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Comparing *in vitro* activity of tigecycline by using the disk diffusion test, the manual microdilution method, and the VITEK 2 automated system

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ABSTRACT

Tigecycline is a broad spectrum antibiotic having activity against multiresistant isolates. *In vitro* susceptibility testing is difficult to perform with the use of traditional microbiological techniques. The aim of this study was to evaluate the disk diffusion test with three different Mueller-Hinton agar brands, and the Vitek 2 automated system in comparison with the standard broth microdilution method against 200 gram-negative isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens* and *Acinetobacter baumannii*). Among *Enterobacteriaceae*, the Becton Dickinson agar had the lowest rate of minor (32.5%) and major errors (3.8%). No very major errors were found. For *A. baumannii*, the rate of minor and major errors was lower. A high rate of agreement (94%) was found between the broth microdilution method and the Vitek 2 system. Our results show that there are important differences between agars used for the disk diffusion test, and that Vitek 2 is a valid tool for susceptibility testing in clinical laboratories.

Key words: tigecycline, antimicrobial susceptibility tests, disk diffusion method, Vitek 2 system, gram-negative bacteria

RESUMEN

Comparación de la actividad *in vitro* de la tigeciclina mediante la prueba de difusión con disco, el método de microdilución manual y el sistema automatizado Vitek 2. La tigeciclina es un antibiótico de amplio espectro con actividad frente a bacterias multirresistentes. Existen dificultades en la determinación de la actividad *in vitro* a través de las técnicas microbiológicas convencionales. El objetivo del estudio fue evaluar tres marcas diferentes de medio agar Mueller-Hinton para utilizar en el método de difusión con disco y el método automatizado Vitek 2, y compararlos con la prueba tradicional de microdilución manual (Paneles Trek) frente a 200 aislamientos de microorganismos gram negativos (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens* y *Acinetobacter baumannii*). Para el grupo de las enterobacterias, el medio con mejor desempeño fue el producido por Becton Dickinson, que tuvo 32,5% de errores menores y 3,8% de errores mayores. No se presentaron errores mayores con ningún medio. Se encontró una alta concordancia (94%) entre el método de microdilución manual y el Vitek 2. Para *A. baumannii*, el medio con mejor desempeño fue el Mueller-Hinton elaborado por Becton Dickinson, con 12,5% de errores menores y 2,5% de errores mayores. Los resultados sugieren que el método Vitek 2 es una herramienta válida en la determinación de la sensibilidad a la tigeciclina y que existen diferencias muy grandes en la prueba de difusión con disco según la marca comercial de medio utilizado.

Palabras clave: tigeciclina, pruebas de sensibilidad a los antimicrobianos, método de difusión con disco, Vitek 2, bacterias gramnegativas

Surveillance of *in vitro* activity of tigecycline against different groups of microorganisms, including multiresistant isolates, have been extensively reported in the literature since this new antibacterial agent was introduced in the market (7). There is no availability of purified antibiotic to perform manual microdilution tests, and other methods

are expensive. The use of antibiotic disks has been marked by discrepancies in the results obtained according to the Mueller-Hinton agar type used, probably as a consequence of a high content of manganese and other cations (1-3). This new antibiotic has recently been included in the Vitek 2 automated system, and it serves

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as an alternative for susceptibility testing of clinical samples against tigecycline.

The purpose of this study is to evaluate three different Mueller-Hinton agar media using disk diffusion tests, and the Vitek 2 automated system against 200 clinical isolates of gram-negative bacteria obtained from hospitalized patients in 13 third-level hospitals in Colombia in 2007 and 2008 (4).

Isolates were randomly selected among the whole range of possibilities. Forty isolates of each bacterial species were selected, including *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, and *Acinetobacter baumannii*.

Three different brands of Mueller-Hinton agar were selected to perform disk diffusion tests: Oxoid (Cambridge, UK), Becton Dickinson (BD) (New Jersey, USA) and bioMérieux (France). Tigecycline disks (15 µg) were used (BD). Minimal inhibitory concentration (MIC) was determined by using the Vitek 2 Compact with gram-negative cards AST N087. This card had tigecycline concentrations between 0.5 µg/ml and 8 µg/ml. Simultaneously, the MIC of all isolates was established by using the broth microdilution technique (Trek Diagnostics System, Cleveland, USA) with increasing concentrations between 0.08 µg/ml and 16 µg/ml (Figure 1). Susceptibility testing was performed according to the Clinical Laboratory Standards Institute (CLSI) recommendations (6). Quality control for both the broth microdilution method and the disk diffusion test, was *E. coli* ATCC 25922. *Pseudomonas aeruginosa* ATCC 27853 was also used as quality control for the disk diffusion method. Susceptibility breakpoints for *Enterobacteriaceae* suggested by the Food and Drug Administration (FDA) were used against *Enterobacteraceae* and *A. baumannii*.

Data analysis of susceptibility testing was done using Whonet software 5.4 (World Health Organization - WHO

- Switzerland). Minor and major errors were established for both the disk diffusion method and Vitek 2, by comparison with the broth microdilution method.

For 160 *Enterobacteriaceae* isolates, a higher percentage of errors was obtained with the Oxoid agar, followed by the bioMérieux and the BD agars. More than 30% of minor errors were found in the agar having the best performance (BD). This same agar had 3.8% of major errors (Table 1). When the analysis was done taking into account microorganisms, global differences were found for the three agar media against *S. marcescens*, followed by *K. pneumoniae* and *E. cloacae*. No major error was observed against *E. coli* with the different agar media used, and no minor error was observed with the BD agar.

In the case of *A. baumannii*, errors were frequently found with the Oxoid agar, followed by the bioMérieux and the BD agars. Discrepancies, presented as minor errors, were over 12% in the BD agar. The same percentage of errors (2.5%) was found when using the BD and the bioMérieux agars (Table 1). Very major errors were not found with any group of microorganisms. Table 2 compares the zone diameters of inhibition in the different agars. There were larger zone diameters with the BD medium than with the bioMérieux and Oxoid media for *Enterobacteriaceae* and *A. baumannii* as well.

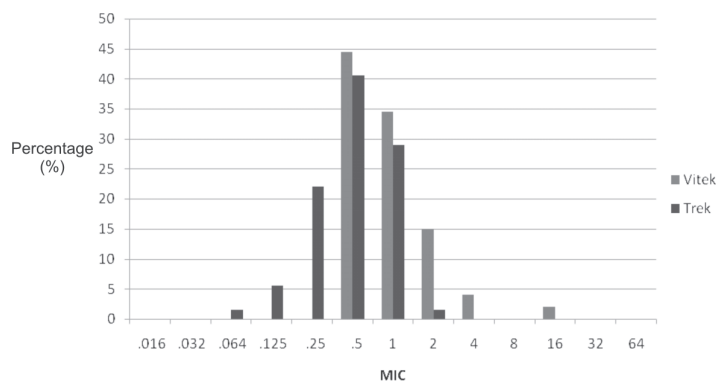
An agreement of 94% was identified when comparing Vitek 2 with the broth microdilution method. Minor errors represented 4%, while major errors were 2%. No errors were found for *E. coli* and *A. baumannii*, and minor errors were found in *E. cloacae* (12%), followed by *S. marcescens* (2.5%) and *K. pneumoniae* (2.5%). The medians and ranges of the MICs obtained by the Vitek 2 method were higher than those obtained by manual microdilution in both *Enterobacteriaceae* and *A. baumannii*.

Table 1. Percentage of error with different susceptibility testing methods against 200 gram-negative isolates

Method	Errors %		
	Minor errors	Major errors	Very major errors
<i>Enterobacteriaceae</i> (n = 160)			
Mueller-Hinton BD	32.5	3.8	0
Mueller-Hinton bioMérieux	58.8	5.6	0
Mueller-Hinton Oxoid	62.5	11.9	0
Vitek 2	5.0	2.5	0
<i>A. baumannii</i> (n = 40)			
Mueller-Hinton BD	12.5	2.5	0
Mueller-Hinton bioMérieux	25.0	2.5	0
Mueller-Hinton Oxoid	37.5	5.0	0
Vitek 2	0.0	0.0	0

Table 2. Comparison of zone diameters, MIC medians, and ranges for each commercial medium and Vitek 2 system against 200 gram-negative isolates

Method	Median	Range
<i>Enterobacteriaceae</i> (n = 160)		
Mueller-Hinton BD	19 mm	11 mm – 27 mm
Mueller-Hinton bioMérieux	18 mm	12 mm – 23 mm
Mueller-Hinton Oxoid	17 mm	9 mm – 24 mm
Vitek 2	1 µg/ml	0.5 µg/ml – 16 µg/ml
Trek	0.5 µg/ml	0.064 µg/ml – 2 µg/ml
<i>A. baumannii</i> (n = 40)		
Mueller-Hinton BD	18 mm	12 mm – 24 mm
Mueller-Hinton bioMérieux	17 mm	12 mm – 22 mm
Mueller-Hinton Oxoid	16 mm	11 mm – 20 mm
Vitek 2	1 µg/ml	0.5 µg/ml – 2 µg/ml
Trek	0.5 µg/ml	0.064 µg/ml – 1 µg/ml

**Figure 1.** Distribution of minimum inhibitory concentrations using manual microdilution (Trek) and Vitek 2 cards.

These results confirmed the evidence found in previous works about limitations of susceptibility testing when using the disk diffusion method (2, 3, 5, 8). A higher rate of errors when using the Oxoid agar was also found by other authors (1, 5, 8), who had systematically reported smaller halo diameters when compared with the BD agar. Higher concentrations of manganese present in the Oxoid agar might offer an explanation for this finding (2).

Since the disk diffusion method is a simple and easily performed technique for gram-negatives, it might be important to select agars with less manganese content when determining tigecycline susceptibility. Alternative techniques such as the Etest in those isolates with intermediate and resistant results might be considered, and resistance should be confirmed by using the broth microdilution technique (3).

According to the results obtained with the Vitek 2 system, and considering the problems arising with the

disk diffusion method, this commercial and automated method could be a valid alternative for tigecycline susceptibility testing in clinical laboratories. However, due to the higher MICs found, especially for *S. marcescens* isolates, surveillance of *in vitro* results should be strengthened, and data produced by local and national laboratories should be carefully followed.

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