

## **Comparison of Meropenem MIC by E Test and VITEK 2 in resistant *Pseudomonas* and *Acinetobacter* isolates**

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### **Abstract**

*Pseudomonas aeruginosa* and *Acinetobacter baumannii* cause serious infections in health care institutions. Many isolates are multidrug resistant and sometimes resistant even to meropenem. The minimum inhibitory concentration (MIC) of an antibiotic is useful to decide on specific treatment and several methods of detecting MIC are in current use. Routine application of such methods is cumbersome for clinical laboratories and the newly introduced VITEK 2 automated method is an attractive alternative. The aims of the study were to compare the performance of the E test and the VITEK 2 system in susceptibility testing of resistant strains of *P.aeruginosa* and *A. baumannii* to meropenem and to compare the MIC of four different carbapenem antibiotics for *P.aeruginosa* and *A.baumannii*. 75 strains of *P.aeruginosa* and 25 of *A.baumannii* were selected randomly from the isolate collection of the Princess Alexandra Hospital, Australia. MIC testing using the E test and the VITEK 2 MIC were performed for each isolate according to the manufacturer's instructions and the CLSI guidelines of June, 2010. MICs obtained by VITEK-2 corresponded closely with those obtained with the E test method. Categorical agreement testing for both organisms was 92% with no major errors and 08% minor errors. We conclude that VITEK 2 is a reliable method to determine MIC to meropenem for *P. aeruginosa* and *A. baumannii*. Doripenem sensitivity results can be extrapolated from the meropenem sensitivity results.

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## Introduction

*Pseudomonas aeruginosa* and *Acinetobacter baumannii* have emerged as major human pathogens because of their ability to cause infections in many clinical scenarios.<sup>1</sup> Infections due to multidrug-resistant *P.aeruginosa* and *A. baumannii* are associated with a prolonged hospital stay and increased cost. *A. baumannii* infections generally affect debilitated patients in intensive care units and are associated with high mortality rates.<sup>2</sup> Further, *A. baumannii* is difficult to control and treat because of its prolonged environmental survival and ability to develop resistance to multiple antimicrobial agents.<sup>3</sup> Inappropriate initial antimicrobial therapy is associated with higher rates of patient morbidity and mortality in infections with *P. aeruginosa*.<sup>4</sup> Several studies have documented increasing resistance rates in these two organisms to many antibiotics.<sup>5,6</sup> As a result, clinicians are left with a restricted choice of therapy, such as the carbapenem antibiotics, including meropenem.

Acquired metallo-beta lactamases (MBL) and Oxa carbapenamases have recently emerged as important resistance mechanisms in *P.aeruginosa* and *A. baumannii* limiting therapy with carbapenem antibiotics.<sup>7</sup> A study by Landman et al<sup>8</sup> found that about 25% of *P.aeruginosa* isolates are resistant to carbapenems and fluroquinolones and over 30% of *A. baumannii* are multi drug resistant. The clinical implications of antibiotic resistance are extremely serious and rapid and sensitive diagnostic methods are urgently needed to guide therapy, monitor resistance development and expedite intervention strategies in the management of serious infections, especially among critical care patients.

Genotypic methods are not suitable for routine clinical testing. Therefore determination of antibiotic susceptibility by quantitative MIC (minimal inhibitory concentration) testing is required. Manual methods of antibiotic sensitivity testing are being replaced with automated systems due to the increasing volumes of clinical specimens. The usage of such systems is motivated by the decrease in laboratory turnaround time compared to that required for standardized methods, cost-effectiveness and convenient interfaces with laboratory and hospital information systems which guides the physicians for efficient antimicrobial therapy. The availability of rapid results, reproducibility, ability to trace results, and potential impact on the workflow too favour the use of automatic systems in the microbiology laboratory. However a few studies have demonstrated errors of various automated systems when several organism-antimicrobial combinations were tested.<sup>9,10</sup>

VITEK 2 (bioMerieux) is able to determine the MIC and the production of carbapenamase during one test cycle. VITEK 2 detects metabolic changes by fluorescence-based methods and identifies bacteria by monitoring the kinetics of bacterial growth. The MIC phenotype detected for the test isolate is interpolated with all the patterns of the database and the best is identified. VITEK 2 systems have recently been adopted by many microbiological laboratories for rapid identification and the determination of antimicrobial susceptibilities of various types of pathogens, including Gram negative organisms.<sup>11</sup> The advanced expert system of the instrument detects infrequent or impossible phenotypes of the organisms.

Given the current increase in infections due to multiresistant *P.aeruginosa* and *A. baumannii* infections, it is necessary to confirm the speed and accuracy of the VITEK 2 system in determining MICs for these organisms by comparing its performance with a standard reference method. Epsilon meter test (E test) serves as an accurate, easy-to-perform and time-saving alternative to the reference agar and broth dilution methods for quantitative antimicrobial susceptibility testing associated with *P. aeruginosa*.<sup>12</sup> Hence this study was designed to compare the E test, as the reference method of detecting minimal inhibitory concentration, with the VITEK 2 system for susceptibility testing of resistant *P. aeruginosa* and *A. baumannii* strains to meropenem and to compare the MICs of four carbapenem antibiotics by the E test method for *P. aeruginosa* and *A. baumannii*.

## Methods

A total of 100 isolates (75 *P. aeruginosa* and 25 *A. baumannii* complex) were randomly selected from the collection of resistant isolates of the Princess Alexandra Hospital, Queensland, Australia. All organisms had been identified at the time of isolation using the VITEK system. The organisms were revived from storage at -70°C and sub cultured twice on horse blood agar.

VITEK 2 MIC testing was performed for all the isolates according to the manufacturer's instructions. Suspensions of the organism were made in 0.45% saline and adjusted to the turbidity of 0.5-0.63 McFarland standard using the VITEK 2 Densi Chek densitometer. Suspensions were placed in a VITEK 2 cassette along with a sterile polystyrene test tube and VITEK 2 AST N149 cards containing serial twofold dilutions of 19 antibiotics. The loaded cassettes were placed in the VITEK 2 instrument which automatically processes the isolate until the MICs were obtained. MICs of carbapenem antibiotics other than meropenem could not be evaluated as the VITEK 2 AST N149 card contains only meropenem. Purity of the organism suspensions was ensured by subculture on solid media. E strips (AB bioMerieux, Solna, Sweden) of meropenem, imipenem, ertapenem and doripenem were placed on different Mueller Hinton agar plates inoculated with a 0.5 McFarland standard suspension of the test isolate. Each E-test strip consists of a predefined gradient of antibiotic, allowing for MIC measurements in the range of 0.002–32 µg/ml. According to the manufacturer's instructions, MIC was determined as the value at which the elliptical growth margin intersected the E test strip. *P.aeruginosa* ATCC 27853 was used as the control for both tests.

MIC's obtained from both methods were interpreted as falling into the susceptible, intermediate or resistant categories as per CLSI document M100-S9, June, 2010. Thus, isolates were considered to be sensitive if the MIC was ≤ 1µg/ml, intermediate if the MIC was 2µg/ml and resistant if the MIC was ≥ 4µg/ml for meropenem, imipenem, and doripenem. The breakpoints for ertapenem were ≤ 0.25 µg/ml, 0.5 µg/ml and ≥ 1 µg/ml respectively.

The MIC results obtained with the VITEK 2 system were compared with those of the reference E test.

## Results

**Table 1 Comparison of MIC obtained using VITEK 2 and E test**

Clinical category	VITEK 2 MIC (meropenem)		MIC determination by E test							
			meropenem		imipenem		doripenem		ertapenem	
	<i>P.aeru</i>	<i>A.bau</i>	<i>P.aeru</i>	<i>A.bau</i>	<i>P.aeru</i>	<i>A.bau</i>	<i>P.aeru</i>	<i>A.bau</i>	<i>P.aeru</i>	<i>A.bau</i>
S	10 (13%)	4 (16%)	10 (13%)	2 (8%)	5 (7%)	2 (8%)	19 (25%)	3 (12%)	1 (1%)	2 (8%)
I	0	2 (8%)	6 (8%)	4 (16%)	15 (20%)	4 (16%)	21 (28%)	3 (12%)	1 (1%)	0
R	65 (87%)	19 (76%)	59 (78%)	19 (76%)	55 (73%)	19 (76%)	35 (47%)	19 (76%)	73 (98%)	23 (92%)

S – sensitive I – intermediate sensitive R - resistant

Of the 75 *P.aeruginosa* isolates, VITEK 2 categorized 10 (13%) as sensitive and 65 (87%) as resistant with no intermediate resistant strains for meropenem. 4 (16%) of the *A. baumannii* strains were sensitive, 19 (76%) were resistant and 2 (8%) were of intermediate resistance. Table 1 compares the Vitek 2 MIC clinical categories of organisms to those obtained by the E test.

Discrepancies between VITEK 2 and the E test reference method were classified as very major errors (E-test result was resistant and VITEK 2 result was susceptible), major errors (E-test result was susceptible and VITEK 2 result was resistant), or minor errors (an intermediate susceptibility result was obtained by E test or VITEK 2 method).<sup>13</sup>

VITEK 2 MIC for determination of MIC to meropenem for *P.aeruginosa* did not show very major errors or major errors in comparison with E test. However 6 (8%) isolates had MIC results that fell into the minor error category. Hence the overall categorical agreement of VITEK 2 meropenem MIC with the reference method was 92%. *A. baumannii* VITEK 2 MIC testing with meropenem followed the same pattern with 8% of minor errors and an overall categorical agreement of 92% with E test MIC.

## Discussion

*Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are multidrug-resistant organisms, increasingly reported as causes of hospital acquired infection worldwide.<sup>14</sup> These multidrug-resistant organisms have diminished susceptibilities to one or more than one of the following groups of antibiotics – antipseudomonal cephalosporins, antipseudomonal carbapenems,  $\beta$  lactam- $\beta$ -lactamase inhibitor combinations, antipseudomonal fluoroquinolones, and aminoglycosides.<sup>15</sup> Carbapenems are considered the gold standard of treatment for multidrug resistant *A. baumannii* and *P. aeruginosa* infections. Imipenem has been recognised as the most active agent for treatment of *A. baumannii* infections.<sup>2</sup> However, recently, strains of these organisms have developed resistance to carbapenems, posing important therapeutic challenges. Peleg and Hooper,<sup>16</sup> in a recent survey, determined that 26.4% of *P. aeruginosa* and 36.8% of

*A. baumannii* causing ventilator-associated pneumonia were resistant to carbapenems (imipenem or meropenem).

Automated antibiotic susceptibility testing systems have been evaluated in numerous studies using several organism-antimicrobial combinations. *P. aeruginosa* has been the organism in which the most performance errors have been reported, especially when tested against the beta-lactam antimicrobial agents.<sup>17,18</sup> Hence, we evaluated the ability of the VITEK 2 system to determine the antimicrobial susceptibility of *P. aeruginosa* and *A. baumannii* isolates accurately using meropenem as the test antibiotic.

A maximum overall category error rate of 10% should be obtained for a susceptibility test to have acceptable performance, including a maximum of 1.5% of very major errors and 3.0% major errors.<sup>19</sup> In our study, most of VITEK 2 meropenem MIC results were in accordance with reference E test MICs. Both *P. aeruginosa* and *A. baumannii* recorded 8% minor error which lies within the acceptable rate of less than 10% and the overall categorical agreement for MIC for the two types of tested isolates was more than expected at 90%.<sup>19</sup>

Our results are in accordance with the results of a study carried out by Providencia Joyanes et al.<sup>20</sup> They reported very major errors for *P. aeruginosa* in only one strain (1.4%) of *A. baumannii*, for imipenem and not for meropenem. Otto Krag<sup>21</sup> tested 224 strains of non-fermentative Gram negatives and their conclusion supports our results that VITEK 2 is suitable for routine clinical use as the overall categorical agreement in antibiotic sensitivity testing was 92.9%. Minor errors were found in 5.1% of strains, and major and very major errors were found in 1.6% and 0.3% of strains, respectively in the same study.<sup>21</sup> Annarita Mazzariol et al<sup>22</sup> demonstrated that VITEK 2 could be used with confidence for identifying resistance to several antimicrobial agents against *P. aeruginosa* including imipenem.

However in contrast to our findings, discordant results were reported in a study using the broth micro dilutions as the reference method, in that VITEK 2 showed very major errors in 19.7% and minor errors in 34.2% of *A. baumannii* isolates for amikacin.<sup>23</sup> Another study demonstrated that the automated systems (MicroScan WalkAway, VITEK 2, and VITEK systems) generally failed to accurately detect piperacillin-tazobactam resistance among clinically significant isolates of *P. aeruginosa*.<sup>13</sup>

In the present study, the MICs of meropenem and imipenem for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were similar, with sensitive, intermediately resistant and resistant rates of *P. aeruginosa* to meropenem being 13%, 8% and 78% respectively and for imipenem 7%, 20% and 73% respectively. For *A. baumannii*, the rates were 8%, 16% and 76% respectively for both antibiotics.

Doripenem is a new carbapenem antibiotic with a spectrum of activity comparable to that of imipenem and meropenem.<sup>24</sup> Considering doripenem MICs in our study, they were more or less similar to the other carbapenems with *P. aeruginosa* strains showing 25% as sensitive, 28% as intermediately resistant and 47% as resistant. 12% of *A. baumannii* strains were sensitive with 12% intermediately resistant and 76% resistant.

Ertapenem, is a carbapenem that is licenced for once daily use. Although it is reported that 93% of ESBL-producing coliform isolates are sensitive to ertapenem,<sup>25</sup> it is not active in infections caused by other resistant organisms, including *A. baumannii* and *P. aeruginosa*.<sup>26</sup> Theoretically ertapenem is the carbapenem least likely to permeate Gram-negative bacteria rapidly. 98% of *P. aeruginosa* and 92% of *A. baumannii* in our study were resistant to ertapenem.

## Conclusion

VITEK 2 meropenem MIC results for *P.aeruginosa* and *A. baumannii* correspond closely with those obtained by the reference E test. Hence it is a reliable method to detect sensitivity to meropenem in the two organisms. Doripenem sensitivity can be extrapolated from that of meropenem. Ertapenem resistance in *P. aeruginosa* and *A. baumannii* is confirmed.

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