RESEARCH ARTICLE

Antibiotic Resistance Patterns and Horizontal Gene Transferring of some Bacterial Species isolated from Tonsillitis in Babylon Province

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ABSTRACT

Tonsillitis or throat infection is one of the most frequent health problems worldwide. In the current study, 100 tonsil swabs were collected from patients, who were suffering from tonsillitis and tonsillectomy and 10 control, who did not take any drugs for both genera (male and female), with age ranging from 3-82 years admitted in Al-Hilla Teaching Hospital and Babylon Hospital for birth and children and people, who are suffering from tonsillitis and living in Hilla during a period from December 2018 to May 2019. The total numbers of gram-positive (G+ve) bacteria were 75 (71.5%), while the number of gram-negative (G-ve) was 30 (28.5%). Antibiotics susceptibility was tested, and results were showed significant differences between antibiotics at ($p < 0.05$). Antibiotic resistance genes (ARGs), in addition associated genetic elements, were distinguished by polymerase chain reaction (PCR) test with definite primers. Conjugation was involved gene transfer, where the PCR test was exhibited only the successful transferring of *blaTEM1A* gene beginning *Klebsiella pneumoniae* to standard strain (*Escherichia coli* Hb101) with the range of conjugation frequency was $2.5 \times 10^{-3} – 1.59 \times 10^{-4}$, while the rest bacteria reveal negative results. In relevant, we observed by transformation, the successful transferring of *erm B* gene from bacteria *Staphylococcus aureus* to *Streptococcus pyogenes*, *tet M* gene from bacteria *S. pyogenes* to *S. aureus* and *blaTEM1A* gene from bacteria *Pseudomonas. aeruginosea* to *K. pneumoniae,* except *blaIMP-1* gene was not transferred from *K. pneumoniae* to *P. aeruginosea*, where the results reveal the transformation frequency of bacteria *S. aureus* was 1.036×10-1, *S. pyogenes* was 1.7, *P. aeruginosea* was 1.43 and *K. pneumoniae* was 2.25×10^{-1}, respectively.

Keywords: Antibiotics susceptibility, Conjugation, Tonsillar inflammation, Transformation.

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INTRODUCTION

Tonsillitis is an infection of the tonsils, characteristically of speedy beginning. It is a kind of pharyngitis. Indications may consist of painful throat, fever, broadening of the tonsils, misfortune swallowing, and great lymph nodes around the neck. Complications contain peritonsillar inflammation.[1] The bacteria, as main causes of tonsillitis, some viruses, and infectious mononucleosis, can be possible causes. Identification of tonsillitis is medical and/or laboratory, though sometimes, it may be challenging to differentiate viral from bacterial infections. As more tests that are accurate take a longer time to deliver the results, speedy antigen testing with an identical low sensitivity is repeating used in the identification of bacterial tonsillitis. Additional sources consist of infectious mononucleosis as of Epstein Barr virus (EBV) infection, cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis A, rubella, and toxoplasmosis.[2]

A symptom is generally caused by a viral or bacterial infection. Respiratory tract infections institute 30–60% of pediatric outpatient attendance, besides 20 to 30% of pediatric hospitalization. Acute tonsillitis and/or pharyngitis are the most important source of upper respiratory infection, which is regularly secondary to viruses and bacteria. The most communal and essential causative agent of bacterial tonsillopharyngitis is *S. pyogenes*, which is selected as group A streptococcal GAS disease, and can create primary and/or late complications, like acute rheumatic fever and post streptococci glomerulonephritis.[3]

Horizontal gene transfer (HGT) efforts the progression of bacteria. Transfer of ARGs shows an essential role in the progress of multidrug resistance (MDR) in bacteria.[4] Present are three “typical” methods of DNA transfer in natural surroundings: bacterial conjugation, natural transformation, and transduction.[5]

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MATERIALS AND METHODS

Samples Collection

One hundred tonsil swabs samples were taken from patients whose pain from tonsillitis and tonsillectomy between 3 and 82 years old attended to be the Al-Hilla Teaching Hospital and Hospital Babylon for Birth and Children and from people suffering from tonsillitis and living in Al-Hilla province from December 2018 to May 2019. Details from a patient parent were possessed, including age and gender. Typical biochemical tests were utilized for the identity of the causative pathogens.

Identification of Bacteria

A colony can show from each one positive culture of bacteria, and it can identify by depending on the morphology properties (colony magnitude, shape, dye, and nature of colors, transparency, edge, raise, and consistency). Then, colonies may stain by gram stain to notice a definite shape, kind of reaction, aggregation, and particular intracellular composites in accordance with WC Winn.[6]

Antibiotic Sensitivity Test

The inoculums utilized in this experiment were prepared by addition 3 to 5 isolated colonies grown-up on a nutrient agar plate to 5 mL. of sterile normal saline and associated with (1.5 × 108 cell/mL) MacFarland standard tube. With sterile swab, the sensitivity Muller Hinton medium was inoculated by rotational the swab above the surface of medium. Formerly using sterile forceps, the antimicrobial discs were located on the inoculum and plates were incubated for 24 hours at 37ºC, by using the disk diffusion method as mentioned by Clinical and Laboratory Standards Institute (CLSI).[7] Then, the zones of inhibition may measure to identify the sensitivity design. Antibiotic sensitivity determined in concerning with the inhibition zones were measured to estimate the sensitivity design.

PCR Detection of Antibiotic-Resistant Genes

The PCR was performed for the detection of resistance gene, according to Sambrook and Russel, (2001).8 The primers were provided from Geneaid company/ Korea as shown in Table 1.

Molecular Test

PCR is a forceful technique towards selective amplification of a specific segment of DNA *in vitro*.[9] The PCR technique was used in this study to confirm the presence of genes, and PCR contains three steps denaturation of dsDNA temperature at 94ºC for 30 seconds, then annealing of primers at 45ºC for 30 seconds also extension of dsDNA molecules at 72ºC for 1 second. These steps are repeated for 40 cycles and use of primers such as tetM to bacteria *S. aureus*.[10] In *S. pyogenes*, denaturation temperature at 94ºC for 30 seconds then annealing at 55ºC 30 seconds also extension at 72ºC for 30 seconds, these steps are repeated for 30 cycles, using of primer *erm B gene* to bacteria *S. pyogenes*,[11] while *P. aeruginosea* PCR includes denaturation temperature at 93ºC for 1-minute, annealing at 61.8ºC for 1-minute and extension at 65ºC for 1-minute, these steps are repeated for 30 cycles, using of primer *blaIMP-1* to bacteria *P. aeruginosea*.[12] Finally, in *K. pneumoniae* denaturation temperature at 94ºC for 1-minute, annealing at 54ºC for 1-minute and extension at 72ºC for 1-minute, these steps are repeating 30 cycles, using of primer *blaTEM1A* to bacteria *K. pneumoniae* and improve their transferring by conjugation as described by J.H. Miller, *et al*.,[14] and transformation process.[8]

Table 1: Primer pairs

Primer name	*Sequence 5-3´*	*Reference*
tetM-F	AGTGGAGCGATTACAGAA	10
tetM-R	CATATGTCCTGGCGTGTCTA	
ermB-F	TGGTATTCCAAATGCGTAATG	11
ermB-R	CTGTGGTATGGCGGTAAGT	
blaIMP-1-F	ACCGCAGCAGAGTCTTTGCC	12
blaIMP-1-R	ACAACCAGTTTGCCTTACC	
blaTEM1A–F	ATGAGTATTCAACATTTCCG	13
blaTEM1B–R	CTGACAGTTACCAATGCTTA	

Statistical Analysis

Data can analyze by utilizing SPSS version 16 and Microsoft Office Excel 2007. Normal data were stated as number and percent. Fischers exact test was utilized for compare of frequency. A p value of less than 0.05 was measured significant.

RESULTS AND DISCUSSION

Microbiological Characterization

One hundred patients suffered from tonsillitis and ten considered as control, were included in this study. The results of this study appeared infections were highly in age groups less than 10 years compared with other age groups, as shown in Figure 1. Results showed that the percentage in age 1–30 years was high, while the less percentage of patients with age 61–90 years. The results of the current study were agreed through Al-Aawadi, *et al*., (2014),[15] who recorded that the age group of < 10 years was the highest infection rate, compared with other age groups, but the result of this study were disagreed with Agrawal *et al*., (2014).[16] That's recorded the age group of 11–20 years was the most affected with tonsillitis and recorded 57 (40.72%) cases.

In the current study, the percentage of males was 53%, while the percentage of females in this study 47%. The ratio male:female 1.3:1 as shown in Figure 2. These results were agreed with a study obtained by Klug, 2014,[17] while this study did not agree with a study obtained by Farooqi *et al*. 2017,[18] who showed tonsillitis was more common among female children as compared to male children. This might be due to differences in population groups.

Microbiological tests results showed that *S. pyogenes* was the first most common of bacteria that isolated from tonsillitis with percentage 42.9% while, the second one was *S. aureus* with percentage 28.5%, *K. pneumoniae* was composed of 19%, and *P. aeruginosa* was 9.5% which the less percentage compare with others as shown in Figure 3. In addition, the

present study agrees with another local study in Kirkuk city in their study where found that *Streptococcus* with percentage (36.73%) while, *Staphylococcus* (30.6%).[19] And also agreed with other studies in Mosul city where their study found that *Streptococcus* (81.53%), while *Staphylococcus* (44.6%).[20]

Antibiotics Susceptibility Characterization

Antibiotics susceptibility were tested and the results showed significant differences at ($p < 0.05$) between the various antibiotics as following: All isolates of *S. pyogenes* were completely resistant (100%) to penicillin. Whereas 80% of isolates were resistant to cefotaxime, 70% of isolates were resistant to erythromycin, 40% of isolates were resistant to tetracycline, 30% of isolates were resistant to vancomycin, 20% of isolates were resistant to azithromycin and 10% of isolates were resistance to chloramphenicol as shown in Figure 4, the results were not agreed with another study while *S. pyogenes* was completely resistant 100% to vancomycin, penicillin 98%, and tetracycline (53%).[21]

Furthermore, *S. aureus* was completely resistant (100%) to penicillin and cefotaxime, whereas 80% of isolates were resistant to azithromycin, 60% of isolates were resistant to tetracycline, and 20% of isolates were resistant to vancomycin, and 10% of isolates were resistant to tobramycin as shown in Figure 5. The present study was not agreeing with another study, while *S. aureus* was resistant to vancomycin (55%), azithromycin (92%), and penicillin (21%),[21] and another study in India, while *S. aureus* was resistance to tetracycline (81.25%).[16]

In relevant, the result of our study shown that *K. pneumoniae* were completely resistance (100%) to piperacillin, whereas 60% of isolates were resistance to cefoxitin and amoxycillin, 50% of isolates were resistant to tetracycline, 40% of isolates were resistant to ampicillin, and 30% of isolates were resistance to trimethoprim 20% of isolates were resistant to aztreonam and 10% of isolates were resistance to chloramphenicol and gentamycin, as shown in Figure 6. The current study who not in agreement of the study by Salih *et al*., (2016),[22] was reported *K. pneumoniae* was completely resistant 100% to cefoxitin, 77.35% to piperacillin, 87.73% to tetracycline, 75% to ampicillin, and 9.43% to amoxicillin.

Finally, *P. aeruginosea* were completely resistant (100%) to chloramphenicol, cefoxitin, ampicillin, piperacillin, and trimethoprim. 60% of isolates were resistant to tetracycline, 40% of isolates were resistant to gentamycin, 30% of isolates were resistance to imipenem and amoxycillin and 20% of isolates were resistance to Aztreonam as shown in Figure 7. In our study, the antimicrobial susceptibility results were agreed with results presented by Bakir and Ali, (2015).[23] Also, from Erbil-Iraq reported that *P. aeruginosea* was (100%) resistance to ampicillin and 27.2% to imipenem. The present study was agreed with another study in Libya by Eldeeb *et al*., 2006,[24] where, 100% resistance to ampicillin but disagreement with our study, the higher sensitivity of 94.28% with *Gentamicin* and lower sensitivity 17.14% with tetracycline and 11.43% with *chloramphenicol*.

Gene Transfer Identification

Five isolates from each bacterial species were chosen for the molecular transferring test. In PCR assay to *blaTEM1A* gene (867 bp), lane 1-5 showed *blaTEM1A* gene in *K. pneumoniae* isolates, lane 6 showed *blaTEM1A* gene in *K. pneumoniae* isolate (donor cell), lane 7 represented to the presence of *blaTEM1A* gene in *E. coli* Hb101 (recipient cell) which transferring from *K. pneumoniae* toward *E. coli* Hb101 during conjugation process as shown in Figure 8. In transformation, we observed that PCR assay confirmed the presence of ermB gene (587 bp) in *S. pyogenes* as shown in lane 5, whereas lane 6 showed *erm B* gene was transferring from *S. aureus* to *S. pyogenes* as shown in Figure 9. As well as, the tetM gene 158bp, whereas lane 1-5 showed tet M gene was represented in *S. aureus* isolated, lane 6 showed tet M gene was transferring from *S. pyogenes* to *S. aureus* as shown in Figures 10. Finally, Figure 11 showed that the PCR assay to the *blaIMP-1* gene (745 bp), where lane 1-5 showed *blaIMP-1* gene was presented in P. aeruginosa isolates, lane 6 referred to that *blaIMP-1* gene failed for transferring from *K. pneumoniae* to *P. aeruginosea.*

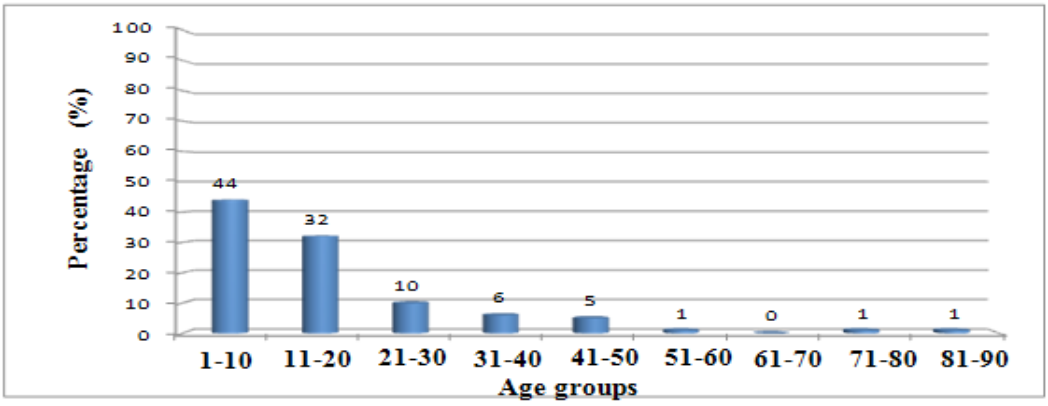

Figure 1: Distribution of tonsillitis patients according to age.

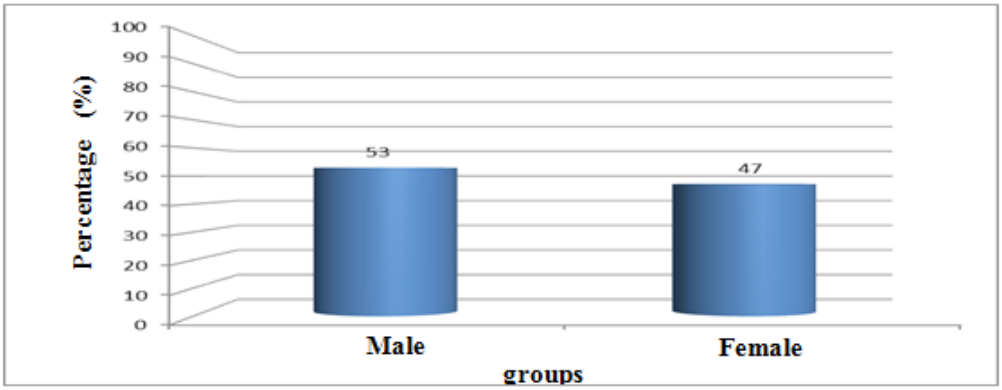

Figure 2: Distribution of tonsillitis patients according to gender

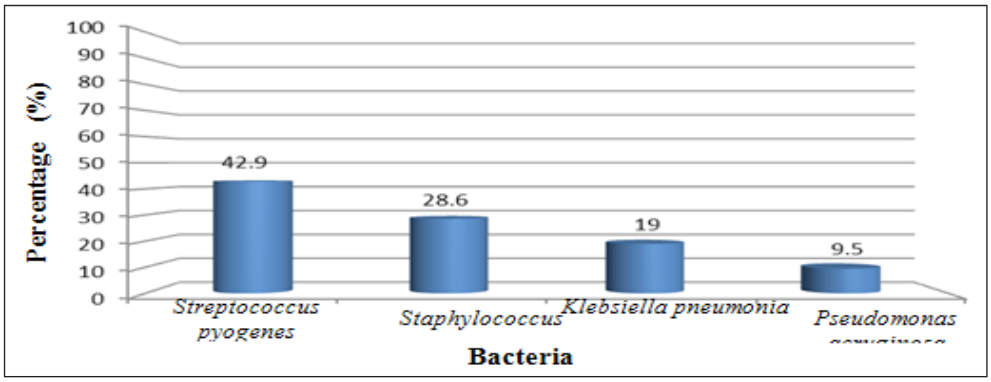

Figure 3: Distribution of tonsillitis patients according to age

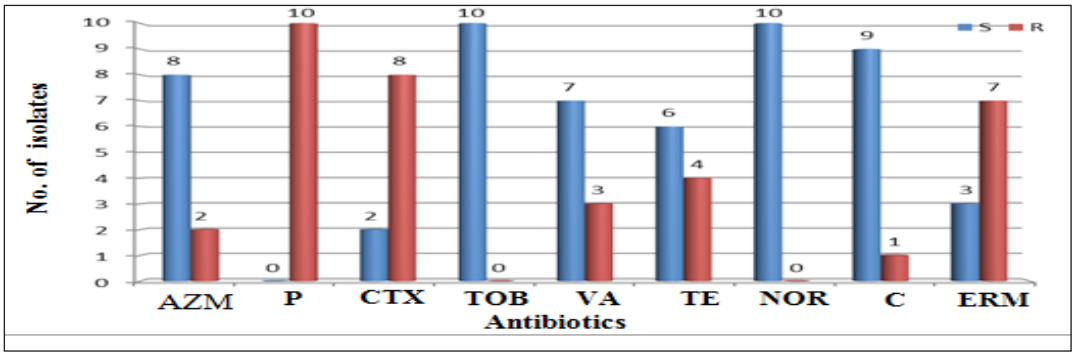

Figure 4: Antibiotics sensitivity of bateria *Streptococcus* pyogenes

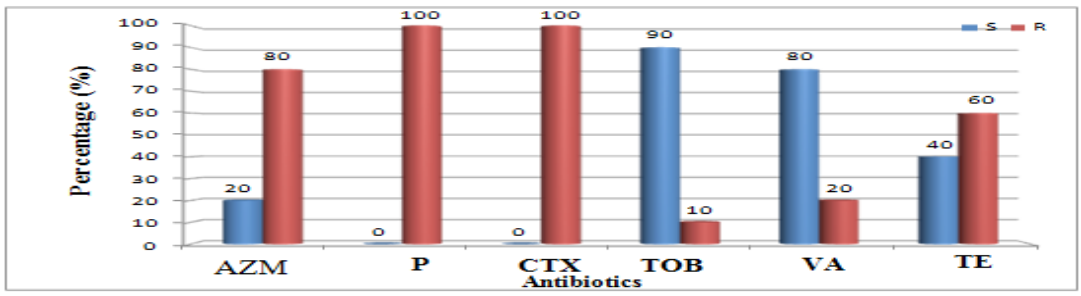

Figure 5: Antibiotics sensitivity of bacteria *Staphylococcus* aureus to some antibiotics

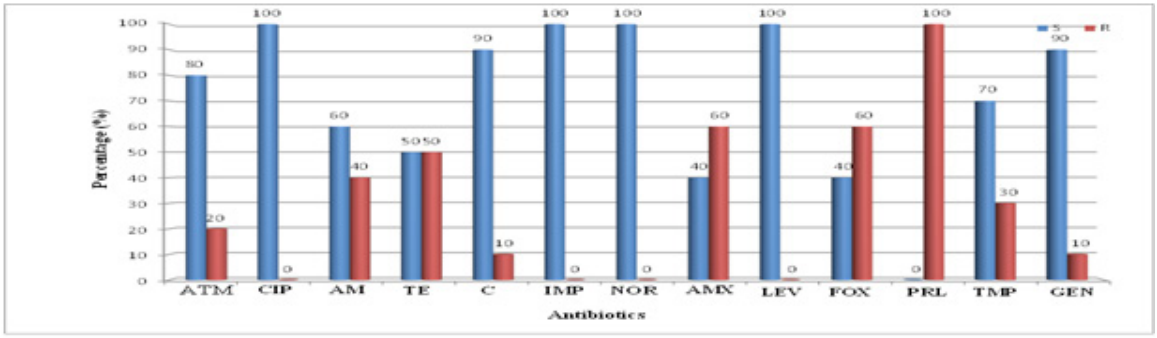

Figure 6: Antibiotics sensitivity of bacteria *Klebsiella pneumonlae* to some antibiotics

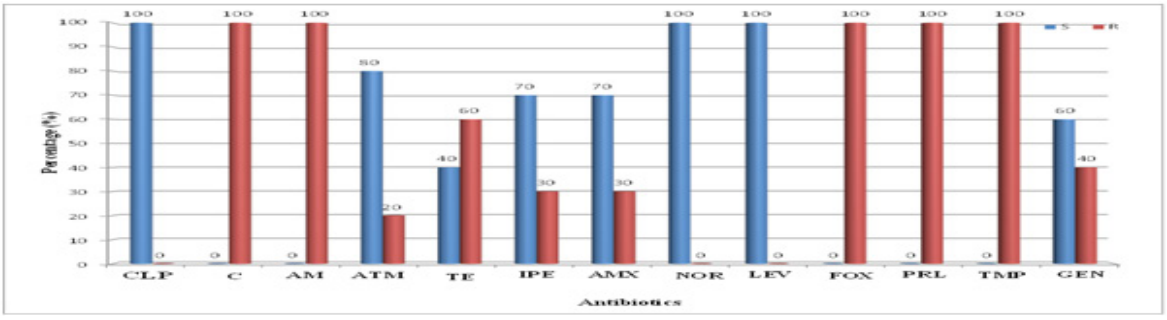

Figure 7: Antibiotics sensitivity of bacteria *Psedomonas aeruginasea* to some antibiotics

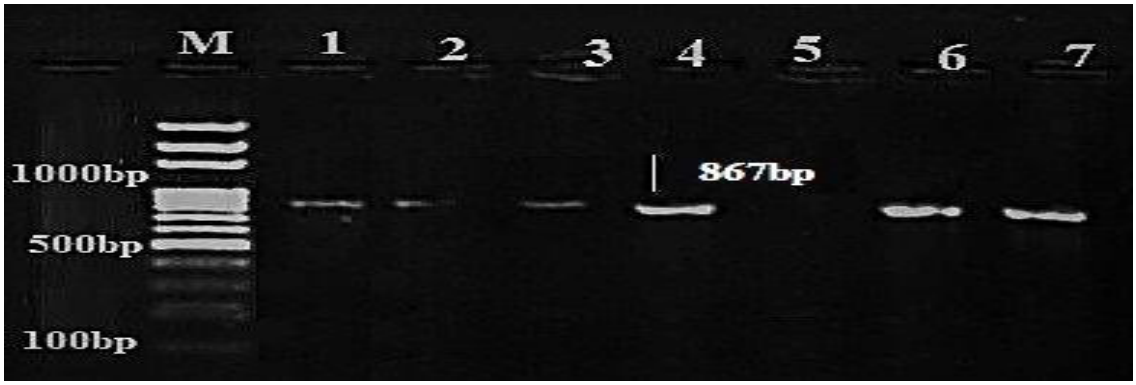

Figure 1-8: Agarose gel electrophoresis (1.5%) at 72 volts for 80 minutes of PCR product of *blaTEM1A* gene (867 bp), lane 1-5 showed the presence of *blaTEM1A* gene in *K. pneumoniae* isolate; lane 6 showed *blaTEM1A* gene in *K. pneumoniae* isolate (donor cell); lane 7 showed presence of *blaTEM1A* gene in *E. coli* Hb101 (recipient cell), which transfer to *E. coli* Hb101 during conjugation process; and lane M [DNA marker size (100 bp)]

The conjugation frequencies (the total number of transconjugants, which divided via the number of recipient cells, where the number of transconjugant cells to bacteria *E. coli* Hb101 was 60 × 102 and the number of recipients cells was 24 × 105 then the conjugation frequency was 2.5×10^{-3} and the transformant cells to *K. pneumoniae* was 27 × 102 and the number of recipient cells was 17 × 106 then the conjugation frequency was 1.59×10^{-4}. The range of conjugation frequency was ($2.5 \times 10^{-3} – 1.59 \times 10^{-4}$) as shown in Table 2.

This study agrees with another study, whereas transfer of wide-ranging- host- range plasmids take place at a changeable frequency (normally in the range from 10-3 to 10-6) dependent on the plasmid and coupling- pair genotype, then mating requirements cocultivation of donor and recipient cells on a solid superficial.[25]

Our results were exhibited the transformant cell of bacteria *S. aureus* was 5.7 × 106, *S. pyogenes* was 4 × 107, *P. aeruginosea* was 4 × 107, and *K. pneumoniae* was 4.5 × 106. In *P. aeruginosa* the *blaIMP-1* gene was not transfer whereas, from successfully even afterward repeating the transformation process numerous times, this might sometimes be due to the huge size of the plasmid may need to noticeable to the breaking it during preparation. Also, the breakage of DNA could be considered as a motivation for the failure of the transformation of this isolated. We also observed the difference in the values of the transformation

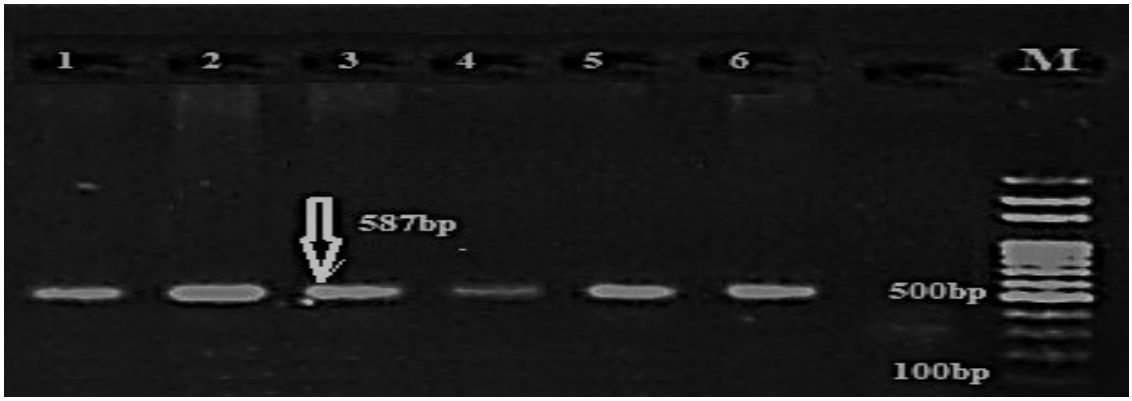

Figure 9: Agarose gel electrophoresis (1.5%) at 72 volt for 80 minutes of PCR to *ermB* gene (587 bp); lane 1-5 showed the presence of *ermB* gene in *S. pyogenes* isolate; lane 6 showed *erm B* gene, which transferred into *S. pyogenes;* lane M [DNA marker size (100bp)]

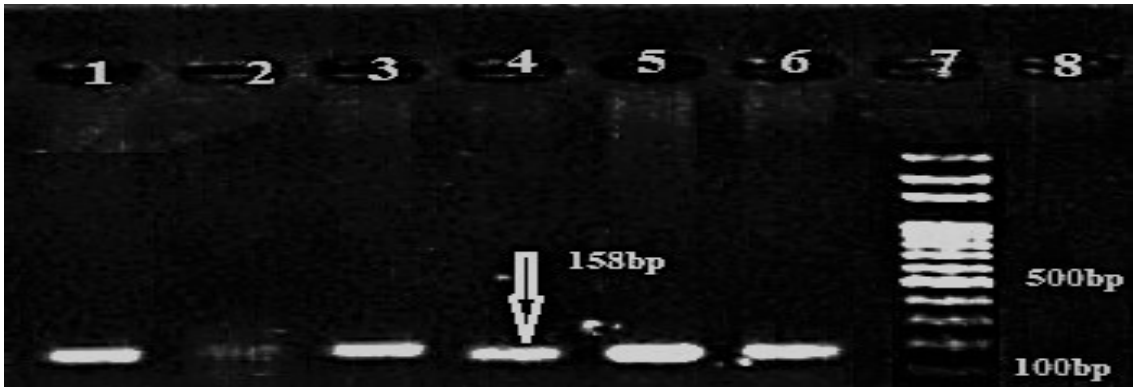

Figure 10: Agarose gel electrophoresis (1.5%) at 72 volt for 80 minutes of PCR to *tetM* gene (18 bp); lane 1-5 showed the presence of *tetM gene* in *S. aureus* isolate; lane 6 showed to *tetM* gene was transferred into *S. aureus* and lane M [DNA marker size (100bp)].

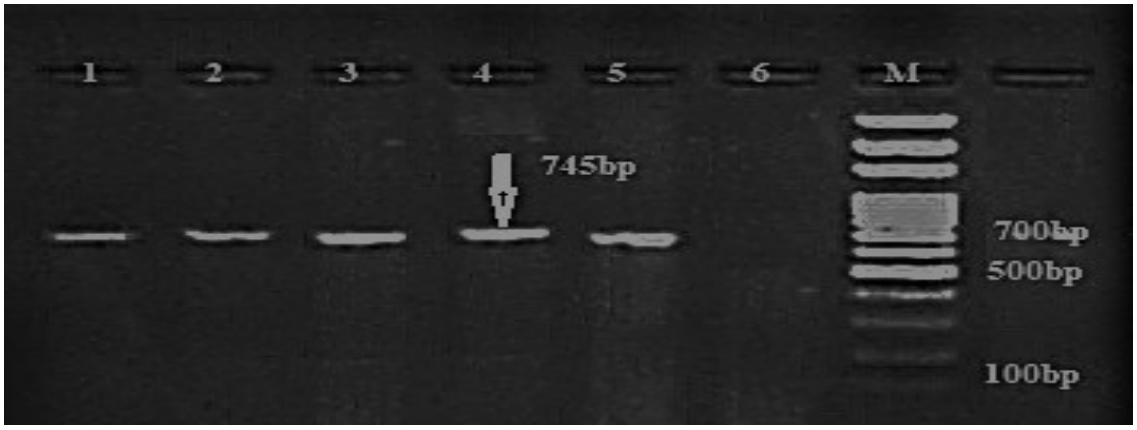

Figure 11: Agarose gel electrophoresis (1.5%) at 72 volts for 80 minutes of PCR product of *blaIMP-1* gene (745 bp); lane 1-5 showed the presence of *blaIMP-1* gene in *P. aeruginosa* isolate; lane 6 showed negative result; lane M [DNA marker size (100bp)]

Table 2: Conjugation frequency between donor (*K. pneumoniae*) and the recipient (*E. coli* Hb101) isolates

Type of bacterial Isolate	*Total no. of isolates*		*Conjugation Frequency*
	Transconjugant	*Recipient*	
E. coli Hb101	60 × 102	24 × 105	2.5×10^{-3}
K. pneumoniae	27 × 102	17 × 106	1.59×10^{-4}

Table 3: Transformation frequency of different bacterial species isolated from tonsillitis

Bacterial species	*Transformant cell*	*Transformation frequency*
S. pyogenes	4 × 107	1.7
S. aureus	5.7 × 106	1.036×10^{-1}
K. pneumoniae	4.5 × 106	2.25×10^{-1}
P. aeruginosea	4 × 107	1.43

frequencies of the local bacterial species, which had been used in transformation experiment, where transformation frequency of bacteria *S. aureus* was 1.036×10^{-1}, *S. pyogenes* was 1.7, *P. aeruginosea* was 1.43 and *K. pneumoniae* was 2.25×10^{-1}, as shown in Table 3.

The difference of values transformation frequency between different local bacterial species is influenced by numerous causes, such as, size of plasmid DNA, while the size of DNA is small, the transformation frequency becomes in height or increased, which refers that the small size of DNA indications to genetic transformation to become successful. The alternative feature is the purity of DNA, which means a high effect on genetic transformation. When DNA is in a high degree of purity, the genetic transformation is improved. Additionally, energy that is required for adherence of the DNA to the competent cell is measured as a feature on energy way of competent cells essential to prevention of the capability of DNA to enter.[26]

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