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Comparative "real world" *in vitro* activity of two new antimicrobials (ceftolozane-tazobactam and ceftazidime-avibactam) against ceftazidime non-susceptible *Pseudomonas aeruginosa* and resistant *Enterobacteriaceae* from california long term acute care hospitals

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Abstract

Objectives: Long term acute care hospitals (LTACHs) cater to chronically, critically ill patients with prolonged durations of stay, significant co-morbidities and high rates of intravenous antibiotic utilization. Transmission dynamics between LTACHs and acute care hospitals (ACHs) have shown significant inter-relatedness. Consequently, LTACHs may act as reservoirs for multi-drug resistant organisms (MDR) organisms including *P. aeruginosa* and *Enterobacteriaceae* compared to ACHs. Choosing appropriate empirical therapy for these MDROs can lower mortality but is unpredictable and problematic. Recently, two new antimicrobials, ceftolozane-tazobactam (C/T) and ceftazidime-avibactam (CZA), both with in vitro activity against *Enterobacteriaceae* and *P. aeruginosa*, have been approved. To ascertain "real world" information about these organisms in LTACHs, we tested C/T and CZA against carbapenem-resistant *Enterobacteriaceae* and ceftazidime non-susceptible *P. aeruginosa* obtained from clinical isolates of infected patients at 12 LTACH's.

Design: Strains were isolated from unique clinical specimens submitted by twelve regional LTACHs to a centralized microbiology laboratory in southern California between March and December 2015. ETests® were performed according to the manufacturers' instructions and interpreted according to FDA criteria.

Results: CZA had very good activity against ESBL and CRE *Enterobacteriaceae*. For carbapenem-resistant *Klebsiella pneumoniae*, the MIC_{90} for CZA was 4/4 mcg/mL. CZA was active in vitro against carbapenem-resistant *Enterobacteriaceae* and to a lesser extent against ceftazidime resistant *P. aeruginosa*. C/T (88% susceptible) appeared to demonstrate better in vitro activity against ceftazidime non-susceptible strains of *P. aeruginosa* than did CZA (72% susceptible). There were no isolates that were susceptible to CZA but resistant to C/T.

Introduction

Multi-Drug Resistant (MDR) Gram-negative aerobic bacteria have become a worldwide concern that has been identified as an urgent global threat by the Centers for Disease Control and Prevention (CDC) [1]. This includes *Enterobacteriaceae* with β-lactam resistance due to extended-spectrum β-lactamases (ESBLs), carbapenem resistance (KPC, NDM, AmpC + porin changes) as well as MDR *P. aeruginosa,* including strains resistant to ceftazidime (AmpC and some OprD lacking isolates). The Infectious Disease Society of America (IDSA) launched two campaigns, "Bad Drugs-No Bugs" and "10 × 20" to encourage the development of new agents to combat this threat [2]. In the past two years, two new antimicrobials, ceftolozane-tazobactam (C/T) and ceftazidime-avibactam (CZA), both with *in vitro* activity against *Enterobacteriaceae* and *P. aeruginosa,* have been approved for complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI) with Phase 3 nosocomial pneumonia trials ongoing [3]. Ceftolozane is a novel β-lactam that was combined with tazobactam, an established penicillanic acid sulfone β-lactamase inhibitor. Ceftazidime is an established cephalosporin combined with avibactam, a new non- β-lactam β-lactamase inhibitor that inactivates select β-lactamases and protects ceftazidime from degradation. Concerns about the development of resistance to these newer agents have been raised with there being limited real-world clinical data available on their *in vitro* activity. This limited information is, in part, due to the lack of FDA approved commercially available tests for these newer antimicrobial agents [4].

Long term acute care hospitals (LTACHs) cater to chronically, critically ill patients who may have prolonged durations of stay, significant co-morbidities and high rates of intravenous antibiotic utilization. Approximately 50% of short term acute care (STACHs) patients with CRE were ultimately discharged to LTACHs so that, not

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surprisingly, there is a "9-fold higher prevalence rate of colonization" at LTACHS compared to STACHs [5]. Within Southern California LTACHs there is a 39.8% respiratory failure rate, 64.8% have tracheostomies and 50.9% have a central line in place [Han et al 2017] [5]. Consequently, LTACHs have an increased potential for serving as reservoirs for infections with MDR organisms, and the heavy exposure to antimicrobials and longer lengths of stay are likely to lead to increased prevalence and development of unique resistance patterns [5,6]. Han et al. [5] recently noted a higher incidence of resistant organisms for LTACHs compared to STACHs. Because of this greater propensity for antimicrobial resistance, we therefore studied the *in vitro* activity of C/T and CZA against clinical isolates of ceftazidime non-susceptible *P. aeruginosa* (tested C/T and CZA) and ESBL or carbapenem-resistant (CRE) *enterobacteriaceae* (tested CZA only) isolates obtained from LTACH patients.

Methods

Test strains, *enterobacteriaceae* isolates that were ESBL or CRE and *P. aeruginosa* isolates non-susceptible to ceftazidime, were isolated from clinical specimens submitted by twelve regional LTACHs to a centralized microbiology laboratory in Southern California between March and December 2015. Specimens were processed, and isolates were identified by standard criteria [7]. Duplicate isolates from the same patient were excluded. All isolates were identified by the VITEK® 2 with the GN card and initial susceptibility testing was performed with the AST-GN67 card according to manufacturer's instructions (bioMerieux, Durham, NC) and interpretations based on established guidelines [8]. C/T and CZA ETESTS® were obtained from Merck & Co, Inc. and Allergan, respectively, through IHMA (labeled for Research Use Only) and susceptibility tests were performed according to the manufacturer's recommendations were tested. Briefly: (a) A 0.5 McFarland suspension was prepared in sterile saline; (b) The inoculum was spread over the entire surface of a Mueller-Hinton II agar plate; (c) ETEST strips were placed onto the surface; (d) Plates were incubated in non-CO_2 at 37°C for 16-24 hours; (e) The MIC was read as the value where the ellipse of inhibition met the ETEST strip. Quality control tests using *escherichia coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853 were performed daily with acceptable results according to manufacturer's recommendations. C/T interpretive criteria for *P. aeruginosa* is ≤ 4/4 mcg/mL susceptible, 8/4 mcg/mL intermediate, and ≥ 16/4 mcg/mL resistant. CZA interpretive criteria for both *Enterobacteriaceae* and *P. aeruginosa* are ≤ 8/4 mcg/mL susceptible and ≥ 16/4 mcg/mL resistant.

Only the activity of CZA was tested against the *enterobacteriaceae* isolates, whereas both the activity of CZA and C/T were tested in *P. aeruginosa* isolates. For *P. aeruginosa* other resistance may be present, but isolates were further characterized only for imipenem resistance.

Results

P. aeruginosa

From the 111 ceftazidime non-susceptible *P. aeruginosa* isolates, there were 41 ceftazidime intermediate strains (16 mcg/mL) and 70 ceftazidime resistant strains (≥32 mcg/mL). All 111 *P.aeruginosa* were also non-susceptible to piperacillin/tazobactam (MIC > 16 mcg/ml). Of the ceftazidime resistant strains, 67/70 (96%) and 59/70 (84%) were also resistant to cefepime and imipenem, respectively; 98 of 111 isolates (88%) were susceptible to C/T. For isolates that were ceftazidime intermediate, 37/41 (90%) were susceptible to C/T and 34/41 (83%) were susceptible to CZA. For ceftazidime resistant isolates, 61/70 (87%) were susceptible to C/T and 46/70 (66%) were susceptible to CZA (Figure 1). There were no isolates that were susceptible to CZA but resistant to C/T. There were 107 isolates with imipenem susceptibility data; of these, 28/41 (68%) of the ceftazidime intermediate isolates were resistant to imipenem; of the 66 ceftazidime resistant isolates, 54 (82%) were imipenem resistant. Of the imipenem resistant isolates, 71/82 (87%) were susceptible to C/T while only 56/82 (68%) were susceptible to CZA. Overall, C/T (88% susceptible) appeared to demonstrate better *in vitro* activity against ceftazidime non-susceptible strains of *P. aeruginosa* than did CZA (72% susceptible). No *P. aeruginosa* isolates were subject to molecular testing to determine resistance mechanisms.

Enterobacteriaceae

All 29 ESBL-producing *enterobacteriaceae* (carbapenem-susceptible) strains were susceptible to CZA. There were 245 *enterobacteriaceae* tested with 242 (98.8%) that were susceptible to CZA (Table 1). Of the 232 carbapenem-resistant *K. pneumoniae* isolates, 99.6% were CZA susceptible. There was a total of 3 resistant isolates (*K. pneumoniae*, 48 mcg/mL; *Citrobacter freundii*, 32 mcg/mL; *E. coli*, 16 mcg/mL), but unfortunately all three were lost for subsequent testing and unavailable to confirm initial resistance or to be available for further evaluation of molecular resistance mechanisms**.** The CZA MIC_{50} and MIC_{90} for the carbapenem-resistant *K. pneumoniae,* respectively, were 2/4 and 4/4 mcg/mL. Overall, CZA had very good activity against ESBL and CRE. In our population of carbapenem-resistant *K. pneumoniae*, the MIC_{90} for CZA was 4/4 mcg/mL.

Table 1. The *in vitro* activity of CZA according to CRE or ESBL resistance, against *Enterobacteriaceae* strains isolated from 12 California LTACHs.

Enterobacteriaceae	Resistance Type	Number of strains Tested	Percent of Susceptible strains	Number of Resistant strains
K. pneumoniae	CRE	232	99.6	1
E. coli	CRE	5	80	1
C. freundii	CRE	3	67	1
E. cloacae	CRE	3	100	0
E. aerogenes	CRE	1	100	0
R. planticola	CRE	1	100	0
K. pneumoniae	ESBL	13	100	0
E. coli	ESBL	13	100	0
P. mirabilis	ESBL	2	100	0
M. morganii	Other	1	100	0

CRE: Carbapenem Resistant Enterobacteriaceae, ESBL: Extended Spectrum Beta-Lactamase, LTACH: Long Term Acute Care Hospitals

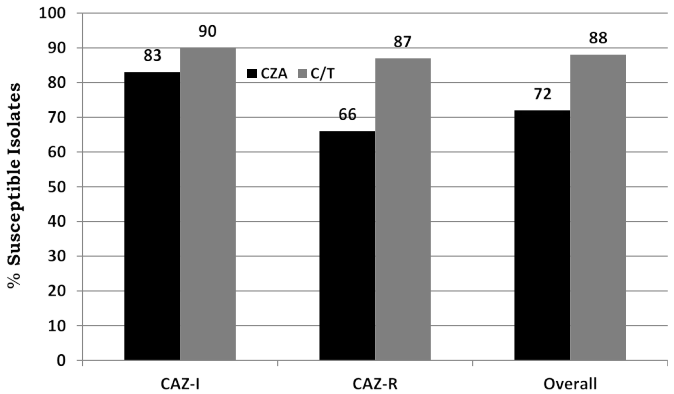

Figure 1. Comparison of percent susceptible to C/T and CZA vs ceftazidime (CAZ) non-susceptible *P. aeruginosa* isolates from 12 California LTACHs.

Discussion

P. aeruginosa has multiple resistance mechanisms, conferring limited therapeutic options choices. The National Healthcare Safety Network (NHSN) estimated that 19.3% of *P. aeruginosa* isolates in their surveillance studies are intermediate or resistant to carbapenems [9]. While this issue is emerging for STACs, the problem is even more dramatic for LTACHs that receive patients who have been heavily pretreated and exposed to multiple antimicrobial agents and often arrive with already developed resistant pathogen infections [5]. Two new agents active against resistant Gram-negative rods have recently been developed: C/T and CZA. Buehrle et al. [10] recently reported on the activity of both agents against 38 meropenem resistant *P. aeruginosa* isolates, none of which had carbapenemases and found C/T to be more active than CZA. They found that for *p. aeruginosa* strains resistant to carbapenems and all β-lactams that C/T was more active than CZA (67% vs 33% susceptible, respectively) [10]. Our study also indicated that C/T appeared more active than CAZ for resistant *P. aeruginosa* isolated from LTACHs. One study testing MDR *P. aeruginosa* indicated susceptibility to C/T at 86% while a second study indicated susceptibility of only 68%; [11,12] however, the latter study was comprised of isolates with higher resistance patterns than the US (Israel and parts of Europe). In one CZA report, 67.4% of *P. aeruginosa* isolates were susceptible if the strains were non-susceptible to ceftazidime, cefepime, piperacillin/tazobactam, and meropenem [13]. In a second study, 97% were susceptible to CZA, but most were ceftazidime susceptible (84%) isolates [14], however, if the isolates were ceftazidime, meropenem, and piperacillin/tazobactam resistant, then the CZA percent susceptible was 72%, but among all MDR *P. aeruginosa* strains, the CZA percent susceptible was 81%. In a more recent study [Humphries et al. 2017] [15], *P. aeruginosa* resistant to ceftazidime were shown to be 62% susceptible to C/T while 46% were susceptible to CZA and those strains with resistance to 5 anti-pseudomonal antimicrobials were 57% susceptible to C/T and 28% susceptible to CZA.

Nichols et al. [16] conducted a global surveillance study of *P. aeruginosa* isolates collected from 2010-2014 and found 8% (563 of 7,062 isolates), including 24.6% of meropenem-non-susceptible isolates, to be resistant to CZA. Miller et al. [17] performed a post-hoc analysis of a randomized phase III double blind trial of C/T plus metronidazole versus meropenem in cIAIs and reported a 100% cure rate (26/26) for C/T treated patient in whom *P. aeruginosa* was isolated.

More recently, Livermore et al. [2017] [18] assessed the activity of C/T against 6080 British Society for Antimicrobial Chemotherapy (BASC) surveillance isolates and 5471 referred problem organisms. They found C/T was active against 99.8% of the *P. aeruginosa* surveillance isolates and 99.7% of the referred isolates. They noted that C/T MICs were 2- to 8-fold lower than those of ceftazidime, except for isolates with metallo-beta-lactamases (MBLs) or ESBL. For *P. aeruginosa* isolates that were resistant to all beta-lactams studied, including ceftazidime and carbapenems, 51.9% were susceptible to C/T and 80.9% if they lacked "ESBL, MB and GES enzymes."

In the US, Shortridge et al. [19] studied 3,851 *P. aeruginosa* isolates from 32 US STACs and found 97% of the isolates were C/T susceptible. For meropenem non-susceptible isolates 87.6% were susceptible to C/T compared to 68% of those resistant to all beta-lactams. In comparison we found C/T to be active against 88% of LTACH *P. aeruginosa* isolates. This suggests the need for continued surveillance of *P. aeruginosa* LTACH isolates.

Our data showed that for *enterobacteriaceae*, CZA had 100% susceptibility to ESBL stains and 99.6% susceptibility to CRE strains. Two recent reports support this data. Livermore et al. [20] reported high CZA susceptibility rates to ESBL and CRE isolates with 87% susceptibility to all ceftazidime-resistant *enterobacteriaceae*. Sader et al. [21] reported 97.5% CZA susceptibility for CRE and > 99% susceptibility for ESBL producing *enterobacteriaceae*.

Conclusion

These two new agents seem to have different potential uses in LTACHs. CZA appears to be an active agent *in vitro* against carbapenem-resistant *enterobacteriaceae* but lesser activity against ceftazidime resistant *P. aeruginosa*. C/T appears to have better *in vitro* activity against ceftazidime non-susceptible *P. aeruginosa*. Continued surveillance and larger studies of LTACH isolates for the emergence of resistance seem warranted.

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