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Original Research Article

Reliability of Kirby-Bauer Disk Diffusion Method for Detecting Doripenem Susceptibility in Oxidase Positive Non-Fermenting Gram Negative Bacilli

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ABSTRACT

Doripenem is a member of the carbapenem family of antibiotics with significant potential for use in *Pseudomonas aeruginosa* infections without acquired resistance. United States Food and Drug Administration (US FDA) approved indications for doripenem include treatment of complicated urinary tract infections (cUTI), complicated intra-abdominal infections (cIAI) and nosocomial pneumonia including ventilator-associated pneumonia (VAP). Non-fermenting Gram negative bacilli (NFGNB) have emerged as important multi-drug resistant nosocomial pathogens and may be associated with poor clinical outcomes. The aim of this study therefore was to determine the susceptibility pattern of doripenem against clinical isolates of *Pseudomonas* spp and other NFGNB. In addition we also used the Kirby Bauer Disc Diffusion method to compare results of Doripenem susceptibility using discs (10µgm) procured from Hi-media and BD Diagnostics. A total of 80 clinical samples of patients with cUTIs and cIAIs were included in the study. Consecutive clinical isolates of oxidase positive NFGNB were identified by routine biochemical examination. Comparison of zone diameters of doripenem discs (10µgm) procured from BD diagnostics and Hi-Media was done with results of bioMérieux Etest. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Susceptibility pattern of isolates was determined by both current CLSI guidelines as well as EUCAST (http://www.EUCAST.org). Discordant results were categorized as very major (sensitive according to KBDD method but resistant by E test), major (resistant according to KBDD method but sensitive by E test) and minor (reported intermediate when resistant or susceptible or vice versa).The isolates were identified as *Pseudomonas aeruginosa* (n=34), other *Pseudomonas* species (n=38) and *Burkholderia cepacia* complex (n=8). Very major errors and major errors were observed with two and one isolates respectively. Minor error rates of 7.5% were observed when results were interpreted as per CLSI break points. No discrepancies were recorded between KBDD and E test when EUCAST break-points were applied to the results obtained.

Keywords: Doripenem, Kirby Bauer Disc Diffusion, E test, Non-fermenting gram negative bacilli.

INTRODUCTION

Non-fermenting Gram negative bacilli (NFGNB) have emerged as important multi-drug resistant nosocomial pathogens and may be associated with poor clinical outcomes. [1] Doripenem is widely being used for the treatment of complicated urinary tract infections (cUTIs) and complicated intra-abdominal infections (cIAIs) caused by these organisms. [2] Accurate susceptibility testing is necessary for guiding appropriate therapeutic options and the Kirby-Bauer Disc Diffusion (KBDD) method is economical for routine testing in any resource limited clinical microbiology laboratory. There are however

few studies on the reliability of testing doripenem against oxidase positive NFGNB by the KBDD method. The aim of this study was to compare the zone diameters of doripenem discs (10µgm) supplied by Hi-Media Laboratories and BD Diagnostics with the bio Mérieux Etest (0.002 to 32µg/ml) on80 non-repeat clinical isolates recovered from urine and pus samples of patients with cUTIs and cIAIs.

MATERIALS AND METHODS

From January to March 2014, a total of 80 non-repetitive clinical *Pseudomonas* spp. and oxidase positive NFGNB isolates recovered from urine and pus samples of patients with cUTIs and cIAIs were included in this study. The isolates were identified by routine biochemical tests as *Pseudomonas aeruginosa* (n=34), other *Pseudomonas* species (n=38) and *Burkholderia cepacia* complex (n=8) by standard bacteriological tests. Sensitivity to doripenem was determined by the KBDD method using discs (10µgm) of both Hi-media and BD diagnostics. The E test was taken as the reference standard. Interpretive criterion were as follows: sensitive at ≥23mm and ≤1µg/ml, intermediate at 20-22mm and 2µg/ml, resistant at ≤19mm and ≥4 µg/ml. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. Susceptibility pattern of the isolates was determined by both current CLSI guidelines [3] as well as EUCAST (http://www.EUCAST.org). Discordant results were categorized as very major (sensitive according to KBDD method but resistant by E test), major (resistant according to KBDD method but sensitive by E test) and minor (reported intermediate when resistant or susceptible or vice versa).

RESULTS AND DISCUSSION

The susceptibility pattern of 80 isolates against doripenem using HM and BD discs as well as E test is summarized in Table 1.

Table 1: Susceptibility pattern for doripenem by KBDD (using BD and Hi-media discs) method and E test

	EUCAST			CLSI		
	Hi-Media discs	**BD doripenem discs**	**bioMerieux E-test**	**Hi-Media discs**	**BD doripenem discs**	**bioMerieux E-test**
Resistant	23	20	12	24	22	16
Intermediate	-	-	-	4	1	2
Susceptible	57	60	64	52	56	61

Very major errors were recorded for four (5%) and six (7.5%) isolates using HM and BD discs respectively as per CLSI guidelines while it was 5% and 11.25% with HM and BD discs respectively as per EUCAST. Major error was recorded for one isolates each (1.25%) by discs of both manufacturers as per both CLSI and EUCAST criterion. As per CLSI guidelines, occurrence of ≥ 1.5% very major errors and ≥ 3% major errors is considered as unacceptable. Minor error rates of 2% and 6% were recorded with HM and BD discs respectively as per CLSI while it was 0% with EUCAST irrespective of the source of the disc.

MIC results in table 1 as per EUCAST are shown for only 76 isolates as there are no intermediate categories. Three isolates had MIC values of 1.5µg/ml while one had a MIC of 3µg/ml. Similarly, one isolate that had a MIC value of 3µg/ml was not included in table 1 while interpreting by CLSI criterion. Its zone diameters were 21mm by HM disc (sensitive by CLSI, resistant by EUCAST) and 27mm by BD disc (sensitive by both CLSI and EUCAST). Higher number of falsely sensitive isolates by KBDD is most likely due to greater potency of the disc. Gautam et al [4] in a similar study have evaluated the reliability of the KBDD method for detecting carbapenem resistance in 124 *Acinetobacter baumannii-calcoaceticus* complex isolates. Doripenem break-points were however not available at the time their study was published. Joseph et al tested [5] 146 NFGNB against meropenem. Their study

included 48 *Pseudomonas aeruginosa* and 18 *Pseudomonas* spp. and they detected 5.6% very major errors and 28.6% major errors. The Clinical Laboratory Standards Institute (CLSI) guidelines do not provide interpretive break-points of doripenem for oxidase positive NFGNB other than *Pseudomonas aeruginosa* while the European Committee for Antimicrobial Susceptibility Testing (EUCAST) has clubbed all isolates under the heading *Pseudomonas* spp.

CONCLUSION

In conclusion, unacceptable error rates were recorded with the KBDD for NFGNB with discs from two separate manufacturers. BD discs though recorded higher very major error rates. The percentage of very major errors was higher when results were interpreted as per EUCAST guidelines.

The limitation of this study is that the E test was used as the reference method for MIC determination and we tested only a small number of isolates. However, for laboratories in developing countries with a high burden of infection and isolate load, broth micro-dilution is impractical and the automated systems are unaffordable. The KBDD method is still a more economical option to test susceptibility to antibiotics.

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