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Antimicrobial activity of ceftazidime–avibactam and comparator agents when tested against bacterial isolates causing infection in cancer patients (2013–2014)

Helio S. Sader *, Mariana Castanheira, Ronald N. Jones, Robert K. Flamm

JMI Laboratories, North Liberty, IA, USA

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ABSTRACT

We evaluated the antimicrobial susceptibility of 623 Gram-negative organisms causing infection in patients with cancer in 52 United States hospitals (2013–2014) as part of the International Network for Optimal Resistance Monitoring (INFORM) program. Isolates were tested for susceptibility by broth microdilution method. β-lactamase encoding genes were evaluated for all *Escherichia coli* and *Klebsiella* spp. with an extended-spectrum β-lactamase (ESBL) phenotype by microarray-based assay. ESBL-phenotype was observed among 17.3 and 9.9% of *E. coli* and *Klebsiella pneumoniae*, respectively; and 25.0% of *Enterobacter cloacae* were ceftazidime-non-susceptible. All Enterobacteriaceae ($n = 486$) were susceptible to ceftazidime-avibactam ($MIC_{50/90}$, 0.12/0.25 μg/mL) with the highest MIC value at 1 μg/mL. Meropenem was active against Enterobacteriaceae overall ($MIC_{50/90}$, ≤0.06/≤0.06 μg/mL; 99.6% susceptible); but showed more limited activity against *Klebsiella* spp. with an ESBL-phenotype (84.6% susceptible) and multidrug-resistant Enterobacteriaceae (93.3% susceptible). The most active agents tested against *Pseudomonas aeruginosa* were colistin (100.0% susceptible), amikacin (97.7% susceptible) and ceftazidime-avibactam (96.6% susceptible).

1. Introduction

Infection remains a significant cause of excess morbidity and premature mortality among cancer patients. The spectrum of infection continues to change, and it is influenced by various factors, including local epidemiology, the use of chemoprophylaxis, and the use of central venous catheters and other medical devices (Nesher and Rolston, 2014). Empirical antimicrobial treatment using broad spectrum agents must be started immediately in neutropenic patients with severe infection. Rapid introduction of effective antimicrobial therapy is decisive and knowledge of local microbiology is crucial for the selection of an empirical antimicrobial agent (Penack et al., 2014).

Avibactam is a novel broad-spectrum non-β-lactam β-lactamase inhibitor with activity against common serine β-lactamase enzymes, including Ambler class A (e.g., ESBL and KPC), class C (Amp C) and some class D (OXA-48) enzymes (Bush, 2015; Li et al., 2015; Zhanel et al., 2013). The addition of avibactam to ceftazidime restores ceftazidime activity against common Gram-negative pathogens, including most of those that are resistant to carbapenem agents (e.g. meropenem) due to the production of β-lactamase enzymes. Ceftazidime-avibactam has recently been approved by the United States (US) Food and Drug Administration (FDA) for the treatment of complicated intra-abdominal infection (cIAI), in combination with metronidazole, as well as complicated urinary tract infections, including pyelonephritis, in patients with limited or no alternative treatment options (Avycaz®, 2015). Ceftazidime-avibactam is also under clinical development for treatment of nosocomial pneumonia (NCT01808092). We evaluated the activity of ceftazidime-avibactam against contemporary (2013–2014) isolates causing infection in patients with cancer in US medical centers.

2. Materials and methods

2.1. Bacterial isolates

A total of 623 Gram-negative organisms were collected from patients hospitalized in the oncology units of 52 US hospitals. Bacterial isolates were consecutively collected (one per patient episode) between January 2013 and December 2014 as part of the International Network for Optimal Resistance Monitoring (INFORM) program (Sader et al., 2014). According the INFORM program protocols, participant medical centers were requested to consecutively collect a defined number of patient unique isolates for certain infection types, including bloodstream infection, pneumonia, skin and skin structure infection and urinary infection, among others. Only bacterial isolates deemed clinically significant by local criteria were included in the program and only Gram-negative organisms from patients with cancer were included in

* Corresponding author. Tel.: +1-319-665-3370; fax: +1-319-665-3371.
E-mail address: helio-sader@jmilabs.com (H.S. Sader).

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this investigation. Species identification was confirmed when necessary by Matrix-Assisted Laser Desorption Ionization-Time Of Flight mass spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, US) by following manufacturer instructions.

2.2. Resistant subsets

An ESBL-screen-positive phenotype was defined according to Clinical and Laboratory Standards Institute (CLSI) (2016), i.e. a MIC of ≥2 μg/mL for ceftazidime and/or ceftriaxone and/or aztreonam. Carbapenem-resistant Enterobacteriaceae (CRE) was defined as resistant (MIC, ≥4 μg/mL (CLSI, 2016) to imipenem (excluding *Proteus mirabilis* and indole-positive Proteeae) or meropenem or doripenem. Further, isolates were categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan drug-resistant (PDR) according to criteria published by Magiorakos et al. (2012); i.e. MDR = nonsusceptible to ≥1 agent in ≥3 antimicrobial classes, XDR = nonsusceptible to ≥1 agent in all but ≤2 antimicrobial classes, and PDR = non-susceptible (CLSI criteria) to all antimicrobial classes tested. Class representatives used in the analysis were: ceftriaxone, meropenem, piperacillin-tazobactam, levofloxacin, gentamicin, tigecycline and colistin (EUCAST criteria) for Enterobacteriaceae; and ceftazidime, meropenem, piperacillin-tazobactam, levofloxacin, gentamicin and colistin for *Pseudomonas aeruginosa*.

2.3. Antimicrobial susceptibility testing

All isolates were tested for susceptibility using the reference broth microdilution method as described by the CLSI (2015). Ceftazidime was combined with avibactam at a fixed concentration of 4 μg/mL. Ceftazidime-avibactam breakpoints approved by the US-FDA (≤8/4 μg/mL for susceptible and ≥16/4 μg/mL for resistant (Avycaz®, 2015)) were applied for all Enterobacteriaceae species and *P. aeruginosa*. Susceptibility interpretations for comparator agents were performed according to breakpoint criteria published by EUCAST (2016) and CLSI (document M100-S26) (CLSI, 2016) or US-FDA when CLSI criteria were not available (Tygacil, 2016). Quality control (QC) was performed using *Escherichia coli* ATCC 25922 and 35,218, *Klebsiella pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853. All QC MIC results were within acceptable ranges as published in CLSI documents (CLSI, 2016).

2.4. Screening for β-lactamases

Enterobacteriaceae isolates displaying an ESBL-phenotype (MIC, ≥2 μg/mL for aztreonam and/or ceftazidime and/or ceftriaxone) were tested for β-lactamase-encoding genes using a microarray based assay (Check-MDR CT101 kit; Check-points, Wageningen, Netherlands). The assay was performed according to the manufacturer's instructions. This kit has the capabilities to detect CTX-M Groups 1, 2, 8 or 25 and 9, TEM wild-type (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMYII, DHA, FOX, KPC and NDM-1. The most common mutations that expand the spectrum of TEM and SHV enzymes are detected by this assay and these mutations include 104 K, 164S/C/H or 123S for TEM and 138S, 238A and 240 K for SHV. Validation of the assay against US isolates was previously performed (Castanheira et al., 2016). Additionally, all Enterobacteriaceae isolates displaying a ceftazidime-avibactam MIC of >4 μg/mL were screened for the presence of metallo-β-lactamase and serine-carbapenemase encoding genes families (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC}, bla_{OXA-48}, bla_{GES}, bla_{IMI}, bla_{NMC-A}, and bla_{SME}) by PCR as previously described (Castanheira et al., 2016). Amplicons were sequenced on both strands and results were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin, USA). Amino acid sequences were compared with those available through the internet using NCBI/BLAST.

3. Results

The isolates were predominantly from bloodstream infections (33.2%), skin and skin structure infections (26.0%), pneumonia (14.9%) and urinary tract infections (14.4%); and the most frequently isolated Gram-negative organisms were *E. coli* (31.5%), *Klebsiella* spp. (20.7%), *P. aeruginosa* (14.1%) and *Enterobacter* spp. (12.7%). ESBL-phenotype was observed among 17.3 and 9.9% of *E. coli* and *K. pneumoniae*, respectively; and 25.0% of *Enterobacter cloacae* were not susceptible to ceftazidime (Table 1).

All Enterobacteriaceae ($n = 486$) were categorized as susceptible to ceftazidime-avibactam ($MIC_{50/90}$, 0.12/0.25 μg/mL), with the highest MIC value at 1 μg/mL (Tables 1 and 2). Ceftazidime-avibactam was particularly active against MDR Enterobacteriaceae ($n = 30$; $MIC_{50/90}$, 0.12/0.5 μg/mL), ESBL-phenotype *E. coli* ($n = 34$; $MIC_{50/90}$, 0.12/0.25 μg/mL) and ESBL-phenotype *K. pneumoniae* ($n = 11$; $MIC_{50/90}$, 0.25/1 μg/mL; Table 1). Ceftazidime-avibactam was very active against two CRE and XDR isolates of Enterobacteriaceae (both *K. pneumoniae*), with MIC values of 0.5 and 1 μg/mL (Table 1). One CRE/XDR *K. pneumoniae* isolate had positive screening test for bla_{KPC-2} and a meropenem MIC value of >8 μg/mL, and the second isolate had a $bla_{CTX-M-15}$ and a meropenem MIC value of 4 μg/mL.

Meropenem was also active against Enterobacteriaceae overall ($MIC_{50/90}$, ≤0.06/≤0.06 μg/mL; 99.6% susceptible), but showed more limited activity against ESBL-phenotype *K. pneumoniae* ($MIC_{50/90}$, ≤0.06/4 μg/mL; 81.8% susceptible; data not shown) and MDR strains ($MIC_{50/90}$, ≤0.06/0.12 μg/mL; 93.3% susceptible; Table 2). Furthermore, ESBL-phenotype *K. pneumoniae* and MDR Enterobacteriaceae exhibited low susceptibility rates for piperacillin-tazobactam (54.5 and 51.7% susceptible, respectively), gentamicin (36.4 and 26.7% susceptible, respectively), levofloxacin (45.5 and 10.0% susceptible, respectively) and colistin (75.0 and 86.4% susceptible [EUCAST], respectively; Table 2).

The most common ESBL observed among *E. coli* was CTX-M-15-like (22 strains [64.7%], including two strains with CTX-M-15-like plus CMY-2-like and one strain with CTX-M-15-like plus DHA-like) and CTX-M-14-like (six strains; 17.6%). The highest ceftazidime-avibactam MIC value among isolates producing either CTX-M-15-like or CTX-M-14-like was only 0.5 μg/mL (MIC_{50}, 0.12 μg/mL; Table 3). CTX-M-15-like was also the most common β-lactamase detected among *K. pneumoniae* (four strains; 36.4%), and bla_{KPC-2} was identified in one strain only, which exhibited a ceftazidime-avibactam MIC of 0.5 μg/mL (Table 3).

Ceftazidime-avibactam inhibited 96.6% of *P. aeruginosa* isolates ($n = 88$) at the US-FDA susceptible breakpoint of ≤8 μg/mL, and MIC_{50} and MIC_{90} values were 2 and 8 μg/mL, respectively. Susceptibility rates for ceftazidime ($MIC_{50/90}$, 2/32 μg/mL), cefepime ($MIC_{50/90}$, 2/16 μg/mL), piperacillin-tazobactam ($MIC_{50/90}$, 4/64 μg/mL) and meropenem ($MIC_{50/90}$, 0.5/8 μg/mL) were 86.4, 88.6, 84.1 and 79.5%, respectively (Table 2). The addition of avibactam to ceftazidime increased the percentage of susceptible *P. aeruginosa* isolates from 86.4% to 96.6% (Table 2). Among non-β-lactam agents, colistin ($MIC_{50/90}$, 2/2 μg/mL; 100.0% susceptible) and amikacin ($MIC_{50/90}$, 2/4 μg/mL; 97.7% susceptible) were the most active compounds tested against *P. aeruginosa* (Table 2).

Ceftazidime-avibactam was also active against *P. aeruginosa* isolates non-susceptible to ceftazidime (75.0% susceptible; $n = 12$), meropenem (83.3% susceptible; $n = 18$) and piperacillin-tazobactam (78.6% susceptible; $n = 14$; Table 1). MDR and XDR phenotypes were observed in 13.6 and 11.4% of *P. aeruginosa* isolates, respectively. Among MDR and XDR *P. aeruginosa*, 75.0 and 70.0% were susceptible to ceftazidime-avibactam, respectively (Table 1); while all isolates were non-susceptible to ceftazidime, piperacillin-tazobactam and meropenem, and 80.0–83.3% of isolates were susceptible to amikacin.

Ceftazidime-avibactam was highly active against *Haemophilus influenzae* ($n = 28$; $MIC_{50/90}$, ≤0.015/0.03 μg/mL; highest MIC, 0.03 μg/mL), and it was four-fold more active than ceftazidime