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INFEZIONI DA BATTERI GRAM-NEGATIVI MDR ASPETTI MICROBIOLOGICI

Dott. Stefano Grandesso

SSD Microbiologia
Dip. di Patologia Clinica
Ospedale dell' Angelo – Mestre
Azienda ULSS 12 Veneziana
Presidente Prof. Enzo Raise

Compiti del Microbiologo Clinico

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- ✓allocando al meglio le (scarse) risorse disponibili
- ✓scegliere il meglio al minor costo

Per raggiungerli:

Ricognizione del mercato

Revisione della letteratura

Prove dirette

Journal of Antimicrobial Chemotherapy (2009) **64**, *Suppl. 1*, i29–i36
doi:10.1093/jac/dkp255

JAC

Has the era of untreatable infections arrived?

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Partiamo dalla definizione …

Journal of Medical Microbiology (2006), **55**, 1619–1629 DOI 10.1099/jmm.0.46747-0

Review

The diversity of definitions of multidrug-resistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

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The review reveals that various definitions have been used for the terms MDR and PDR A. baumannii and P. aeruginosa, a fact that causes confusion to researchers and clinicians. The authors believe that at least a widely accepted definition for PDR A. baumannii and P. aeruginosa should be uniformly used worldwide.

Risolviamo il problema …

ORIGINAL ARTICLE BACTERIOLOGY

Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance

A.-P. Magiorakos[1], A. Srinivasan[2], R. B. Carey[2], Y. Carmeli[3], M. E. Falagas[4,5], C. G. Giske[6], S. Harbarth[7], J. F. Hindler[8], G. Kahlmeter[9], B. Olsson-Liljequist[10], D. L. Paterson[11], L. B. Rice[12], J. Stelling[13], M. J. Struelens[1], A. Vatopoulos[14], J. T. Weber[2] and D. L. Monnet[1]

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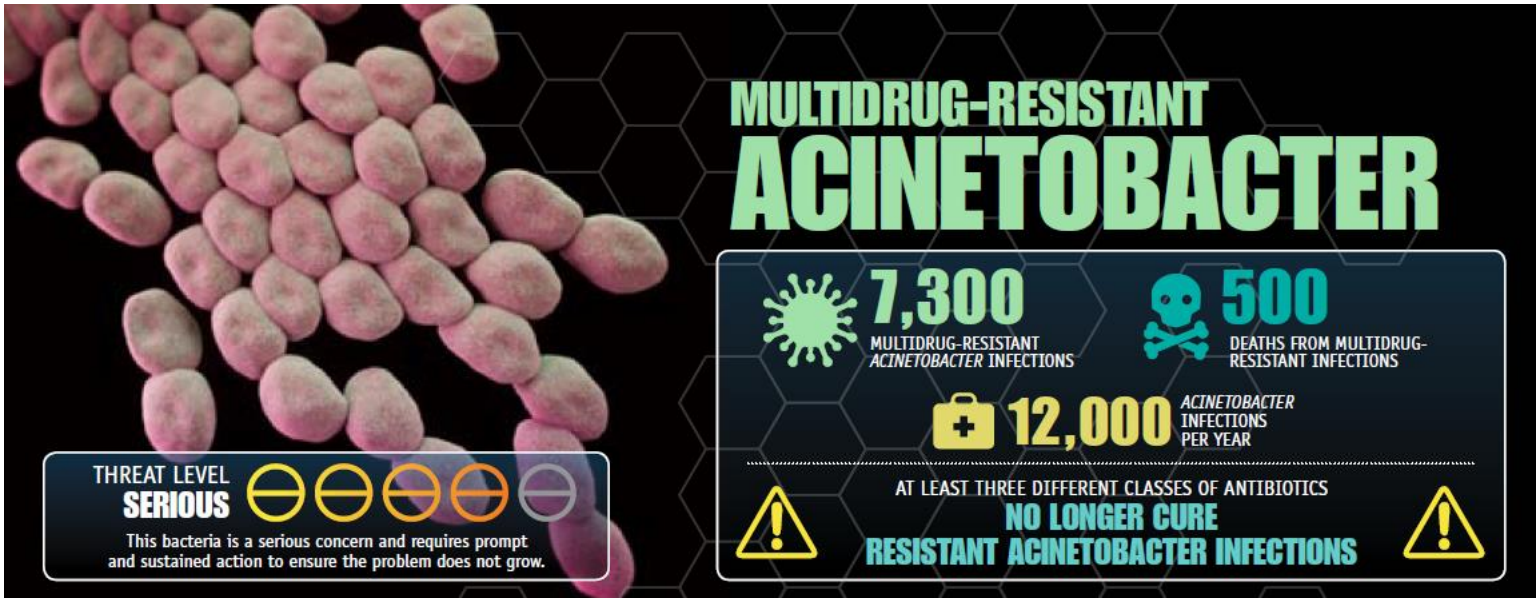
Clin Microbiol Infect 2012; 18: 268–281

TABLE 5. *Acinetobacter* spp.; antimicrobial categories and agents used to define MDR, XDR and PDR (worksheet for categorizing isolates)

Antimicrobial category	Antimicrobial agent	Results of antimicrobial susceptibility testing (S or NS)
Aminoglycosides	Gentamicin	
	Tobramycin	
	Amikacin	
	Netilmicin	
Antipseudomonal carbapenems	Imipenem	
	Meropenem	
	Doripenem	
Antipseudomonal fluoroquinolones	Ciprofloxacin	
	Levofloxacin	
Antipseudomonal penicillins + β-lactamase inhibitors	Piperacillin-tazobactam	
	Ticarcillin-clavulanic acid	
Extended-spectrum cephalosporins	Cefotaxime	
	Ceftriaxone	
	Ceftazidime	
	Cefepime	
Folate pathway inhibitors	Trimethoprim-sulphamethoxazole	
Penicillins + β-lactamase inhibitors	Ampicillin-sulbactam	
Polymyxins	Colistin	
	Polymyxin B	
Tetracyclines	Tetracycline	
	Doxycycline	
	Minocycline	

Criteria for defining MDR, XDR and PDR in *Acinetobacter* spp.
MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories.
XDR: non-susceptible to ≥1 agent in all but ≤2 categories.
PDR: non-susceptible to all antimicrobial agents listed.


MULTIDRUG-RESISTANT
ACINETOBACTER
7,300
MULTIDRUG-RESISTANT ACINETOBACTER INFECTIONS
500
DEATHS FROM MULTIDRUG-RESISTANT INFECTIONS
12,000
ACINETOBACTER INFECTIONS PER YEAR
AT LEAST THREE DIFFERENT CLASSES OF ANTIBIOTICS
NO LONGER CURE
RESISTANT ACINETOBACTER INFECTIONS
THREAT LEVEL
SERIOUS
This bacteria is a serious concern and requires prompt and sustained action to ensure the problem does not grow.

International Journal of Infectious Diseases (2009) 13, e217–e220

http://intl.elsevierhealth.com/journals/ijid

Investigation of colistin sensitivity via three different methods in *Acinetobacter baumannii* isolates with multiple antibiotic resistance

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Corresponding Editor: J. Peter Donnelly, Nijmegen, the Netherlands

Results: In all studied A. baumannii strains, susceptibility to colistin was determined to be 100% with the disk diffusion, E-test, and broth microdilution methods. Results of the E-test and broth microdilution method, which are accepted as reference methods, were found to be 100% consistent with the results of the disk diffusion tests; no very major or major error was identified upon comparison of the tests. The sensitivity and the positive predictive value of the disk diffusion method were found to be 100%.
Conclusions: Colistin resistance in A. baumannii was not detected in our region, and disk diffusion method results are in accordance with those of E-test and broth microdilution methods.

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Comparative Evaluation of the VITEK 2, Disk Diffusion, Etest, Broth Microdilution, and Agar Dilution Susceptibility Testing Methods for Colistin in Clinical Isolates, Including Heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* Strains▽

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a. **<u>Disk diffusion</u> is an <u>unreliable method to measure susceptibility to colistin</u>.**
b. **High error rates and low levels of reproducibility were observed in the disk diffusion test.**
c. **The colistin Etest, agar dilution, and the VITEK 2 showed a high level of agreement with the broth microdilution reference method.**

Comparative Evaluation of Tigecycline Susceptibility Testing Methods for Expanded-Spectrum Cephalosporin- and Carbapenem-Resistant Gram-Negative Pathogens

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We evaluated the Vitek2, Etest, and MIC Test Strip (MTS) methods of tigecycline susceptibility testing with 241 expanded-spectrum cephalosporin-resistant and/or carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* clinical isolates by using dry-form broth microdilution (BMD) as the reference method. The $MIC_{50/90}$s were as follows: BMD, 1/4 μg/ml; Vitek2, 4/≥8 μg/ml; Etest, 2/4 μg/ml; MTS, 0.5/2 μg/ml. Vitek2 produced 9.1/21.2% major errors, Etest produced 0.4/0.8% major errors, and MTS produced no major errors but 0.4/3.3% very major errors (FDA/EUCAST breakpoints). Vitek2 tigecycline results require confirmation by BMD or Etest for multidrug-resistant pathogens.

Journal of Clinical Microbiology November 2012 Volume 50 Number 11 p. 3747–3750

TABLE 1 Tigecycline susceptibilities of the study isolates and MIC_{50}s and MIC_{90}s determined by BMD, Vitek2, Etest, and MTS

Test method and isolate group	No. (%) of isolates						MIC (μg/ml)	
	Susceptible		Intermediate		Resistant			
	FDA	EUCAST	FDA	EUCAST	FDA	EUCAST	50%	90%
BMD								
All isolates	201 (83.4)	150 (62.2)	35 (14.5)	51 (21.2)	5 (2.1)	40 (16.6)	1	4
CR *K. pneumoniae*	105 (84.0)	80 (64.0)	18 (14.4)	25 (20.0)	2 (1.6)	20 (16.0)	1	4
CR *A. baumannii*	42 (75.0)	25 (44.6)	12 (21.4)	17 (30.4)	2 (3.6)	14 (25.0)	2	4
ESCR *Enterobacteriaceae*	54 (90.0)	45 (75.0)	5 (8.3)	9 (15.0)	1 (1.7)	6 (10.0)	0.5	2
Vitek2								
All isolates	103 (42.7)	53 (22.0)	84 (34.9)	50 (20.7)	54 (22.4)	138 (57.3)	4	≥ 8
CR *K. pneumoniae*	50 (40.0)	12 (9.6)	50 (40.0)	38 (30.4)	25 (20.0)	75 (60.0)	4	≥ 8
CR *A. baumannii*	10 (17.9)	3 (5.4)	27 (48.2)	7 (12.5)	19 (33.9)	46 (82.1)	4	≥ 8
ESCR *Enterobacteriaceae*	43 (71.7)	38 (63.3)	7 (11.7)	5 (8.3)	10 (16.7)	17 (28.3)	1	≥ 8
Etest								
All isolates	198 (82.2)	108 (44.8)	33 (13.7)	89 (36.9)	10 (4.1)	44 (18.3)	2	4
CR *K. pneumoniae*	105 (84.0)	48 (38.4)	17 (13.6)	56 (44.8)	3 (2.4)	21 (16.8)	2	4
CR *A. baumannii*	39 (69.6)	16 (28.6)	11 (19.6)	23 (41.1)	6 (10.7)	17 (30.4)	2	4
ESCR *Enterobacteriaceae*	54 (90.0)	44 (73.3)	5 (8.3)	10 (16.7)	1 (1.7)	6 (10.0)	0.5	2
MTS								
All isolates	229 (95.0)	190 (78.8)	9 (3.7)	39 (16.2)	3 (1.2)	12 (5.0)	0.5	2
CR *K. pneumoniae*	124 (99.2)	106 (84.8)	1 (0.8)	18 (14.4)	0 (0)	1 (0.8)	1	2
CR *A. baumannii*	47 (83.9)	32 (57.1)	6 (10.7)	15 (26.8)	3 (5.4)	9 (16.1)	1	4
ESCR *Enterobacteriaceae*	58 (96.7)	52 (86.7)	2 (3.3)	6 (10.0)	0 (0)	2 (3.3)	0.25	2

TABLE 2 EA, CA, and types of errors produced when testing tigecycline susceptibility by Vitek2, Etest, and MTS compared to BMD

Test method and isolate group	No. (%) of isolates with:								
		CA		VME		ME		mE	
	EA	FDA	EUCAST	FDA	EUCAST	FDA	EUCAST	FDA	EUCAST
Vitek2									
All isolates	148 (61.4)	116 (48.1)	95 (39.4)	0 (0)	0 (0)	22 (9.1)	51 (21.2)	103 (42.7)	95 (39.4)
CR *K. pneumoniae*	65 (52.0)	57 (45.6)	34 (27.2)	0 (0)	0 (0)	10 (8.0)	32 (25.6)	58 (46.4)	59 (47.2)
CR *A. baumannii*	31 (55.4)	14 (25.0)	18 (32.1)	0 (0)	0 (0)	7 (12.5)	16 (28.6)	35 (62.5)	22 (39.3)
ESCR *Enterobacteriaceae*	52 (86.7)	45 (75.0)	43 (71.7)	0 (0)	0 (0)	5 (8.3)	3 (5.0)	10 (16.7)	14 (23.3)
Etest									
All isolates	229 (95.0)	220 (91.3)	173 (71.8)	0 (0)	0 (0)	1 (0.4)	2 (0.8)	20 (8.3)	66 (27.4)
CR *K. pneumoniae*	121 (96.8)	117 (93.6)	81 (64.8)	0 (0)	0 (0)	1 (0.8)	1 (0.8)	7 (5.6)	43 (34.4)
CR *A. baumannii*	52 (92.9)	47 (83.9)	39 (69.6)	0 (0)	0 (0)	0 (0)	1 (1.8)	9 (16.1)	16 (28.6)
ESCR *Enterobacteriaceae*	56 (93.3)	56 (93.3)	53 (88.3)	0 (0)	0 (0)	0 (0)	0 (0)	4 (6.7)	7 (11.7)
MTS									
All isolates	187 (77.6)	208 (86.3)	165 (68.5)	1 (0.4)	8 (3.3)	0 (0)	0 (0)	32 (13.3)	68 (28.2)
CR *K. pneumoniae*	103 (82.4)	105 (84.0)	80 (64.0)	1 (0.8)	6 (4.8)	0 (0)	0 (0)	19 (15.2)	39 (31.2)
CR *A. baumannii*	50 (89.3)	48 (85.7)	35 (62.5)	0 (0)	1 (1.8)	0 (0)	0 (0)	8 (14.3)	20 (35.7)
ESCR *Enterobacteriaceae*	34 (56.7)	55 (91.7)	50 (83.3)	0 (0)	1 (1.7)	0 (0)	0 (0)	5 (8.3)	9 (15.0)

Comparative Evaluation of Tigecycline Susceptibility Testing Methods for Expanded-Spectrum Cephalosporin- and Carbapenem-Resistant Gram-Negative Pathogens

Olympia Zarkotou,[a] Spyros Pournaras,[b] George Altouvas,[a] Vassiliki Pitiriga,[c] Maria Tziraki,[a] Vassiliki Mamali,[a] Katerina Themeli-Digalaki,[a] and Athanassios Tsakris[c]

Department of Microbiology, Tzaneio General Hospital, Piraeus, Greece[a]; Department of Microbiology, Medical School, University of Thessaly, Larissa, Greece[b]; and Department of Microbiology, Medical School, University of Athens, Athens, Greece[c]

We evaluated the Vitek2, Etest, and MIC Test Strip (MTS) methods of tigecycline susceptibility testing with 241 expanded-spectrum cephalosporin-resistant and/or carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* clinical isolates by using dry-form broth microdilution (BMD) as the reference method. The $MIC_{50/90}$s were as follows: BMD, 1/4 μg/ml; Vitek2, 4/≥8 μg/ml; Etest, 2/4 μg/ml; MTS, 0.5/2 μg/ml. Vitek2 produced 9.1/21.2% major errors, Etest produced 0.4/0.8% major errors, and MTS produced no major errors but 0.4/3.3% very major errors (FDA/EUCAST breakpoints). Vitek2 tigecycline results require confirmation by BMD or Etest for multidrug-resistant pathogens.

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Since tigecycline is commonly used against infections with CR pathogens, reliable susceptibility results are important for therapeutic decisions. Our study underlines the shortcomings of automated and manual susceptibility testing methods, which may falsely restrict the available treatment options or lead to inappropriate antimicrobial therapy. Clinical laboratories should be aware of the interpretive problems. **Confirmation of susceptibility results by a reference method is therefore recommended, particularly when tigecycline administration is deemed necessary.**

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Effect of Manganese in Test Media on *In Vitro* Susceptibility of *Enterobacteriaceae* and *Acinetobacter baumannii* to Tigecycline

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We assessed the effect of increasing manganese concentrations in test media (0.001 to 1,024 mg/liter) on MICs of tigecycline. For both broth microdilution (BMD) and Etests, this effect was negligible for physiological concentrations, but MICs increased when concentrations exceeded 8 mg/liter. Susceptibility testing should be performed on media with standardized low manganese content.

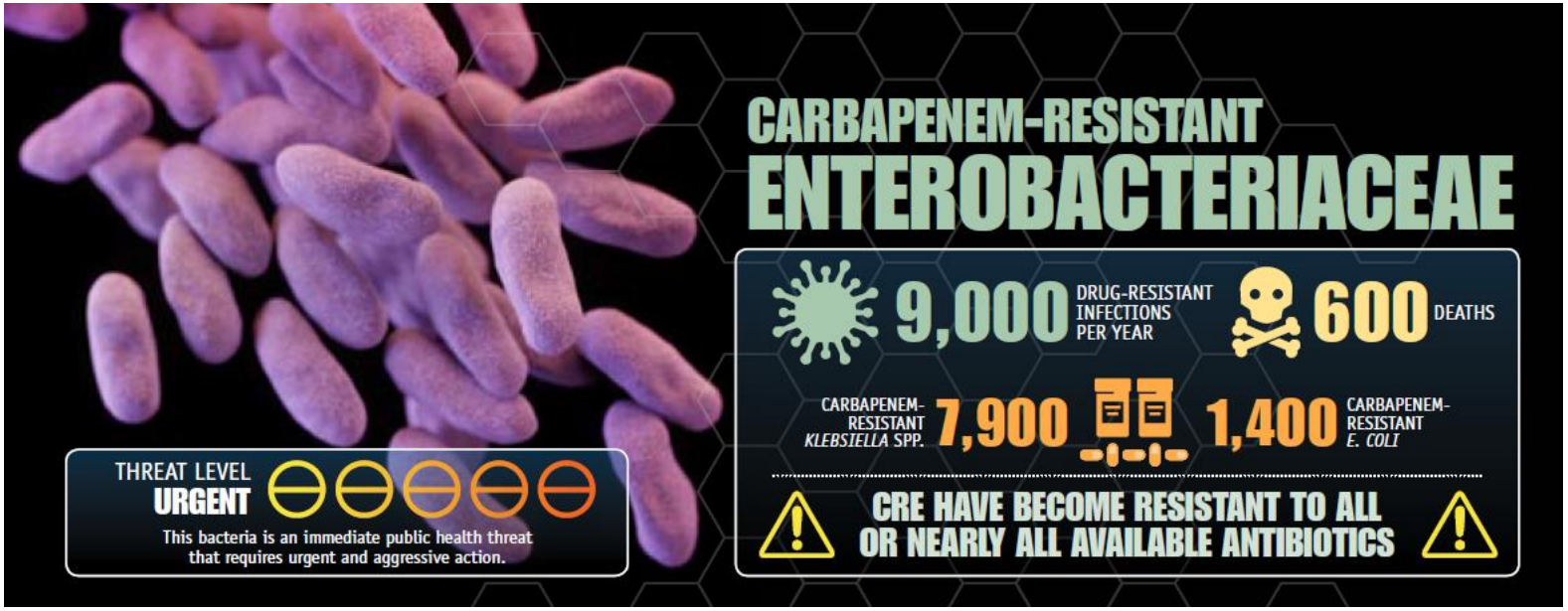
Other divalent cations may have **similar effects** on susceptibility test results, and because we did not use the same medium for the Etests and for the BMD, it is possible that differences in the concentrations of minerals other than manganese may partly explain the observed differences in MICs between these 2 methods. Further studies are needed to identify causal factors involved. Meanwhile, **results of tigecycline susceptibility testing by Etest should be interpreted with caution**.

Journal of Clinical Microbiology September 2012 Volume 50 Number 9 p. 3077–3079

Are E-test and Vitek2 good choices for tigecycline susceptibility testing when comparing broth microdilution for MDR and XDR *Acinetobacter baumannii*?

	N. of isolates (%)		M.I.C. (mg/L)	
	Sensible	Resistant	50%	90%
BMD	95,2	4,8	0,25	1,00
Vitek2	63,0	37,0	1,00	8,00
E-test	10,7	89,3	2,00	16,00

Count Total %	BMD R	BMD S	
Vitek 2	4	27	31
R	4,76	32,14	36,90
Vitek2	0	53	53
S	0,00	63,10	63,10
	4	80	84
	4,76	95,24	
E-test	4	71	75
R	4,76	84,52	89,29
E-test	0	9	9
S	0,00	10,71	10,71
	4	80	84
	4,76	95,24	


CARBAPENEM-RESISTANT
ENTEROBACTERIACEAE
9,000 DRUG-RESISTANT INFECTIONS PER YEAR
600 DEATHS
CARBAPENEM-RESISTANT *KLEBSIELLA* SPP. 7,900
1,400 CARBAPENEM-RESISTANT *E. COLI*
CRE HAVE BECOME RESISTANT TO ALL OR NEARLY ALL AVAILABLE ANTIBIOTICS
THREAT LEVEL
URGENT
This bacteria is an immediate public health threat that requires urgent and aggressive action.

ARTICULO ORIGINAL

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Evaluación de diversos métodos fenotípicos para la detección de carbapenemasas KPC en *Klebsiella pneumoniae*

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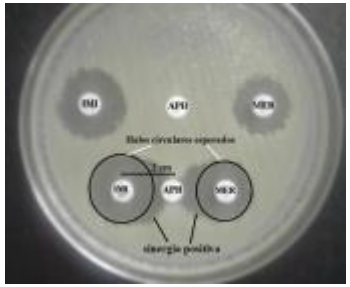
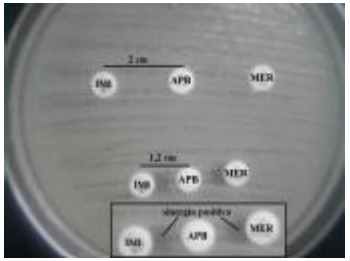
**Correspondencia. E-mail: fnicola@cemic.edu.ar*





a. The double disk diffusion test using **boronic acid** could detect all kPc-positive isolates, but adjustment of disk distance was necessary for achieving such performance.
b. The simulation of combined disks by our pre-diffusion technique detected all kPcpositive strains for all 3 carbapenems when using boronic acid as inhibitor, clavulanic acid was less susceptible and specific as compared with boronic acid.
c. The modified Hodge test using any carbapenem was clearly positive for all kPc-producing isolates. This test was negative for all kPc-negative strains when imipenem or meropenem were used, but 2/14 isolates yielded a weak positive result when using ertapenem.

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0095-1137/10/$12.00 doi:10.1128/JCM.00267-10

Comparison of Meropenem MICs and Susceptibilities for Carbapenemase-Producing *Klebsiella pneumoniae* Isolates by Various Testing Methods[▽]

Catharine C. Bulik,[1] Kathy A. Fauntleroy,[3] Stephen G. Jenkins,[3] Mayssa Abuali,[4] Vincent J. LaBombardi,[4] David P. Nicolau,[1,2] and Joseph L. Kuti[1]*

Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, Connecticut[1]; Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut[2]; New York Presbyterian Hospital, Weill Cornell Medical Center, New York, New York[3]; and Mount Sinai Medical Center, New York, New York[4]

TABLE 1. Interpretive results for 46 KPC-producing *K. pneumoniae* isolates[a]

Testing method	No. (%) of isolates with indicated result		
	Susceptible	Intermediate	Resistant
	2010 CLSI meropenem interpretive criteria[b]		
BMD	0 (0)	1 (2.2)	45 (97.8)
Etest	1 (2.2)	0 (0.0)	45 (97.8)
Vitek 2	11 (23.9)	19 (41.3)	16 (34.8)
Sensititre	4 (8.7)	11 (23.9)	31 (67.4)
MicroScan	1 (2.2)	0 (0.0)	45 (97.8)

TABLE 2. Frequency of very major, major, and minor errors[a]

Testing method	No. (%) of isolates with indicated result		
	Very Major	Major	Minor
	2010 CLSI meropenem interpretive criteria		
Etest	1 (2.2)	0 (0)	1 (2.2)
Vitek 2	11 (23.9)	0 (0)	18 (39.1)
Sensititre	3 (6.5)	0 (0)	12 (26.1)
MicroScan	0 (0)	0 (0)	1 (2.2)

JOURNAL OF CLINICAL MICROBIOLOGY, July 2010, p. 2402–2406 Vol. 48, No. 7
0095-1137/10/$12.00 doi:10.1128/JCM.00267-10

Comparison of Meropenem MICs and Susceptibilities for Carbapenemase-Producing *Klebsiella pneumoniae* Isolates by Various Testing Methods▽

Catharine C. Bulik,[1] Kathy A. Fauntleroy,[3] Stephen G. Jenkins,[3] Maryssa Abuali,[4] Vincent J. LaBombardi,[4] David P. Nicolau,[1,2] and Joseph L. Kuti[1]*

Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, Connecticut[1]; Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut[2]; New York Presbyterian Hospital, Weill Cornell Medical Center, New York, New York[3]; and Mount Sinai Medical Center, New York, New York[4]

Clinical Laboratory Standards Institute (CLSI) interpretative criteria using 2010 susceptibility breakpoints.

Based on broth microdilution, 0%, 2.2%, and 97.8% of the KPC isolates were classified as susceptible, intermediate, and resistant to meropenem, respectively.

Results from MicroScan demonstrated the most agreement with those from broth microdilution, with 95.6% agreement based on the MIC and 2.2% classified as minor errors, and no major or very major errors.

Etest demonstrated 82.6% agreement with broth microdilution MICs, a very major error rate of 2.2%, and a minor error rate of 2.2%.

Vitek 2 MIC agreement was 30.4%, with a 23.9% very major error rate and a 39.1% minor error rate.

Sensititre demonstrated MIC agreement for 26.1% of isolates, with a 3% very major error rate and a 26.1% minor error rate.

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0095-1137/07/$08.00+0 doi:10.1128/JCM.00015-07

Evaluation of Methods To Identify the *Klebsiella pneumoniae* Carbapenemase in *Enterobacteriaceae*▽

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Centers for Disease Control and Prevention, Division of Healthcare Quality Promotion, Atlanta, Georgia

TABLE 1. Performance of susceptibility testing methods for detecting KPC-mediated resistance

Method	Sensitivity (%)/specificity (%) of:					
	Intermediate or resistant susceptibility result[a]			Carbapenem MIC of >1 μg/ml		
	Meropenem	Imipenem	Ertapenem	Meropenem	Imipenem	Ertapenem
Reference BMD	94/98	94/93	97/89	100/93	100/93	100/89
Etest	58/96	55/96	90/84	84/91	90/89	100/84
Disk diffusion	71/96	42/96	97/87	NA[b]	NA	NA
Vitek Legacy	52/98	55/96	NA[d]	NA[c]	NA[c]	NA[d]
Vitek 2	48/96	71/96	94/93	71/93	94/89	94/89
MicroScan	84/98	74/96	100/89	100/93	100/93	NA[c]
Phoenix	61/98	81/96	NA[d]	74/96	87/93	NA[d]
Sensititre	42/98	29/96	NA[d]	81/96	NA[c]	NA[d]

[a] Interpretive criteria were based upon CLSI criteria.
[b] NA, not applicable.
[c] Not applicable because the MIC range tested was not low enough (e.g., lowest dilution tested was either 2 μg/ml or 4 μg/ml) for the identification of a carbapenem MIC of >1 μg/ml.
[d] Not applicable because ertapenem was not available on a panel.

Ertapenem was a more sensitive indicator of KPC resistance than meropenem and imipenem independently of the method used.

Carbapenemase production could be confirmed with the modified Hodge test.

Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing *Klebsiella pneumoniae* with the EUCAST and CLSI breakpoint systems

M. Vading[1], Ø. Samuelsen[2], B. Haldorsen[2], A. S. Sundsfjord[2,3] and C. G. Giske[1]|

Clin Microbiol Infect 2011; 17: 668–674

- All carbapenemase producers were detected with EUCAST disk diffusion breakpoints for ertapenem and meropenem, and four strains were susceptible to imipenem.
- CLSI disk diffusion breakpoints characterized 18 (imipenem), 14 (meropenem) and three (ertapenem) isolates as susceptible.
- When cards with a single carbapenem were used, detection failures with VITEK2 were four for imipenem, none for meropenem and one for ertapenem.
- Cards containing all three carbapenems had one to two failures.
- All carbapenemase producers were detected with the clinical EUCAST breakpoint for ertapenem.
- EUCAST disk diffusion breakpoints for meropenem and ertapenem detected all carbapenemase producers. VITEK2 had between none and four failures in detecting carbapenemase producers, depending on the antibiotic card.

J Antimicrob Chemother 2010; **65**: 1319–1321
doi:10.1093/jac/dkq124 Advance Access publication 15 April 2010

Journal of Antimicrobial Chemotherapy

Inhibitor-based methods for the detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice by using boronic acid compounds

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[1]Department of Microbiology, Medical School, University of Thessaly, Larissa, Greece; [2]Department of Microbiology, Serres General Hospital, Serres, Greece; [3]Department of Microbiology, Medical School, University of Athens, Athens, Greece

Currently, the detection of putative carbapenemase production is based on an initial phenotypic screen for carbapenem resistance followed by the modified Hodge test (MHT) as a confirmatory test.
However, the MHT is often difficult to interpret, is not specific for carbapenemase activity due to KPC and there are reports of false-positive results with CTX-M-positive or AmpC-hyperproducing Enterobacteriaceae.
Boronic acid compounds have also been evaluated for the differentiation of KPC-producing Enterobacteriaceae.
In that respect, combined disc tests using carbapenems with and without phenylboronic acid (PBA) have been proposed as the most accurate phenotypic tests for detecting KPC production.
When these disc tests are extended to include carbapenem discs with EDTA or both PBA and EDTA on the same plate, the production of metallo-b-lactamase (MBL) or both KPC and MBL, respectively, can also be accurately detected.
They are very easy to perform and interpret, and may be applied from the first day of isolation of the suspected resistant Enterobacteriaceae.
They could effectively replace MHT for the convenient and early detection of KPC carbapenemases in regions where these enzymes are common.

K. pneumoniae CRE
(22 ceppi)

	BMD	Vitek	E-test	
ERTAPENEM				2 ceppi : Sensi >2 - Vitek <=0.5 1 ceppo : Sensi 0.25 - Vitek 1
MIC50	≥2	≥8		
MIC90	≥2	≥8		
MEROPENEM				2 ceppi : Sensi 4-32 - Vitek <=0.25 2 ceppi : Sensi 0.25-0.5 - Vitek >=16
MIC50	16	≥16		
MIC90	32	≥16		

K. pneumoniae CRE
(22 ceppi)

	BMD	Vitek	E-test
GENTAMICINA			
MIC50		4	2
MIC90		≥16	8
AMIKACINA			
MIC50		≥64	≥16
MIC90		≥64	≥16
TIGECICLINA			
MIC50	0,5	2	1,5
MIC90	1	≥8	3
COLISTINA			
MIC50	≤0,25	≤0,5	
MIC90	≥4	≥16	

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0066-4804/11/$12.00 doi:10.1128/AAC.00387-11

Comparative Effectiveness of Aminoglycosides, Polymyxin B, and Tigecycline for Clearance of Carbapenem-Resistant *Klebsiella pneumoniae* from Urine▽

Michael J. Satlin,[1]* Christine J. Kubin,[2] Jill S. Blumenthal,[3] Andrew B. Cohen,[3] E. Yoko Furuya,[4] Stephen J. Wilson,[1] Stephen G. Jenkins,[5] and David P. Calfee[1]

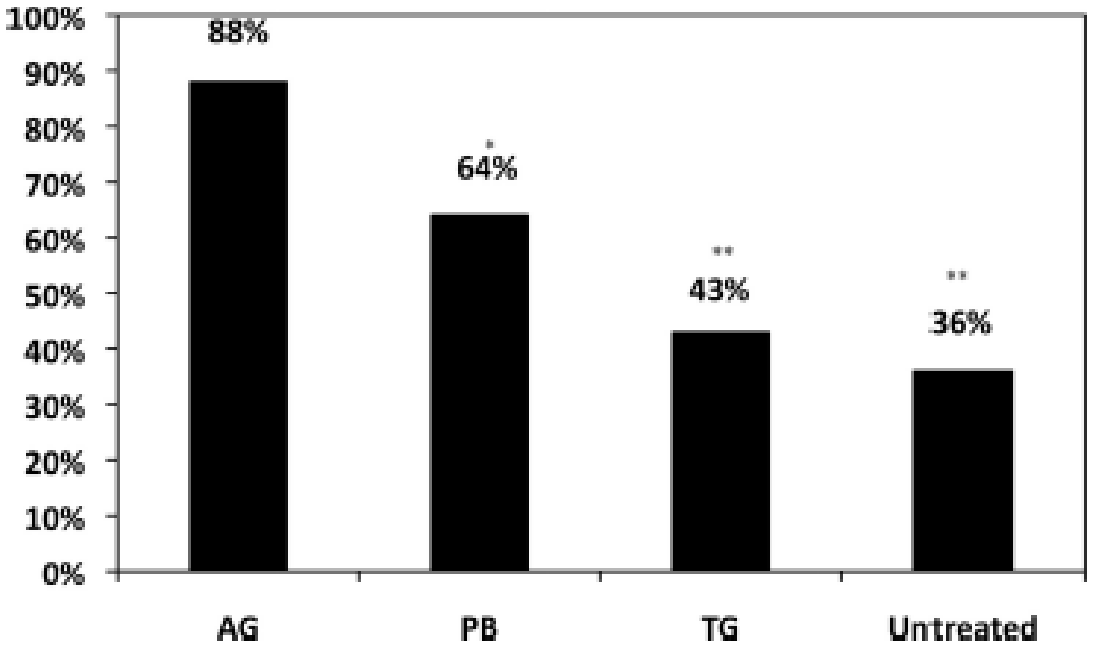


FIG. 2. Microbiologic clearance rates by the antimicrobial treatment cohort. AG, aminoglycoside; PB, polymyxin B; TG, tigecycline; *,

Aminoglycosides, **when active in vitro**, were associated with a significantly higher rate of microbiologic clearance of carbapenem-resistant K. pneumoniae in the urine compared to polymyxin B or tigecycline

JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 1795–1798 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.02534-10

Comparison of Polymyxin B, Tigecycline, Cefepime, and Meropenem MICs for KPC-Producing *Klebsiella pneumoniae* by Broth Microdilution, Vitek 2, and Etest[▽]

Asma Lat,[1]* Sarah A. Clock,[2] Fann Wu,[1,2] Susan Whittier,[1] Phyllis Della-Latta,[1,2] Kathy Fauntleroy,[1,3] Stephen G. Jenkins,[1,3] Lisa Saiman,[1,2] and Christine J. Kubin[1,2]

New York-Presbyterian Hospital, New York, New York[1]; Columbia University Medical Center, New York, New York[2]; and Weill Cornell Medical College, New York, New York[3]

TABLE 3. Incidence of errors for selected testing methods[a]

Testing method	No. (%) of isolates with the indicated errors											
	Polymyxin B			Tigecycline			Cefepime			Meropenem		
	Very major	Major	Minor	Very major	Major	Minor	Very major	Major	Minor	Very major	Major	Minor
Etest	1 (2)	11 (23)	NA[b]	0 (0)	0 (0)	10 (21)	3 (6)	0 (0)	12 (25)	0 (0)	0 (0)	1 (2)
Vitek 2	NA	NA	NA	0 (0)	5 (10)	12 (25)	32 (67)	0 (0)	5 (10)	13 (27)	0 (0)	13 (27)

[a] Incidence of very major, major, and minor errors compared to BMD results.
[b] NA, not applicable.

We suggest that laboratories consider supplemental use of reference BMD or Etest for cefepime and meropenem for KPC-producing *K. pneumoniae* susceptibility testing, as Vitek 2 did not provide reliable results for these agents.

Stenotrophomonas maltophilia

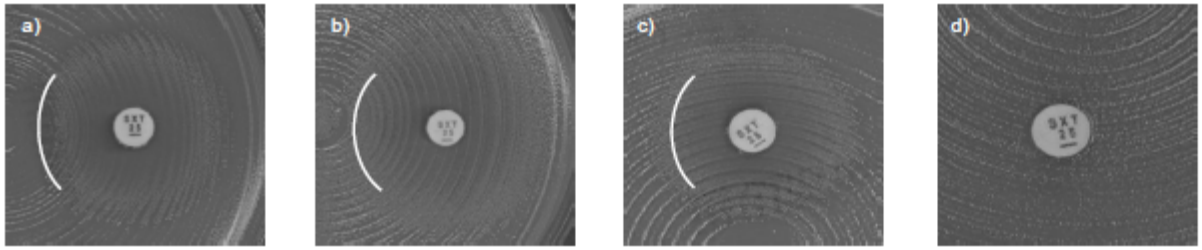
EUCAST 2013

Stenotrophomonas maltophilia

EUCAST Clinical Breakpoint Table v. 3.1, valid from 2013-02-11

Disk diffusion (EUCAST standardised disk diffusion method)
Medium: Mueller-Hinton agar
Inoculum: McFarland 0.5
Incubation: Air, 35±1°C, 18±2h
Reading: Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
Quality control: *Escherichia coli* ATCC 25922

Miscellaneous agents	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
Trimethoprim-sulfamethoxazole[1]	4	4	1.25-23.75	16[A]	16[A]	1. Trimethoprim:sulfamethoxazole in the ratio 1:19. Breakpoints are expressed as the trimethoprim concentration. A. Ignore haze or fine growth within the inhibition zone (see pictures below).



Examples of inhibition zones for *Stenotrophomonas maltophilia* with trimethoprim-sulfamethoxazole.
a-c) An outer zone can be seen. Report susceptible if the zone diameter ≥ 16 mm.
d) Growth up to the disk and no sign of inhibition zone. Report resistant.

E' sufficiente??

Stenotrophomonas maltophilia

CLSI 2013

Table 2B-4. Zone Diameter and MIC Interpretive Standards for *Stenotrophomonas maltophilia*

Testing Conditions

Medium: Disk diffusion: MHA
Broth dilution: CAMHB
Agar dilution: MHA

Inoculum: Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard

Incubation: 35±2°C; ambient air; all methods, 20 to 24 hours

Routine QC Recommendations (See Tables 3A and 4A for acceptable QC ranges.)

Escherichia coli ATCC® 25922
Pseudomonas aeruginosa ATCC® 27853
Escherichia coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)

General Comments

(1) For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth) and measure the more obvious margin to determine the zone diameter.

NOTE: Information in boldface type is new or modified since the previous edition.

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS									
B	Ticarcillin-clavulanic acid	–	–	–	–	≤16/2	32/2–64/2	≥128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)									
B	Ceftazidime	–	–	–	–	≤8	16	≥32	
TETRACYCLINES									
B	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
FLUOROQUINOLONES									
B	Levofloxacin	5 µg	≥17	14–16	≤13	≤2	4	≥8	
FOLATE PATHWAY INHIBITORS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥ 16	11–15	≤ 10	≤2/38	–	≥4/76	
PHENICOLS									
B	Chloramphenicol	–	–	–	–	≤8	16	≥32	(2) Not routinely reported on isolates from the urinary tract.

Abbreviations: ATCC, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

Stenotrophomonas maltophilia

- **Effetti collaterali**
- **Eventi avversi**

✓ disturbi gastrointestinali (nausea, vomito, diarrea)

✓ discrasie ematiche (trombocitopenia, neutropenia, etc.)

✓ reazioni di ipersensibilità lieve (orticaria) o, più raramente, grave (sindrome di Stevens-Johnson)

- **Controindicazioni**

✓ nei soggetti allergici a uno o a entrambi i componenti dell'associazione

✓ durante il primo trimestre di gravidanza per evitare il rischio teorico di teratogenesi (osservato su animali di laboratorio)

✓ nei soggetti con deficit di glucosio-6-fosfato deidrogenasi (favismo) per evitare fenomeni di anemia emolitica

OPEN ACCESS Freely available online PLoS one

Stenotrophomonas maltophilia Infections in a General Hospital: Patient Characteristics, Antimicrobial Susceptibility, and Treatment Outcome

George Samonis[1], Drosos E. Karageorgopoulos[2,3], Sofia Maraki[4], Panagiotis Levis[2], Dimitra Dimopoulou[1], Nikolaos A. Spernovasilis[1], Diamantis P. Kofteridis[1], Matthew E. Falagas[2,5,6]*

1 Department of Internal Medicine, University Hospital of Heraklion, Heraklion, Crete, Greece, 2 Alfa Institute of Biomedical Sciences (AIBS), Marousi, Athens, Greece, 3 Hellenic Center for Disease Control and Prevention, Marousi, Athens, Greece, 4 Department of Clinical Microbiology, University Hospital of Heraklion, Heraklion, Crete, Greece, 5 Department of Medicine, Henry Dunant Hospital, Athens, Greece, 6 Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts, United States of America

Table 4. Susceptibility pattern of the 68 tested *Stenotrophomonas maltophilia* isolates.

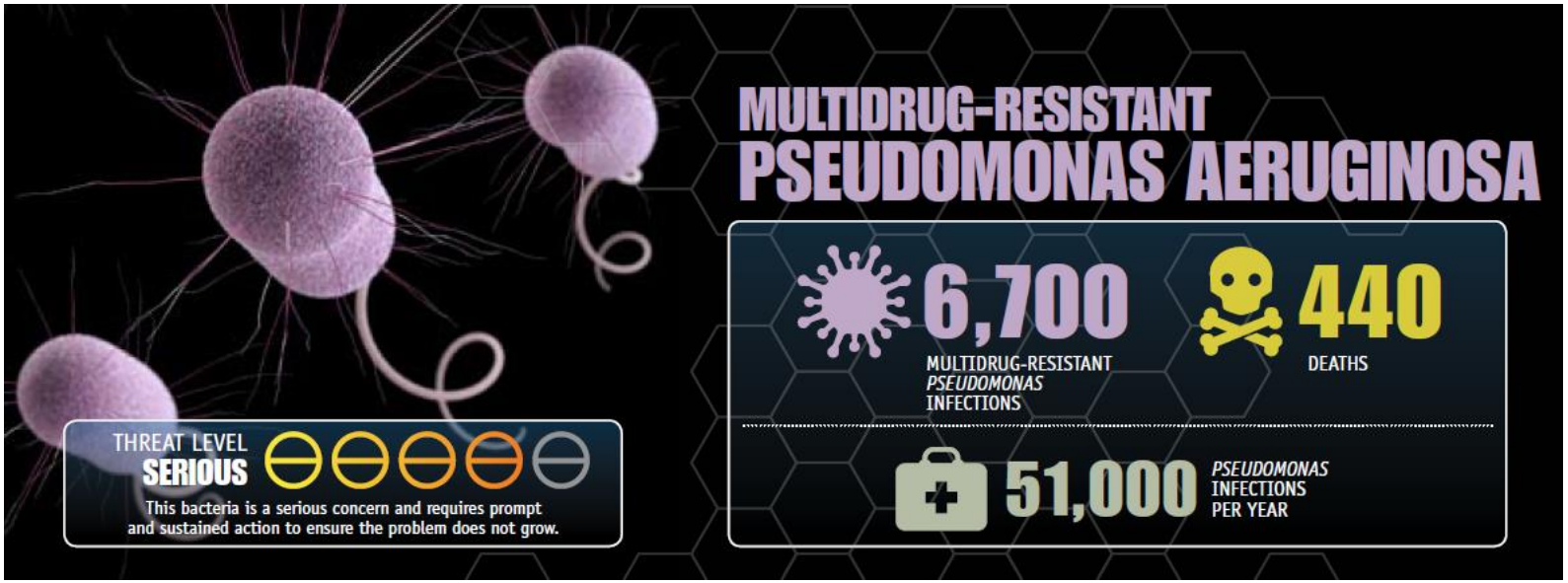
Antimicrobial agents	S (%)	I (%)
Colistin	62 (91.2)	0 (0.0)
Netilmicin	58 (85.3)	4 (5.9)
Trimethoprim/sulfamethoxazole	58 (85.3)	1 (1.5)
Chloramphenicol	57 (83.8)	7 (10.3)
Amikacin	56 (82.4)	3 (4.4)
Ciprofloxacin	56 (82.4)	5 (7.4)
Gentamicin	56 (82.4)	3 (4.4)
Tobramycin	48 (70.6)	1 (1.5)
Tetracycline	47 (69.1)	8 (11.8)
Ceftazidime	18 (26.5)	6 (8.8)
Ticarcillin/clavulanic acid	18 (26.5)	10 (14.7)

I: intermediately susceptible, S: susceptible.

Stenotrophomonas maltophilia:
le nostre resistenze 2012-2013

	Sensibile	Intermedio	Resistente
Ceftazidime		0.5	99.5
Levofloxacina	19.9	15.5	64.6
Cotrimossazolo	94.7		5.3
Tigeciclina*	85.8	11.9	2.3

***BP EUCAST per Enterobacteriaceae:**
S ≤1 ; R>2


MULTIDRUG-RESISTANT
PSEUDOMONAS AERUGINOSA
6,700
MULTIDRUG-RESISTANT
PSEUDOMONAS
INFECTIONS
440
DEATHS
51,000
PSEUDOMONAS
INFECTIONS
PER YEAR
THREAT LEVEL
SERIOUS
This bacteria is a serious concern and requires prompt
and sustained action to ensure the problem does not grow.

Pseudomonas aeruginosa

TABLE 4. ***Pseudomonas aeruginosa*; antimicrobial categories and agents used to define MDR, XDR and PDR (worksheet for categorizing isolates)**

Antimicrobial category	Antimicrobial agent	Results of antimicrobial susceptibility testing (S or NS)
Aminoglycosides	Gentamicin	
	Tobramycin	
	Amikacin	
	Netilmicin	
Antipseudomonal carbapenems	Imipenem	
	Meropenem	
	Doripenem	
Antipseudomonal cephalosporins	Ceftazidime	
	Cefepime	
Antipseudomonal fluoroquinolones	Ciprofloxacin	
	Levofloxacin	
Antipseudomonal penicillins + β-lactamase inhibitors	Ticarcillin–clavulanic acid	
	Piperacillin–tazobactam	
Monobactams	Aztreonam	
Phosphonic acids	Fosfomycin	
Polymyxins	Colistin	
	Polymyxin B	

Criteria for defining MDR, XDR and PDR in *Pseudomonas aeruginosa*
MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories.
XDR: non-susceptible to ≥1 agent in all but ≤2 categories.
PDR: non-susceptible to all antimicrobial agents listed.

Comparison of different methods for determining beta-lactam susceptibility in *Pseudomonas aeruginosa*

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TABLE 2 - *Comparison with E-test to evidence very major errors (VM = false susceptibility), major errors (MA = false resistant) and minor errors (MI = errors with intermediate results).*

		Kirby-Bauer (%)	*Sensititre (%)*	*VITEK 2 (%)*
ATM	VM	0	0.0	0.0
	MA	0	0.0	1.3
	MI	7.8	10.4	20.8
CAZ	VM	0	0.0	1.3
	MA	0	3.9	1.3
	MI	18.2	7.8	11.7
IMI	VM	0	0.0	0.0
	MA	0	1.3	0.0
	MI	20.8	22.1	29.9
MEM	VM	1.3	5.2	7.8
	MA	0	2.6	0.0
	MI	3.9	6.5	2.6
TZP	VM	0	5.2	9.1
	MA	0	0.0	0.0

ATM = aztreonam, CAZ = ceftazidime, IMI = imipenem, MEM = meropenem, TZP = piperacillin+tazobactam.

Journal of Medical Microbiology (2009), 58, 625–629 DOI 10.1099/jmm.0.005587-0

Comparison of different methods of determining β-lactam susceptibility in clinical strains of *Pseudomonas aeruginosa*

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Germán Bou
germanbou@canalejo.org

Very major errors (false susceptible) were only detected for ATM and FEP with DD and for IMP with three methods. Major errors (false resistant) were generally acceptable for all antibiotics except TZP.
VITEK 2 yielded a high level of minor errors (trends toward false susceptibility), mainly with CAZ and FEP.

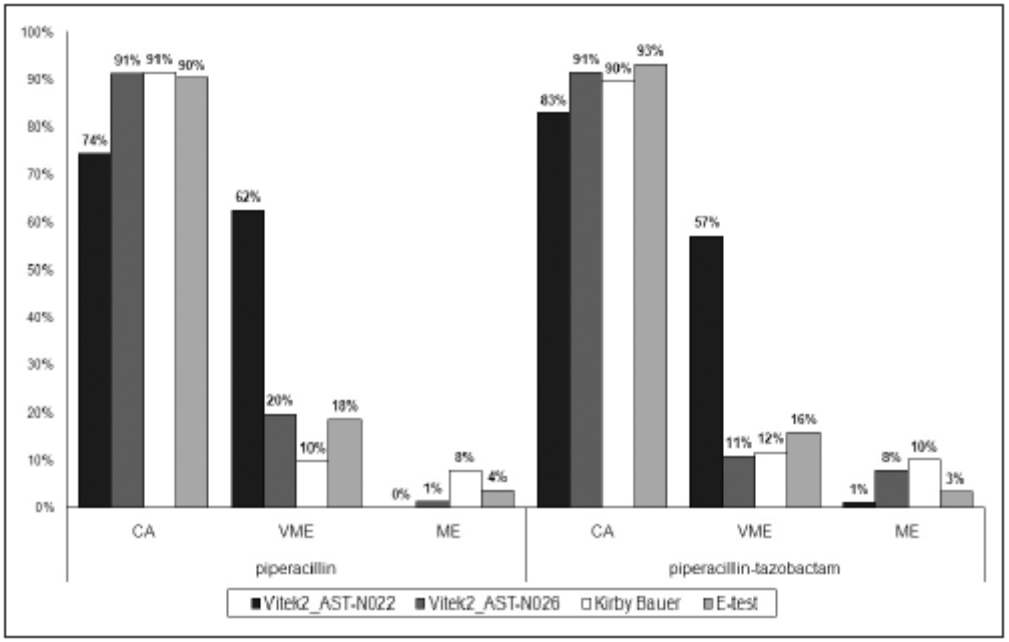
Table 4. Essential agreement (EA), agreement with clinical category (ACC) and errors in clinical categories between VITEK 2, Etest, DD and the RM

Antibiotic	VITEK 2 (%)	Etest (%)	DD (%)
PIP			
Very major error	0	0	0.99
Major error	0.99	0.99	0.99
EA	79.21	94.06	
ACC	99.0	99.0	98.02
TZP			
Very major error	0	0	0.99
Major error	11.88	10.89	5.94
EA	84.16	92.08	
ACC	98.02	89.11	93.07
CAZ			
Very major error	0.99	0	0
Major error	0	4.95	0
Minor error	39.60	9.9	22.77
EA	89.11	86.14	
ACC	95.04	93.07	99.01
FEP			
Very major error	0.99	0	4.95
Major error	0	1.98	0
Minor error	58.42	16.83	28.71
EA	71.29	90.1	
ACC	94.06	91.09	91.09
ATM			
Very major error	0	0	2.97
Major error	0	0	0
Minor error	36.63	30.69	30.69
EA	97.03	89.11	
ACC	91.09	96.03	93.07
IMP			
Very major error	8.9	0.99	6.93
Major error	0	0	0
Minor error	9.9	3.96	3.96
EA	92.08	100	
ACC	89.11	97.03	91.09

New Microbiologica, 34, 97-99, 2011

Accuracy of automated and manual systems for susceptibility testing of *Pseudomonas aeruginosa* to piperacillin and piperacillin-tazobactam

Carlo Gagliotti[1], Mario Sarti[2], Carla Sabia[3], Raffaele Gargiulo[2], Gian Maria Rossolini[4], Carmelina Carillo[5], Carla Cassani[6], Antonio Paolo Cipolloni[7], Federica Pedna[8], Maria Rita Rossi[9], Silvia Storchi Incerti[10], Giovanna Testa[11], Claudia Venturelli[12], Maria Luisa Moro[1]



Vitek2 (card AST-N022) showed the worst performance; the other three methods (Vitek2 card AST-N026, Kirby-Bauer and E-test) performed comparably but never fulfilled the minimal standard proposed by FDA.

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2007, p. 1339–1342 Vol. 45, No. 4
0095-1137/07/$08.00+0 doi:10.1128/JCM.01716-06

Accuracies of β-Lactam Susceptibility Test Results for *Pseudomonas aeruginosa* with Four Automated Systems (BD Phoenix, MicroScan WalkAway, Vitek, and Vitek 2)[▽]

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TABLE 1. Types of intermethod errors produced when testing 30 *P. aeruginosa* isolates by four commercial automated systems in seven laboratories[a]

System and antimicrobial agent (no. of strains tested)	Percentage of indicated type of error					
	Compared to BMD result[b]			Compared to consensus result[c]		
	Very major	Major	Minor	Very major	Major	Minor
BD Phoenix						
Aztreonam (60)[g]	0.0	1.7	33.3[d]	0.0	1.7	36.7[d]
Cefepime (60)	0.0	1.7	18.3[d]	0.0	1.7	18.3[d]
Ceftazidime (60)	1.7[d]	0.0	18.3[d]	1.7[d]	0.0	16.7[d]
Imipenem (60)	0.0	0.0	3.3	0.0	0.0	1.7
Piperacillin (30)[e]	0.0	6.7[d]	NA[f]	0.0	3.3[d]	NA[f]
Piperacillin-tazobactam (60)	1.7[d]	6.7[d]	NA[f]	1.7[d]	5.0[d]	NA[f]
MicroScan WalkAway						
Aztreonam (60)	0.0	3.3[d]	21.7[d]	0.0	3.3[d]	23.3[d]
Cefepime (60)	0.0	3.3[d]	48.3[d]	0.0	3.3[d]	45.0[d]
Ceftazidime (60)	1.7[d]	6.7[d]	23.3[d]	0.0	6.7[d]	20.0[d]
Imipenem (60)	0.0	1.7	11.7[d]	1.7[d]	1.7	10.0
Piperacillin (60)	10.0[d]	3.3[d]	NA[f]	15.0[d]	3.3[d]	NA[f]
Piperacillin-tazobactam (60)	5.0[d]	1.7	NA[f]	10.0[d]	0.0	NA[f]
Vitek						
Aztreonam (60)	0.0	3.3[d]	18.3[d]	0.0	5.0[d]	31.7[d]
Cefepime (60)	1.7[d]	0.0	36.7[d]	1.7[d]	0.0	36.7[d]
Ceftazidime (60)	1.7[d]	0.0	20.0[d]	1.7[d]	3.3[d]	16.7[d]
Imipenem (60)	8.3[d]	0.0	13.3[d]	6.7[d]	0.0	10.0
Piperacillin (60)	0.0	8.3[d]	NA[f]	0.0	6.7[d]	NA[f]
Piperacillin-tazobactam (60)	15.0[d]	5.0[d]	NA[f]	15.0[d]	5.0[d]	NA[f]
Vitek 2						
Aztreonam (60)	1.7[d]	0.0	28.3[d]	0.0	0.0	33.3[d]
Cefepime (60)	0.0	0.0	13.3[d]	1.7[d]	0.0	16.7[d]
Ceftazidime (60)	3.3[d]	0.0	23.3[d]	1.7[d]	0.0	21.7[d]
Imipenem (60)	6.7[d]	0.0	25.0[d]	5.0[d]	0.0	26.7[d]
Piperacillin (60)	5.0[d]	0.0	NA[f]	6.7[d]	0.0	NA[f]
Piperacillin-tazobactam (60)	21.7[d]	1.7	NA[f]	20.0[d]	0.0	NA[f]

Unacceptable levels of error (minor, major, and very major) were detected, some with systematic biases toward false susceptibility (piperacillin-tazobactam and imipenem) and others toward false resistance (aztreonam, cefepime, and ceftazidime).

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Accuracy of Three Automated Systems (MicroScan WalkAway, VITEK, and VITEK 2) for Susceptibility Testing of *Pseudomonas aeruginosa* against Five Broad-Spectrum Beta-Lactam Agents

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TABLE 3. Evaluation of accuracies of automated systems for susceptibility testing of 100 *P. aeruginosa* strains against β-lactam antimicrobial agents

Antimicrobial and error type[a]	Error rate (%)		
	MicroScan WalkAway	VITEK 2	VITEK
Aztreonam			
Very major	0	0	2
Major	0	1	2
Minor	28	31	28
Cefepime			
Very major	0	0	0
Major	3	1	0
Minor	32	18	26
Ceftazidime			
Very major	0	1	2
Major	0	1	0
Minor	13	9	11
Imipenem			
Very major	0	1	0
Major	2	2	2
Minor	10	8	11
Piperacillin-tazobactam			
Very major	19	27	21
Major	1	0	0

[a] Error rates were calculated based on the consensus result among the broth microdilution (frozen dry-form panels), agar dilution, and disk diffusion methods.

All systems tested exhibited a high, unacceptable level of very major (false-susceptible) errors for piperacillin/tazobactam (19 to 27%). Major (false-resistant) error rates were generally acceptable (0 to 3%), but minor error rates were elevated (8 to 32%) for cefepime (VITEK 2 and VITEK) and for aztreonam (all three systems), leading to consistent trends toward false resistance.

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Strain-Tailored Double-Disk Synergy Test Detects Extended-Spectrum Oxacillinases in *Pseudomonas aeruginosa*▽

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The prevalence of class D extended-spectrum oxacillinases (ES-OXAs) in ceftazidime-resistant strains of *Pseudomonas aeruginosa* is often underestimated by double-disk synergy tests (DDST) using clavulanate. A DDST with a customized distance between a disk of ceftazidime or cefepime and inhibitors (clavulanate and imipenem) detected 14 out of 15 different ES-OXAs.

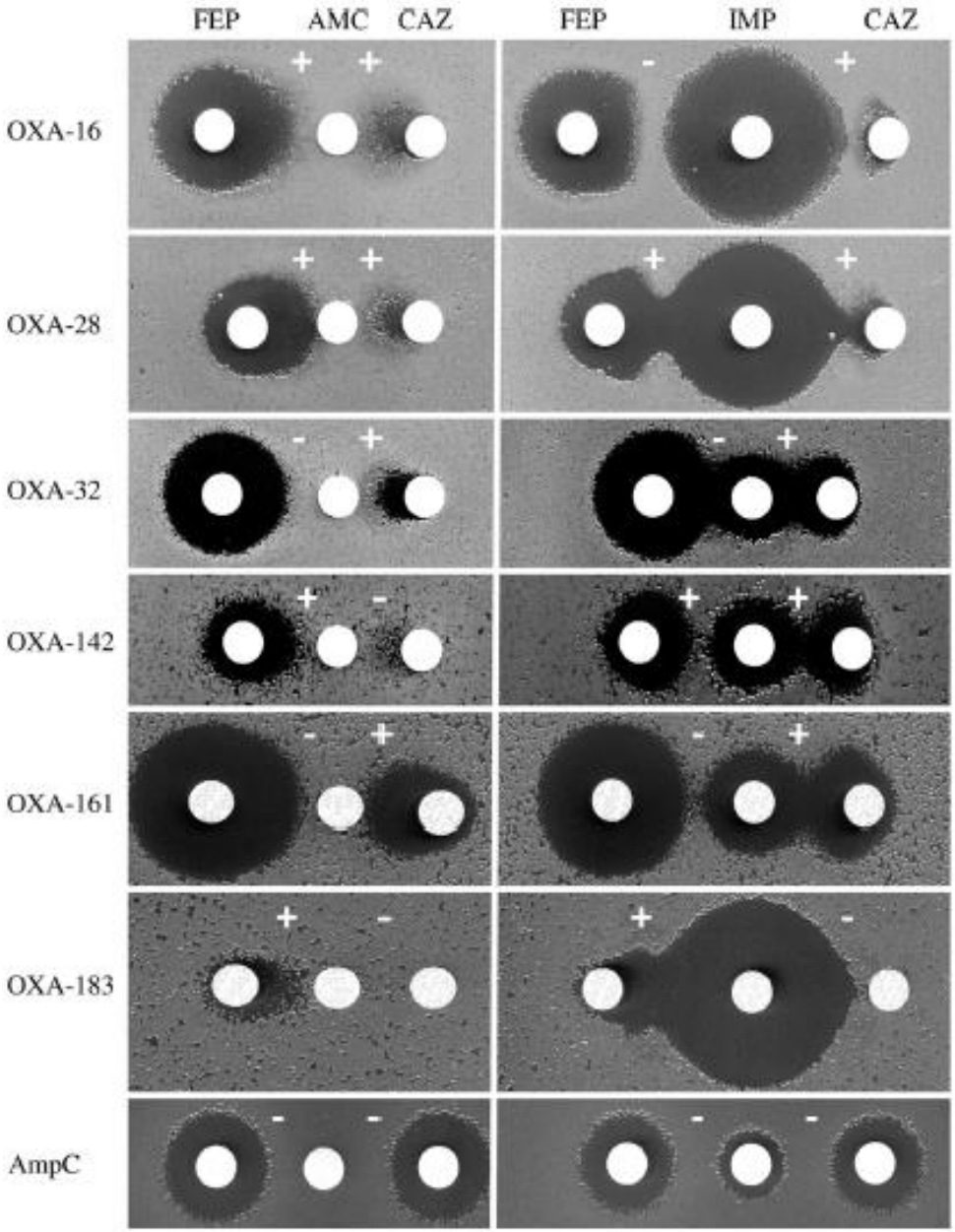

FIG. 1. Double-disk synergy test with *P. aeruginosa* isolates producing the extended-spectrum oxacillinases OXA-16 and OXA-142 (OXA-10 derived), OXA-28 and OXA-183 (OXA-13 derived), or OXA-32 and OXA-161 (OXA-2 derived) or overproducing the cephalosporinase AmpC (AmpC). Distances between the disks were adapted to each strain, based on the inhibition zone diameter around disks containing each compound tested separately. For instance, if no inhibition zone was noticed around clavulanate- and ceftazidime-containing disks, the distance between their two disks is 5 ± 1 mm. Abbreviations: FEP, cefepime (30 μg); AMC, amoxicillin-clavulanate (20/10 μg); CAZ, ceftazidime (30 μg); IMP, imipenem (10 μg). Interpretative results are given (see Table 1).

Concludendo…

… tante idee (forse), ma ben confuse (sicuramente) !!!

Mi dispiace che FORSE vi ho IO aiutato a confondervele ancora di più …