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Microbiological characterisation of *Enterobacterales* baseline qualifying urinary pathogens obtained from adult patients participating in the ALLIUM Phase 3 study comparing cefepime/enmetazobactam to piperacillin/tazobactam for the treatment of complicated urinary tract infections/acute pyelonephritis

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INTRODUCTION

- Treatment options for infections caused by extended spectrum β-lactamase (ESBL)-producing Gram-negative pathogens are limited and typically involve the use of carbapenems, a class of antibacterial agents considered of 'last-resort' [1].
- Increased carbapenem usage has led to increased prevalence of carbapenem-resistance determinants such as *Klebsiella pneumoniae* carbapenemases (KPC), metallo-β-lactamases and OXA-type carbapenemases [2,3].
- There is a critical need for novel carbapenem-sparing therapies exhibiting potent activity against ESBL-producing pathogens.
- The 4th generation cephalosporin cefepime combined with the novel extended spectrum β-lactamase (ESBL) inhibitor enmetazobactam is intended as a carbapenem-sparing option to treat serious infections caused by ESBL-producing pathogens [4,5].
- Superior treatment outcomes with cefepime/enmetazobactam (FPE) compared to piperacillin/tazobactam (PTZ) was demonstrated in the ALLIUM Phase 3 study of adult patients with cUTI/AP [6].
- In this study, the in vitro susceptibility profile and β-lactamase genotypes of *Enterobacterales* baseline qualifying urinary pathogens obtained from ALLIUM patients were investigated.

METHODS

- A total of 1034 cUTI/AP patients randomized 1:1 in a double-blind, multicenter trial received either 2 g cefepime/0.5 g enmetazobactam or 4 g piperacillin/0.5 g tazobactam q8h by 2h infusion for 7-14 days.
- Ninety sites in Europe (Belarus, Bulgaria, Croatia, Estonia, Georgia, Hungary, Latvia, Lithuania, Poland, Russia, Serbia, Slovakia, Spain, and Ukraine), North and Central America (United States and Mexico), South America (Argentina and Peru) and South Africa enrolled patients. The study was designed according to current regulatory guidelines and was conducted in accordance with the Declaration of Helsinki, all applicable country laws and regulations, and in compliance with Good Clinical Practice Guidelines.
- Urine samples were collected using local standard practices and target baseline pathogens (BP) were sent to the central laboratory (IHMA, Schaumburg, IL) for confirmation of identification, antimicrobial susceptibility testing, and select molecular characterization. Isolates were identified to the species level using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltronics, Bremen, Germany; library version MBT Compass 4.1.90.; version 4.1.100 after 12/2019) [5].
- *Enterobacterales* urinary baseline pathogens were analysed from both the primary efficacy population mMITT (n=663 of 690 total BP) and the secondary population mMITT+R (n=704 of 787 total BP):
  - mMITT included pathogens with FPE MICs≤8 µg/ml or PTZ MICs≤64 µg/ml
  - mMITT+R included pathogens in mMITT as well as those resistant to either agent (FPE MIC ≥16 µg/ml; PTZ MIC≥128 µg/ml) or with a missing MIC determination
- Broth microdilution MIC testing was performed according to CLSI guidelines [7]. Quality control testing was performed with *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NCTC 13358, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 on each day to ensure appropriate assay performance [8]. Susceptibility was determined using CLSI and EUCAST breakpoints [9,10]. FPE breakpoints have not yet been assigned and for comparative purposes, the CLSI breakpoints for cefepime at 2 µg/ml and the susceptible-dose-dependent breakpoint of 8 µg/ml were used. FPE MIC was determined with a fixed enmetazobactam concentration of 8 µg/ml [7].
- Clinical isolates of *Enterobacterales* with MIC ≥1 µg/ml to ceftazidime, ceftriaxone, cefepime, meropenem, or FPE were genotyped for β-lactamases. Genotyping was performed by multiplex PCR on *Enterobacterales* isolates followed by sequencing to identify variants of genes encoding class A ESBLs (CTX-M, SHV, TEM, VEB, PER and GES) and KPC, class B metallo-β-lactamases (IMP, VIM, NDM, GIM and SPM), class C AmpCs (ACC, CMY I/MOX, CMY II, DHA, FOX, ACT-MIR, PDC) and class D β-lactamases (OXA-type) [5].

RESULTS

1. *E. coli* and *K. pneumoniae* are the predominant *Enterobacterales* baseline pathogens in ALLIUM

Table 1. *Enterobacterales* baseline pathogens obtained in the mMITT and mMITT+R populations

Enterobacterales species	mMITT (n=663)	mMITT+R (n=704)
	n (%)	n (%)
Escherichia coli	518 (78.1)	526 (74.7)
Klebsiella pneumoniae	66 (10.0)	92 (13.1)
Proteus mirabilis	38 (5.7)	40 (5.7)
Enterobacter cloacae	10 (1.5)	14 (2.0)
Serratia marcescens	7 (1.1)	7 (1.0)
Klebsiella oxytoca	6 (0.9)	6 (0.9)
Citrobacter koseri	3 (0.5)	3 (0.4)
Citrobacter freundii	2 (0.5)	2 (0.3)
Enterobacter bugandensis	2 (0.5)	2 (0.3)
Klebsiella variicola	2 (0.5)	2 (0.3)
Morganella morganii	2 (0.5)	2 (0.3)
Providencia rettgeri	2 (0.5)	2 (0.3)
Citrobacter amalonaticus	1 (0.2)	1 (0.1)
Citrobacter braakii	1 (0.2)	1 (0.1)
Enterobacter, non-speciated	1 (0.2)	1 (0.1)
Enterobacter xianfangensis	0 (0.0)	1 (0.1)
Proteus vulgaris	1 (0.2)	1(0.1)
Serratia liquefaciens	1 (0.2)	1 (0.1)

2A. Of the *Enterobacterales* baseline pathogens in the primary efficacy population (mMITT), 21.0% encoded an ESBL.

Table 2A. β-lactamase genotypes of the *Enterobacterales* baseline pathogens obtained in the mMITT population

Organism	% (n) of isolates within species producing β-lactamases (n=160)	% (n) of species with β-lactamase genotype			
		ESBL (±OSBL)[1]		AmpC	OSBL
		Only	+AmpC		
All *Enterobacterales* (n=663)	24.1 (160)	20.7 (137)	0.8 (5)	1.2 (8)	1.5 (10)
E. coli (n=518)	23.4 (121)	20.5 (106)	0.6 (3)	0.8 (4)	1.6 (8)
K. pneumoniae (n=66)	36.4 (24)	30.3 (20)	0.0 (0)	3.0 (2)	3.0 (2)
P. mirabilis (n=38)	21.1 (8)	15.8 (6)	2.6 (1)	2.6 (1)	0.0 (0)
E. cloacae (n=10)	50.0 (5)	40.0 (4)	0.0 (0)	10.0 (1)	0.0 (0)
C. braakii (n=1)	100 (1)	100 (1)	0.0 (0)	0.0 (0)	0.0 (0)
C. freundii (n=2)	50 (1)	0.0 (0)	50 (1)	0.0 (0)	0.0 (0)

[1]*OSBL refers to original spectrum ß-lactamases such as non-ESBL variants of SHV or TEM.*

2B. In the mMITT+R population, 22.9% of the *Enterobacterales* baseline pathogens encoded an ESBL whereas 1.8% encoded a β-lactamases conferring resistance to carbapenems (MBL, OXA-48 or KPC).

Table 2B. β-lactamase genotypes of the *Enterobacterales* baseline pathogens obtained in the mMITT+R population

Organism	% (n) of isolates within species producing β-lactamases (n=195)	% (n) of species with β-lactamase genotype							
		ESBL (±OSBL)[1]					AmpC	KPC	OSBL
		Only	+AmpC	+AmpC+MBL[2]	+MBL	+OXA			
All *Enterobacterales* (n=704)	27.7 (195)	22.2 (156)	0.7 (5)	0.7 (5)	0.4 (3)	0.6 (4)	1.3 (9)	0.1 (1)	1.7 (12)
E. coli (n=526)	23.6 (124)	20.5 (108)	0.6 (3)	0.0 (0)	0.0 (0)	0.0 (0)	1.0 (5)	0.0 (0)	1.5 (8)
K. pneumoniae (n=92)	54.3 (50)	34.8 (32)	0.0 (0)	4.3 (4)	3.3 (3)	4.3 (4)	2.2 (2)	1.1 (1)	4.3 (4)
P. mirabilis (n=40)	22.5 (9)	14.6 (6)	2.4 (1)	2.4 (1)	0.0 (0)	0.0 (0)	2.4 (1)	0.0 (0)	0.0 (0)
E. cloacae (n=14)	64.3 (9)	57.1 (8)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	7.1 (1)	0.0 (0)	0.0 (0)
E. xiangfangensis (n=1)	100 (1)	100 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
C. braakii (n=1)	100 (1)	100 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
C. freundii (n=2)	50.0 (1)	0.0 (0)	50.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)

[1]*OSBL refers to original spectrum ß-lactamases such as non-ESBL variants of SHV or TEM.* [2]*MBL refers to metallo-ß-lactamases.*

3. Cefepime/enmetazobactam exhibited comparable *in vitro* antibacterial activity to meropenem and was more potent than piperacillin/tazobactam against the *Enterobacterales* baseline pathogens.

Table 3A. Summary of MIC and susceptibility results of the *Enterobacterales* baseline pathogens and selected organism groups in the mMITT population

Organism group/antibacterial agent	MIC (µg/ml)			CLSI			EUCAST	
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%R
All *Enterobacterales* (n=663)								
Cefepime[1]	0.06	64	≤0.008->64	79.5	4.7	15.8	77.7	18.4
Meropenem	≤0.015	0.03	≤0.015-0.5	100.0	0.0	0.0	100.0	0.0
Piperacillin-tazobactam	1	8	≤0.25-64	96.1	3.9	0.0	92.5	7.5
Cefepime-enmetazobactam[2]	0.03	0.12	≤0.008-4	*99.7*	*0.3*	*0.0*	*99.2*	*0.0*
Enterobacterales, ESBL positive (n=142)								
Cefepime	64	>64	0.03->64	12.7	16.2	71.1	5.6	81.7
Meropenem	0.03	0.06	≤0.015-0.25	100.0	0.0	0.0	100.0	0.0
Piperacillin-tazobactam	4	16	≤0.25-64	90.8	9.2	0.0	81.7	18.3
Cefepime-enmetazobactam	0.06	0.12	0.015-4	*98.6*	*1.4*	*0.0*	*97.2*	*0.0*
E. coli (n=518)								
Cefepime	0.03	64	≤0.008->64	80.1	5.0	14.9	78.4	17.8
Meropenem	≤0.015	0.03	≤0.015-0.06	100.0	0.0	0.0	100.0	0.0
Piperacillin-tazobactam	1	8	≤0.25-64	97.3	2.7	0.0	93.6	6.4
Cefepime-enmetazobactam	0.03	0.06	≤0.008-4	*99.6*	*0.4*	*0.0*	*99.0*	*0.0*
K. pneumoniae (n=66)								
Cefepime	0.06	>64	0.03->64	69.7	3.0	27.3	69.7	28.8
Meropenem	0.03	0.06	≤0.015-0.5	100.0	0.0	0.0	100.0	0.0
Piperacillin-tazobactam	2	32	0.5-64	89.4	10.6	0.0	83.3	16.7
Cefepime-enmetazobactam	0.03	0.12	0.015-1	*100.0*	*0.0*	*0.0*	*100.0*	*0.0*

Table 3B. Summary of MIC and susceptibility results of the *Enterobacterales* baseline pathogens and selected organism groups in the mMITT+R population

Organism group/antibacterial agent	MIC (µg/ml)			CLSI			EUCAST	
	MIC_{50}	MIC_{90}	Range	%S	%I	%R	%S	%R
All *Enterobacterales* (n=704)								
Cefepime[1]	0.06	>64	≤0.008->64	75.9	4.7	19.5	74.0	21.9
Meropenem	≤0.015	0.06	≤0.015->32	97.9	0.6	1.6	98.4	1.3
Piperacillin-tazobactam	1	16	≤0.25->256	91.1	3.8	5.1	87.5	12.5
Cefepime-enmetazobactam[2]	0.03	0.12	≤0.008->64	*98.2*	*0.4*	*1.4*	*97.3*	*1.4*
Enterobacterales, ESBL positive[3] (n=165)								
Cefepime	64	>64	0.03->64	11.5	13.9	74.5	4.8	83.6
Meropenem	0.03	0.12	≤0.015->32	95.8	2.4	1.8	98.2	1.2
Piperacillin-tazobactam	4	>256	≤0.25->256	78.2	8.5	13.3	70.3	29.7
Cefepime-enmetazobactam	0.06	0.5	0.015-16	*97.6*	*1.8*	*0.6*	*95.2*	*0.6*
E. coli (n=526)								
Cefepime	0.06	64	≤0.008->64	79.5	5.3	15.2	77.8	18.1
Meropenem	≤0.015	0.03	≤0.015-0.06	100.0	0.0	0.0	100.0	0.0
Piperacillin-tazobactam	1	8	≤0.25->256	96.2	2.7	1.1	92.6	7.4
Cefepime-enmetazobactam	0.03	0.06	≤0.008-4	*99.6*	*0.4*	*0.0*	*98.9*	*0.0*
K. pneumoniae (n=92)								
Cefepime	0.25	>64	0.03->64	53.3	2.2	44.6	52.2	45.7
Meropenem	0.03	4	≤0.015->32	83.7	4.3	12.0	88.0	9.8
Piperacillin-tazobactam	4	>256	0.5->256	64.1	8.7	27.2	59.8	40.2
Cefepime-enmetazobactam	0.06	4	0.015->64	*89.1*	*1.1*	*9.8*	*89.1*	*9.8*

Abbreviations: S, susceptible; I, intermediate; R, resistant. [1]Addition of %S and %I provides susceptibility at the cefepime susceptible-dose dependent breakpoint of 8 µg/ml. [2]Cefepime/enmetazobactam MIC was determined using a fixed enmetazobactam concentration of 8 µg/ml. Breakpoints for cefepime-enmetazobactam have not been established and utilized the cefepime breakpoints. [3]Excludes ESBL-producing isolates that co-express metallo-β-lactamases.

CONCLUSIONS

- Urinary *Enterobacterales* baseline pathogens in ALLIUM were typical of those causing cUTI /AP, with a majority being *E. coli* and *K. pneumoniae.*
- ESBL were the predominant β-lactamases encoded by more than 20% of the *Enterobacterales* baseline pathogens, emphasising the critical need for novel carbapenem-sparing therapies.
- Unlike piperacillin/tazobactam, cefepime/enmetazobactam *in vitro* antibacterial activity was unaffected by the presence of ESBL thereby supporting its development as a novel carbapenem-sparing therapy.

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