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COMPARATIVE EFFICACY OF IMIPENEM AND CEFTOLOZANE/TAZOBACTAM IN TREATING MDR-PSEUDOMONAS AERUGINOSA URINARY TRACT INFECTIONS

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ABSTRACT:

Background: UTI, one of the most prevalent illnesses in modern medicine, it affects patients of all ages, from newborns to the elderly**.** Urinary tract pathogens possess virulence characteristics that facilitate their attachment to mucosal surfaces and consequent infection. Sepecifically we chose Pseudomonas aeruginosa (P.aeruginosa) in our study, it is an obligate gram negative, rod-shaped bacilli, which impart blue-green color in a culture plate. Our research drugs are Imipenem(I) and Ceftolozane are abroad-spectrum carbapenem antibiotic that is effective against many Gram-negative and Gram-positive bacteria, including Pseudomonas aeruginosa.

Objective: To compare the Efficacy of Imipenem and Ceftolozane/Tazobactam in Treating MDR-Pseudomonas aeruginosa Urinary Tract Infections.

Methods: An in-vitro pre-clinical experimental study was carried out at University of Karachi, from December 2022 to May 2023. A total of 40 isolates per group were collected for evaluation of antibacterial activity. The experimental samples of MDR-*P* were collected from Microbiology Laboratory, University of Karachi. Antibacterial activity of antibiotics was determined using Disc Diffusion method.

Conclusion: MDR P. aeruginosa strains exhibit significant resistance to imipenem, but CT, a novel antibiotic, shows increased efficacy against these multidrug-resistant bacteria, indicating a potential alternative to imipenem in treating UTI.

Keywords: Imipenem (I), Ceftolozane/Tazobactum (CT), UTI, MDR-Pseudomonas aeruginosa

INTRODUCTION

Acute cystitis, prostatitis, pyelonephritis, urethritis, and other clinical entities range in severity from asymptomatic infection to UTIs. One of the most prevalent illnesses in modern medicine, it affects patients of all ages, from newborns to the elderly(Chen et al., 2023). The most often identified bacteria that cause urinary tract infections (UTTIs) include Escherichia coli, Pseudomonas spp., Streptococcus spp., Proteus spp., Klebsiella spp., Staphylococcus spp., Neisseria gonorrhoeae, Chlamydia trachomatis, and Candida spp. These bacteria typically begin in the intestine. Urinary tract pathogens possess virulence characteristics that facilitate their attachment to mucosal surfaces and consequent infection(Dougnon et al., 2020). A major contributor to nosocomial illness and an increasing hazard to public health worldwide is the rise of MDR species of these bacteria, which are prevalent in both hospital and community settings(Morris and Cerceo, 2020).

Sepecifically we chose Pseudomonas aeruginosa (P.aeruginosa) in our study, it is an obligate gram negative, rod-shaped bacilli, which impart blue-green color in a culture plate. P. aeruginosa is 1.5um long and 0.5- 1.0um wide and monoflagellated bacterium that has an incredible nutritional versatility. P. aeruginosa has a cellular envelop made up of lipopolysaccharide, this layer gives protection against many drugs(Gautam, 2019).

The primary therapeutic tool used in medicine to treat a wide range of bacterial illnesses including UTI is an antimicrobial agent. One of the most significant scientific discoveries of the modern era is thought to be the discovery of antibiotics(Varela et al., 2021). Millions of lives have been saved by antibiotics. Antibiotic resistance is one of the largest threats the world is now facing(Salam et al., 2023). The overuse or misuse of antibiotics can lead to the formation of bacterial strains that are resistant to treatment. Antimicrobial resistance is a significant global public health issue, affecting human health and hindering advancements(Hamdani et al., 2020). Broad-spectrum antibiotics, effective against both Gram-positive and Gram-negative bacteria, are commonly used in cases of unknown infection causatives or mixed bacterial infections(Bassetti et al., 2022).

Urinary tract infections (UTIs) are commonly treated with a range of antibiotics, depending on the severity of the infection, the specific bacteria involved, and the patient's medical history. First line antibiotics for UTI are Nitrofurantoin,Trimethoprim/Sulfamethoxazole (TMP/SMX) and Fosfomycin. The second line treatment includes , Fluoroquinolones Beta-Lactams (Amoxicillin-clavulanate, Cefdinir, Cefpodoxime)(Butler et al., 2022).

Antibiotics for Complicated UTIs and Pyelonephritis includes Extended-Spectrum Cephalosporins (Ceftriaxone, Cefepime) Carbapenems (Imipenem, Meropenem) Ceftolozane/Tazobactam Aminoglycosides (Gentamicin, Amikacin)(Bielen and Likic, 2019).

Specifically, Pseudomonas aeruginosa has an innate resistance to several popular antibiotics, which makes treating a urinary tract infection (UTI) caused by this bacterium difficult. Susceptibility testing typically serves as a guidance for selecting an antibiotic(AL-Khikani and Ayit, 2022). However Ciprofloxacin and Levofloxacin are the first line antibiotics used in Pseudomonas causing UTI. Further more Second-Line and Intravenous Antibiotics for Severe or Resistant Infections includes Piperacillin/Tazobactam Ceftazidime Cefepime (Daikos et al., 2021) while in case of MDR (Multidrug-Resistant Pseudomonas) Ceftolozane/Tazobactam , Imipenem/Cilastatin and Aminoglycosides **(Gentamicin and Amikacin) are used(Galani et al., 2020).**

Among various factors for antibiotic resistance, specifically in developing countries, physicians prescribed antibacterial drugs without performing diagnostic susceptibility test(Godman et al., 2021). It has been reported that clinicians prescribed broad spectrum antibiotics in circumstances where more appropriate or specific narrow spectrum antibiotics can be used(Allen et al., 2022).

Another factor contributing to antibiotic resistance is noncompliance with prescribed therapy; patients may stop taking antibiotics for any reason or neglect to take their meds(Mohiuddin, 2023).

Efflux pumps, target site modification, enzyme destruction or inactivation, and changes to porin channels are some of the cellular mechanisms of bacterial resistance(Garcia et al., 2022) while chromosomal-mediated, plasmid-mediated, and transposon-mediated resistance are the three genetic

bases of resistance(Alrahmany and Ghazi, 2020).

Due to the development of antibiotic resistance among developing countries we selected two antibiotics Imipenem and Ceftolozane/Tazobactam to compare their efficacy in Treating Pseudomonas aeruginosa Urinary Tract Infections.

Our first research drug Imipenem(I) is a broad-spectrum carbapenem antibiotic that is effective against many Gram-negative and Gram-positive bacteria, including Pseudomonas aeruginosa. It works by inhibiting bacterial cell wall synthesis, leading to cell death. It is often used in severe or complicated infections due to its broad spectrum of activity(Armstrong et al., 2021).

Our second drug was, Ceftolozane(CT) also known as Tazobactam, Ceftolozane is a cephalosporin antibiotic combined with Tazobactam, a β-lactamase inhibitor, which extends its spectrum of activity against β-lactamase-producing bacteria. This combination is specifically designed to target multidrug-resistant P. aeruginosa(Lizza et al., 2021).

MATERIALS AND METHODS

An in-vitro pre-clinical experimental study was carried out at University of karachi, from December 2022 to May 2023. A total of 40 isolates per group were collected for evaluation of antibacterial activity. The experimental samples of MDR-*P* were collected from Microbiology Laboratory,University of karachi. Antibacterial activity of antibiotics was determined using Disc Diffusion method. Included were UTI samples that demonstrated MDR-P growth. Agar plates on which other species were growing were excluded. The institutional Ethical Review Committee granted the study exemption.

Isolation of Bacteria from Clinical Specimen

Isolates were identified by gram stain, microbiological analysis on Cetrimide agar medium, and biochemical testing.

Isolation of MDR Pseudomonas Aeruginosa by Modified Kirby-Bauer Disk Diffusion Test

With the use of antibiotic discs containing ampicillin (10 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), cefuroxime (30 µg), and gentamicin (10 µg), isolates were found to be multidrug resistant if they demonstrated resistance to at least one antibiotic in at least three distinct antibiotic groups. Furthermore, Mueller Hinton agar plates were swabbed with the bacterial colonies, and they were incubated for 24 hours at 37 ºC.

Antibiotic Susceptibility Testing by Kirby Bauer Disc Diffusion Technique

This is among the most often used techniques for assessing antibiotic susceptibility. Using this technique, a 150 mm Mueller Hinton Agar plate was covered with a lawn of bacterial inoculum. Agar plates were covered with commercially made fixed concentration paper antibiotic discs. Before the findings were determined, the plates were incubated at 35 ºC for 16 to 24 hours. The DMSO was used as negative control. Following CLSI recommendations (2020), the zones of growth inhibition surrounding each antibiotic disc were quantified and classified as either sensitive or resistant(CLSI 2020).

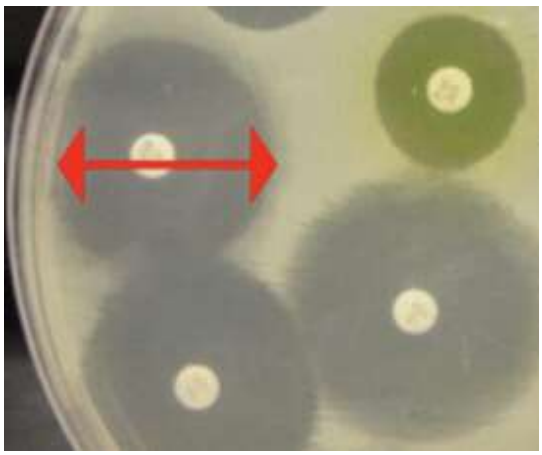

Figure 1: Zone Diameters of Antibiotic Disc by Disc Diffusion Method

Tab 1: Mic Interpretive Criteria (Ug/Ml) According to CLSI Guidelines (2020)

ANTIMICROBIAL AGENTS	DISK CONTENT	SENSITIVE	INTERMEDIATE	RESISTANT
Ceftolozane/Tazobactum	30/10ug	>21mm	8mm	<16
Imipenem	10ug	>19mm	4mm	<15

Data Analysis

Data was analyzed using SPSS version 22. Mean and standard deviation was calculated for ZOI.Descriptive analysis for numerical variables were mentioned as Mean with standard deviation. The zone of inhibition of antibiotics was categorized into resistant and sensitive on the basis of cutoff values present. Chi square test was applied to measure the association between sensitivity and resistance patterns of drugs. P value of less than 0.05 was considered as significant.

RESULTS

Results of Disc Diffusion Assay Against MDR P. Aeruginosa

Tab 2 indicates the results of disc diffusion assay. The MDR-*P* strains were evaluated at 2 concentrations of antibiotics that is Ceftolozane/tazobactum (30/10 ug) and Imipenem 10ug while DMSO as negative control. The mean ZOI of Ceftolozane/tazobactum was found to be 18.3mm while that of Imipenem (10ug) was found to be 10.3mm.Negative Control showed no antibacterial activity. Experiment was performed in triplicates.

Results of antimicrobial susceptibility testing are summarized in Table 3. Of the 40 tested isolates, 50% (n 20) were resistant to Ceftolozane/tazobactum and 62% (n 25) were resistant to Imipenem Susceptibility of CT was found to be 20(20%), whereas susceptibility of imipenem was 25(15%). The p-value was found to be highly significant 0.0001.

Tab-2 -ZOI of Imipenem and Ceftolozane/Tazobactum Against MDR P.Aeruginosa

S.No.	Concentration of Antibiotics)	Mean ZOI (mm)	Mean ZOI (mm)	SD	P Value
1	Ceftolozane/Tazobactum 30/10 ug	19	20mm	0.57	.001
		20			
		22			
2	Imipenem 10ug	15	15mm	0.57	
		14			
		15			
3	Control	-	-	-	

*Comparison of ZOIs of Antibiotics by ANOVA

Tab-3- Resistance Rates of Investigated MDR Pseudomonas Aeruginosa Isolates

Drug	R	I	S%	R%	P value
Ceftolozane/tazobactum	20/40	0	20%	50%	.0001
Imipenem	25/40	0	15%	62%	

DISCUSSION

Gram-negative bacteria P. aeruginosa has been linked to a number of illnesses, including bacteremia, pneumonia, urinary tract infections, skin and soft tissue infections, and more, particularly in those with impaired immune systems. P. aeruginosa clinical isolates have the potential to exhibit resistance to many antibiotic classes, hence providing doctors with limited alternatives for therapeutic antibacterial medications or regimens to treat infectious illnesses.

A number of β-lactam antibiotics, such as cephalosporins, monobactams, carbapenems, and

antipseudomonal penicillins, are suggested as part of this antibacterial regimen to treat potentially fatal nosocomial infections brought on by P. aeruginosa(Paprocka et al., 2022). Imipenem is still utilized as a first line empirical therapy for MDR infections, either in combination or alone. According to a recent review of the literature, resistance to this miracle medication has been steadily rising over the past few years and is now posing a serious worldwide therapeutic risk(Bassetti et al., 2019).

Currently available drugs against MDR P. aeruginosa include Fluoroquinolones (ofloxacin, ciprofloxacin) antipseudomonal penicillins (ticarcalin, piperacllin) cephalosporins (ceftazidime, cefepime), aminoglycosides (amikacin, gentamicin) and carbapenems (Imipenem, meropenum).

P. aeruginosa shown increased resistance to both medications in our study. Imipenem showed the highest level of resistance (62%) whereas C/T showed the lowest level of resistance (50%).

In this study the susceptibility rates of MDR P. aeruginosa to both CT and I are com-parable to one another, at 20% and 15% respectively. Of note, other studies reported higher susceptibility to these antibiotics as well.

According to study results from 2023, resistance to gentamycin, imipenem, amikacin, ceftazidime, ciprofloxacin, and piperacllin/tazobactam was 7.3%, 9.7%, 17%, 12%, and 64%, respectively(Hafiz et al., 2023).

Nonetheless, a number of research carried out in several Pakistani contexts have shown evidence of this tendency. A 2022 study found a significant increase in resistance compared to earlier years. tazobactam (56%), on the other hand, were resistant to MDR strains in this investigation, but imipenem was 40% resistant(Gomis-Font et al., 2022).

According to a study, imipenem was 100% resistant to MDR strains of pseudomonas, while resistance to this nosocomial pathogen was 98% for gentamycin, 61.1% for ciprofloxacin, 77.8% for amikacin, and 68.1% for piperacillin/tazobactam(Makled et al., 2023).

It is clear that Pakistan is seeing an increase in MDR pseudomonas strains. Our community's rising resistance to these medications is a result of their widespread prescription use in secondary and tertiary care facilities. Accepted chosen theory states that the usage of antibiotics and the emergence of resistance are causally related. The World Health Organization has noted that the extensive use of antibiotics in medicine is contributing significantly to the rise of resistant bacteria by creating a number of resistance mechanisms, such as the synthesis of enzymes called beta lactamases, which break down these drugs(Farooq et al., 2019).

On the other hand, research conducted in a tertiary care hospital in Pakistan revealed that imipenem exhibited 100% sensitivity to P. aeruginosa, while piperacllin/tazobactum demonstrated 72% sensitivity (Farooq et al., 2019).

According to another study , imipenem was 95% sensitive to P.aeruginosa, while ceftazidime was 100% resistant (Hasan et al., 2020)

In vitro activity of C/T was found to be better 20% as compared to imipenem 15.6%, when susceptibility tests were performed by disc diffusion assay. These results were similar to a study conducted in UK in 2020 which reported that C/T was found to be more efficacious antimicrobial drug as compared to imipenem when tested against MDR P. aeruginosa by the same method (Alvarez-Buylla et al., 2020).

A study conducted in USA against MDR P. aeruginosa revealed that 96.6% of MDR P. aeruginosa isolates were susceptible to C/T and 78% to imipenem (Goodlet and Nailor, 2017).

A mega study conducted by D.J Farrell et al in year 2014 explored that in vitro activity of C/T was better than imipenem and other comparator antimicrobial agents when tested against P. aeruginosa in fourteen European and Israeli Hospitals by E-strip method (Sader et al., 2014). C/T may demonstrate as an excellent choice in treatment of MDR P. aeruginosa infections. This is due to the fact that C/T has a low MIC and more specifically is unaffected by efflux pumps or loss of porins channels that may affect the other antibiotics (Takeda et al., 2007b, Takeda et al., 2007a).

CONCLUSION

MDR P. aeruginosa strains exhibit significant resistance to imipenem, but C/T, a novel antibiotic, shows increased efficacy against these multidrug-resistant bacteria, indicating a potential alternative to imipenem in treating UTI.

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