A Comparison of Ceftazidime-avibactam Antimicrobial Susceptibility Testing Methods (Disk Diffusion, Gradient Diffusion and Broth Micro-dilution) amongst *Klebsiella pneumoniae* OXA-48-like producing isolates.

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Article summary:

Ceftazidime-avibactam is widely used for treating infections caused by serine carbapenemase-producing Enterobacterales. However, Sub-Saharan African data on *bla*$_{\text{OXA-48-like}}$ isolate testing is lacking. This study compared disk diffusion and gradient diffusion to BMD, finding both methods reliable for determining susceptibility in *Klebsiella pneumoniae* *bla*$_{\text{OXA-48-like}}$ isolates.

Declaration of Work

I declare that this research project for the submission of the degree: Masters of Medicine (Microbiology) has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where stated otherwise by reference or acknowledgment, the work presented is entirely my own.

Recoverable Signature

X 

VMD CLOETE

Signed by: b6499085-b103-4007-8a09-daf6fed044f8 16.07.2024

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Abstract:

Background:

The use of ceftazidime-avibactam for the treatment of infections caused by Enterobacterales harbouring serine carbapenemases, most notably *Klebsiella pneumoniae,* is well established worldwide. This agent is particularly useful for *bla*$_{\text{OXA-48-like}}$ infections. However, there is currently no data from the South African setting for the comparison of manual susceptibility testing methods against reference method for this agent. It was the aim of this study to determine the performance of disk diffusion and gradient diffusion antibiotic susceptibility testing methods compared to broth microdilution (BMD) in a collection of OXA-48-like producing *Klebsiella pneumoniae* isolates.

Methods:

De novo ceftazidime-avibactam susceptibility testing was performed on 100 stored *Klebsiella pneumoniae bla*$_{\text{OXA-48-like}}$ clinical isolates collected between 2017 and 2023. Disk diffusion and gradient diffusion testing was conducted as per Clinical Laboratory and Standards Institute guidelines and BMD using Sensititre™ (Thermo Fischer, Massachusetts, USA). Categorical and essential agreement as well as precision were compared to BMD.

Results:

Categorical agreement for disk diffusion and gradient diffusion was 100% (100/100) while essential agreement was 97% (86/88) for gradient diffusion versus BMD. No major or very major errors were observed. All isolates tested susceptible to ceftazidime-avibactam, with the BMD $MIC_{50/90}$ at 1/4 and 2/4 µg/mL and gradient diffusion $MIC_{50/90}$ at 0.5/4 and 1/4 µg/mL, respectively.

Conclusions:

Disk diffusion and gradient diffusion are reliable alternative methods for determining ceftazidime-avibactam susceptibility in susceptible isolates of *Klebsiella pneumoniae* OXA-48-like producers.

Keywords:

Ceftazidime-avibactam

Klebsiella pneumoniae

Antimicrobial susceptibility testing

Carbapenemases

Antimicrobial resistance

1. Introduction

1.1 Background

Antimicrobial resistance poses a significant challenge to the management of Gram negative sepsis worldwide. The increased incidence of resistant Gram negative infections has led to the development of novel antibiotics to target these infections. Sub-Saharan African populations remain vulnerable to these infections due to multiple factors including: excessive cost of newer agents, limited access and supply of drugs, poorly manufactured drugs leading to substandard chemotherapeutic efficacy, counterfeit drugs, absence of or poor implementation of standardised infection prevention and control measures as well as poor sanitation and hygiene practices. [1]

Carbapenemase producing Enterobacterales (CPE) are of concern globally and in South Africa (RSA), as they threaten the efficacy of recommended empiric antibiotic therapies, increase hospital costs and contribute increasingly to morbidity and mortality. The spread of CPE has been well documented in hospital acquired infections. However, as has been shown by community acquired extended spectrum beta-lactamase (ESBL) producing infections, there is growing concern for the potential of community transmission of CPE. [2] Assessing the global burden of antimicrobial resistance (AMR) by systematic review, Murray *et al* showed that 70% of deaths attributed to AMR were caused by resistance to beta-lactams and fluoroquinolones. Specifically, the burden of carbapenem resistant *Klebsiella pneumoniae* was notably higher in low to-middle income (LMIC) regions when compared to high income regions, where the predominant AMR burden is attributed to *Staphylococcus aureus* and *Escherichia coli*. [3] In RSA, the most recent AMR report (2021) noted *Klebsiella pneumoniae* to be the most isolated bloodstream infection organism, with 70% non-susceptible to third generation cephalosporins and 40% non-susceptible to at least one carbapenem. [4] Furthermore, while different carbapenemase enzymes exist within local populations of *Klebsiella pneumoniae* isolates, $bla_{\text{OXA-48-like}}$ carbapenemase production was the most frequent enzyme detected. It accounted for more than 70% of enzymes detected in CPEs by epidemiological studies in RSA [5,6]

The treatment options for CPE are limited, with no single therapeutic agent having activity against all enzymes. LMIC guidelines rely heavily on the usage of potentially toxic and sub-optimal combination therapies for treatment of CPE infections. Antibiotics recommended for use in combination in RSA include polymyxins, aminoglycosides, meropenem/imipenem-cilastatin or tigecycline, depending on the source of infection and patient co-morbidities. [7] While the use of novel beta-lactam/beta-lactamase inhibitor combination drugs such as ceftazidime-avibactam (CAZ-AVI) has been widespread in high income countries, their use in LMIC has often been precluded by high costs compared to older antimicrobials, and delayed registration through regulatory bodies. CAZ-AVI was only registered for use in RSA in 2021.[8]

In view of the predominance of *bla*$_{\text{OXA-48-like}}$ CPE in RSA, CAZ-AVI is a suitable therapeutic option. [7]

Comparisons of the manual methods for CAZ-AVI antimicrobial susceptibility testing (AST) in these drug resistant Gram negative bacterial populations have been addressed by international authors. [9–11] Broth microdilution (BMD) remains the current reference method to determine the MIC. [12] BMD is labour intensive, costly and requires specialised skills to perform. This often precludes it from routine laboratory practice, particularly in LMIC. CAZ-AVI disks (30/20µg or 10/4µg) and gradient diffusion strips are commercially available, inexpensive, easy to perform and interpret, allowing for routine testing in such settings.

Data from *in vitro* testing of this drug has been published from high income countries given its availability in these settings. Data from LMIC is currently limited to publications from China, India and Turkey [9,13,14]. It was the aim of this study to assess (de novo) CAZ-AVI AST using gradient diffusion and disk diffusion compared to BMD amongst a collection of *Klebsiella pneumoniae bla*$_{\text{OXA-48-like}}$ isolates on the African continent.

1.2 Objectives

We present an *in vitro* evaluation of disk diffusion and gradient diffusion testing compared to BMD in a collection of South African *bla*$_{\text{OXA-48-like}}$ *Klebsiella pneumoniae* isolates as well as performance of disk diffusion, gradient diffusion and BMD testing for CAZ-AVI in these isolates.

2. Materials and Methods

2.1 Isolate selection

As per Clinical Laboratory Standards Institute (CLSI) M52 recommendation, AST was undertaken for 100 *bla*$_{\text{OXA-48-like}}$ *Klebsiella pneumoniae* isolates from a bank of CPE *Klebsiella pneumoniae* populations. [15] Convenience sampling was used. These were clinical isolates from blood cultures collected at or referred to Charlotte Maxeke Johannesburg Academic Hospital Clinical Microbiology laboratory between 2017 and 2023. Duplicate isolates from the same patient had been removed. Carbapenemase production was confirmed by polymerase chain reaction, RESIST-4™ (Coris BioConcept, Gembloux, Belgium) or RESIST-5™ (Coris BioConcept, Gembloux, Belgium) lateral flow assays. Isolates were stored at -70 degrees Celsius in Microbank™ (Pro-Lab Diagnostics, Toronto, Canada) storage.

2.2 Isolate processing

All AST was performed at the National Institute of Communicable Diseases' Centre for Antimicrobial Resistance and Mycology (CHARM). Isolates were sub-cultured to MacConkey agar (Media Mage, Johannesburg, RSA) with a meropenem 10µg disk in the first quadrant to encourage carbapenemase phenotypic expression.

Pure sub-cultured colonies were used to inoculate de-ionised water to a MacFarland 0.5 standard using a digital turbidometer. The same bacterial suspension was utilised for all

methods. Isolates were tested in batches and included the CLSI recommended CAZ-AVI quality control (QC) strain (*Klebsiella pneumoniae* ATCC700603) in each batch for all methods.

Disk diffusion and gradient diffusion were performed using CAZ-AVI 30/20µg disks (bioMérieux, Marcy-l'Ètoile, France) and E-test (bioMérieux, Marcy-l'Ètoile, France) on Mueller-Hinton Agar (Media Mage, Johannesburg, RSA). BMD was conducted using Sensititre™ DKMGN BMD plates ™ (Thermo Fischer, Massachusetts, USA) with a minimum inhibitory concentration (MIC) range of 0.5/4 – 16/4 µg/mL and in accordance with manufacturer's specifications. Panels were inoculated using an automated inoculation delivery system Sensititre AIM™ (Thermo Fischer, Massachusetts, USA) to minimise manual error. No prior verification of these BMD plates had been undertaken and this study served as part of a verification process in the laboratory for this BMD plate.

2.3 Endpoint reading

Isolate zone of inhibition or MIC for all methods was interpreted according to CLSI M100 (2023) Enterobacterales breakpoints for CAZ-AVI. [16] Results from all methods were read by two individuals independently. In the case of inter-reader discordance, a third blinded reader was consulted. The final value was determined by the agreement of two out of three readers' results. In cases where all three readers' results differed, the isolate was retested. Disk diffusion and gradient diffusion methods were evaluated by the naked eye using transmitted light against a dark background. MIC values falling between two-fold dilutions were rounded up to the higher MIC value. BMD MICs were read using a manual viewbox and recorded at the lowest concentration that completely inhibited growth of the organism.

2.4 Precision testing

Two randomly selected isolates and a QC strain were repeatedly tested over 5 days. MIC values within 3 $\log_2$ dilutions with uniform categorical classification was considered

precise.[12] For disk diffusion, uniform categorical classification upon retesting was considered precise.

2.5 Data analysis

Categorical and essential agreement, as well as very major error and major error rates were defined according to CLSI criteria. [15] Data was captured manually and entered into Microsoft Excel spreadsheets where data analysis was conducted to calculate rates and agreement.

3. Results

All 100 isolates tested susceptible to CAZ-AVI by the three AST methods. 100 isolates were included in the final analysis for categorical agreement. In cases where gradient diffusion MIC was <0.25/4 µg/mL, these isolates were excluded from data analysis for essential agreement as the lowest MIC on the BMD was 0.5/4 µg/mL. 88 isolates were included in the final analysis for essential agreement. QC strains in each batch passed according to CLSI M100 guidelines. [16]

3.1 Disk diffusion

Categorical agreement between disk diffusion and BMD was 100% (n=100/100). No very major errors or major errors were recorded. Zones of inhibition ranged from 23 – 33mm with a median of 26mm (IQR 25 - 27mm). No isolates produced zones in the region of 20-22mm. Zone size in relation to BMD MIC and gradient diffusion MIC is presented in Table 1 and Table 2, respectively.

3.2 Gradient diffusion

For BMD, the $MIC_{50/90}$ was 1/4 µg/mL and 2/4 µg/mL. For gradient diffusion, the $MIC_{50/90}$ was 0.5/4 µg/mL and 1/4 µg/mL, respectively. BMD MIC had a range of ≤0.5/4 to 4/4 µg/mL and

gradient diffusion MIC ranged from 0.064/4 to 4/4 µg/Ml. MIC data and distribution for gradient diffusion and BMD can be found in Table 3 and Figure 1. Categorical agreement was 100% for gradient diffusion testing versus BMD. Essential agreement was 97% (n=86/88). On average, the gradient diffusion showed a single doubling dilution difference with a median of 1 (IQR 0 - 1) compared to the BMD. When this occurred, the MIC on gradient diffusion was more often lower than the reference BMD. The isolates that failed to show essential agreement tested susceptible by both methods. No very major errors or major errors were recorded. Of interest, there were 3 isolates tested by gradient diffusion method that demonstrated an unusual pattern of inhibition whereby the shape of the ellipse was not clearly demarcated at the point of intersection, as shown in Figure 2. These isolates had a narrow “necked” zone of inhibition that extended to lower MIC values. Isolates demonstrating this phenomenon were retested using the same bacterial population and new Mueller-Hinton media. All retested isolates had resolution of this issue following retesting. Documentation of a similar phenomenon has been described for clavulanate in gradient diffusion testing [17]. However, it is unknown whether the same mechanism observed for clavulanate explains the phenomenon observed in CAZ-AVI in this study.

3.3 Precision

Upon repeated testing of 3 isolates (1 QC strain and 2 clinical isolates) over 5 days, precision was demonstrated. The CLSI reference guide M52 refers to precision in commercial AST testing as no more than 1 $\log_2$ dilution variation for MIC testing methods between repeatedly tested isolates. It also notes that the presence of certain beta-lactamases can result in variations up to 4 $\log_2$ dilutions or more for MIC testing methods [15]. Universal categorical agreement was demonstrated in all three test methods. Between batch reproducibility for BMD was demonstrated with no MIC beyond 1 $\log_2$ dilution for repeated batches. Gradient diffusion testing varied between 1-2 $\log_2$ dilutions between repeated batches. 2 isolates demonstrated 2 $\log_2$ dilutions difference on 1 day when compared to other days of testing. Disk diffusion was categorised as susceptible for each

isolate upon repeated testing. The QC strain included with every batch demonstrated no difference in MICs beyond 2 $\log_2$ dilutions for the BMD or gradient diffusion strips and both repeatedly demonstrated values within the CLSI recommended MIC range for the ATCC strain. QC strain zone diameter also repeatedly demonstrated measurements within the recommended CLSI 21-27mm range. [16]

4. Discussion

To the authors' knowledge, the findings of this study are the first to examine testing methods for CAZ-AVI for South African OXA-48-like producing *Klebsiella pneumoniae* isolates. CAZ-AVI is the only novel beta-lactam-beta-lactamase inhibitor agent that is effective for the treatment of *bla*$_{\text{OXA-48-like}}$ CPE infections. A total of 100 isolates was chosen based on CLSI M52 probability associated with sample size recommendations and best practices in AST documented by Humphries *et al* [12,15]. The increasing pattern and prevalence of CPE infections has had implications for treatment options available, most notably in the public sector. *Klebsiella pneumoniae* is the most commonly isolated Gram negative pathogen from bacteraemic patients in the country according to national surveillance [5,6]. The most recent AMR data for *Klebsiella pneumoniae* collected by the National Institute for Communicable Diseases for public healthcare facilities in South Africa for 2023 shows that 34.5% of isolates show non-susceptibility to ertapenem, 25.9% for imipenem and 25.4% for meropenem. Colistin resistance in *Klebsiella pneumoniae* is currently at 3.2%. [18]

Using CAZ-AVI as a therapeutic option for these infections forms part of a larger antimicrobial stewardship (AMS) framework in the healthcare setting. The World Health Organisation (WHO) created the AWaRE (Access, Watch, Reserve) classification of antibiotics (most recently updated in 2021) as a tool to optimise the usage of narrow spectrum antibiotics in prescribing practices. Access antibiotics are narrow spectrum agents that have a lower cost, side effect profile and have lower selection pressure. Watch

antibiotics, on the other hand, are broader spectrum agents reserved for critically ill patients in the hospital setting with a focus on limiting overuse. Finally, restriction antibiotics are last resort agents reserved for critical patients with multi-drug resistant pathogens. CAZ-AVI is categorised as a restricted antibiotic.[19] Using the antimicrobial susceptibility pattern of isolated organisms, the role of the clinical microbiologist/infectious disease specialist for the guidance in appropriate selection of patients to treat with this agent is crucial.

Accurate, affordable and rapid antimicrobial susceptibility testing is required from microbiology laboratories at all levels, considering the lack of automated AST panels that include CAZ-AVI in RSA currently.

This study demonstrated that the results for manual methods of testing for CAZ-AVI show good correlation compared to BMD. The findings are in keeping with studies conducted in other geographical regions of the world. Similar categorical and essential agreement was reported for a population of 200 *Klebsiella pneumoniae* isolates producing both serine and metallo-beta lactamases tested in Turkey by Isler *et al.* However, it is important to note that Isler *et al* used 10/4 µg disks and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria for testing as well as a different Sensititre™ BMD panel. [9] Additionally, Sherry *et al* tested 50 isolates of mixed serine carbapenemase producing Gram negative isolates. Sherry *et al* also showed that gradient diffusion MIC testing was 0.9 doubling dilutions lower than reference BMD testing. [10] This finding is in keeping with what was observed in our study, suggesting that the gradient diffusion may under-call the MIC.

The ATLAS Global Surveillance Programme recently described susceptibility and molecular profiles of Enterobacterales to CAZ-AVI in the Sub-Saharan region. It is worth noting that *bla*$_{\text{OXA-48-like}}$ was most commonly isolated in Enterobacterales from the South African set of isolates across six laboratories. This is in keeping with data from the national epidemiology data described previously. Furthermore, all CPE isolates that had serine producing

carbapenemases without metallo-beta-lactamase production were noted to be susceptible to CAZ-AVI.[20]

Our analyses verified that disk diffusion and gradient diffusion are appropriate testing methods for these isolates [9,10]. Data by Wenzler *et al* suggest that 10/4 µg disks (as detailed in EUCAST) outperform 30/20 µg disks (as detailed in CLSI M100), as the 30/20 µg disks may overcall resistance [11]. This could be investigated by retesting isolates that measure in the 20-22mm zone diameter range to prevent reporting false resistance.

These findings carry financial implications for microbiology laboratories when choosing an appropriate method of AST for this agent. Disk diffusion testing is considerably cheaper and can be conducted at the routine laboratory in low resource settings without the need for referral to reference laboratories. The disk diffusion categorical agreement rate in this study supports this and reassures laboratories wishing to introduce this method of testing. Furthermore, the high essential agreement supports the continued use of gradient diffusion testing for MIC determination – such as for isolates measuring between 20-22mm as per CLSI M100 recommendations.

It is important to note that the MIC distribution for the entirety of this tested population was well below the established breakpoints for susceptibility in Enterobacterales. The MIC distribution for BMD and gradient diffusion is shown in Figure 1. MIC_{90} for both BMD and gradient diffusion were more than 2 doubling dilutions below the susceptible breakpoint. The lack of access to CAZ-AVI during the study period may have attributed to the absence of resistant strains in the population tested. Resistance to CAZ-AVI has been described where *bla*$_{KPC}$ is the dominant carbapenemase enzyme detected, such as in the USA, Greece, Italy as well as Israel and Latin America. [21] These *bla*$_{KPC}$ variants have shown adaptive potential through mutations, insertions and deletions conferring resistance to the agent. [21]

Although the proportion of resistant isolates currently remains low, continued surveillance is important to ensure resistance is detected early and investigated. Furthermore, the potential for resistance development in settings with *bla*$_{\text{OXA-48-like}}$ predominance remains unknown. There is evidence to show that Enterobacterales with dual carbapenemase enzymes are circulating in RSA, specifically a mixture of serine and metallo-beta-lactamase enzymes [4]. Isolates that test resistant to CAZ-AVI should be carefully scrutinised by pathologists and technical staff to ensure that rigorous testing methods have been followed and that metallo-beta-lactamase production has been ruled out before describing an isolate as resistant. This may require retesting of the isolate and additional carbapenemase confirmation. Due to the inherent challenges in determining MICs, there was an expectation that major and very major errors might occur in the tested population, although we did not observe this. [22] Additionally, commercial testing methods have previously demonstrated poor reproducibility, which could have resulted in major and very major errors. [23]

4.1 Limitations of this study

There were limitations in this study that warrant further research and broader applicability. The first was that this study was conducted on a single genus and species with a particular carbapenemase producing profile. More data is needed to describe the categorical and essential agreement for other Enterobacterales harbouring *bla*$_{\text{OXA-48-like}}$ carbapenemases as well as other serine carbapenemases.

The ATLAS surveillance study in Sub-Saharan Africa demonstrated good *in vitro* susceptibility to CAZ-AVI by BMD for non-Enterobacterales, such as drug resistant *Pseudomonas aeruginosa*.[20] These organisms contribute to the burden of hospital acquired infections in RSA. [4] More data is required for comparison of disk diffusion and gradient diffusion testing in these organisms.

Due to limitations in funding for this research, a bacterial population of only 100 isolates could be tested. A larger contemporary bacterial population needs to be tested since the introduction of CAZ-AVI in RSA. All isolates in this study were tested by CAZ-AVI de novo with no prior knowledge of the AST profiles of the isolates. Hence, a mix of susceptible and resistant isolates and those with zone diameters between 20 – 22mm could not be specifically included.

Commercial BMD plates were chosen over manual BMD due to the potential high rate of manual error associated with this method and difficulty in accessing CAZ-AVI powder. Although commercial BMD is not the reference gold standard, manual BMD can be time consuming, labour intensive and out of the spectrum of routine testing for some microbiology laboratories. Commercial BMD is a viable alternative to manual BMD, but does not replace the gold standard. [12] These panels are an alternative for routine laboratories to efficiently meet turnaround time for BMD MIC. The significant cost of these panels often precludes it from testing in many LMIC, where the burden of resistant Gram negative infections is greatest. [3] Furthermore, only one manufacturer of disks and E-tests was utilised in this study, and the comparison of different manufacturers would be important.

5. Conclusion

The findings of this study are the first from the African continent demonstrating that disk diffusion and gradient diffusion testing are acceptable methods for CAZ-AVI AST amongst susceptible strains of *Klebsiella pneumoniae* OXA-48-like producing isolates. Laboratories wishing to introduce testing for this agent must perform internal verification studies prior to commencement of offering this method of testing to the facility for which they serve. With the high burden of AMR on the African continent, further data on the susceptibility of this agent from the region is essential, as are surveillance programmes focused on AST of novel antimicrobial agents for Gram negative pathogens.

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MC drafted protocol, conducted testing, analysed data and reported for final manuscript. VC and TN supervised testing processes and edited protocol and final manuscript.

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Charlotte Maxeke Johannesburg Academic Hospital Microbiology Laboratory

Infection Control laboratory at Charlotte Maxeke Johannesburg Academic Hospital

Data available in supplementary material.

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8. Ethics

Ethics clearance to undertake this study was approved by the Wits Human Research Ethics Committee (HREC) [M220944]

9. Conflicts of Interest

All authors declare that they have no conflicts of interest.

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APPENDIX A – ETHICS CLEARANCE

APPENDIX B – UNIVERSITY APPROVAL

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

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25 October 2022
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Dr VMD Cloete
P O Box 9052
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Dear Dr Vernon Cloete

Master of Medicine in Microbiological Pathology: Approval of Title

We have pleasure in advising that your proposal entitled *A Comparison of ceftazidime-avibactam Antimicrobial Susceptibility Testing methods (Disk Diffusion, Gradient Diffusion and Broth Micro-dilution) amongst Klebsiella pneumoniae OXA-48-like producing isolates* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

TABLE 1

Table 1: Number of isolates zone of inhibition (mm) vs BMD MIC (µg/mL)

BMD MIC (µg/mL)	**4**		1									
	2	2	6	2	1				1			
	1	4	8	16	10	11	2	1				
	<=0.5		1	6	10	3	8	5		1		1
		23	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
	ZONE OF INHIBITION (mm)											

Key:
BMD = Broth microdilution ; MIC = Minimum inhibitory concentration

TABLE 2

Table 2: Number of isolates Zone of Inhibition (mm) vs Gradient Diffusion MIC (µg/mL)

GRADIENT DIFFUSION MIC (µg/mL)	**4**			1								
	2	2	4	1								
	1	3	9	10	7	5	1		1			
	0.5		3	11	11	7	3	2				
	0.25			1			2	4				
	0.125	1			3	2	3			1		1
	0.064						1					
	0.032											
		23	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
		ZONE OF INHIBITION (mm)										

Key:
MIC = Minimum inhibitory concentration

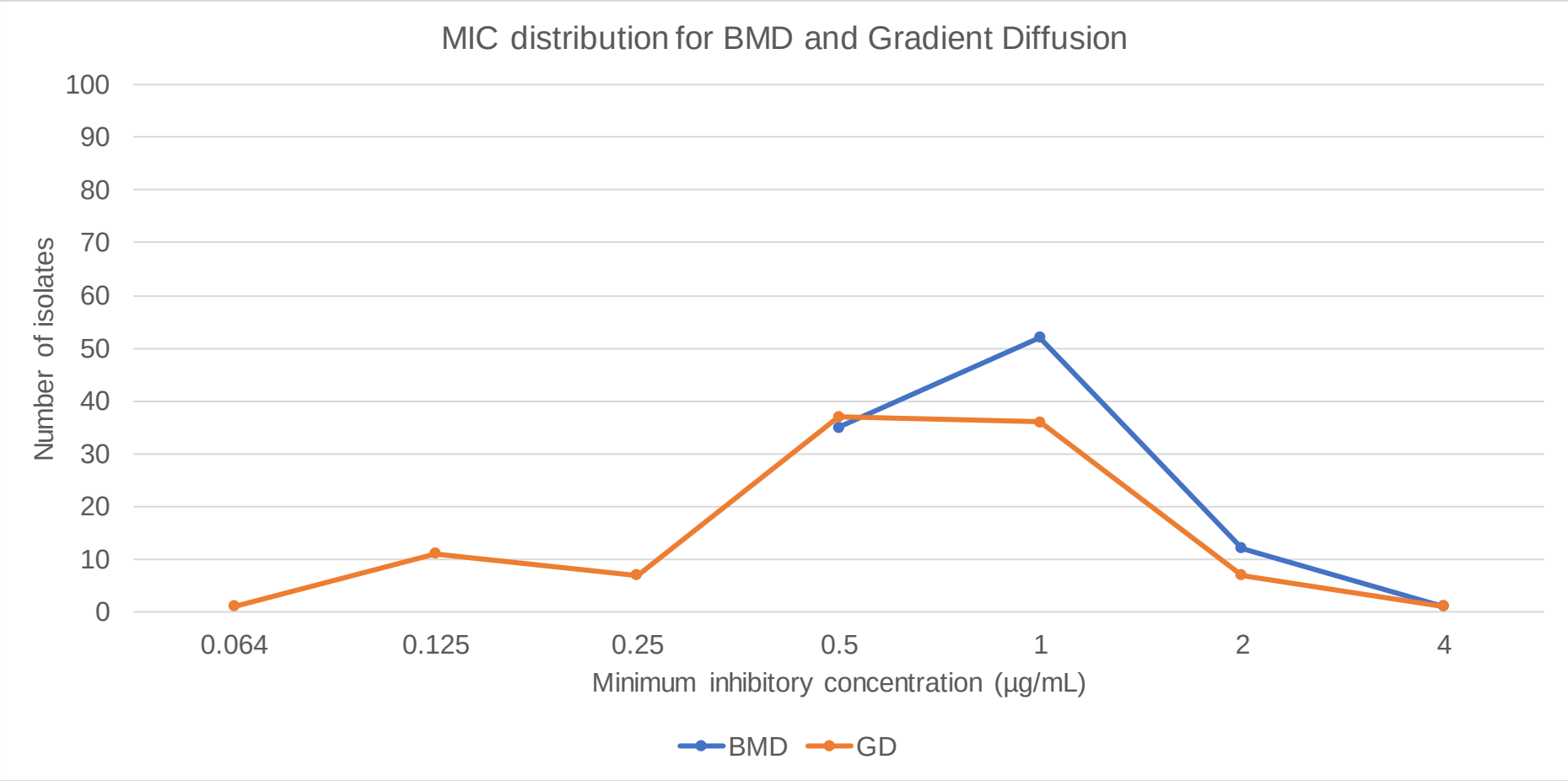
TABLE 3

Table 3: Gradient Diffusion MIC (µg/mL) vs BMD MIC (µg/mL)

BMD MIC (µg/mL)	**4**						1	
	2				1	8	3	
	1		1		23	24	3	1
	<=0.5	1	10	7	13	4		
		0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**
		GRADIENT DIFFUSION MIC (µg/mL)						

Key:
BMD = Broth microdilution ; MIC = Minimum inhibitory concentration

FIGURE 1



Key:
BMD = Broth microdilution ; GD = Gradient diffusion ; MIC = Minimum inhibitory concentration

FIGURE 2

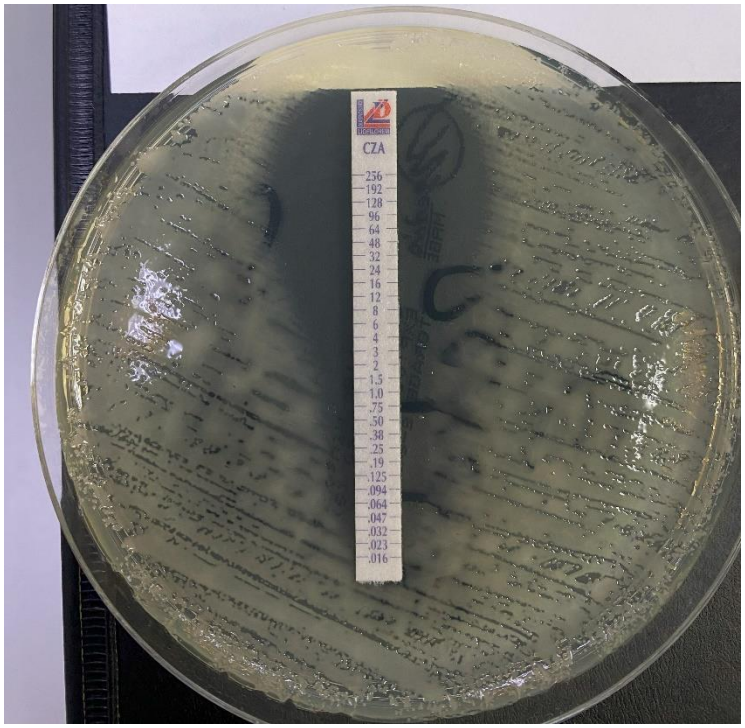


Image A

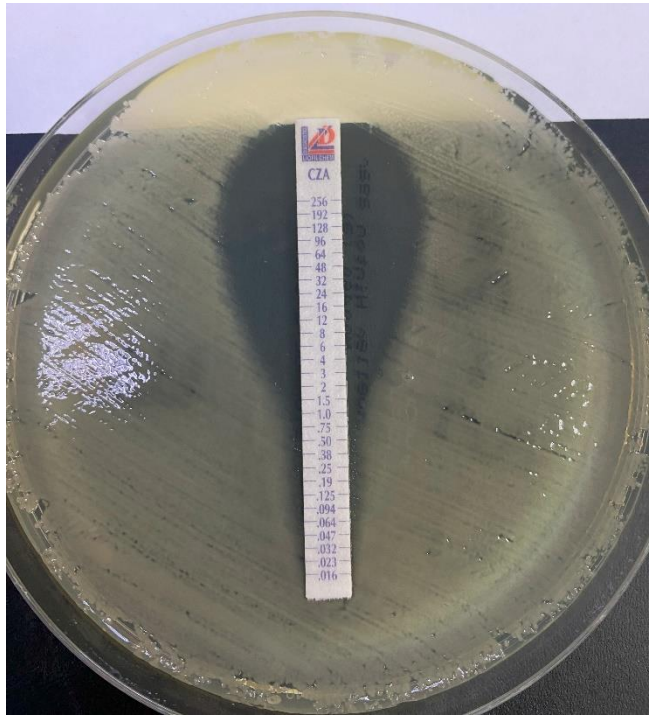


Image B

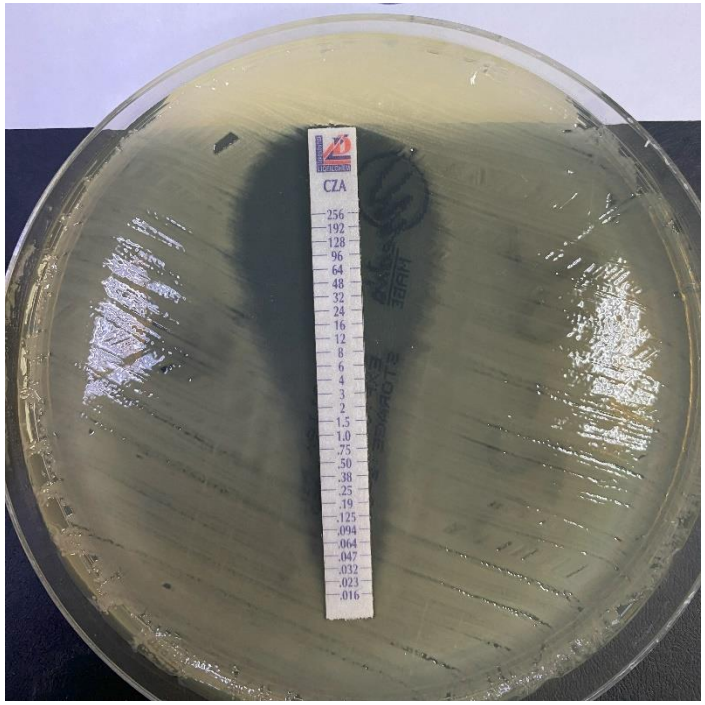


Image C

Image A, B, C: Gradient diffusion testing revealed unusual “necked” zones of inhibition in some isolates. These isolates were retested.

SUPPLEMENTARY DATA – RAW DATA

Batch number	Isolate number	Purity Plate (Pass/ Fail)	Meropenem diameter (mm)	CAZ/AVI diameter (mm)	Interpretation (S/R)	Gradient diffusion MIC	Interpretation (S/R)	BMD MIC	Interpretation S/R)	OXA CONFIRMED	CAT DD AGREE	CAT GD AGREE	ESSEN AGREE
1	1	P	24	26	S	0.5	S	0.5	S		YES	YES	YES
1	2	P	13	24	S	2	S	4	S		YES	YES	YES
1	3	P	14	24	S	1	S	1	S		YES	YES	YES
1	4	P	20	25	S	1	S	1	S		YES	YES	YES
1	5	P	0	25	S	1	S	2	S		YES	YES	YES
1	6	P	13	30	S	1	S	2	S		YES	YES	YES
1	7	P	16	26	S	1	S	0.5	S		YES	YES	YES
1	8	P	21	25	S	1	S	1	S		YES	YES	YES
1	9	P	14	24	S	2	S	1	S		YES	YES	YES
1	10	P	14	23	S	2	S	2	S		YES	YES	YES
2	1	P	23	25	S	0.5	S	0.5	S	YES	YES	YES	YES
2	2	P	20	24	S	1	S	1	S		YES	YES	YES
2	3	P	24	28	S	0.25	S	0.5	S	YES	YES	YES	YES
2	4	P	13	25	S	2	S	1	S		YES	YES	YES
2	5	P	0	25	S	0.5	S	0.5	S		YES	YES	YES
2	6	P	11	26	S	1	S	1	S		YES	YES	YES
2	7	P	0	26	S	0.5	S	1	S		YES	YES	YES
2	8	P	13	24	S	1	S	2	S		YES	YES	YES
2	9	P	24	28	S	0.5	S	0.5	S	YES	YES	YES	YES
2	10	P	25	26	S	0.5	S	0.5	S	YES	YES	YES	YES
2	11	P	26	31	S	0.125	S	0.5	S	YES	YES	YES	EXCLUDED
2	12	P	0	25	S	0.5	S	1	S		YES	YES	YES

2	13	P	14	24	S	2	S	2	S		YES	YES	YES
2	14	P	15	27	S	0.5	S	1	S		YES	YES	YES
2	15	P	9	27	S	1	S	1	S		YES	YES	YES
2	16	P	23	29	S	0.5	S	1	S	YES	YES	YES	YES
2	18	P	25	26	S	0.5	S	1	S	YES	YES	YES	YES
2	19	P	15	26	S	0.5	S	1	S		YES	YES	YES
2	20	P	11	25	S	0.5	S	1	S		YES	YES	YES
2	21	P	13	24	S	2	S	2	S		YES	YES	YES
2	22	P	12	25	S	0.5	S	1	S		YES	YES	YES
2	23	P	20	25	S	1	S	1	S		YES	YES	YES
2	24	P	16	25	S	0.5	S	1	S		YES	YES	YES
2	25	P	24	28	S	0.5	S	1	S	YES	YES	YES	YES
2	26	P	20	26	S	1	S	0.5	S		YES	YES	YES
2	27	P	0	26	S	0.5	S	1	S		YES	YES	YES
2	28	P	22	27	S	0.5	S	1	S		YES	YES	YES
2	29	P	22	29	S	0.5	S	0.5	S		YES	YES	YES
3	1	P	25	29	S	0.25	S	0.5	S	YES	YES	YES	YES
3	2	P	14	23	S	2	S	1	S		YES	YES	YES
3	3	P	22	26	S	0.5	S	0.5	S		YES	YES	YES
3	4	P	14	26	S	0.5	S	0.5	S		YES	YES	YES
3	5	P	26	27	S	0.5	S	1	S	YES	YES	YES	YES
3	6	P	28	27	S	1	S	1	S	YES	YES	YES	YES
3	7	P	0	27	S	0.5	S	1	S		YES	YES	YES
3	8	P	14	25	S	0.5	S	0.5	S		YES	YES	YES
3	9	P	6	27	S	0.5	S	1	S		YES	YES	YES
3	10	P	20	27	S	0.5	S	0.5	S		YES	YES	YES
3	11	P	11	25	S	1	S	1	S		YES	YES	YES
3	12	P	0	26	S	0.5	S	1	S		YES	YES	YES
3	13	P	21	25	S	0.5	S	0.5	S		YES	YES	YES
3	14	P	17	25	S	4	S	1	S		YES	YES	**NO**

3	15	P	28	26	S	1	S	1	S	YES	YES	YES	YES
3	16	P	16	28	S	0.5	S	1	S		YES	YES	YES
3	17	P	0	25	S	1	S	1	S		YES	YES	YES
3	18	P	27	26	S	1	S	0.5	S	YES	YES	YES	YES
3	19	P	0	25	S	1	S	1	S		YES	YES	YES
3	20	P	11	26	S	1	S	1	S		YES	YES	YES
3	21	P	0	25	S	0.5	S	1	S		YES	YES	YES
3	22	P	0	24	S	1	S	2	S		YES	YES	YES
3	23	P	0	27	S	0.5	S	1	S		YES	YES	YES
3	24	P	0	24	S	1	S	1	S		YES	YES	YES
3	25	P	25	26	S	0.125	S	0.5	S	YES	YES	YES	EXCLUDED
3	26	P	17	26	S	0.5	S	1	S		YES	YES	YES
3	27	P	25	28	S	0.125	S	0.5	S	NO RESIST-5	YES	YES	EXCLUDED
3	28	P	13	24	S	1	S	2	S		YES	YES	YES
3	29	P	21	24	S	1	S	1	S		YES	YES	YES
3	30	P	13	23	S	1	S	1	S		YES	YES	YES
4	1	P	13	27	S	1	S	1	S		YES	YES	YES
4	3	P	28	28	S	1	S	0.5	S	YES	YES	YES	YES
4	4	P	0	26	S	1	S	1	S		YES	YES	YES
4	5	P	27	29	S	0.25	S	0.5	S	YES	YES	YES	YES
4	6	P	27	33	S	0.125	S	0.5	S	YES	YES	YES	EXCLUDED
4	7	P	0	27	S	1	S	1	S		YES	YES	YES
4	8	P	22	28	S	0.064	S	0.5	S		YES	YES	EXCLUDED
4	10	P	14	26	S	0.5	S	2	S		YES	YES	**NO**
4	12	P	14	25	S	1	S	2	S		YES	YES	YES
4	15	P	21	25	S	0.25	S	0.5	S		YES	YES	YES
4	16	P	27	29	S	0.25	S	0.5	S	YES	YES	YES	YES

4	17	P	28	28	S	0.125	S	0.5	S	YES	YES	YES	EXCLUDED
4	19	P	12	25	S	0.5	S	0.5	S		YES	YES	YES
5	1	P	10	25	S	1	S	1	S		YES	YES	YES
5	2	P	24	24	S	0.5	S	1	S	NO RESIST-5	YES	YES	YES
5	3	P	27	28	S	0.25	S	0.5	S	RECENTLY TESTED	YES	YES	YES
5	5	P	8	23	S	1	S	2	S	RECENTLY TESTED	YES	YES	YES
5	6	P	24	24	S	0.5	S	0.5	S	RECENTLY TESTED	YES	YES	YES
5	8	P	0	24	S	1	S	1	S		YES	YES	YES
5	9	P	0	27	S	1	S	1	S		YES	YES	YES
5	10	P	25	27	S	0.125	S	0.5	S	RECENTLY TESTED	YES	YES	EXCLUDED
5	11	P	24	23	S	0.125	S	1	S	NO RESIST-5	YES	YES	EXCLUDED
5	12	P	23	27	S	0.125	S	0.5	S		YES	YES	EXCLUDED
6	2	P	15	25	S	1	S	1	S		YES	YES	YES
6	3	P	15	24	S	0.5	S	1	S		YES	YES	YES
6	4	P	0	24	S	1	S	2	S		YES	YES	YES
6	5	P	23	23	S	1	S	1	S	RECENTLY TESTED	YES	YES	YES
6	6	P	24	28	S	0.125	S	0.5	S	RECENTLY TESTED	YES	YES	EXCLUDED
6	7	P	25	29	S	0.25	S	0.5	S	RECENTLY TESTED	YES	YES	YES
6	8	P	13	25	S	0.5	S	1	S		YES	YES	YES
6	9	P	23	26	S	0.125	S	0.5	S	RECENTLY TESTED	YES	YES	EXCLUDED
6	10	P	24	26	S	0.125	S	0.5	S	RECENTLY TESTED	YES	YES	EXCLUDED