Brief Communication

In silico and *In vitro* Activity of Ceftolozane/Tazobactam Against *Pseudomonas aeruginosa* Collected Across Indian Hospitals

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Abstract

Ceftolozane/tazobactam is a novel antimicrobial agent with activity against *Pseudomonas aeruginosa* and other common Gram-negative pathogens. In this study, we determined the antimicrobial susceptibility for a total of 149 clinical isolates of *P. aeruginosa* for the most commonly used antimicrobials including the new agent ceftolozane/tazobactam (C/T). Broth microdilution was performed to determine the minimum inhibitory concentration against various antimicrobials including C/T. Among the β-lactam/β-lactamase inhibitor, overall susceptibility was 67%, 55% and 51% for C/T, Piperacillin/Tazobactam (P/T) and Cefoperazone/Sulbactam, respectively. The variations in the susceptibility rates were noted among the three different β-lactam/β-lactamase inhibitors. Interestingly, 33% susceptibility was noted for C/T against isolates that were resistant to P/T, indicating the higher activity of C/T. This finding suggests about 33% of the P/T-resistant isolates can still be treated effectively with C/T. C/T could be a better alternative for the treatment of ESBL-producing organism, and thereby usage of higher antimicrobials can be minimised.

Keywords: Antimicrobials, ceftolozane/tazobactam, India, *Pseudomonas aeruginosa,* susceptibility

Introduction

The increasing antimicrobial resistance is a worrisome scenario in developing countries, especially in India. Around 30% of the infections caused by *Pseudomonas aeruginosa* are resistant to multiple classes of antibiotics. Most of these infections are treated by the higher antimicrobial agents such as carbapenem and colistin. To minimise the use of higher agents, β-lactam/β-lactamase inhibitor combinations are alternative for treating extended spectrum beta lactamase (ESBL) producers. Of these, the frequently used combinations for *P. aeruginosa* at present are piperacillin/tazobactam (P/T) and cefoperazone/sulbactam (C/S). Recently, in 2014 and 2015, two novel combinations were approved by the Food and Drug Administration (FDA) are ceftolozane/tazobactam (C/T) and ceftazidime/avibactam (C/A), respectively.[1] While aztreonam/avibactam and Imipenem/relebactam are undergoing clinical trials (clinicaltrials.Gov)

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Ceftolozane is a novel cephalosporin, active against pseudomonal *ampC* enzymes, efflux system and membrane impermeability. However, it can be hydrolysed by ESBLs and carbapenemases.[2] The addition of tazobactam to ceftolozane broadens the spectrum of activity against ESBL producers. FDA licenses this novel combination for use in adults for the treatment of complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI) including pyelonephritis. This combination covers infections caused by *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Proteus mirabilis*, *Bacteroides fragilis and Streptococcus* spp. C/T has been shown to

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demonstrate better *in vitro* activity against isolates resistant to ceftazidime, cefepime and P/T studied across the world.[2] In this study, we evaluated the *in vitro* efficacy of C/T against *P. aeruginosa* collected from Indian hospitals. Further *in silico* analysis was performed to support the *in vitro* findings.

Materials and Methods

In vitro analysis

In this study, we determined the antimicrobial susceptibility for a total of 149 invasive clinical isolates of *P. aeruginosa* for the most commonly used antimicrobials including C/T. Isolates collected across various centers in India were tested. The participating center includes Christian Medical College, Vellore, Tamil Nadu; Manipal Hospital, Karnataka; Fortis Hospital, Kolkata, West Bengal; The Calcutta Medical Research Institute, West Bengal; Choithram hospital, Madhya Pradesh; Hinduja Hospital, Mumbai and Sanjay Gandhi Postgraduate Institute of medical science, Lucknow, Uttar Pradesh.

These isolates were sourced from the bloodstream, intra-abdominal and UTIs collected between the year 2013 and 2014. Broth microdilution was performed to determine the minimum inhibitory concentration (MIC) against various antimicrobials and results were interpreted according to the CLSI 2016 breakpoint interpretative criteria.[3] *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853 were used as quality control organisms.

In silico analysis

The 3D structure of *P. aeruginosa ampC* was retrieved from the Protein Data Bank (PDB) with the PDB ID 4GZB.[4] The SMILES of C/T, and Piperacillin were retrieved from PubChem database with CIDs 53234134, 123630 and 43672, respectively. The 3D structures were converted to PDB format structure using OpenBabel. Molecular docking was performed using AutoDock 4.2. Hydrogen and necessary charges were charges added to the protein followed by torsion to the drugs. The grid was fixed around the active site, and AutoGrid was performed. Finally, AutoDock was performed using Lamarckian Genetic Algorithm in 10 runs. The docking was performed thrice, and average binding energy was calculated.

Results

In vitro analysis

Antimicrobial susceptibility profiles of the study isolates against the tested agents are summarised in Table 1. MIC_{50} and MIC_{90} for *P. aeruginosa* were 1/4 μg/ml and ≥16/4 μg/ml, respectively. Among the β-lactam/β-lactamase inhibitor, overall susceptibility was 67%, 55% and 51% for C/T, P/T and C/S, respectively. The variations in the susceptibility rates were 16% for C/T versus C/S and 12% for C/T versus P/T, respectively. Interestingly, 33% susceptibility was noted for C/T against isolates that were resistant to P/T, indicating the higher activity of C/T. However, the number of isolates analysed are less. Hence, a large number of isolates needs to be tested to prove the superiority of C/T over other agents.

In silico analysis

The docking analysis has shown higher binding affinity for *ampC* with piperacillin and least binding affinity for *ampC* with ceftolozane. However, the binding affinity of-6.47 kcal/mol for *ampC* and tazobactam was noted, which was found to be in between the *ampC* and ceftolozane (−4.38) and *ampC* and piperacillin (−7.22). *AmpC* found to interact with ceftolozone through 13 amino acids (GLN120, THR290, SER289, ASN288, SER64, THR320, ASN321, SER319, ASN344, ASN347, PRO346, ALA348 and ARG350) and seven polar contacts. While for piperacillin, *ampC* found to react with 12 amino acids (TYR223, GLN129, ASN153, SER64, LYS67, LEU119, ASN344, TYR131, ALA293, ARG350, ASN347 and THR290) and three polar contacts. However, *ampC* with tazobactam showed interaction with 12 amino acids (SER319, ALA293, THR317, ASN344, TYR345, THR290, PRO346, ASN347, ASN288, SER289, ARG350 and ALA348) and eight polar contacts [Figure 1a-c].

Table 1: Antimicrobial Susceptibility profile to various agents as determined by Broth microdilution for *Pseudomonas aeruginosa*

Antimicrobials	Overall study isolates (*n*=149), *n* (%)	Piperacillin/tazobactam		Ceftazidime		Cefepime	
		Susceptible (*n*=82), *n* (%)	Non-susceptible (*n*=67), *n* (%)	Susceptible (*n*=90), *n* (%)	Non-susceptible (*n*=59), *n* (%)	Susceptible (*n*=95), *n* (%)	Non-susceptible (*n*=54), *n* (%)
Ceftazidime	90 (60)	75 (91)	15 (22)	90 (100)	0	84 (88)	6 (11)
Cefepime	95 (64)	78 (95)	17 (25)	84 (93)	11 (19)	95 (100)	0
C/S	79 (53)	74 (90)	5 (7)	71 (79)	8 (14)	77 (81)	2 (4)
P/T	82 (55)	82 (100)	0	75 (83)	7 (12)	78 (82)	4 (7)
C/T	100 (67)	78 (95)	22 (33)	85 (94)	15 (25)	91 (96)	9 (17)
Imipenem	100 (67)	75 (91)	25 (37)	78 (87)	22 (37)	82 (86)	18 (33)
Meropenem	93 (62)	73 (89)	20 (30)	75 (83)	18 (31)	83 (86)	10 (19)
Ciprofloxacin	91 (61)	76 (93)	15 (22)	80 (89)	11 (19)	86 (91)	5 (9)
Levofloxacin	91 (61)	76 (93)	15 (22)	80 (89)	11 (19)	86 (91)	5 (9)
Amikacin	98 (66)	79 (96)	19 (28)	83 (92)	15 (25)	91 (96)	7 (13)
Colistin	140 (94)	79 (96)	61 (91)	86 (96)	54 (92)	92 (97)	48 (89)

Percentage susceptible is mentioned in parentheses. C/T: Ceftolozane/tazobactam, C/S: Cefoperazone/sulbactam, P/T: Piperacillin/tazobactam

Table 2: Susceptibility of *Pseudomonas aeruginosa* to ceftolozane/tazobactam reported by various studies

Study	Number of isolates (*n*)	Study	Period	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Source of specimen
Farrell *et al*., 2013[5]	1971	US census region surveillance collection	2011-2012	0.5	2	BSI, PNM, SSSI, UTI, IAI
Sader *et al*., 2014[6]	2191	European Countries, Turkey, Israel surveillance collection	2011-2012	1	>32	BSI, PNM, SSTI, UTI, IAI and others
Farrell *et al*., 2014[7]	1019	US and Europe	2012	0.4	4	PNM
Buehrle *et al*., 2016[8]	38	University of Pitsburg Medical Center, USA	-	-	4	BSI, RTI
This study	149	India	2013-2014	1	≥16	BSI, IAI, UTI

BSI: Bloodstream infection, PNM: Pneumonia, SSTI: Skin and soft-tissue infection, UTI: Urinary tract infection, IAI: Intra-abdominal infection, RTI: Respiratory tract infection, MIC: Minimum inhibitory concentration

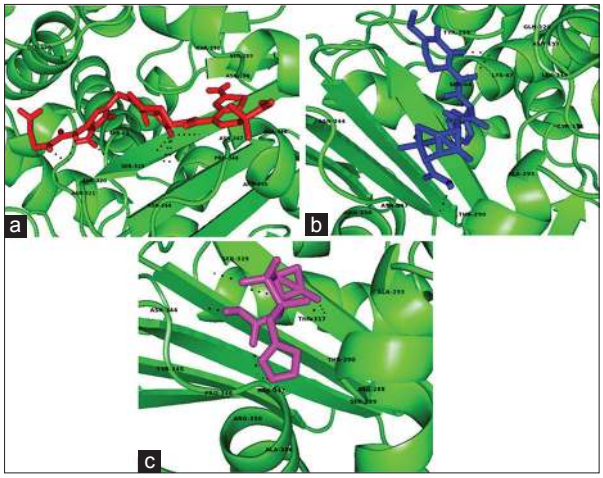

Figure 1: (a-c) PyMOL visualization showing the interaction between *AmpC* and antimicrobials. (a) Interaction with ceftolozone (b) Interaction with piperacillin (c) Interaction with tazobactam

Discussion

This study finding is in concurrence with the previously published *in vitro* studies; wherein, the susceptibility rates and MIC_{50} were almost similar as summarised in Table 2.

Further, this study data suggest that C/T will be effective than P/T and C/S, especially for managing infections due to drug-resistant *P. aeruginosa*. Buehrle *et al*. have attributed improved C/T activity, as it evades *ampC*-mediated hydrolysis in *P. aeruginosa*.[8] Further, ceftolozane escapes from the efflux mechanisms. It is well known that majority of the drug resistance in *P. aeruginosa* is due to over expression of efflux pumps. The notable difference in the activity of C/T over P/T and C/S could be due to overcoming these resistance mechanisms such as *ampC* and efflux. This finding has to be validated with testing a large number of isolates with known resistance mechanisms. However, the studies have shown mutations in the *ampC* confer resistance to C/T. Although it is intrinsic, mutations in the ampC have variable effects in the MIC of other cephalosporin agents as well, including C/T.[9,10]

This is the first report of testing C/T against clinical isolates from India showing 30% incremental increase in the susceptibility for beta-lactamase producers. This finding suggests about 33% of the P/T-resistant isolates can still be treated effectively with C/T. From the docking analysis, hydrolysis rate of piperacillin by *ampC* was higher due to the higher binding affinity, leading to hydrolysis of piperacillin. However, ceftolozane is less prone to hydrolysis by *ampC* due to the less binding affinity, results in the effective activity of ceftolozane. This concurs with the *in vitro* susceptibility testing, wherein ceftolozane activity is superior to piperacillin. To conclude, as the development of newer agents are minimal, the utility of higher antibiotics (carbapenems and colistin) can be minimised using C/T when available in India and found susceptible against the beta-lactamase producing *P. aeruginosa*.

Conclusion

Antimicrobial resistance has become a major concern across the world. C/T is a new beta-lactam/beta-lactamase inhibitor-based antimicrobial agent, which has shown to have an excellent antipseudomonal activity, as it by passes *ampC*-mediated resistance. Among the β-lactam/β-lactamase inhibitor, the activity of C/T was observed to be superior to P/T and C/S. This overall *in vitro* and *in silico* analysis elucidates that the C/T could be a better alternative to P/T for the treatment of infections due to *P. aeruginosa*.

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Conflicts of interest

There are no conflicts of interest.

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