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Evaluation of Three Automated Systems for Susceptibility Testing of Enterobacteria Containing *qnrB*, *qnrS*, and/or *aac(6′)-Ib-cr*▽

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The accuracy of the MicroScan WalkAway, BD Phoenix, and Vitek-2 systems for susceptibility testing of quinolones and aminoglycosides against 68 enterobacteria containing *qnrB*, *qnrS*, and/or *aac(6′)-Ib-cr* was evaluated using reference microdilution. Overall, one very major error (0.09%), 6 major errors (0.52%), and 45 minor errors (3.89%) were noted.

Previous reports indicate that automated systems for susceptibility testing are reliable in detecting quinolone-resistant enterobacteria (4, 7, 9, 12), but there is very limited information on the accuracy of these systems with organisms expressing plasmid-mediated quinolone resistance (PMQR) mechanisms. PMQR genes determine the low level of resistance to quinolones and may favor or complement the selection of additional mechanisms (5, 6, 10). They code for Qnr proteins, the acetyltransferase Aac(6′)-Ib-cr, or the efflux systems QepA and OqxAB. Aac(6′)-Ib-cr also confers resistance to tobramycin and amikacin.

Detection of strains harboring PMQR mechanisms usually depends on genotypic assays (often PCR amplification and sequencing of these genes), as we currently lack reliable phenotypic methods to detect these organisms. Qnr proteins and Aac(6′)-Ib-cr seem to be the most relevant PMQR mechanisms in Spain and other European countries, as the plasmid locations of the *oqxAB* (present in the chromosomes of most *Klebsiella pneumoniae* strains) and *qepA* genes have uncommonly been described in this geographical location. Also, most enterobacteria with plasmid-mediated *qnr* genes contain *qnrA*, *qnrB*, or *qnrS* alleles, while those with *qnrD* and *qnrC* still seem to be exceptional.

A previous study had evaluated four clinical strains of *K. pneumoniae* and the corresponding *Escherichia coli* transconjugants carrying the *qnrA1* gene with four automated systems (11). In this study, the performance of three automated instruments for susceptibility testing of quinolones and aminoglycosides against bacteria containing *qnrB*, *qnrS*, and/or *aac(6′)Ib-cr* was evaluated.

We tested 68 clinical isolates (one per patient), collected at two centers in northern (Hospital Universitario Marqués de Valdecilla, Santander) and southern (Hospital Virgen Macarena, Seville) Spain, as indicated in Table 1.

qnrB, *qnrS*, and *acc(6′)-Ib-cr* were detected by multiplex PCR and sequencing of the obtained amplicons, as described elsewhere (1). In total, 47 isolates produced a *qnr* determinant (7 *qnrB*-related alleles, 40 *qnrS1*-related alleles), and 26 produced *acc(6′)-Ib-cr*, with 5 isolates producing both types of genes.

Reference MIC values for the tested organisms were determined by a broth microdilution assay according to CLSI guidelines (2). The following antimicrobial agents and concentrations (mg/liter) were tested: nalidixic acid (0.5 to 1,024), ciprofloxacin (0.015 to 32), norfloxacin (0.015 to 32), levofloxacin (0.015 to 32), gentamicin (0.06 to 128), tobramycin (0.06 to 128), and amikacin (0.06 to 128). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

The following systems and corresponding panels were tested: BD Phoenix (BD Diagnostic Systems, Sparks, MD; panel UNMIC/ID-62), MicroScan WalkAway (Siemens, West Sacramento, CA; urine combo 37), and Vitek-2 (bioMérieux-Vitek, Hazelwood, MO; AST-N058). The relevant antimicrobial agents and concentrations (mg/liter) evaluable in the used panels or cards were as follows: for UNMIC/ID-62, ciprofloxacin (0.125 to 2), norfloxacin (2 to 8), gentamicin (2 to 8), tobramycin (2 to 8), and amikacin (8 to 32); for urine combo 37, nalidixic acid (4, 16), ciprofloxacin (0.12, 1, 2), norfloxacin (1, 4, 8), levofloxacin (0.25, 2, 4), gentamicin (4 to 8), tobramycin (4 to 8), and amikacin (8 to 32); and for AST-N058, nalidixic acid (2 to 32), ciprofloxacin (0.25 to 4), gentamicin (1 to 16), tobramycin (4 to 8), and amikacin (8 to 32).

The MICs obtained with the reference method or with the automated systems were translated into clinical categories (susceptible, intermediate, or resistant) according to the interpretive criteria of the CLSI (3). Percentages of agreement in clinical categories were calculated. The following types of disagreements were considered: very major errors (resistant by the reference method but susceptible by the test method), major errors (susceptible by the reference method but resistant by the test method), and minor errors (susceptible or resistant by either the reference or the test method but intermediate by the other method). Essential agreement was defined as when

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TABLE 1. Enterobacteria isolates containing *qnrB*, *qnrS*, and/or *aac(6′)-Ib-cr* that were evaluated in this study

Species (no. of isolates)	PMQR gene(s) expressed[a]	No. of isolates
Escherichia coli (32)	*qnrB*	1
	qnrS1	9
	aac(6′)-Ib-cr	21
	aac(6′)-Ib-cr + qnrA1	1
Enterobacter cloacae (29)	*qnrB5*	1
	qnrB2	1
	qnrS1	25
	qnrS1 + aac(6′)-Ib-cr	2
Citrobacter freundii (2)	*qnrB*	2
Klebsiella pneumoniae (2)	*qnrB*	1
	qnrS1	1
Klebsiella oxytoca (2)	*qnrS1*	1
	qnrS1 + aac(6′)-Ib-cr	1
Enterobacter aerogenes (1)	*qnrB*	1

[a] The exact allele was not determined for the *qnrB* gene of some isolates because of incomplete gene amplification.

the same MIC values (within ±1 dilution) were obtained by the automated systems and the reference method; in this case, when both MICs determined with the automated panels and by reference microdilution were under or over the limit concentrations in the automated panels, these results were not considered to disagree.

The percentages of agreement in clinical categories and of essential agreement and the number of errors made by the automated methods, relative to the reference results obtained by microdilution, are presented in Table 2.

The agreement in clinical categories was ≥90% for all combinations of agents and automated systems. Essential agreement was also ≥90% in all except two cases (nalidixic acid and ciprofloxacin results with MicroScan). In most cases where essential agreement did not reach the highest theoretical value of 100%, it was because the MICs obtained with the corresponding system for the considered agent were ≥2 times higher than the reference MIC, and only in a minority of cases was this due to lower MICs obtained with the automated system than with the reference method.

A total of 1,156 results (17 antibiotic/automated system combinations by 68 isolates) were obtained in this study, corresponding to 476 for MicroScan, 340 for BD Phoenix, and 340 for Vitek-2. Overall, one very major error (0.09%; for nalidixic acid and MicroScan), 6 major errors (0.52%; 2 for nalidixic acid, 1 for ciprofloxacin, 2 for gentamicin, 1 for tobramycin), and 45 minor errors (3.89%) were noted (Table 2). These minor errors were evenly distributed between results for quinolones (4% of errors) and aminoglycosides (3.8%). When considering the three systems independently, the percentages of errors presented small differences. Very major errors were 0.21% of the total number of results for MicroScan. Major errors were 0.21% for MicroScan, 0.88% for BD Phoenix, and 0.59% for Vitek-2. Finally, minor errors were 3.57% for MicroScan, 5.00% for BD Phoenix, and 2.06% for Vitek-2.

When specifically considering organisms producing Aac(6′)-Ib-cr, 5 minor errors were observed for tobramycin, including 3 with MicroScan and 2 with Vitek-2.

TABLE 2. Summary of the results obtained with automated methods compared to the results with reference microdilution

Antimicrobial agent	System (concns in mg/liter)	% agreement in clinical categories	% essential agreement	No. of errors of indicated type		
				Very major	Major	Minor
Nalidixic acid	MicroScan (4, 16)	99	88	1	0	0
	Vitek-2 (2, 4, 8, 16, 32)	97	93	0	2	0
Ciprofloxacin	MicroScan (0.12, 1, 2)	91	75	0	0	6
	BD Phoenix (0.12, 0.25, 0.5, 1, 2)	90	91	0	1	6
	Vitek-2 (0.25, 0.5, 1, 2, 4)	96	96	0	0	3
Norfloxacin	MicroScan (1, 4, 8)	96	90	0	0	3
	BD Phoenix (2, 4, 8)	96	100	0	0	3
Levofloxacin	MicroScan (0.25, 2, 4)	99	99	0	0	1
Gentamicin	MicroScan (4, 8)	93	97	0	1	4
	BD Phoenix (2, 4, 8)	91	97	0	1	5
	Vitek-2 (1, 2, 4, 8, 16)	97	97	0	0	2
Tobramycin	MicroScan (4, 8)	90	99	0	0	7
	BD Phoenix (2, 4, 8)	94	97	0	1	3
	Vitek-2 (4, 8)	97	99	0	0	2
Amikacin	MicroScan (8, 16, 32)	100	99	0	0	0
	BD Phoenix (8, 16, 32)	100	100	0	0	0
	Vitek-2 (8, 16, 32)	100	91	0	0	0

It has been recommended that the performance of susceptibility tests is considered adequate when the total error rate is <10%, with ≤1.5% of errors being very major errors and ≤3.0% being major errors, and when the overall essential MIC agreement is >90% (13). Taking these values as a reference, the three systems that we have evaluated in this study (MicroScan, BD Phoenix, and Vitek-2) can be considered reliable for susceptibility testing of quinolones and aminoglycosides against enterobacteria with the *qnrB*, *qnrS*, and/or *aac(6′)Ib-cr* gene.

Because of the predefined number of wells available in the panels or cards of these systems, only a limited number of concentrations (sometimes corresponding to discontinuous scales) of both quinolones and aminoglycosides can be tested, which often precludes obtainment of concrete MIC values. In the case of quinolones, it should be considered that PMQR genes, by themselves, cause only low-level resistance; for this reason, it would be convenient to increase the number of wells

with low concentrations of these compounds. This will also help in recognizing enterobacteria susceptible to nalidixic acid and enterobacteria with decreased susceptibility to fluoroquinolones, a phenotype often associated with the presence of PMQR genes in organisms lacking other mechanisms of quinolone resistance (6, 10). Similarly, including more wells with low concentrations of quinolones would also be helpful in presumptively recognizing strains overexpressing the AcrAB-TolC efflux pump and presenting elevated MICs of nalidixic acid in comparison to those of other quinolones (8).

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