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Short Communication

Susceptibility to cephalosporin combinations and aztreonam/avibactam among third-generation cephalosporin-resistant Enterobacteriaceae recovered on hospital admission

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ABSTRACT

As part of the multicentre Antibiotic Therapy Optimisation Study (ATHOS), minimum inhibitory concentrations (MICs) were determined for cephalosporins alone and in combination with the β-lactamase inhibitors tazobactam, clavulanic acid and avibactam against third-generation cephalosporin-resistant *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. isolates collected in German hospitals. $MIC_{50/90}$ values were 0.25–4 mg/L for cefepime/tazobactam, 0.25–2 mg/L for ceftazidime/avibactam, 0.125–0.5 mg/L for ceftaroline/avibactam, 0.5–4 mg/L for cefpodoxime/clavulanic acid and 0.25–1 mg/L for aztreonam/avibactam, depending on the underlying resistance mechanism and organism. Based on in vitro testing, β-lactam antibiotics play an important role in the treatment of infections due to β-lactamase-producing organisms.

1. Introduction

Resistance to β-lactam antibiotics among Gram-positive and -negative organisms remains one of the most significant threats to the efficacy of this class of antimicrobial agents [1]. Such Gram-negative pathogens mainly include *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [2]. Third-generation cephalosporin-resistant Enterobacteriaceae (3GCREB) have spread dramatically over the last decades. Among 3GCREB, resistance is mostly due to β-lactam-hydrolysing enzymes, particularly extended-spectrum β-lactamases (ESBLs), which is a heterogeneous group of enzymes. Inducible AmpC-type β-lactamases occur on the chromosome of *Enterobacter*, *Citrobacter*, *Morganella* and *Serratia* spp. [3]. AmpC-type β-lactamases may also be localised on transmissible plasmids, including in *Escherichia coli*, *K. pneumoniae*, *Salmonella* spp. and *Proteus mirabilis* [4]. Although clinical outcome with the use of several β-lactams, particularly third- and fourth-generation cephalosporins and aminopenicillins, in the treatment of infections caused by ESBL- and AmpC-producing isolates remains to be fully evaluated, isolates may be reported susceptible to one or more β-lactam agents according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, which is supported by several clinical studies and observations, pharmacokinetic/pharmacodynamic data, Monte Carlo simulations and animal model studies [5,6].

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The aim of the present study was to evaluate the minimum inhibitory concentration (MIC) distributions of cephalosporins alone and in combination with various β-lactamase inhibitors in a collection of German 3GCREB isolates from different tertiary care hospitals across the country. The study is part of the multicentre Antibiotic Therapy Optimisation Study (ATHOS), the largest prevalence study of 3GCREB carriage upon hospital admission in Europe, and delivers important insight into susceptibility distributions among German multidrug-resistant organisms (MDROs) [7].

2. Materials and methods

2.1. Study design

Isolates were collected in 2014 at six large tertiary care hospitals covering the North, West, East, Southwest and Southeast of Germany as part of ATHOS. Centres had between 1300 and 3200 inpatient beds. Patients aged ≥18 years from general wards who had been admitted between June and December 2014 and who gave their informed consent were included in the study. Patients from intensive care units, dermatology, obstetrics, ophthalmology, otorhinolaryngology and psychiatry were excluded. The study was approved by the institutional ethics committees.

2.2. Selection of isolates

Study patients were screened for 3GCREB colonisation within 72 h of admission using rectal swabs or stool samples. Screening of patients for 3GCREB was done by plating rectal swabs or stool samples on ChromID™ ESBL agar (bioMérieux, Marcy-l'Étoile, France). Species identification of isolates growing on ESBL agar was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) or using the VITEK®2 GN ID card (bioMérieux). Susceptibility testing was carried out using a VITEK®2 system (bioMérieux). All isolates that were non-susceptible to cefotaxime, ceftriaxone or ceftazidime according to EUCAST breakpoints were included in the study and were further characterised. Phenotypic determination of ESBL production was performed with the combination disk test as recommended by EUCAST, using cefotaxime, ceftazidime and cefepime ± clavulanate acid, and isolates were tested for AmpC production by cefoxitin–cloxacillin disk test [8,9].

2.3. Antimicrobial susceptibility testing

Study isolates underwent susceptibility testing for carbapenems (meropenem and ertapenem), cephalosporins (cefotaxime, ceftazidime, cefepime, ceftaroline and ceftobiprole) and the combinations cefepime/tazobactam, ceftazidime/avibactam, ceftaroline/avibactam, cefpodoxime/clavulanic acid and aztreonam/avibactam. Isolates were tested by broth microdilution antimicrobial susceptibility testing using a MICRONAUT system (Merlin Diagnostika, Bornheim-Hersel, Germany) [10] according to standard procedures (ISO 20776-1:2006). The following concentration ranges were included: meropenem, 0.5–64 mg/L; ertapenem, 0.125–16 mg/L; cefotaxime, 0.25–32 mg/L; ceftazidime, 0.25–32 mg/L; cefepime, 0.25–32 mg/L; ceftaroline, 0.125–1 mg/L; ceftobiprole, 0.0625–0.5 mg/L; cefepime/tazobactam, 0.25/4–32/4 mg/L; ceftazidime/avibactam, 0.25/4–32/4 mg/L; ceftaroline/avibactam, 0.125/4–1/4 mg/L; cefpodoxime/clavulanic acid, 0.5/4–2/4 mg/L; and aztreonam/avibactam, 0.25/4–32/4 mg/L. Results were interpreted according to EUCAST breakpoints (http://www.eucast.org; accessed 24 July 2016), except for cefepime/tazobactam, ceftazidime/avibactam, ceftaroline/avibactam, cefpodoxime/clavulanic acid and aztreonam/avibactam, for which no EUCAST clinical breakpoints are currently available for systemic infections.

3. Results

A total of 328 *Escherichia coli*, 35 *Klebsiella* spp. (1 *Klebsiella oxytoca* and 34 *K. pneumoniae*) and 16 *Enterobacter* spp. (1 *Enterobacter aerogenes* and 15 *Enterobacter cloacae*) non-susceptible to third-generation cephalosporins were available for susceptibility testing. Isolates had been molecularly characterised previously, which allowed allocation of the tested MIC to the resistance mechanisms (Tables 1 and 2) [7]. Carbapenemase-producing isolates from the ATHOS prevalence study were not included in the current study. All tested isolates were fully susceptible to meropenem and ertapenem (Table 3). Results of MIC tests are shown in Tables 1–3.

4. Discussion

Among the 3GCREB in this study, the dominant β-lactamase was CTX-M-type ESBLs, whilst the prevalence of AmpC and non-CTX-M ESBL (mainly TEM and SHV) is generally low in Europe [11,12]. All of the isolates were susceptible to meropenem and ertapenem as shown by the low MICs of these two antimicrobial agents against

Table 1
Minimum inhibitory concentrations (MICs) of cefepime/tazobactam, ceftazidime/avibactam and ceftaroline/avibactam.[a]

Isolate/phenotype	MIC (mg/L)														
	Cefepime + tazobactam (4 mg/L)					Ceftazidime + avibactam (4 mg/L)					Ceftaroline + avibactam (4 mg/L)				
	≤0.25	0.5–1	2–4	>4	$MIC_{50/90}$	≤0.25	0.5–1	2–4	>4	$MIC_{50/90}$	≤0.125	0.25	0.5	>0.5	$MIC_{50/90}$
Escherichia coli (*n* = 328)															
Hyperproduced AmpC (*n* = 5)	4	1			0.25/1	5				0.25/0.25	5				0.125/0.125
CTX-M (*n* = 303)	296	6	1		0.25/0.25	302	1			0.25/0.25	299	4			0.125/0.125
SHV ESBL (*n* = 14)	14				0.25/0.25	14				0.25/0.25	14				0.125/0.125
TEM ESBL (*n* = 2)	1	1			0.5/0.5	2				0.25/0.25	2				0.125/0.125
CTX-M + hyperproduced AmpC (*n* = 2)	1		1		4/4	1		1		2/2				2	0.5/0.5
CTX-M + TEM ESBL (*n* = 2)	2				0.25/0.25	2				0.25/0.25	2				0.125/0.125
Klebsiella spp. (*n* = 35)															
CTX-M (*n* = 28)	28				0.25/0.25	27	1			0.25/0.25	28				0.125/0.125
SHV ESBL (*n* = 5)	5				0.25/0.25	5				0.25/0.25	5				0.125/0.125
CTX-M + SHV ESBL (*n* = 2)	2				0.25/0.25	2				0.25/0.25	2				0.125/0.125
Enterobacter spp. (*n* = 16)															
Hyperproduced AmpC (*n* = 12)	4	4	4		0.5/4	3	9			0.5/0.5	4	4	4		0.25/0.5
CTX-M + hyperproduced AmpC (*n* = 4)	2	1	1		0.5/2	4				0.25/0.25	1	2	1		0.25/0.5

$MIC_{50/90}$, MICs for 50% and 90% of the isolates, respectively; ESBL, extended-spectrum β-lactamase.
[a] European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints are not available for any of the combinations.