reported that Pseudomonas spp. and Proteus spp. They do not usually inhabit the upper respiratory tube, so their presence in chronic suppurative middle ear infections is not through the Eustachian tube, so these microbes are a secondary attacker from the external auditory tube when the resistance of the middle ear is low, as they enter the middle ear through the hole resulting from acute inflammation in the membrane eardrum.

2- Water and soil of the infected environment

1-Distribution bacterial species according to gram stain acceptance

Table (7) Distribution bacterial species according to gram stain acceptance.

The type of sample	Number of Positive bacteria	%	Number of Negative bacteria	%
Water	14	60.9	12	42.9
Soil	9	39.1	16	57.1

The table (7) showed Distribution bacterial species according to gram stain acceptance, the number of positive bacteria represented 14 (60.9%), while the number of negative bacteria was 12 (42.9%). this study agreed with what was reached (Hamed *et al.*, 2015).

As for the soil, the number of isolates was 9 (39.1%) for positive bacteria, while16(57.1%) for negative bacteria.

2- bacterial type according environmental sample type

Table (8) bacterial type according environmental sample type.					
Water Isolates			Soil isolates		
Bacterial isolates	numbers	%	Bacterial isolates	numbers	%
S.aureus	8	30.7	S.aureus	4	16.0
S.homonis	2	7.6	Klebsiella pneumoniae	4	16.0
E.coli	8	30.7	Bacillus subtilis	3	12.0
S.epidermidis	2	7.6	Proteus vulgaris	1	4.0
P.aeruginosa	1	3.8	p.aeruginosa	5	20.8
Salmonella enterica	1	3.8	E. coli	5	20.8
Streptoccus faecalis	2	7.6	Micrococcus lutes	2	8.0
Proteus mirabilis	2	7.6	Proteus mirabilis	1	4.0
Total	26	100	Total	25	%100

The highest percentage of bacterial isolates obtained in water was S.aureus and E.coli, the same number of isolates was at 8 (30.8%), this agree with study conducted that E.coli had the highest infection rate (Mohsen et al., 2019).

As for the soil, the dominance of E.coli, p.aeruginosa, and the number of isolates was 5 (20.%), as in a study conducted by the researcher (Saleh, 2015).

3-The relationship of bacterial isolates that cause otitis media with isolates taken from the infected environment (soil water)

Table (9) The relationship between bacterial types of otitis media and isolates taken from the environment of infected people						
Clinical samples of patients			Environmental samples			
Middle ear			water		Soil	
Bacterial types	number	%	number	%	number	%
S.aureus	28	30.7	8	66.6	4	33.3
P.aeruginosa	14	15.4	1	8.3	5	41.6
Klebsiella pneumoniae	9	9.9	0	0	4	33.3
E. coli	8	8.8	8	66.6	5	41.6
Proteus mirabilis	6	6.6	2	16.6	1	8.3
S.epidermidis	5	5.5	2	16.6	0	0
Streptoccus faecalis	2	2.2	2	16.6	0	0

Through the table (9), there is not a relationship between the bacterial species that cause otitis media with bacterial species taken from soil and water samples for the environmental areas of the infected people, as these environmental factors (soil water) had a very significant impact on people with otitis media. From the results in this study and as clarify in table (9) It turned out that the biggest cause of infection was due to these factors, the highest percentage among species was S.aureus, where the number of isolates for otitis media was 28 (30.7%). The number of isolates with a percentage of water was 8 (66.6%), while the number of soil isolates was 4(33.3%). p. aeruginosa was followed, it was 14 (15.4%). The numbers of bacteria Streptoccus faecalis that isolated from the patients with otitis media and water were similar, but it different in their percentages it was 2.2% with otitis media sample and 16.6 with water sample.

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