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New Agent Ceftolozane/Tazobactam for the Treatment of Superbug - *Pseudomonas aeruginosa*: Restoring the Miracle

Lubna Jahanzeb[1*], Zahida Memon[1], Faisal Afridi[2], M. Owais Ismail[1] and Bina Fawad[3]

[1]Department of Pharmacology, Ziauddin University, Karachi, Pakistan.
[2]Department of Microbiology, Ziauddin Hospital, Nazimabad Campus, Pakistan.
[3]Department of Community Health Sciences, Ziauddin University, Karachi, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. Authors LJ and ZM conceived and designed the study. Author FA provided microbiological setup and contributes in material and methods writing. Authors MOI and LJ approved the protocol, managed the literature searches, wrote the manuscript and managed the analyses of the study. Author BF performed statistical analysis of the data. Author ZM submitted her intellectual input in this study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: To compare in vitro activity of Ceftolozane/tazobactam and Imipenem against multi-drug resistant (MDR) *Pseudomonas aeruginosa* from various clinical specimens.
Place of Study: Study was conducted in department of Pharmacology, Ziauddin University and isolates were collected from Microbiology department of Ziauddin hospital, Nazimabad campus, Karachi.
Methodology: It was a Quasi experimental study in which total 176 isolates of P.aeruginosa was collected from which 97 isolates was MDR *P.aeruginosa* MDR *Pseudomonas aeruginosa* (*P.aeruginosa*) isolates were collected from various specimens such as pus, tracheal aspiration, wound swab, blood and urine. Samples were processed as per procedures interpreted by Clinical

**Corresponding author: E-mail: jjahanzeb85@gmail.com;*

Laboratory Standard Institute (CLSI) guidelines 2018. Susceptibility of MDR *P. aeruginosa* against Ceftolozane/tazobactam (C/T) and Imipenem was performed by E-test strip method. Results were interpreted by (CLSI) guidelines. Data were analyzed by using Statistical Package for Social Sciences (SPSS) version 21.

Results: The maximum number of MDR P. aeruginosa was isolated from pus (33.1%) followed by tracheal aspiration (20.6%). C/T showed 60% susceptibility whereas Imipenem showed 19% susceptibly to *P.aeruginosa*. In vitro activity of C/T was found to be superior as compared to Imipenem against MDR *P. aeruginosa* with E-test strip method (P-value = <0.0001).

Conclusion: *In vitro* activity of C/T was found to be better against MDR *P. aeruginosa* compared to Imipenem. This combination has a low tendency to induce resistance, especially against Gram-negative organisms, so it is an initiative of a new phase in the world of complicated infections.

Keywords: Imipenem; infection; gram-negative bacteria; Pseudomonas aeruginosa.

1. INTRODUCTION

Drug-resistant infections and related morbidity and mortality are on the rise around the globe. The World Health Organization has identified antimicrobial resistance as one of the greatest threats to human health [1]. Excessive antibiotic use as a part of treatment of common viral infections is one of the causes of enhancement of antibacterial resistance [2]. Various other factors are also responsible for the emergence of resistance such as misuse or abuse of antibiotics, inappropriate prescriptions by the physicians, self-medications, use of broad spectrum antibiotics, and lack of awareness with the new guidelines recommended for antimicrobial testing etc [3].

P. aeruginosa is often resistant to multiple antibiotics and consequently has joined the rank of superbug due to its enormous capacity to produce resistance [4].

P. aeruginosa is an opportunistic as well as nosocomial pathogen. It is included among the major causes of life-threatening infections in immunocompromised patients [5]. Data presented by the Center for Disease Control and Prevention (CDC) revealed that *P. aeruginosa* is responsible for the nosocomial Respiratory tract infections, Bloodstream infections, Cardiovascular infections, Central nervous system infections, Ear infections, Eye infections, Urinary tract infections, Gastro intestinal tract infections and Skin infections [6]. Hospital associated *P. aeruginosa* isolates are usually multidrug resistant, which may be due to either continuous or previous exposure to antimicrobials in some clinical settings [7].

There are several mechanisms for resistance development in *P. aeruginosa*, including enzymes production, outer membrane protein loss and targets site alteration. Acquired resistance is mediated through multi-drug efflux pump system that play a key role in development of multidrug resistance against *P. aeruginosa* [8].

Carbapenems are considered to be the most potent anti-pseudomonal beta-lactam agents that inhibit bacterial cell wall synthesis. These drugs inactivate penicillin-binding proteins PBP1 and PBP2 that results in lysis of cell wall [9]. A serious challenge due to excessive use of carbapenems against *P. aeruginosa* is emergence of resistance during treatment. To compensate this mechanism of resistance Pharmaceuticals developed a novel antimicrobial agent that is C/T, an anti-pseudomonal cephalosporin with a β-lactamase inhibitor, which showed a vast activity against gram positive as well as gram negative organisms [10].

P. aeruginosa showed less resistance to ceftolozane as compared to other beta lactams. C/T showed a significant stability against class AmpC beta-lactamase. The synergistic effect with tazobactum makes it more stable against extended spectrum beta lactamase (ESBL) [11]. More specifically, C/T is unaffected by efflux pumps or loss of porins channels that may affect the other antibiotics. However C/T has maintained its activity against imipenem-resistant clinical isolates of *P. aeruginosa* which showed resistance with mutational change in OprD [12].

To the best of our knowledge, there is no present data available in Pakistan regarding the susceptibility and comparison of C/T with imipenem against MDR *P. aeruginosa*. The rationale of this study was to diversify the treatment options against MDR *P. aeruginosa* infections, which is quite rampant in our set up.

The objective of this study was to compare *in vitro* activity of C/T and imipenem against MDR *P. aeruginosa* from various clinical specimens.

2. MATERIALS AND METHODS

2.1 Study Period and Sampling

This was a Quasi experimental study and samples were collected using non probability technique. Study was conducted from October 2017 to April 2018 at Ziauddin University Karachi. Samples were collected from the indoor and outpatients clinics, afterwards submitted to Microbiology Lab. of Ziauddin hospital, Nazimabad campus, for culture and sensitivity. Routine specimens were taken including pus, wound swab, blood, and tracheal aspiration and urine.

2.2 Culture

Samples were processed as per microbiological procedures CLSI Guideline 2018. After a written informed consent detailed information of the patients and isolates was recorded on a separate questionnaire.

2.3 Biochemical Identification of *P. aeruginosa*

All nonlactose fermenting colonies on MacConkey Agar were picked up for Gram's stain and biochemical identification, such as motility, pigment production, citrate, catalase and oxidase test. MDR *P. aeruginosa* is defined by European center for disease prevention and control which stated that resistance to at least three or more than three antibiotics such as aminoglycoside, anti-pseudomonal penicillin, carbapenems, cephalosporins and fluoroquinolones.

2.4 E Test Strip Method

E-test strip method was performed by first thawing and subculturing the stored micro-organisms on a non-inhibitory medium like blood agar (Oxoid, UK). MDR *P. aeruginosa* colonies were emulsified into 5 ml of sterile normal saline to achieve a turbidity equivalent to 0.5 McFarland standard. A sterile swab was dipped into the inoculum suspension and the entire Mueller Hinton Agar (MHA) (Oxoid, UK) surface was swabbed 3 times to ensure an even distribution of inoculum. E-strips containing C/T and imipenem (Oxoid, UK) were applied separately on the bacterial suspension of MHA and incubated at 35°C ±2 for 16-20 hours. The minimum inhibitory concentration (MIC) values were read where the respective inhibition 0ellipses intersected the strip.

Data was analyzed by using Statistical Package for Social Sciences (SPSS) version 21. Descriptive analysis for numerical variable has been mentioned as Mean with standard deviation. Frequencies and percentages were calculated for susceptibility of antimicrobials and isolation of MDR P. aeruginosa from various clinical specimens. Zones of inhibition for E-test strip methods were interpreted as per CLSI guidelines. Chi square test was applied to measure the association between sensitivity and resistance patterns of drugs. A *P*-value <0.05 was considered as statistically significant.

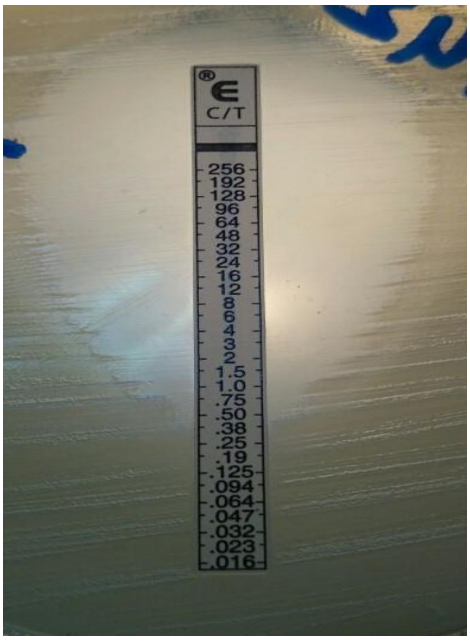

Fig. 1. E test strip of C/T

3. RESULTS

A total of 176 strains of *Pseudomonas* were isolated out of which, 97 (55.1%) were MDR *P. aeruginosa*. The predominant source of MDR *P. aeruginosa* isolates was obtained from pus (33.1%) followed by tracheal aspiration (20.6%) and urine (18.6%) and least by sputum (14.4%), swab (4.2%) and blood (10.1%) as shown in Fig. 2.

Majority of the cases were isolated in age group of 61-80 years, (46.1%) and the least in patients eighty years or above (2.1%) patients as shown in (Table 1). Gender wise distribution of MDR isolates showed that (46.4%) were males and (51.6%) were females.

By E-test strip method, it was found that (60.0%) of MDR *P. aeruginosa* isolates were susceptible to C/T compared to (19.6%) with imipenem. P value was found to be highly significant statistically (p value = 0.0001). MIC ranges of imipenem were lower as compared to C/T by E-test strip method shown in Table 2.

4. DISCUSSION

P. aeruginosa is intrinsically resistant to several antibiotics due to the low permeability of its outer membrane, over expression of several efflux pumps, and production of antibiotics inactivating enzymes [13]. Several antibiotic regimens are proposed for treatment of MDR *P. aeruginosa*. Imipenem is still being used as a first line empirical therapy in the treatment of MDR pathogens. Literature survey revealed that resistance against this wonder drug has gradually been increased since last few years that has become a major therapeutic threat globally [14,15].

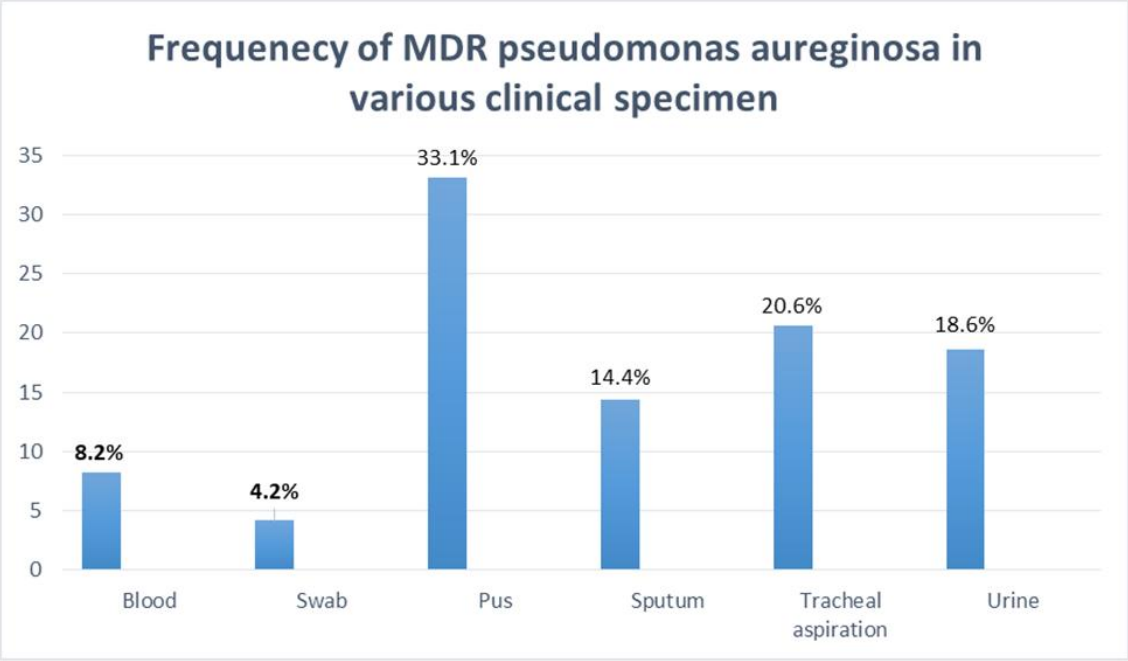

Fig. 2. Frequency of MDR *P. aeruginosa* in various clinical specimen

Table 1. Age distribution of MDR *P .aeruginosa* strains (N=97)

Age	MDR strain N (%)	P- value
1 month – 20 years	4 (4.1)	
21- 40 years	23 (23.7)	
41- 60 years	22 (22.7)	0.25
61- 80 years	46 (47.4)	
80 years above	2 (2.1)	

Table 2. Susceptibility results of Imipenem and C/T by E-test method

Antibiotics	Susceptible N (%)	Resistant N (%)	P value
Imipenem	19 (19.6)	78 (80.4)	0.0001
C/T	58 (60.0)	39 (40.2)	

In our study, MDR P. aeruginosa isolates were mainly recovered from pus (33.1%) and tracheal aspiration (20.6%) followed by urine (18.6%). The justification of presence of highest number of isolates in pus is due to the fact that majority of patients had postoperative wound complications and prolong duration of hospitalization in our setup. Inadequate antiseptic measures and substandard wards dynamics at particular point of time are other possible contributory factors in acquiring the resistant strains. Recent study done by Fouzia Khan et al in year 2014 also reported maximum number of Pseudomonas isolates recovery from pus that was (33.3%) [16,17]. On the contrary, a study conducted in Islamabad in year 2011 showed that the highest number of such isolates recovered from urine that was (32%) followed by sputum (19.5%) [18].Similar, Study done in Egypt in year 2007 by Gamal F Gad et al reported highest number (29%) of *Pseudomonas* isolates in urine [19]. This conflict is might be due to the variation in environmental dynamics and techniques in different clinical setups.

Our study showed MDR *P. aeruginosa* were predominant in females that was (54.6%) as compared to males that was (46.4%). This is similar to the study done in Nepal in year 2013 by Chander aneil et al, which showed (55.1%) of isolated in females [20]. In comparison of different age groups, the majority of the MDR strains were identified in patients in age group of 61-80 years that was (47.4%) as shown in Table-1. This finding is in contrast to a study conducted by Indu Biswal et al in year 2014 who reported majority of resistant isolates (33.93%) in age group of 21-30 years [21]. This contrast exists probably because patients who are in higher age group are immunocompromised and might be admitted to hospitals, from where they are acquiring resistant pathogens due to their low immunity.

In our study in vitro activity of C/T was found to be better in (60%) and potent as compared to imipenem (19.6%) when susceptibilities were performed by E-test strip method. These results were similar to a study conducted by Damien Fournie al in 2017 which reported that C/T was found to be the more potent antimicrobial as compare to imipenem when tested against MDR *P. aeruginosa* by the same method [22]. A study conducted in USA against MDR *P. aeruginosa* by Kellie J. Good et alin year 2017 revealed that (96.6%) of MDR *P. aeruginosa* isolates were susceptible to C/T and (78%) to imipenem [23]. 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* From 14 European and Israeli Hospitals by E-strip method [24].

C/T has a broad spectrum of activity against variety of bacteria including MDR Gram-negative bacteria. It is indicated as a single antimicrobial agent for the treatment of complicated infections including ventilator-associated bacterial pneumonia, nosocomial pneumonia, complicated urinary tract infections and complicated intra-abdominal infections that are either because of Gram-positive or Gram-negative bacteria plus some of the MDR strains [25]. The most important difference between C/T and the other antibiotics is the superior activity noted in several in vitro studies for C/T against MDR *P. aeruginosa* [26]. 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 low MICs and more specifically is unaffected by efflux pumps or loss of porins channels that may affect the other antibiotics [12].

5. CONCLUSION

On the basis of literature facts and figures it is concluded that increasing resistance patterns and its influence on clinical utility of conventional antibiotics are most concerning and challenging problems globally to optimal care of infected patients especially in tertiary care units. To date, C/T has demonstrated an excellent safety profile and low MIC as compare to imipenem. Further to it, C/T exhibited an inherently low tendency to inducing resistance in general and especially against Gram-negative organisms so it is an initiative of a new phase in the world of complicated infections.

Keeping in view above discussion, it can be recommended that the therapeutic use of C/T and imipenem for treatment of MDR *P. aeruginosa* should be reserved only for severe and life threatening infections. This is particularly true where the infection is polymicrobial, anaerobic or Pseudomonas resistant to other antimicrobial drugs.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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