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Article

Antibiotic Resistance Patterns of Bacteria Isolated from Otitis Patients

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Abstract: Otitis media is an inflammation of the cavity behind the eardrum. The inflammation may be acute or otitis with effusion, and it may also lead to chronic otitis media, the most important symptoms of which are severe pain, swelling, and redness, and it may lead to hearing loss. Two hundred swabs were taken from otitis media. Swabs obtained from patients were cultured on appropriate media such as McConkey and blood and then incubated at 37°C for 24 hours. After which bacterial characteristics appeared and were identified by Gram stain, light microscopy and biochemical tests, most notably mannitol fermentation, oxidase and catalase. To confirm bacterial species, Enterosystem 18 R and Vitek-2 compact were used. The results of this study showed that the number of bacteria isolated from both sexes amounted to 128 bacterial isolates, most of which were Staphylococcus aureus with 49 isolates. The results also showed that the antibiotic resistance of bacteria was relatively high among them, appeared that the Pseudomonas aerogenosa isolates were the most resistant to ciprofloxacin by a percentage of 63.15 %, while the Staph. aureus bacteria appeared more sensitive to the antibiotic Ciprofloxacin by a percentage of 61.22 %. Staphylococcus aureus isolates were the most sensitive to the antibiotic amoxicillin-clavulanate by 91.83%, while Escherichia coli appeared more resistant to the antibiotic amoxicillin-clavulanate by 85.71%. While the P. aerogenosa appeared resistant by 3% Tetracycline antibiotics, where Strep. pneuminiae was the most sensitive with a percentage of 66.66% while all Enterococus spp. isolats sensitive 100% for Gentamycin Staph. aureus more sensitive 63.26% and P. aerogenosa 57.89%. The results during the study presented that the isolates of *P. seudomonas, Klebsiella pneumoniae, Enterococus spp.* and *E. coli* carried the CTX-M gene.

Keywords: antibiotic resistance, otitis media, CTX-M gene

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1. Introduction

Otitis is a disease of the ear caused by a microbial pathogen that infects the eardrum and has three types acute otitis media, effusion otitis media, and chronic otitis media [1]. Respiratory infections are the most common in patients with otitis media and can lead to hearing loss. Otitis media is also ranked fifth in the world as well as there are data approved by the World Health Organization (WHO) that there are approximately 65-330 million patients per year affected by chronic suppurative otitis media (CSOM) and 50% of them suffer from hearing loss [2,3]. The pathogens of ear infections are usually bacteria and viruses, and the most important bacteria causing the disease are *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, and Escherichia coli*, as these bacterial species are among the most common bacteria causing infections [4].

A lot of significant changes have occurred recently. There has been a correlation between a decrease in the occurrence of amoxicillin resistance and the limitation of treatment indication for specific patients [5] the accurate microbiological identification of infections and the assessment of their susceptibility to antibiotics Profile is crucial for continuing monitoring, diagnosing, and managing antibiotic use [6]. There are limitations to the choice of antibiotics used for treatment against bacteria because they possess ESBL enzyme which gives bacteria more resistance against antibiotics [7]. In cases where treatment fails, the antimicrobial agents are identified, especially those strains that have complications, and the sensitivity results of the antibiotic are started as empirical antibiotic therapy. Also, taking antibiotics repeatedly leads to the occurrence of resistant strains of bacteria [8]. The ability of bacteria to produce biofilms in many pathological cases increases the resistance of the pathogen. Therefore, biofilms provide protection against any threat to the microorganism. Also, the bacteria that form biofilms possess a genetic system that increases their resistance [9,10]. Aim of the study is know the bacterial species causing otitis, their resistance to commonly used antibiotics and the ability of these bacterial isolates to β-lactamases producing.

2. Materials and Methods

Work plane and sampling

The study included collecting 200 ear swab samples from patients with otitis in Dhi-Qar province, where the study period was from February to May 2024.

Microbial culture

The swabs were taken from otitis media, were cultured on MacConkey agar and blood agar using a streaking technique and incubated at 37°C for 24 hours. Bacterial isolates were identified by appearance, Gram stain, light microscopy and biochemical tests, the most important of which are catalase, oxidase, mannitol fermentation and hemolysis. Enterosystem 18 R and Vitek-2 compact were used to confirm bacterial isolates.

Antibiotic susceptibility testing

Using Kirby-Bauer and Vitec-2 disk diffusion, we performed antibiotic susceptibility testing procedures where the most important antibiotics were used such as ciprofloxacin, amoxicillin-clavulanate, tetracycline and ceftriaxone, which are among the most important antibiotics used to treat ear infections. In order to determine the effectiveness of the antibiotics, the test was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [11].

Molecular analysis

Using primers specific to the Enterobacteriaceae family, PCR was performed. CTX-M, gene production was performed according to of the Genomic DNA Mini-Kit Geneaid, and then DNA was extracted. The final volume of the PCR reaction was 20 µl, which included 20 µl of the mixture, and 2 µl of the template for the isolate, and 1 µl of primers. In order to measure the reaction conditions, we used a thermal cycler (A&B Singapore). Specific primer sequences F: CGCTTTGCGATGTGCAG, R: ACCGCGATATCGTTGGT (550 bp product size) were used in PCR experiments as for CTX-M was 20 µl [12].

Statistical analysis

Statistical examination was used by Excel 2016 workbook to find out the presence of statistical differences or variation, and a probability p-value below 0.01 is statistically significant.

3. Results and Discussion

The study was designed on the basis of 200 patients, 126 males and 74 females, with otitis media. The patients' ages ranged from 1 to 80 years and were divided into four age groups as shown in Table 1.

Table 1. Shows age and gender groups of patients with otitis media

Age groups	Female	%	Male	%	Total	Grand %
1-21	37	48.68	46	36.50	83	**41.5**
22-41	23	30.26	39	30.95	62	**31**
42-61	12	15.78	35	27.77	47	**23.5**
62-80	2	2.70	6	4.76	8	**4**
Grand total	74		126		200	**100**

X^2 (df=3, N=200) = 5.1912, p<0.001

The study showed that the age group 1-21 years was the group with the most patients suffering from ear infection. This study also showed that there were not significant statistical differences between the different age groups and genders. The presence of bacterial isolates according to age group, where the highest age group infected with the largest number of pathogens was 42-61, where the number of bacteria was 46, most of which were *staph. auareus* bacteria, while the group 62-80 had the lowest number of 17 isolates, as show in Table 2. The analysis of distribution of bacteria across different age groups statistically not significant differences, particularly in the younger age groups (1-21 and 22-41 years) with *p<0.001*.

Table 2. Distribution of bacterial isolates by age group

Age Group (Years)	*Staph. aureus*	*E. coli*	*K. pneumoniae*	*H. influenza*	*Enterococus spp.*	*P. aeruginosa*	*S. pneumoniae*	Total grand
1-21	10	5	3	5	0	3	1	**27**
22-41	15	8	1	8	0	6	0	**38**
42-61	20	6	3	7	1	7	2	**46**
62-80	4	2	2	2	1	3	3	**17**
Total	**49**	**21**	**9**	**22**	**2**	**19**	**6**	**128**

X^2 (df=3, N=128) = 5.2999, p<0.001

The number of samples during this study reached is 200 for both genders and isolated bacteria was 128 bacterial isolates. *Staphylococcus aureus* with 49 isolates, then *Escherichia coli* 21 isolates, as well as H. influenza 22, then *Klebsiella Pneumoniae*, and *Enterococus* *spp.* isolates accounting 9 isolates and 2 isolates for These results were in line with the results obtained by [13].

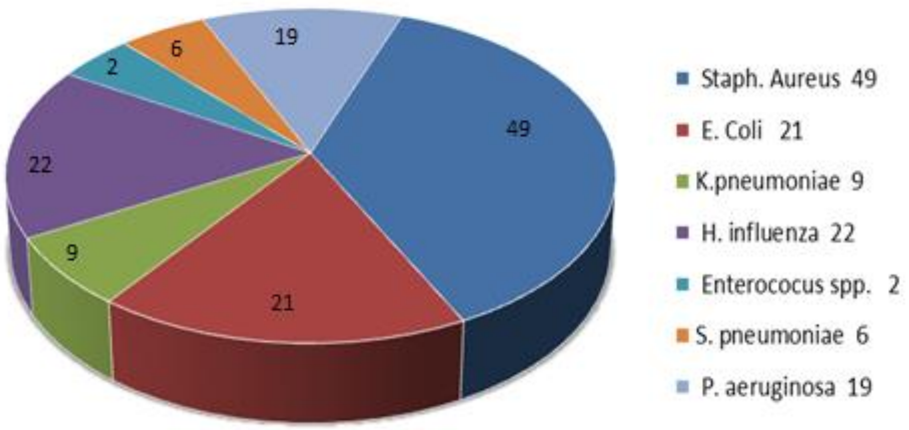

Figure 1. Percentages of bacterial species isolated from patients

According to the results obtained, it was clear that most of the isolates appeared resistant to different types of antibiotics, as it appeared that the *Pseudomonas aerogenosa* isolates were the most resistant to ciprofloxacin by a percentage of 63.15 %, while the *Staph. aureus* bacteria appeared more sensitive to the antibiotic Ciprofloxacin by a percentage of 61.22 % (Fig 2). This result was close to the results obtained by [14].

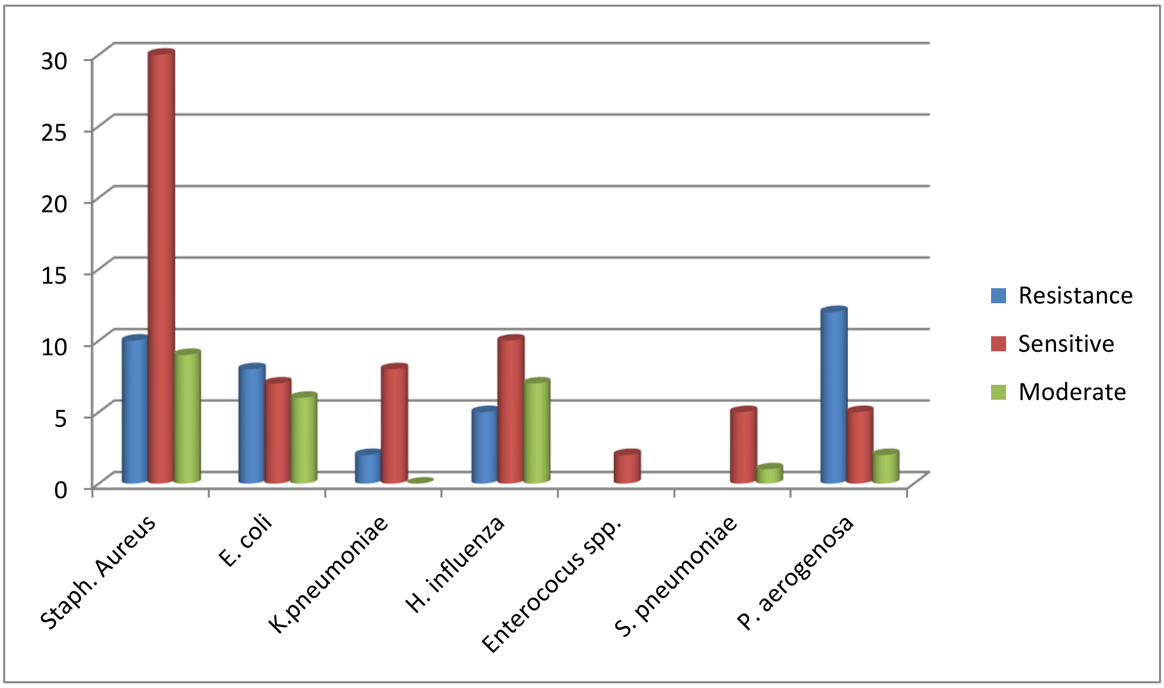

Figure 2. Resistance patterns to the antibiotic ciprofloxacin of bacterial isolates

The study showed that most of the isolates appeared more sensitive to the antibiotic amoxicillin-clavulanate, as it appeared that Staphylococcus aureus isolates were the most sensitive to the antibiotic amoxicillin-clavulanate by 91.83%, while Escherichia coli appeared more resistant to the antibiotic amoxicillin-clavulanate by 85.71%. While the *P. aerogenosa* appeared resistant by 3% and all Enterococus spp. appeared sensitive by 100% to the antibiotic amoxicillin-clavulanate (Fig 3). This result was agreement with results reported by [15].

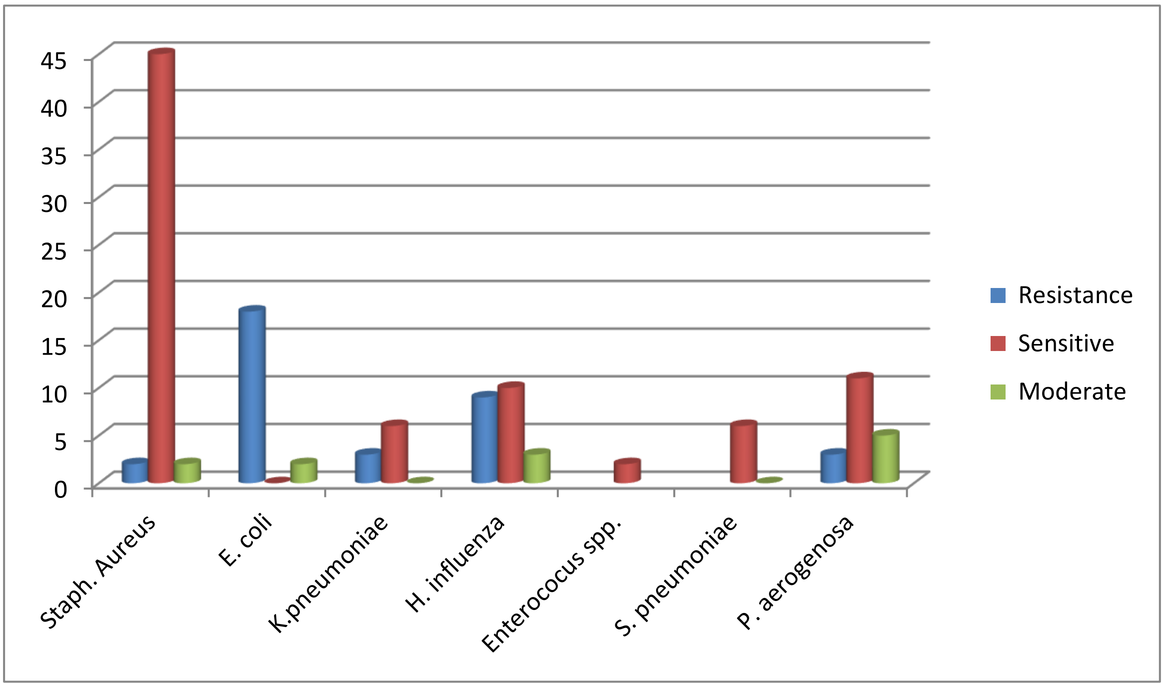

Figure 3: Resistance patterns to the antibiotic amoxicillin-clavulanate of bacterial isolates

Tetracycline antibiotics, where Strep. pneuminiae was the most sensitive with a percentage of 66.66%, which is close to the results in [16], where it appeared that Staphylococcus aureus isolates were the most sensitive to the antibiotic tetracycline with a percentage of 59.18%, while P. aerogenosa isolates appeared resistant with a percentage of 73.68 % and Klebsiella pneumonia bacteria appeared resistant with a percentage of 77.77 to the antibiotic tetracycline (Figure 4). This agreed with results of [17]. Gentamycin antibiotic where Staph. aureus more resistance 48.97% and P. aerogenosa 52.63 % while all Enterococus spp. isolates sensitive 100% for Gentamycin antibiotic (Fig 5) and this results in line with result by [18].

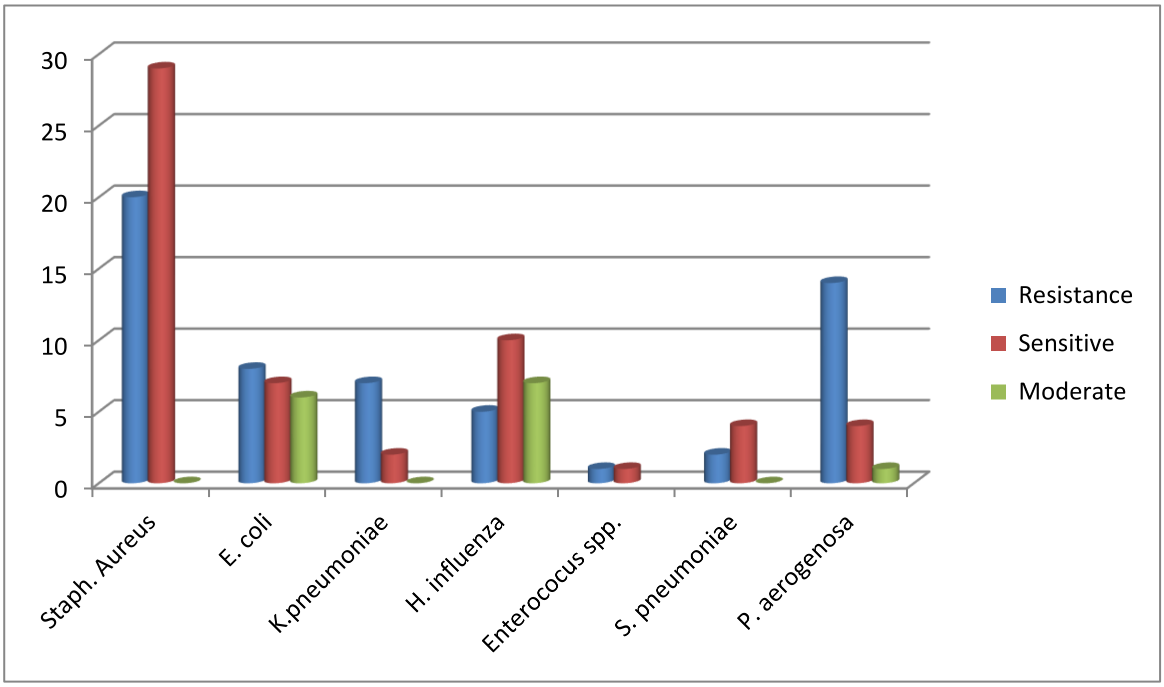

Figure 4. Resistance patterns to the antibiotic Tetracycline of bacterial isolates

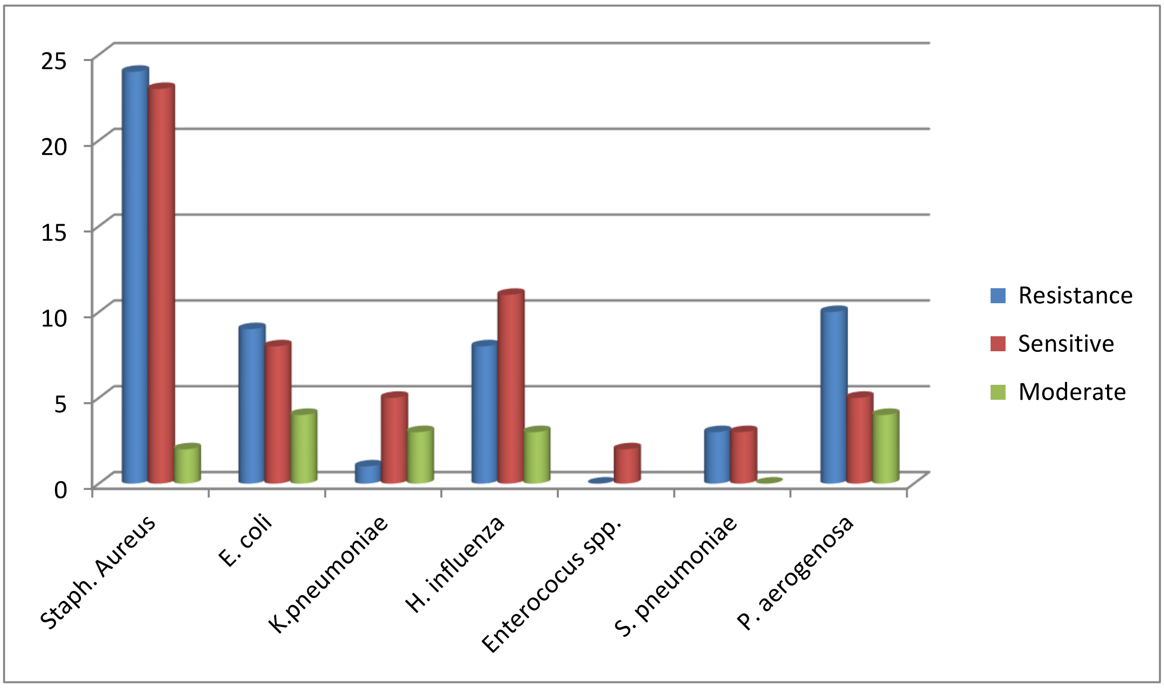

Figure 5. Resistance patterns to the antibiotic Gentamycin of bacterial isolates

Ceftriaxone antibiotic where Staph. aureus more sensitive 63.26% and P. aerogenosa 57.89% this result close with result by [19]. As for the rest of the isolates, there was a clear difference in resistance to ceftriaxone antibiotic, as shown in the (Fig 6).

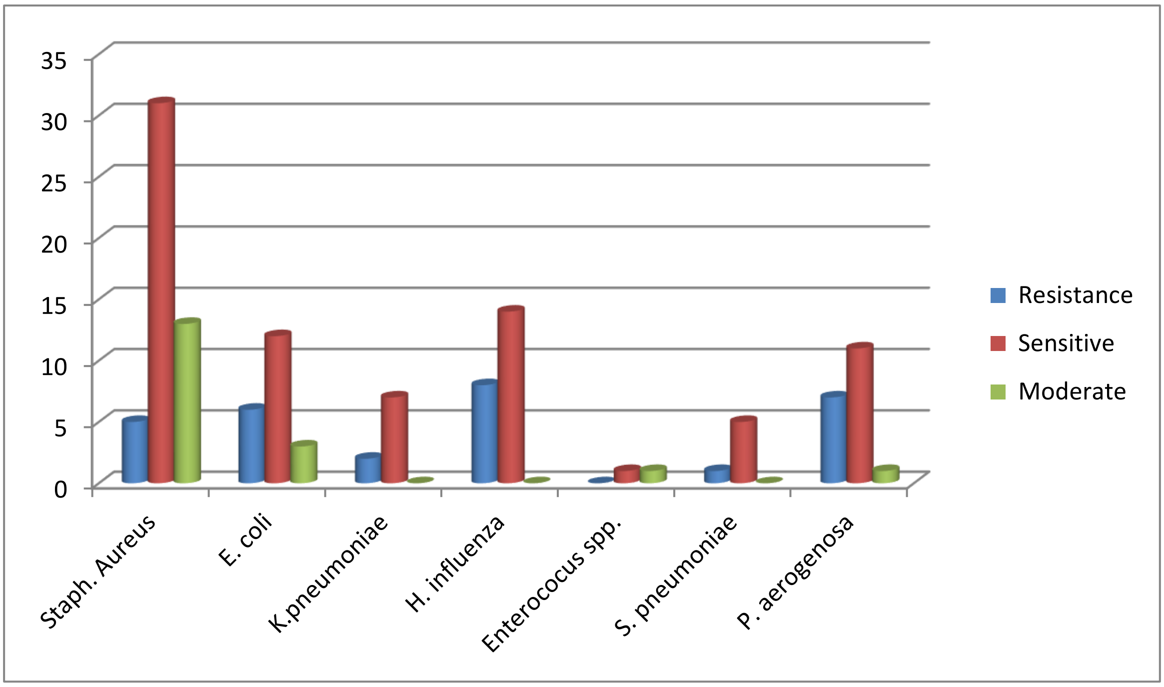

Figure 6. Resistance patterns to the antibiotic ceftriaxone of bacterial isolates

Antibiotics also have different effects on the bacterial cell, some of them work to change the permeability, some of them destroy the cell wall of the cell, and others denature the wall proteins, and there are antibiotics that work to denature the DNA as well as the cell enzymes [20]. The relevance of regional surveillance studies in antibiotic prescriptions has been highlighted by the reporting of geographic disparities in antimicrobial susceptibility patterns [21]. Antibiotics used for otitis media Data are limited, with some studies suggesting that staph resistance to penicillin increased in the post-vaccination period, particularly in Greece [22]. The resistance was even more pronounced in the age group 42-61 years ($P < 0.01$), indicating a potential age-related factor in resistance. For the older age group (62-80 years), the differences were not statistically significant ($P > 0.05$), which may suggest other influencing factors.

The CTX-M gene was identified because it is associated with giving the organism greater resistance to ESBLs. The results during the study showed that the isolates of *P. seudomonas, Klebsiella pneumoniae, Enterococus spp.* and *E. coli* carried the CTX-M gene, as it appeared that all isolates carried the CTX-M gene (Fig. 7, 8) of this study, which was close to the results recorded by [23,24].

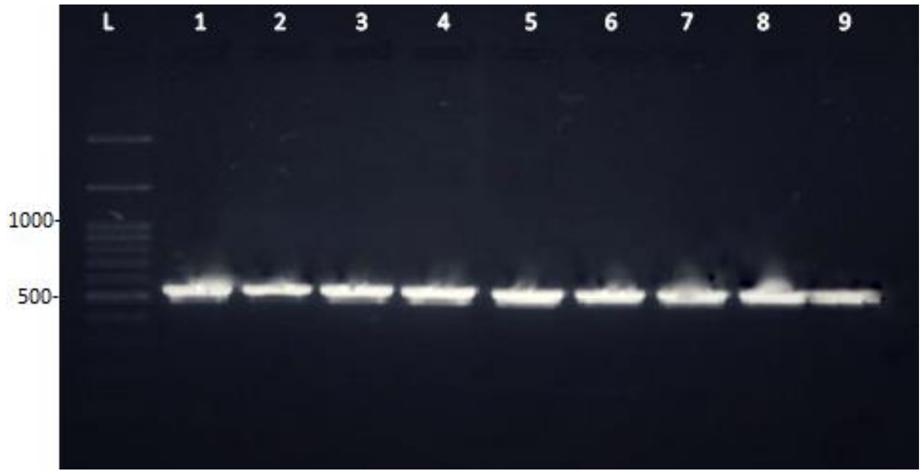

Figure 7. Gel electrophoresis of PCR CTX-M gene (550 bp.) of K. *pneumoniae* Lane L. DNA marker (3000 bp). 1-9 result positive

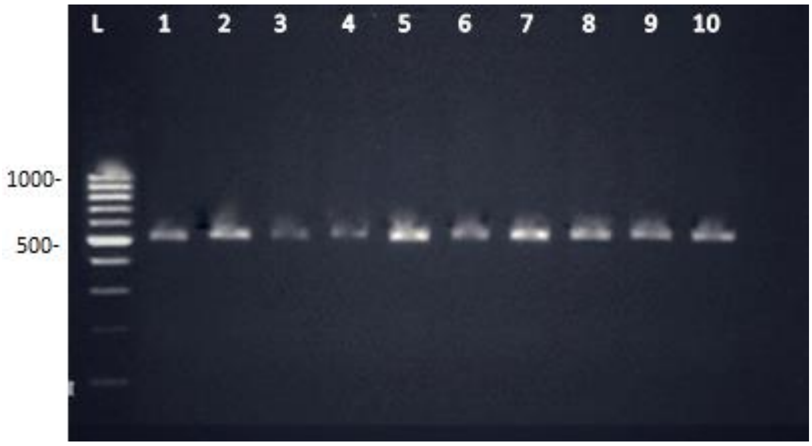

Figure 8. Gel electrophoresis of PCR CTX-M gene (550 bp.) of *P. aeruginosa* Lane L. DNA marker (3000 bp). 1-10 result positive

4. Conclusion

The present study showed that *Staphylococcus aureus* was the most prevalent bacteria among the patients, followed by *Pseudomonas aeruginosa* and K. Pneumoniae. The best antibiotics that were effective against *Staphylococcus aureus* diagnosed with Otitis were ciprofloxacin and gentamicin. The results of the antibiotic analysis from the present study showed that almost all *Staphylococcus aureus* were drug resistant to at least one antibiotic. During this study, it was found that the most prevalent pathogen was *Staphylococcus aureus* bacteria, followed by *Enterococus spp*. there was also a variation in resistance and sensitivity to antibiotics, as *Staphylococcus aureus* were more resistant to tetracycline antibiotics, while *P. aerogenosa* were more resistant to tetracycline antibiotics compared to other isolates. The study also showed that all the bacteria of the Enterobacteriaceae family were carriers of the genes, which gives the bacteria more resistance to antibiotics.

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