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RESEARCH NOTE

Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin

T. Y. Tan and S. Y. Ng

Laboratory Medicine Services, Changi General Hospital, Singapore

ABSTRACT

In total, 172 isolates of Enterobacteriaceae, *Acinetobacter* spp., *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were tested for susceptibility to colistin by agar dilution, Etest and the Vitek 2 system. Isolates with a colistin MIC ≤2 mg/L were considered to be susceptible. Fifty-four (31%) Gram-negative isolates were resistant to colistin. Categorical agreement between agar dilution and Etest was 87%, and between agar dilution and Vitek 2 was 82%. Based on the data obtained, the Vitek 2 system was unreliable for detecting colistin resistance, and results obtained by Etest may require confirmation by a standard MIC susceptibility testing method.

Keywords Agar dilution, colistin, Etest, resistance, susceptibility testing, Vitek 2

Original Submission: 26 October 2006; **Revised Submission:** 15 December 2006; **Accepted:** 20 December 2006

Clin Microbiol Infect 2007; **13**: 541–544
10.1111/j.1469-0691.2007.01708.x

Increasing antibiotic resistance in Gram-negative bacilli, coupled with a shortage of new antimicrobial agents, has led to renewed interest in the use of polymyxins for treating multidrug-resistant infections [1]. There are few guidelines for antibiotic susceptibility testing of polymyxins. The CLSI issued interpretative breakpoints for *Acinetobacter* spp. only in 2005 [2], and disk susceptibility testing generally yields poor results with colistin [3]. Determination of colistin MICs is

Corresponding author and reprint requests: T. Y. Tan, Laboratory Medicine Services, Changi General Hospital, 2 Simei Street 3, Singapore 529889
E-mail: thean_yen_tan@cgh.com.sg

considered to be the optimum method, but is impractical for most clinical microbiology laboratories. Alternative methods include the Etest (AB Biodisk, Solna, Sweden) and automated susceptibility testing systems, e.g., the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). This study compared these three methods for testing colistin susceptibility.

In total, 172 non-duplicate isolates of Enterobacteriaceae, *Acinetobacter* spp., *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were collected from clinical specimens over a 24-month period. Bacterial suspensions were prepared from overnight cultures using a nephelometer (bioMérieux) to give an initial density of 0.5 × McFarland standard for Etest and agar dilution, and 0.6 × McFarland standard for Vitek 2, followed by a dilution step to yield a test inoculum of 10^4 CFU for agar dilution.

MICs of colistin were determined by agar dilution [4], in which colistin sulphate powder (Sigma-Aldrich, Singapore) in solution was added to molten Mueller–Hinton agar (Becton-Dickinson, Franklin Lakes, MD, USA) to provide two-fold dilutions ranging from 0.25 to 128 mg/L. Bacterial suspensions were applied to agar plates using a multipoint inoculator (Mast Diagnostics, Bootle, UK). Results were read following incubation at 35°C for 16–20 h for Enterobacteriaceae, and for 20–24 h for *Acinetobacter* spp.

Susceptibility tests were performed on the Vitek 2 system using AST-N032 test cards, inoculated according to the manufacturer's guidelines. MICs were determined without application of the expert rule base.

Colistin Etests (AB Biodisk) were performed on Mueller–Hinton agar plates according to the manufacturer's guidelines. Etest strips were applied when the agar surface was completely dry, and were incubated at 35°C for 18 h. The MIC was determined at the point where inhibition of growth intersected the Etest strip. The highest MIC intersect was recorded if microcolonies were present within the zone of inhibition, or if the ellipse was asymmetrical. For comparison purposes, Etest MICs were rounded up to the next highest two-fold dilution. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as quality control organisms for all testing methods. Quality control findings were in line with published standards [2].

Essential agreement between agar dilution and Etest was defined as MICs that were within ±1 $\log_2$ dilution of each other. MIC agreement between agar dilution and Etest was only evaluated for isolates with an agar dilution MIC of 0.5–64 mg/L, and between agar dilution and Vitek 2 for isolates with a Vitek MIC of 1–8 mg/L, as the MIC ranges for Etest (0.06–1024 mg/L) and Vitek 2 (1–8 mg/L) differed from that available for agar dilution. Organisms with a colistin MIC of ≥4 mg/L were considered to be resistant, and categorical agreement was calculated using this breakpoint. The agar dilution result was taken as the reference method against which results from the other two methods were compared. A very major error denoted a false-susceptible result, and a major error denoted a false-resistant result.

In total, 54 (31%) Gram-negative isolates were resistant to colistin, with resistance detected in *Morganella* spp. (3/3, 100%), *Serratia* spp. (9/9, 100%), *S. maltophilia* (9/9, 100%), *Enterobacter* spp. (15/20, 75%), *P. aeruginosa* (15/47, 32%) and *Klebsiella* spp. (3/18, 17%). No resistance was detected in *Acinetobacter* spp. and *E. coli*.

There was categorical agreement between the Etest and agar dilution for 149 (86.6%) tests. There were eight (4.7%) very major errors and 15 (8.7%) major errors; 19 (83%) of the errors were detected when testing *P. aeruginosa*, two when testing Enterobacteriaceae, and one error each when testing *Acinetobacter* spp. and *S. maltophilia* (Table 1). Based on the isolates tested, the Etest had a sensitivity of 85% and a specificity of 87% for detecting colistin resistance, with a positive predictive value of 75%, and a negative predictive value of 93%. There was categorical agreement between the Vitek 2 system and agar dilution for

Table 1. Errors in comparison with agar dilution when testing susceptibility to colistin by Etest and the Vitek 2 system

		Etest		Vitek	
Organism tested	No. tested	Major error (% of total)	Very major error (% of total)	Major error (% of total)	Very major error (% of total)
Acinetobacter spp.	58	1 (2)	0	0	0
Enterobacteriaceae	58	0	2 (4)	0	15 (26)
Pseudomonas aeruginosa	47	14 (30)	5 (11)	0	14 (30)
Stenotrophomonas maltophilia	9	0	1 (11)	0	2 (22)
Total	172	15 (8.7)	8 (4.7)	0	31 (18.0)

Table 2. Essential agreement of colistin MICs among three test methods

Organism tested	No. of isolates (%) showing difference in $\log_2$ dilutions							Total
	>−2	−2	−1	0	+1	+2	>+2	
Etest compared to agar dilution[a]								
Acinetobacter spp.	0	20 (36)	32 (57)	3 (5)	1 (2)	0	0	56
Enterobacteriaceae	2 (7)	2 (7)	14 (47)	10 (33)	2 (7)	0	0	30
Pseudomonas aeruginosa	2 (4)	0	18 (38)	10 (21)	12 (26)	5 (11)	0	47
Stenotrophomonas maltophilia	1 (25)	0	0	1 (25)	0	1 (25)	1 (25)	4
Vitek 2 compared to agar dilution[b]								
Acinetobacter spp.	0	0	0	1 (33)	1 (33)	1 (33)	0	3
Enterobacteriaceae	0	0	2 (50)	0	0	1 (25)	1 (25)	4
Pseudomonas aeruginosa	0	0	0	14 (60)	5 (22)	2 (9)	2 (9)	23
Stenotrophomonas maltophilia	0	0	0	0	1 (33)	2 (66)	0	2

[a]Calculated for isolates for which the MIC by agar dilution was 0.5–64 mg/L (inclusive).
[b]Calculated for isolates for which the MIC by Vitek 2 was 1–8 mg/L (inclusive).

141 (82%) tests. There were 31 very major errors, but no major errors were detected. The Vitek 2 system failed to detect colistin resistance in some isolates of *P. aeruginosa*, *Enterobacter* spp., *Klebsiella* spp. and *S. maltophilia* (Table 1). The Vitek 2 system had a sensitivity of 43% and a specificity of 100% in detecting colistin resistance, with a positive predictive value of 100%, and a negative predictive value of 79%. Essential agreement between Etest and agar dilution was achieved in 129 (75%) tests (Table 2), and between Vitek 2 and agar dilution in 24 (75%) tests.

The isolates used in this study were nosocomial and are unlikely to be representative of the general population. No colistin-resistant *Acinetobacter* spp. were detected in the present study, and it is therefore not possible to draw definitive conclusions concerning the efficacy of the Etest or Vitek 2 systems in detecting colistin resistance in this genus. Based on the overall results, the Vitek 2 system was unreliable for detecting colistin resistance in Gram-negative bacilli. The Etest produced unacceptable error rates for *P. aeruginosa* and *S. maltophilia*.

A previous study comparing colistin Etests with broth microdilution for *A. baumannii* reported a very major error rate of 1.7% [5]. Susceptibility results obtained by colistin Etests may require confirmation by reference dilution methods. MIC results for colistin Etests were dependent on the manufacturer and batch of Mueller–Hinton agar used, while a 4-h difference in the incubation time could alter MIC values for *P. aeruginosa* by 0.5 $\log_2$ dilution. In the present study, MICs for *P. aeruginosa* were read after incubation for exactly 18 h. The narrow ellipse of inhibition produced by Etests requires close scrutiny to determine MIC endpoints, and high-level resistance in *Enterobacter* spp. manifests as scanty single colonies within a clear ellipse of inhibition. Because of these factors, it would be advisable to perform concurrent quality control with ATCC strains.

The best reference method for testing susceptibility to the polymyxins remains to be defined. Limited data suggest good concordance between agar dilution and microbroth dilution [6], although higher MICs have been reported using agar dilution [7]. Agar dilution and Etest are both agar-based methods, while Vitek 2 uses interpolated data based on growth in liquid media. There remain many unresolved questions regarding polymyxin susceptibility testing, including the effect of the sulphate vs. sulphomethyl derivatives, anion content and pH on susceptibility test results [8]. In addition, there is no consensus regarding the interpretative breakpoints for polymyxin susceptibility; e.g., the CLSI specifies susceptibility breakpoints of <4 mg/L for *Acinetobacter* spp., whereas the guidelines of the British Society for Antimicrobial Chemotherapy specify a susceptibility breakpoint of <8 mg/L (http://www.bsac.org.uk/_db/_documents/version_5_.pdf).

With increasing use of polymyxins for the treatment of multiresistant Gram-negative infections, further and more extensive studies are needed to clarify susceptibility testing methods for these compounds.

ACKNOWLEDGEMENTS

This work was funded by the National Medical Research Council, Singapore. This work was presented, in part, at the 43rd Annual Meeting of the Infectious Diseases Society of America (San Francisco, 2005).

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RESEARCH NOTE

Human alveolar echinococcosis in Slovenia

J. Logar[1], B. Šoba[1], T. Lejko-Zupanc[2] and T. Kotar[2]

[1]Department of Parasitology, Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana and [2]Department of Infectious Diseases and Febrile Illnesses, University Medical Centre Ljubljana, Ljubljana, Slovenia

ABSTRACT

Between January 2001 and December 2005, 1263 patients suspected of having echinococcosis were screened serologically by indirect haemagglutination assay (IHA). IHA-positive patient sera were then retested by western blot for confirmation and differentiation between *Echinococcus granulosus* and *Echinococcus multilocularis* infection. Of 43 sera confirmed as *Echinococcus*-positive, nine appeared to be specific for alveolar echinococcosis (AE) caused by *E. multilocularis*. AE-positive serological results corresponded to the clinical and/or imaging findings concerning the patients' liver cysts. The detected incidence of AE was $0.45/10^5$ inhabitants, which suggests that clinicians and health authorities in Slovenia should give greater attention to AE in the future.

Keywords Alveolar echinococcosis, diagnosis, *Echinococcus*, indirect haemaglutination assay, Slovenia, western blot

Original Submission: 4 September 2006; **Revised Submission:** 10 November 2006; **Accepted:** 18 December 2006

Clin Microbiol Infect 2007; **13**: 544–546
10.1111/j.1469-0691.2007.01701.x

Human echinococcosis is caused mainly by the larvae of the tapeworm *Echinococcus granulosus*, which cause cystic echinococcosis (CE), and by the larvae of *Echinococcus multilocularis*, which can cause alveolar echinococcosis (AE). *E. granulosus* occurs worldwide, but *E. multilocularis* is found only in the temperate northern hemisphere. The main endemic areas of this tapeworm are Alaska, Canada, central North America, some parts of central western Europe, western Turkey, Russia, China, central Asia and northern Japan. At the adult stage, *E. multilocularis* is 1.2–3.7 mm in length and is harboured in the intestine of definitive hosts, typically foxes, and also domestic dogs and cats. The tapeworm eggs are excreted with the faeces. Following accidental ingestion of these eggs, the larval stage of AE may develop in many species of small rodents, and sometimes in humans as intermediate hosts, usually in the liver. AE is potentially fatal and is chronically progressive as a tumour-like hepatic disease [1–6]. The aim of the present study was to examine serologically whether patients in Slovenia suspected of having echinococcosis had been infected by the larvae of *E. multilocularis*.

Between 1 January 2001 and 31 December 2005, 1263 patients suspected of having echinococcosis

Corresponding author and reprint requests: J. Logar, Department of Parasitology, Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Zaloška 4, Ljubljana, Slovenia
E-mail: jernej.logar@mf.uni-lj.si