Journal Pre-proof

In vitro fosfomycin study on concordance of susceptibility testing methods against ESBL and carbapenem-resistant Enterobacteriaceae

Ausilia Aprile, Guido Scalia, Stefania Stefani, Maria Lina Mezzatesta

PII: S2213-7165(20)30254-X

DOI: https://doi.org/10.1016/j.jgar.2020.09.022

Reference: JGAR 1375

To appear in: *Journal of Global Antimicrobial Resistance*

Received Date: 13 June 2020

Revised Date: 1 September 2020

Accepted Date: 26 September 2020

Please cite this article as: Aprile A, Scalia G, Stefani S, Mezzatesta ML, *In vitro* fosfomycin study on concordance of susceptibility testing methods against ESBL and carbapenem-resistant Enterobacteriaceae, *Journal of Global Antimicrobial Resistance* (2020), doi: https://doi.org/10.1016/j.jgar.2020.09.022

In vitro fosfomycin study on concordance of susceptibility testing methods against ESBL and carbapenem-resistant Enterobacteriaceae

Ausilia Aprile [1], Guido Scalia[1,2], Stefania Stefani [1] and Maria Lina Mezzatesta [1*] mezzate@unict.it

[1]Department of Biomedical and Biotechnological Sciences, section of Microbiology, University of Catania, Italy

[2]U.O.C. Laboratory Analysis Unit, A.O.U. "Policlinico-Vittorio Emanuele", Via S. Sofia 78, Catania 95123, Italy.

*Corresponding author:

Maria Lina Mezzatesta

Department of Biomedical and Biotechnological Sciences, section of Microbiology, University of Catania, Italy

Address: Via Santa Sofia n.97 – 95123 Catania

Phone: +39.095.4781240

Highlights

- 88.6% of the ESBL *Escherichia coli* strains were susceptible to fosfomycin while 76.0% of carbapenemase-producing *Klebsiella pneumoniae* showed fosfomycin susceptibility
- For the ESBL-producing *E.coli*, the reference agar dilution method showed a categorical agreement of 100% with the microdilution broth and gradient test
- In cases of KPC-producing *K.pneumoniae*, there was a categorical agreement of 92% and 94%; and an essential agreement of 64% and 68% between the agar dilution with microdilution broth and gradient test, respectively
- The BD Phoenix system exhibits a categorical agreement > 90% for all isolates

- The agar dilution method remains the only reference method for carbapenem-resistant *K. pneumoniae* isolates

ABSTRACT

Objectives.

The increasing emergence and diffusion of multidrug-resistant pathogenic bacteria, both in hospital and community settings, are inducing clinicians to reconsider old antibiotics, such as fosfomycin, to overcome the difficulties posed by these microorganisms. Recent studies reported the good *in vitro* activity of fosfomycin against ESBL and carbapenem-resistant Enterobacteriaceae. The aim of this study was to assess the *in vitro* activity of fosfomycin by different methods against 120 clinical MDR isolates.

Methods: Fosfomycin MICs were determined by using the Agar Dilution reference method (AD), Gradient-test (GT), broth microdilution method (BMD), according to CLSI recommendations, and automated systems (VITEK 2 and BD Phoenix) against 85 carbapenem-resistant *Klebsiella pneumoniae* and 35 ESBL-producing *Escherichia coli*. Agreement and discrepancies between the evaluated methods and the reference method were calculated.

Results. Fosfomycin showed very good activity against ESBL-producing *E.coli* (88.6%). Excellent agreement (100%) between the three (AD, BMD and GT) susceptibility methods was found for *E. coli.* No major errors were observed. The fosfomycin resistance rate ranged from 24% (KPC-producing) to 100% (NDM-OXA-48 co-producing) *K.pneumoniae*. For all carbapenem-resistant *Klebsiella pneumoniae* strains, categorical agreement was >90% for all methods except for Vitek2, which was 84%.

Conclusions. When ESBL *E. coli* isolates are found to be susceptible to fosfomycin with automated systems, it is not necessary to verify these results with the AD reference method; while for resistant strains, the gradient test can be used. In cases of KPC *K.pneumoniae* resistant to fosfomycin, the agar dilution method is the only reference method.

Key words: ESBL, Carbapenem-resistant, Enterobacteriaceae, Fosfomycin, Agar dilution, Automated system

1. Introduction

Antimicrobial resistance is globally recognized as one of the greatest threats to public health. Of particular concern are infections caused by resistant Gram-negative bacilli. Gram-negative antimicrobial resistance is largely due to ß-lactamases, which are enzymes that bind and deactivate ß-lactam antibiotics, rendering them ineffective (1). For years, carbapenems have been used successfully to treat infections due to resistant Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae*, including those producing extended spectrum ß-lactamases (ESBLs).

However, carbapenemase-producing Enterobacteriaceae commonly reported as carbapenem-resistant Enterobacteriaceae have recently emerged, which confer broad resistance to most ß-lactam antibiotics including "last-resort" carbapenems (2). CRE can cause a number of serious infections and currently there are a limited selection of treatment options for these infections. Clinicians have been forced to re-evaluate the use of agents, which have been historically rarely used due to efficacy and/or toxicity concerns, such as polymyxins, aminoglycosides and fosfomycin (3).

A number of *in vitro* studies have demonstrated that fosfomycin has an excellent activity against many multidrug-resistant Gram-negative bacteria, including extended spectrum β-lactamase (ESBL) and carbapenem-resistant Enterobacteriaceae, isolated from patients with UTIs (4). The clinical use of fosfomycin requires *in vitro* testing of the drugs in order to be categorized correctly in clinical reports. According to EUCAST (The European Committee on Antimicrobial Susceptibility Testing, 2018), the only approved MIC method for testing fosfomycin susceptibility is the agar dilution

method (AD), whereas broth microdilution (BMD) MIC testing should not be performed. However, agar dilution is cumbersome and not routinely performed, and BMD, though not advisable, is the base method used in automatic systems.

Moreover, different studies have shown disagreement in fosfomycin susceptibility among BMD, Gradient Test (GT) and disk diffusion method (DDM) if compared with the reference AD method, above all in Klebsiella, Enterobacter and other Gram-negative bacteria (5,6).

In this study we determined the susceptibility to fosfomycin for selected MDR Gram-negative isolates with agar dilution and we compared the results with those obtained using automated, broth microdilution and gradient diffusion systems.

2 Materials and methods

2.1. Bacterial isolates

A total of 120 Gram-negative bacteria, isolated from different clinical sources obtained from hospitalized patients at the "Policlinico-Vittorio Emanuele" Hospital (Catania, Italy), were studied. The identification and antimicrobial susceptibility testing of the 120 isolates were preliminarily performed by the VITEK 2 (bioMerieux, Marcy l'Etoile, France) and BD Phoenix systems.

They comprised ESBL-producing *Escherichia coli* strains (n.35) and carbapenem-resistant *Klebsiella pneumoniae* strains (n.85).

Phenotypic screening for the presence of ESBLs in *E. coli* and carbapenemases in *K. pneumoniae* strains was performed according to the Clinical and Laboratory Standards Institute criteria (CLSI, 2018) (7), by using a commercial synergy test (Rosco Diagnostica, Taastrup, Denmark).

In order to fully characterize the resistance profile of these strains, amplification and sequencing for detection of carbapenemases was performed using previously described primers [8-10].

2.2. Antimicrobial susceptibility testing

Fosfomycin antimicrobial susceptibility testing was performed according to three methods: agar dilution (AD), used as the reference method, broth microdilution (BMD) and the gradient test (GT), in accordance with CLSI guidelines. Fosfomycin MICs were determined by the agar dilution and

broth microdilution methods in Mueller Hinton agar or broth medium, respectively, containing 25 µg/mL of glucose-6-phosphate (G6P; Sigma Aldrich Co, Italy) and fosfomycin in concentrations from 0.5 mg/L to 2048 mg/L.

Fosfomycin was supplied by Nordic Pharma S.r.l, (Milan, Italy). The gradient test was carried out on Mueller Hinton agar with strips containing fosfomycin (0.064 mg/L-1024 mg/L) and G6P (Liofilchem, Roseto degli Abruzzi, Italy). The inoculated plates were incubated in ambient air at 35°C for 16 to 18 h. For *Escherichia coli*, isolated colonies, within the inhibition zone, were ignored as recommended by the manufacturer (Liofilchem).

Furthermore, fosfomycin MICs were determined using the BD Phoenix™ (Becton Dickinson, USA; panel NMIC/ID-402) and VITEK 2 (bioMérieux, Inc., Marcy-l'Etoile, France; card AST-N378) automated microbiology systems following manufacturers' instructions. The fosfomycin concentrations evaluable in the used panels or cards were as follows: for NMIC/ID-402 (16 to 64 mg/L), and for AST-N378 (16 to 256 mg/L).

Fosfomycin breakpoints for the interpretative criteria were used according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019 (susceptible ≤ 32 µg/mL, resistant >32 µg/mL) (11). *E. coli* ATCC 25922 was used as the quality control strain.

2.3. Analysis of results

Susceptibility results of fosfomycin obtained with the microdilution broth, gradient test, and both automated systems were compared with those obtained from the agar dilution method, used as the reference method.

Essential agreement (EA) was calculated by the percentage of isolates with MICs within 1 doubling dilution from the reference method MIC, and categorical agreement (CA) was calculated by the percentage of isolates producing the same category result (S I R) as compared to the reference method. Disagreements were classified as very major errors (VME) when isolates were resistant by agar dilution but susceptible by all other evaluated methods and major errors (ME) when isolates were susceptible by agar dilution but resistant by all other evaluated methods (12).

3. Results

3.1. *Fosfomycin antimicrobial susceptibilities*

Fosfomycin MIC distribution data and percentage of resistance obtained with agar dilution reference method against 35 ESBL *E. coli* and 85 *K.pneumoniae* strains are presented in Table 1.

All *K.pneumoniae* were carbapenemase-producing: KPC (n.50), OXA-48 (8), NDM-1 and OXA-48 co-producers (27).

3.1.1. ESBL-producing E.coli

Fosfomycin inhibited 88.6% of the ESBL-producing *E. coli* ESBL strains, with MIC values ≤ 16 mg/l in most cases. The concentrations of fosfomycin inhibiting 50 and 90% of the isolates were 1 and 32 mg/L, respectively.

3.1.2. Carbapenemase-producing Klebsiella pneumoniae

Among the carbapenemase-producers, the NDM-OXA-48 co-producer *K.pneumoniae* was 100% resistant to fosfomycin, whereas the percentage in KPC-producing (KPCKp) was much lower (24%). The MIC_{90} of all KPC and NDM-OXA-48 producers was >2048 mg/L.

3.2. *Comparison of Agar dilution with Vitek2 and BD Phoenix*

3.2.1. ESBL-producing E.coli

Categorical agreement and error rates of agar dilution reference method compared to those of the VITEK 2 and BD Phoenix systems for fosfomycin against 120 Enterobacteriaceae are displayed in table 2.

Using the automated broth microdilution method (VITEK 2) 11/35 *E. coli* isolates (31.4%) were resistant to fosfomycin (MIC ≥ 64 mg/L); six isolates had MICs of ≥ 64 mg/L by using BD Phoenix™ (17.1%).

The comparison between the agar dilution method with the VITEK 2 and BD Phoenix systems for ESBL *E.coli* showed a categorical agreement of 80% and 94%, respectively. No cases of VME were

detected. There were 7 cases of ME in the comparison with the VITEK 2 and 2 cases with the BD Phoenix systems.

3.2.2. Carbapenemase-producing Klebsiella pneumoniae

Among carbapenem-resistant *K.pneumoniae,* more than 90% of NDM-OXA-48 co-producer *K. pneumoniae* had very high MICs (>64 mg/l) by both automated systems.

By the VITEK 2 system, 36% of KPC-producing isolates were resistant to fosfomycin: most of them had MIC 32 mg/L (31 of 50 strains), while 39 of isolates had MIC ≤ 16 mg/L by using BD Phoenix™ (data not shown).

Categorical agreement of 84% and 98% were observed in KPCKp comparing agar dilution with the VITEK 2 and BD Phoenix systems, respectively. Moreover, one case of VME between the AD method both with the VITEK 2 and with BD Phoenix systems and 7 cases of ME with the VITEK 2 system.

Finally, for the NDM and OXA-48 coproducing *K.pneumoniae* there was a categorical agreement of 93% and 96% between agar dilution with the VITEK 2 and the BD Phoenix systems, respectively. 2 cases of VME between agar dilution and the VITEK 2 system were observed. No errors for both systems were found in OXA-48 *K.pneumoniae*.

3.3. *Comparison of Agar dilution with the microdilution broth and gradient test*

3.3.1. ESBL-producing E.coli

Table 3 compares the categories obtained by the microdilution broth and gradient test methods with those obtained by the agar dilution method, used as reference.

Fosfomycin was highly active (11.4% R) against ESBL-*E.coli* by both the microdilution broth and gradient test methods (4/35 isolates had MIC >64 mg/L).

All methodologies (AD, BMD and GT) showed a categorical agreement of 100% for ESBL-producing *E.coli* and no evaluation errors were observed.

With the gradient test, the inner colonies, which were ignored for reading the MIC, were observed in 8 of 25 (32%) strains.

3.3.2. Carbapenem-resistant Klebsiella pneumoniae

The activity of fosfomycin against KPC- producing *K. pneumoniae* (28%) was lower than that against NDM and OXA-48 coproducing *K.pneumoniae* (100% R). Some strains (50%) of *K. pneumoniae* had MICs ≤ 16 mg/L by both microdilution broth and gradient test

In cases of KPCKp, there was a categorical agreement of 92% and 94%; and an essential agreement of 64% and 68% between the agar dilution method with microdilution broth and gradient test, respectively, revealing a very low concordance with agar dilution. Some errors were found with respect to the reference method: one strain showed resistance to fosfomycin by the agar dilution method (MIC 64 mg/L) and susceptibility to fosfomycin by the microdilution broth and gradient test methods (MIC values 16–32 mg/L). Three MEs were noted by AD (MIC 16 mg/L) compared to BMD (MIC 64 mg/L).

Essential agreement of 59% and 55% were observed in comparison with microdilution broth and gradient test, respectively, against NDM-OXA-48 co-producers *K.pneumoniae*.

In addition, for the OXA-48 producing strains an essential agreement of 75% and 87% in comparison with microdilution broth and gradient test, respectively, were observed. No evaluation errors were found for NDM-OXA-48 co-producer *K.pneumoniae* as well as for OXA-48 producing strains.

Sporadic colonies, within the inhibition zone, were reported in 25.8% of the isolates.

4. Discussion

The current epidemiological situation in Italy and in most European countries, where the diffusion of MDR Gram-negative isolates is increasing, discourages the use of the empirical antibiotic therapy for severe infections (13). Fortunately, some reports, based on susceptibility data, suggest that fosfomycin could be considered for the treatment of patients with infections due to multidrug-resistant bacteria. For example, the antibiotic is fully active against the most important MDR

Enterobacteriaceae responsible for urinary tract infections (UTIs), such as ESBL-positive *E. coli*, but also *Proteus mirabilis* and MR *Staphylococcus saprophyticus*; and it has demonstrated a marked activity against a good percentage of KPC *K. pneumoniae* (12,14). In agreement with other studies, our results demonstrated the very high activity of fosfomycin against all ESBL-producing strains and showed an excellent agreement between the three susceptibility methods (AD, BMD and GT) for *E. coli* (5,15). Moreover, the automated systems reported a very high CA. On the contrary, most discrepancies were observed for KPC *K.pneumoniae*, with cases of VME and ME between the reference method and BMD and GT, but also with the automated systems (16). The three methods are in agreement for the OXA-48 producing strains and NDM-OXA-48 co-producers *K. pneumoniae*; furthermore, the majority of these isolates are resistant to fosfomycin.

Our data, according to Endimiani et al., suggest that the result of fosfomycin susceptibility testing is dependent on the method used and the microorganisms tested (17).

In fact, if *E. coli* ESBL isolates are susceptible to fosfomycin with automated systems, it is not necessary to verify these results with the AD reference method; while for resistant strains, the gradient test can be used, because AD is not suitable for use in routine susceptibility testing. In cases of KPC *K.pneumoniae* resistant to fosfomycin, the agar dilution method is the only reference method, but a higher CA was observed between AD and BD Phoenix and also for OXA-48 producing strains and NDM-OXA-48 co-producers *K. pneumoniae,* there were good CA between AD and automatic systems.

With the gradient test, the difficulty in reading and interpreting the inhibition zone is usually because colonies can appear inside (15). Therefore, further studies are needed to for a consensus on this topic.

Finally, fosfomycin may be a reliable empirical therapeutic option for a number of severe infection types caused by ESBL and carbapenem-resistant Enterobacteriaceae, but the result of fosfomycin susceptibility testing obtained with methods other than agar dilution should be considered with caution.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests: None declared.

Ethical approval: Not required.

Acknowledgements:

The authors wish to thank the Scientific Bureau of the University of Catania for language support.

References

1 Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med 2012; 18:263–72.

2 Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. Treatment options for carbapenem-resistant enterobacteriaceae infections. Open Forum Infectious Diseases 2015;2(2):ofv050.

3 Keating GM. Fosfomycin trometamol: a review of its use as a single-dose. oral treatment for patients with lower urinary tract infections and pregnant women with asymptomatic acute bacteriuria. Drugs 2013;73:1951-66.

4 Bielen L, Likić R, Erdeljić V, Mareković I, Firis N, GrgićMedić M, et al. Activity of fosfomycin against nosocomial multiresistant bacterial pathogens from Croatia: a multicentric study. Croat Med J 2018;59:56-64.

5 de Cueto M, López L, Hernández JR, Morillo C, Pascual A. *In vitro* activity of fosfomycin against extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: comparison of susceptibility testing procedures. Antimicrob Agents Chemother 2006;50:368-70.

6 Lu CL, Liu CY, Huang YT, Liao CH, Teng LJ, Turnidge JD et al. Antimicrobial Susceptibilities of Commonly Encountered Bacterial Isolates to Fosfomycin Determined by Agar Dilution and Disk Diffusion Methods. Antimicrob Agents Chemother 2011; 55: 4295-301

7 CLSI (Clinical and Laboratory Standards Institute. Performance standard for antimicrobial susceptibility testing. M100-Ed28Approved Standard 28th ed.; 2018.

8 Falcone M, Perilli M, Mezzatesta ML, Mancini C, Amicosante G, Stefani S, Venditti M. Prolonged bacteraemia caused by VIM-1 metallo-beta-lactamase-producing *Proteus mirabilis*: first report from Italy. Clin Microbiol Infect 2010; 16:179-81

9 Gona F, Bongiorno D, Aprile A, Corazza E, Pasqua B, Scuderi MG, Chiacchiaretta M, Cirillo DM, Stefani S, Mezzatesta ML. Emergence of two novel sequence types (3366 and 3367) NDM-1- and OXA-48-co-producing *K. pneumoniae* in Italy. Eur J Clin Microbiol Infect Dis 2019 Sep;38(9):1687-1691

10 Gugliandolo A, Caio C, Mezzatesta ML, Rifici C, Bramanti P, Stefani S, Mazzon E. Successful ceftazidime-avibactam treatment of MDR-KPC-positive *Klebsiella pneumoniae* infection in a patient with traumatic brain injury: A case report. Medicine (Baltimore). 2017 Aug;96(31):e7664.

11 EUCAST (The European Committee on Antimicrobial Susceptibility Testing). Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0, 2019. http://www.eucast.org

12 Mezzatesta ML, La Rosa G, Maugeri G, Zingali T, Caio C, Novelli A, et al. *In vitro* activity of fosfomycin trometamol and other oral antibiotics against multidrug-resistant uropathogens. Int J Antimicrob Agents 2017; 49: 763-766.

13 Perdigão-Neto LV, Oliveira MS, Rizek CF, Carrilho CMDM, Costa SF, Levin AS. Susceptibility of *multiresistant* gram-negative bacteria to fosfomycin and performance of different susceptibility testing methods. Antimicrob Agents Chemother 2014;58:1763–7.

14 Cottell JL, Webber MA Experiences in fosfomycin susceptibility testing and resistance mechanism determination in *Escherichia coli* from urinary tract infections in the UK. J Med Microbiol. 2019 Feb;68(2):161-168.

15 Kaase M, Szabados F, Anders A, Gatermann SG. Fosfomycin susceptibility in carbapenem-resistant Enterobacteriaceae from Germany. J Clin Microbiol 2014;52:1893–7

16 van den Bijllaardt W, Schijffelen MJ, Bosboom RW, Cohen Stuart J, Diederen B, Kampinga G, Le TN, Overdevest I, Stals F, Voorn P, Waar K, Mouton JW, Muller AE. Susceptibility of ESBL *Escherichia coli* and *Klebsiella pneumoniae* to fosfomycin in the Netherlands and comparison of several testing methods including Etest, MIC test strip, Vitek2, Phoenix and disc diffusion. J Antimicrob Chemother 2018;73(9):2380-2387.

17 Endimiani A, Patel G, Hujer KM, Swaminathan M, Perez F, Rice LB et al. *In vitro* activity of fosfomycin against blaKPC-containing *Klebsiella pneumoniae* isolates, including those non susceptible to tigecycline and/or colistin. Antimicrob Agents Chemother 2010;54: 526-9.

Table 1. Minimum inhibitory concentration (MIC) distribution and susceptibility rates of fosfomycin by agar dilution method against n.120 Enterobacteriaceae

SPECIES	**N.**	**MIC distribution of fosfomycin (mg/L)**														**MIC_{50} (mg/L)**	**MIC_{90} (mg/L)**	**% R**
		≤0.5	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**>2048**			
ESBL *E. coli*	**35**	16	4	0	0	1	7	3	0	0	0	3	1	0	0	1	32	11.4
KPC *K. pneumoniae*	**50**	0	0	0	0	4	10	24	1	1	2	0	0	2	6	32	>2048	24
NDM-OXA-48 *K. pneumoniae*	**27**	0	0	0	0	0	0	0	1	5	4	3	2	1	11	512	>2048	100
OXA-48 *K. pneumoniae*	**8**	0	0	0	0	0	1	4	1	1	1	0	0	0	0	32	128	37.5

Legend: ESBL, extended-spectrum β-lactamase-producing; KPC, *Klebsiella pneumoniae* carbapenemase;

Table 2. Categorical agreement and error rates of AD compared to those of VITEK 2 and BD Phoenix™ for fosfomycin against 120 Enterobacteriaceae

Species	**N.**	**FOSFOMYCIN**				**% R**
		AGREEMENT N. (%) of isolates				
		Methods	**Categorical agreement**	**Very major error**	**Major error**	
ESBL *Escherichia coli*	**35**	Vitek 2	28 (80)	0	7 (20)	31.4
		BD Phoenix	33 (94)	0	2 (6)	17.1
KPC *Klebsiella pneumoniae*	**50**	Vitek 2	42 (84)	1 (2)	7 (14)	36
		BD Phoenix	49 (98)	1 (2)	0	22
NDM-OXA-48 *Klebsiella pneumoniae*	**27**	Vitek 2	25 (93)	2 (7)	0	92.5
		BD Phoenix	26 (96)	1 (4)	0	96.2

OXA-48 *Klebsiella pneumoniae*	**8**	Vitek 2	8 (100)	0	0	37.5
		BD Phoenix	8 (100)	0	0	37.5

Legend: ESBL, extended-spectrum β-lactamase producing; KPC, *Klebsiella pneumoniae* carbapenemase; CA, Categorical agreement; VME, very major error; ME, major error.

Table 3. Agreement and error rates of the broth microdilution and gradient tests with the reference agar dilution method against 120 Enterobacteriaceae

Species	**FOSFOMYCIN**						
	N.	**AGREEMENT N. (%) of isolates**					**% R**
		Methods	**Essential agreement**	**Categorical agreement**	**Very major error**	**Major error**	
ESBL *Escherichia coli*	**35**	Microdilution broth	14 (40)	35 (100)	0	0	11.4
		Gradient test	23 (66)	35 (100)	0	0	11.4
KPC	**50**	Microdilution broth	32 (64)	46 (92)	1 (2)	3 (6)	28

Klebsiella pneumoniae		Gradient test	34 (68)	47 (94)	1 (2)	2 (4)	28
NDM-OXA-48 *Klebsiella pneumoniae*	**27**	Microdilution broth	16 (59)	27 (100)	0	0	100
		Gradient test	15 (55)	27 (100)	0	0	100
OXA-48 *Klebsiella pneumoniae*	**8**	Microdilution broth	6 (75)	8 (100)	0	0	50
		Gradient test	7 (87)	8 (100)	0	0	37.5

Legend: ESBL, extended-spectrum β-lactamase producing; KPC, *Klebsiella pneumoniae* carbapenemase; EA, essential agreement; CA, Categorical agreement; VME, very major error; ME, major error.