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Assessment of the Phoenix™ automated system and EUCAST breakpoints for antimicrobial susceptibility testing against isolates expressing clinically relevant resistance mechanisms

T. Giani[1], M. I. Morosini[2], M. M. D'Andrea[1], M. García-Castillo[2], G. M. Rossolini[1] and R. Cantón[2]

1) Dipartimento di Biotecnologie, Sezione di Microbiologia, Siena University Hospital, 53100-Siena, Italy and 2) Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), 28034-Madrid, Spain

Abstract

EUCAST breakpoint criteria are being adopted by automatic antimicrobial susceptibility testing systems. The accuracy of the Phoenix Automated System in combination with 2012 EUCAST breakpoints against recent clinical isolates was evaluated. A total of 697 isolates (349 Enterobacteriaceae, 113 *Pseudomonas* spp., 25 *Acinetobacter baumannii*, 11 *Stenotrophomonas maltophilia*, 95 *Staphylococcus aureus*, 6 coagulase negative staphylococci, 77 enterococci and 21 *Streptococcus pneumoniae*) with defined resistance phenotypes and well-characterized resistance mechanisms recovered in Spain (n = 343) and Italy (n = 354) were tested. Comparator antimicrobial susceptibility testing data were obtained following CLSI guidelines. Experimental agreement (EA), defined as MIC agreement ±1 $\log_2$ dilution, category agreement (CA) and relative discrepancies (minor (mD), major (MD) and very major discrepancies (VMD)) were determined. The overall EA and CA for all organism-antimicrobial agent combinations (n = 6.294) were 97.3% and 95.2%, respectively. mD, MD and VMD were 4.7%, 1.3% and 2.7%, all of them in agreement with the ISO (ISO20776-2:2007) acceptance criteria for assessment of susceptibility testing devices. VMD were mainly observed in amoxicillin-clavulanate and cefuroxime in Enterobacteriaceae and gentamicin in *Pseudomonas aeruginosa*, whereas MD were mainly observed in amoxicillin-clavulante in Enterobacteriaceae. mD were mainly observed in Enterobacteriaceae but distributed in different antimicrobials. For *S. aureus* and enterococci relative discrepancies were low. The Phoenix system showed accuracy assessment in accordance with the ISO standards when using EUCAST breakpoints. Inclusion of EUCAST criteria in automatic antimicrobial susceptibility testing systems will facilitate the implementation of EUCAST breakpoints in clinical microbiology laboratories.

Keywords: Antimicrobial susceptibility testing, automatic system, EUCAST breakpoints, resistance mechanism
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Corresponding author: R. Cantón, Servicio de Microbiología, Hospital Universitario Ramón y Cajal, Madrid, Spain
E-mail: rcanton.hrc@salud.madrid.org

Introduction

Accuracy determination of antimicrobial susceptibility testing (AST) in clinical laboratories is essential not only for guiding the antimicrobial treatment in a specific patient but also from a general perspective for compiling data for antimicrobial guidance [1,2]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST), in agreement with the European Medicines Agency (EMA) and the European Centre for Disease Prevention and Control (ECDC), has defined clinical breakpoints to allow European microbiology laboratories to use harmonized criteria for the interpretation of AST results (http://www.eucast.org). These criteria are being adopted by a number of European Union (EU) member states and it is likely that in a short time period they will be used by all EU countries. Automatic diagnostic systems currently present in the market and commonly used for AST in clinical laboratories will therefore have to incorporate these criteria in their instruments to meet the needs of European microbiology laboratories.

The Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, MD, USA) is designed for the rapid bacterial identification at the species level and determination of AST of clinically significant human bacterial pathogens [3]. Performance of this system has been previously evaluated using the CLSI breakpoints but not the EUCAST ones [4–11]. These evaluations are commonly established using Food and Drug Administration (FDA) criteria but not with those from the International Standard Organization [12,13]. In addition, the Phoenix system has demonstrated accuracy in identification of isolates with resistance mechanisms, including extended-spectrum β-lactamases (ESBL), acquired AmpC β-lactamases, certain carbapenemases in gram-negatives, PBP2a in *Staphylococcus aureus* and vancomycin resistance determinants in *Enterococcus* [8,14,15]. The objective of this work was to evaluate the performance of the Phoenix system for the determination of bacterial AST using the EUCAST standards in two different centres. A library of selected isolates with well-defined phenotypes and well-characterized resistance mechanisms was used.

Materials and Methods

Bacterial isolates

A total of 697 isolates were tested, 343 from laboratory A (Ramón y Cajal University Hospital, Madrid, Spain) and 354 from laboratory B (University of Siena, Siena, Italy). Both of these laboratories selected clinical isolates with well-defined resistance phenotypes obtained from routine clinical sample processing (150 from laboratory A and 175 from laboratory B) during 2009 and isolates with well-characterized resistance mechanisms (193 from laboratory A and 179 from laboratory B). Details of the organisms tested are shown in Table 1. Resistance mechanisms were characterized by molecular methods, including PCR and sequencing, described elsewhere.

Antimicrobial susceptibility testing

The BD Phoenix™ (Phoenix) Automated Microbiology System (Becton-Dickinson Diagnostic Systems, Sparks, MD, USA), equipped with software suitable for interpretation of AST results using EUCAST breakpoints (document V1.3, 2011, http://www.eucast.org), was used for performing the antimicrobial susceptibility testing. The panels selected to perform the evaluation were NMIC/ID-76 for gram-negatives, PMIC/ID-67 for staphylococci and enterococci, and SMIC/ID-9 for *Streptococcus pneumoniae*. These panels, introduced into the market in 2009, contained a range of doubling dilutions of different antimicrobials to cover the breakpoints

TABLE 1. Performance of the PHOENIX automated system using EUCAST breakpoints in recent routine clinical isolates and isolates with well-characterized resistance mechanisms

Organisms	No. of recent routine isolates	Isolates with well-known resistance mechanisms		No. of tests[a]	Essential agreement (%)	Category agreement (%)
		No.	Resistance mechanisms/phenotype (No.)			
Enterobacteriaceae[b]	123	226	ESBL (151), metallo-carbapenemase (53), serine-carbapenemase (3), acquired ampC (16), other β-lactamases (3)	3,534	3427 (97.0)	3338 (94.4)
Pseudomonas spp.[c]	64	49	ESBL (5), metallo-carbapenemase (43), other β-lactamases (1)	934	899 (96.2)	868 (92.9)
Acinetobacter baumannii[d]	–	25	Metallo-carbapenemase (1), serine-carbapenemase (24)	200	198 (99.0)	171 (97.7)
Stenotrophomonas maltophilia[e]	11	–	–	11	11 (100)	11 (100)
Staphylococcus aureus[f]	56	39	*mecA*-positive (22), VISA (6), hVISA(2), VSSA (8), MSSA (1)	1,025	1008 (98.3)	1005 (98.0)
Coagulase-negative staphylococci[f]	4	2	hVISA (1), VSSA (1)	63	63 (100)	61 (96.8)
Enterococcus spp.[g]	52	25	*vanA*-positive (15), *vanB*-positive (10)	413	401 (97.1)	402 (97.3)
Streptococcus pneumoniae[h]	15	6	*mefA*-positive (1), *ermB*-positive (1)	114	112 (99.1)	112 (98.2)
Total	325	372		6,294	6120 (97.3)	5968 (95.2)

[a]Organism-antimicrobial agent combination tested.
[b]Evaluated antibiotics are as follows: amoxicillin-clavulanate (AXC), piperacillin-tazobactam (TZP), cefuroxime (CXM), cefotaxime (CTX), ceftazidime (CAZ), imipenem (IPM), meropenem (MEM), ertapenem (ETP), ciprofloxacin (CIP), gentamicin (GM), tobramicin (NN) and trimethoprim-sulphamethoxazole (SXT).
[c]Aztreonam (ATM), TZP, CAZ, IPM, MEM, CIP, GM, NN and colistin (CL).
[d]IPM, MEM, CIP, GM, SXT and CL.
[e]SXT.
[f]Cefoxitin (FOX), CIP, clindamycin (CLI), daptomycin (DAP), erythromycin (EM), GM, linezolid (LZD), moxifloxacin (MXF), oxacillin (OXA), penicillin (PEN), teicoplanin (TEC), tetracycline (TET), vancomycin (VAN), and fusidic acid (FUS).
[g]GM, LZD, ampicillin (AM), TEC, VAN and nitrofurantoin (NIT)
[h]PEN, EM, CTX, MXF, CLI and TET.

recommended by EUCAST (document V1.3, 2011, http://www.eucast.org/). Selected antibiotics for each group of microorganisms are shown in Table 1.

Isolates to be tested were plated at least once on sheep blood Columbia agar and incubated overnight at 35–37°C. An initial suspension was made in Phoenix-ID broth and adjusted to a density corresponding to MacFarland 0.5. Twenty-five microlitres of the initial inoculum were then transferred into 8 mL Phoenix AST broth. This final suspension was then poured into the panel, which was then placed in the instrument.

MIC results in recent clinical isolates with well-defined resistance phenotypes and isolates with well-characterized resistance mechanisms were previously obtained following CLSI criteria for each bacterial species [16]. Mueller-Hinton broth, inoculum of 5×10^5 CFU/mL and incubation period of 18 h were used.

Data evaluation

To assess the performance of the Phoenix system, essential agreement (EA), category agreement (CA) and relative discrepancies were determined considering different antimicrobial agents for each microorganism (Table 1).

Essential agreement was defined when the MICs obtained with the Phoenix system and by the CLSI method were identical or ±1 $\log_2$ dilution. CA was defined as clinical interpretive category agreement between the Phoenix system and the standard CLSI method after applying the EUCAST breakpoints published in 2012 (document V2.0, 2012, http://www.eucast.org/). MIC results previously obtained in recent clinical isolates with well-defined resistance phenotypes and in isolates with well-characterized resistance mechanisms with the CLSI microdilution method were re-interpreted for the susceptible, intermediate and resistant categories using the 2012 EUCAST breakpoints. In addition, relative discrepancies were determined for each antimicrobial-organism combination. Discrepancies were classified as very major discrepancy (VMD) when the MIC obtained with the Phoenix system was categorized as susceptible and that obtained with the standard method was categorized as resistant. A major discrepancy (MD) occurred when the comparator system result (Phoenix) was resistant and that obtained with the standard method was susceptible. A minor discrepancy (mD) occurred when the Phoenix system result was resistant or susceptible and the standard method result intermediate, and also when the Phoenix system result was intermediate and the standard method result susceptible or resistant. mD were not recorded when the intermediate category was not recognized by EUCAST. When computing the discrepancy errors, the number of resistant isolates, the number of susceptible isolates and the total number of tests were used as denominators for VMD, MD and mD, respectively. In the case of VMD or MD, we tried to resolve discrepancies by retesting the isolate in duplicate using both Phoenix and Etest or by using the CLSI microdilution method. Unsolved discrepancies were maintained in the database for calculation of the discrepancies.

Acceptance criteria for accuracy assessment were those defined by the ISO standards: EA ≥90%, VMD ≤3% and MD ≤3% [13].

Quality control (QC) testing

For QC purposes, six ATCC reference isolates were tested in each run: *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853. These isolates were used as daily quality controls at both sites for the entire duration of the study. If the results for one antimicrobial were not within the expected range, all results for the specific drug obtained during that study day were excluded from the dataset and repeated upon resolution of the issue.

Results

A total of 6294 organism-antimicrobial agent combinations were analysed. The studied organisms include 349 isolates of the family Enterobacteriaceae, 113 *Pseudomonas* spp., 25 *Acinetobacter baumannii*, 11 *Stenotrophomonas maltophilia*, 95 *S. aureus*, 6 coagulase negative staphylococci, 77 enterococci and 21 *S. pneumoniae* (Table 1). Detailed results for major groups of organisms and antibiotics can be observed in Tables 2–5. The overall EA in MIC (±1 $\log_2$ dilution) for all the organism-antimicrobial agent combinations was 97.3%; 1.7% (n = 105) of the organism-antimicrobial combination results with the Phoenix system were 2 or more dilutions lower than the standard values and 1.0% (n = 67) of the organism-antimicrobial combination results were 2 or more dilutions higher than the standard values. In both cases, the majority of discrepancies were due to ESBL- and carbapenemase-producing isolates (see below).

The overall CA of the Phoenix system was 95.2%, ranging from 92.9% in *Pseudomonas* spp. to 100.0% in *S. maltophilia*. Overall mD, MD and VMD were 4.7%, 1.3% and 2.7%, respectively, all of them in agreement with the ISO acceptance criteria for accuracy assessment of susceptibility test devices [13]. When considering all resistant test results (n = 2184) for relevant bacterial groups (Tables 2–5), the VMD (n = 62) were mainly concentrated in amoxicillin-clavulanate

TABLE 2. Performance of the Phoenix automatic system and EUCAST breakpoints in *Enterobacteriaceae* isolates

Antimicrobial agents[a]	No. of tests	No. of organism-antimicrobial agent combinations categorized as		Essential agreement (%)	Category agreement (%)	Discrepancies[b]		
		Resistant	Susceptible			mD (%)	MD (%)	VMD (%)
AXC	349	235	114	328 (94.0)	318 (91.1)	–[c]	23 (20.2)	8 (3.4)
TZP	349	110	221	345 (98.8)	332 (95.1)	14 (4)	3 (1.4)	0 (0.0)
CXM	197	155	42	187 (94.9)	184 (93.4)	–[c]	2 (4.8)	11 (7.1)
CTX	349	249	100	340 (97.4)	344 (98.5)	4 (1.1)	0 (0.0)	1 (0.4)
CAZ	349	196	119	342 (98.0)	328 (93.9)	20 (5.7)	0 (0.0)	1 (0.5)
IPM	349	3	305	337 (96.6)	330 (94.6)	19 (5.4)	0 (0.0)	0 (0.0)
MEM	348	7	320	336 (96.5)	332 (95.4)	16 (4.6)	0 (0.0)	0 (0.0)
ETP	152	23	129	152 (100.0)	151 (99.3)	1 (0.7)	0 (0.0)	0 (0.0)
CIP	349	160	164	327 (93.7)	321 (92)	27 (7.7)	0 (0.0)	1 (0.6)
GM	349	101	224	345 (98.8)	322 (92.3)	25 (7.2)	1 (0.4)	1 (1.0)
NN	197	75	116	194 (98.5)	185 (93.9)	11 (5.6)	0 (0.0)	1 (1.3)
SXT	197	72	122	194 (98.5)	191 (96.9)	5 (2.5)	0 (0.0)	1 (1.4)
Total	3534	1386	1976	3427 (97.0)	3338 (94.5)	142 (4.7)	29 (1.4)	25 (1.8)

[a]Abbreviations for antibiotics, see Table 1.
[b]mD, minor discrepancies; MD, major discrepancies; VMD, very major discrepancies.
[c]No intermediate category has been defined for these antibiotics.

TABLE 3. Performance of the Phoenix automatic system and EUCAST breakpoints in *Pseudomonas aeruginosa* isolates

Antimicrobial agents[a]	No. of tests	No. of organism-antimicrobial agent combinations categorized as		Essential agreement (%)	Category agreement (%)	Discrepancies[b]		
		Resistant	Susceptible			mD (%)	MD (%)	VMD (%)
ATM	56	15	9	46 (82.1)	40 (71.4)	16 (28.6)	0 (0.0)	0 (0.0)
TZP	110	50	60	110 (100.0)	105 (95.5)	–[c]	3 (5.0)	2 (4.0)
CAZ	110	51	59	104 (94.5)	108 (98.2)	–[c]	1 (1.7)	1 (2.0)
IPM	110	47	59	108 (98.2)	102 (92.7)	7 (6.4)	1 (1.7)	0 (0.0)
MEM	110	46	53	101 (91.8)	97 (88.2)	13 (11.8)	0 (0.0)	0 (0.0)
CIP	110	61	46	108 (98.2)	106 (96.4)	4 (3.6)	0 (0.0)	0 (0.0)
GM	110	63	47	105 (95.4)	94 (85.5)	–[c]	0 (0.0)	16 (25.4)
NN	50	12	38	50 (100.0)	49 (98.0)	–[c]	0 (0.0)	1 (8.3)
CL	109	0	109	108 (99.1)	109 (100)	–[c]	0 (0.0)	0 (0.0)
Total	875	345	480	840 (96.0)	810 (92.5)	40 (10.4)	5 (1.0)	20 (5.7)

[a]Abbreviations for antibiotics, see Table 1.
[b]mD, minor discrepancies; MD, major discrepancies; VMD, very major discrepancies.
[c]No intermediate category has been defined for these antibiotics.

TABLE 4. Performance of the Phoenix automatic system and EUCAST breakpoints in *Staphylococcus aureus*

Antimicrobial agents[a]	No. of tests	No. of organism-antimicrobial agent combinations categorized as		Essential agreement (%)	Category agreement (%)	Discrepancies[b]		
		Resistant	Susceptible			mD (%)	MD (%)	VMD (%)
FOX	45	25	20	41 (91.1)	43 (95.6)	–	2 (10.0)	0 (0.0)
CIP	42	24	18	42 (100.0)	42 (100.0)	–	0 (0.0)	0 (0.0)
CLI	95	16	79	95 (100.0)	95 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
DAP	92	4	88	92 (100.0)	89 (96.7)	–[c]	1 (1.1)	2 (50.0)
EM	95	46	46	92 (96.8)	92 (96.8)	3 (3.1)	0 (0.0)	0 (0.0)
GM	95	27	68	94 (98.9)	93 (97.9)	–[c]	0 (0.0)	2 (7.4)
LZD	95	0	95	94 (98.9)	95 (100.0)	–[c]	0 (0.0)	0 (0.0)
MXF	42	23	17	41 (97.6)	41 (97.6)	1 (2.4)	0 (0.0)	0 (0.0)
OXA	95	47	48	92 (96.8)	91 (95.8)	–[c]	4 (8.3)	0 (0.0)
PEN	95	88	7	94 (98.9)	95 (100.0)	–[c]	0 (0.0)	0 (0.0)
TEC	45	13	32	45 (100.0)	41 (91.1)	–[c]	2 (6.2)	2 (15.4)
TET	44	12	31	43 (97.7)	43 (97.7)	0 (0.0)	0 (0.0)	1 (8.3)
VAN	95	0	95	93 (97.9)	95 (100.0)	–[c]	0 (0.0)	0 (0.0)
FUS	50	3	47	50 (100.0)	50 (100.0)	–[c]	0 (0.0)	0 (0.0)
Total	1025	328	691	1008 (98.3)	1005 (98.0)	4 (1.4)	9 (1.3)	7 (2.1)

[a]Abbreviations for antibiotics, see Table 1.
[b]mD, minor discrepancies; MD, major discrepancies; VMD, very major discrepancies.
[c]No intermediate category has been defined for these antibiotics.

TABLE 5. Performance of the Phoenix automatic system and EUCAST breakpoints in enterococci

Antimicrobial agents[a]	No. of tests	No. of organism-antimicrobial agent combinations categorized as		Essential agreement (%)	Category agreement (%)	mD (%)	MD (%)	VMD (%)
		Resistant	Susceptible					
GM	77	61	16	69 (89.6)	68 (94.8)	–[c]	0 (0.0)	9 (14.8)
LZD	77	0	77	74 (96.1)	77 (100.0)	–[c]	0 (0.0)	0 (0.0)
AM	76	23	52	76 (100.0)	76 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
TEC	77	14	63	76 (98.7)	76 (98.7)	–[c]	1 (1.6)	0 (0.0)
VAN	77	26	51	77 (100.0)	76 (97.4)	0 (0.0)	0 (0.0)	1 (3.8)
NIT	29	1	28	29 (100.0)	29 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	413	125	287	401 (97.1)	402 (97.3)	0 (0.0)	1 (0.3)	10 (8.0)

[a]Abbreviations for antibiotics, see Table .
[b]mD, minor discrepancies; MD, major discrepancies; VMD, very major discrepancies.
[c]No intermediate category has been defined for these antibiotics.

(*n* = 8) and cefuroxime (*n* = 11) in Enterobacteriaceae and gentamicin (*n* = 16) in *P. aeruginosa*. The MD (*n* = 44) were mainly concentrated in amoxicillin-clavulanate (*n* = 23) in *Enterobacteriaceae*. Most of the mD were observed in *Enterobacteriaceae* but distributed in different antimicrobials (Table 2).

When considering the *Enterobacteriaceae* isolates, EA values were all higher than 95%, except for ciprofloxacin (93.7%), amoxicillin-clavulanate (94.0%) and cefuroxime (94.9%) (Table 2). In this group of organisms, CA ranged from 91.1% for amoxicillin-clavulanate to 99.3% for ertapenem. The amoxicillin-clavulanate CA value was reflected in error discrepancies as this combination had the highest MD values (20.2%). VMD (overall *n* = 25, 1.8%) were concentrated in amoxicillin-clavulate (*n* = 8, 3.4%) and cefuroxime (*n* = 11, 7.1%). These discrepancies were mainly due to ESBL-producing isolates; 17/23 (7 *E. coli* and 10 *K. pneumoniae*) MD as well as 7/8 (4 *E. coli* and 3 *K. pneumoniae*) VMD were observed in isolates with well-characterized ESBLs (data not shown in Tables).

In *P. aeruginosa* isolates (Table 3), EA values were also higher than 95%, except for aztreonam (82.1%), ceftazidime (94.5%) and meropenem (91.8%). The CA ranged from 71.4% for aztreonam to 100.0% for colistin. Discrepancies were unusual but concentrated in VMD for gentamicin (*n* = 16, 25.4%) and mD for aztreonam (*n* = 16, 28.6%) and meropenem (*n* = 13, 11.8%). These discrepancies mainly occurred in carbapenemase-producing isolates. Regarding VMD for gentamicin, 6/16 were recorded with *P. aruginosa* isolates with well-characterized resistance mechanisms, and 5 were VIM positive (in all five cases, the Phoenix system gave -1 MIC result, 4 instead 8 mg/L). Moreover, 10/16 mD for aztreonam were obtained with VIM- or IMP-producing *P. aruginosa* isolates and mD for meropenem, nine of them with isolates with well-characterized resistance mechanisms (six VIM- or IMP- and three PER-producing isolates) (data not shown in Tables).

In *S. aureus* (Table 4), CA was always higher than 95%, including oxacillin. Moreover, EA always exceeded 96%, with the exception of cefoxitin (91.1%). Only 15 discrepancies were recorded when analysing the relative discrepancies and four (8.3%) of them were MD for oxacillin. In enterococci (Table 5) and with the exception of gentamicin (94.8%), CA was higher than 95%. Only 11 discrepancies were recorded in enterococci, nine (14.8%) of them were VMD for gentamicin.

Discussion

Antimicrobial susceptibility testing interpretation in Europe has been traditionally driven by local committees or by the CLSI standards but nowadays most European countries are moving to EUCAST breakpoints. The EUCAST committee was formed in 1996 and reorganized in 2001 with the aim of harmonization of existing breakpoints [17]. The harmonization process was finished in 2009 and EUCAST became the breakpoint committee of EMA and ECDC. EUCAST breakpoints are being introduced into devices for automated susceptibility testing but with some limitations, depending on the system (http://www.eucast.org).

The Phoenix automatic system has included adequate antimicrobial concentrations in most of its panels to implement the EUCAST breakpoints as well as definitions of susceptible and resistant clinical categories (http://www.bd.com/resource.aspx?IDX=10841, last accession June 25, 2012). The Phoenix system suppresses from the final report the drugs with MIC values interpreted with a dash ('-') (susceptibility testing is not recommended as the species is a poor target for therapy with the drug) as well as with the acronym 'IE', which indicates that there is insufficient evidence that the species in question is a good target for therapy with the drug. In both cases, the MIC remains visible at the laboratory level.

Different studies of the accuracy of different automatic susceptibility tests have been published, most of them using CLSI breakpoints. In our study, we evaluate the accuracy of the Phoenix system with current EUCAST breakpoints (document 2.0, http://www.eucast.org) using a panel of isolates representing relevant resistance mechanisms such as ESBL- and carbapenemase-producing isolates, as well as methicillin resistance in *S. aureus* or vancomycin resistance in enterococci. To our knowledge, this is the first time that a susceptibility testing device using EUCAST breakpoints has been evaluated. Acceptance criteria for accuracy assessment were in agreement not only with those described by the ISO standard [13] but also with those required by the FDA in the USA [12]. The Phoenix system was previously evaluated using CLSI breakpoints and different panels of organisms, including specific resistance mechanisms [4–11,14,15].

In our evaluation, overall MD and VMD discrepancies were below 3%, with a figure of 4.7% for mD. In general, the number of discrepancies in gram-negative organisms was higher than that found in gram-positive bacteria. This can be associated with the complexity of the resistance mechanism in the selected gram-negative isolates for this evaluation, most of them having an enzymatic resistance mechanism affecting β-lactam antibiotics. When discrepancies were specifically analysed, amoxicillin-clavulanate in Enterobacteriaceae was responsible for a majority of them, with a high proportion of MD and VMD discrepancies. AST of β-lactam-β-lactamase inhibitor combinations has been previously reported as problematic in certain automatic systems related to the instability of the β-lactamase inhibitors [18,19]. The effect of specific enzymatic resistance mechanisms that can be present in the microorganisms, including ESBL, and potential fluctuation of ±1 $\log_2$ MIC value in the absence of an intermediate EUCAST clinical category in Enterobacteriaceae, could be responsible for these discrepancies. Most of the discrepant isolates were within the Spanish isolates. Unlike amoxicillin-clavulanate, carbapenems showed mD but not MD or VMD. Heteroresistance and variable expression of the carbapenemase-mediated carbapenem resistance mechanism can be responsible for these discrepancies [20]. Gentamicin discrepancies in Enterobacteriaceae and *P. aeruginosa* with a high proportion of mD and VMD, respectively, are also of note. The former can be related to a narrow range of concentrations in the EUCAST intermediate category and the latter to the absence of a EUCAST intermediate category for this antibiotic. An antibiotic with a high proportion of discrepancies is ciprofloxacin. They were mainly concentrated in Enterobacteriaceae (nearly all mD). The inclusion of a single antimicrobial concentration in the EUCAST intermediate category for ciprofloxacin accounts for these mD.

An interesting feature of the Phoenix system is its excellent accuracy in gram-positive isolates. The studied collection of *S. aureus* included a high proportion of methicillin-resistant isolates and also isolates with decreased susceptibility to glycopeptides. Despite this fact, CA for these antibiotics was high and discrepancies not noticeable. This was also the case for glycopeptides and enterococci. The robustness of the Phoenix system for AST of these organisms has been previously described [4,8,15].

In summary, we have evaluated the accuracy of the Phoenix AST system when using EUCAST breakpoints. Despite the inclusion of a panel of isolates with complex resistance mechanisms from two different laboratories, EA, CA and discrepancy rates were in agreement with the acceptance criteria established by the ISO standard [12]. Interpretive category discrepancies observed in specific organism-antimicrobial combinations were mainly due to particular breakpoints, such as those for amoxicillin-clavulanate, gentamicin or ciprofloxacin, or the heterogeneous expression of resistant mechanisms such as metallo β-lactamases affecting carbapenems. Inclusion of EUCAST criteria in automatic systems for susceptibility testing will facilitate the implementation of EUCAST breakpoints in clinical microbiology laboratories.

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Transparency Declaration

RC is currently Chairman of the European Commitee on Antimicrobial Susceptibility Testing (EUCAST) and has participated in educational workshops organized by BD, Siemens and BioMérieux. GMR has participated in educational workshops organized by BD, Siemens and BioMérieux. The other authors have no conflict of interests.

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