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Multicentre evaluation of the VITEK 2 Advanced Expert System for interpretive reading of antimicrobial resistance tests

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Interpretive reading analyses the complete resistance profiles of bacteria to multiple antibiotics and infers the resistance mechanisms present; it aids therapeutic choice and enhances surveillance data. We evaluated the Advanced Expert System (AES), which interprets MICs generated by the VITEK 2. Ten European laboratories tested 42 reference strains and 76–106 of their own strains, representing important resistance genotypes. Interpretive reading by the VITEK 2 AES achieved full agreement with genotype data for 88–89% of strains, with the correct mechanism identified as one of two possibilities for a further 5–6%. Mechanisms inferred with ≥90% agreement with reference data included methicillin resistance in staphylococci, glycopeptide resistance in enterococci, quinolone resistance in staphylococci and Enterobacteriaceae, AAC(6′)-APH(2″)-mediated aminoglycoside resistance in Gram-positive cocci, *erm*-mediated macrolide resistance in pneumococci, extended-spectrum β-lactamases (ESBLs) in Enterobacteriaceae and *Pseudomonas aeruginosa*, and acquired penicillinases in Enterobacteriaceae. VanA, VanB and VanC phenotypes of enterococci were distinguished reliably, and ESBL production was accurately inferred in AmpC-inducible species as well as *Escherichia coli* and *Klebsiella* spp. Mechanisms identified, but only as possibilities among several, included IRT-type β-lactamases and individual aminoglycoside-modifying enzymes in Enterobacteriaceae. Most disagreements with reference data concerned pneumococci found to have high-level penicillin resistance by the VITEK 2 AES but previously determined, phenotypically, to have intermediate resistance. When ESBL production was inferred in *E. coli* and klebsiellae, the VITEK 2 AES edited susceptible results for cephalosporins (except cefoxitin) to resistant; when an acquired penicillinase was inferred in Enterobacteriaceae, piperacillin results were edited to resistant; and when staphylococci were found methicillin resistant, resistance was reported for all β-lactams. Further editing may be desirable (e.g. of cephalosporin results for salmonellas inferred to have ESBLs).

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Introduction

Laboratories routinely record the results of susceptibility tests as if bacterial susceptibility or resistance to any one antimicrobial was independent of all other susceptibilities and resistances. It has been argued, particularly by both Courvalin and Livermore that this premise is irrational, given that resistances to multiple related agents often depend on single mechanisms.[1–3] This criticism is more than academic: recording susceptibility results individually, rather than as patterns, wastes data that are potentially valuable for both surveillance and patient care. If, instead, the patterns of resistance to panels of related antimicrobials are considered, then the underlying mechanisms of resistance can often be inferred. Such a strategy allows identification of further antimicrobial agents that merit testing, for example, those β-lactams known to be stable to whichever β-lactamase is inferred to be produced. Secondly, the approach allows unlikely combinations of resistance phenotype and species to be recognized, so that the isolate can be retested or sent to a reference laboratory. Thirdly, individual results that are dubious in the context of the inferred mechanism can be identified [e.g. susceptibility to cefotaxime in an isolate inferred to have an extended-spectrum β-lactamase (ESBL)], and the clinician can be advised to use alternative treatments.

Reviewing resistance patterns rather than individual results was dubbed 'interpretive reading' by Courvalin,[1] and is followed most widely in France. It is followed in a rudimentary form elsewhere, for example in reporting oxacillin-resistant staphylococci as resistant to all β-lactams on the inference that they have *mecA*, and reporting ceftazidime- or cefpodoxime-resistant klebsiellae as resistant to other cephalosporins, on the inference that they have ESBLs.[3,4] Wider adoption has been constrained by the need for laboratory staff to be familiar with an increasing array of different mechanisms and phenotypes, but this problem can be overcome by using a computerized expert system to review the susceptibility data. We describe here an international evaluation of the Advanced Expert System (AES) of the VITEK 2 (bioMérieux, Marcy l'Étoile, France). The VITEK 2 is an automated susceptibility testing system enabling rapid (4–7 h) determination of MICs by the analysis of growth kinetics of bacteria with antibiotics in test cards.[5–7] The AES provides standardized interpretive reading of these MICs.[8,9] Briefly, it comprises a database of MIC distribution for different combinations of antibiotics and prevalent resistance mechanisms in different species, together with a series of algorithms. The MIC phenotype found for the isolate by the VITEK 2 is compared with all the patterns in the database and the best match is identified. Unlikely combinations of phenotype and species are highlighted and the user alerted; likewise the user is alerted when the inferred mechanism predicts clinical resistance to drugs to which the bacteria appeared susceptible at breakpoint. For example, the MICs found for an *Escherichia coli* isolate might be found to be: ampicillin 1 mg/L; cefalothin 0.5 mg/L; cefoxitin 2 mg/L; cefotaxime 0.125 mg/L; and ceftazidime 0.5 mg/L. All these values are compatible with a wild phenotype without significant β-lactamase activity, none is compatible with an AmpC-hyperproducing phenotype, and only the cefotaxime MIC is potentially compatible with an ESBL-producing phenotype (since ESBL production does not consistently cause obvious cefotaxime resistance). The isolate is consequently inferred to lack acquired resistance, this phenotype being the best match to *all* the data. For another *E. coli* isolate, the recorded MICs might be: ampicillin 128 mg/L; cefalothin 32 mg/L; cefoxitin 4 mg/L; cefotaxime 0.25 mg/L; and ceftazidime 32 mg/L. In this case, only the cefotaxime value is compatible with a wild phenotype, and only the ampicillin, cefalothin and ceftazidime MICs are compatible with an AmpC-hyperproducing phenotype, whereas all the results are compatible with ESBL production. ESBL production is therefore inferred and, based on this inference, the VITEK 2 AES recommends editing of the cefotaxime result to 'resistant', despite the low MIC. In all cases the VITEK 2 AES prints a report indicating the actual MIC, raw categorizations, and the categorizations after interpretation. Reasons for any editing are stated, allowing review.

Materials and methods

Strains and evaluation strategy

VITEK 2 systems were installed at 10 European laboratories by bioMérieux technicians (Table 1). Each laboratory was asked to test the same set of 50 reference strains distributed by bioMérieux (LBM strains), together with 100 distinct strains with known resistance mechanisms from their own collections (evaluators' strains). None of the LBM strains had been used for the initial development of the VITEK 2 AES. The evaluators were informed of the species, but not of the genotypes or anticipated resistance phenotypes of the LBM strains. The evaluators' strains were chosen as non-replicate organisms to represent resistance types that present significant clinical problems worldwide (Table 1). The strains were selected as having known resistance genotypes, except that penicillin-resistant pneumococci were accepted on the basis of phenotype, as were strains with hyperproduction of AmpC chromosomal β-lactamases and some ESBL producers (Table 1).

Confirmation of key resistances

Etests (AB Biodisk, Solna, Sweden) were used to confirm retention of key resistances. These tests, performed in accordance with the manufacturer's directions, were run in parallel with MIC determinations using the VITEK 2. Which Etest was used was dependent on the reason for the inclusion of a particular isolate in the study: enterococci

Table 1. Resistance mechanisms of isolates tested at the evaluation sites

Resistance mechanisms	No. strains tested											
	Oporto	Madrid	Lausanne	Bochum	London	Linz	Genoa	Brussels	Leuven	Utrecht	LBM strains[a]	total
Aminoglycoside-resistant enterobacteria	10[b]				5[b]					18[c]	5[b]	38
Aminoglycoside-resistant staphylococci	4[d]							11[d]	9[d]			24
Enterococci with high-level aminoglycoside resistance					3[e]	16[e]		25[e]				44
Enterobacteriaceae with known β-lactamases	24[f]	55[g]	24[f]	63[g]	41[h]				20[f]	18[g]	12[f,g]	257
P. aeruginosa with known β-lactamases					10[i]						2[f,g]	12
Macrolide-resistant *S. pneumoniae*							40[j]		25[j]			65
Oxacillin-resistant staphylococci	20[k]	15[k]		33[k]		63[k]	20[k]	30[k]	25[k]		7[k]	213
Penicillin-resistant *S. pneumoniae*	19[f]	13[f]	44[f]		17[f]		30[f]		17[f]		8[f]	148
Quinolone-resistant Enterobacteriaceae							10[l]	10[m]		24[n]		44
Quinolone-resistant staphylococci								13[o]				13
Vancomycin-resistant enterococci	18[p]	14[p]	18[p]		30[p]			1[p]		16[p]	8[p]	105
Total	95	97	86	96	106	79	100	90	96	76	42	963[q]

[a]The LBM strains were tested at each of the 10 evaluation sites.

Methods used to identify the resistance mechanisms present in the test strains: [b]PCR for *aac*(3)-I, *aac*(3)-II, *ant*(4′)(4″), *aac*(6′)-Ib, *aac*(6′)-Ic; [c] PCR for *ant*(2″)-Ia, *ant* (3″)-I, *aac*(3′)-III, *aac*(3′)-IV, *aac*(6′)-I, *aph*(3′)-I, *aph*(3′)-II; [d]PCR for *aac*A–*aph*D, *aph*A3 and *aad*C; [e]PCR for *aac*A–*aph*D; [f]phenotype; [g]isoelectric focusing, PCR and sequencing; [h]isolectric focusing, PCR single strand conformational polymorphism of bla_{SHV} and PCR RFLP of bla_{TEM}; [i]gene sequencing; [j]PCR detection of *ermB* and/or *mefAE*; [k]PCR of *mec*A; [l]PCR for *gyr*A and *gyr*B and complementation with plasmids pBP 517 and pBP 548; [m]PCR for *gyr*A RFLP analysis after restriction with *Hin*fI ; [n]PCR and sequence and analysis of *gyr*A and *par*C;[o]PCR of *gyr*A and *gyr*1A (*par*C) and RFLP analysis after restriction with *Hin*fI ; [p]PCR of *van*A and *van*B.

[q]Comprising 921 evaluators' strains and 42 LBM strains.

selected for glycopeptide resistance were tested with teicoplanin and vancomycin; those selected for high-level resistance to aminoglycosides were tested with high-content gentamicin Etests. Staphylococci chosen as methicillin resistant were tested with oxacillin; those selected as aminoglycoside resistant were tested with gentamicin and tobramycin. Pneumococci were tested with penicillin and ceftriaxone. Enterobacteriaceae selected for resistance to β-lactams were tested with amoxicillin, co-amoxiclav, cefalothin, cefotaxime and ceftazidime; those selected for resistance to aminoglycosides were tested with amikacin, gentamicin, netilmicin and tobramycin. *Pseudomonas aeruginosa* strains were tested with ticarcillin and ceftazidime.

Susceptibility tests with the VITEK 2

Strains were subcultured twice, then grown for 18–24 h at 35°C on Columbia agar containing 5% sheep blood; streptococci were grown with 5% CO_2, other bacteria were grown in air. Suspensions of these cultures were made in 0.45% saline, adjusted to the turbidity of a 0.5 McFarland standard, and used to load the test cards for the VITEK 2, which was used in accordance with the manufacturer's directions (bioMérieux). The drugs contained in the antibiotic susceptibility test cards are listed in Table 2. Each agent was included at two to four different concentrations. The function of the VITEK 2 has been described in detail elsewhere.[6,7,9] Briefly, for each antibiotic-containing test well, a turbidity signal is automatically measured every 15 min for up to 18 h. These data are used to generate growth curves and, by comparison with a control, the MIC of each antibiotic is estimated. This calculation is done with an algorithm specific for each antibiotic but independent of the species. *E. coli* ATCC 25922 and 35218 and *P. aeruginosa* ATCC 27853 were used as control strains for enterobacterial and pseudomonal test cards; *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 35218 and *E. faecalis* ATCC 51299 for staphylococcal and enterococcal test cards; and *Streptococcus pneumoniae* ATCC 49619 for the pneumococcal card.

Data analysis

For each strain, the evaluators completed a data sheet, indicating the MICs found by the VITEK 2, the AES interpretation of the phenotype, the Etest results and the laboratory's previous susceptibility and genotype data. These records were sent to a central laboratory, then merged and analysed using Microsoft Excel tools. The mechanisms inferred by the VITEK 2 AES were compared with previ-

Table 2. Composition of antimicrobial susceptibility test cards

Staphylococci AST-P515 (product 21015)	Pneumococci AST-P506 (product 21530)	Enterococci AST-P516 (product 21016)	Enterobacteriaceae AST-N010 (product 21018)	Non-fermenters AST-N008 (product 21012)
Clindamycin	amoxicillin	ampicillin/sulbactam	amikacin	amikacin
Erythromycin	cefotaxime	ampicillin	co-amoxiclav	aztreonam
Fosfomycin	ceftriaxone	cefuroxime	ampicillin	cefepime
Fusidic acid	chloramphenicol	ciprofloxacin	cefalothin	cefpirome
Gentamicin	erythromycin	clindamycin	cefepime	ceftazidime
Kanamycin	imipenem	erythromycin	cefotaxime	ciprofloxacin
Lincomycin	ofloxacin	gentamicin (high level)	cefoxitin	colistin
Minocycline	penicillin	imipenem	cefpodoxime	fosfomycin
Nitrofurantoin	pristinamycin	kanamycin (high level)	ceftazidime	gentamicin
Norfloxacin	tetracycline	levofloxacin	cefuroxime	imipenem
Ofloxacin	co-trimoxazole	nitrofurantoin	ciprofloxacin	isepamicin
Oxacillin screen	vancomycin	norfloxacin	gentamicin	meropenem
Oxacillin MIC		ofloxacin	meropenem	netilmicin
Penicillin		penicillin	nitrofurantoin	pefloxacin
Pristinamycin		quinupristin/dalfopristin	norfloxacin	piperacillin
Rifampicin		streptomycin (high level)	ofloxacin	piperacillin/tazobactam
Teicoplanin		teicoplanin	piperacillin	ticarcillin
Tetracycline		tetracycline	piperacillin/tazobactam	ticarcillin/clavulanate
Tobramycin		co-trimoxazole	tobramycin	tobramycin
Co-trimoxazole		vancomycin	co-trimoxazole	co-trimoxazole
Vancomycin				

ous conclusions based on biochemical or genetic analysis (Table 1). The results were graded as 'Agreement' when the VITEK 2 AES inferred the same mechanism(s) found previously; 'Partial agreement' when the AES suggested more than one possible mechanism including that found previously; 'Disagreement' when the AES indicated a different mechanism(s) to that found previously; and 'Uninterpretable' when the VITEK 2 gave no MIC, or the AES no interpretation.

Statistical methods

The Sign test[10] was used to establish whether geometric mean MICs based on results generated by the VITEK 2 were above or below those found with Etests. In some cases the Sign test was also used to compare standard deviations on the logarithms of MICs.

Results

Data were accepted for analysis when the principal investigators (the first two authors) were satisfied, based on the Etest results, that the strains retained and expressed the requisite resistance mechanisms. This screening left 42 of the 50 LBM strains and 921 out of 1000 evaluators' strains available for analysis. Each LBM strain had been tested 10 times (once at each of 10 sites), whereas each evaluator's strain had been tested once.

Detection of resistance mechanisms

Results for the evaluators' strains are summarized in Table 3 and those for the LBM strains in Table 4; both tables indicate the degree of agreement with reference results and, since the LBM strains were tested at 10 separate sites, Table 4 also tests inter-site reproducibility.

By interpreting phenotypes, the VITEK 2 AES achieved agreement with reference data in 88–89% of tests, with partial agreement in a further 5–6%, and disagreements in only 5–7%. It achieved near-complete agreement with genotypic data for the detection of methicillin resistance in *S. aureus* (99%) and coagulase-negative staphylococci (100%), and for detection of glycopeptide resistance in enterococci (99%). VanA, VanB and VanC forms of glycopeptide resistance in enterococci were discriminated, with only one disagreement among 177 tests (97 with the evaluators' strains and 80 with the 10 LBM organisms). Excellent agreement with genotypic analysis was also found for quinolone resistance mechanisms in Entero-

Table 3. Agreement between reference genotype data and VITEK 2 AES phenotypic interpretations for evaluators' strains

	No. tests	Agreement	Partial agreement	Disagreement	Uninterpretable
Enterobacteriaceae, any β-lactamase	245	205	18	19	3
Enterobacteriaceae, ESBL	137	126	4	6	1
E. coli, TEM/SHV/PER ESBL	28	26		2	
Klebsiella spp., TEM/SHV ESBL	99	90	4	4	1
E. cloacae/C. freundii, TEM/SHV ESBL	6	6			
Salmonella, TEM/SHV ESBL	3	3			
Enterobacter gergoviae, CTX-M ESBL	1	1			
Enterobacteriaceae, acquired penicillinase	62	56	2	4	0
E. coli, TEM/SHV/OXA acquired penicillinase	29	24	1	4	
Klebsiella spp., TEM/SHV/OXA acquired penicillinase	33	32	1		
Enterobacteriaceae, IRT	13	6	6	1	
E. coli, IRT	13	6	6	1	
Enterobacteriaceae, AmpC cephalosporinase	9	5	2	2	
E. coli, AmpC cephalosporinase	2	2			
Enterobacter/*C. freundii*, hyperproduced AmpC	7	3	2	2	
Enterobacteriaceae, other β-lactamases	20	9	3	6	2
Klebsiella oxytoca, K1 enzyme	3	3			
P. vulgaris, cefuroximase	6	1		3	2
P. vulgaris, low cefuroximase	1			1	
Citrobacter koseri, cefuroximase	10	5	3	2	
Enterobacteriaceae, multiple β-lactamases	4	3	1		
E. coli, penicillinase + cephalosporinase	3	3			

Table 3. (*Continued*)

	No. tests	Agreement	Partial agreement	Disagreement	Uninterpretable
E. cloacae, TEM/SHV ESBL + hyperproduced AmpC	1		1		
P. aeruginosa, any β-lactamase	10	9		1	
P. aeruginosa, PSE/OXA acquired penicillinase	4	3		1	
P. aeruginosa, OXA/PER/IMP ESBL	6	6			
All aminoglycoside-resistant Gram-negative bacilli	33	8	21	4	
Gram-negative bacilli, AAC(3)	10	2	7	1	
Gram-negative bacilli, AAC(6′)	5	1	2	2	
Gram-negative bacilli, AAC(3) + AAC(6′)	2		1	1	
Gram-negative bacilli, AAC(6′) + ANT(2″)	3	2	1		
Gram-negative bacilli, ANT(2″)	5		5		
Gram-negative bacilli, ANT(3″)	4		4		
Gram-negative bacilli, APH(3′)	2	2			
Gram-negative bacilli, APH(3′) + ANT (2″)	1		1		
Gram-negative bacilli, AAC(3) + AAC(6) + ANT(2″)	1	1			
All quinolone-resistant Gram-negative bacteria	44	43			1
E. coli, *gyrA* mutants	20	19			1
Enterobacter, *parC*, *gyrA* mutants	24	24			
All β-lactam-resistant staphylococci	206	206			
S. aureus, penicillinase	1	1			
S. aureus, *mecA*	205	205			
All aminoglycoside R staphylococci	24	20	4		
S. aureus, APH(2″) + AAC(6′)	7	7			
Staphylococcus epidermidis, APH(2″) + AAC(6′)	5	5			
S. aureus, APH(2″)+AAC(6′) + more	12	8	4		
All quinolone-resistant staphylococci	13	13			
Quinolone-resistant staphylococci	13	13			
All vancomycin-resistant enterococci	97	96		1	
All VanA enterococci	66	65		1	
All VanB enterococci	25	25			
E. faecalis, VanA	18	17		1	
E. faecalis, VanB	9	9			
Enterococcus faecium, VanA	32	32			
E. faecium, VanB	1	1			
Enterococcus spp., VanA	15	15			
Enterococcus spp., VanB	15	15			
Enterococcus gallinarum, VanC	6	6			
Enterococcus durans, VanA	1	1			
Enterococcus spp. APH(2″)+AAC(6″)	44	42		2	
Enterococcus APH(2″)+AAC(6″)	44	42		2	
All penicillin-non-susceptible pneumococci	140	106		34	
S. pneumoniae, penicillin intermediate	66	34		32	
S. pneumoniae, penicillin resistant	74	72		2	
All macrolide-resistant pneumococci	65	62		3	
S. pneumoniae, *erm*	63	60		3	
S. pneumoniae, *mefAE*	2	2			
Total (all strains, all mechanisms)	921	810 (87.9%)	43 (4.7%)	64 (6.9%)	4 (0.4%)

Rows shaded in grey summarize the unshaded blocks below them, categorizing the organisms into larger groups.

Table 4. Agreement between reference genotype data and VITEK 2 AES interpretations for the LBM strains, based on tests of each strain at 10 sites

	No. test results, with one test per strain at each of 10 sites				
	Total	Agreement	Partial agreement[a]	Disagreement[a]	Uninterpretable
Enterobacteriaceae, any β-lactamase	120	106	11 (4)	3 (3)	
Enterobacteriaceae, TEM/SHV ESBL	40	40			
E. coli, TEM/SHV ESBL	10	10			
Klebsiella, TEM/SHV ESBL	20	20			
Salmonella spp., TEM/SHV ESBL	10	10			
Enterobacteriaceae, acquired penicillinase	40	36	3 (2)	1 (1)	
E. coli, TEM/SHV/OXA acquired penicillinase	30	26	3 (2)	1 (1)	
Klebsiella spp., classic TEM/SHV acquired penicillinase	10	10			
Enterobacteriaceae, IRT	20	12	8 (2)		
E. coli, IRT	20	12	8 (2)		
Enterobacteriaceae, cephalosporinase	10	9		1 (1)	
Enterobacter/C. freundii, hyperproduced AmpC	10	9		1 (1)	
Enterobacteriaceae, cephalosporinase	10	9		1 (1)	
K. oxytoca, high K1	10	9		1 (1)	
P. aeruginosa any β-lactamase	20	10		10 (1)	
P. aeruginosa, penicillinase	10			10 (1)	
P. aeruginosa, hyperproduced AmpC	10	10			
All aminoglycoside R GNB	50	37	13 (3)		
Gram-negative bacilli, AAC(3)	10	9	1 (1)		
Gram-negative bacilli, AAC(6′)	20	20			
Gram-negative, AAC(3) + AAC(6′)	10	8	2 (1)		
Gram-negative bacilli, ANT(2″)	10		10 (1)		
Any oxacillin staphylococcus	70	67		3 (1)	
S. aureus, *mecA*	20	17		3 (1)	
S. epidermidis, *mecA*	40	40			
Staphylococcus warneri, *mec*A	10	10			
All VRE	80	80			
E. faecalis, VanA	10	10			
E. faecalis, VanB	10	10			
E. faecium, VanA	40	40			
E. faecium, VanB	20	20			
All penicillin-resistant pneumococci	77[b]	73		4 (3)	
S. pneumoniae, PenI	38[b]	36		2 (2)	
S. pneumoniae, PenR	39[b]	37		2 (1)	
Total (all tests, all mechanisms)	417[b]	373 (89.4%)	24 (5.8%)	20 (4.8%)	0 (0%)

Rows shaded in grey summarize the data in the unshaded rows immediately below them.
[a]Numbers in parentheses indicate the numbers of different individual strains for which disagreements or partial agreements were recorded.
[b]Do not equal multiples of 10 since one or two pneumococci failed to grow at individual sites.

bacteriaceae (98%), for AAC(6′)-APH(2″)-mediated gentamicin resistance in enterococci (95%) and staphylococci (83%), and for *erm*-mediated macrolide resistance in *S. pneumoniae* (95%).

Detection of most β-lactamase-mediated resistance types was also in good agreement with the genotype data, although the AES evidently could not identify individual ESBL variants. The ESBL producers among the LBM strains were identified with complete agreement at all 10 sites (Table 4) and, among the evaluators' strains, ESBL production was detected with agreement for 126 of 137 strains (92%) and partial agreement in another four (3%).

The ESBL producers were predominantly klebsiellae and *E. coli*, but also included *Salmonella* spp., *Enterobacter cloacae* and *Citrobacter freundii* (Tables 3 and 4). The six disagreements (and one uninterpretable result) for ESBL producers concerned *E. coli* and *Klebsiella* spp., and do not indicate a problem with less frequent hosts of these enzymes. For Enterobacteriaceae with acquired penicillinases (predominantly *E. coli* and *Klebsiella* spp.), the VITEK 2 AES achieved 90% agreement with genotype analysis for the evaluators' and LBM strains, with partial agreement for a further 3% of the evaluators' strains and 7.5% of tests with the LBM strains. The enzymes produced by these isolates included various TEM, SHV and OXA types. For Enterobacteriaceae with inhibitor-resistant TEM enzymes (IRT), the VITEK 2 AES achieved agreement with genotypic analysis for six of 13 evaluators' strains, with partial agreement for a further six (Table 3). Similarly, 12 of 20 results for two IRT producers among the LBM strains were in full agreement with reference data, whereas eight of 20 were in partial agreement. Where agreement was only partial for IRT producers, penicillinases were indicated as an alternative cause of the observed phenotypes. Hyperproduction of chromosomal AmpC and K1 β-lactamase types was inferred with 95% agreement with reference data among the LBM strains (Table 4), but agreement was achieved for only three of the evaluators' seven AmpC-hyperproducing *E. cloacae* and *C. freundii* strains (with partial agreement for a further two) and for one of six *Proteus vulgaris* strains with chromosomal cefuroximase (functional Group 2e enzyme[2,11]).

Agreement between the VITEK 2 AES interpretation and genotype data was seen for 90% of the evaluators' *P. aeruginosa* strains with extended-spectrum (OXA-11, -14, -16, IMP-1 and PER-1) and classical (OXA-10 and PSE-4) β-lactamases (Table 3). However, the VITEK 2 AES consistently failed to infer an acquired penicillinase in one LBM *P. aeruginosa* strain (Table 4); this failure seemingly reflected an anomalously high cefepime MIC for the organism (>64 mg/L), perhaps contingent on efflux or impermeability.

For Enterobacteriaceae with aminoglycoside-modifying enzymes, disagreements were rare (12% among the evaluators' strains; none among tests with the LBM strains) but agreement was often only partial, particularly for the evaluators' strains, with the VITEK 2 AES identifying the correct aminoglycoside-modifying enzyme, but only as one possibility among two or more.

Almost half of all the frank disagreements recorded (Tables 3 and 4 combined) concerned evaluators' pneumococci found to have penicillin resistance (MIC $\geq$ 2 mg/L) by the VITEK 2 but previously recorded as having intermediate resistance (MIC 0.12–1 mg/L) as determined by various reference MIC methods. Such disagreements arose for 32 of 66 evaluators' strains previously found to have intermediate resistance. However, the VITEK 2 AES achieved 95% categorization agreement with reference data for the penicillin-intermediate and -resistant pneumococci in the LBM set, where MICs had previously been determined with NCCLS methodology.

Editing of antibiograms, based on inferred mechanisms

When ESBL production was inferred in *E. coli* and klebsiellae, the AES edited the reported results for cephalosporins, except cefoxitin, to resistant. Wherever oxacillin resistance was recorded in staphylococci the user was advised to avoid all β-lactam therapies. This editing is in accordance with NCCLS guidelines.[4] In addition, although not advised by the NCCLS, piperacillin results were edited to resistant when acquired penicillinases were inferred in Enterobacteriaceae. Other editing was not done in the absence of official guidelines, but may be appropriate (see Discussion).

Agreement of Etest and VITEK 2 MICs

All the strains were tested with limited ranges of Etests (see Materials and methods) at the same time as they were processed through the VITEK 2, primarily to confirm retention of key resistances. MICs as determined by the two methods are briefly compared in Table 5: in >94% of cases there was agreement within one dilution. Significant divergence, with the VITEK 2 and Etest MIC results at least two dilutions apart, only occurred for $\geq$10% of strains or tests in the cases of cefotaxime for Enterobacteriaceae, oxacillin for MRSA and erythromycin for pneumococci. In the case of cefotaxime, most divergence was for ESBL producers.

MICs of penicillin for pneumococci by the two methods were within one dilution of each other in 95–97% of cases; but there was a consistent and significant ($P < 0.001$, Sign test) trend for the VITEK 2 to indicate two-fold higher MICs than the Etests.

A further comparison between the Etests and VITEK 2—of reproducibility—was possible for the 42 LBM strains, since these were tested by each method at the 10 different sites. Based on these data, geometric mean MICs (and standard deviations) were calculated by each method for each combination of antibiotic and strain. The Sign test was then performed to compare the results. No significant difference was found between the mean MICs from the VITEK 2 and Etest determinations ($P = 0.1945$), but the standard deviations were significantly greater with the Etests ($P < 0.0001$), indicating greater consistency with the VITEK 2.

Discussion

The case for interpretive reading was outlined in the Introduction and has been argued more fully elsewhere.[1–3] It is a strategy with benefits for patient care and surveillance of resistance. This study examined whether the VITEK 2

Table 5. Agreement between MICs by VITEK 2 AES and Etests

		Evaluators' strains: one test per site				LBM strains: one test per each of 10 sites[a]			
Antibiotic	Bacterial species or group	agreement ±1 dilution	VITEK 2 MIC ≥2 dilutions higher	VITEK 2 MIC ≥2 dilutions lower	total	agreement ±1 dilution	VITEK 2 MIC ≥2 dilutions higher[b]	VITEK 2 MIC ≥2 dilutions lower[b]	total
Amikacin	Enterobacteriaceae	31 (93.9%)	0	2	33	47 (94%)	0	3 (2)[b]	50
Gentamicin	Enterobacteriaceae	33 (100.0%)	0	0	33	49 (98%)	0	1 (1)	50
Tobramycin	Enterobacteriaceae	31 (93.9%)	0	2	33	49 (98%)	0	1 (1)	50
Gentamicin	staphylococci	24 (100.0%)	0	0	24				
Tobramycin	staphylococci	22 (91.7%)	0	2	24				
Gentamicin, high level	enterococci	44 (100.0%)	0	0	44				
Co-amoxiclav	Enterobacteriaceae	225 (91.8%)	18	2	245	110 (92%)	9 (5)[b]	0	119
Cefalothin	Enterobacteriaceae	235 (96.3%)	2	7	244	98 (98%)	0	2 (2)	100
Cefotaxime	Enterobacteriaceae	203 (82.9%)	17	25	245	106 (89%)	10 (2)	3 (3)	119
Ceftazidime	Enterobacteriaceae	230 (94.3%)	2	12	244	114 (97%)	0	4 (4)	118
Ceftazidime	*P. aeruginosa*	10 (100.0%)	0	0	10	17 (85%)	3 (1)	0	20
Ticarcillin	*P. aeruginosa*	10 (100.0%)	0	0	10	20 (100%)	0	0	20
Erythromycin	pneumococci	58 (89.2%)	0	7	65				
Oxacillin	staphylococci	202 (98.1%)	2	2	206	46 (66%)	22 (5)	2 (1)	70
Ceftriaxone	pneumococci	131 (95.6%)	3	3	137	73 (92%)	5 (2)	1 (1)	79
Penicillin	pneumococci	133 (97.1%)	4	0	137	72 (91%)	7 (2)	0	79
Ofloxacin	Gram-negative bacilli	44 (100.0%)	0	0	44				
Norfloxacin	Gram-negative bacilli	44 (100.0%)	0	0	44				
Ciprofloxacin	Gram-negative bacilli	44 (100.0%)	0	0	44				
Ofloxacin	staphylococci	12 (92.3%)	0	1	13				
Norfloxacin	staphylococci	13 (100.0%)	0	0	13				
Teicoplanin	enterococci	92 (94.8%)	3	2	97	62 (89%)	0	8	70
Vancomycin	enterococci	97 (100.0%)	0	0	97	70 (100%)	0	0	70
Total, all antibiotics	all species	1708 (94.1%)	49 (2.7%)	58 (3.2%)	1815 (100%)	887 (94%)	34 (3.6%)	23 (2.4%)	944 (100%)

[a]Numbers of tests are not always multiples of 10, owing to the death of a few strains at individual sites.
[b]Numbers in parentheses indicate the number of different LBM strains for which discrepancies of ≥2 dilutions were recorded.

AES could accurately detect and interpret resistance phenotypes, and whether its susceptibility reports were edited appropriately based on the mechanisms inferred. To provide a rigorous evaluation, we challenged the AES with clinically important resistance types and used, as far as possible, unique strains with known genotypes. In general we did not attempt to relate the MICs from the VITEK 2 to the evaluators' historic MIC data, because these latter results had been obtained by a variety of different methods. Limited comparison with MICs determined by Etests was, however, undertaken here, and a fuller comparison of MICs obtained by the VITEK 2 with reference MIC data is published elsewhere.[5,6]

By recording and interpreting phenotypes the VITEK 2 AES accurately inferred the presence or absence of *mecA*-mediated methicillin resistance in staphylococci; VanA-, VanB- or VanC-mediated glycopeptide resistance in enterococci; *gyrA*- and *parC*-mediated quinolone resistance in staphylococci and Enterobacteriaceae; AAC(6′)-APH(2″)-mediated gentamicin resistance in enterococci and staphylococci; *erm*-mediated macrolide resistance in pneumococci; ESBL-mediated cephalosporin resistance in Enterobacteriaceae and *P. aeruginosa*; and acquired penicillinases in Enterobacteriaceae. Enterococci with the VanA, VanB and VanC determinants were distinguished reliably. ESBL production was inferred accurately not only in *E. coli, Klebsiella* spp. and *Salmonella* spp., but also in those species—*E. cloacae* and *C. freundii*—where its detection is complicated by the presence of inducible AmpC enzymes.[2,12] Hyperproduction of K1 β-lactamase in *Klebsiella oxytoca* and *mefA-E*-mediated macrolide resistance in *S. pneumoniae* also appeared to be detected reliably, although too few representatives were tested for a rigorous assessment (Tables 3 and 4).

Production of aminoglycoside-modifying enzymes was accurately inferred in Enterobacteriaceae, but individual enzyme types were often only identified with partial agreement with reference data. This lack of discrimination reflected the fact that many evaluators' strains had multiple modifying enzymes. Such combinations are increasingly frequent, and the individual enzymes involved can only be identified by molecular methods or with non-clinical aminoglycoside analogues (e.g. 2′ and 6′ *N*-ethyl netilmicin and epi-sisomicin derivatives).[13] Agreement was also often only partial for producers of IRT β-lactamases, with the VITEK 2 AES indicating acquired penicillinases as another possible mechanism in the strains harbouring these enzymes. This limitation is not surprising, since hyperproduced TEM penicillinases often confer resistance to inhibitor combinations, as do some OXA penicillinases that have poor susceptibility to β-lactamase inhibitors.[2,3]

The study only tested a few strains that hyperproduced AmpC β-lactamases. This mechanism was accurately referred in nine of 10 tests with the one representative LBM strain (Table 4), but was only inferred with full agreement for three of seven evaluators' strains and with partial agreement in a further two. Using a larger sample of AmpC-derepressed *E. cloacae* and *C. freundii*, Sanders and others[7] found that the VITEK 2 AES achieved accurate inference of this mechanism from phenotype data for 25 of 27 strains; so we do not believe that there is any fundamental detection problem. The two AmpC-depressed isolates for which the VITEK 2 AES gave disagreements were misidentified as having ESBLs. One was an *Enterobacter aerogenes* strain that was anomalous in being susceptible to co-amoxiclav (MIC 8 + 4 mg/L, both with the VITEK 2 and with Etests); the other was an *E. cloacae* strain that was broadly resistant to cephalosporins, and the reasons for its miscategorization are unclear. In contrast to Sanders *et al.*,[7] we found that the VITEK 2 AES could accurately predict AmpC hyperproduction in *E. coli*, albeit based on just two strains.

Most disagreements between the VITEK 2 AES and reference data concerned penicillin resistance in pneumococci, where about half (48%) of the 66 strains previously categorized as intermediately resistant (MIC 0.12–1 mg/L) were found to be fully resistant (MIC $\geqslant$2 mg/L) by the VITEK 2 (Table 3). MICs of 1 mg/L had previously been recorded for 70% of these penicillin-intermediate isolates, meaning that the discrepancy between the VITEK 2 AES and previous data was only one dilution; moreover, the previous tests had been by a variety of different MIC methods. The MICs of penicillin for pneumococci determined with the VITEK 2 were also within one dilution of those obtained with Etests (Table 5), although there was a trend for the VITEK 2 to indicate MICs one dilution higher than the Etests ($P < 0.001$). More generally, there was good agreement between MICs obtained with the VITEK 2 AES and Etests, with 94% of the results falling within one dilution of each other (Table 5), and with most of the wider discrepancies concerning cefotaxime against ESBL producers and oxacillin against MRSA. These are both cases where MICs notoriously vary with the inoculum size and test conditions.

The editing of susceptibility by the AES was judged to be appropriate, but further editing may be desirable. Specifically, and irrespective of the MICs found, the VITEK 2 AES edited categorizations for cephalosporins (except cefoxitin) to resistant for all *E. coli* and klebsiellae inferred to have ESBLs. This editing accords with widespread advice, based on clinical experience,[1,2,4,12] and is a considerable improvement over the present situation, where >30% of ESBL-positive klebsiellae continue to be reported as susceptible to one or more third-generation oxyimino cephalosporins in European laboratories.[14,15] However, in the absence of an NCCLS guideline, comparable editing of cephalosporin results was *not* undertaken when ESBL production was inferred in *Salmonella* spp., *Enterobacter* spp. and *C. freundii*, although there is no reason to suppose that an ESBL would be any less protective against cephalosporin therapy in these species than in a *Klebsiella* strain. As a second example, susceptible (MIC $\leqslant$ 16 mg/L) or intermediate (32–64 mg/L) results for piperacillin were

edited to resistant for Enterobacteriaceae isolates inferred to have acquired penicillinases. Clinical data indicate a poor response to piperacillin for Enterobacteriaceae with acquired penicillinases, even when the MIC is low,[16] and so support such editing. However, comparable editing of piperacillin results was not performed for Enterobacteriaceae inferred to have ESBLs, nor for *P. aeruginosa* strains inferred to have either ESBLs or acquired penicillinases. It seems unlikely that ESBLs would be any less protective than classical penicillinases against piperacillin in Enterobacteriaceae, or that either enzyme type would fail to protect *P. aeruginosa*, and we believe that editing of 'susceptible' to 'resistant' would be appropriate in these cases too. Amikacin presents another case where more editing may be desirable: isolates correctly inferred to have AAC(6′) were reported as amikacin susceptible if the MIC was at or below the NCCLS breakpoint of 16 mg/L. There are no substantive data to indicate whether infections caused by strains with AAC(6′) and low amikacin MICs respond to amikacin therapy *in vivo*, but it seems prudent not to use the drug against producers if alternatives are available. Finally, the VITEK 2 AES accurately recognized Enterobacteriaceae strains that had reduced susceptibility to all quinolones, and correctly inferred these to have DNA gyrase modifications, but then categorized individual fluoroquinolone results as susceptible, intermediate or resistant based on NCCLS breakpoints. Strains were reported resistant to one quinolone, based on an MIC two-fold above breakpoint, and susceptible to another based on an MIC at breakpoint, or two-fold below. Allowing the single-tube error accepted in MIC determinations, it might arguably be better to interpret all quinolone results as intermediate in these circumstances. None of these criticisms reflects on the VITEK 2 AES itself, which inferred these mechanisms with >90% agreement with reference data; rather, they reflect the absence of official guidelines, principally from the NCCLS. Other guidelines are available, and some 'phenotypic guidelines' advocate more comprehensive editing of categorizations.[1,3] It should be added that the VITEK 2 could be reconfigured to accommodate these suggestions and the breakpoints of other organizations such as the BSAC or Comité Antibiogramme de la Societé Française de Microbiologie. Each user is then free to select the guidelines he or she prefers and can customize the editing of reports. Used in this way, VITEK 2 AES potentially provides a tool to assist the development of a consensus among microbiologists regarding antibiotic susceptibility interpretation.

In summary, this study demonstrated the capacity of VITEK 2 to detect and interpret resistance mechanisms with a high level of accuracy and standardization. Only 64 of 963 interpretations at 10 European centres were discrepant, with half of these concerning *S. pneumoniae* isolates that were intermediately resistant to penicillin by previous methods, but which crossed the threshold into the resistant category with the VITEK 2 AES.

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