Diagnostic Microbiology and Infectious Disease 104 (2022) 115745

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Antimicrobial Susceptibility Studies

Comparison of ceftazidime-avibactam susceptibility testing methods against OXA-48-like carrying *Klebsiella* blood stream isolates

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ARTICLE INFO

Article history:
Received 17 March 2022
Revised in revised form 25 May 2022
Accepted 10 June 2022
Available online 18 June 2022

Keywords:
Ceftazidime-avibactam
Carbapenem resistant *Klebsiella*
OXA-48-like
OXA-232
Sensititre plate
Etest

ABSTRACT

Ceftazidime-avibactam exhibits good in vitro activity against carbapenem resistant *Klebsiella* carrying OXA-48-like enzymes. We tested two hundred unique carbapenem resistant *Klebsiella* blood stream isolates (71% with single OXA-48-like carbapenemases, including OXA-48, n = 62; OXA-232, n = 57; OXA-244, n = 17; OXA-181, n = 5) that were collected as part of a multicentre study against ceftazidime-avibactam using Etest (bioMérieux, Marcyl'Étoile, France), 10/4 μg disc (Thermo Fisher) and Sensititre Gram Negative EURGNCOL Plates (Lyophilized panels, Sensititre, Thermo Fisher) with the aim of comparing the performances of the Etest and disc to that of Sensititre. Ceftazidime-avibactam $MIC_{50/90}$ was 2/>16 mg/L for the entire collection and was 2/4 mg/L for single OXA-48-like producers. Categorical and essential agreements between the Etest and Sensititre were 100% and 97%, respectively. Categorical agreement between the disc and Sensititre was 100%. Etest and 10/4 μg discs are suitable alternatives to Sensititre for ceftazidime-avibactam sensitivity testing for OXA-48-like producers.

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https://doi.org/10.1016/j.diagmicrobio.2022.115745
0732-8893/

1. Introduction

Ceftazidime-avibactam demonstrates good in vitro activity against KPC and OXA-48 carbapenemases, extended-spectrum beta-lactamases and AmpC beta-lactamases, and is the preferred antimicrobial for the treatment of OXA-48 producing carbapenem-resistant *Klebsiella* (CRK) infections [1,2]. Accurate susceptibility testing is important for optimizing ceftazidime-avibactam use. Broth microdilution (BMD) is the reference ceftazidime-avibactam susceptibility testing method, while gradient and disc diffusion tests are more frequently used in routine practice. More recently, Sensititre plates replaced traditional BMD for clinical and research use due to their practicality [3]. Ceftazidime-avibactam discs recommended by EUCAST and Clinical and Laboratory Standards Institute (CLSI) differ by their ceftazidime (10 μg vs 30 μg, respectively) and avibactam (4 μg vs 20 μg, respectively) contents. A handful of studies compared the performances of the Etest and mainly 30/20 μg discs to BMD for a small number carbapenem resistant Gram-negative isolates, and demonstrated resistance overcall with 30/20 μg discs [4–7] The aim of this study was to compare the performance of ceftazidime-avibactam 10/4 μg disc and gradient diffusion assay to that of Sensititre plates against a large collection of CRK blood stream isolates with mainly OXA-48-like carbapenemases.

2. Materials and methods

Two hundred unique carbapenem and ceftazidime resistant *Klebsiella* blood stream isolates were collected as part of a multicentre observational cohort study conducted in 13 tertiary care centres in Turkey between June 2018 and June 2019 [8]. Isolates were identified using MALDI-TOF MS. *Klebsiella pneumoniae* was the predominant species (n = 190) followed by *Klebsiella variicola* (n = 7), *Klebsiella quasipneumoniae* (n = 2), and *Klebsiella oxytoca* (n = 1). Carbapenemase genes were detected on whole bacterial genomes using Abricate v0.8.10 (https://github.com/tseemann/abricate) with the NCBI database [9]. OXA-48 (n = 62), OXA-232 (n = 57), OXA-244 (n = 17) and OXA-181 (n = 5) constituted the majority of carbapenemases (71%) followed by metallo-beta-lactamases (MBLs) (i.e., MBL plus OXA-48-like, MBL plus KPC-2 and single MBL) (21%). The remainder (7%) of the isolates did not carry any carbapenemases (7%) (Supplementary Table 1). All MBLs belonged to NDM class, except for a single VIM.

Ceftazidime-avibactam BMD was performed using Sensititre Gram Negative EURGNCOL Plates (lyophilized panels, Sensititre, Thermo Fisher) according to the manufacturer's instructions [10]. Gradient diffusion test was performed using ceftazidime-avibactam Etest (bioMérieux, Marcyl'Étoile, France) according to the manufacturer's instructions using Mueller-Hinton agar plates (Thermo Fisher Scientific Australia Pty Ltd). Disc diffusion was performed using ceftazidime-avibactam 10/4 μg disc (Thermo Fisher) and Mueller-Hinton agar plates (Thermo Fisher Scientific Australia Pty Ltd) as per EUCAST 2021 guidelines [11]. *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used for quality control.

Ceftazidime-avibactam MIC range was <1 to >16 mg/L for the Sensititre plates and 0.038 to >256 mg/L for the Etest. When the Etest MIC value was between two standard values, result was rounded up to the higher value. Isolates with an MIC of ≤8 mg/L or the disc diffusion zone diameter of ≥13 mm were categorized as ceftazidime-avibactam susceptible, and those with an MIC of >8 mg/L and zone diameter of <13 mm were categorized as resistant as per EUCAST 2021 interpretive criteria.

Essential and categorical agreement (EA and CA, respectively) along with major and very major error (ME and VME, respectively) rates were determined according to CLSI guidelines [12]. Specifically, CA was defined as the agreement between the BMD and Etest or disc diffusion interpretive results, and EA was defined as Etest MIC within one doubling dilution of the BMD MIC. ME was defined as misclassification of a susceptible isolate as resistant and VME was defined as misclassification of a resistant isolate as susceptible. Correlation between MICs and disc diffusion zones of inhibition (ZOI) was assessed using Spearman correlation coefficient (ρ) (Stata v.16.1, StataCorp LP, College Station, TX).

3. Results

3.1. General characteristics of the isolates

Ceftazidime-avibactam Sensititer and Etest $MIC_{50/90}$ were 2/>16 and 2/>256 mg/L, respectively, for 200 CRK isolates. All OXA-48-like producers and the isolates without carbapenemases were susceptible to ceftazidime-avibactam, and 35/42 (83%) of MBL producers were resistant to ceftazidime-avibactam (Supplementary Table 1).

Ceftazidime-avibactam Sensititer MICs were ≤ 1, 2, 4 or 8 mg/L for 51 (25.5%), 97 (48.5%), 14 (7%), and three (1.5%) of 200 isolates, respectively. There were no isolates with a Sensititer MIC of 16 mg/L. There were 35 (17.5%) isolates with Sensititer MICs of >16 mg/L, all of which were MBL producers. Ceftazidime-avibactam Etest MICs were ≤ 1, 2, 4 mg/L for 58 (29%), 95 (47.5%), and 12 of 200 (6%) isolates, respectively. There were no isolates with Etest MICs of 8 to 256 mg/L. There were 35 (17.5%) isolates with Etest MICs of >256 mg/L.

3.2. Comparison of Etest and Sensititer MICs

Ceftazidime-avibactam MICs obtained by Etest and Sensititre were strongly correlated (ρ = 0.98, $P < 0.0001$). CA between the Etest and Sensititre was 100% and the EA was 97% (194/200) (Table 1).

Of the 58 isolates with an Etest MIC of ≤ 1 mg/L, 38 (66%) had the same Sensititer MIC; 16 (28%), 3 (5%) and 1 (2%) had Sensititer MICs

Table 1
MIC value variation and summary of essential agreement rates between Etest and Sensititer.

	Number of isolates with $\log_2$ MIC variation (compared to Sensititer)					
Carbapenemase type (n)	-3	-2	-1	0	+1	%EA
Total (200)	1	5	22	152	20	97
OXA-48-like (141)	0	5	19	99	18	96
OXA-48 (62)	0	2	13	43	4	97
OXA-232 (57)	0	3	6	37	11	95
OXA-244 (17)	0	0	0	14	3	100
OXA-181 (5)	0	0	0	5	0	100
MBL[a] (42)	0	0	1	41	0	100
KPC-2 (4)	0	0	1	3	0	100
No carbapenemase (13)	1	0	1	9	2	92

[a] All NDM, except one VIM. 31/42 isolates coharbour an OXA-48.

>8	35												
8							1	1					1
4					1	1	1	7	2	1			1
2			1	1		2	10	37	30	8	4	2	2
≤1							1	8	10	9	7	2	14
	6	13	14	15	17	18	19	20	21	22	23	24	≥25

Fig. 1. Distribution of ceftazidime-avibactam MICs by Sensititer versus 10/4 μg disc diffusion zone diameters against all 200 carbapenem-resistant Klebsiella isolates. X axis: Zone diameters (mm), Y axis: Sensititer MIC values (mg/L). Dashed lines represent the susceptibility breakpoints for ceftazidime-avibactam.

of 2, 4, and 8 mg/L, respectively. Of the 95 isolates with an Etest MIC of 2 mg/L, 74 (78%) had the same Sensititer MIC; 13 (14%), 6 (6%), and 2 (2%) had Sensititer MICs of ≤1, 4 and 8 mg/L, respectively. Of the 12 isolates with an Etest MIC of 4 mg/L, five (42%) had the same Sensititer MIC and seven (58%) had a Sensititer MIC of 2 mg/L.

For 22 isolates (19 with OXA-48-like carbapenemases), Etest MICs were 1 doubling dilution lower than the Sensititer MICs (16 isolates with BMD MICs of 2 mg/L and Etest MIC of ≤1mg/L, and 6 isolates with BMD MICs of 4 mg/L and Etest MICs of 2 mg/L), and for 20 isolates (18 OXA-48-like producers), Etest MICs were 1 doubling dilution higher than the Sensititer MICs (13 isolates with BMD MICs of ≤1 mg/L and Etest MICs of 2 mg/L, and 7 isolates with BMD MICs of 2 mg/L and Etest MICs of 4 mg/L) (Table 1).

There were six isolates that did not meet the EA criteria by having Etest MICs that were less than 1 doubling dilution of the BMD MICs. Five of them were OXA-48-like producers and one of them did not harbour a carbapenemase. For three of the five isolates, Sensititer MICs were 4 mg/L and Etest MICs were 1 mg/L, and for two of them Sensititer MICs were 8 mg/L and Etest MICs were 2 mg/L. There was one isolate with no carbapenemase, for which Sensititer MIC was 8 mg/L and Etest MIC was 1 mg/L. Disc ZOI for these six isolates ranged between 19 and 25 mm.

3.3. Comparison of disc diffusion and Sensititer MICs

Median ceftazidime-avibactam disc ZOI was 21 mm (IQR 20−22 mm). Disc ZOI and Sensititre MICs were strongly correlated (ρ = -0.96, $P < 0.0001$). CA between the disc and Sensititre was 100% (Fig. 1). There were two isolates with low ceftazidime-avibactam MICs (i.e., Sensititre 2 mg/L and Etest 1 mg/L), but narrow ceftazidime-avibactam ZOIs (i.e., 14 and 15 mm). Both were OXA-48-like producers.

4. Discussion

In this study, all OXA-48-like producing CRK including OXA-48, OXA-232, OXA-244, and OXA-181 were susceptible to ceftazidime-avibactam. Ceftazidime-avibactam sensitivities by Etest and 10/4μg disc correlated well with Sensititre, with 100% CA for both methods and no error rates.

Previous studies compared the performance of Etest and BMD for mainly KPC producing small number of isolates and demonstrated CA rates of 97%-100% with no error rates [4–7]. This study corroborated these findings in a larger collection with mainly OXA-48-like producing CRK. In this study, MIC values obtained by Etest were at least one doubling dilution lower for 14% of the isolates, and one doubling dilution higher for 10% of the isolates as compared to the Sensititer MIC values. Higher Etest MICs were mainly observed for the isolates with lower Sensititer MICs, whereas lower Etest MICs were mainly observed for the isolates with higher Sensititer MICs. Similar results were observed by Sherry et al, where Etest MICs were generally 0.9 doubling dilutions lower than that of BMD [7]. We were not able to find an association between the carbapenemase type and the Etest-Sensititer MIC difference. Molecular background and the clinical implications of the MIC difference between the Sensititer and Etest remains to be further characterized.

Ceftazidime-avibactam discs used by EUCAST and CLSI differ in their ceftazidime and avibactam contents. In this study, we used EUCAST-recommended 10/4 μg discs and observed a CA rate of 100% with no errors, similar to the findings of Wenzler et al, where 10/4 μg discs were used against 69 CRK isolates with a CA rate of 98.5% CA and ME rate of 1.5%. Previous studies demonstrated an overestimation of ceftazidime-avibactam resistance with 30/20 μg discs, with ME rates of 18.5% to 24.3% [5,6].

Interestingly, 7/42 (17%) of the MBL producers (all NDM) tested susceptible to ceftazidime-avibactam with all three susceptibility testing methods. Similar results were observed with a small number of IMP producers previously [13]. Most likely explanation for this is the drop out of MBL carrying plasmids on repeat colony passages due to fitness cost [14]. Another possibility is that the identification of a beta-lactamase gene by genomic sequencing does not provide quantitative or functional information related to its ability to hydrolyse beta-lactams. We were unable to test the functionality of MBL genes in this study.

One of the limitations of this study is that commercially prepared Sensititre plates were used as the reference standard for susceptibility testing of ceftazidime-avibactam rather than the custom made plates. It is reassuring, on the other hand, that Sensititre panels for ceftazidime-avibactam produced accurate ceftazidime-avibactam MIC results and have been widely used [3]. Second, we have not performed EDTA synergy testing to confirm MBL activity.

In conclusion, this study demonstrated on a large collection of unique bloodstream isolates that ceftazidime-avibactam is active against OXA-48, OXA-232, OXA-244, and OXA-181 producers. Etest and 10/4 μg discs are suitable alternatives to Sensititre for ceftazidime-avibactam sensitivity testing.

Authors contributions

Conceptualization: B.I., S.K., O.D., O.E., F.C.; Methodology: B.I., B.O., C.V., C.F., B.F., O.D., F.C.; Software: V.B. and M.G.; Formal Analysis: B.I. C.F., B.F.; Investigation and data curation: B.I., B.O., C.V., G.C., A.T.A, F. S., N.T., H.D., S.M., H.A., I.I.B., M.A., E.T.T., S.K.D., M.K., S.K., O.D., C.A., S. Y., G.H., N.S., A.A., O.A., M. A.; Resources: B.I., P.H., O.E., D.P. F.C.; Data Curation: B.I.; Writing – Original Draft Preparation: B.I., A.S., P.S.; Writing – Review & Editing: B.I., B.O., C.V., A.S., G.C., A.T.A, C.F., B.F., P. S., F.S., N.T., H.D., S.M., H.A., I.I.B., M.A., E.T.T., S.K.D., M.K., S.K., O.D., C. A., S.Y., G.H., N.S., A.A., O.A., M.A., P.H., O.E., D.P., F.C.; Supervision: P. H., O.E., D.P., F.C.; Project Administration: B.I., B.O., C.V., O.D., F.C., O. E.; Funding Acquisition: B.I., D.P."

Funding

This work was supported by Pfizer Global Medical Grants (grant number 56644459). Dr Brian Forde's research is supported by Advance Queensland Industry Research Fellowship from the Queensland Government.

Institutional review board statement

The study was approved by the Koc University Institutional Review Board (approval number: 2018.151.IRB1.018)

Declaration of competing interest

Dr. Paterson reports research grants from Merck, Pfizer and Shionogi. David Paterson has received honoraria for advisory board membership from Merck, Pfizer, Shionogi, GSK, QPex, Entasis, VenatoRx, BioMerieux, and Accelerate. Dr Harris has received research grants from Sandoz, Merck/MSD and Shionogi, speaker's fees from Pfizer and honoraria for advisory board membership from Merck and Sandoz, paid to the University of Queensland. All others have no conflict of interest to declare.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2022.115745.

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