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

Barbara Sapino, Sandra Mazzucato, Maria Solinas, Massimo Gion, Stefano Grandesso

Clinical Microbiology Unit, Department of Clinical Pathology, Ospedale dell'Angelo, Mestre, Italy

SUMMARY

This study compared the results of antimicrobial susceptibility testing of 77 clinical strains isolated for *Pseudomonas aeruginosa* to five beta-lactam agents: aztreonam, ceftazidime, imipenem, meropenem and piperacillin+tazobactam. Four different methods were employed: two automated systems (VITEK 2 and Sensititre) and two standardized manual methods (Kirby-Bauer and E-test).

The concordances for the susceptibility categories were better for Kirby-Bauer (medium value =89.6%), followed by Sensititre (medium value =87.0%) and VITEK 2 (medium value =82.8%). The disk diffusion method did not present very major errors in comparison to the two automated systems.

KEY WORDS: Pseudomonas aeruginosa, Antibiotic susceptibility, E-test, Vitek 2, Sensititre, MIC.

Received June 06, 2012 *Accepted* August 13, 2012

Automated or semiautomated systems have been widely used for species identification and susceptibility testing in many laboratories. However, in many studies different methods for testing *Pseudomonas aeruginosa* susceptibility have not resulted in the same accuracy, especially for beta-lactam agents (Steward *et al.* 2003; Sader *et al.* 2006; Juretschko *et al.* 2007; Torres *et al.* 2009). Between October 2008 and June 2009, 77 different consecutive clinical strains of *Pseudomonas aeruginosa* were collected from different units in our hospital, predominantly from the Intensive Care Unit and Pneumology. All isolated strains were identified with card GN VITEK 2 (bioMérieux, Inc., Durham, North Carolina). Results from all the methods were validated using the quality control *Pseudomonas aeruginosa* ATCC 27853 strain. A single physiological suspension of 0.5 McFarland (0.45%) was used for each of 77 *Pseudomonas aeruginosa* strains for biochemical identification and antimicrobial susceptibility tests to five beta-lactam agents. We used cards AST-N022 (bioMérieux, Inc., Durham, NC, USA) for VITEK 2 antimicrobial susceptibility tests. The concentrations of the agents in the cards were: aztreonam =2, 8 and 32 µg/ml; ceftazidime =1, 2, 8 and 32 µg/ml; imipenem =2, 4 and 16 µg/ml; meropenem =0.5, 4 and 16 µg/ml; piperacillin+tazobactam 4/4, 16/4 and 128/4 µg/ml. Sensititre antimicrobial susceptibility tests were performed using MIC plates EMIZA9EF (Trek Diagnostics Systems, Cleveland, OH, USA). The concentrations of the agents in the plates were: aztreonam =4, 8 and 16 µg/ml; ceftazidime =0.25, 0.5, 1, 2, 4, 8 and 16 µg/ml; imipenem =0.25, 0.5, 1, 2, 4 and 8 µg/ml; meropenem =0.5, 1, 2, 4 and 8 µg/ml; piperacillin+tazobactam =8/4, 16/4, 32/4 and 64/4 µg/ml. E-test susceptibility testing was performed in accordance with the manufacturer's instructions (AB BIODISK, Solna, Sweden) on Mueller-Hinton Agar plates (bioMérieux). Antimicrobial susceptibilities testing results for

Corresponding author
Stefano Grandesso
Clinical Microbiology Unit
Department of Clinical Pathology
Ospedale dell'Angelo
Via Paccagnella, 11 - 30174 Mestre, Italy
E-mail: stefano.grandesso@ulss12.ve.it

TABLE 1 - *Susceptibilities of 77 clinical isolates of Pseudomonas aeruginosa according to CLSI 2011, calculated for 4 methods.*

		E-test (%)	*Kirby-Bauer (%)*	*Sensititre (%)*	*VITEK 2 (%)*
ATM	S	71.4	74.0	71.4	57.1
	I	11.7	9.1	11.7	19.5
	R	16.9	16.9	16.9	23.4
CAZ	S	68.8	87.0	64.9	72.7
	I	16.9	1.3	16.9	13.0
	R	14.3	11.7	18.2	14.3
IMI	S	63.6	84.4	79.2	83.1
	I	20.8	/	3.9	9.1
	R	15.6	15.6	16.9	7.8
MEM	S	79.2	81.8	83.1	88.3
	I	1.3	2.6	5.2	1.3
	R	19.5	15.6	11.7	10.4
TZP	S	84.4	84.4	89.6	93.5
	R	15.6	15.6	10.4	6.5

S = susceptible, I = intermediate, R = resistant; ATM = aztreonam, CAZ = ceftazidime, IMI = imipenem, MEM = meropenem, TZP = piperacillin+tazobactam.

Susceptibility Categories according to criteria CLSI 2011 are shown in Table 1. For the E-test method, piperacillin+tazobactam had major activity with a susceptibility of 84.4%, followed by meropenem (79.2%), aztreonam (71.4%) and ceftazidime (68.8%). Imipenem had the worst susceptibility (63.6%). The concordance of the susceptibility categories was calculated with three different methods: Kirby-Bauer, Sensititre and VITEK 2 compared with E-test, chosen as the reference method. The agar disk-diffusion method showed a good agreement with piperacillin+tazobactam, meropenem and aztreonam (SIR category values were 100%, 94.8% and 92.2% respectively). The SIR categories for ceftazidime and imipenem were 81.8% and 79.2% respectively. The calculated percentages for this agent were lower for each method whereas only the E-test reported a high intermediate susceptibility of 20.8%. The medium value calculated for all agents with Kirby-Bauer method was 89.6%, particularly for the results of imipenem testing. The Sensititre results were in agreement with E-test for piperacillin+tazobactam (94.8%), being slightly lower than the limit for aztreonam (89.6%), ceftazidime (88.3%), meropenem (85.7%), and imipenem (76.6%). The medium value calculated for all agents with Sensititre was 87.0%, resulting to be slightly lower than the disk-diffusion calculated result. The comparison of the automated system VITEK 2 SIR categories with E-test resulted in lower percentages: piperacillin+tazobactam 90.9%, meropenem 89.6%, ceftazidime 85.7%, aztreonam 77.9% and imipenem 70.1%. The medium value calculated for all agents with VITEK 2 was 82.8%, resulting to be the lowest for all three methods compared.

The automated methods and Kirby-Bauer results were compared with E-test results and different types of errors were revealed: very major error (VM), major error (MA) and minor error (MI). The results were considered acceptable when VM was ≤3%, MA+MI was <7% and categories concordance was <90% (Jorgensen 1993). The agar disk-diffusion as shown in Table 2 did not present very major errors (false susceptibility) with the exception of meropenem which still demonstrated an acceptable level (1.3%) with no major errors (false resistant) for any molecule, confirming the good correlation with E-test. Sensititre reported 5.2% VM errors for meropenem and for piperacillin+tazobactam and presented MA errors for ceftazidime (3.9%), meropenem (2.6%) and imipenem (1.3%), thus further disclosing many MI errors for imipenem (22.1%). VITEK 2 showed high percentages of VM errors, particularly for piperacillin+tazobactam (9.1%) and meropenem (7.8%); VITEK 2 presented acceptable MA errors for aztreonam and ceftazidime (1.3%) and numerous MI errors for aztreonam (20.8%) and imipenem (29.9%). It is important to remember that there is not an intermediate category for piperacillin+tazobactam, so therefore only a difference of ±1 dilution of MIC could be considered a "very major error". From the results reported in Table 2, we can deduce that the Kirby-Bauer demonstrated good performances. We found piperacillin+tazobactam to be the most critical antimicrobial agent for both automated systems, followed by meropenem and no imipenem as was expected from the data previously described. We analyzed MICs distribution calculated for all five agents and confirmed the most potent *in vitro* activity of meropenem *ver-*

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.

sus imipenem against Gram-negative microorganisms (MICs medium values was 0.5-1 µg/ml for meropenem against 1-2 µg/ml for imipenem). We noted many clustered concentrations for imipenem near the breakpoint of 4 µg/ml. Many concentrations for meropenem were distant from breakpoints with low minor errors reported; 4 strains with Sensititre and 6 with VITEK 2 were incorrectly reported as susceptible instead of resistant and MICs were 3-4 times lower dilutions than those reported by E-test for the isolated strains. The Kirby-Bauer and E-test results were compared and Pearson correlation coefficients (r values) were calculated for each antimicrobial agent. Four agents presented good correlation: aztreonam and meropenem (r=-0.93), ceftazidime (r=-0.92), piperacillin+tazobactam (r=-0.89). As expected, we found the lowest but still acceptable correlation value for imipenem (r=-0.81).

We did not use pre-selected bacterial strains on the basis of the MICs values to disclose more differences and errors among different systems as reported in previous studies, but chose them randomly from our routine work to monitor the methods' performance in our laboratory. For imipenem we found strains with MICs distributions near to the clinical breakpoint.

As can be seen in Table 1, only E-test imipenem demonstrated high intermediate susceptibility (20.8%). Different studies have disclosed more delicacy and degradability of imipenem than meropenem, emphasizing that attention must be given to storage of material. Imipenem is more unstable than meropenem and humidity could alter the quality of the strips thus modifying the results.

The results of our study confirm the difficulties encountered in testing beta-lactam susceptibility in automated systems, particularly with piperacillin+tazobactam (Gagliotti *et al.* 2011) and requires more attention to meropenem. During elaboration of results for only piperacillin+tazobactam in April 2011 all card bioMérieux users received a communication that confirmed the*" performance of TZP test degraded… because of no good correlation with broth-dilution reference method…"*. It could be very useful to have plates that test low dilutions (0.125-0.250 µg/ml) for meropenem.

In conclusion, this study has demonstrated a better accuracy of Sensititre compared with VITEK 2 for *Pseudomonas aeruginosa* susceptibility testing for 4 agents (aztreonam, ceftazidime, imipenem and piperacillin+tazobactam).

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