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

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SUMMARY

This study aimed to evaluate the accuracy of routine systems (Vitek2 cards AST-N022 and AST-N026; Kirby Bauer; E-test) for susceptibility testing of *Pseudomonas aeruginosa* to piperacillin and piperacillin-tazobactam. 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.

KEY WORDS: Diagnostic tests; antimicrobial resistance; *Pseudomonas aeruginosa*; very major error

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Data on the accuracy of automated and manual systems for testing the antimicrobial susceptibility of *Pseudomonas aeruginosa* have been reported (Burns *et al.,* 2000; Juretschko *et al.,* 2007; Sader *et al.*, 2006; Steward *et al.*, 2003). High rates of false susceptibility (very major errors: VMEs) of the automated systems have been observed, especially for piperacillin and piperacillin-tazobactam, where the interpretative categories of the results are only two: susceptible and resistant without the intermediate susceptibility (Juretschko *et al.,* 2007; Sader *et al.,* 2006). The present study aimed to compare the accuracy of one automated system (Vitek2 (V2)), considering both the card AST-N022 and the recently released AST-N026, and two manual systems (Kirby-Bauer (KB) and E-test (ET)) for susceptibility testing of *P. aeruginosa* to piperacillin and piperacillin-tazobactam. Nine laboratories in the Emilia-Romagna region (Italy) were recruited on a voluntary basis to participate to the study. These laboratories (7 hospital laboratories and 2 teaching hospital laboratories) collected all the consecutive clinical isolates of P. aeruginosa; identified between January and February 2008, for which V2 (AST-N022) estimated a Minimum inhibitory concentration (MIC) ≥8 μg/ml for piperacillin and/or piperacillin-tazobactam. A further sample of 40 consecutive isolates with MICs≤4 μg/ml for both

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agents was collected in the same period in the referral centre (Nuovo Ospedale S. Agostino Estense, Modena).
All isolates included were retested, in the referral centre, using V2, bioMérieux Italia S.p.A. (cards AST-N022 and AST-N026), KB and ET (AB Biodisk, Solna, Sweden) and compared to Agar dilution (AD), as reference test. The measure of antibiotic zone size was performed manually or with the Osiris system (Bio-Rad). The standard AD method was performed on Mueller Hinton medium (BD) with a Denley multipoint inoculator (Denley Instruments LtD., Billingshurst, UK; the range of concentrations for both antimicrobial agents was 2-256 μg/ml (Sigma Chemical Co., St. Louis, MO, USA). Quality control was monitored using the *P. aeuriginosa* ATCC 27853. All tests were performed in compliance with the Clinical and Laboratory Standards Institute (CLSI, 2007) and/or as recommended by the manufactures' package inserts with the products.
The category agreement and the errors rates of the four methods were calculated according to the Food and Drug Administration recommendations (FDA, 2007). This institution considers the following criteria to assess the acceptability of the performance of a susceptibility test: category agreement (CA) >89.9%, false resistance (major

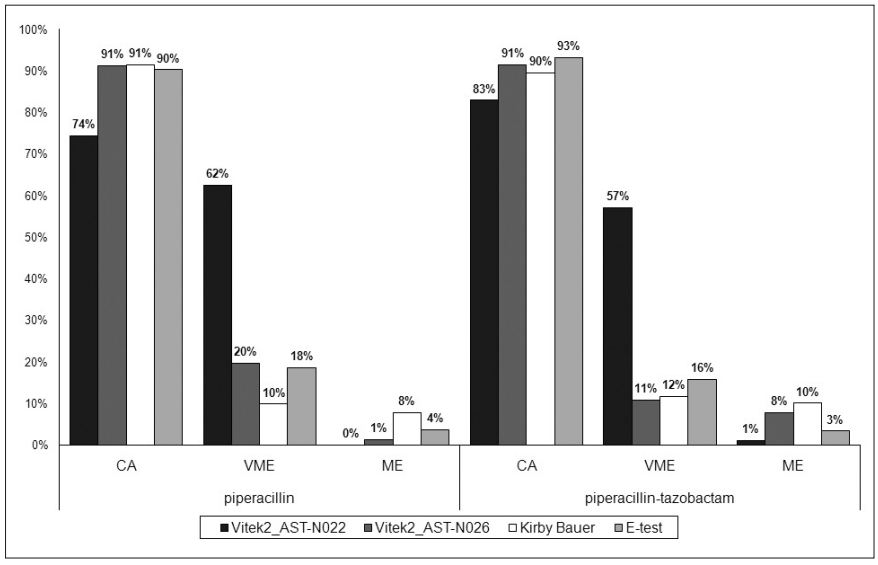

FIGURE 1 - *Susceptibility test of Pseudomonas aeruginosa to piperacillin and piperacillin-tazobactam: rates of Category Agreement (CA), Very Major Errors (VME) and Major Errors (ME) of Vitek2 (AST-N022 and AST-N026), Kirby Bauer and E-test.*

TABLE 1 - *Proportion of Very Major Errors (VME) of Vitek2 (AST-N022 and AST-N026) and E-test according to the Minimum inhibitory concentrations (MICs) as estimated by the three systems for the susceptibility test of Pseudomonas aeruginosa to piperacillin and piperacillin-tazobactam.*

	Vitek2 AST-N022			*Vitek2 AST-N026*			*E-test*		
	n.	*VME*	*% VME*	*n.*	*VME*	*% VME*	*n.*	*VME*	*% VME*
piperacillin (MICs)									
<=4 μg/ml	47	2	4.3%	24	0	0.0%	65	1	1.5%
8-16 μg/ml	147	9	6.1%	149	4	2.7%	123	5	4.1%
32-64 μg/ml	161	97	60.2%	105	30	28.6%	82	26	31.7%
128- μg/ml	65	-		142	-		150	-	
piperacillin-tazobactam (MICs)									
<=4 μg/ml	52	0	0.0%	21	0	0.0%	109	1	0.9%
8-16 μg/ml	156	5	3.2%	172	2	1.2%	138	7	5.1%
32-64 μg/ml	157	64	40.8%	96	11	11.5%	61	11	18.0%
128- μg/ml	55	-		131	-		112	-	

error: ME) rate, based on the number of susceptible organisms, ≤3% and false susceptibility (VMEs) rate, based on the number of resistant organisms, presenting a 95% confidence interval below a fixed threshold both in the upper limit (<7.5%) and in the lower limit (<1.5%). Data analysis was performed by Stata v.8.2 (Stata Corp., College Station. TX, USA).

The study included 420 isolates. Percentages of resistance were 41% (173/420) for piperacillin and 29% (121/420) for piperacillin-tazobactam. V2 (AST-N022) showed a category agreement below 90% for both considered agents while the other three methods were within the range of 90%-91% for piperacillin and within the range of 90%-93% for piperacillin-tazobactam. KB had the lowest rate of VMEs for piperacillin (10%; 95%CI 6%-15%), while V2 (AST-N026) had the lowest for piperacillin-tazobactam (11%; 95%CI 6%-18%). KB obtained the highest rates of MEs for both antimicrobial agents (Figure 1). The MIC values of 32 and 64 μg/ml, as measured by V2 and ET, accounted for most VMEs (Table 1).

The results from this study show that the accuracy of the four methods considered never fulfilled the minimal standard proposed by FDA for false susceptibility (VMEs). V2 (AST-N022) showed the highest rate of VMEs, while the performance of the three other methods appears to be comparable with a slightly better accuracy of V2 (card AST-N026) for piperacillin-tazobactam and of KB for piperacillin (Figure 1). These findings are not biased by the method of strains selection (undersampling of susceptible isolates with MICs≤4 μg/ml), which is unlikely to have any impact on the estimation of error frequencies, especially for the rate of false susceptibility (VMEs) calculated on the number of resistant organisms. Another important observation refers to the relatively high MIC values reported by V2 and ET methods for most strains and wrongly interpreted as susceptible (VMEs) (Table 1).

This is the first paper comparing routine manual systems and the old and new cards of Vitek2. The other two available studies on this topic reported problems of Vitek2 performance for the susceptibility testing of *P. aeruginosa* but were based on the old cards since the AST-N026 was introduced later. Moreover, they included a lower number of isolates (100 and 30 respectively) with selection methods leading to a more pronounced bias toward the breakpoints than the present study (Juretschko *et al.,* 2007; Sader *et al.,* 2006).

The findings from this study are relevant because they highlight the fact that a reliable routine test for the susceptibility of P. aeruginosa is missing for piperacillin and piperacillin-tazobactam. A possible way to reduce the clinical impact of routine susceptibility testing inaccuracy is to include a warning comment with the laboratory results sent to the physicians when the MIC values of V2 or ET are high (32-64 μg/ml), even if within the interpretation category of susceptibility.

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