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Susceptibility Testing and Resistance Phenotypes Detection in Bacterial Pathogens Using the VITEK 2 System

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A b s t r a c t

A set of well characterized strains, collected in Polish hospitals, including Gram-negative (n = 93) and Gram-positive (n = 90) isolates was used in the study. The VITEK 2 AST-cards were used in the analysis according to the manufacturer's recommendations. Comparison of the susceptibility data obtained by the standard method and by VITEK 2 cards proved concordant in 99% of cases. Clinically important mechanisms were revealed by the VITEK 2 AES with >95% agreement with reference data including methicillin resistance in staphylococci (98%), high-level aminoglycoside resistance in enterococci (100%), VanA and VanB phenotypes in enterococci (100%), and ESBLs in *Enterobacteriaceae* (93.8%). The VITEK 2 AES System appears a reliable tool for the detection and interpretive reading of clinically important mechanisms of resistance and can be recommended for routine work.

K e y w o r d s: bacterial pathogens, antimicrobial agents, susceptibility testing, resistance mechanisms, VITEK 2

Introduction

Increasing resistance in bacterial pathogens of high clinical relevance requires proper and rapid detection of the resistance phenotype. Dilution methods are the reference methods used in determining microorganisms susceptibility which allow the determination of the minimal inhibitory concentration of the drug (MIC) and thus the proper choice of antimicrobial agents for therapy. Following the emergence of many mechanisms of resistance special methods for their detection, allowing full expression of resistance mechanisms *in vitro*, are more often applied. Different phenotypic methods are used, supplemented by molecular biology techniques. However the main drawback in the majority phenotype methods used is the time required to obtain results.

For many years the automation of microbiological diagnostics has been developing dynamically. New automated microbiology systems create opportunities for quickly obtaining complete microbiological results. Changes in the spectrum of etiological agents and their susceptibility profiles demand the continous improvement of such systems and their adjustment to new epidemiological situations. The VITEK 2 System (bioMérieux, USA) belongs to the new generation of automated microbiology systems designed to identify bacterial isolates to the species level and to determine their drug susceptibility. The system provides different types of card used for identification tests and for drug susceptibility determination.

The aim of this study was to evaluate the VITEK 2 Automated System for antimicrobial susceptibility determination of the most important clinical pathogens and the Advanced Expert System (AES) for its interpretation of resistance mechanisms. The results of the analyses were compared with those obtained by reference microbiological methods.

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Experimental

Materials and Methods

Bacterial strains. A total of 183 clinically significant Gram-negative (n = 93) and Gram-positive (n = 90) bacterial pathogens were included in the study. Gram-negative organisms represented seven of the most commonly encountered species of the family *Enterobacteriaceae* (*Escherichia coli* n = 23, *Klebsiella pneumoniae* n = 18, *Klebsiella oxytoca* n = 3, *Proteus mirabilis* n = 2, *Serratia marcescens* n = 9, *Enterobacter cloacae* n = 13, *Citrobacter freundii* n = 5) and two species of nonfermenting rods (*Acinetobacter baumannii* n = 10, *Pseudomonas aeruginosa* n = 10). Gram-positive isolates belonged to four *Staphylococcus* species (*S. aureus* n = 42, *S. epidermidis* n = 4, *S. haemolyticus* n = 3, *S. hominis* n = 1), two species of *Enterococcus* (*E. faecalis* n = 10, *E. faecium* n = 10) and *Streptococcus pneumoniae* (n = 20). The isolates were recovered from specimens collected from patients hospitalised in different medical centers in Poland from 1996 to 2002. All strains were collected by the National Institute of Public Health. They were not epidemiologically related and represented one isolate per patient. A wide variety of clinically important antimicrobial resistance mechanisms were represented by the isolates, including extended-spectrum β-lactamases (ESBLs) in *Enterobacteriaceae* (48 isolates), methicillin resistance in staphylococci (37 isolates), decreased pneumococcal susceptibility to penicillin (8 isolates) and resistance to vancomycin and to high concentrations of aminoglycosides in enterococci (8 and 20 isolates, respectively).

E. coli ATCC 25922, *E. coli* ATCC 35218, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *K. pneumoniae* ATCC 700603 *P. aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 and *S. aureus* MR3 from the collection of the National Insitute of Public Health in Warsaw were used as reference strains.

Identification methods. Gram-negative isolates were identified to the species level by API 20 E (*Enterobacteriaceae*) or API 20 NE (non-fermenters) biochemical tests (bioMérieux, Marcy l'Etoile, France). *S. aureus* was identified by coagulase production (bound and free), supplemented by DN-ase test, and coagulase-negative staphylococci were speciated by the API Staph test (bioMérieux, Charbonnieres-les-Bains, France). Identification of the enterococcal isolates was performed according to the method of Facklam and Collins (Facklam and Collins, 1989) and by the API 20 Strep test (bioMérieux, Charbonnieres-les-Bains, France), supplemented by potassium tellurite reduction, motility, and pigment production tests (Facklam and Collins, 1989). *S. pneumoniae* was identified using standard microbiology techniques including agar morphology, optochin test and bile solubility test (Ruoff *et al*., 1999).

MIC evaluation. MICs of various antimicrobials were evaluated by agar or broth dilution methods in accordance with the Clinical and Laboratory Standards Institute (CLSI/ NCCLS – National Committee for Clinical Laboratory Standards) guidelines (NCCLS, 2003a.; CLSI, 2005). The same antimicrobial agents were tested as those that were present in the VITEK 2 cards used in the study, and specific sets of agents were selected for each of the species analysed. The following antimicrobials were included in the study: penicillin, cefotaxime, streptomycin and gentamicin (Polfa Tarchomin, Poland); amoxicillin, clavulanic acid and oxacillin and ceftazidime (Glaxo SmithKline, UK); piperacillin, and tazobactam (Wyeth, USA); cefepime and amikacin (Bristol-Myers Squibb, USA); meropenem (Zeneca, UK), ciprofloxacin (Bayer, Germany); vancomycin (Eli Lilly, USA); teicoplanin (Marion Merrell, UK). In β-lactam-inhibitor combinations, concentrations used were in accordance with the CLSI (CLSI, 2005).

Detection of methicillin resistance. Methicillin resistance was detected in staphylococcal isolates by two methods recommended by the CLSI, using a 30mg cefoxitine disc and the agar screening method (CLSI, 2005; NCCLSb., 2003). The screening method was used only for *S. aureus* isolates. Results of the analysis were confirmed by the PCR amplification of the *mecA* gene in all methicillin-resistant isolates as described previously (Murakami *et al*., 1991).

Detection of the high-level aminoglycoside resistance (HLAR) and vancomycin resistance in *Enterococcus* spp. Enterococcal isolates were tested for the presence of the HLAR phenotype using the agar screening method as described by CLSI (CLSI, 2005; NCCLSb., 2003). Vancomycin-resistant enterococci (VRE) were identified by the CLSI agar screening procedure (NCCLS, 2003a.; CLSI, 2005) and positive results of the test were confirmed by PCR detection of *vanA* or *vanB* genes as described previously (Dutka-Malen *et al.*, 1995; Dahl *et al.*, 1999).

Detection of penicillin resistance in *S. pneumoniae.* Penicillin-resistant *S. pneumoniae* strains were identified by two methods recommended by CLSI: screening method with the 1mg oxacillin disc and the reference broth dilution method (NCCLS, 2003a.; CLSI, 2005).

ESBL detection. ESBL activity was detected in *Enterobacteriaceae* isolates by the double disc synergy (DDS) test (Jarlier *et al.,* 1988) with discs containing cefotaxime, ceftazidime and amoxicillin/clavulanate. The 48 DDS test-positive isolates included 15 *Klebsiella pneumoniae*, 2 *Klebsiella oxytoca*, 17 *Escherichia coli*, 5 *Serratia marcescens*, 7 *Enterobacter cloacae* and 2 *Citrobacter freundii* isolates. These were recovered in 7 different Polish hospitals in 1996–98, and were confirmed to be ESBL producers by biochemical and molecular methods (isoelectric focusing, bioassay, PCR and sequencing) published before (Pałucha *et al.*, 1999; Fiett *et al.*, 2000).

VITEK 2 tests. Analysis was conducted with the use of antibiogram cards VITEK 2 (for Gram-positive cocci: AST-524, -526 and -GP56; for Gram-negative rods: AST-N019, -N020, -N021). The tests are in the form of small, waterproof cards with 64 wells containing antibacterial drugs (antibiogram tests) in different dilutions suitable for testing microorganisms according to the CLSI recommendations. Tests were performed according to the manufacturer's instruction.

Evaluated parameters. Characteristic parameters of the automatic method of drug susceptibility determination were defined: total concordance of resistance categories (CA), accuracy, sensitivity, specificity and type of errors (mE, ME, VME). Minimal error – mE was recognised when one of the methods – reference or evaluated (in this study – automatic method) identified the tested strain as "intermediate", where the other method classified it as "resistant" or "sensitive". Major error – ME – reference method – "sensitive" and automatic method – "resistant". "Very major error" VME – reference method – "resistant", whereas automatic method – "sensitive". Additional parameters were also evaluated such as time taken to obtain results, functionality and level of difficulty in using the system.

Results

MIC values and interpretation. A high correlation was found between the results (n = 1882 susceptibility tests) obtained by the aid of VITEK 2 and by the dilution reference method. The total accordance of resistance interpretation was 99%. For the species of *Enterobacteriaceae* family CA was 98.8%. Single minor errors (mE) in MIC values were observed for gentamicin, amikacin and piperacillin. One major error (ME) appeared when testing *E. cloacae* susceptibility to piperacillin with tazobactam.

In the group of nonfermenting rods CA obtained was 96.4% for *A. baumannii* (4 mE), and 99.2% for *P. aeruginosa* (1 mE). The interpretation of the carbapenems MIC value for nonfermenting rods was in 100% consistent with the interpretation of the MIC value defined with the reference methods.

Susceptibility tests for Gram-positive cocci exhibited an accordance variation from 97.8% for pneumococci, 98% and 99% respectively for *E. faecium* and *E. faecalis*, to 99.5% for staphylococci. In this group of microorganisms one very major error VME (for *S. aureus* and oxacillin) was noted. Comparative analysis of the interpretation of glycopeptide MICs (vancomycin and teicoplanin) obtained for enterococci, and penicillin MIC values for pneumococci, showed one minor error in both cases. All types of errors encountered in the respective species are presented in Table I.

Table I
Discrepanciens between the VITEK 2 results and reference methods results

Species [No of isolates with errors/no of all isolates]	Antimicrobial agent / No and kind of errors	Reference method MIC [mg/l] / category of susceptibility		VITEK 2 MIC [mg/l] / category of susceptibility	
S. pneumoniae [n = 3/20]	Penicillin / 1 × mE	1	I	≥ 2	R
	Ceftriaxon / 2 × mE	2	I	≥ 4	R
	Cefotaxime / 1 × mE	0.5	S	1	I
S. aureus [n = 1/50]	Oxacillin /1×VME	64	R	2	S
E. faecalis [n = 1/10]	Ciprofloxacin /1 × mE	2	I	≤ 0.5	S
E. faecium [n = 2/10]	Ciprofloxacin / 1 × mE	4	R	2	I
	Teicoplanin / 1 × mE	32	R	16	I
E. coli [n = 3/23]	Piperacillin / 1 × mE	256	R	64	I
	Gentamicin / 1 × mE	32	R	8	I
	Amikacin / 2 × mE	64	R	32	I
		16	S	32	I
K. pneumoniae [n = 3/18]	Gentamicin / 1 × mE	8	I	≥ 16	R
	Amikacin / 2 × mE	32	I	16	S
		16	S	32	I
K. oxytoca [n = 1/3]	Gentamicin / 1 × mE	8	I	≥ 16	R
E. cloacae [n = 2/13]	Piperacillin/Tazobactam / 1 × ME	2	S	≥ 128	R
	Gentamicin / 1 × mE	4	S	8	I
S. marcescens [n = 2/9]	Gentamicin / 1 × mE	8	I	≥ 16	R
	Cefoxitin / 1 × mE	16	I	32	R
A. baumannii [n = 3/10]	Piperacillin/Tazobactam / 1 × mE	128	16	64	I
	Cefotaxim / 1 × mE	32	R	≥ 64	R
	Cefepime / 2 × mE	16	I	32	R
		16	I	8	S
P. aeruginosa [n = 2/10]	Cefepime / 1 × mE	16	I	8	S

mE – minor error; ME – major error; VME – very major error; S – sensitive; I – intermediate; R – resistant

Resistance phenotype detection and interpretation. ESBLs detection was not achieved by the VITEK 2 system in 3 isolates only. Two of these were *E. cloacae* and one *C. freundii*. The strains of these species produce cefalosporinase that is AmpC specific for those individual species, which when produced at a high level can mask the ESBL phenotype. In general good results were obtained, with 93.8% sensitivity, 95.9% accuracy and 100% specificity of detection of ESBL production by *Enterobacteriaceae* strains (for *E. coli*, *K. pneumoniae* and *K. oxytoca* – all parameters reached the level of 100%).

In the case of staphylococci 97.3% sensitivity, 98% accuracy and 100% specificity in the detection of MRS strains (methicillin resistant *Staphylococcus*) was achieved. One isolate of *S. aureus* characterised

by the presence of *mecA* gene showed an MIC value for oxacillin of 64 mg/l by the reference method and 2 mg/l by the VITEK 2 system. For enterococci, 100% accuracy, sensitivity and specificity was achieved for HLAR and VRE by VITEK 2 detection. The difference between teicoplanin MIC values described above had no influence on the susceptibility category.

Detection of the resistance phenotype by the VITEK 2 system seldom resulted in a change of clinical interpretation of the sensitivity results for the respective drugs. High agreement between the results obtained with the automatic method and reference methods was achieved, as shown in Table II.

Table II
Characteristic features of VITEK 2 method in detection of selected resistance mechanisms

	MRSA	HLAR	VRE	ESBL (all species)	ESBL (*E. cloacae*, *K. pneumoniae*, *K. oxytoca*)
Reference methods positive / VITEK 2 positive	36	20	8	45	35
Reference methods negative / VITEK 2 negative	13	0	12	25	9
Reference methods positive / VITEK 2 negative	1	0	0	3	0
Reference methods negative / VITEK 2 positive	0	0	0	0	0
Accuracy (%)	98	100	100	95.9	100
Sensitivity (%)	97.3	100	100	93.8	100
Specificity (%)	100	100	100	100	100

Times taken to obtain results by VITEK 2. The average time for results to be obtained using the VITEK 2 automatic system was 8 hours from the installation of the antibiogram cards. For Gram-positive cocci it required 9 hours and for Gram-negative rods – 7 hrs 45 minutes. The shortest time recorded was for *Klebsiella pneumoniae* (5.5 hours) and the longest – up to 17 hours for coagulase-negative staphylococci.

Discussion

The usefulness of microbiological diagnostics for patient care has been limited by the time taken for patient specimen processing and obtaining results. Thus a shortening and an improvement of this process is urgently needed in order to introduce etiologic agent specific therapy as early as possible. In this study VITEK 2, the new system offered by bioMérieux, was evaluated in regard to its usefulness for antibiotic susceptibility testing, one of the most important steps in routine diagnostic microbiology. The great majority of isolates tested in this study, representing the most clinically relevant bacterial species, showed a high concordance of the results with the reference methods. However, they were obtained in a much shorter time, on average in 8 hours. This compared with 16 to 24 hrs needed for the reference methods. Although some molecular techniques are able to detect resistance genes in less than 4 hrs they have several limitations. They can only detect mechanisms of resistance that are already known and need to use several primers since more and more bacterial pathogens show multiple drug resistance. Favourable results when testing the VITEK 2 system have been also obtained by several other workers. Aissa and Horstkotte reported the high sensitivity and specificity of the system in the detection of methicillin resistance in staphylococcal strains as well as in *S. aureus* and coagulase-negative staphylococci (Aissa *et al.*, 2004; Horstkotte *et al*., 2002). All strains but one (MRSA) were properly identified in respect to methicillin resistance. Ligozzi also obtained good agreement between results obtained by the reference methods and the VITEK 2 system, ranging from 90–100% for staphylococci, pneumococci and enterococci with an average of 96% (Ligozzi *et al*., 2002). In our study even higher concordance was obtained, 97.8% for pneumococci, 98–99% for enterococci and 99.5% for staphylococci.

The results published by Blondell-Hill demonstrated a high agreement of 96.2% between the VITEK 2 system and reference method in the interpretation of antimicrobial susceptibility for 300 isolates of the *Enterobacteriacae* family (Blondell-Hill *et al.*, 2003). Similar results were obtained in this study, with an even higher agreement of 98.8% achieved between the two methods. It should be stressed that in the case of the isolates of the *Enterobacteriacae* family, not even a single VME was encountered, and only one ME was reported for piperacillin/tazobactam combinations (*E. cloacae*). Similar results for *E. cloacae* and

piperacillin/tazobactam susceptibility were reported by Karlovsky on a very large number of isolates (Karlovsky *et al.*, 2003).

An important function of VITEK 2 is the interpretation of susceptibility data through the AES in reference to a major resistance mechanism. In our study, when ESBLs producing strains were tested, the mechanism was not identified in only 3 strains; all 3 strains belonged to AmpC producing species, with two representing *E. cloacae* and one representing *C. freundii*. The same problem was encountered in our previous study with BD Phoenix which was unable to detect ESBL in two strains of AmpC producing species (*E. cloacae*) (Stefaniuk *et al*, 2003). It seems that the presence of AmpC in those isolates makes detection of ESBL by AES impossible. Usually, ESBLs production is easily detectable in *E. coli* and *Klebsiella* spp. (Stefaniuk *et al*, 2003, Livermore, 1995, Leverstein-van Hall *et al.*, 2002). However, Levenstein-van Hall comparing several methods of ESBL detection, such as E-test ESBL, BD Phoenix, Vitek 1 and Vitek 2, in 74 isolates of *E. coli* and *K. pneumoniae,* found an accuracy detection rate of 78% for the Vitek 2 (Leverstein-van Hall *et al.*, 2002). This is in contrast to our study, which showed an excellent result of 100% ESBLs detection. The discrepancy between those two reports may result from the different bacterial populations under study, reflecting the complexity and diversity of these enzymes.

The shorter time involved in getting susceptibility results, as compared to conventional methods, and their good correlation with those methods make the VITEK 2 automated microbiological system a useful device in clinical microbiological laboratory work.

In addition, the VITEK 2 automated microbiological system is an aesthetically pleasing and user-friendly device. It should be operated by a qualified member the microbiological laboratory staff who has been trained by an experienced microbiologist. Accurate *in vitro* susceptibility testing methods are important for optional patient therapy, particularly for hospitalized patients, and for epidemiology study. As far as sensitivity, accuracy and specificity are concerned, antibiogram tests of the VITEK 2 system are comparable to other reference methods used to detect particular resistance mechanisms of clinically important bacterial strains.

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