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Comparison the sensitivity of pathogenic bacteria isolated from patients infected with otitis media and bacteria isolated from infected environment (water and soil)

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ABSTRACT

From a total of 235 samples different sources, 115 samples (males and females) in different ages were taken from patients attended the consulting clinic at AL- Tarmiyah General Hospital were infected with Otitis media bacterial infection and (120) samples from environmental sources of same patients which infected with Otitis Media (O.M) which included water and soil environments from the infected environment). 15 bacterial type were isolated from patients with (O.M) while 10 bacterial types were from water and soil environents. all bacterial isolates were tested for sensitivity to 10 antibiotics, included (Amoxicillin (30µg), Ampicillin(25 µg), Trimethoprim(10 µg), Cefixime (5 µg), Cefepime (10 µg), Gentamicin(10 µg), Amoxicillin(25 µg), Azithromycin(15 µg), Ciprofloxacin(10 µg), Meropenem(10 µg). Bacterial isolates from different sources were showed different sensitivity results toward antibiotics that were used in this study, bacterial species were taken from O.M. (*Staph.aureus, P.aeruginosa ,Acinetobacter baumanni comlex, Klebsiella pneumoniae E.coli, Proteus mirabilis, Staph .epidermidis ,Streptococcus pneumoniae ,Enterobacter faecalis Enterobacter cloca comples , Kocuria kristinae , Gemella bacter, Shigilla group ،Morgenal morgan ssp morganii ,Burkholder cepacia group*) showed high resistant to ampicillin at(100)% and showed in same time high sensitivity to Meropeneme at (95)%, while bacterial isolates(water(S.*aureus , S. homonis , S.epidermidis , Proteus mirabilis, Streptoccus faecalis, P.aeruginosa Salmonella enterica*,),soil(*E.coli , p.aeruginosa, Klebsiella pneumonia, S.aureus, Micrococcus lutes, Proteus vulgaris , Proteus mirabilis*) were isolated from water and soil showed high resistant to Ampicillin at (100)% and show high sensitivity towards Meropeneme at (95)% . most isolates were showed high sensitivity to Meropeneme and ciprofloxacin while were showed high resistant to Ampicillin and Amoxicillin.

Introduction

Antibiotics are used to treat various bacterial infections, The bacterial infection cause a major effect on the public health and it may occur in any site of body. but the increase in bacterial resistance to antibiotics has become very difficult to treat various infections. The treatment of otitis media depends on treating the infection of the upper respiratory tract, as well as on topical treatment, which includes cleaning the ear by withdrawing pus from the ear and then using antibiotics, and the selection of the appropriate antibiotic depends on the bacteriological culture (Lieberthal *et al*, 2013; Nayyef and Thalij, 2020).

In previous studies related to the treatment of otitis media, they indicate that B-lactam antagonists, such as ampicillin, were used as the main treatment for this condition, but with the development of bacterial resistance to this class of antibiotics, it became ineffective as its use is now very rare in the treatment of otitis media. The middle ear and for this reason, the search for alternative antibiotics was very important for the purpose of treating the condition, as new types and varieties with high effectiveness against bacteria that cause otitis media have been discovered, such as aminoglycoside, quinolone and carbapenem antibiotics, but they differ among themselves in terms of the percentage of antibacterial activity (Crocco, 2011).

Soil is a good place to look for new organisms, since bacteria naturally compete for resources and use a range of strange chemical compounds to kill each other. Notably, the lack of new antibiotics over the past years has raised dire warnings that the world is approaching the cusp of a "post-post" era. Antibiotics, if the bacteria persist. Scientists say finding new antibiotics is the key to staying one step away from the threats of drug-resistant bacteria, as only one new natural antibiotic, teixobactin, has been discovered in the past 33 years.

Note that the problem of bacteria resistant to antibiotics is one of the important challenges for

workers in the field of environmental and medical microbiology, as studies confirm a continuous increase in this type of isolates, and there is still difficulty in accurately explaining the existence, development and transmission of resistance in these bacteria isolated from water due to the different sources of these isolates as well as The different concentrations and sources of arrival of antibacterial substances, including antibiotics, and their contact with germs in water and other interactions, but in all cases, these resistant isolates pose an additional danger in terms of the disease they cause and the difficulty of treating them (Acar and Rosetel, 2001; Kapil, 2005; Ibezim, 2005).

Materials and Methods

The Samples Collection

Otitis Media Bacterial Samples:

115 samples were taken from patients (males and females) in different ages were attended to ENT (Ear Nose Tonsillitis) consultant clinic at AL- Tarmiyah General Hospital were infected with Otitis media bacterial infection in the period between January to April 2022. (15 bacterial type were isolated from patients with (O.M) The external ear was cleaned with 70% alcohol solution (Trullas *et al*., 2004; Nayyef and Thalij, 2020). Then the medical sterile swab was inserted with sterile ear speculum after the clinical diagnosis of the patient's infected

Environmental Samples (Water and Soil): (120) samples from environmental sources of same patients which infected with Otitis Media (O.M), included water and soil environments from the infected environment were collected during 6 months between (1st December to 30 April) , 10 bacterial types were collected from water and soil environments.

a) The Soil Samples: 60 soil samples were collected taken after a depth of 1 cm from the surface of the soil, then 1-5 cm was determined, then the samples were placed in sterile nylon bags, and then transferred to the laboratory for the purpose of preparing a series of dilutions for the purpose of later diagnosing bacterial isolates.(Hassan, 2008).

b) The Water samples: 60 water Samples were collected and in sterile glass container and then transferred to the laboratory to preparing a series of dilutions(Ismail,2009). as in soil samples .

The Identification of bacterial isolates: Colonies of the bacterial isolates were identified at first with the morphology of isolates depending on the color with consistency and the formed on the nutrient, maCconkey's agar medium and also according to the type of hemolytic on blood agar medium. (Alfred, 2005).

The microscopic examination: Microscopic examination were used to identified the bacterial isolates according to shapes and clusters under the microscope. (Alfred, 2005).

The biochemical tests: The biochemical tests were performed for the purpose to detect some the biochemical parameters that special for the each species of isolates (Alfred, 2005). In addition, the confirm results were conducted by used the (Vitec-2 compact system) technique.

Media preparation:

It was prepared by dissolving (38 g) of the powder in one liter of distilled water, then sterilized with an oxidizer and using the medium for the purpose of examining the sensitivity of antibiotics, as it is one of the best media for this test, as the inhibition area is clear. (MacFaddin, 2000).

Solutions preparation:

(0.5): Macfarlands standared solution1-

Consist from two solutions were prepared according to (NCCL 2003):

A-Barium chloride (BaCl2) solution:

The solution was prepared by dissolving (1.175) of barium chloride in (50) milliliters of sterile distilled water and completing the volume to (100) milliliters to obtain a concentration of (0.048 ml) of BaCl2.

B-Sulfuric acid solution (H2SO4

The solution was prepared by slowly adding (18) ml of sulfuric acid to (50) ml of sterile distilled water, and the volume was completed to (100) ml to obtain a concentration of 18.0 ml of (H2SO4).

0.5 ml. of solution (A) was added to 99.5 ml of solution (B) and adjusted to read standard turbidity to (6.08-0.10) at wavelength (625 nm) and this reading represents approximately (1.5 $x10^8$) ml (bacterial density or turbidity) The solution was distributed on sterile glass tubes with tight closed and kept in sterile places at room temperature, the tube was used for the purpose of comparing the density of bacterial growth in the used inoculum with the density of the solution in the tube.

Antibiotic sensitivity test:

The sensitivity of bacterial isolates toward antibiotics was tested using the Kirby-Baure method) as stated in (Vandepitte *et al*., 2003) and as follows:

1- loop full of the bacterial isolates under study were transferred to a tube containing (5) ml of normal saline and the turbidity of the solution was controlled with the turbidity of McFarland solution 0.5, which is equivalent to 1.5 x 10^8 cells/ml.

2- The sterile cotton swab was inserted into the tube containing the bacterial suspension and was rotated and pressed on the inner sides of the tube to remove the excess inoculum, and then the bacteria were spread on (Muller Hinton - Agar medium) by spreading more than once while rotating the plate at an angle 45° each time the dish is brushed for the purpose of ensuring the good spread of bacteria.

3-After that, the antibiotics disc were placed on the surface of the culture medium, and then the plates were incubated at a temperature of 37 C° for 24 hours. Resistant and sensitive to antibiotics, according to Clinical and Laboratory Standards Institute (CLSI, 2018).

Table 1: The antibiotic types

Antibiotic	Symbol	Disc concentrations (µg/disc)	The manufacture company
Amoxycillin	AMC	30	Bioanalyse Turkey
Azithromycin	AZM	15	
Gentamicin	CN	10	
Cefixime	CFM	5	
Amoxicillin	AX	25	
Ampicillin	AM	25	
Trimethoprim	TMP	10	
Cefepime	FEP	10	
Ciprofloxacin	CIP	10	
Meropenem	MEM	10	

Results and discussion

Comparison of antibiotics sensitivity for bacterial types from different sources (otitis media, water and soil environment)

Table 2: Antibiotics sensitivity for bacterial types from different sources (otitis media, water and soil environment)

Bacterial types	Antibiotics sensitivity (from O.M. source)																				
	Number	AMC		AM		TMP		CFM		FEP		CN		AX		AZM		CIP		MEM	
		S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Staph .aureus	28	6 21.4	22 75.5	0	28 100	4 14.2	24 85.7	0	28 100	25 89.2	3 10.7	18 64.2	10 35.7	4 14.2	24 85.7	2 7.1	26 92.8	28 100	0	28 100	0
Ps. Aeruginosa	14	0	14 100	0	14 100	0	14 100	0	14 100	0	14 100	0	14 100	0	14 100	0	14 100	6 42.8	8 57.1	14 100	0
Acinetobacter baumanni comlex	9	0	9 100	0	9 100	4 44.4	5 55.5	0	9 100	0	9 100	7 77.7	2 22.2	0	9 100	4 44.4	5 55.5	8 88.1	1 11.1	9 100	0
Klebsiella pneumonia	9	8 88.8	1 11.1	0	9 100	7 77.7	2 22.2	0	9 100	0	9 100	8 88.8	1 11.1	0	9 100	0	9 100	9 100	0	9 100	0
E.Coli	8	0	8 100	0	8 100	3 37.5	5 62.5	0	8 100	6 75	2 25	5 62.5	3 37.5	0	8 100	2 25	6 75	7 87.5	1 12.5	8 100	0
Proteus mirabilis	6	0	6 100	0	6 100	4 66.6	2 33.3	0	6 100	1 16.6	5 83.3	6 100	0	0	6 100	5 83.3	1 16.6	6 100	0	5 83.3	1 16.6
Staph .epidermidis	5	0	5 100	0	5 100	4 80	1 20	0	5 100	0	5 100	4 80	1 20	3 60	2 40	3 60	2 40	5 100	0	5 100	0
Streptococcus pneumonia	3	2 66.6	1 33.3	0	3 100	2 66.6	1 33.3	2 66.6	1 33.3	0	3 100	3 100	0	3 100	0	0	3 100	3 100	0	3 100	0
Enterobacter faecalis	2	0	2 100	0	2 100	2 100	0	0	2 100	0	2 100	0	2 100	1 50	1 50	1 50	1 50	2 100	0	2 100	0
Enterobacter cloca complex	2	1 50	1 50	0	2 100	2 100	0	0	2 100	0	2 100	0	2 100	2 100	0	1 50	1 50	2 100	0	2 100	0
Kocuria kristinae	1	0	1 100	0	1 100	1 100	0	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	1 100	0	1 100	
Gemella bacter	1	0	1 100	0	1 100	1 100	0	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	1 100	0	1 100	0
Shigilla group	1	0	1 100	0	1 100	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0
Morgenal morgan ssp morganii	1	0	1 100	0	1 100	1 100	0	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	100 1	0
Burkholder cepacia group	1	0	1 100	0	1 100	1 100	0	1 100	0	0	1 100	0	1 100	1 100	0	0	1 100	1 100	0	1 100	0
Bacterial types	**Antibiotics sensitivity (from water source)**																				
	Number	AMC		AM		TMP		CFM		FEP		CN		AX		AZM		CIP		MEM	
		R%	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**
S.aureus	8	0	8 100	0	8 100	2 25	6 75	3 37.5	5 62.5	2 25	6 75	5 62.5	3 37.5	0	8 100	6 75	2 25	5 62.5	3 37.5	8 100	0
S.homonis	2	0	2 100	0	2 100	1 50	1 50	0	2 100	1 50	1 50	0	2 100	0	2 100	1 50	1 50	2 100	0	1 50	1 50
E.coli	8	1 12.5	7 87.5	0	8 100	2 25	6 75	0	8 100	8 100	0	6 75	2 25	0	8 100	8 100	0	8 100	0	8 100	0
S.epidermidis	2	1 50	1 50	0	2 100	2 100	0	1 50	1 50	0	2 100	0	2 100	0	2 100	0	2 100	2 100	0	2 100	0
P.aeroginosa	1	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	1 100	0	1 100	0
Salmonlla entericea	1	0	1 100	0	1 100	0	1 100	1 100	0	0	1 100	0	1 100	0	1 100	1 100	0	1 100	0	1 100	0
Streptoccus faecalis	2	0	2 100	0	2 100	1 50	1 50	1 50	1 50	0	2 100	2 100	0	0	2 100	1 50	1 50	2 100	0	2 100	0
Proteus mirabilis	2	1	1	0	2	1	1	0	2	0	2	0	2	0	2	0	2	1	1	2	0

		50	50		100	50	50		100		100		100		100		100	50	50	100	
Bacterial types		**Antibiotics sensitivity (from soil source)**																			
	Number	AMC		AM		TMP		CFM		FEP		CN		AX		AZM		CIP		MEM	
		R%	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**	**S%**	**R%**
S.aureus	4	2 50	2 50	0	4 100	3 75	1 25	1 25	3 75	0	4 100	4 100	0	0	4 100	2 50	2 50	4 100	0	4 100	0
Klebsiella pneumonia	4	0	4 100	0	4 100	3 75	1 25	0	4 100	0	4 100	3 75	1 25	0	4 100	3 75	1 25	4 100	0	4 100	0
E.coli	5	3 60	2 40	0	5 100	4 80	1 20	1 20	4 80	0	5 100	0	5 100	0	5 100	3 60	2 40	4 80	1 20	5 100	0
Bacillus Subitils	3	0	3 100	0	3 100	3 100	0	2 66.6	1 33.3	0	3 100	2 66.6	1 33.3	0	3 100	2 66.6	1 33.3	3 100	0	3 100	0
Proteus Vulgaris	1	0	1 100	0	1 100	1 100	0	0	1 100	0	1 100	1 100	0	0	1 100	0	1 100	1 100	0	1 100	0
Proteus mirabilis	1	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	0	1 100	1 100	0
P.aeroginosa	5	0	5 100	0	5 100	0	5 100	0	5 100	0	5 100	0	5 100	0	5 100	0	5 100	3 60	2 40	5 100	0
Micrococcus lutes	2	0	2 100	0	2 100	2 100	0	0	2 100	0	2 100	1 50	1 50	0	2 100	1 50	1 50	2 100	0	1 50	1 50

The effect of some antibiotics commonly used in the treatment of otitis media on some bacterial isolates that were isolated and diagnosed in this study. The results of the current study showed, and as shown in Table (2), *S.aureus* was showed a high sensitivity 100% to Ciprofloxacin(10 µg) and Meropenem (10 µg) while they were resistant to Ampicillin (25 µg) and these results are consistent with the findings (Ali, 2011and Abdullah, 2006) and also showed a high resistance 100% to Cefixime (5 µg), this resistance is due to the possession of enzymes that break down these antibiotics (Shadman, 2010) .

As for *P. aeruginosa*, which was ranked second among all the genera of bacteria that were isolated, this bacterium is considered one of the most resistant to various antibiotics and the least sensitive, such as (Amoxicillin, Ampicillin, Azithromycin) and other antibiotics, and this is agreed with what was found by (Worlitzsch *et al*., 2002), as the cause of resistance is due to *P. aeruginosa* having many mechanisms of resistance to various antibiotics, the most important of which is that its outer membrane contains a special protein called PER-1), which is characterized by a high ability to release various antibiotics to the outside of the bacterial cell wall at the same speed of its entry, which does not make the concentration of the antibiotic inside the bacterial cell sufficient to eliminate it(bacteria), and also the resistance may be attributed to the presence of the resistance plasmid (R-plasmid), which plays an important role in resistance to many antibiotics (Rashid *et al*., 2007). these bacteria showed a high sensitivity to meropenem at a concentration (10 µg) at a rate of 100%, while the resistance was very high at 100% to Ampicillin at a concentration (25 µg), Amoxicillin (30 µg), Azithromycin (15 µg), and also showed 100% high resistance to Gentamicin at a concentration (10 µg), as this study agrees with the findings of the researchers (Asghar and Ahmed, 2018) in Saudi Arabia.

As for *Acinetobacter baumanni comlex*, which showed high resistance to various types of antibiotics, where the resistance ratio was very high, such as Amoxicillin (30 µg), Cefepime (10 µg), Ampicillin (25 µg), Cefixime (5 µg). Azithromycin (15 µg) at 100%. this resistance is due to the rapid acquisition of resistance genes for various types of antibiotics such as penicillins and cephalosporins (Valencia *et al*., 2009), in the same time these bacteria showed a high sensitivity to the antibiotic such as Meropenem (10 µg) at 100% and Ciprofloxacin (10 µg) at 95%. As for *Klebsiella pneumoniae* bacteria showed high resistance to the ampicillin (25 µg) and all isolates of *Klebsiella* showed a high resistance 100% to the antibiotics ampicillin (25 µg), Amoxicillin (25 µg), Cefpime (10 µg) and Cefixime at 100% to the antibiotic, and this result is close to what was reached (Zaidan, 2021and Ahmed *et al*., 2021).. The percentage of its resistance to Ampicillin 25 µg was reached to 96.1. (Dao-Ming *et al*., 2022) indicated that the resistance of *Klebsiella* bacteria to this antibiotic ranges between (96-100%).The reason for resistance is related to bacterial ability to produce beta-lactamase enzymes which breakdown or cleave beta lactam antibiotics like amoxicillin, Ampicillin and cefepime (Hassan and Nizam, 2012).

As for *E.coli*, these bacteria showed high resistance against many types of antibiotics: Ampicillin (25 µg), Amoxicillin (25 µg) and Amoxicillin (30 mg) 100%, which was relatively consistent with what was found (Al-Nuaimi, 2018). While it showed sensitivity to Meropenem (10 µg) at 100% and Ciprofloxacin (10 µg) at 95%. . Resistance to ampicillin (25 µg), Amoxicillin (25 µg), Cefixime (5 µg) and Amoxicillin (30 µg) in a high percentage is 100% , These bacteria showed high sensitivity to ciprofloxacin and meropenem at 100%.

Enterobacter faecalis Their intrinsic resistance to many commonly used antibiotics has been noted as one of the main reasons for their survival in the hospital environment, and perhaps more importantly, their ability to acquire resistance to most available antibiotics, either through mutation or via gene exchange through plasmids (Brooks *et al* .,2004) .It was resistant to ampicillin (25 µg), amoxicillin (30 µg), Cefixime (5 µg), and cefepime (10 µg) with a high rate of 100%(Ben-Ami R *et al* .,2003).

Bacteria Gimella, Cloca enterobacter, Shigella group, Morgan group ssp morganii and Burkholder group cepacia. Were showed 100% sensitive toward meropenem (10 µg) in and ciprofloxacin (10 µg) .

As for the bacterial isolates taken from environmental sources, which included the same environment as those patients infected with the Otitis Media(water) ,*Staphylococcus aureus* produce rapid resistance to many antibiotics and cause various problems as a result of this resistance (Jawets *et al*,.2019). Where this bacteria was highly resistant For many antibiotics, including Ampicillin and Amoxicillin at 100%, sensitive to meropenem at 100% .

Pseudomonas aeruginosa, showed high resistance to ampicillin (25 µg) amoxicillin (30 µg), azithromycin (15 µg), gentamicin (10 mg), cefixime (5 µg), trimethoprim (10 µg) and cefepime (10 µg) this bacterium is one of the most natural and resistant species resulting from the many mutations that assist to develop resistance toward antibiotics. (Muthanna Hassan, 2008) by genetic mutations in chromosomal genes, or by horizontal transfer of antibiotic resistance genes from one cell to another (Yoko *et al*., 2005). *Pseudomonas aeruginosa* is characterized by its low sensitivity and sensitivity to antibiotics, and this advantage is due to pumps located at the level of the cell membrane, which serve to pump many drugs, including antibiotics, out of the cell.

Escherichia coli is also highly resistant100% to many antibiotics, including ampicillin (25 µg), amoxicillin (25 µg), and cefixime (5 µg). Many studies indicate that the increased resistance of *Escherichia coli* to antibiotics may be due to the develope new

mechanisms of antibiotic resistance that assist into resistance to multiple antibiotics, through the responsible genetic material such as resistance enzyme or mutation (Dunn *et al*., 2004; Yoko *et al*., 2005). In addition to this resistance this bacteria showed sensitive to azithromycin (15 µg), meropenem at(10 µg), and ciprofloxacin (10 µg) at a high rate

P. mirabilis showed high resistance to ampicillin, (amoxicillin at concentrations 30 µg and 25 µg), cefepime (10 µg). while it showed 100% sensitive to meropenem (10 µg). Resistance may come as a result of modification of the target sites of the antibiotic. penicillin-binding proteins (PBPs) bind these antibiotic and convert the antibiotic to ineffective form (Nelson *et al*., 2000). It was

salmonella has shown very high resistance, according to several studies(Lamboro *et al*,. 2016), salmonella has become more aggressive and mutates a lot, the stronger type prevails and spreads, which means that it will turn into a bacteria that is very tolerant to many antibiotics used nowadays, so it showed a high resistance of 100% to both ampicillin Amoxicillin 25 µg at a concentration (15 µg) at 100%.

As for the bacterial isolates taken from environmental sources, which included the same environment as those patients infected with the Otitis Media (Soil), Where these bacteria were highly resistant 100%to many antibiotics, including Ampicillin and Cefepime, while they were 100% sensitive to Meropenem and Ciprofloxacin. *S.aureus* show ability to rapidly produce resistance to many antibiotics and causes various problems as a result of this resistance (Jawets *et al*.,2019)

The results of this study indicate the extent of resistance of the bacterium *K.pneumoniae* to antibiotics of various kinds, and this was agreed with different previous studies. (Jones *et al*., 1997), as this bacterium possesses many resistance mechanisms, the most important of which is the production of beta-lactamase enzymes, where studies have proven its possession of many types. Of these enzymes that work to break down a wide range of beta-lactams, they may also have enzymes that break down other antibiotic types (Welle *et al*., 1997). These traits of resistance are often carried on plasmids that are transmissible even to other types of bacteria, and perhaps in this way the resistance to antibiotics increases and spreads among species. , Amoxicillin at 100%, and they were very sensitive to meropenem and Ciprofloxacin at 100%.

E.coli strains have acquired this resistance against antibiotics due to the random and without medical advice to use of these drugs, and this helped bacteria to develop resistance to such antibiotics (Khezerlou *et al*., 2018). Also the isolates of this bacteria showed high resistance to Amoxicillin at a concentration of (25 µg), Ampicillin 25 µg, and Gentamicin (10 µg) at a concentration of 100%. With the resistance of most beta-lactam isolates, the reason for resistance to beta-lactam antigens may be due to the isolates producing beta-lactamase enzymes that break the amide bond in the beta-lactam ring in the antigen, so the antibody becomes ineffective in the form of penicillonic acid in penicillins. It was 100% sensitive to Meropenem and 95% to Ciprofloxacin.

As for *Micrococcus lutes* bacteria, they are also resistant to Ampicillin at a concentration of 25 µg Amoxicillin and a concentration of 25 Cefepime at a concentration of 10 Cefixime at a concentration of 5 mg at a rate of 100%. In the same time it sensitive to Ciprofloxacin (10 µg) by 100%.

In which the presence of antibiotics is a source for the emergence of multi-resistant strains, and this is what is observed in the atmosphere of hospitals, (Hobson *et al*., 1996), and the wrong diagnosis of the pathogen leads to the development of resistance to the antibiotic and thus delayed recovery, and this indicates the importance of preliminary diagnosis of the bacteria causing the infection and knowing the nature of their sensitivity to the antibiotics before starting treatment.

P. vulgaris and P. mirabilis bacteria showed high resistance to Ampicillin at a concentration of (25, mg Amoxicillin, (25 µg Amoxicillin), (25 µg) Cefixime at a concentration 5 µg Cefixime, while it 100% sensitive to meropenem at a concentration (10 µg). 100%..may come Resistance due to modification of the target sites of the antagonist, which are Penicillin-binding proteins (PBPs) that are designated to bind these antagonists, renders the antagonist inactive (Nelson *et al*., 2000).

Through the table, we notice that the types of bacteria taken from patients with otitis media(*Staph .aureus* 28 (30,7)% , *E.coli*, 8 (8,8)% , *Proteus mirabilis* ,6 (6,6)% , *Pseudomonas aeruginosa*, 14 (15.4)% ,and the environment of the infected (water) *Staph .aureus* 8 (30,8)%, *E.coli* 8 (30,8)%, *Proteus mirabilis*, number 2 (7.7)%, *Pseudomonas aeruginosa* 1 (3,8)% ,and(soil) for the same bacterial type *Staph .aureus* 4 (16,0)%, *E.col* and *Pseudomonas aeruginosa* at 5 (20)%, *Proteus mirabilis* 1 (4)%. All the bacterial types showed the same percentage of resistance towards Amoxicillin (25 and 30 µg) at 100% and sensitivity to Meropenem (10 µg) at 100%.

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