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IN VITRO ANTIMICROBIAL ACTIVITY OF CEFTOLOZANE/TAZOBACTAM AGAINST P. AERUGINOSA AND OTHER NON-FERMENTING GRAM-NEGATIVE ORGANISMS IN ADULTS WITH CYSTIC FIBROSIS

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HIGHLIGHTS

- A novel cephalosporin with activity towards non fermenting Gram-negative bacteria.
- Good *in vitro* antimicrobial activity against CF clinical isolates of *P. aeruginosa* in comparison to other antimicrobials.
- No activity against *B. cenocepacia* and *A. xylosoxidans*.

ABSTRACT

INTRODUCTION. Pulmonary exacerbations in people with Cystic Fibrosis (CF), with chronic Gram-negative pathogens, are associated with reduced survival. These pathogens are usually treated with repeated courses of systemic antibiotics. However there is a linked emergence of multidrug resistant (MDR) pathogens. Ceftolozane/tazobactam is a new cephalosporin/beta-lactamase inhibitor combination that has been demonstrated to have good activity against MDR *Pseudomonas aeruginosa*.

MATERIAL & METHODS. In this study ceftolozane/tazobactam was compared to other commonly used intravenous antibiotics against 193 non-fermenting Gram-negative bacteria isolated from CF sputum specimens, including *P. aeruginosa*, *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia* and *Burkholderia*. MICs

to ceftolozane/tazobactam were determined by standard E-test assay and interpreted according to current EUCAST guidelines.

RESULTS. Ceftolozane/tazobactam had good *in vitro* antimicrobial activity against CF clinical isolates of *P. aeruginosa* in comparison to other antimicrobials with the exception of colistin. Ceftolozane/tazobactam also had activity against *S. maltophilia*, but was not active against *B. cenocepacia* and *A. xylosoxidans*.

DISCUSSION. ceftolozane/tazobactam showed excellent *in vitro* activity against *P. aeruginosa* from CF clinical isolates. This antibiotic is a potential therapeutic option when presented with challenging MDR *P. aeruginosa* and *S. maltophilia* exacerbations. Further clinical experience and trials in CF are required to determine the place of this antimicrobial in clinical practice.

KEYWORDS: Ceftolozane/tazobactam; *P. aeruginosa*; *S. maltophilia*; respiratory infection; cystic fibrosis; antibiotic resistance.

INTRODUCTION

Pulmonary exacerbations of chronic respiratory infections significantly contribute to the morbidity and mortality among adults with Cystic Fibrosis (CF) [1]. These infections are usually dominated by non-fermenting Gram-negative bacteria (NF-GNB), e.g. *Burkholderia cenocepacia*, *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia* and especially *Pseudomonas aeruginosa* [2].

Aggressive antibiotic management of acute pulmonary exacerbations has contributed to improvements in the median expectation of life for adults affected by CF with a median predicted survival of 41 years [3]. However, the repeated use of antibiotics

aligns with the emergence of antimicrobial resistance [4]. The persistence of MDR bacterial strains in the lung is associated with poorer clinical outcomes [3].

Ceftolozane/tazobactam is a new extended-spectrum beta-lactam/beta-lactamase inhibitor combination approved by the European Medicines Agency (EMA) for the treatment of complicated urinary and intra-abdominal infections in adults [5].

Ceftolozane has a similar mechanism of action to other cephalosporin antibiotics. The addition of tazobactam increases the activity of this combination to include several extended-spectrum beta-lactamase-producing bacteria [6]. This new compound has demonstrated promising results in comparison with other beta-lactams against *P. aeruginosa* strains with various resistance patterns in non-CF isolates [7]. The purpose of this study was to evaluate the *in vitro* antibacterial activity of ceftolozane/tazobactam against *P. aeruginosa* and other NF-GNB isolates from CF patients.

METHODS

This study was performed on an anonymized historical collection of non-duplicated bacterial isolates from patients with CF attending the Northern Ireland Regional Adult CF Centre, Belfast City Hospital, UK. One hundred and twenty *P. aeruginosa* isolates, 34 *S. maltophilia* isolates and 39 *B. cenocepacia* isolates were recovered from CF sputum specimens between January 2001 to December 2010 (www.microark.com). *P. aeruginosa* reference strain NCTC 10662 was also included. Minimum inhibitory concentration (MIC) to ceftolozane/tazobactam for each isolate was determined by standard E-test assay based on the manufacturer's recommendations [8]. The results were interpreted according to current EUCAST

guidelines [9]. A clinical breakpoint of 4 mcg/mL was employed for *P. aeruginosa*. However as no species-specific breakpoint is currently available for other NF-GNB, the same breakpoint of ≤ 4 mcg/mL was used.

Susceptibilities for all others antimicrobials were determined by the modified Bauer–Kirby disc diffusion methodology following the EUCAST criteria [9]. Comparator antimicrobials were piperacillin/tazobactam (30/6 mcg), aztreonam (30 mcg), ceftazidime (30 mcg), meropenem (10 mcg), ciprofloxacin (5 mcg), tobramycin (10 mcg) and colistin (10 mcg). Testing of trimethoprim/sulphamethoxazole (25 mcg) and minocycline (30 mcg) were added for *S. maltophilia* and *B. cenocepacia* isolates.

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial classes, XDR was defined as susceptibility to only one or two categories and pan-Resistant was defined as non-susceptibility to all agents in all antimicrobial classes [10].

RESULTS

The median MIC for ceftolozane/tazobactam against *P. aeruginosa* was 1.5 mcg/mL; MIC₅₀ and MIC₉₀ were 1.5 mcg/mL and 8 mcg/mL, respectively. A total of 101/120 (84.2%) *P. aeruginosa* clinical isolates were sensitive to ceftolozane/tazobactam and in only two cases isolates were highly resistant (MIC >256). The percentage of resistant *P. aeruginosa* isolates for ceftolozane/tazobactam and other antimicrobial comparators is reported in Figure 1.

In this collection of strains, percentage of resistance for ceftolozane/tazobactam were lower than those for piperacillin/tazobactam, meropenem and aztreonam (Figure 1). In particular, ceftolozane/tazobactam was effective in most of meropenem-resistant *P. aeruginosa* isolates (20/34, median MIC 3).

Ceftolozane/tazobactam median MICs were significantly higher in ceftazidime and meropenem resistant isolates in comparison to susceptible isolates ($p=0.003$ and 0.013 , respectively); in particular, the 57/71 (80.3%) ceftazidime-resistant isolates were sensitive to ceftolozane/tazobactam, whilst all the isolates resistant to ceftolozane/tazobactam ($n=14$) were also resistant to ceftazidime. Antibiotic resistance among *P. aeruginosa* (defined as both MDR and XDR isolates) was observed in 51 isolates (42.5%), while pan-resistant isolates were 3 (2.5%). Ceftolozane/tazobactam median MIC in *P. aeruginosa* isolates increased markedly as the number of resistant antibiotic classes increased (Table 1).

The median MIC for ceftolozane/tazobactam against *S. maltophilia* was 1.75 mcg/ml; MIC₅₀ and MIC₉₀ were 1.5 mcg/ml and >256 mcg/ml, respectively. A specific clinical breakpoint for *S. maltophilia* has not been validated yet. However assuming a value of ≤ 4 mcg/mL, 20/34 (58.8%) of CF isolates were susceptible to ceftolozane/tazobactam. The percentage of isolates within the susceptible range for other antibiotics was as follows: 1/34 (2.9%) for ceftazidime, 27/34 (79.4%) for levofloxacin, 22/34 (64.7%) for trimethoprim/sulfamethoxazole and 31/34 (91.2%) for minocycline.

A. xylosoxidans and *B. cenocepacia* were resistant to ceftolozane/tazobactam (median MICs were 32 mcg/ml and >256 mcg/ml for *A. xylosoxidans* and *B. cenocepacia*, respectively) as well as to all the others antimicrobial comparators

DISCUSSION

This study demonstrates that ceftolozane/tazobactam has the lowest percentage of *in-vitro* antimicrobial resistances against *P. aeruginosa* within the beta-lactam group. In *P. aeruginosa* strains ceftolozane/tazobactam resistance was less than for the majority of antimicrobials, including aminoglycosides and fluoroquinolones. Only colistin had a better *in vitro* performance. Ceftolozane/tazobactam is a new antipseudomonal compound and not yet widely used in CF. A recent case report demonstrated the first successful use of ceftolozane/tazobactam to treat an acute respiratory exacerbation of CF caused by MDR *P. aeruginosa* [11, 12]. Our CF related-data is in agreement with previously published non-CF data [13], demonstrating that ceftolozane/tazobactam was active in the majority of meropenem-resistant *P. aeruginosa* isolates.

A subanalysis for *P. aeruginosa* showed that MICs for ceftolozane/tazobactam were higher as isolates became resistant to one, two or more antimicrobial classes. All isolates resistant to all antimicrobial classes, including colistin, were also resistant to ceftolozane/tazobactam. These results suggest that resistance mechanisms for ceftolozane/tazobactam may already exist in MDR isolates and such mechanisms are probably shared with other antibiotics.

Antimicrobial resistance to common beta-lactams in *S. maltophilia* was consistent with data published for non-CF patients [14]. However, it is encouraging to note ceftolozane/tazobactam performed well against *Stenotrophomonas* with almost 60% of isolates susceptible at a surrogated clinical breakpoint. In contrast ceftolozane/tazobactam had little *in vitro* effect against other NF-GNB such as *B. cenocepacia* and *A. xylosoxidans*.

This is the largest sample of NF-GNB tested for ceftolozane/tazobactam and the data suggests that it has a role in the treatment of *P. aeruginosa* resistant to commonly used beta-lactams and *S. maltophilia* isolates. Additionally, this is the first study to perform antibiotic susceptibility testing of ceftolozane/tazobactam on *B. cenocepacia* isolates confirming the highly resistant pattern of this group of bacteria.

The most notable limitation of this study is the data was generated from a single centre. Further large-scale studies including clinical NF-GNB isolates from different centres/countries are warranted to further corroborate our findings.

This collection of isolates dates back to the period 2001-2010 and the percentage of resistant pathogens may have changed since this epoch.

Our results should be interpreted under the understanding that : 1) the susceptibility profiles studied here may not reflect the *in vivo* activity in patients with CF, since microorganisms tested may be in a biofilm-mode of growth and we are not able to report any differences between mucous versus non-mucous strains; 2) the current clinical breakpoint for ceftolozane/tazobactam is calculated for abdominal or complicated urinary tract infections in non-CF population. A definite breakpoint for *P. aeruginosa* isolated from CF sputum as well as other non-fermenting pathogens has not been determined as yet.

Lastly, although microdiffusion is usually preferred to test MIC for colistin, in this study MIC values have been obtained following disc diffusion methodology.

In conclusion, ceftolozane/tazobactam showed excellent *in vitro* activity against *P. aeruginosa* from CF clinical isolates. This antibiotic is a potential therapeutic option

when presented with challenging MDR *P. aeruginosa* and *S. maltophilia* exacerbations. Further clinical experience and trials in CF are required to determine the place of this antimicrobial in clinical practice.

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Ethical Approval: Not applicable

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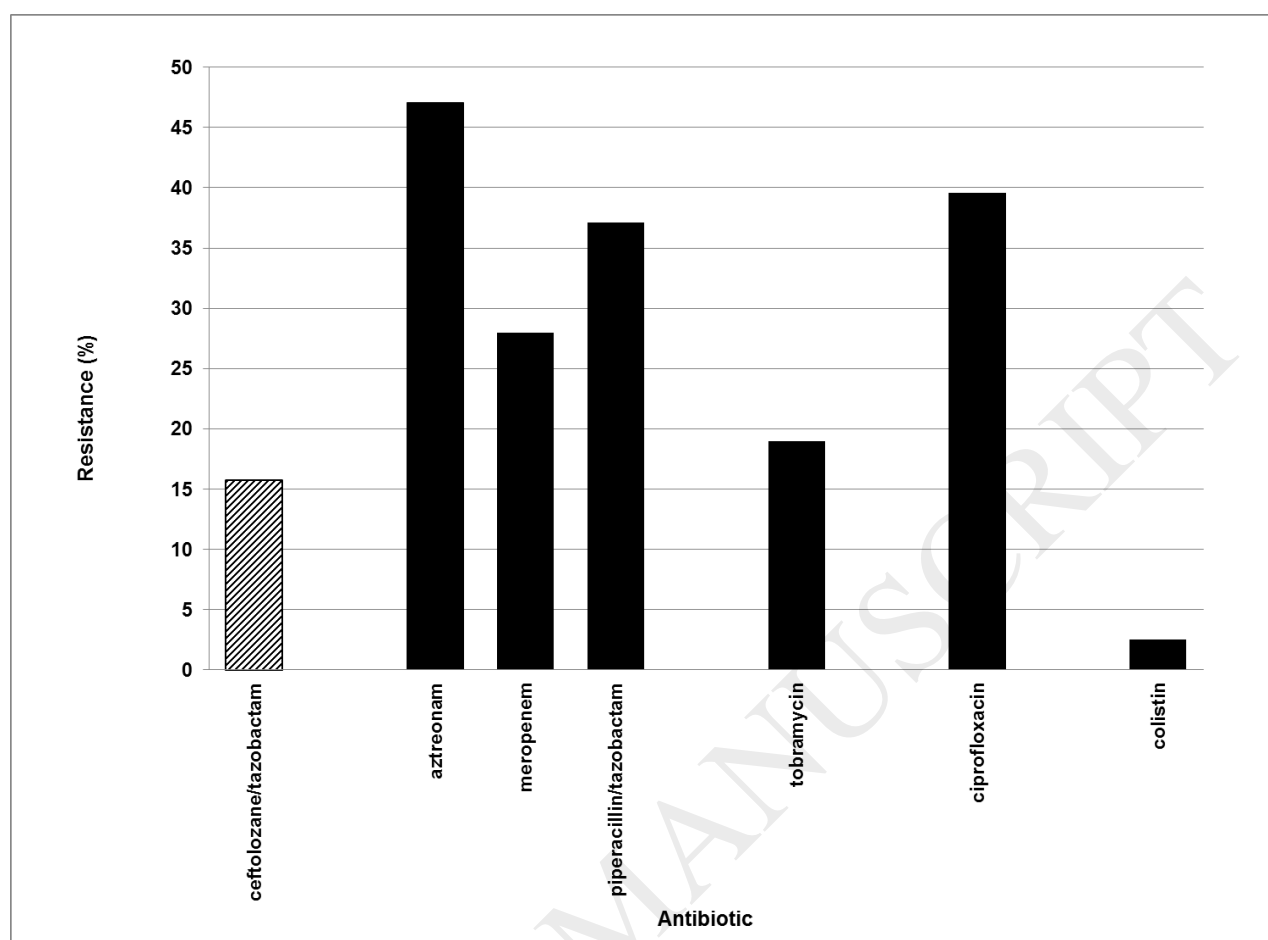


Figure 1: Percentage of *Pseudomonas aeruginosa* isolates resistant to ceftolozane/tazobactam and other commonly used anti-pseudomonal antibiotics.

Table 1 – Antibiotic susceptibility of *Pseudomonas aeruginosa* isolates to ceftolozane/tazobactam according to resistance to other antimicrobials

<i>Pseudomonas aeruginosa</i> isolates	MIC (range) [mcg/mL]
MDR <i>Pseudomonas</i> isolates (n=29)	1.5 (0.75 - >256)
XDR <i>Pseudomonas</i> isolates (n=22)	3.5 (0.75 - >256)
Pan-Resistant <i>Pseudomonas</i> isolates (n=3)	256 (>256 - >256)