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A comparison of Neo-Sensitabs™ tablets and paper discs in disc diffusion antimicrobial susceptibility testing

Branislava Kocic and Snezana Mladenovic-Antic*

University of Nis School of Medicine, Institute for Public Health Nis, Serbia.

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A comparative evaluation of Neo-Sensiitabs™ (Rosco, Taastrup, Denmark) and BBL paper discs (Becton Dickinson, Cockeysville, MD) according to the CLSI Performance Standards for Antimicrobial Susceptibility Testing was done. A total of 303 clinical isolates were included: 60 *Enterobacteriaceae* (including ESBLs isolates), 63 *Pseudomonas aeruginosa*, 63 *Staphylococcus aureus*, 33 *Streptococcus pneumoniae,* 51 *Enterococcus faecalis* and 33 *Beta haemolytic streptococci.* Strains were tested using the disc diffusion method, including both discs and tablets. minimal inhibitory concentrations (MICs) were determined by E test (AB Biodisk, Sölna, Sweden). The results were analyzed by linear regression and Pearson's correlation coefficient. The Pearson correlation coefficient of the inhibition zone diameter, related to the discs and tablets, had a high value (0.824 to 0.998). Based on the CLSI categorization of antimicrobial susceptibility (S-I-R), agreement was found within the range of 78.79 to 100%. Overall percentage of 3.31% of minor error and 0.38% of major error was observed. All of the 12 major errors occurred when there was no intermediate category. The major errors were found within isolates with SR discrepancies, and minor errors (1,64/1,67% for Neosensitabs and BBL, respectively) within isolates with IS-IR discrepancies. Antibiotic tablet sensitivity for ESBL detection was 97.14% compared to paper discs. Neo-Sensitabs™ tablets showed a high inter-correlation with BBL paper discs which indicated the possibility of using tablets as an alternative to paper discs.

Key words: Disc diffusion, paper discs, Neo-Sensitabs™ tablets.

INTRODUCTION

The disc diffusion method, used for determining antimicrobial susceptibility, is rapidly being replaced by more sensitive and specific methods such as determining the minimal inhibitory concentrations with the help of the E-test or by using an automatized system. In routine work, nevertheless, the method is still necessary. Either paper discs or tablets can be used in this method. The dilemma of whether and to which extent antibiotic-impregnated tablets or paper discs influence the results of susceptibility when testing different types of bacterial isolates from various types of clinical material led us to carry out this study.

Clinical and Laboratory Standards Institute (CLSI) has published and updated zone diameter breakpoints every year and this protocol only accepts standard paper discs (6 mm diameter). Rosco diagnostics has standardized the zone sizes of Neo-Sensitabs™ to “CLSI potency”, to correlate with minimal inhibitory concentrations (MIC), so they should produce zone sizes equivalent to those of standard paper discs. Laboratories that use this protocol could start using them as an alternative to paper discs. Neo-Sensitabs are tablets manufactured by a process

*Corresponding author. E-mail: kocicb@yahoo.com.

using crystalline antimicrobials. The tablets can be stored at room temperature (up to 25°C) without degradation of the antimicrobial agent, no need for acclimatization to room temperature and no need for special storage or refrigerator facilities (Rosco Diagnostica, 2008). They have been used for over 35 years in the several European countries (Lauwers et al., 1991).

Data from several studies comparing Neo-Sensitabs and paper discs demonstrated a very good correlation between them. Rodríguez-Villalobos et al. (2012) demonstrated an excellent correlation between Neo-Sensitabs and Oxoid paper discs in a study including 175 Gram-negative isolates, *Enterobacteriaceae* (n0150) and non-fermenters (n025) (Rodríguez-Villalobos and Boeras, 2012), and Justesen et al. (1785) compared Neo-Sensitabs with Oxoid paper disks using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) on 351 clinical isolates which were included to cover a broad range of species, as well as resistance mechanisms, including Gram positive bacteria (Justesen et al., 1785).

Our aim was to compare Neo-Sensitabs with BBL paper disks in disc diffusion susceptibility testing, according to the recommendations of the CLSI.

MATERIALS AND METHODS

A total of 303 clinical isolates, including: the *Enterobacteriaceae* (60), *Escherichia coli* (29), *Klebsiella spp.* (17), *Enterobacter sp*. (12) and *Proteus mirabilis* (2), *Pseudomonas aeruginosa* (63), *Staphylococcus aureus* (63), *Streptococcus pneumoniae* (33), *Enterococcus faecalis* (51) and *Beta haemolytic streptococci* (33) were tested with both BBL paper discs and Neo-Sensitabs using the disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) recommendations, standard 2008. The minimum inhibitory concentration (MIC) was determined by E-test (AB Biodisc, Sölna, Sweden), according to the manufacturer's specifications. Seven quality control tests were included: (S. aureus ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *E. coli* ATCC35218, *P. aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619, and *Klebsiella pneumoniae* ATCC 700603). The ATCC strains were tested according to CLSI recommendations with the same antimicrobial agents as clinical isolates (Table 1). This was repeated with each testing of the clinical isolates from this study, including all the used tablets, discs and E tests. The test results of the control strains (the zone diameters with each antimicrobial agent - tablet and disc) were within CLSI control ranges.

A Neo-Sensitabs™ tablet (Rosco, Taastrup, Denmark) and a BBL paper disc (Becton Dickinson, Cockeysville, MD) of the same antimicrobial agent were placed on the same MH agar plate (Müeller Hinton II agar and Müeller Hinton II agar and 5% sheep blood -MH+B, Biomedics). A maximum of six tablets or discs were placed on each 9-cm MH agar plate. Each antimicrobial agent was tested in duplicate from one single 0.5 McFarland suspension. To allow comparison, zones around BBL disks with diameters below 9 mm were read as 9 mm. The inhibition zones were measured and expressed in milimeters. If the interpreted test results between the BBL disc and Neo-Sensitabs were categorized differently (SR, IS or IR discrepancy), according to the CLSI criteria, it was resolved with MIC testing (Etest, AB Biodisk)

The results were analyzed by 1) Pearson's correlation between two parameters: the degree of the inhibition zone correlation expressed in millimeters and the degree of agreement within the susceptibility category (S-I-R). The degree of agreement of the inhibition zones expressed in millimeters was presented by means of Pearson's correlation coefficient and the degree of the overall agreement was used to compare the degree of agreement within the susceptibility categories (S-I-R). The sample size was determined based on the frequency of occurrence of the difference between the inhibition zone larger than 4 mm in the pilot sample, and larger than 580 combinations isolate- antimicrobial drug - AMD (10 isolates of all the tested bacteria to all the recommended AMDs). The level of error was estimated at less than 5% and the power study of more than 80%. The data was analyzed by SSPS 8.0; 2) SR and IS-IR discrepancies between the zone diameters of discs and tablets according to the CLSI breakpoints; 3) in isolates which were categorized differently, the discrepancy was resolved with MIC testing. Very major errors, major errors and minor errors in the interpretation were defined as recommended in the CLSI document, M23-A2; 3) Scatterplots of the measured zone diameters and linear regression lines were constructed using Excel 2003.

RESULTS

A total of 3048 antimicrobial drug (AMD) - isolate combinations were analyzed: *Enterobacteriaceae* (60) to 14 AMD, *Pseudomonas aeruginosa* (63) to 10 AMD, *Staphylococcus aureus* (63) to 11 AMD, *Streptococcus pneumoniae* (33) to 8 AMD, *Enterococcus faecalis* (51) to 7 AMD and *Beta haemolytic streptococci* (33) of 8 AMD.

Correlation and the degree of agreement for the isolates of the *Enterobacteriaceae* family (n=60) (Table 2) showed that there were 25 SI-RI discrepancies in combinations of 840 bacteria - AMD. Following the retesting of the isolates which showed signs of dis-agreement (25 isolates) along with an E-test comparison, the overall percentage of MI errors was 2.97% (25 combinations); no VM or M errors were found. The E test was performed in 25 cases and categorization was correct in 13 cases with Neo-Sensitabs and in 12 cases with BBL discs.

A total of 35 isolates of the *Enterobacteriaceae* which were phenotypically confirmed to be ESBL producers were also tested. *Klebsiella spp.* was isolated in 17 specimens, followed by *Enterobacter spp.* in 12, *E. coli* in 4 and *Proteus mirabilis* in 2 specimens. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls respectively for ESBL production. All isolates were simultaneously tested using ceftazidime (30 µg) - ceftazidime/clavulanic acid (30/10 µg) and cefotaxime 30 µg (CTX30) - cefotaxime - clavulanate (30/10 µg) Neo-Sensitabs and BBL paper discs. Isolates showing a difference of >5 mm were interpreted as positive for ESBL production. This method was treated as standard for purpose of comparison.

Among 35 isolates, Neo-Sensitabs detected 34 ESBL-producing isolates, while when using paper discs, 35 isolates were detected to produce ESBLs. Antibiotic tablet sensitivity for ESBL detection was 97.14% compared to filter paper discs.

Table 1. Species, number of clinical isolates and all tested antimicrobial drugs.

Antimicrobial drugs (AMD)	*Enterobacteriaceae*	*Enteroccoccus faecalis*	*Streptococcus pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptoccoccus β haemolyticus*	Total
Amikacin (30µg)	60			63			123
Amoxicillin-Clavulanic acid (20/10 µg)	59						59
Ampicillin (10 µg)	60	51				33	144
Cefepime (30 µg)	60			63			123
Cefotaxime (30 µg)				63			63
Ceftazidime (30 µg)	60			63			123
Ceftriaxone (30 µg)	60					33	93
Cefuroxime (30 µg)	60						60
Ciprofloxacin (5 µg)	60	51		63	63		237
Erythromycin (15 µg)		51	33		63	33	180
Gentamicin (10 µg)	60			63	63		186
Chloramphenicol (30 µg)		51	33		63		147
Imipenem (10 µg)	60			63			123
Clindamycin (2 µg)			33		63	33	129
Meropenem (10 µg)	60			63			123
Ofloxacin (5 µg)	60		33	63	63	33	252
Oxacillin (1 µg)			33		63		96
Penicillin (10 units)		51			63	33	147
Piperacillin-Tazobactam (100/10 µg)	60			63			123
Tetracycline (30 µg)		51	33		63	33	180
Trimethoprim-sulfametoxazole (1.25/23.75 µg)	60		33		63		156
Vankomycin (30 µg)		51	33		63	33	180
							3048

The correlation and the degree of agreement for the isolates of the *Staphylococcus aureus* (n=60), (Table 3) showed that there was 1 SR and 16 SI-RI discrepancies in combinations of 660 bacteria - AMD. Following the retesting of the isolates, which indicated disagreement (17 isolates) along with an E-test comparison, the total number of MI errors was 0.24% (16 combinations), for both the paper discs and tablets, and of M errors was 0.01% (only in the test involving penicllin-1 combination) while no VM errors were found. The E-test was performed in 17 cases and categorization was correct in nine cases with Neo-Sensitabs and in eight cases with BBL discs.

The correlation and degree of agreement for the *Streptococcus pneumoniae* isolates (n=33) (Table 4). All 33 isolates of *S. pneumoniae* were tested by means of oxacillin disc diffusion and penicillin and ceftriaxone E-test, for all of the isolates. There was disagreement between the disc and the tablet methods in five isolates, where we found a discrepancy of only 1 ml with oxacillin, where Neo-sensitabs had 20 mm and the BBL disc 19 mm.

The degree of agreement of the percentage of isolates susceptible to oxacillin with the percentage of isolates susceptible to penicillin and ceftriaxone was 100% (all of the isolates susceptible to oxacillin were also confirmed to be susceptible to penicillin and ceftriaxone by means

Table 2. Correlation and the degree of agreement for the isolates of the *Enterobacteriaceae* family (n=60).

Antibacterial drugs	Pearson correlation coefficient	Agreement (%)
		Based on the CLSI breakpoint categorization
Ampicillin	0.991	95.00
Amoxycillin + Clavulanate	0.966	83.33
Piperacillin+Tazobactam	0.811	98.33
Cefuroxime	0.960	86.67
Ceftriaxone	0.976	100.00
Ceftazidime	0.956	98.33
Cefepime	0.956	98.33
Imipenem	0.843	100.00
Meropenem	0.731	100.00
Gentamicin	0.974	98.33
Amikacin	0.928	90.00
Ciprofloxacin	0.985	96.67
Ofloxacin	0.983	98.33
Trimethoprim-sulfamethoxazole	0.994	100.00

Table 3. The correlation and the degree of agreement for the isolates of the *Staphylococcus aureus* (n=60).

Antibacterial drugs	Pearson correlation coefficient	Agreement (%)
		Based on the CLSI breakpoint categorization
Penicillin	0.981	93.65
Oxacillin	0.893	96.83
Erythromycin	0.975	84.13
Clindamycin	0.838	80.95
Ciprofloxacin	0.881	93.65
Ofloxacin	0.880	100.00
Tetracyclin	0.991	98.41
Chloramphenicol	0.859	98.41
Trimethoprim-sulfamethoxazole	0.936	100.00
Gentamicin	0.903	98.41
Vancomycin	0.824	100.00

Table 4. The correlation and degree of agreement for the *Streptococcus pneumoniae* isolates (n=33).

Antibacterial drugs	Pearson correlation coefficient	Agreement (%)
		Based on the CLSI *breakpoint* categorization
Oxacillin	0.995	84.85
Erythromycin	0.991	100.00
Clindamycin	0.992	100.00
Tetracyclin	0.993	90.91
Ofloxacin	0.808	100.00
Trimethoprim + Sulfamethoxazole	0.987	100.00
Chloramphenicol	0.905	96.97
Vancomycin	0.963	100.00

of the E-test). There were disagreements between the diffusion methods and E-test, and it was manifested among the isolates which were not susceptible to oxacillin, but following the results of the E-test were

Table 5. The correlation and degree of agreement for the *Enterococcus faecalis* isolates (n=51).

Antibacterial drugs	Pearson correlation coefficient	Agreement (%)
		Based on the CLSI *breakpoint* categorization
Penicillin	0.958	82.35
Ampicillin	0.899	88.24
Erythromycin	0.972	98.04
Tetracyclin	0.991	96.08
Ciprofloxacin	0.981	92.16
Chloramphenicol	0.971	84.31
Vancomycin	0.909	100.00

Table 6. The correlation and degree of agreement for the *Beta haemolytic streptococci* isolates (n=33).

Antibacterial drugs	Pearson correlation coefficient	Agreement (%)
		Based on the CLSI breakpoint categorization
Penicillin	0.921	93.94
Ampicillin	0.869	81.82
Ceftriaxone	0.856	78.79
Erythromycin	0.936	84.85
Clindamycin	0.959	87.88
Tetracyclin	0.979	96.97
Ofloxacin	0.772	100.00
Vancomycin	0.906	93.94

classified in the S category for penicillin and ceftriaxone. The fact that in the tests which involved oxacillin, only 10 (30.3%) of the isolates with Neosensitabs and 5(15.15%) isolates of BBL discs had an inhibition zone greater than 20 mm, and the results of the E-test indicated that 29 (88%) of them were susceptible, indicated the poor predictive ability of the test involving oxacillin for the beta lactam antibiotics with both discs and tablets.

There were 1 SR, 5 S-ND* (not defined without MIC) and 6 SI-RI discrepancies in combinations of 264 bacteria - AMD. Following the retesting of the isolates which indicated disagreement (12 isolates), along with an E-test comparison, the overall percentage of MI errors was 2.2% (6 combinations), and of M error, one was found (chloramphenicol) while no VM errors were found. The E-test was performed in 12 cases and categorization was correct in four cases with Neo-Sensitabs and in three cases with BBL discs for MI and M errors. The E-test confirmed susceptibility to penicillin and ceftriaxone in the remaining five cases, with 19 mm to oxacillin for BBL discs and 20 mm for Neo-Sensitabs (S-ND* discrepancy).

The correlation and degree of agreement for the *Enterococcus faecalis* isolates (n=51) (Table 5) showed that there were six SR and 11 SI-RI discrepancies in combinations of 357 bacteria - AMD. Following the retesting of the isolates which showed disagreement, along with an E-test comparison, the total number of MI errors was 3. 08% (11 combinations - categorization was correct in six cases for BBL, five for Neo-Sensitabs), and M errors - 6 (1.6%) combination and categorization was correct in six cases for Neo-Sensitabs, while no VM errors were found.

The correlation and degree of agreement for the *Beta haemolytic streptococci* isolates (n=33) **-** (Table 6). There was one SR and 20 SI-RI discrepancies in combinations of 264 bacteria - AMD. Following the retesting of the isolates which displayed disagreement, along with an E-test comparison, the total number of MI was 7.5%, (20 combinations). Categorization was correct in ten cases for Neo-Sensitabs and in ten cases for BBL disks), and M errors in one case -vancomycin, categorization was correct for Neo-Sensitabs.

All seven S-NS* (non-susceptible) discrepancies were related to testing susceptibility to beta lactam antibiotics. Here, the lack of I and R categories, a minor error which was considered a discrepancy, occurred in cases where the sensitive isolate on the E-test showed a zone below the range for the S category (≤ 24) with Neo-Sensitabs and BBL discs, when the isolates, according to protocol, could not be reported as sensitive. The E-test confirmed susceptibility to penicillin in all of these cases. Categorization was correct in five cases for Neo-Sensitabs and in two cases for BBL discs.

In addition, the test included five isolates of *Beta*

Table 7. The correlation and degree of agreement for the *Pseudomonas aeruginosa* isolates (n=63).

Antibacterial drugs	Pearson correlation coefficient	Agreement (%)
		Based on the CLSI breakpoint categorization
Piperacillin+Tazobactam	0.963	95.24
Ceftazidime	0.971	90.48
Cefepime	0.929	82.54
Imipenem	0.981	95.24
Meropenem	0.982	96.83
Gentamicin	0.990	100.00
Amikacin	0.986	92.06
Ciprofloxacin	0.998	100.00
Ofloxacin	0.998	100.00
Cefotaxime	0.989	92.06

Table 8. SR, S/NS and S/ND discrepancies between Neo-Sensitabs and BBL discs.

Isolate	Tablet/Disc	Neo-Sensitabs	BBL	Number of isolates	Comment difference in mm
Staphylococcus aureus	Penicillin (10 units)	R	S	1	2 mm, no intermediate category
Streptococcus pneumoniae	Oxacillin (1 µg)	S	ND*	5	1 mm, no intermediate category
	Chloramphenicol (30 µg)	R	S	1	3 mm, no intermediate category
Enterococcus faecalis	Penicillin (10 units)	R	S	3	1,3,2 mm, respectively, no intermediate category
	Ampicillin (10 µg)	R	S	3	1,1,2 mm, resp ectively, no intermediate category
Streptococcus β haemolyticus	Penicillin (10 units)	NS*	S	3	1 mm, no intermediate category
	Ampicillin (10 µg)	NS*	S	1	2 mm, no intermediate category
	Ampicillin (10 µg)	S	NS*	1	2 mm, no intermediate category
	Ceftriaxone (30 µg)	NS*	S	1	2 mm, no intermediate category
	Ceftriaxone (30 µg)	S	NS*	1	2 mm, no intermediate category
	Vancomycin 30 µg	R	S	1	1 mm, no intermediate category
Pseudomonas aeruginosa	Piperacillin-tazobactam 100/10µg	S	R	3	3,2,3 mm, respectively, no intermediate category

*NS, non-susceptibility, categorization only with MIC; *ND, not defined susceptibility against beta lactam antimicrobial agents without MIC.

haemolytic streptococci B which had an MIC greater than sensitive (intermediate isolate), and where none of them had a zone of inhibition ≥ 24 mm with both Neo-Sensitabs and BBL discs.

The correlation and degree of agreement for the *Pseudomonas aeruginosa* isolates (n=63) - (Table 7) showed that there were three SR and 23 SI-RI discrepancies in combinations of 630 bacteria - AMD. Following the retesting of the isolates which displayed disagreement, along with an E-test comparison, the overall percentage of MI errors was 3.65%. The E-test was performed in 23 IS-IR cases and categorization was correct in 11 cases for Neo-Sensitabs and in 12 cases for BBL discs. Also, the E-test was performed in 3 SR cases and categorization was correct in three cases for BBL (M errors).

Overall number of 12 of major error and 101 of minor error was observed in 3048 antimicrobial drug (AMD) - isolate combinations. SR, S/NS and S/ND discrepancies between Neo-Sensitabs and BBL discs showed in Table 8, IS - IR discrepancies between Neo-Sensitabs and BBL discs showed in Table 9.

All of the 12 major errors occurred when there was no intermediate category (Table 10).

Table 9. IS - IR discrepancies between Neo-Sensitabs and BBL discs.

Tablet/Disc	Isolate	Number of isolates	Average (mm)
Oxacillin (1 µg)	*Staphylococcus aureus*	1	2
Ampicillin (10 µg)	*Enterobacteriaceae*	3	2
Amoxycillin clavulanic acid	*Enterobacteriaceae*	7	2.28
Piperacillin-tazobactam (100/10 µg)	*Enterobacteriaceae*	1	3
Ceftriaxone (30 µg)	*Enterobacteriaceae*	1	1
Ceftazidime (30 µg)	*Pseudomonas aeruginosa*	6	3
Cefepime (30 µg)	*Pseudomonas aeruginosa*	10	2.7
Cefuroxime (30 µg)	*Enterobacteriaceae*	3	3
Meropenem (10 µg)	*Pseudomonas aeruginosa*	1	3
Tetracycline (30 µg)	*Staphylococcus aureus*	1	1.3
	Streptococcus β haemolyticus	1	1
	Streptococcus pneumoniae	3	1.7
	Enterococcus faecalis	3	1.3
Ciprofloksacin (5 µg)	*Staphylococcus aureus*	3	2.7
	Enterococcus faecalis	4	2
	Enterobacteriaceae	3	2
Ofloksacin (5 µg)	*Streptococcus β haemolyticus*	7	1.2
	Enterobacteriaceae	1	1
Gentamicin (10 µg)	*Enterobacteriaceae*	2	2
Amikacin (30 µg)	*Enterobacteriaceae*	4	1.5
	Pseudomonas aeruginosa	6	2
Erithromycin (15 µg)	*Staphylococcus aureus*	3	2
	Streptococcus pneumoniae	3	3
	Streptococcus β haemolyticus	6	2
	Enterococcus faecalis	1	1
Clindamycin (2 µg)	*Staphylococcus aureus*	7	3
	Streptococcus β haemolyticus	6	2
Chloramphenicol (30 µg)	*Staphylococcus aureus*	1	1
	Enterococcus faecalis	2	1.5
Vancomycin (30 µg)	*Enterococcus faecalis*	1	1

Table 10. Number of minor errors and major errors.

Isolate	No. of minor errors		No. of major errors	
	Neosensitabs	BBL	Neosensitabs	BBL
Enterobacteriaceae	13	12	0	0
Staphylococcus aureus	8	8	1	0
Streptococcus pneumoniae	3	3	1	0
Enterococcus faecalis	5	6	6	0
Beta haemolytic streptococci	10	10	1	0
Pseudomonas aeruginosa	11	12	0	3
	50	51	9	3
Overall number of combination of bacteria-AMD	3048	3048	3048	3048

Table 11. Percent of minor errors and major errors.

Combination of bacteria-AMD	Minor error		Major error	
	% of combination of bacteria-AMD		% of combination of bacteria-AMD	
	Neo-Sensitabs	BBL	Neo-Sensitabs	BBL
3048/100%	1.64%	1.67%	0.29%	0.09%

Overall percentage of 3.31% of minor error and 0.38% of major error was observed. The major errors were within isolates with SR discrepancies, and minor errors (1.64/1.67% for Neosensitabs and BBL, respectively) were within isolates with IS-IR discrepancies (Table 11)

DISCUSSION

The testing of the antimicrobial susceptibility of the Neo-Sensitabs™ tablets in everyday routine work was made possible following an adjustment to the concentration of the antimicrobial drugs and the inhibition zones of the Neo-Sensitabs™ tablets according to the CLSI criteria. Thus, within the same testing conditions it was possible to compare tablets to paper discs in the evaluation of the relevance of the results obtained by means of the disc diffusion method. The evaluation included: 1) the determination of the inhibition zones (in millimeters) for the discs and tablets; 2) the definition of the susceptibility categories (S-I-R); 3) the determination of the MIC: all combinations of bacteria/antimicrobial drugs which gave conflicting results, where one manufacturer's disc indicated the organism to be susceptible and another resistant or intermediate, were retested using the E-test (AB Biodisk, Solna, Sweden), following the manufacturer's instructions. Very major errors, major errors and minor errors in interpretation were defined using the results of the E-test as a reference method. Very major errors (false susceptibility) indicate that the isolates were susceptible by disc diffusion and resistant by the reference method; Major errors, resistant by the E-test method but susceptible by the reference test and minor error indicates that the isolates were intermediate by one method and resistant or susceptible by the other (Metzler and DeHaan, 1974). The statistical analysis encompassed the determination of 4) the Pearson correlation coefficient for the inhibition zones expressed in millimeters for the discs and tablets, as well as 5) the overall agreements of the susceptibility categories (S-I-R).

Most SR discrepancies were found in combinations of bacteria/AMD without intermediary categories (penicillin and staphylococci and enterococci, ampicillin and enterococcus, piperacillin-tazobactam and *Pseudomonas aeruginosa*. also, streptococci and vancomycin and chloramphenicol for *Streptococcus pneumoniae*). In those cases, a difference of only 1 ml indicates a different category. Also, when the susceptibility of the tested strains was near breakpoint value, only a 2 mm divergence in the zone sizes made the difference in interpretation. In the case of piperacillin-tazobactam, false resistance results might be a result of drug instability. All of the major errors in the testing were found in cases where there were no intermediary categories.

A great percentage of S/NS discrepancies were found in the tests involving the susceptibility of beta hemolytic streptococci to penicillin and cephalosporins (susceptibility was also defined in terms of susceptible/non-susceptible).

Studies evaluating various methods must ideally validate their test with broth microdilution (reference method). The concordance of the test with broth microdilution must be calculated with respect to the errors produced. The unacceptable levels are > 1.5% for very major errors, > 3% for major errors and 10% for minor errors, as recommended in the CLSI document, M23-A2. We did not test isolates with broth microdilution. In this study, the E-test was treated as the reference method. IS - IR discrepancies correlate with minor errors, and total percent of minor and major errors were within the acceptable ranges.

The testing of the susceptibility of staphylococcus to penicillin and clindamycin which indicated the occurrence of minor errors, gave similar interpretative results as in the studies of other authors (Skov et al., 2006), while in one case we did not define the category of susceptibility to oxacillin, because we could not confirm methicillin resistance with polymerase chain reaction (PCR). Recent studies also indicate the need for the confirmation of the resistance to methicillin and molecular methods as the most relevant ones (Antunes et al., 2007). The same data were obtained in the susceptibility testing of *Streptococcus pneumoniae* to beta lactam antibiotics with the help of oxacillin discs. Reduced susceptibility to penicillin has adequately been detected by means of the oxacillin impregnated discs - no penicillin intermediary or resistant isolates were noted which were susceptible to oxacillin. Contrary to this, there were many susceptible isolates which could not be detected by means of an oxacillin test, as it displayed a zone of less than 20mm. Our results with testing oxacillin in pneumococci found five cases with the use of discs, when a difference of 1mm did not allow the isolate to be defined as susceptible (Manninen et al., 1998).

Extended-spectrum ß lactamases (ESBLs) are increas-

ingly being detected in members of family Enterobacteriaceae. A total of 35 isolates of *Enterobacteriaceae,* phenotypically confirmed to be ESBL producers, were tested. The isolates were confirmed to be an ESBL producer by means of the double disc diffusion test (cefepime-cefepime clavulanate, ceftazidime-ceftazidime clavulanate, Neo-Sensitabs and BBL. (The Comparative study of antibiotic-impregnated discs and tablets. Pathology in practice nov 2008. Available from: http://www.pathologyinpractice.com/2008).

Antibiotic tablet sensitivity for ESBL detection was 97.14% compared to paper discs. Nayar et al. (2012) recently found that antibiotic tablet sensitivity for ESBL detection was 93.9% compared to filter paper discs (Nayar et al., 2012). Routine screening for these resistance mechanisms should be implemented in laboratories to control the spread of infections by these microorganisms. Sensitivity tablets as well as antibiotic impregnated discs can be used for this purpose.

In the categorization of susceptibility of the *Pseudomonas aeruginosa*, the results indicate a high degree of agreement in terms of the use of the discs and tablets. The Pearson correlation coefficient had a high value (from 0.929 to 0.998), as did the agreement within the susceptibility category (92.06 to 100.0), except in the case of cefepime (82.54). These results did not confirm the results obtained by other authors, where the complete agreement is 95.1% for cefepime and 97.5% for ceftazidime. Other authors have determined an insufficient precision in the detection of isolates that are intermediary to beta lactam antibiotics, independent of whether the disc or tablet was used (Stes et al., 1996). This indicates an insufficient reliability of testing by means of the disc diffusion method for the given strains of bacteria/fungi and the selected antimicrobials, irrespective of the type of carrier for the antibiotic - tablets or discs (Espinel-Ingroff and Canton 2008; Manninen et al., 2008; Rodriguez-Villalobos 2008). In this study, during the repeat testing of such isolates with the introduction of the E-test, we confirmed major errors within isolates with SR discrepancies, and at the same time, in an almost equal number of cases with IS-IR discrepancies (in relation to the E-test), we noted minor errors of both manufacturers (1.64/1.67% Neo-Sensitabs and BBL, respectively), which was interpreted as the insufficient precision of the disc diffusion method.

Many studies have provided support for the fact that the type of carrier of the antibiotic has no influence on the results of the testing by means of the disc diffusion method in the case of identical concentrations of the tested drugs. The difference in the zones is related solely to the carrier of the antibiotic and is related to technical difficulties: breakage of the tablet during application and the deformation of the obtained zone, and an incorrect adhesion of the paper disc during application by means of a dispenser (Kauppila et al., 2008). Disagreement within the zones (and inadequately reported categories) during routine work with paper discs is noted in the cases of inadequate transport and storage, due to the unreliability of the antimicrobial medication at room temperature.

Conclusion

The testing of antimicrobial susceptibility by means of the disc diffusion method using paper discs and Neo-Sensitabs™ tablets indicated a high inter-correlation, as well as reliability (in relation to the E-test). Disagreement in the inhibition zones and categories to a greater extent depended on the testing protocol - the absence of an I and R category, technical difficulties and accuracy of the disc diffusion method - than on the type of used tablets/discs. A high correlation between the tablets and discs indicated the possibility of using tablets as an alternative to paper discs.

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