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Identification of the Resistance Gene of Bacteria Isolated From Children with Respiratory Tract Infections

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Abstract: **Objective:** The resistance gene for bacterial species isolated from juvenile respiratory tract infections was the goal of the current investigation. **Methods:** The pcr approach was utilized to detect the resistance gene of two bacterial strains from respiratory tract infections in pediatric patients at the Children's Hospital in Karbala. The current study's findings demonstrated that four of the six isolates of Klebsiella pneumonia and five of the ten isolates of Pseudomonas aeruginosa exhibited the antibiotic Cephotaxime (CTX-M) resistance genes. Both two Klebsiella pneumonia isolates out of six and five Pseudomonas aeruginosa isolates out of ten both exhibited the antibiotic Temocillin (TEM) resistance gene. **Conclusion:** According to the results of the current experiment, the isolates that caused respiratory tract infections in children had the gene for antibiotic resistance to temocillin (TEM) and cefotaxime (CTX-M).

Key points: Gene Resistance, Pseudomonas aeruginosa, Klebsiella pneumonia, CFX-M, TEM.

Introduction

The most frequent infections of the upper respiratory tract include tonsillitis, sinusitis, pharyngitis, nasopharyngitis, and otitis media. These serious problems are usually caused by viruses, such as influenza viruses, adenoviruses, rhinoviruses, and human respiratory viruses. Fever and chronic obstructive lung disease are caused by subsequent bacterial infections that develop after the viral invasion. Streptococcus pneumoniae, Moraxella catarrhalis, Staphylococcus aureus, Hemophilus influenzae, and Streptococcus pyogenes are the microbes that cause upper respiratory tract infections (RTIs).[1] The initial swelling that upper respiratory tract infections produce might jeopardize the airways and lead to asthma or difficulties swallowing, which also causes significant dehydration, even though these infections are rarely serious. Respiratory infections are more common in underdeveloped countries, where they are the second most common cause of pneumonia-related fatalities in children, behind diarrhea [1,2].

According to statistics, there are 5.6 million cases of community-acquired pneumonia (CAP) in the US annually, which directly contributes to the 14% annual death rate [2,3].

Once-effective antibiotics are no longer efficient against bacteria. Gram-positive bacteria, especially vancomycin-resistant enterococci and methicillin-resistant Staphylococcus aureus, were the main cause for worry ten years ago [3]. But many clinical microbiologists now concur that the biggest hazard to public health is multidrug-resistant Gram-negative bacteria [4].

Only a few number of drug research initiatives will be sufficient to provide therapeutic coverage in the next ten to twenty years because of the sharp rise in Gram-negative bacteria's resistance to both existing and emerging antibiotics. The primary cause of the rise in antibiotic-resistant bacteria is the mobile genes on plasmids, which proliferate and disperse among bacterial species [5].

One of the primary causes of the development of antibiotic resistance in bacteria is the overuse and abuse of antibiotics to treat respiratory infections [6].

Another component that contributes to bacterial resistance to antibiotics is the production of biofilms during the quorum sensing process, which releases the beta-lactamase enzyme that breaks down many antibiotics [7].

Haemophilus influenzae was shown to be resistant to ampicillin, Streptococcus pneumoniae to both penicillin and erythromycin, and isolates of Streptococcus pyogenes to erythromycin. Erythromycin resistance was observed in S. pyogenes [8].

With a rise in death rates from severe illnesses brought on by different bacteria in respiratory infections in China, bacterial resistance to currently available antibiotics has reached a serious level globally. Thus, further research is required to identify novel bacterial strains that are resistant to various antibiotics. In order to improve the effectiveness of currently available medications, restore the efficacy of current medications, and create a new class of antibiotics tailored to the novel antibiotic resistance mechanisms, a potential strategy to target the regulation of bacterial resistance mechanisms will be developed using the isolated resistant bacterial strains [9].

The purpose of this study was to discover bacterial species from sick individuals of all ages residing in various regions of China that are resistant to commonly given antibiotics.

Materials and Methods

Samples

The current study was carried out on pediatric patients with respiratory tract infections at Children's Hospital in Karbala between April 2024 and September 2024. Where bacteria were isolate and diagnosis as well as antibiotic resistance test by Vitek.

Identification of antibiotic resistance genes in isolated bacteria:

1. Molecular detection

DNA was extracted using the heat lysis technique then use monoplex-PCR [10]. Table 1, lists the primer utilized in this study.

Table 1: The primers used in this study are listed

Organism	Target gene	Primer Sequence 5´- 3´	Size (bp)	Temp 0C	Reference
Pseudomonas aerogenoza	CTX-M-F CTX-M-R	GTTACAATGTGTGAGAAGCA GCCGTTTCCGCTATTACAAAC	550	55	[11]
	TEM-F TEM-R	TTCTTGAAGACGAAAGGG CACGCTCAGTGGAAAAC	1150	55	[12]
Klebsilla pneumonia	CTX-M-F CTX-M-R	GTTACAATGTGTGAGAAGCA GCCGTTTCCGCTATTACAAAC	550	55	[11]
	TEM-F TEM-R	TTCTTGAAGACGAAAGGG CACGCTCAGTGGAAAAC	1150	55	[12]

In monoplex-PCR, the conditions for PCR amplification: The primers were created by first annealing for 1.5 minutes at 56 ºC, then extending for 1 minute at 95 ºC, and then extending for 10 minutes at 95 ºC. Five minutes of denaturation at 95 ºC, followed by thirty-five cycles of one minute of natural denaturation at 95 ºC.

After the PCR results were analyzed using electrophoresis on an agarose gel, they were visualized using a UV transilluminator.

Results and Discussion

Out of the numerous bacterial species gathered from the Children's Hospital in Karbala, 16 isolates of two different types of bacteria were found from children who had respiratory tract infections. These two species of bacteria, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, were resistant to the medications temocillin (Temocillin - TEM) and cephotaxime (Cephotaxime - CTX), as shown in Table 2.

Table 2: Bacterial isolates from children with respiratory tract infections.

Total isolation sum	Total isolation sum	Number of isolates resistant to the antibiotic timosiline	The number of isolates resistant to the antibiotic cefotaxime	Bacteria
	10	5	5	*Pseudomonas aerogenoza*
	6	2	4	*Klebsilla pneumonia*

Cephotaxime

In Figures 1 and 2, Following their showed in gel electrophoresis, the results of the PCR-discovered genes for the ceftazidime-resistant bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumonia* revealed that CTX_M was present and appeared in the range of 550 bp range.

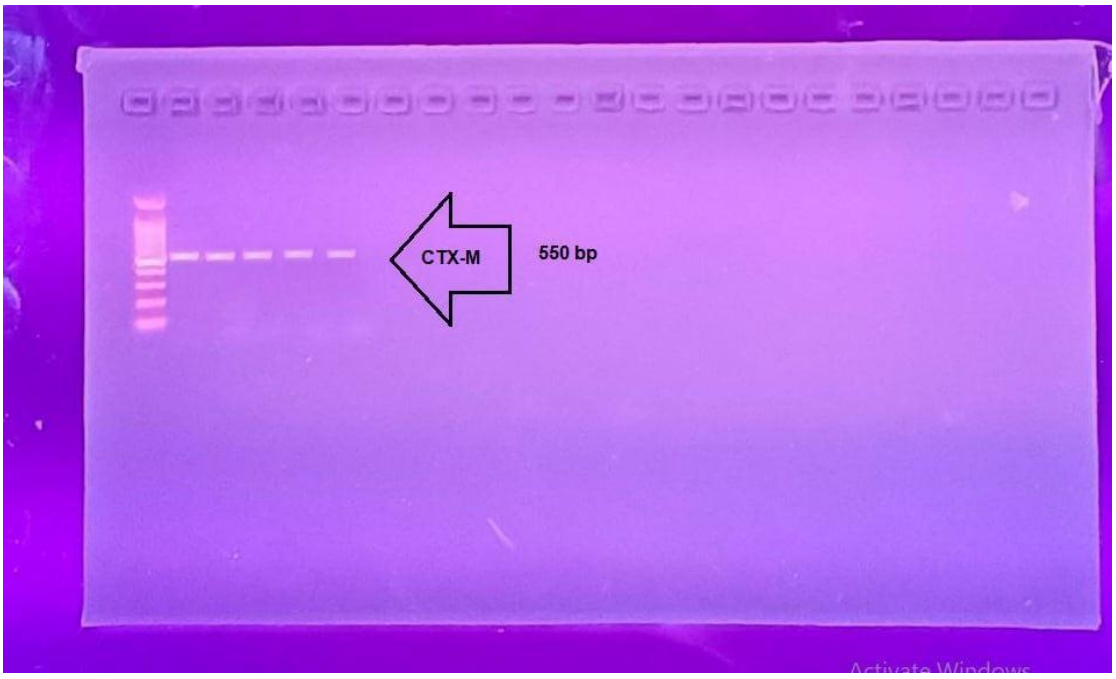

Figure 1: Range of UV results for the electrophoretic migration of the gel for the cefotaxime resistance gene.

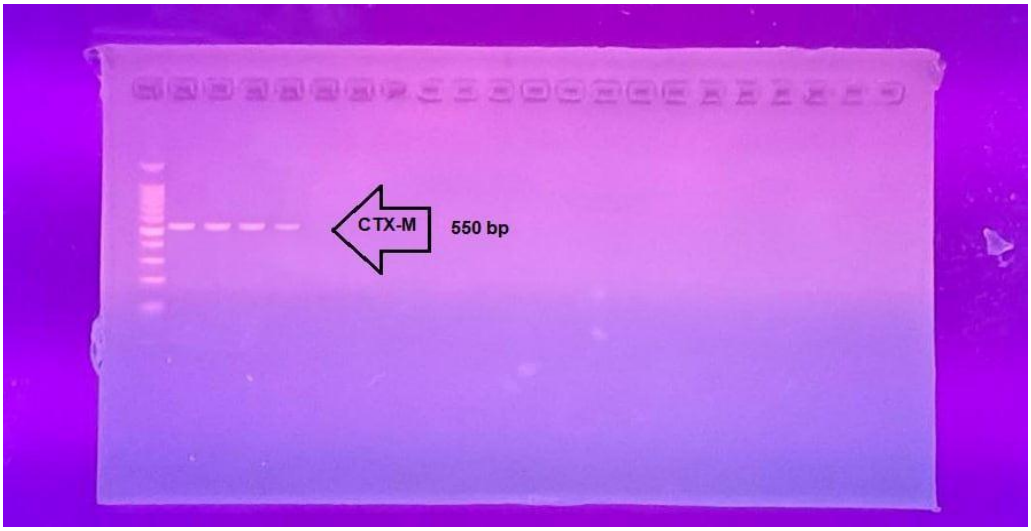

Figure 2: UV range results for the electrophoretic mobility of the gel for the cefotaxime resistance gene.

The resistance gene for the antibiotic temocillin (Temocilin-TEM)

In Figures 3 and 4, Following their presence in gel electrophoresis, the PCR results of the genes for timosiline-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacteria revealed that TEM was present and appeared in the range of 1150 bp.

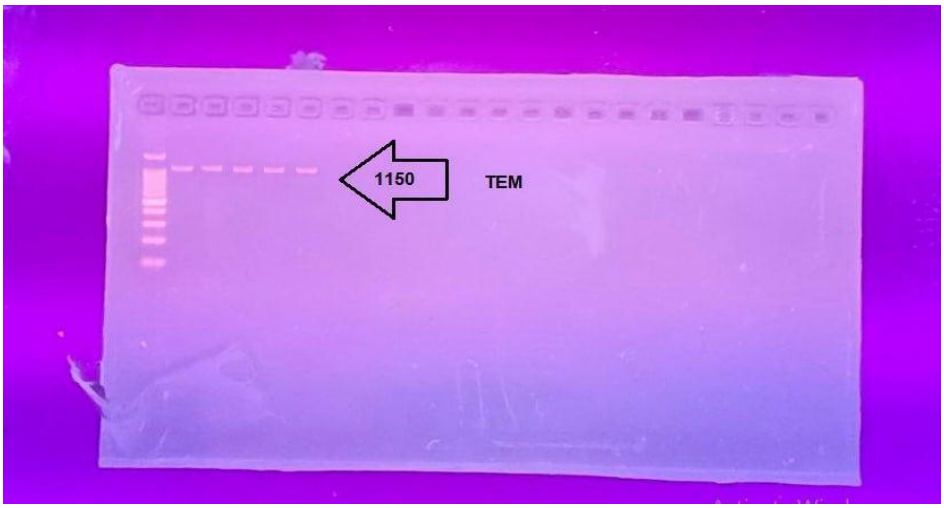

Figure 3: Range of UV results for the electrophoretic migration of the gel for the temozolomide resistance gene. *Pseudomonas aeruginosa* is a bacterium. (Temocilin-TEM).

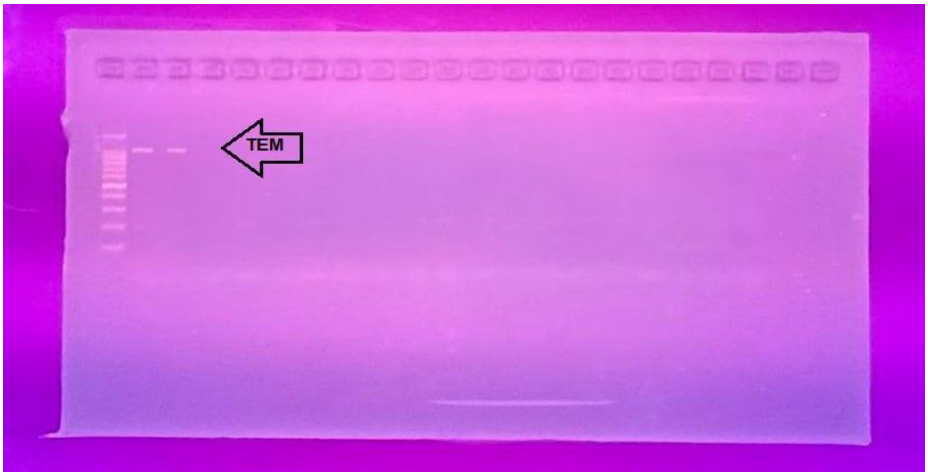

Figure 4: range of UV outcomes for the temozolomide resistance gene electrophoretic migration of the gel.

Multi-drug resistance bacterial infections caused by *Klebsiella pneumoniae* (Temocilin-TEM) are one of the biggest issues that patients worldwide deal with, since they raise hospital expenses and mortality rates. Antibiotic resistance is a serious hazard to public health, according to the World Health Organization and the Centers for Disease Control and Prevention [13].

P. aeruginosa is one of several bacterial infections that are classified. Pathogens include Acinetobacter baumannii, Staphylococcus aureus, Enterobacter, Klebsiella pneumoniae, and Enterococcus fecium. Extremely drug-resistant and a leading cause of infections acquired in hospitals. One of the most common bacteria, P. aeruginosa, is also the cause of hospital-acquired infections and treatment resistance. As a result, certain strains of P. aeruginosa are able to successfully evade antibiotic treatment [14].

In recent years, carbapenems, imipenem, and meropenem have been identified as successful therapies for *P. aeruginosa* infections. But in recent years, bacteria have become resistant to various antibiotics, and colistin or polymyxin B has been recognized as a last choice for treating *P. aeruginosa* that is very drug-resistant. However, colistin resistance has also been reported recently. Furthermore, it explains. Eleven percent of hospital-acquired infections are caused by *P. aeruginosa*, which has high mortality and morbidity rates, particularly in patients with compromised immune systems. Colonization, surgical wound infections, urinary tract infections, pneumonia, and bacteremia can occur in the kidneys, urinary tract, and upper respiratory tract. Among the primary virulence agents are adhesions, hemolysins, exotoxins, proteases, and iron carriers [17].

Reduced expression or damage to the OprD porin results in lower antibiotic permeability and increased expression of the MexAB-OprM pump, which is one of the documented mechanisms of antibiotic resistance for *P. aeruginosa*. This in turn leads to a rise in the flow of antibiotics, as well as the creation of aminoglycosides and beta-lactam inactivating enzymes due to gyrase and topoisomerase mutations that induce fluoroquinolone resistance. The procedure outlined may be a factor in multidrug resistance [18,19].

One of the main obstacles preventing antibiotics from penetrating *Pseudomonas aeruginosa* bacteria is their outer membrane. *P. aeruginosa* creates a number of pores, including OprD and OprF. Accordingly, imipenem resistance is linked to decreased or absent expression of (OprD)[20]

Conclusions

Through the results of the current study, the following conclusions can be reached:

1. Among the most dangerous kinds of bacteria that infect children's respiratory tracts is Both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are among the most harmful bacterial species that cause respiratory tract infections in children.
2. Cefotaxime and ticarcillin resistance genes were detected in the isolates.

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