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Expert Systems in Clinical Microbiology

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INTRODUCTION

John Naisbett, a well-known futurist, once said, "We are drowning in information, but starving for knowledge" (180)—a situation well known to the modern-day clinician. There are a number of artificial intelligence (AI) systems in routine clinical use, and some are specific to medicine (see http://www.coiera.com/ailist/list-main.html, http://www.generation5.org/content/2005/Expert_System.asp, and http://www.sci.brooklyn.cuny.edu/~kopec/cis718/fall_2005/1/jiang_hwl.htm).

Very early on, scientists and doctors alike were captivated by the potential that computer technology might have in medicine (153). With intelligent computers able to store and process vast amounts of knowledge, the hope was that they would become perfect doctors, assisting or surpassing clinicians with tasks like diagnosis. Medical AI is concerned primarily with the construction of AI programs that perform diagnosis and make therapy recommendations. Unlike medical applications based on other programming methods, such as purely statistical and probabilistic methods, medical AI programs are based on symbolic models of disease entities and their relationship to patient factors and clinical manifestations. Many of the problems with medical AI are associated with the poor way in which they have fitted into clinical practice, either solving problems that were not perceived to be an issue or imposing changes in the ways in which clinicians worked. What is now being realized is that when they fill an appropriate role, intelligent programs do

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indeed offer significant benefits. One of the most important tasks now facing developers of AI-based systems is to characterize accurately those aspects of medical practice that are best suited to the introduction of artificial intelligence systems. Expert or knowledge-based systems are the commonest type of AIM (artificial intelligence in medicine) system in routine clinical use. They contain medical knowledge, usually about a very specifically defined task, and are able to reason with data from individual patients to come up with reasoned conclusions. Although there are many variations, the knowledge within an expert system (ES) is typically represented in the form of a set of rules. Expert systems can be applied to various tasks of medicine domains, including prediction, design, monitoring, instruction, control, generation of alerts and reminders, diagnostic assistance, therapy critiquing and planning, information retrieval, image recognition, and interpretation.

Clinical decision support systems (CDSSs) form a significant part of the field of clinical knowledge management, supporting the clinical process, making better use of knowledge, and helping in diagnosis, investigation, treatment, and long-term care. CDSSs are the new-generation clinical support tools that "make it easy to do it right." Despite promising results, these systems are not common practice, although experts agree that the necessary revolution in health care will depend on their implementation. Few diagnostic decision support systems are in routine clinical use, mainly because these systems typically require time-consuming manual data entry. However, some clinical decision support systems are linked to electronic medical and health records (30, 34, 89), while others are embedded in laboratory information systems (LISs) (53) and in other laboratory systems (50).

Probably the most widely used aspect of expert systems in the hospital environment is decision support for antibiotic prescribing and antibiotic stewardship. These topics have been well reviewed by Miller (176), Sintchenko et al. (228), and Thursky (261). Many studies described improved antibiotic use, more appropriate use of guidelines, improved patient care, reduced costs, and stabilization of antibiotic resistance (198, 262). Expert systems have been used for clinical case simulations. For example, ALERT (29) was used to assist in the training of general practitioners regarding the control of serious, communicable, and rare diseases, such as anthrax, plague, and smallpox. In one study, clinicians using an expert system (compared with conventional practice) ordered fewer laboratory tests during the diagnostic process, completed the diagnostic workup with fewer sample collections, generated lower laboratory costs, shortened the time required to reach a diagnosis, showed closer adherence to established clinical practice guidelines, and exhibited a more uniform and diagnostically successful investigation (231). Expert systems have been used to diagnose a variety of clinical conditions, including community-acquired pneumonias (9, 279), septicemia (203), female genital disease and abdominal pain (263), urinary tract infection (51, 281), viral (121) and infantile (90) meningitis, febrile tropical diseases (27), chronic prostatitis (31), infective endocarditis (76), and infectious diseases (266). Some systems have used fuzzy-logic methods (14); others have used the World Wide Web (76).

Expert systems have also been used to identify bacteria (195), and a prototype of an expert system for the identification of β-galactosidase-positive *Enterobacteriaceae* has been developed for use with the API 20 EC kit (bioMérieux). The system is implemented in Prolog on an IBM personal computer (PC) with 640 K of central memory and 20 megabytes of secondary memory. Its objectives are to highlight errors that can occur when the kit is in use. It can indicate the presence of new groups or species and give advice or suggest additional tests for the differentiation of the new species from those included in the kit (96). Expert systems have also been used for parasite identification (256).

Hospital-acquired infections represent a significant cause of prolonged inpatient days and additional hospital charges. As the demands on hospital infection control teams increase, it becomes less efficient for them to use paper-based surveillance methods, and several expert systems have been developed. Early systems included Help (37) and Germwatcher (133, 134). Hospital information system-based alerts can play an important role in the surveillance and early prevention of methicillin-resistant *Staphylococcus aureus* (MRSA) transmission and can help to recognize patterns of colonization and transmission (202). Some systems, e.g., Mercurio, make use of knowledge discovery approaches (150). Others support multiple hospitals, e.g., the Doherty system (72), the remodeled Germwatcher (71), and Moni (2). The debugIT (Detecting and Eliminating Bacteria Using Information Technology) (http://www.debugit.eu/) project collects routinely stored data from clinical systems, learns by applying advanced data-mining techniques, stores the extracted knowledge, and then applies it for decision support and monitoring.

A major application of expert systems to microbiology is the interpretation of an organism's antibiogram. This was facilitated by the concept of interpretive reading, driven largely by one of the coauthors of this review (55–58).

In 1947, Alan Turing (271) considered artificial intelligence to be activity carried out by a machine that if carried out by a human would be considered intelligent. An expert system is a computer program designed to simulate the problem-solving behavior of a human who is an expert in a narrow task domain or discipline, and the terms expert system and knowledge-based system are often used synonymously; the terms knowledge application system and decision support system are also used. Expert systems are best suited to problems requiring experience, knowledge, judgment, and complex interactions to arrive at a solution. Conventional software processes passive data, using algorithms to solve problems, and expert systems process active factual knowledge that can be used to infer new information from what is already known; they also process less rigorous, more experiential, and more judgmental knowledge known as heuristics. Expert systems can deliver quantitative information and interpret qualitatively derived values.

Whether or not the problem merits the use of an expert system is dependent on several criteria: the need for a solution must justify the costs involved in development, and human expertise may not be available in all situations where it is needed, but cooperative and articulate experts should exist. The problem must be solvable by using symbolic reasoning techniques, it must be well structured and not require much "common-sense knowledge," it must not be easily solved by using more traditional computing methods, and it must be of a proper size and scope.

That said, expert systems offer a number of advantages over conventional approaches. They provide consistent answers for repetitive decisions, processes, and tasks; they can hold and maintain significant levels of information; and they can combine knowledge from many domain experts. Expert systems can also reduce training costs, centralize decision-making processes, increase efficiency, reduce time, and reduce human errors and omissions.

Expert systems are limited by the lack of human common sense needed in some decision-making processes, and they lack the creative response and flexibility of humans. Also, domain experts may be unable to explain logic and reasoning. There is often a challenge in automating complex processes: expert systems are often unable to recognize when there is no answer. When interpreting antibiograms, it is vital that the correct antibiotics be tested (55).

A simple expert system normally comprises a knowledge base, an inference engine, and the end-user interface. Almost all expert systems have an explanation subsystem; some have a knowledge base editor to facilitate updating and checking by the domain expert or knowledge engineer (see below). The case-specific data include both data provided by the user and partial conclusions (along with certainty measures) based on these data; these are elements in working memory.

Development generally proceeds through problem selection, knowledge acquisition, knowledge representation, programming, testing, and evaluation. The power of an expert system lies in its bank of domain knowledge. Most developers employ a knowledge engineer to explicitly "tease out" highly compiled expert knowledge, as the domain expert may be too familiar with the subject. An alternative approach would be to train the domain expert in knowledge acquisition and representation. A further, attractive approach would be to use computer programs to implicitly derive rules by examining test data produced by domain experts. Such neural networks are self-replicating and may often derive rules not seen by a knowledge engineer.

Knowledge representation formalizes and organizes the knowledge. Knowledge bases can be represented by production rules that use Boolean operators. These consist of one or more conditions or premises followed by an action or conclusion (IF condition…AND condition…OR condition…THEN action…AND print message). Production rules permit the knowledge base to be broken down to facilitate management and organization; rules may also be deleted or added without affecting other rules. Breaking the rule base down into contextual segments permits segments to be paged into and out of the expert system as required. Another widely used representation, based on a more passive view of knowledge, is the unit (frame, schema, or list structure). In microbiology, examples of rule-based expert systems are those employed by the Vitek Legacy (bioMérieux, La Balme les Grottes, France), Phoenix (Becton Dickinson, Oxford, United Kingdom), and MicroScan (Siemens Healthcare Diagnostics, Deerfield, IL) systems.

In a microbiology expert system, the use of hierarchical grouping permits rules to be applied to groups of organisms or of antibiotics, for example. Drug test groups may be used to define the antimicrobials to be tested as well as their reporting priority, although the specimen source is usually not considered. Expert systems may be constructed to trigger rules following certain guidelines. Although many of these are enforced by the logic of the rules themselves, there will often be a relative priority for rules, as a rule can use the expert result obtained by the action of a previously triggered rule. Generally, there will be some protective mechanism to prevent one rule from changing a result that has already been changed by another rule. Also, rules that will have no effect on a result change generally do not fire.

The use of rules with different certainty values or confidence factors (assigned by the expert during knowledge acquisition) allows the system to address imprecise, uncertain, and incomplete data. Confidences are similar to probabilities but are meant to imitate human reasoning rather than to be mathematical definitions. An important subclass of such reasoning with uncertainty is called evidence theory or fuzzy logic. Currently, only one commercial system (Vitek 2; bioMérieux) uses a pattern-based expert system along the lines of, "Overall, the pattern of resistances and susceptibilities best matches. . . ."

An inference engine is a program that interprets the rules in the knowledge base in order to form a line of reasoning and to draw conclusions: it uses either forward or backward chaining or both strategies. A backward-chaining inference engine is goal driven and tries to prove a rule conclusion by confirming the truth of all its premises, e.g., MYCIN (237). A forward-chaining inference engine is data driven and examines the current state of the knowledge base, finds those rules whose premises can be satisfied, and adds the conclusion of those rules to the database; it then reexamines the complete knowledge base and repeats the process, e.g., CLIPS (192). Users may question the credibility of an expert system that uses uncertain and heuristic knowledge. For this reason, most expert systems can trace the line of reasoning and provide explanations for conclusions drawn. This also helps the user to understand system behavior.

Expert systems usually separate domain-specific knowledge from general-purpose reasoning and representation techniques. The user interface, the explanation subsystem, the inference engine, and the knowledge base editor comprise the shell (the skeletal system or AI tools). There are basically two ways to write an expert system: from first principles or by using a shell. The use of shells to write expert systems greatly reduces the cost and time of development; all that is required is domain-specific knowledge. Certain programming languages, such as LISP and Prolog, facilitate symbol manipulation. Expert systems are developed iteratively from a prototype by consultation with both experts and users.

Informational messages can be in the form of footnotes and warnings from standard guidelines. Intrinsic rules detect atypical susceptibility or resistance in an isolate with a known identification (ID). Resistance marker rules may change susceptible (S) or intermediate (I) interpretations to resistant (R). Antibiotic-specific rules can be of several types. A promotion rule may promote an antibiotic for a resistant organism. Similarly, a suppression rule may suppress a single antibiotic or a class, e.g., fluoroquinolones in children. Drug class rules allow representative antimicrobials to be tested as markers of antibiotic classes. Hierarchical rules are more specific; e.g., oxacillin may be the class representative of other penicillinase-stable penicillins.

NONCOMMERCIAL SYSTEMS

Comby et al. (52) developed a model of an expert system by using Prolog language to verify the coherence of the results of the antibiotic susceptibility tests. Biological knowledge was formalized in three different ways: a credibility coefficient based on epidemiological data was assigned to known observed resistances; coexistent resistances were described with lists of "implicit" resistances, reflecting phenotypes commonly observed within some antibiotic groups; and every single or "implicit" resistance was connected to a "gregarious" status, expressing the plasmidic nature of the resistance. In a feasibility study applied to *Staphylococcus aureus*, the expert system was able to detect the inconsistencies of the antibiotic susceptibility test and to identify required knowledge, thereby permitting a phenotypic interpretation of results.

As stated above, artificial intelligence is a part of computer science that deals with programs mimicking the intelligence of humans. Artificial intelligence can be used to check the quality of the determination of the antibiotic susceptibility of bacteria. This application is useful because susceptibility testing is subject to biological and technical variations that have to be detected. Three types of reasoning are used either by the biologist or by expert systems: low-level quality checking, dealing with individual results; microbiological interpretation of the whole set of results; and medical interpretation of the results. The use of artificial intelligence in these fields is sustained by the structured nature of the knowledge. Bacterio-expert is a simple expert system for assisting in the validation of antibiotic sensitivity testing. This system is incorporated into a data acquisition and editing program for bacteriology tests (Bacterio was written in Turbo-Pascal for personal-computer users). Of 4,053 antibiotic sensitivity tests on *S. aureus*, approximately 10% required corrections (158). The main problem was, as usual for artificial intelligence applications, to transfer human expertise into an adapted knowledge base. The advantages of expert systems over humans are their reproducibilities of answers and their availability (87).

Interpretive reading of antibiotic disc agar diffusion tests indicates the resistance mechanisms, if any, expressed by a bacterium. Vedel et al. (277) developed an expert system for determining resistance mechanisms by using rapid automated antibiotic susceptibility tests. The β-lactam susceptibilities of 300 strains of clinically significant species of *Enterobacteriaceae*, displaying natural and acquired resistance mechanisms, were determined by disc diffusion and by a rapid automated method with an expert system. For every strain, the conclusion of the expert analysis of the automated test was compared with the commonly accepted interpretation of disc diffusion tests. Of the 300 strains studied, 275 were similarly interpreted (91.7% agreement). The susceptible and naturally β-lactam-resistant phenotypes (wild phenotypes) were equally recognized by both methods. Similarly, the results of the two methods concurred for most of the acquired resistance phenotypes. However, for 25 strains (8.3%), the results diverged. The expert system proposed an erroneous mechanism (5 strains); several mechanisms, including the correct one (17 strains); or no mechanisms (1 strain). For 2 strains the natural resistance mechanism was not detected at first by the automated method but was subsequently deduced by the expert analysis according to the bacterial identification. These results demonstrate that a satisfactory interpretive reading of automated antibiotic susceptibility tests is possible in 4 to 5 h but requires a careful selection of the antibiotics tested as phenotypic markers. Janeckova and Janecek (120) described digital documentation in the microbiology laboratory using the BACMED 4i system, an analyzer of inhibition zones and equivalence of MICs with the BEES expert system.

Manual review of antibiotic susceptibility testing results is an essential component of a microbiology laboratory's quality control (QC) process. Such a review is tedious and prone to human error, however. Jackson et al. (118) described an expert system that remembers which susceptibility patterns are considered typical or atypical by expert reviewers and then uses these patterns to prescreen future isolates. It uses a similarity function to allow matching against this library when two patterns are close but not identical. The use of this system allows a more efficient and reliable review of the laboratory's antibiotic susceptibility testing results. Those authors pointed out several limitations of the system: the challenge of keeping knowledge up to date, the quality of knowledge entered, the need for a knowledge engineer, and the fact that the system is retrospective (not incorporated into the laboratory information system). Lamma et al. (149) introduced the concept of data mining. In a project jointly run by the University of Bologna and Dianoema, those researchers used data-mining techniques to automatically discover association rules from microbiological data and to obtain from them alarm rules for data validation by the ESMIS expert system. To our knowledge, there are two expert systems capable of interpreting antibiograms which are available on the World Wide Web. The first is Assistant Software for Antimicrobial Susceptibility Interpretation (ASISI; version 0.61, build 1, 2003), which can be downloaded as freeware (http://member.hitel.net/~chleeymc/ynasasi.html). As its rule base, it refers to CLSI (formerly NCCLS) guidelines, the Advanced Expert System (AES) of Vitek 2, and the rule tables described previously by Livermore et al. (161, 164) and Courvalin et al. (56), and users are asked to contribute rules. The second expert system is actually functional over the Web and can be found at http://memiserf.medmikro.ruhr-uni-bochum.de/ResId/index_en.html. It was written by Sören Gatermann from the Ruhr Universität in 2007, addresses four groups of organisms (*Enterobacteriaceae*, staphylococci-enterococci, *Pseudomonas-Acinetobacter-Stenotrophomonas*, and other nonfermenters); as a rule base, it uses data from various sources (55, 161, 164, 280).

Seven sets of antimicrobial MIC breakpoints are used in Europe. There are 6 active European National Breakpoint Committees: the British Society for Antimicrobial Chemotherapy (BSAC; United Kingdom), Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM; France), Commissie Richtlijnen Gevoeligheidsbepalingen (CRG; the Netherlands), Deutsches Institut für Normung (DIN; Germany), the Norwegian Working Group on Antibiotics (NWGA; Norway), and Swedish Reference Group for Antibiotics (SRGA; Sweden), and since many of the other countries, in the absence of a national system, subscribe to breakpoints reported by the CLSI, the divergence in interpretations is prominent. Furthermore, almost all the breakpoint committees produce varied and often conflicting expert rules. To achieve a harmonization

of clinical breakpoints and expert rules, the six national committees have now organized themselves into the EUCAST (European Committee on Antimicrobial Susceptibility Testing), convened and financed by the ESCMID (European Society for Clinical Microbiology and Infectious Diseases). EUCAST's main objectives are to set common European breakpoints for the surveillance of antimicrobial resistance, to harmonize clinical breakpoints for existing and new antimicrobial drugs, to encourage internal and external national and international quality assessment schemes, and to work with groups outside Europe (e.g., the CLSI) to achieve an international consensus on susceptibility testing. EUCAST-agreed breakpoints are now available (http://www.eucast.org). The method described by International Standard ISO 20776 (100) is essentially the same as the broth microdilution method (BMD) reported by the EUCAST. A disc diffusion method based on the Kirby-Bauer procedure but with zone diameter breakpoints calibrated to EUCAST MIC breakpoints has now been developed, and the European method was finalized in December 2009.

The EUCAST definition of an expert rule is "a description of action to be taken, based on current evidence, in response to specific antimicrobial susceptibility test results." Interpretive reading, one type of expert rule, is the "inference of resistance mechanisms from susceptibility test results and interpretation of clinical susceptibility on the basis of the resistance mechanism." A EUCAST expert rule subcommittee (Chairman, R. Leclercq) was established early in 2006 with the purpose to prepare tables of expert rules for antimicrobial susceptibility testing (AST) in order to assist microbiologists in the interpretation of results. The subcommittee was comprised of Roland Leclercq (Laboratoire de Microbiologie, CHU Côte de Nacre, Caen Cedex, France), Rafael Cantón (Servicio de Microbiología, Hospital Universitario Ramón y Cajal, Carretera de Colmenar, Madrid, Spain), Christian Giske (Department of Clinical Microbiology L2:02, Karolinska University Hospital, Solna, Stockholm, Sweden), Peter Heisig (Institute of Biochemistry and Molecular Biology & Microbiology, Institute of Pharmacy, University of Hamburg, Hamburg, Germany), Patrice Nordmann (Service de Bactériologie-Virologie, Hôpital de Bicêtre, Le Kremlin-Bicêtre Cedex, France), Gian Maria Rossolini (Dip. di Biotecnologie, Sezione di Microbiologia, Policlinico Le Scotte, Siena, Italy), and Trevor Winstanley (Department of Microbiology, Royal Hallamshire Hospital, Sheffield, United Kingdom).

The EUCAST Expert Rules in Antimicrobial Susceptibility Testing (http://www.srga.org/eucastwt/EUCAST%20Expert%20rules%20final%20April_20080407.pdf) are divided into intrinsic resistances, exceptional phenotypes, and interpretive rules. Intrinsic (natural and inherent) resistance, as opposed to acquired resistance, is a characteristic of all, or almost all, representatives of the bacterial species. The antimicrobial activity of the drug is clinically insufficient or antimicrobial resistance is innate or so common as to render it clinically useless. Antimicrobial susceptibility testing is therefore unnecessary, although it may be done as a part of panels of test agents. For these species, "susceptible" results should be viewed with caution, as they most likely indicate an error in identification or susceptibility testing. Even if susceptibility is confirmed, the drug should preferably not be used, or when no alternatives are available, it should be used with caution. In some cases, intrinsic resistance to an antibiotic may be expressed at a low level, with MIC values close to the susceptible breakpoint, although the antibiotic is not considered clinically active. There are also situations where the antibiotic appears fully active *in vitro* (MIC values cannot be separated from those of the wild-type population) but is inactive *in vivo*. These situations are generally not mentioned in the tables, since they are rather a matter of therapeutic recommendations. Examples of intrinsic resistances are strains of the *Enterobacteriaceae* resistant to glycopeptides or linezolid, *Proteus mirabilis* strains resistant to nitrofurantoin and colistin, *Serratia marcescens* strains resistant to colistin, *Stenotrophomonas maltophilia* strains resistant to carbapenems, Gram-positive organisms resistant to aztreonam, and enterococci resistant to fusidic acid (see Tables 1 to 4 of EUCAST Expert Rules in Antimicrobial Susceptibility Testing).

Exceptional resistance phenotypes are the resistances of some bacterial species to particular antimicrobial agents which have not yet been reported or are very rare. Exceptional resistance phenotypes should be checked, as they may also indicate an error in identification or susceptibility testing. If these phenotypes are confirmed locally, the isolate should be further studied and sent to a reference laboratory for independent confirmation. Exceptional resistance phenotypes may change with time, as resistance may develop and increase over time. There may also be local, regional, or national differences, and a very rare resistance in one hospital, area, or country may be more common in another. Examples of exceptional phenotypes are *Streptococcus pyogenes* strains resistant to the penicillins, *S. aureus* strains resistant to vancomycin, *Enterococcus faecalis* strains resistant to ampicillin, *Enterococcus faecium* strains susceptible to ampicillin, strains of the *Enterobacteriaceae* resistant to carbapenems (rare but increasing), and anaerobes resistant to metronidazole (see Tables 5 to 7 of EUCAST Expert Rules in Antimicrobial Susceptibility Testing).

Interpretive reading is another type of expert rule and involves the inference of resistance mechanisms from susceptibility test results and the interpretation of clinical susceptibility on the basis of the resistance mechanism. The applicability of such rules is limited by the range of agents tested, so individual laboratories will need to choose which agents to test for their local requirements. The applicability of any rule will also depend on the MIC breakpoints used to define the rule. EUCAST interpretive rules may be simple (e.g., if an *S. aureus* strain is resistant to oxacillin or cefoxitin, then it should be reported as being resistant to all β-lactams) or more complicated (e.g., if a strain of the *Enterobacteriaceae* is intermediate to tobramycin, resistant to gentamicin, and susceptible to amikacin, then it should be reported as being resistant to tobramycin). The evidence supporting interpretive rules is often not conclusive, and there may be differences of opinion regarding the most appropriate clinical action. Hence, these rules should be based on current published evidence, the quality of evidence should be assessed, and exceptions to any rules should be noted.

It must be recognized that evidences of the clinical significance of interpretive rules vary and that in these tables, the evidence for rules has been graded as follows. (i) There is

clinical evidence that the reporting of the test result as susceptible leads to clinical failure. (ii) Evidence is weak and based only on a few case reports or on experimental models. It is presumed that the reporting of the test result as susceptible may lead to clinical failure. (iii) There is no clinical evidence, but microbiological data suggest that the clinical use of the agent should be discouraged. Actions indicated by EUCAST expert rules include recommendations on reporting (i) inference of susceptibility, (ii) editing of results (from S to I or R or from I to R but never from I or R to S), and (iii) suppression of results, addition of comments, advice on further tests, and advice on the referral of isolates. After the second draft was open for consultation via the EUCAST public website, EUCAST national breakpoint committees, EUCAST national representatives, industry networks, and experts, version 1.0 was published in April 2008, and version 2.0 was ratified in February 2011. The EUCAST intends that these rules be applied to routine antimicrobial susceptibility tests and that they will make a significant contribution to the quality of reported results. The application of EUCAST expert rules may impose some testing requirements on clinical laboratories. Many rules require the full identification of the organism, even if it is not essential for clinical management. There may be a need to test an extended range of appropriate antibiotics, as interpretive rules may require the testing of agents, which may not be required clinically. There is also a clinical need for access to a set of expert rules, as there are many expert rules, and few individuals are able to remember them all and to apply them consistently.

There are few publications on expert rules, and these publications are more likely to be used as a reference source than for everyday application. The wide range of expert rules means that they are likely to be applied consistently and widely only if they are available as a published set of rules that can be incorporated into computer systems. Rules may be incorporated into laboratory information systems (LISs), but this is limited by the capabilities of the LIS and the ability and interest of individual laboratories in incorporating rules into the LIS. Expert systems are, however, incorporated into several automated susceptibility and inhibition zone reading systems. The purpose of the EUCAST expert rules is to provide a written description of current expert rules. The rules are a comprehensive collection that may be applied manually or incorporated into automated systems. Rules should not conflict with EUCAST MIC breakpoints, but it is appreciated that some antimicrobial agents are not included in EUCAST breakpoints, and many rules have developed over the years in conjunction with other breakpoint systems. Hence, the first version was amended as EUCAST breakpoints were developed and in light of experience with the application of the rules.

One of the authors of this review (T.W.) together with D. Drew wrote the computer program HALMOS in 2008, primarily to handle EUCAST expert rules. The program was written in Visual Basic as a stand-alone, compiled executable file that will run on any recent version of Microsoft (MS) Windows. Parameters are stored in an MS Access database, although MS Access need not itself be installed (but would be required if any of the parameters need to be changed). The user manually selects the organism name or organism group, any antibiotic tested, and the result obtained (S, I, or R). The program edits any intrinsic resistance (S→I, I→R, or S→R but never R→I, I→S, or R→S); with manual input, the program will ask the user whether they wish to accept any edits. The program examines the complete antibiogram, performs an interpretive reading, and then "back-chains" to suggest further antibiotics that should be tested in order to differentiate between possible resistance mechanisms. The program issues warnings and alerts users of exceptional phenotypes. The complexity of functionality is determined only by the number and complexity of the rules in the database. The program is written as a "shell" and, although populated with EUCAST rules, can accept any rule base. Results (and reasoning) are displayed on screen and logged to a file. The program will optionally support local codes for organisms, organism classes, and antibiotics. Its natural language is English, although screen prompts and titles can be altered. The program may optionally process a file (or collection of files) of results (e.g., output from an analyzer) in an agreed format (i.e., it may need to be transformed before use). It is intended that the program be distributed freely when EUCAST, version 2.0, expert rules are published formally.

One of the spin-offs from EUCAST's harmonizing breakpoints is that, if they are set correctly, they could obviate expert rules altogether. As an example, the need for extended-spectrum β-lactamase (ESBL) detection is under challenge based on the supposition that it is possible to set breakpoints of injectable cephalosporins and aztreonam that accurately discriminate which ESBL-producing isolates can and cannot be reliably treated with these drugs. This approach is controversial (132) but has been adopted in slightly different forms by the EUCAST and the CLSI. It is based on limited therapeutic outcome data, pharmacokinetic/pharmacodynamic (PK/PD) data, and the concept that the lower the cephalosporin MIC, the greater the likelihood of successful therapy. Brun-Buisson et al. (35) observed that when isolates had low-level resistance to expanded-spectrum cephalosporins (mode MIC of cefotaxime, 2 μg/ml), cefotaxime was effective in cases of uncomplicated urinary tract infection but failed in major infections at other sites. Rice et al. (208) studied 16 patients with infection with ceftazidimase-producing strains of the *Enterobacteriaceae* (ceftazidime MICs, 64 to 256 μg/ml; cefotaxime MICs, 0.5 to 1 μg/ml). Four patients treated with cefotaxime (including *Escherichia coli* septicemia) were cured. Paterson et al. (194) related the MIC to failure of therapy and noted that if the MIC was ≤2 μg/ml, deaths occurred in 2/14 patients, whereas if the MIC was ≥8 μg/ml, 100% of patients failed treatment and 33% died. Wong-Beringer et al. (290) studied 36 episodes of bloodstream infection with isolates from 21 episodes (ceftazidime MIC ≥ 2 μg/ml) available for analysis. For non-ESBL producers, one failure was seen (ceftazidime monotherapy, MIC of >32 μg/ml), one partial response was seen (ceftazidime monotherapy, MIC of 32 μg/ml), and one success was seen (cefotaxime monotherapy, MIC of 0.5 μg/ml). For ESBL producers, failure was observed with ceftazidime monotherapy in one case (MIC of 32 μg/ml). Kang et al. (135) also related MIC to treatment failure and noted that if the MIC was ≤2 μg/ml, 1/6 patients failed therapy, whereas if the MIC was ≥8 μg/ml, 14/18 failed; the mortalities at 30 days were 1/6 patients if the MIC was ≤2 μg/ml and 7/18 if the MIC was ≥8 μg/ml. Andes and Craig (6) noted that animal model studies suggested that the pharmacodynamic target associated with

efficacy in the treatment of infection by ESBL-producing organisms is the same as that in therapy against non-ESBL-producing bacteria (the drug concentration remains above the MIC for the organism for 50% of the dosing period [50% T > MIC]). Outcomes in relation to MIC in bloodstream infection (42 cases, with monotherapy with cephalosporin and infection with *Klebsiella* spp. or *E. coli*) were as follows: 81% success at 1 μg/ml, 67% success at 2 μg/ml, 27% success at 4 μg/ml, and 11% success at 8 μg/ml. Monte Carlo simulation with ceftriaxone at 2 g every 24 h (q24h) showed 100% target attainment (>50% T > MIC) with MICs of 1 μg/ml and 99% target attainment if the MIC was 2 μg/ml. Bhavnani et al. (20) reported that three patients with ESBL-producing organisms (MICs of 2, 4, and 8 μg/ml) all responded to treatment with cefepime. Bin et al. (23) carried out a prospective controlled clinical study of 22 consecutive cases of bacteremia due to CTX-M-type ESBL-producing *E. coli* with ceftazidime MICs of ≤8 μg/ml. Seven patients were treated with ceftazidime, 8 were treated with imipenem, and 7 were treated with cefoperazone-sulbactam after detection of bacteremia. The treatment success rates were 85.7% with ceftazidime, 87.5% with imipenem-cilastatin, and 71.4% with cefoperazone-sulbactam. All seven patients who received ceftazidime survived, and six of them were cured, although the treatment of one patient with a strain with a ceftazidime MIC of 2 μg/ml failed because of abdominal abscess. Bhat et al. (19) assessed 176 episodes of bacteremia caused by Gram-negative organisms for which patients received cefepime (typically 1 to 2 g every 12 h) as the primary mode of therapy. The outcome (28-day mortality) was dependent on the MIC: 23.3% if the MIC was ≤1 μg/ml, 27.8% if the MIC was 2 μg/ml, 27.3% if the MIC was 4 μg/ml, 56.3% if the MIC was 8 μg/ml, and 53.3% if the MIC was ≥16 μg/ml. For ESBL producers, 2/3 patients died (MIC of 2 μg/ml), 2/3 died (MIC of 4 μg/ml), 1/2 died (MIC of 8 μg/ml), and 0/2 died (MIC of 16 μg/ml). Chin et al. (49) suggested that subgroup analysis excluding *Pseudomonas* spp. and *Acinetobacter* spp. might support a higher susceptible breakpoint for cefepime, i.e., S at ≤4 μg/ml. Taking MIC results into account also negates the delay over a susceptibility result while a supplementary ESBL detection test is performed.

At variance with this approach, there have been reports of therapeutic failures with cefepime associated with MICs of 2 μg/ml or lower (132, 194) and 4 μg/ml in a pediatric patient (20) and with a cefotaxime MIC of 0.75 μg/ml (136). Suankratay et al. (247) evaluated the therapeutic outcome of ceftriaxone treatment of acute pyelonephritis caused by ESBL-producing *E. coli*, *Klebsiella pneumoniae*, or *P. mirabilis* strains and recorded that both clinical (65% and 93%) and microbiological (67.5% and 100%) responses at 72 h after ceftriaxone treatment were poorer in the ESBL-producing group than in the non-ESBL-producing group, respectively ($P < 0.0002$). There is also the concern that instead of the simplicity of cephalosporin and aztreonam susceptibility results being automatically changed to a result of resistant for positive isolates, laboratories face the impossible task of having to overcome the inherent variability of testing of the ESBL-labile drugs to provide precise and accurate results. The problem is that the usual 2-fold error of the MIC test can be greatly amplified in tests with ESBL producers (260). This introduces an enormous potential for an inaccurate reporting of susceptibility results. Several experts remain unconvinced that routine laboratory workers can consistently determine susceptibility to the requisite standards to distinguish MICs of, e.g., 1 versus 2 μg/ml or 4 versus 8 μg/ml of ceftazidime or even that a precisely determined MIC of 2 or 4 μg/ml for ceftazidime or cefepime is predictive of clinical success even if used at high doses. This is especially important in the United Kingdom, because MICs of ceftazidime for *E. coli* ST131 CTX-M-15 strain A (a common lineage) are usually 2 to 8 μg/ml, meaning that some representatives of the lineage would be reported as intermediate, implying that they are "susceptible at high dosage." For some, the paucity and inconsistency of current human data create an impression that infected patients will become experimental guinea pigs to prove or refute a hypothesis, and the care of patients should continue to be based on the proven approach of ESBL detection and editing of susceptibility results.

Machine learning methods have not yet been applied to the inference of antibiotic resistance mechanisms. To date, all expert systems have used rule-based or pattern-based decision trees: the associations between factors and their levels are predefined and fixed (neural nets determine new patterns in data in addition to using prior knowledge); the expert systems find it hard to cope with missing data, and these missing data normally result in a rule failing to be triggered (neural nets compensate for missing data); specific rules must be written to capture ambiguous results (neural nets can detect and correct ambiguous results); rules are triggered in a predefined sequence, and data are processed in a linear fashion (neural nets process data in parallel); and expert systems cannot learn from or comment on previously seen data (neural nets perform pattern recognition and can report how many times a particular antibiogram has been encountered). One of the authors of this review (T.W.) has carried out a successful feasibility study using neural net technology to interpret full MIC profiles. MIC profiles (input data) were analyzed by using Alyuda Forecaster XL embedded into a Microsoft Excel interface, and the system proved to be as robust as the quality of the target data. For this reason, a larger project in collaboration with the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL), Centre for Infections, Health Protection Agency, Colindale, London, United Kingdom, has begun. Neural networks trained using antibiogram and gene profiling data should be able to predict coresident resistance mechanisms, highlight nonexpressed resistance genes, and identify isolates with novel resistance mechanisms, and it is the intention to train neural nets using data from Identibac (Veterinary Laboratories Agency [VLA]) microarrays. The completed software (working title of VIGIL) will be a valuable tool for inferring resistance mechanisms from MIC antibiograms. If successful, the project will be rolled out to interpret disc diffusion results using the new European method.

COMMERCIAL SYSTEMS

Instrumentation in antimicrobial susceptibility testing has been reviewed by Felmingham and Brown (82). Some systems also include so-called "expert" software to improve the quality of interpretations by the filtering of results according to a set of rules (164). Systems known to us include Accuzone (AccuMed International Inc., West Lake, OH) (139); Wider (Francisco

Soria Melguizo, Spain); Aura Image (Oxoid, Basingstoke, United Kingdom) (7); Biomic Vision/V3 (Giles Scientific, New York, NY) (145); BioVideobact (Launch, Longfield, United Kingdom); Mastascan Elite (Mast, Bootle, United Kingdom); Osiris (Bio-Rad, Hemel Hempstead, United Kingdom) (220); ProtoZONE (Don Whitley Scientific, Shipley, United Kingdom), Trek/Sensititre ARIS (Trek Diagnostic Systems, Cleveland, OH); Mini API, ATB Plus Expert, and Vitek Legacy (bioMérieux, La-Balme, France); and SIRSCAN (Becton Dickinson, Oxford, United Kingdom) (171). The three main commercial instruments running expert systems are Phoenix (Becton Dickinson), MicroScan (Siemens), and Vitek 2 (bioMérieux).

ATB Plus Expert

An expert system (cadi-yac), written in Turbo-Prolog and working on IBM PC and Bacanal+ (a Microbiology management software), was used to recognize and correct the phenotypes of antibiotic sensitivity. The knowledge was adapted from two reference works. A routine use of the expert system gave a correct recognition of the enzymatic profile in more than 80% of cases for the β-lactams and more than 98% cases for the aminoglycosides. The mistakes detected by cadi-yac were often interpreted as a deficiency of the API system by human experts. The expert system mistakes (1.5%) were due to composite phenotypes (200).

ATB Plus Expert was tested with 217 strains of the *Enterobacteriaceae*. The strains were selected in order to cover a maximum number of bacterial species and resistance mechanisms. The isolates were tested on Rapid ATB E, Rapid ATB G−, Rapid ATB Ur, ATB G−, and ATB Ur strips. In parallel, a disc diffusion test was performed with five discs of aminoglycosides (kanamycin, gentamicin, tobramycin, netilmicin, and amikacin), and the interpretation was carried out according to the criteria usually followed. Of the 217 strains tested, 122 showed a resistance phenotype. Only the rapid ATB E strips included kanamycin and allowed the detection of APH(3′) phenotypes. Amikacin was not included in the ATB Ur strip; consequently, it was impossible to discriminate AAC(3)-II and AAC(6′) plus AAC(3)-I phenotypes. Twelve strains did not grow within 5 h using the rapid ATB methodology. Not taking into account the problems previously encountered, different phenotypes between the 6 susceptibility tests were found for 16 strains. In 5 cases, the expert system detected an anomaly instead of the correct phenotype, and in 3 cases of unknown phenotypes, the answers were variable. In the other cases, the main difficulty was the detection of isolated resistance to gentamicin [AAC(3)-I phenotype]. The expert system automatically corrected the susceptibility test result according to the phenotype observed (199).

Other studies were aimed at analyzing resistance to some β-lactams among *E. coli* and *K. pneumoniae* clinical isolates and at evaluating ESBL production. One analysis included 137 *E. coli* and 52 *K. pneumoniae* isolates. In an evaluation of ESBL production-detecting tests, the double-disc test (DDT) was found to be more reliable than the ATB ESBL test (8). In another study, 22 ESBL-producing strains of *Enterobacteriaceae* recovered in the authors' hospital were tested by using the Rapid ATB E test coupled with the API V2.1.1 expert system. The expert system detected 90.9% of ESBL-producing strains. Two strains producing SHV-2 or CTX-1 escaped detection by the expert system despite concomitant resistance to aminoglycosides (108).

By using the API ATB 24H system, Ronco and Migueres (214) found that the system was not fully able to detect acquired resistance to oxyiminocephalosporins in strains of the *Enterobacteriaceae* producing ESBLs (CTX-1, SHV-3, and SHV-4). However, the frequency of detection varied with the type of API system (ATB G− or ATB PSE), the nature of the β-lactam (cefotaxime or ceftazidime), and the type of β-lactamase produced. Considering the fact that this new mechanism of resistance must be taken into account, those authors suggested that the most simple method for the detection of oxyimino-β-lactamases is a double-disc synergy test (DDST) between clavulanic acid (CA) plus amoxicillin (Augmentin) and an oxyiminocephalosporin.

Gram-negative pathogens harboring ESBLs are becoming an increasing therapeutic problem in many wards. Tuchilus et al. (270) studied ESBL production by strains of the *Enterobacteriaceae* from Eastern Romania and their antimicrobial resistances. Those authors selected 54 clinical isolates among 1,068 strains of *Enterobacteriaceae* according to their susceptibility spectra (183a). Susceptibility tests were performed by using the Rapid ATB E gallery of the Mini API system (bioMérieux) and by a macrodilution method with Mueller-Hinton agar according to standard procedures of the CLSI. ESBL production was established by using both DDT and the Expert computer program of the Mini API system. The isoelectric points (pIs) of the enzymes were determined. The Expert computer program of the Mini API system confirmed the positive DDT results for all strains. Almost all strains displayed resistance to ampicillin, ampicillin-sulbactam, expanded-spectrum cephalosporins, and aztreonam. By isoelectric focusing, those authors identified 51 strains that had a unique enzyme and 3 *E. coli* strains with two enzymes. According to those results, TEM-type ESBLs were the most common ESBLs.

Wider

Sorlozano et al. (235) found the positive and negative predictive values (NPVs) for the Wider system to be 81% and 98.5%, respectively. They stressed the high incidence of ESBLs in their setting, the predominance of cases in the outpatient setting, and the acceptable detection of ESBLs in *E. coli* and *Klebsiella* spp. by means of the Wider system.

Osiris

Osiris (Bio-Rad) is a system for the reading and interpretation of inhibition zone sizes by disc diffusion (184, 220). It reads, interprets, and is packaged with the Extended Expert Module. This system can identify over 2,700 clinically significant resistance phenotypes, including ESBL, MRSA, and vancomycin-resistant enterococci (VRE), and can expertize and comment on susceptible/intermediate/resistant (SIR) results. It is regularly updated according to CLSI guidelines and according to data from the literature and from microbiology experts.

Bert et al. (18) evaluated the efficacy of the Osiris extended

expert system (EES) for the identification of β-lactam susceptibility phenotypes of *Pseudomonas aeruginosa*. Thirteen β-lactams were tested in four laboratories by disc diffusion with 53 strains with well-characterized resistance mechanisms, including the production of 12 ESBLs. The plates were read with the Osiris system, the results were interpreted with the EES, and the phenotype identified by the EES was compared with the resistance mechanism. The strains were also screened for the presence of ESBLs by DDT. Overall, the EES accurately identified the phenotypes of 88.2% of the strains and indicated an association with several mechanisms for 3.8% of them. No phenotypes were identified for four strains with low-level penicillinase production. Misidentification was observed for two penicillinase-producing strains: one with partially derepressed cephalosporinase production and one overexpressing the MexAB-OprM efflux system. These results indicate that the Osiris EES is an effective tool for the identification of *P. aeruginosa* β-lactam resistance phenotypes, although a specific DDT with reduced disc distances is necessary for the detection of ESBL production by this organism.

In a second study, Bert et al. (17) evaluated the efficacy of EES for the identification of β-lactam susceptibility phenotypes of 50 *E. coli* strains. Overall, the EES accurately identified the phenotype for 78% of the strains, indicated an inexact phenotype for 17%, and could not find a matching phenotype for the remaining 5%. The percentages of correct identification for each resistance mechanism were 100% for inhibitor-resistant TEM and for TEM plus cephalosporinase, 89% for TEM and for ESBL, 71% for cephalosporinase overproduction, and 25% for oxacillinase. The main cause of discrepancies was the misidentification of oxacillinase as an inhibitor-resistant TEM enzyme. The conventional DDT failed to detect ESBL production in two strains, one producing VEB-1 and one producing CTX-M-14, but synergy between cefepime and amoxicillin-clavulanic acid was visible after the distance between the discs was reduced to 20 mm. After the interpretative guidelines of the EES were updated, the percentage of correct phenotype identification increased from 78 to 96%.

Shells

One of the authors of this review (T.W.) contributed to the expert system shells used with the Oxoid Aura (7, 103) and Mastascan Elite systems. The Mastascan Elite expert system is an example of how shells may be used in microbiology, and the system has been in use in the author's laboratory for many years. The expert system is written in Microsoft Access, a relational database, and comprises a series of tables: rules, rule condition groups, rule conditions, rule action groups, and rule actions. The expert system has two components: the maintenance module is used to design, build, and maintain rules, and the application module is used to apply rules to results. Winstanley et al. (289) populated the expert system and evaluated it by using 120 genotypically characterized Gram-negative organisms resistant to oxyiminocephalosporins by a variety of mechanisms. Susceptibility was determined by an agar incorporation method, and putative genotypes were suggested by an interpretive reading of phenotypes. The expert system was able to identify the correct β-lactamase in a single choice for 98 of the 120 isolates (82%) and for an additional 15 isolates within two, or more, choices (12.5%). The detected phenotype was incorrect for 7 isolates (6%), but 3 of these were not inherent to the expert system.

Barry et al. (11) carried out an evaluation of the Vitek 2 system in five United Kingdom laboratories, comparing results with "gold standard" agar dilution MIC data, assessing its ability to recognize resistant phenotypes, and comparing results with those generated by routine antimicrobial susceptibility testing methods. In comparison with the reference MIC method, Vitek 2 gave essential agreements of 304/315 (enterococci), 1,619/1,674 (staphylococci), and 2,937/3,074 (Gram-negative bacilli) isolates, with 96% agreement overall. Corresponding clinical category (SIR) agreements with Vitek 2 were 247/252, 1,496/1,561, and 2,478/2,626 isolates, respectively (95% agreement overall). By use of the Mastascan Elite expert system, category agreements were 58/63, 222/232, and 333/372 isolates for the three organism groups, respectively, with an overall agreement of 95%. In contrast to the Vitek 2 AES, routine microbiology laboratories did not attempt to detect resistance mechanisms for every antibiotic studied. The Vitek 2 AES detected all 19 resistance mechanisms in enterococci; where applicable, Mastascan detected 14. Of 30 resistance mechanisms in staphylococci, the Vitek 2 AES detected 25, compared with 23 detected by Mastascan. Finally, of 44 resistance mechanisms in Gram-negative bacilli, the Vitek 2 AES detected 30, compared with 30 detected by Mastascan.

Vitek 2

Vitek 2 (bioMérieux) is an automated susceptibility testing system enabling rapid (4 to 7 h) determinations of MICs (152, 197, 223). Its improved performance over those of earlier rapid systems is due to the larger number of wells in each card, enhanced optics, and new algorithms based on kinetic analyses of growth data. The Advanced Expert System (AES) provides standardized interpretive reading of these MICs. Unlike previous expert systems, the AES is based upon an extensive knowledge base that comprises over 2,000 phenotypes and 20,000 MIC distributions obtained from published reports, human experts with their own databases on phenotypes, and in-house data at bioMérieux. For each of the recognized phenotypes, a range of MICs is determined and an MIC distribution is defined (93, 128).

In the biological validation phase, the AES examines the antimicrobial susceptibility data and determines if the MICs obtained are consistent with the species identification. If a single error is found, the AES recommends either a change in the identification that will make the outlying MIC consistent or a numerical change in the MIC that will make it consistent with the identification. The AES presumes that (i) an error has occurred in the data generated by the Vitek 2 system, (ii) results were atypical due to the strain, (iii) a "falsely" negative result has occurred (e.g., noninduced β-lactamase), or (iv) an incorrect result was entered manually. A biological correction is recommended by the AES if it detects only a single MIC inconsistency. The AES will recommend the retesting of the isolate if more than one biological correction would be needed to bring the susceptibility in line with the identification or to match phenotypes. The AES may also recommend biological correction based on the phenotype of the organism.

During biological validation, the AES examines the MIC data for each class of antibiotic and determines a phenotype for the isolate by comparing them with MICs held within the database. If the MICs fall within the range expected for a specific phenotype, that phenotype is assigned; if the MICs fall within the ranges expected for more than one phenotype, the AES lists all phenotypes but does not suggest the most likely one.

In the therapeutic interpretation step, the AES assigns an interpretive clinical category (susceptible, intermediate, or resistant) by utilizing one of the five default interpretation guidelines: CLSI, DIN, CA-SFM, phenotypic resistance, or natural resistance. With certain phenotypes, the AES may also suggest a therapeutic correction. Here, the species name and numerical value of the MIC are not altered, but the interpretation is. Since therapeutic corrections do not imply errors in the data, the AES may suggest multiple therapeutic corrections for a single isolate.

Isolates for which any biological or therapeutic corrections have been suggested require human intervention to decide whether corrections should be accepted. The AES also deduces susceptibility to antibiotics not tested based upon the phenotype and susceptibility to antibiotics that have been tested.

As an example, the MICs found for an *E. coli* isolate might be 1 μg/ml for ampicillin, 0.5 μg/ml for cephalothin, 2 μg/ml for cefoxitin, 0.125 μg/ml for cefotaxime, and 0.5 μg/ml for ceftazidime. All these values are compatible with a wild phenotype without significant β-lactamase activity, none are 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, since this phenotype is the best match to all the data. For another *E. coli* isolate, the recorded MICs might be 128 μg/ml for ampicillin, 32 μg/ml for cephalothin,4 μg/ml for cefoxitin, 0.25 μg/ml for cefotaxime, and 32 μg/ml for ceftazidime. In this case, only the cefotaxime value is compatible with a wild phenotype, and only the ampicillin, cephalothin, 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 the editing of the cefotaxime result as "resistant," despite the low MIC. In all cases the Vitek 2 AES prints a report indicating the actual MIC, raw categorization, and the categorization after interpretation. Reasons for any editing are stated, allowing review.

The Vitek 2 system has at least a yearly software update, which includes modifications of the expert system. Depending on the numbers of modifications and when the new guidelines are published, it can take 1 to 2 years from publication before it is released to the field (G. Zambardi, personal communication).

BD Phoenix

The Phoenix system uses a rule-based expert system called BDXpert. The rule base comprises data from current scientific literature as well as from the CLSI, EUCAST, and CA-SFM. Since the introduction of the EUCAST, the DIN standard (Germany) is no longer included among the available standards because it is aligned with EUCAST standards. BDXpert offers expert advice on specific test results, MICs, overall phenotypes, or a combination of these. Before results are evaluated by the inference engine, MICs are transcribed to clinical categories based upon interpretive breakpoints for broth microdilution methods. EpiCenter utilizes two expert systems, BDXpert and BD EpiCARE, to ensure the rapid and accurate reporting of Phoenix identification (ID) and antimicrobial susceptibility testing (AST) results as well as monitoring for emerging resistance. The BDXpert system is a "best-practice" rule set that expertizes the full doubling-dilution MIC results produced by the Phoenix AST system. The BDXpert system is a rule-based software tool that provides expert advice based on the organism ID and AST results obtained by broth microdilution with the BD Phoenix automated microbiology system (Phoenix). BDXpert may alter certain interpretations according to the selected standard, but MIC results are never altered. Most BDXpert rules can be enabled or disabled and set to fire automatically or manually; 1,500 critical rules, e.g., resistance markers, cannot be disabled. In addition, ID/AST results obtained from other systems can be expertized via BD EpiCenter. The distribution of the expertized final report through the laboratory information system (LIS) interface facilitates timely communication to assist the clinician in the selection of appropriate drug therapy.

The BDXpert system is updated 2 to 3 times a year to incorporate changes advocated by various committees around the world. Generally, for each update, there is focus on a specific standard, and this follows the order of release. It takes approximately 6 months from the moment when the standard is released by the committee to the time when this standard is translated into a software update for Phoenix/EpiCenter and is released to the market. Each year, updates include both breakpoints and interpretive recommendations (i.e., expert rules). For the CLSI only, FDA-concordant breakpoints are incorporated by default into the BDXpert system, since manufacturers of AST systems are required by U.S. law to use FDA breakpoints. Nevertheless, customization is possible and easily implemented. Besides what is strictly included in the guidelines, a number of rules are also included to enhance the detection of resistance mechanisms and unusual phenotypes, etc. (T. Payne, personal communication).

MicroScan WalkAway and autoScan

The Siemens WalkAway-40 and -96 SI and autoScan 4 systems utilize broth microdilution trays to determine bacterial identification and susceptibility. Synergies Plus panels combine a rapid (2.5-h) bacterial identification with both read-when-ready (4.5 to 16/18 h) and overnight susceptibility tests. Other panels determine breakpoint susceptibilities or confirm the presence of ESBLs; chromogenic panels are used for the identification of yeasts and fastidious organisms. LabPro Alert System software complements the LabPro Information Management system by automating the detection of atypical results or conditions that warrant infection control or physician review. Rules are customizable, and Alert RuAlert Resolution History

keeps a record of actions taken by the laboratory to confirm and finalize atypical results.

Expert rules are updated with each software and panel update. The alert system (without an expert part) was introduced in version 1.1 and updated in versions 1.5, 1.55 (introduction in Europe), 1.6, and 2.0. The expert system capability was added in version 3. The cefoxitin/inducible clindamycin screen for *Staphylococcus* spp. was added next, which also required an update, and the upcoming panel/software update for EUCAST breakpoints and the EUCAST expert system was released in April 2010. The aim of Siemens is to capture as much of what is a current antibiotic resistance concern in the next available update. There are currently four versions, FDA, two non-FDA, and Japanese versions, and each version is updated every 12 to 18 months. Interpretations are also updated as necessary when a panel is added; comments are retained, but new ones are added occasionally. The user can add, edit, and/or update any of above-described features, and user edits are never overridden (B. Zimmer, personal communication).

GRAM-POSITIVE COCCI

There is a marked contrast in the literature between the extended discussion of resistance detection in Gram-negative organisms using expert systems and the detection of resistance in Gram-positive organisms. To some degree, this results from expert systems being more focused on the challenge of detecting resistance in the former organisms. However, the detection of methicillin resistance in staphylococci, for example, relies mainly on the ability of the instrument to detect resistance to a single antibiotic rather than the interpretation of an antibiogram by use of an expert system.

Staphylococci and Methicillin

A number of papers have addressed the abilities of automated instruments to detect resistance to methicillin (or surrogate marker antibiotics) in staphylococci. Results for the Automicrobic, Vitek Legacy, and Vitek 2 (3, 12, 40, 54, 66, 83, 91, 105, 106, 112, 115, 116, 123, 129, 131, 140, 141, 146, 148, 165, 168, 169, 186, 205, 213, 219, 230, 249, 250, 264, 278, 294, 297, 298, 300, 301); Phoenix (44, 111, 129, 172, 204, 238, 249), and autoScan/MicroScan (4, 28, 39, 40, 54, 69, 104, 116, 131, 205, 211, 230, 233, 249, 250, 295, 296) systems are shown in Table 1. Many of these reports refer specifically to difficult-to-detect strains with borderline resistance or to clones characteristic of different countries. For the majority of these systems, the expert system is not central to the performance of the instrument; i.e., the effectiveness is related directly to the ability to detect resistance. Some papers referred to the expert system altering oxacillin results based upon cefoxitin resistance. Earlier papers used phenotypic resistance to methicillin or oxacillin as a comparator; later papers relied on the detection of the *mecA* gene. Vitek Legacy, Vitek 2, MicroScan, and Phoenix all demonstrated satisfactory sensitivities and specificities, and, not surprisingly, these data improved with improvements to both hardware and software.

Staphylococci and Vancomycin or Linezolid

The reporting of vancomycin resistance in *Staphylococcus* spp. has enormous therapeutic and epidemiological consequences. During the last several years, a series of staphylococcal isolates that demonstrate reduced susceptibility to vancomycin (or other glycopeptides) or to linezolid have been reported, and several papers addressed the abilities of automated instruments to detect this resistance. Tenover et al. (254) selected 12 staphylococci for which the vancomycin MICs were $\geq$4 μg/ml or the teicoplanin MICs were $\geq$8 μg/ml and 24 control strains for which the vancomycin MICs were $\leq$2 μg/ml or the teicoplanin MICs were $\leq$4 μg/ml to determine the abilities of commercial susceptibility testing procedures and vancomycin agar screening (VScr) methods to detect reduced glycopeptide susceptibility. By PCR analysis, none of the isolates with decreased glycopeptide susceptibility contained known *van* vancomycin resistance genes. Broth microdilution tests incubated for a full 24 h were best at detecting strains with reduced glycopeptide susceptibility. Disc diffusion did not differentiate the strains inhibited by 8 μg/ml vancomycin from more susceptible isolates. MICs were reflected correctly in Sensititre MD panels read visually and Combo 6 panels on the MicroScan WalkAway system (4 to 8 μg/ml) but not in Rapid POS Combo 1 panels or Vitek GPS-101 cards (version R05.01), where Vitek results were 4 μg/ml for all strains for which the vancomycin MICs were $\geq$4 μg/ml. Thus, strains of staphylococci with reduced susceptibility to glycopeptides, such as vancomycin, are best detected in the laboratory by nonautomated quantitative tests incubated for a full 24 h. Commercial vancomycin agar screening plates can be used to detect these isolates, although there are no commercially available vancomycin screen plates that can be used to detect *S. aureus* strains for which the vancomycin MIC is 4 μg/ml in the United States.

Webster et al. (282) studied a series of 10 *S. aureus* isolates with vancomycin MICs from 2 to 8 μg/ml: they were detected by Phoenix and Etest but not by MicroScan, PASCO, Vitek 2, or Sensititre.

Hsu et al. (114) showed that the screening of MRSA isolates by use of modified Etest-based methods detected potential hetero-glycopeptide-intermediate *Staphylococcus aureus* (hGISA) phenotypes for 9% (8/92) of the isolates. Almost all isolates with an hGISA phenotype had a high MIC, as determined by Etest, MicroScan, and Vitek. In contrast, a high MIC was observed for only 4/8 isolates (50%) by broth microdilution.

Behera and Mathur (13) evaluated Vitek software, version 2.01. Of 105 isolates of staphylococci tested, the Vitek, version 2.01, software gave 16 (15%) false vancomycin-intermediate/resistant phenotypes. Laboratories using automated systems for routine microbiological susceptibility testing must confirm such resistance results by validated methods.

Swenson et al. (248) compared the results obtained with six commercial MIC test systems (Etest, MicroScan, Phoenix, Sensititre, Vitek Legacy, and Vitek 2) and three reference methods (agar dilution, disk diffusion, and VScr) with the results obtained by the CLSI broth microdilution (BMD) reference method for the detection of vancomycin-intermediate *S. aureus* (VISA). A total of 129 *S. aureus* isolates (vancomycin MICs by

TABLE 1. Detection of resistance to methicillin (or surrogate marker antibiotics) in staphylococci by Automicrobic, Vitek Legacy, Vitek 2, Phoenix, and autoScan/MicroScan

Instrument and/or card	Organism	% (no.) of isolates with methicillin resistance status[a]					Reference
		Methicillin-oxacillin R	Methicillin-oxacillin S	Methicillin-oxacillin R (*mecA*$^+$)	Methicillin-oxacillin S (*mecA* negative)	Raised oxacillin MIC (*mecA* negative)	
GPS-MIC cards	CoNS	99 (104)	100 (102)				298
	S. aureus	100 (26)	100 (167)				
GPS-MIC cards	*S. aureus*	88 (105)	100 (52)				66
GPS-MIC cards	*S. aureus*	85–88 (100)					148
GPS cards	*S. aureus*	23–95 (100)					
GPS cards	CoNS (*S. epidermidis*)	81 (62)					294
GPS-MIC cards	CoNS (*S. epidermidis*)	33 (49)	100 (10)				54
	S. aureus	71 (21)	100 (7)				
GPS cards	*S. aureus*	99.5 (29)	99.8 (134)				106
GPS-MIC cards	*S. aureus*	100 (222)	84 (176)				105
Not stated	CoNS	100 (79)	100 (21)				278
GPS-SA	*S. aureus*			86 (254); 93.7 after ES			230
GPS-SA	*S. aureus*	100 (67)					140
	CoNS	100 (47)					
GPS-SA	*S. aureus*					86 (51)	141
GPS-503	*S. aureus*			95.3 (64)			91
	CoNS			80.3 (76); all not detected had oxacillin MICs ≤ 2.0 μg/ml			
GPS-SA Gram-Positive (software version VTK-R03.01)	CoNS		93.1 (131) (9 isolates were *mecA*)				205
GPS-SA	CoNS			89.9 (99)			116
Not stated	CoNS			98 (99)			168
GPS-107	*S. aureus*			95 (19)	97 (36)		250
GPS-SV	CoNS			98 (84)			165
GPS-107				100 (84)			
GPS-105	CoNS			96.2 (79)	100 (61)		169
VTK-RO7.01 software GPS-106	CoNS			95.8 (95)	85.7 (28)		300
VTK-RO7.01 software GPS-106	*S. aureus*			99.0 (98)	100 (101)		301
GPS-106 (Vitek 1)	*S. aureus*			99.0 (203)	100 (107)		219
AST-GP55 (Vitek 2)				99.5 (203)	97.2 (107)		
Not stated	*S. aureus*			94 (83)			83
AST-P515	CoNS			96.0 (124)			112
GPS-105	CoNS			99.4 (158)	92.5 (134)		115
Not stated	*S. aureus*			96.1 (51)	92.9 (14)		146
GPS-105	CoNS			100 (89)	100 (104)		297
VTK-RO7.01 software							
AST-P515	CoNS			91 (70)			3
AST-P507	CoNS, *S. aureus*			99.2 (265)	96.2 (53)		186
GPS-105	CoNS			90 (70)			40
GPS-109 (Vitek Legacy)	*S. aureus*			75 (79)	92.9 (56)		249
AST-GP55/61 (Vitek 2)				91.1 (79)	75 (56)		
AST-P549	*S. aureus*			97.5 (157)	100 (56)		213
AST-P559	*S. aureus*			98.8 (250)	100 (51)		264
AST-GP66	*S. aureus*			99.8 (448); in 7 cases, ES changed result of S to oxacillin to R based on cefoxitin result)			129
AST-P549	*S. aureus*			99.0 (104); in 5 cases, ES changed result of S to oxacillin to R based on cefoxitin result			131
AST-GP66	CoNS, *S. aureus*						123
Oxacillin				93.8 (259)	77.9 (540)		
Cefoxitin				94.6 (259)	93.5 (540)		

Not stated	CoNs (*S. lugdunensis*)				96.7 (60)	12
MicroScan	*S. aureus*	88 (25)				4
MicroScan	*S. aureus*	100 (73)				28
MicroScan	*S. aureus*	86 (49)				104
MicroScan	*S. aureus*	76 (21)				54
Pos MIC	*S. epidermidis*	92 (49)				
MicroScan	*S. aureus*	68 (100) at 24 h				211
Gram-Positive panel		85 (100) at 48 h				
MicroScan Pos MIC	*S. aureus*	100 (23) at 24 h (additional 7 isolates at 48 h)				296
	CoNS	99.4 (162)				
MicroScan						230
Rapid Positive MIC1	*S. aureus*			93.3 (254)	99.2 (252)	
MicroScan	*S. aureus*	96.7 (92)				295
Rapid Positive MIC1	CoNS	72 (100) (22 did not grow in the panels)				
MicroScan	*S. aureus*					69
Rapid Positive MIC1		100 (83)	100 (169)			
Overnight Combo type 6		100 (83)	95.5 (169)			
MicroScan	CoNS			78.8 (99) at 24 h		116
Gram-Positive Combo type 6				86.9 (99) at 48 h		
Autoscan-4 Gram-Positive Combo type 6	CoNS		87.7 (57) (7 were *mecA*)			205
MicroScan						250
Pos Combo 10 panels	*S. aureus*			74 (19)	97 (36)	
MicroScan rapid panels				90 (19)	86 (36)	
MicroScan Conventional Pos Combo 12	*S. aureus*			100 (22)		233
MicroScan Positive Combo 13	CoNS			99.1 (121)	85.1 (54)	39
MicroScan panel PC-13	CoNS			88.57 (70)		40
MicroScan WalkAway Pos MIC type 20A, oxacillin	*S. aureus*			88.6 (79)	96.4 (56)	249
MicroScan WalkAway Pos MIC 24 panel	*S. aureus*			94.2 (104)		131
Phoenix PMIC/ID-6	CoNS			99.2 (124)	98.7 (76)	111
Phoenix	*S. aureus*			100 (96)	100 (127)	238
PMIC/ID-14	CoNS			99 (210)	91.7 (60)	
Phoenix PMIC/ID-33	*S. aureus*	97.5 (157); 98.1 after ES				44
Phoenix	CoNS			91.5 (71), oxacillin	96.8 (64), oxacillin	204
PMIC/ID-52				93 (71), cefoxitin	100 (64), cefoxitin	
				100 (71), moxalactam	100 (64), moxalactam	
Phoenix	*S. aureus*			97.5 (79), cefoxitin >8 μg/ml	100 (56), cefoxitin >8 μg/ml	249
PMIC/ID-25				91.1 (79), cefoxitin >16 μg/ml	100 (56), cefoxitin >16 μg/ml	
				67.1 (79), oxacillin	96.4 (56), oxacillin	
Phoenix	*S. aureus*			99.2 (448), oxacillin	99.4 (172), oxacillin	129
PMIC/ID-102				100 (448), cefoxitin	100 (172), cefoxitin	
Phoenix	*S. aureus*			100 (347), cefoxitin + oxacillin	99.86 (719), cefoxitin + oxacillin	172
PMIC/ID	CoNS			100, oxacillin	88.4, oxacillin	

[a] Abbreviations: CoNS, coagulase-negative staphylococci; ES, expert system; R, resistant; S, susceptible.

a previous BMD test less than or equal to 1 μg/ml [$n = 60$ strains], 2 μg/ml [$n = 24$], 4 μg/ml [$n = 36$], or 8 μg/ml [$n = 9$]) were tested. The results of BMD with Difco Mueller-Hinton broth (MHB) were used as the standard for data analysis. Essential agreement (percent ± 1 dilution) ranged from 98 to 100% for all methods except for the Vitek Legacy system, for which it was 90.6%. Of the six commercial MIC systems tested, Sensititre, Vitek Legacy, and Vitek 2 tended to categorize VISA isolates as susceptible (i.e., they undercalled resistance); MicroScan, Phoenix, and Etest tended to categorize susceptible strains as VISA; and Vitek Legacy tended to categorize VISA strains as resistant (i.e., it overcalled resistance). Disc diffusion categorized all VISA strains as susceptible. No susceptible strains (MICs ≤2 μg/ml) grew on the VScr, but all strains for which the vancomycin MICs were 8 μg/ml grew on the VScr. Only 12 (33%) strains for which the vancomycin MICs were 4 μg/ml grew on the VScr. The differentiation of isolates for which the vancomycin MICs were 2 or 4 μg/ml was difficult for most systems and methods, including the references. Nadarajah et al. (179) compared 4 methods to detect VISA. Of the 20 VISA strains, susceptible endpoints of 2 μg/ml were found for 7 strains by the CLSI BMD method, for 2 strains by MicroScan, for 1 strain by Trek Sensititre, and for no strains by Etest. Comparison with the CLSI method showed essential agreements of 95% or more for Etest, MicroScan, and Trek; categorical agreement was as follows: 60% for Etest, 65% for MicroScan, and 60% for Trek. Reliance on a single automated method for the determination of vancomycin MICs could lead to a misclassification of some VISA isolates as being vancomycin susceptible. Those authors concluded that at least 2 methods, including the Etest, should be used when confirming a VISA result because of slight differences in results from different methods around the endpoints of 2 and 4 μg/ml.

Tenover et al. (255) studied 17/50 enterococci and 15/50 staphylococci not susceptible to linezolid: MicroScan results showed the highest category agreement (96%). The overall categorical agreement levels for Vitek 2, Etest, Phoenix, disc diffusion, and Vitek were 93%, 90%, 89.6%, 88%, and 85.9%, respectively. The essential agreement levels (results within ±1 doubling dilution of the MIC determined by the reference method) for MicroScan, Phoenix, Vitek 2, Etest, and Vitek were 99%, 95.8%, 92%, 92%, and 85.9%, respectively. The very-major-error rates for staphylococci were the highest for Vitek (35.7%), Etest (40%), and disc diffusion (53.3%), although the total number of resistant isolates tested was small. The very-major-error rate for enterococci with Vitek was 20%.

Enterococci and Vancomycin

Again, improvements in instruments, cards, and software have resulted in a better detection of vancomycin resistance conferred by different mechanisms (VanA, VanB, and VanC) in enterococci. Results for Vitek Legacy and Vitek 2 (1, 74, 75, 77, 95, 105, 113, 143, 182, 189, 217, 218, 275, 286–288, 299, 304) and for MicroScan (47, 64, 77, 113, 117, 286) are shown in Table 2.

Jett et al. (122) identified factors contributing to the inability of the Vitek Gram-positive susceptibility (GPS) system to reliably detect VanB-type resistance among enterococci. To some extent, the accuracy of the GPS system depended on a particular strain's level of resistance. Growth medium had the most notable effect on the detection of resistance. Medium-based strategies should be explored for the enhancement of resistance detection among commercial systems.

Biochemical identification of enterococci to the species level is an important step in distinguishing VanA- and VanB-type resistances (*E. faecalis* and *E. faecium*) from VanC-type resistance (*Enterococcus casseliflavus* and *Enterococcus gallinarum*). Ramotar et al. (206) compared several routine phenotypic tests to determine the species identity of clinical enterococci, and a PCR assay for the *van* ligase gene was used to confirm the identification of VanC-type VRE. The Vitek Gram-positive identification card identified 53/60 (88%) *E. faecalis* and *E. faecium* strains and 81/141 (57%) VanC-type VRE without additional testing. Another 32 VanC-type VRE required additional testing (e.g., motility and pigmentation) for correct identification. However, 7 of these 32 VanC-type VRE were nonmotile. Acidification by1% methyl-alpha-D-glucopyranoside (μGP) is suggested as a simple and less costly test for the identification of these isolates.

Recently, *Enterococcus casseliflavus* and *Enterococcus gallinarum* strains were isolated from two different urine samples from a patient, and they were reported as being resistant to teicoplanin, although the MIC was less than 1.0 μg/ml (16). Upon examination of the preliminary reports, a change from susceptible to resistant for teicoplanin by the automated Phoenix BDXpert system in accordance with rule 1099 of that system was observed. Rule 1099 under the expert trigger rules states that "*E. casseliflavus* or *E. gallinarum* is intrinsically low-level resistant to vancomycin and teicoplanin (VanC)," and because of the identified bacteria, the exchange of the teicoplanin result from susceptible to resistant was made according to this rule. *E. casseliflavus* and *E. gallinarum* strains have the chromosomal nontransferable *vanC* operon and are intrinsically low-level resistant to vancomycin; however, they are susceptible to teicoplanin, and the editing of the teicoplanin result to resistant was therefore inappropriate.

In a study by Pendle et al. (196), isolates of *E. faecium* from urine, tested by the CLSI disc diffusion method, were apparently susceptible or intermediate to vancomycin upon primary testing. Phoenix 100 identified all isolates as being vancomycin resistant, although the MICs, measured by Etest, were in the susceptible range for 3 of 16 isolates. A reduction of the vancomycin concentration in screening media substantially increased the sensitivity for the detection of VRE. Isolates were characterized as being of the *vanB* genotype by PCR and were indistinguishable from each other by pulsed-field gel electrophoresis. VRE with low-level inducible resistance can be missed by routine screening methods.

Raponi et al. (207) tested the susceptibilities of 30 *E. faecium* strains to teicoplanin, vancomycin, and linezolid by Vitek 2, Phoenix, Etest, broth microdilution, and disc diffusion. The *vanA* and *vanB* resistance genes and the 23S rRNA G2576T mutation were detected by PCR and PCR-restriction fragment length polymorphism (RFLP) analysis, respectively. Rates of resistance to teicoplanin ranged from 3% for Vitek 2 to 57.6% for the Phoenix test, and rates of resistance to vancomycin ranged from 56.7% for Vitek 2 to 86.7% for Phoenix. Only 2 out of 25 strains carrying the *vanA* gene were unequivocally recognized as being of the VanA type (resistant to both van-

TABLE 2. Detection of vancomycin resistance in enterococci by Vitek Legacy, Vitek 2, and MicroScan

Instrument/card	% (no.) of strains with vancomycin resistance status[a]		Reference
	R	S	
GPS-M		99.0 (398)	105
GPS-A MIC 32-64 μg/ml	0 (3)		217
GPS-A *E. gallinarum* (MIC 16–32 μg/ml)	0 (2)		218
E. faecalis, *E. faecium* (MIC 128–256 μg/ml)	100 (3) with 10× inoculum		
E. faecium (MIC 2048 μg/ml)	100 (1)		
GPS-TA	72 (98)	100 (136)	286
GPS-TA software version 7.1	98 (252)	95 (122)	287
GPS-TA	91.5 (47)	96.2 (53)	304
GPS-TA			299
vanA	81.3 (27)		
vanB	42 (31)		
GPS-TA			77
vanA	100 (50)		
vanB	47 (15)		
vanC1	72 (50)		
vanC2	67 (30)		
GPS-101			
vanA	100 (50)		
vanB	100 (15)		
vanC1	88 (50)		
vanC2	73 (30)		
GPS-TA	100 (39), clonally related		113
GPS-TB R05.03 software	97.9 (97)	99.4 (313)	288
GPS-418			189
vanB	95 (20)		
AST-P516	93.9 (99)		95
AST-P516		100 (50)	275
vanA	100 (50)		
vanB	93 (15)		
vanC1	88 (50)		
vanC2	93 (30)		
AST-P516, software version 1.02	100 (35)		143
Vitek 2 (card not stated)		100 (20)	74
vanA	96.8 (31)		
vanB	95.8 (24)		
vanC1	100 (20)		
vanC2	100 (10)		
AST-P524			75
vanA	100 (62)		
vanB	100 (9)		
vanC	100 (4)		
AST-534 software version 4.01			1
vanA	98.5 (66)		
vanB	100 (14)		
vanC	100 (40)		
AST-P546 Vitek2 Compact software version V2C 1.01			182
vanA	100 (25)		
vanB	100 (25)		
vanC	100 (4)		
Microscan	93 (98), WalkAway	98 (136), WalkAway	287
Pos MIC 6	99 (98), visual	96 (136), visual	

Continued on following page

TABLE 2—*Continued*

Instrument/card	% (no.) of strains with vancomycin resistance status[a]		Reference
	R	S	
Microscan Pos MIC 6	64 R isolates correct 19 I isolates called S (motile enterococci misidentified, additional tests required)	8/315 isolates called I	286
Microscan Pos MIC 8	98.8 (40)		47
Microscan Overnight Pos Combo type 6			77
vanA	100 (50)		
vanB	100 (15)		
vanC1	76 (50)		
vanC2	7 (30)		
Rapid Pos Combo type 1			77
vanA	100 (50)		
vanB	53 (15)		
vanC1	86 (50)		
vanC2	90 (30)		
Microscan GP-6	100 (39), clonally related		113
MicroScan WA96; Positive Combo Panel type 11	100 (14)	100 (362)	64

[a] Abbreviations: CoNS, coagulase-negative staphylococci; ES, expert system; R, resistant; S, susceptible.

comycin and teicoplanin). The strain with the G2576T mutation (in multiple alleles carrying 23S rRNA) showed resistance to linezolid by disc diffusion, Vitek 2, and broth dilution (MIC of >8 μg/ml) but was susceptible when tested by Phoenix and Etest (MIC ≤4 μg/ml).

Enterococci and Aminoglycosides

Several workers have evaluated the ability of the Automicrobic and Vitek 2 (38, 48, 95, 113, 166, 173, 215, 216, 251, 276, 283) or MicroScan (47, 64, 92, 113, 166, 178, 185, 240, 251, 283, 286, 293) system to detect high-level resistance to gentamicin and streptomycin in enterococci (Table 3). With more recent studies at least, sensitivity and specificity data were acceptable for both gentamicin and streptomycin.

Resistance to Macrolides, Lincosamides, and Streptogramins B

Tang et al. (252) carried out a prospective study of erythromycin and clindamycin resistance with 304 consecutive group B streptococci (GBS). According to two automated susceptibility testing systems, Vitek Legacy and Vitek 2, and double-disc agar diffusion, 80% were susceptible to both erythromycin and clindamycin. However, for inducibly macrolide-lincosamide-streptogramin B (MLS_B) ($iMLS_B$)-resistant isolates, the accuracies of the Vitek Legacy and Vitek 2 systems were 5.6% and 94.4%, respectively. In light of these results, those authors recommended that GBS be routinely tested by Vitek 2 or by double-disc diffusion (DDD) (rather than by Vitek Legacy).

Turng et al. (272) tested a total of 182 *Staphylococcus* strains (148 *S. aureus* strains, 12 *S. epidermidis* strains, and 22 other coagulase-negative staphylococci) in Phoenix panels containing erythromycin and clindamycin. CLSI, CA-SFM, or DIN breakpoints were used to interpret Phoenix MIC results. The double-disc diffusion D-zone test was used as the reference for the determination of the inducibly MLS_B-resistant phenotype. The Phoenix erythromycin and clindamycin MIC values were interpreted based on the standard selected. The BDXpert rules were executed, and applicable expert messages were displayed. The Phoenix system correctly detected 38 out of 43 constitutive MLS_B ($cMLS_B$) phenotypes compared to the D-zone test results. Four $cMLS_B$ strains were interpreted by the BDXpert as having potential $iMLS_B$/efflux phenotypes. A total of 72 $iMLS_B$ and 22 efflux phenotype isolates were all reported by the BDXpert system as having the $iMLS_B$/efflux phenotype, and the users were alerted to perform the D test before reporting of the clindamycin results. The clindamycin interpretation was suppressed in these isolates. The CLSI, CA-SFM, or DIN criteria showed identical detections and interpretations of the MLS_B-resistant phenotype by the Phoenix and BDXpert systems.

Bemer et al. (15) evaluated the performance of the Vitek 2 system versus agar dilution for testing the susceptibilities of *S. aureus* and *S. epidermidis* strains to MLS_B. Eighty clinical isolates were selected according to their resistance phenotypes and genotypes. Results for erythromycin and clindamycin showed 100% agreement; results for lincomycin showed an agreement of 78%, with 1 very major error and 17 minor errors; and results for pristinamycin showed an agreement of 46%, with 1 major error and 43 minor errors. Most isolates resistant to lincomycin and streptogramin A (LS_A phenotype) were falsely susceptible to lincomycin and intermediately resistant or resistant to pristinamycin by Vitek 2. No resistance genes were detected. Most (80%) isolates resistant constitutively to MLS_B ($cMLS_B$ phenotype) were falsely intermediately resistant to pristinamycin with the Vitek 2 system. The *erm*(A)

TABLE 3. Detection of high-level resistance to gentamicin and streptomycin in enterococci by Automicrobic, Vitek 2, and MicroScan

Instrument/card	% (no.) of isolates				Reference(s)
	Gentamicin at 500/1,000 μg/ml		Streptomycin at 2,000 μg/ml		
	R	S	R	S	
AMS-Vitek GPS-TA	81 (32)	100 (51)	100 (35)	94 (48)	215, 216
AMS-Vitek GPS-TA	90 (63)		78 (86)		251
AMS-Vitek GPS-TA	82 (112)	100 (122)	90 (136) 100 (52 ribosomal) 83 (84 enzymatic)	96 (98)	283
AMS-Vitek GPS-TA	97.3 (37)		79.2 (34)		173
AMS-Vitek GPS-TA	95 (41)		82 (66)		166
AMS-Vitek GPS-TA	100 (31)	100 (177)	100 (51)	96.8 (157)	38
AMS-Vitek GPS-TA	100 (107)	100 (141)	99 (96)	100 (152)	48
AMS-Vitek GPS-TA	100 (63)	99.1 (227)	100 (91)	79.4 (199)	276
GPS-TA	100 (53)	97 (35) (1 isolate failed to grow)	97.6 (41) (1 isolate failed to grow)	100 (47)	113
Vitek 2 AST-P516	98.7 (47)		100 (68)		95
MicroScan Pos-MIC2 panels	15 (13)		33 (18)		240
MicroScan Type 2 aminoglycoside synergy	84 (63)		31 (86)		92
MicroScan Type 5 aminoglycoside synergy	90 (63)		41 (86)		
MicroScan Pos-MIC6 panels	95 (63)		85 (86)		251
MicroScan Type 5 panels with MHB	100 (112)	100 (123)	93 (137)	100 (98)	283
MicroScan Modified type 5 panels with dextrose phosphate broth	100 (112)	100 (123)	98 (137)	100 (98)	283
MicroScan Pos-MIC6 panels	42 (41), automated 80 (41), visual at 18 h 97 (41), visual at 48 h		64 (66), automated 77 (66), visual at 18 h 84 (66), visual at 48 h		166
MicroScan Pos-MIC6	45 (106), WalkAway 78 (106), visual	100 (128), WalkAway 100 (128), visual	49 (126), WalkAway 82 (126), visual	99 (108), WalkAway 99 (108), visual	286
MicroScan Pos MIC	100 (25), rapid 100 (25), overnight	96, rapid 100, overnight	100 (24), rapid 96 (24), overnight	100, rapid 100, overnight	185
MicroScan Pos Combo type 6	90.2 (41) at 18 h 95.1 (41) at 48 h		64.6 (82) at 18 h 90.2 (82) at 48 h		293
Rapid Pos Combo type 1	97.5 (41)		97.5 (82)		
MicroScan Pos MIC 8	96.9 (34)		94.8 (68)		47
GP-6	100 (53)	100 (35)	100 (41)	100 (47)	113
MicroScan WA96; Positive Combo Panel type 11	96.4 (83)	98.9 (296)	90.6 (53)	98.4 (328)	64
MicroScan POS Combo Panel type 13	96 (124)	99.9 (691)	98 (255)	98.9 (560)	178

gene was more common than *erm*(C). Resistance to pristinamycin alone (SgA SgB PT phenotype) or associated with either lincomycin resistance (L SgA SgB PT phenotype) or constitutive MLS_B resistance (MLS_{BC} SgA PT phenotype) was well characterized without discordant results. Resistance to pristinamycin was always associated with resistance to streptogramin A, encoded by the *vga*(A), *vga*(B), *vgb*(A), and *vat*(A) genes in association with the *erm*(A) or *erm*(C) gene.

Lavallee et al. (151) studied inducible clindamycin resistance in *Staphylococcus* spp. and showed that the sensitivity and specificity of the Vitek 2 card were 93 and 100%, respectively, not as sensitive as the double-disc diffusion method at 24 h.

GRAM-NEGATIVE BACILLI

It is clear that the most informative discussion in the literature is associated with studies using the most fully characterized test strains. It also follows that the use of strains characterized by less-than-perfect confirmatory tests (particularly the early studies) will have a direct bearing on conclusions, and one must be aware of the caveats of determining sensitivity and specificity values using these test strains. Some studies merely confirm ESBLs detected by automated instruments (e.g., see references 62 and 175), and negative predictive values and specificity cannot be determined from these studies. Different tests are often used as reference methods to confirm a presumptive ESBL producer. Some workers (e.g., see references 175 and 277) used phenotypic methods to detect ESBL production. Others, e.g., Thomson et al. (259), used enzyme characterization as the reference, whereas Wiegand et al. (285) used biochemical and molecular characterization. Genotypic determination of the *bla* gene family is the most reliable procedure to identify ESBL-producing *Enterobacteriaceae*, but its integration into the routine diagnostic process is not feasible because of its cost and labor-intensiveness. Hence, in most microbiology laboratories, the ESBL Etest has become the most commonly employed confirmatory test. It has an accuracy of about 94% compared with that of the molecular identification of ESBLs (285). In a study by Farber et al. (81), the ESBL Etest had false-positive results for 6% of the genotypically confirmed ESBL-positive specimens. Mismatches were found for two CTX-M-positive *Klebsiella oxytoca* isolates and for strains carrying two or more *bla* genes (TEM and SHV or TEM, SHV, and OXA). Most performance analyses of automated systems have largely stressed their sensitivity by challenging them with known ESBL producers; Hope et al. (109) tested the predictive value of a positive result, assessing what proportion of the isolates identified as being ESBL producers could be confirmed by reference testing. The majority of evaluations of expert systems and the *Enterobacteriaceae* have centered on ESBLs and AmpC enzymes only.

Detection of Extended-Spectrum β-Lactamase in Gram-Negative Organisms Producing No or Low Levels of AmpC

A number of studies have sought to determine the reliability of automated systems for ESBL detection in the *Enterobacteriaceae*, most with satisfactory results. These studies, however, have involved primarily *E. coli* and *K. pneumoniae*, i.e., organisms producing no or very low levels of the AmpC enzyme.

Vitek. Vedel et al. (277) determined the β-lactam susceptibilities of 300 strains of clinically significant species of the *Enterobacteriaceae* displaying intrinsic and acquired resistance mechanisms (according to disc diffusion tests) by using a rapid automated susceptibility method associated with an expert system. For every strain, the conclusion of the expert analysis was compared with the commonly accepted interpretation of disc diffusion results. Of the 300 strains, 275 were similarly interpreted (91.7% agreement). The susceptible and intrinsic β-lactam-resistant phenotypes (wild phenotypes) were equally recognized by both methods. Similarly, the results of the two methods concurred for most of the acquired resistance phenotypes. However, for 25 strains (8.3%) the results diverged. The expert system proposed an erroneous phenotype (5 strains); several phenotypes, including the correct one (17 strains); or no phenotype (1 strain). For two strains, intrinsic resistance was not detected at first by the automated method but was subsequently deduced by the expert analysis according to bacterial identification. These results demonstrate that a satisfactory interpretive reading of automated antibiotic susceptibility tests is possible in 4 to 5 h but requires careful selection of the antibiotics tested as phenotypic markers, e.g., cefpodoxime, ceftazidime and cefotaxime, cefoxitin, cefepime or cefpirome, and cephalosporins with or without β-lactamase inhibitors, etc.

Midolo et al. (175) compared three methods of confirming the presence of an ESBL with initial detection by the Vitek AutoMicrobic System (AMS). Gram-negative bacteria that were flagged as being ESBL positive in the Vitek GNS card, or were suspected of harboring an enzyme, were further tested by (i) a combination disc test using cefpodoxime, ceftazidime, and cefotaxime with and without clavulanate; (ii) a cefotaxime ESBL Etest; and (iii) the Jarlier keyhole method with cefpodoxime (10 μg), cefotaxime (5 μg), and aztreonam (30 μg) placed 15 mm away from an amoxicillin-clavulanate (co-amoxiclav) (30-μg) disc. Of the 52 isolates investigated, 50 were positive by Vitek. Twenty-eight (56%) were confirmed by other methods (true positives). Of the 44% Vitek-positive/confirmatory test-negative isolates (false positives), eight were *E. coli* (53% of all *E. coli* strains tested). The majority of other false-positive isolates were *Klebsiella oxytoca* isolates (24% overall), which were all Vitek and Etest positive but negative by the combination disc test. Those workers concluded that all strains that were ESBL positive by Vitek should be confirmed by the combination disc test using all three antibiotics. This will enable the differentiation of "true" ESBL-positive organisms from false-positive organisms, including K1 hyper-β-lactamase-producing *K. oxytoca* and AmpC-producing organisms. The cefpodoxime combination discs gave the best differentiation in this study, with only one ESBL organism being missed. While there is a phenotypic method to distinguish K1 overproducers from plasmid-mediated ESBL producers, it seems valid to treat K1-overproducing *K. oxytoca* isolates as true false-positive isolates when they are identified as ESBL-producing isolates.

Sanders et al. (221) assessed the abilities of the Vitek ESBL test (ceftazidime and cefotaxime alone at 0.5 μg/ml and in combination with clavulanic acid at 4 μg/ml) and a double-disc test (cefotaxime, ceftazidime, aztreonam, and ceftriaxone with and without clavulanic acid) to detect ESBLs in strains of *Enterobacteriaceae* (mainly *E. coli* and *K. pneumoniae*). Using 157 strains possessing well-characterized β-lactamases, sensitivity and specificity were found to be 99.5 and 100%, respectively, for the Vitek ESBL test, compared to 98.1 and 99.4%, respectively, for the 2-disc test. When used to detect ESBLs in 295 clinical isolates (of which 176 were *E. coli* and 157 were *K. pneumoniae* isolates), there was only one false-positive result (Vitek ESBL test). In contrast to data described by Midolo et al. (175), those workers found the Vitek ESBL test to also be capable of detecting K1 β-lactamase in *K. oxytoca* strains. The contrasting results of Midolo et al. (175) and Sanders et al. (221) may also be explained by the choice of test organism: the

former workers chose isolates flagged as being ESBL positive by Vitek, whereas the latter workers used well-characterized strains. Although cefpodoxime is a very sensitive marker of ESBL production, it is somewhat nonspecific, as illustrated by Gibb and Chrichton (98), who used a Vitek custom card to detect a cefpodoxime MIC of >2 μg/ml as a screen for ESBL production in *E. coli* and *Klebsiella* sp. isolates. Of 2,873 organisms tested, 60 were positive, but only 3 were confirmed to be ESBL producers. Furthermore, although the inclusion of cefotaxime-clavulanate and ceftazidime-clavulanate combinations in Vitek systems (138, 221) enhanced the accuracy of ESBL detection, the interpretation of the corresponding phenotype in the Vitek 2 system is based on the analysis of MIC distributions for several β-lactam antibiotics rather than synergy between expanded-spectrum cephalosporins and clavulanate (25).

Sanders et al. (223) used this MIC-based method to great effect. The Vitek 2 system plus the Advanced Expert System (AES) was employed to ascertain the β-lactam phenotypes of 196 isolates of the family *Enterobacteriaceae* and the species *P. aeruginosa*. These isolates represented a panel that had been collected from laboratories worldwide and whose β-lactam phenotypes had been characterized by biochemical and molecular techniques. Overall, the AES was able to ascertain a β-lactam phenotype for 183 of the 196 (93.4%) isolates tested. For 111 of these 183 (60.7%) isolates, the correct β-lactam phenotype was identified definitively in a single choice by the AES, while for an additional 46 isolates (25.1%), the AES identified the correct β-lactam phenotype provisionally within two or more choices. For the remaining 26 isolates (14.2%), the β-lactam phenotype identified by the AES was incorrect. However, for a number of these, the error was due to remediable problems. These results suggest that the AES is capable of an accurate identification of the β-lactam phenotypes of Gram-negative isolates and that certain modifications can improve its performance even further.

Similarly, Canton et al. (42) evaluated Vitek 2 plus AES by using 86 ESBL and 6 inhibitor-resistant-TEM (IRT) β-lactamase-producing isolates of the *Enterobacteriaceae* (genotypically characterized). The Vitek 2 MICs of 12 β-lactams were compared with those obtained by the CLSI microdilution technique. The overall essential agreement (±1 log dilution) was 88%. Discrepancies were observed mainly with cefepime (30% of the total number of discrepancies), ceftazidime (21%), and cefotaxime (15%). Rates of MIC discrepancies were slightly higher for CTX-M-type (14.4%) than for TEM-type (12.5%) or SHV-type (12%) ESBL producers and were rare in IRT producers (1.4%). The overall interpretive agreement was 92.5%, and rates of minor, major, and very major errors were 5.4%, 1.7%, and 2.1%, respectively. The AES was able to identify an ESBL phenotype for 85 out of 86 isolates (99%) and an IRT phenotype for all 6 isolates harboring these enzymes, thus reducing very major errors to 1%. The Vitek 2 system, in conjunction with the AES software, is a reliable tool for the detection of ESBL- or IRT-producing *Enterobacteriaceae*.

Livermore et al. (163) also evaluated the Vitek 2 AES. Ten European laboratories tested 42 reference strains and 76 to 106 of their own strains, representing clinically important resistance genotypes. AST-N010 cards were used for members of the *Enterobacteriaceae*, and AST-N008 cards were used for nonfermenters. Those researchers reported the successful detection of 126 of 137 ESBL producers (92%). ESBL production was accurately inferred for AmpC-inducible species as well as *E. coli* and *Klebsiella* spp. Mechanisms identified, but only as possibilities among several, included IRT-type β-lactamases. Vitek 2 identified any β-lactamase in strains of the *Enterobacteriaceae* in 205/245 cases and partially identified a further 18 cases. When ESBL production was inferred for *E. coli* and *Klebsiella* strains, the Vitek 2 AES edited susceptible results for cephalosporins (except cefoxitin) to resistant; when an acquired penicillinase was inferred for strains of the *Enterobacteriaceae*, piperacillin results were edited to resistant. Further editing may be desirable (e.g., of cephalosporin results for *Salmonella* spp. inferred to have ESBLs).

Giordano et al. (99) evaluated the performance of the Vitek 2 AES for the testing of Gram-positive and Gram-negative bacteria. The strains were well characterized with regard to resistance mechanisms, and the MICs were determined by agar dilution. The resistance mechanisms associated with each resistance pattern were determined by the AES for 79.6% of strains, although it is unclear whether these very strains were used to "train" the AES.

Barry et al. (11) carried out an evaluation of Vitek 2 in five United Kingdom laboratories, comparing results with gold standard agar dilution MICs, assessing its ability to recognize resistant phenotypes, and comparing results with those generated by routine antimicrobial susceptibility testing methods. Laboratories tested a collection of 82 strains selected on the basis of their challenging and characterized resistance mechanisms. Vitek 2 was able to detect the production of penicillinase (19/19 strains), inhibitor-resistant penicillinase (2/2), ESBLs (16/21 plus a further 4 suggested), and cephalosporinase (4/12 plus a further 4 suggested) in Gram-negative organisms. Vitek 2 performed susceptibility tests accurately, and the AES detected and interpreted resistance mechanisms appropriately.

Sorlozano et al. (236) used the disc approximation method, Etest, and Vitek 2 system (AST-N020) to study ESBL-producing (115 strains) and non-ESBL-producing (284 strains) *E. coli* strains. They recorded a Vitek 2 sensitivity of 98.3% and a specificity of 100%. These values are somewhat better than those reported by Leverstein-van Hall et al. (100% sensitivity and 87% specificity) (159), Sanders et al. (91% sensitivity) (223), and Livermore et al. (93% sensitivity) (163) and reflect the different species studied.

Stefaniuk et al. (241) studied a set of well-characterized strains collected in Polish hospitals, including 93 Gram-negative strains. Comparison of the susceptibility data obtained by the standard method and by Vitek 2 showed concordant results in 99% of cases. The Vitek 2 AES detected ESBLs in isolates of the *Enterobacteriaceae* (93.8%) and appears a reliable tool for the detection and interpretive reading of clinically important mechanisms of resistance.

Dashti et al. (62) examined the epidemiology of ciprofloxacin-resistant, ESBL-producing *K. pneumoniae* strains. Strains flagged as being ESBL positive by the integrated ESBL screen on the Vitek GNS-532 card were subjected to isoelectric fo-

cusing. The results suggested that all 69 isolates harbored at least one ESBL, which was later confirmed by PCR with bla_{TEM} and/or bla_{SHV} primers.

Skippen et al. (229) compared two combination disc methods (Oxoid and Mast Diagnostics) containing cefpodoxime with and without clavulanate with Vitek 2 for the routine detection of ESBLs in *E. coli* and *Klebsiella* sp. strains isolated from blood cultures. A total of 58 potential ESBL-producing strains (resistant to cefotaxime and/or ceftazidime) by BSAC disc susceptibility guidelines were tested by the combination discs and Vitek 2. This study detected 7.4% more ESBL-producing isolates by Vitek 2 than by Oxoid disc testing (95% confidence interval [CI], 0.15 to 14.7%; $P < 0.2$), and 31.6% more ESBL-producing isolates were detected by Vitek 2 than by Mast disc testing (95% CI, 16.2 to 46.96%; $P < 0.001$).

A study by Dashti et al. (62) illustrates how refinements in software can improve the specificity of the AES. Those researchers collected *K. pneumoniae* (123), *E. coli* (114), *K. oxytoca* (7), *Enterobacter cloacae* (5), and *Citrobacter freundii* (2) strains flagged as being ESBL positive by the Vitek system (GNS-526 card). Etest-negative strains (15 *E. coli* strains) were retested with GNS-532 cards (in the course of the study, GNS-532 cards superseded GNS-526 cards, with a subsequent software upgrade), and 14 were found to be ESBL negative, despite being originally flagged as ESBL positive.

Spanu et al. (239) examined a total of 1,129 clinically relevant *Enterobacteriaceae* isolates, including 218 that had been previously characterized (only 144 *Enterobacter* and 36 *C. freundii* isolates). These isolates produced at least 21 different ESBL types. The ESBL classification furnished by the Vitek 2 ESBL test system (N045 card, with six wells containing cefepime at 1.0 μg/ml, cefotaxime at 0.5 μg/ml, and ceftazidime at 0.5 μg/ml, alone and in combination with clavulanate) was concordant with that of the comparison method (molecular identification of β-lactamase genes) for 1,121 (99.3%) isolates. ESBL production was correctly detected in 306 of the 312 ESBL-producing organisms (sensitivity, 98.1%; positive predictive value, 99.3%). False-positive results emerged for 2 of the 817 ESBL-negative isolates (specificity, 99.7%; negative predictive value, 99.3%). Vitek 2 ESBL testing took 6 to 13 h (median, 7.5 h; mean ± standard deviation [SD], 8.2 ± 2.39 h).

Dashti et al. (63) studied the efficacy of Vitek 2 for the identification of ESBLs in clinical isolates of *E. coli* and related this to agar dilution. The presence of the major ESBLs groups was confirmed by PCR. Seventy-one isolates from 65 patients were screened for ESBL activity by the Vitek 2 system. Isolates showing positive results were further tested with Etest ESBL strips and by disc approximation methods. All the isolates were flagged as being ESBL positive by the Vitek 2 AES and detected as being ESBL positive by the Etest, only if both ESBL strips were used. The double-disc approximation test using five antibiotics could detect the presence of ESBLs in isolates from only 46 patients. In this test, the synergy with cefepime was the most sensitive for ESBL detection, showing their presence in 41 strains. PCR with primers for bla_{TEM} and bla_{SHV} demonstrated one or both of these enzymes in all isolates.

Nakasone et al. (182) reported that the newly redesigned colorimetric Vitek-2 Compact system with an updated AES correctly detected 98% of 51 ESBLs (TEM, SHV, and CTX-M) in *E. coli* and *K. pneumoniae*, with a specificity of 100%.

Donaldson et al. (73) evaluated a Vitek 2 antibiotic susceptibility testing (AST) card, AST N-054, introduced for aerobic Gram-negative bacilli in 2007 and widely adopted for routine use in the United Kingdom. Results were interpreted by the software version WSVT2-R04.03. ESBL-producing fecal isolates of *E. coli* ($n = 137$) from residents in nursing homes were tested by using the AST N-054 card on the Vitek 2 system and with Mastdiscs ID ESBL detection disc diffusion tests (Mast Diagnostics, Bootle, United Kingdom). The susceptibility result recommended by the Vitek 2 software was also recorded. The AST N-054 card detected ESBL production in 93 of the 137 isolates tested (test sensitivity, 67.9% [95% CI, 59.7 to 75.1%]). *E. coli* strain A, a widespread lineage in the United Kingdom with low-level CTX-M production, accounted for most of the detection failures, with 35/73 A strain isolates being incorrectly reported, versus 9/64 non-A strain isolates ($P < 0.0001$). The Mastdiscs correctly detected ESBLs in 135/137 isolates (test sensitivity, 98.5% [95% CI, 94.5 to 99.9%]). Of the 44 isolates found to be negative by Vitek 2, the AES misreported 29 as being susceptible to cefotaxime and all isolates as being susceptible to ceftazidime and aztreonam. These data suggest that the AST N-054 card is less reliable than other previous cards for the detection of CTX-M β-lactamase-producing *E. coli* strains circulating in the United Kingdom, particularly strain A isolates. Strain A, one of the five related *E. coli* ST131 clones with the CTX-M-15 enzyme, is nationally distributed in the United Kingdom and is dominant in some areas. Expression is reduced by an IS*26* insertion between the $bla_{CTX\text{-}M\text{-}15}$ gene and its promoter in IS*Ecp1* (299). The sensitivity of ESBL detection by Vitek 2 may thus depend on both the AST card used and the ESBLs present. Concerns have been raised previously regarding the ability of the Vitek 2 AES to detect ESBL-producing organisms with low MICs when the AST cards contained neither cefpodoxime nor a specific ESBL test (223, 245), as is the case with the AST N-054 card. The ability of the Vitek 2 AES to detect ESBL production in *E. coli* with the AST N-054 card (sensitivity, 67.9%) was poorer than that previously reported by other investigators who used mostly a heterogeneous mix of ESBL producers and other AST cards with different combinations of cephalosporins (159, 163, 223, 245). The Vitek 2 AST N-010 card, which, unlike AST N-054, includes cefpodoxime, successfully detected ESBL production in 5/5 *E. coli* strain A isolates, 4/4 non-A *E. coli* isolates producing CTX-M-15, and 4/4 *E. coli* isolates with CTX-M-9 (H. Jones and D. M. Livermore, unpublished results).

Gagliotti et al. (94) studied a sample of *E. coli*, *Klebsiella* sp., and *P. mirabilis* isolates from 5 laboratories in the Emilia-Romagna Region of Italy. They concluded that Vitek 2 was an accurate tool to detect ESBL phenotypes of *E. coli* isolates but expressed concern over its performance with other bacterial species, especially *P. mirabilis*. Nyberg et al. (187) included a total of 123 clinical *E. coli* and *Klebsiella* sp. isolates in a study to evaluate the Vitek 2 AST-N029 (Nordic) card for the detection of ESBLs and to compare the results with results of genotypic ESBL verification. The results were also compared to results of alternative phenotypic methods, i.e., agar dilution

and disc diffusion. The strains that were ESBL positive according to the AST-N029 card were further analyzed with the Vitek 2 AST-N041 ESBL test card. Using the genotype as a reference, the Vitek 2 AES had the highest accuracy of the tested methods in classifying the strains as being ESBL positive or negative (91%). When Vitek 2 gave an ESBL as the only option for *E. coli* or *K. pneumoniae*, 44 of 45 (98%) strains had an ESBL structural gene. Vitek 2 achieved an accuracy of 95% and disc diffusion achieved an accuracy of 96% compared to agar dilution as the reference method for *E. coli* and *K. pneumoniae*. For *K. oxytoca*, Vitek 2 achieved the highest level of accuracy (84%) of the methods used.

Lee et al. (156) expressed concerns over some of the editing employed by Vitek 2. According to the AES, the piperacillin susceptibility of all *Klebsiella* spp. is converted to resistance, even though the automated MICs determined for the isolates are low. In contrast, other automated systems do not convert the piperacillin susceptibilities of *Klebsiella* spp. to resistance. In addition, there is no explanation in the CLSI guidelines in this regard, and the U.S. FDA package insert lists *Klebsiella* as an organism for which piperacillin use is indicated for injection (package insert; Pfizer Pharmaceuticals Inc., Philadelphia, PA). Livermore et al. reported that the use of any penicillin, except temocillin, against *Klebsiella* spp. should be discouraged, because *Klebsiella* spp. produce low levels of SHV-1 or K1 β-lactamase (164), and that an inoculum effect was observed with piperacillin and other ureidopenicillins (161). However, in that study, the inoculum effect of piperacillin was not related to the species or the presence of bla_{SHV}, bla_{TEM}, or bla_{OXA}. Although more studies, including the use of an animal infection model, are needed, the conversion of piperacillin susceptibility to resistance for *Klebsiella* spp. by Vitek 2 must be reconsidered or used with caution.

Pitout et al. (201) designed a study to determine the *in vitro* activities of several antimicrobial agents against well-characterized CTX-M-producing *E. coli* strains. MICs were determined for 202 ESBL-producing *E. coli* strains using microbroth dilution and Vitek methods according to CLSI criteria. Molecular characterization was performed by using isoelectric focusing and PCR with sequencing, while strain relatedness was determined by pulsed-field gel electrophoresis. Of the 202 ESBL-producing *E. coli* strains, 2 produced VEB-1, 12 produced TEM-52, 32 produced SHV types (including SHV-2 and -12), and 156 produced CTX-M types (including CTX-M-2, -3, -14, -15, -24, -27, and -30). Vitek Legacy and Vitek 2 failed to detect piperacillin-tazobactam (TZP) resistance in 91 (90%) and 75 (74%) of 101 TZP-resistant ESBL-producing strains, respectively, especially CTX-M-15-producing isolates that coproduced OXA-1. Those authors recommended that laboratories using Vitek should employ alternative susceptibility testing methods for TZP before reporting of the activity of this agent against ESBL-producing *E. coli* strains.

Lefevre et al. (157) compared the phenotypic resistances of strains of the *Enterobacteriaceae* to β-lactams by using Vitek 2 AIX and Vitek 2 PC and concluded that, despite the use of different rules for phenotypic interpretations, the results were essentially identical.

BD Phoenix. The two papers in this section of the review provide little information regarding system evaluation but are included for completeness. The first is limited in that only 12 isolates of 4 species were studied. The second, although more robust, addresses only 6 confirmed ESBL producers.

Pagani et al. (191) collected 12 isolates of the *Enterobacteriaceae* (1 *K. pneumoniae*, 8 *E. coli*, 1 *P. mirabilis*, and 2 *Proteus vulgaris* isolates) classified as being ESBL producers according to the ESBL screen flow application of the BD Phoenix system (NMIC/ID 4) and for which the cefotaxime MICs were higher than those of ceftazidime. By PCR and sequencing, a CTX-M-type determinant was detected in six isolates, including three *E. coli* isolates (carrying $bla_{\mathrm{CTX\text{-}M\text{-}1}}$), two *P. vulgaris* isolates ($bla_{\mathrm{CTX\text{-}M\text{-}2}}$), and one *K. pneumoniae* isolate ($bla_{\mathrm{CTX\text{-}M\text{-}15}}$).

Carroll et al. (45) evaluated the accuracy of the BD Phoenix system (NMIC/ID-26 software, versions V3.34A and V3.54A) for the identification and antimicrobial susceptibility testing of 251 isolates of the family *Enterobacteriaceae*, representing 31 species. Agar dilution, performed according to CLSI guidelines, was the reference method. The essential and categorical agreements were 99% and 98%, respectively. The very-major-error, major-error, and minor-error rates were 0.4%, 0.3%, and 2%, respectively. Six isolates (three *E. coli* and three *Klebsiella* isolates) were ESBL producers. All six isolates were flagged by the Phoenix system expert rules. The Phoenix system compares favorably to traditional methods for ID and AST of the *Enterobacteriaceae*.

MicroScan. Jorgensen et al. (126) compared the performances of two MicroScan dried panels with CLSI reference broth microdilution and disc diffusion on a collection of genetically characterized ESBL-producing isolates. These isolates included 64 *Enterobacteriaceae* isolates that produced CTX-M-8, -14, -15, or -16 based upon PCR and sequencing of the *bla* gene; 17 isolates that produced a SHV or TEM ESBL; and 19 isolates with both CTX-M and SHV. Each isolate was tested by a frozen reference microdilution panel, MicroScan ESBL Plus Confirmation, and a routine MicroScan dried panel containing streamlined ESBL confirmation dilutions (Neg MIC type 32) that included cefotaxime and ceftazidime tested alone or with a fixed concentration of clavulanate (4 μg/ml) as well as by the CLSI double-disc confirmation tests. Disc diffusion detected all ESBL-producing isolates, the frozen reference panel detected 90% of isolates (10 could not be determined because of off-scale MICs that exceeded the clavulanate combination concentrations in the panel), the ESBL Plus system detected 98% of isolates (1 missed and 1 off-scale), and the streamlined ESBL system detected 95% of isolates (5 off-scale). Very high MICs for a few strains that produced SHV or both CTX-M and SHV ESBLs precluded noting the required three 2-fold dilution differences with clavulanate needed to confirm an ESBL primarily in the reference and Neg MIC type 32 panels.

Comparative studies. Katsanis et al. (138) introduced plasmids encoding ESBLs of the TEM (TEM-3, -7, -12, and -26) and SHV (SHV-2 and -4) families and AmpC (MIR-1) into *E. coli* and *K. pneumoniae* to create a homogeneous panel for evaluation of the ability of five systems to detect resistance to eight β-lactams. Although MICs, as determined by agar dilution or Etest, were increased and disc diffusion zone diameters were diminished, breakpoints for resistance were often not reached, and neither approach was sensitive for the detection of resistance to oxyimino-β-lactams. The Vitek AutoMicrobic system with GNS-DE, GNS-DF, and GNS-F4 cards and R06.4

software performed poorly with aztreonam, cefotaxime, cefotetan, cefoxitin, ceftriaxone, cefuroxime, and cephalothin; ceftazidime was the best antibiotic for the detection of ESBL production. TEM-7 and -12 were particularly difficult to detect. Those authors noted that, as β-lactamase genes are often found on plasmids encoding resistance to aminoglycosides, sulfonamide, tetracyclines, and other antibiotics, the finding of unusual resistances to these agents should alert the microbiologist to perform further studies and that such rules could be incorporated into expert systems (108). They also commented that cefotaxime and ceftazidime, with and without clavulanate or sulbactam, have been incorporated into experimental Vitek panels (85). Those authors found MicroScan 18-h microdilution Neg/Urine MIC type 6 panels to be similarly insensitive. Because of such difficulties, the prevalence of ESBLs is likely to be greater than is currently appreciated.

Leverstein-van Hall et al. (159) recovered 74 multiresistant *E. coli* and *Klebsiella* sp. strains during a 3-year period. These strains, and 17 control strains with genotypically identified β-lactamases, were tested for the production of ESBLs by using the Etest and the Vitek 1 (Legacy) (GNS-522 with AMS R09.1 software), Vitek 2 (GNS AST-N010 with VTK R01.02 software), and Phoenix (NMIC/ID-5) systems with a confirmatory ESBL test. The accuracy of the Etest was 94%. With the Etest as the reference for the clinical strains and the genotype as the reference for the control strains, the automated instruments detected the ESBL-producing strains with accuracies of 78% (Vitek 2), 83% (Vitek Legacy), and 89% (Phoenix). No significant differences between the systems with regard to the control strains were detected. The Vitek 2 system did, however, perform less well than the Phoenix system ($P = 0.03$) on the clinical isolates, mainly because of its high percentage of indeterminate results (11%). No significant difference between the performances of the Vitek Legacy and either the Vitek 2 or the Phoenix systems was found. However, because of its associated BDXpert system, the Phoenix system showed the best performance. The outcome was indeterminate with Phoenix for 4 isolates, but the ES suggested that further confirmatory tests be carried out; the ES was also able to compensate partially for false-negative results. However, the Vitek 2 system lacked an ESBL confirmatory test, and the reference test for the clinical isolates was the Etest ESBL test and not enzyme characterization. Since the clinical isolates were uncharacterized, it is unknown if the Etest was an accurate reference, how many types of ESBLs were encountered, or if the types of organisms for which it is difficult to detect ESBLs were included.

Sturenburg et al. (245) compared the abilities of two rapid susceptibility and identification instruments, Vitek 2 (no ESBL confirmatory tests) and BD Phoenix, to detect ESBLs from 34 ESBL-producing clinical isolates of *E. coli* and *Klebsiella* species. The ESBL content was previously characterized on the basis of PCR and sequencing, which were used as the reference. BD Phoenix (NMIC/ID-6) correctly determined the ESBL outcomes for all strains tested (100% detection rate), whereas Vitek 2 was not able to detect the ESBL statuses of 5 isolates (85% detection rate). A detailed analysis revealed that the discrepancies were observed mainly with "difficult-to-detect" strains. Misidentification either was due to low oxyiminocephalosporin MICs for these strains or was associated with pronounced "cefotaximase" or "ceftazidimase" phenotypes. *K. oxytoca* chromosomal β-lactamase (K1) is phenotypically quite similar to ESBL enzymes. In order to evaluate whether the K1 and ESBL enzymes could be discriminated, the analysis was extended to eight *K. oxytoca* strains with a K1 phenotype. Vitek 2 gave an excellent identification of these strains, whereas 7 out of 8 were falsely labeled as being ESBL positive by BD Phoenix. The insufficient discrimination of K1 hyperproducers from ESBL producers by Phoenix was described previously (224) and is attributed to an incorrect placement of ceftazidime in the ESBL test algorithm in the present study.

Linscott and Brown (160) tested 20 previously characterized strains and 49 clinical isolates suspected of ESBL production by four ESBL phenotypic confirmatory methods for accuracy and ease of use. The tests included Dried MicroScan ESBL plus ESBL Confirmation panels, Etest ESBL, Vitek GNS-120, and BD BBL Sensi-Disc ESBL Confirmatory Test discs. Results were compared to frozen microdilution panels prepared according to CLSI specifications, and discrepant isolates were sent for molecular testing. The sensitivities for the ESBL phenotypic confirmatory methods were 100% for MicroScan ESBL plus ESBL Confirmation panels, 99% for Vitek Legacy GNS-120, 97% for Etest ESBL, and 96% for BD BBL Sensi-Disc ESBL Confirmatory Test discs. The specificities were 100% for BD BBL Sensi-Disc ESBL Confirmatory Test discs, 98% for MicroScan ESBL plus ESBL Confirmation panels and Vitek Legacy GNS-120, and 94% for Etest ESBL.

Thomson et al. (259) evaluated the Vitek 2 and Phoenix ESBL systems, which comprise confirmatory tests and expert systems, for their abilities to discriminate between 102 well-characterized strains of ESBL-positive or -negative *E. coli*, *K. pneumoniae*, and *K. oxytoca* strains. At least 38 distinct ESBLs were included. The strains were chosen to include some strains known to cause false-positive and false-negative CLSI ESBL confirmatory test results. Therefore, enzyme characterization, rather than CLSI tests, was the reference method. A third arm of the study was conducted with Phoenix using two normally inactive expert rules intended to enhance ESBL detection, in addition to the use of the currently available software. The Phoenix ESBL confirmatory test and unmodified expert system exhibited 96% sensitivity and 81% specificity for ESBL detection. The activation of the two additional rules increased the sensitivity to 99% but reduced the specificity to 58%. The Vitek 2 AST-GN13 card was run with software version WSVT2-R04.01. The Vitek 2 ESBL confirmatory test exhibited 91% sensitivity, which was reduced to 89% by its expert system, while its specificity was 85%. Many of the expert system interpretations of both instruments were helpful, but some were suboptimal. The Vitek 2 expert system was potentially more frustrating because it provided more inconclusive interpretations of the results. Considering the high degree of diagnostic difficulty posed by the strains, both ESBL confirmatory tests were highly sensitive. It was, however, necessary to include some laboratory strains of *E. coli* that produced certain β-lactamases, some of which grew poorly in the Vitek 2 system and contributed to the lower sensitivity of its ESBL confirmatory test. The Phoenix system was able to sustain the growth of these strains, suggesting that it may use a more robust growth medium. In general, the Vitek 2 expert system offered more-complex interpretations and more choices for the user and

suggested that more tests be repeated. It also suggested more often that the laboratory should select which resistance mechanism was present. This is likely to cause frustration, particularly for small laboratories where a microbiologist with sufficient expertise may not be available to make the required decisions. Frustration is also likely when isolates are encountered for which the software keeps looping back to suggest that the laboratory keep repeating the test. In conclusion, the ESBL confirmatory tests of both systems exhibited a high capacity to detect a wide range of ESBLs. However, both expert systems require modification to update and enhance their utility. In this regard, the Vitek 2 expert system was considered potentially more frustrating, as it provided more inconclusive interpretations of the results. The seemingly high percentages of false-positive test results obtained with ESBL-negative strains reflected the challenging nature of the strains and the high mathematical impact of an incorrect result when only 26 strains were tested. Also, certain organisms harboring specific ESBLs failed to grow in the Vitek 2 system, and this contributed to the lower sensitivity of its ESBL confirmatory test.

Snyder et al. (232) studied clinical and challenge strains of the *Enterobacteriaceae* ($n = 150$) and nonfermentative Gram-negative bacilli (NFGNB) (45 clinical and 8 challenge isolates). For AST of the *Enterobacteriaceae*, the rate of complete agreement between the Phoenix and MicroScan results was 97%; the rates of very major, major, and minor errors were 0.3%, 0.2%, and 2.7%, respectively. For NFGNB, the rate of complete agreement between the Phoenix and MicroScan results was 89%; the rates of very major, major, and minor errors were 0%, 0.5%, and 7.7%, respectively. Following the confirmatory testing of nine clinical isolates initially screened by the MicroScan system as being possible ESBL-producing organisms (seven *K. pneumoniae* and two *E. coli* isolates), complete agreement was achieved for eight strains (one ESBL positive and seven negative); one false-positive result was obtained with Phoenix. The MicroScan system correctly detected the 10 ESBL challenge isolates, versus 6 detected by Phoenix. Overall, there was good correlation between Phoenix and MicroScan systems for the ID and AST of the *Enterobacteriaceae* and common NFGNB. The Phoenix system is a reliable method for the ID and AST of the majority of clinical strains encountered in the clinical microbiology laboratory. Until additional performance data are available, results for all *K. pneumoniae* or *K. oxytoca* and *E. coli* isolates screened and confirmed as being ESBL producers by any automated system should be confirmed by alternate methods prior to the release of final results.

Trevino and coworkers (267, 268) compared the performances of Vitek 2 and BD Phoenix for confirmatory testing of ESBL production. A total of 193 clinical isolates of phenotypically confirmed ESBL producers (174 *E. coli* and 19 *K. pneumoniae* isolates) were assayed by the Vitek 2 and BD Phoenix systems using AST-N058 cards and UNMIC/ID-62 panels, respectively. The double-disc synergy test and the Etest were used as reference methods. Twelve strains characterized by genotyping were used as positive and negative controls. For the clinical isolates, the sensitivities of the tests were 99.5% for Vitek and 95.3% for Phoenix. There were no significant differences for the control strains. The execution of the expert system raised the sensitivity of Phoenix to 100%. However, the Vitek 2 expert system considered the results obtained for 7 strains with ESBL-positive tests to be incoherent. Confirmatory testing for ESBL production with Vitek 2 (AST-N058 card) showed a higher sensitivity than that of Phoenix (UNMIC-ID 62 panel). Nevertheless, the performances of the expert systems in the two automated tests were similar for ESBL detection in *E. coli* and *K. pneumoniae* strains.

Dashti et al. (60) collected *K. pneumoniae*, *E. coli*, *K. oxytoca*, and *E. cloacae* isolates that were flagged as being ESBL positive by the Vitek 2 system. The isolates were retested by the Vitek 2 system and also tested by double-disc diffusion (DDD), the disc approximation test (DAT), Etest, and MicroScan. Retesting with Vitek revealed 100% compatibility with the results of the source hospitals. MicroScan, DDD, disc approximation, and Etest could detect ESBLs in 199, 192, 178, and 205 isolates, respectively. Technically, MicroScan and Vitek 2 were the least demanding methods to detect ESBLs, as they are an integral part of the routine susceptibility test card. Etest strips were reliable but the most expensive of all the techniques used. The DDD test and disc approximation, while relatively inexpensive, were technically subjective. The Vitek system may be very suitable for clinical laboratories but would be better if accompanied by another test for the detection of ESBL bacteria.

Detection of Extended-Spectrum β-Lactamases in Gram-Negative Organisms Producing AmpC

ESBL detection in *Enterobacter* spp. (for example) by automated systems is more complicated because of the production of chromosomally encoded AmpC-type enzymes, which, unlike ESBLs, are not inhibited by clavulanate and may even be induced by it. Therefore, this may nullify the ability of the Vitek system to identify ESBL production based on the effect of clavulanic acid. From a clinical point of view, the discrimination between ESBLs and overproduced class C β-lactamases may not be critical, since the therapeutic options for infections caused by organisms that possess any of these mechanisms of resistance are similarly limited. Nevertheless, the detection of such "hidden" ESBLs is still of epidemiological importance in the hospital environment.

Vitek. Sanders et al. (221) found Vitek to be efficient with *E. coli* and *Klebsiella* spp. but that its reliability with *Enterobacter* spp. and *Serratia* spp. remained questionable. Tzouvelekis et al. (274) reported that Vitek ESBL detection tests and the conventional double-disc synergy test (DDST) were both unable to detect SHV-5 in a *K. pneumoniae* isolate that produced plasmid-borne AmpC. The ESBL was successfully detected by a DDST that combined clavulanate with cefepime.

Sanders et al. (223) later found a high degree of accuracy of the Advanced Expert System (AES) (Vitek 2 AST-N009 card) in resistance mechanism detection in *Enterobacter* strains (92%), but insufficient data precluded a determination of the accuracy of the AES in ESBL detection specifically. Canton et al. (42) studied the testing accuracy of the Vitek 2 system (AST-N010 card, software V1.01) and the ability of the AES to provide interpretive readings. All *Enterobacter* sp. strains (10) and a single *C. freundii* strain harbored AmpC as well as ESBL genes; 31 *E. coli* strains, 38 *K. pneumoniae* strains, and a single *Salmonella* sp. strain produced ESBLs; and 6 *E. coli* strains

harbored inhibitor-resistant-TEM (IRT) β-lactamases. Vitek 2 MICs of 12 β-lactams were compared with those obtained by the CLSI microdilution technique. The overall essential agreement (±1 log dilution) was 88%. Discrepancies were observed mainly with cefepime (30% of the total number of discrepancies), ceftazidime (21%), and cefotaxime (15%). MIC discrepancies were slightly higher for CTX-M-type (14%) than for TEM-type (12.5%) or SHV-type (12%) ESBL producers and were rare in IRT producers (1.4%). The overall interpretive agreement was 92.5%, and rates of minor, major, and very major errors were 5.4%, 1.7%, and 2.1%, respectively. The AES was able to identify an ESBL phenotype in 99% of 86 isolates and an IRT phenotype in all 6 isolates harboring these enzymes, thus reducing very major errors to 1%. Livermore et al. (163) stated that ESBL production was accurately inferred for AmpC-inducible species as well as *E. coli* and *Klebsiella* spp. Thus, two groups have reported ≥90% agreement between the Vitek 2 AES and reference genotype data in ESBL detection overall in strains of the *Enterobacteriaceae*, including in AmpC-inducible species, although in each study, only a few *Enterobacter* isolates were tested.

In one study looking specifically at ESBL detection in *Enterobacter* spp., of 31 ESBL-producing isolates, the Vitek detection test, using cefotaxime and ceftazidime alone and in combination with clavulanic acid, was positive for only 2 (6.5%) of them (273). DDST with amoxicillin-clavulanate and with expanded-spectrum cephalosporins and aztreonam was positive for 5 (16%) strains. Modification of the DDST consisting of a closer application of the discs (at 20 instead of 30 mm), the use of cefepime, and both changes increased the sensitivity of this test to 71%, 61%, and 90%, respectively.

Other studies have addressed AmpC-producing organisms, but numbers have been low. Sanders et al. (222) studied the impact of the Vitek 2 automated system (T01.01.0038) and the Advanced Expert System (AES X01.00P) on the clinical laboratory of a typical university-based hospital. A total of 259 consecutive isolates, including 170 nonduplicate *Enterobacteriaceae* (AST-N009 card), comprising 78 *E. coli*, 29 *K. pneumoniae*, 7 *K. oxytoca*, 15 *E. cloacae*, 3 *Enterobacter aerogenes*, 5 *Citrobacter koseri*, 1 *Citrobacter amalonaticus*, 4 *C. freundii*, 13 *P. mirabilis*, 3 *Morganella morganii*, 4 *Providencia stuartii*, and 8 *S. marcescens* isolates, 41 *P. aeruginosa* (AST-N008 card), and 48 *S. aureus* isolates, were collected and tested by Vitek 2 for identification and antimicrobial susceptibility testing, and the results were analyzed by the AES and also by a human expert. The human expert thought that most of these corrections were appropriate and that some resulted from a failure of the Vitek 2 system to detect certain forms of resistance. Antimicrobial phenotypes assigned to the strains by the AES were similar to those assigned by the human expert for 96 to 100% of strains. Of the 259 isolates, 95% were definitively identified by Vitek 2, and 75% had no inconsistencies between identification and antimicrobial susceptibility. Of the 65 strains for which a correction was identified by the AES, 58.5% required only a therapeutic change to the susceptibility results. Most of these were due to the failure of the Vitek 2 system to detect β-lactam resistance in organisms possessing an intrinsic β-lactamase, a failure common to test systems that are rapid or involve small inocula (258). When false susceptibility results occurred with two or more antibiotics, the AES suggested retesting, and this occurred with a number of the *Enterobacter* sp. and *C. freundii* isolates. There was very good agreement between the human expert and the AES in recognizing inconsistencies in this study. For only 5 (8%) of the 65 strains identified by the AES as needing corrections to the data did the human expert disagree with the AES about whether an inconsistency existed or how to correct the inconsistency. The major limitation of the AES noted in the biological validation phase of the data analysis was its inability to recognize a single pattern of inconsistency and correct it. For example, false susceptibility to ampicillin, amoxicillin-clavulanate, cephalothin, and/or cefoxitin occurred with a few strains of *Enterobacter* spp. or *C. freundii*. The design of the AES prevents it from making corrections if two or more inconsistencies are identified. Thus, it cannot recognize single-source problems that lead to multiple inconsistencies. The overall agreement across the different drug groups varied from 96 to 100%. The major limitation noted for the AES was its inability to rank in order the various phenotypes among the possible phenotypes when more than one matched the MIC distribution.

Blondel-Hill et al. (24) evaluated the applicability and adaptability of the Vitek 2 system (AES) to the customized interpretive susceptibility guidelines used at Dynacare Kasper Medical Laboratories (DKML). Three hundred isolates of the *Enterobacteriaceae* (not more than 30% *E. coli* isolates) were tested on the Vitek 2 system and the API 20E system for identification. Susceptibility testing was performed with the Vitek 2 system and Pasco broth microdilution panels. Of 287 isolates, interpretations by the AES and DKML guidelines were compared for 10 antibiotics. The overall correlation between interpretations was 96%. Those authors commented on several limitations of the AES: (i) incompatible results may be suggested since the system does not compare antibiotics within the same class after specific phenotypes are identified; (ii) when more than one biological correction is required, the AES does not identify the antibiotics that are giving inconsistent results, which would be helpful to infer resistance mechanisms; (iii) the knowledge base is limited to published literature, which may not always reflect actual susceptibility patterns of all geographic regions and patient populations, and (iv) while customization is an attractive feature, it still requires significant expertise.

Jamal et al. (119) determined the prevalence of ESBL-producing *Enterobacteriaceae* by using Vitek 2 (VTK-R01.02 software) and Etest. Consecutive clinically relevant Gram-negative isolates (a single isolate per patient) of the *Enterobacteriaceae* (AST-N020) and *Pseudomonas* (AST-N022) were studied for ESBL production over a period of 1 year at Mubarak Al-Kabeer Hospital, Kuwait. Of the 3,592 bacterial isolates, 264 (7.5%) and 185 (5%) were positive for ESBL production by the Vitek 2 system and Etest, respectively. All the ESBL-producing *P. aeruginosa* isolates identified by Vitek 2 gave indeterminate results by Etest. Prevalent ESBL producers identified by the Vitek 2 system versus the Etest were *Citrobacter* sp. (15% versus 3.2%), *K. pneumoniae* (12.2% versus 11.4%), *Enterobacter* sp. (12% versus 3%), *E. coli* (6.5% versus 5.6%), *P. aeruginosa* (6.5% versus 0%), and *Morganella* sp. (2% versus 1%) isolates.

Linscott and Brown (160) tested 20 previously characterized strains and 49 clinical isolates suspected of ESBL production.

The Vitek Legacy automated system using the GNS-120 card was performed in accordance with the guidelines of the manufacturer for ESBL detection. VTK R07 software or higher was needed in order to interpret the reduction in growth due to cefotaxime-clavulanic acid or ceftazidime-clavulanic acid compared to the growth of either cefotaxime or ceftazidime alone. The sensitivity for the ESBL phenotypic confirmatory test methods was 99%, and the specificity was 98%. The Vitek Legacy ESBL system correctly identified 21 of the 32 challenge isolates for ESBL production and detected 59 of 90 ESBL producers of the clinical isolates. One discrepant isolate, identified as *E. coli*, was shown to contain both SHV-5 and AmpC enzymes. The overall sensitivity and specificity of the Vitek Legacy test method were 99% and 98%, respectively.

Nakamura and Takahashi (181) investigated whether or not the AES correctly categorized the β-lactamases derived from the Vitek 2 results using the AST-N025 card and software (VT2-R03.02) on strains of the *Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter baumannii*. They studied 131 strains with determined genotypes. The AES analysis matched the genotype in 120 (92%) of the 131 strains. Incorrect findings were found for six strains, including three *S. marcescens* strains. The mechanism could not be determined for five strains, including three *Providencia rettgeri* strains. Results of the analysis agreed for 34 (97%) of 35 strains with ESBLs and for 27 (96%) of 28 strains with high-level cephalosporinase. The AES also incorrectly identified one *E. coli* strain producing high-level cephalosporinase as an ESBL-producing strain (ceftazidime MIC $>$ 32 μg/ml).

Diamante and Camporese (68) carried out an evaluation of the performance of the Vitek 2 AES in the identification of ESBLs in members of the *Enterobacteriaceae* other than *E. coli*, *P. mirabilis*, and *Klebsiella* spp. by comparing results obtained with the Etest and those obtained by double-disc diffusion. Seventy isolates of *Enterobacteriaceae* were tested for the production of ESBLs with Etest as the gold standard. The AES produced 19 ESBL warnings, of which only 5 were classified as "major misunderstandings," especially for strains of the *Enterobacteriaceae* other than *E. coli*, *P. mirabilis*, and *Klebsiella* spp., which produced plasmid-mediated AmpC (pAmpC) β-lactamases. The Etest, together with the cefoxitin sensitivity test, was found to be the best method to confirm ESBLs and distinguish AmpC from ESBLs.

Schwaber et al. (226) identified 40 clinical isolates of *Enterobacter* spp. as being ESBL producers by disc diffusion and genotypic methods. The Vitek 2 AES identified the ESBL phenotype in only 25 isolates (62.5%) and erroneously reported cephalosporin susceptibility for 11 isolates (28%). Refinements in the AES are required in order to improve ESBL detection in *Enterobacter* spp. Those authors suggested that the inclusion of cefepime or cefpirome alone and with a β-lactamase inhibitor in susceptibility testing may improve performance, as these agents are less efficiently hydrolyzed by AmpC enzymes than are narrow-spectrum cephalosporins (273). Also, those authors suggested that tazobactam may be a more appropriate β-lactamase inhibitor than clavulanic acid, as it is a weaker inducer of AmpC enzymes. They concluded that additional studies are required before the Vitek 2 AES can be used as a sole method of detection of ESBL production in *Enterobacter* spp. The reliability of the results depends on the creation of the ESBL screen and the expert systems, on the setting of the antibiotics of the panels, on the MIC ranges, and, finally, on the resistance mechanisms.

Song et al. (234) isolated a total of 16 ceftriaxone- and cefoxitin-resistant *K. pneumoniae* isolates from 15 patients. These isolates showed negative results for ESBLs by the Vitek system and were highly resistant to ceftazidime, aztreonam, and cefoxitin (MIC $\geq$ 128 μg/ml). The $bla_{\text{SHV-2a}}$ and $bla_{\text{DHA-1}}$ genes were detected by PCR and sequence analysis, and the pulsed-field gel electrophoresis profiles of the isolates were indistinguishable. Disc tests for AmpC enzymes as well as double-disc tests and CLSI confirmatory disc tests for ESBLs yielded positive results for all the isolates. However, only three isolates (19%) were shown to produce ESBLs by CLSI confirmatory tests using broth microdilution. In addition, this report presented problems associated with ESBL detection using broth microdilution for isolates that coproduce an ESBL and an AmpC β-lactamase: the presence of the DHA-1 AmpC enzyme might mask the effect of the SHV-2a ESBL on the Vitek system and the CLSI confirmatory test by broth microdilution.

Thomson et al. (259) reported some falsely positive ESBL results associated with AmpC β-lactamases. The ESBL test classified correctly the MIR-1 plasmid-mediated AmpC β-lactamase of an *E. coli* strain as a non-ESBL, but the AES overrode this result and classified it incorrectly as an ESBL and twice suggested that the test be repeated.

Savini et al. (225) examined only four isolates but reported that inducible β-lactam resistance in *Hafnia alvei* may remain undetected by the Vitek 2 AES. They suggested the routine performance of a disc approximation assay, together with conventional susceptibility tests, in order to define the susceptibility profile of *H. alvei* and to screen for the expression of inducible cephalosporinases to avoid *in vivo* antimicrobial failures.

Robin et al. (210) assessed the Vitek-2 ESBL test (AST-N041) with 94 ESBL-positive and 71 ESBL-negative nonduplicate isolates of the *Enterobacteriaceae*. The test comprised a panel of six wells containing ceftazidime at 0.5 μg/ml, cefotaxime at 0.5 μg/ml, and cefepime at 1.0 μg/ml, alone and in combination with clavulanic acid (4, 4, and 10 μg/ml, respectively). The isolates produced a wide diversity of β-lactamases, including 61 different ESBLs, two class A carbapenemases, and various species-specific β-lactamases. ESBL detection was performed by using (i) the conventional synergy test as recommended by the CA-SFM, (ii) the CLSI test for ESBLs, and (iii) the Vitek-2 ESBL system. For *E. coli* and *Klebsiella*, the sensitivity and specificity values were 97% and 97%, respectively, for the synergy test; 92% and 100%, respectively, for the CLSI test; and 92% and 100%, respectively, for Vitek-2 ESBL. For other organisms, the sensitivity and specificity values were 100% and 97%, respectively, for the synergy test; 90.5% and 100%, respectively, for the CLSI phenotypic confirmatory test (CLSI-PCT); and 90.5% and 100%, respectively, for the Vitek 2 ESBL test. The Vitek 2 ESBL system seemed to be an efficient method for the routine detection of ESBL-producing isolates of the *Enterobacteriaceae*, including isolates producing AmpC-type enzymes (although there were only 8 such isolates in this study). That study was the first to include five CMT-type ESBLs in an evaluation of the ESBL detection test, i.e.,

TEM-50 (CMT-1), TEM-109 (CMT-5), TEM-125 (CMT-6), TEM-151 (CMT-7), and TEM-152 (CMT-8). The use of three oxyiminocephalosporins, including cefepime, could explain the higher sensitivity (87.5%) of the Vitek-2 ESBL test among AmpC-producing organisms. Cefepime use has also been shown to improve the sensitivity of the synergy test among AmpC producers (102, 246). ESBL detection in the AmpC-producing group of the *Enterobacteriaceae* is particularly useful because of the numerous infections caused by bacteria belonging to this group (65, 167, 303).

Chen et al. (46) tested 317 *K. pneumoniae* and 291 *E. coli* nonduplicate isolates by the Vitek 2 system (AST-GN13) to evaluate its capability to detect ESBLs among putative ESBL-producing isolates, in particular those with a coproduction of AmpC enzymes. The sensitivity and specificity for ESBLs were 99% and 98.5%, respectively. Ninety of the isolates were AmpC (CMY-2, CMY-8, or DHA-1) and ESBL (SHV and/or CTX-M) coproducers, and 74 (82%) of them were flagged as being ESBL producers. This study indicated that Vitek 2 is acceptable for ESBL detection among *K. pneumoniae* and *E. coli* isolates with both imported AmpC and ESBLs. The relatively low negative predictive value (NPV) for *K. pneumoniae* can be attributed, in part, to the small number of non-AmpC- and non-ESBL-producing strains in this study. A majority of false-negative results was observed for isolates producing both CMY-8 and CTX-M-3 (six isolates) or DHA-1- and SHV-5-like enzymes (six strains, including one with an additional CTX-M enzyme). Only 1 of 30 isolates coproducing CTX-M ESBLs and DHA-1 was not detected by Vitek.

The Vitek 2 ESBL test including ceftazidime, cefotaxime, and cefepime in the presence and absence of clavulanic acid was developed as an integral part of routine susceptibility test cards and has been shown to be acceptable for ESBL detection in *E. coli* and *Klebsiella* sp. strains. The test exhibits a comparable capability to detect ESBL production among AmpC producers with the use of cefepime, which is usually not inhibited by AmpC enzymes (210, 239).

BD Phoenix. Sanguinetti et al. (224) used an algorithm based on phenotypic responses to a panel of cephalosporins (ceftazidime plus clavulanic acid, ceftazidime, cefotaxime plus clavulanic acid, cefpodoxime, and ceftriaxone plus clavulanic acid) to test 510 *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. mirabilis*, *P. stuartii*, *M. morganii*, *E. aerogenes*, *E. cloacae*, *S. marcescens*, *C. freundii*, and *C. koseri* isolates. Of these isolates, 319 were identified as being ESBL producers based on the results of current phenotypic tests. The combined use of isoelectric focusing, PCR, and/or DNA sequencing demonstrated that 288 isolates possessed $bla_{\text{TEM-1}}$- and/or $bla_{\text{SHV-1}}$-derived genes and that 28 isolates possessed a $bla_{\text{CTX-M}}$ gene. Among the 191 non-ESBL-producing strains, 77 synthesized an AmpC-type enzyme; 110 synthesized TEM-1, TEM-2, or SHV-1 β-lactamases; and the remaining 4 (all *K. oxytoca* strains) hyperproduced K1 chromosomal β-lactamase. The Phoenix ESBL system gave positive results for all 319 ESBL-producing isolates (12 different enzymes) and also for 2 of the 4 K1-hyperproducing *K. oxytoca* isolates. Compared with the phenotypic tests and molecular analyses, Phoenix displayed 100% sensitivity and 99% specificity. These findings suggest that Phoenix ESBL can be a rapid and reliable method for the detection of ESBLs in Gram-negative bacteria.

Park et al. (193) evaluated the Phoenix ESBL test with chromosomal AmpC-producing *E. cloacae*, *E. aerogenes*, *C. freundii*, and *S. marcescens* isolates. The study was conducted with 72 nonrepetitive ESBL producers (33 *E. cloacae*, 13 *E. aerogenes*, 14 *C. freundii*, and 12 *S. marcescens* isolates) and 77 non-ESB-producing isolates (33 *E. cloacae*, 9 *E. aerogenes*, 6 *C. freundii*, and 29 *S. marcescens* isolates). The organisms were selected as suspected ESBL producers based on the double-disc synergy test (DDST) and confirmed by PCR amplification of $bla_{\text{TEM-1}}$, $bla_{\text{SHV-1}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, and $bla_{\text{CTX-M-9}}$. The Phoenix ESBL system, using a 5-well confirmatory test, and the BDXpert system were evaluated. Of the 72 ESBL producers based on the DDST, 46 harbored CTX-M-type enzymes, 21 harbored TEM-type enzymes, and 31 harbored SHV enzymes. Phoenix identified ESBL production in only 15 isolates. Of the 77 non-ESBL-producing isolates, Phoenix identified ESBLs in 4 isolates, 3 of which were confirmed to be ESBL producers. In that study, Phoenix was highly specific (76/77 isolates; 99%), and it identified 3 additional ESBL producers that were not detected by DDST. However, the Phoenix sensitivity was very low (15/72 isolates; 21%). Considering the increasing prevalence of ESBL production among AmpC producers, Phoenix cannot be considered a reliable stand-alone ESBL detection method for the strains tested in that study.

Thomson et al. (259) studied 102 well-characterized strains of ESBL-positive or -negative *E. coli*, *K. pneumoniae*, and *K. oxytoca* isolates. At least 38 distinct ESBLs were included. The strains were chosen to include some strains known to cause false-positive and false-negative CLSI ESBL confirmatory test results. Therefore, enzyme characterization, rather than the CLSI test, was the reference method. A third arm of the study was conducted with Phoenix using two normally inactive expert rules intended to enhance ESBL detection, in addition to using the currently available software. The Phoenix ESBL confirmatory test (NMIC/ID-108 was run with software version 5.02H/V4.11B) and the unmodified expert system exhibited 96% sensitivity and 81% specificity for ESBL detection. The activation of the two additional rules increased the sensitivity to 99% but reduced the specificity to 58%. Many of the expert system interpretations were helpful, but some were suboptimal. Considering the high degree of diagnostic difficulty posed by the strains, ESBL confirmatory tests were highly sensitive. The expert system requires modification to update and enhance its utility. The Phoenix expert system performed better than Vitek 2 with some strains that produced multiple β-lactamases. For example, it correctly deduced ESBL production in an SHV-5-like-enzyme-producing *K. pneumoniae* isolate that produced four β-lactamases, including a FOX-like AmpC enzyme, whereas the Vitek 2 system initially suggested retesting due to irresolvable correction possibilities and, upon retesting, incorrectly suggested carbapenem resistance. As mentioned above, the Phoenix expert system utilizing rules 345 and 1437 increased the ESBL detection rate from 96% to 99% but was associated with an unacceptable 42% false-positive rate. The reduced specificity was due mostly to rules interpreting high-level AmpC production as evidence of the presence of an ESBL. The currently available Phoenix expert system was in conflict with CLSI recommendations in that, for ESBL-producing strains, it converted susceptible and intermediate re-

sults for amoxicillin-clavulanate to resistant results. It also changed a cefotaxime-susceptible result to resistant for a K1-hyperproducing *K. oxytoca* isolate. It did not correct some false-positive ESBL results associated with KPC or the high-level production of AmpC, K1, and SHV-1 β-lactamases. Two *K. oxytoca* isolates were incorrectly identified as *K. pneumoniae* isolates, making interpretations relevant to the K1 β-lactamase of *K. oxytoca* impossible. In addition, a *K. pneumoniae* isolate was misidentified as *E. cloacae*, which would incorrectly direct the expert system to include the production of a chromosomally mediated AmpC β-lactamase as a possible interpretation. The seemingly high percentages of false-positive tests obtained with the ESBL-negative strains reflected the challenging nature of the strains and the high mathematical impact of an incorrect result when only 26 strains were tested. It was, however, necessary to include some laboratory strains of *E. coli* that produced certain β-lactamases, some of which grew poorly in the Vitek 2 system and contributed to the lower sensitivity of its ESBL confirmatory test. The Phoenix system was able to sustain the growth of these strains, suggesting that it may use a more robust growth medium.

Lee et al. (154) compared the BD Phoenix (NMIC/ID-108 Combo panel) ESBL test with the CLSI ESBL phenotypic confirmatory test by disc diffusion for 224 *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* isolates. For the isolates showing discordant results between the two tests, a boronic acid disc test was performed to differentiate AmpC and ESBLs. Among the 224 isolates, 75 and 79 were positive for ESBL by the CLSI ESBL and Phoenix tests, respectively. Having detected four more isolates as being ESBL producers, Phoenix showed a 98% agreement with a 100% sensitivity and a 97% specificity compared with the CLSI ESBL test. Among the four false-positive results, three were AmpC positive but ESBL negative. The BD Phoenix ESBL test was sensitive and specific and can be used as a rapid and reliable method to detect ESBL production in *E. coli*, *Klebsiella* species, and *P. mirabilis*.

Fisher et al. (86) noted that the phenotypic identification of AmpC and ESBLs among the *Enterobacteriaceae* remains challenging. That study compared the Phoenix system (instrument version 5.15A and software version 5.10A/V4.31A) with the CLSI confirmatory disc method to identify ESBL production and a double-disc boronic acid inhibitor method to detect AmpC production among 200 *E. coli* and *K. pneumoniae* isolates That paper did not state whether the AmpCs were plasmidic (although none were found in *K. pneumoniae*) or whether they were hyperproduced. Phoenix reported an ESBL-positive result for 46/48 ESBL-producing strains, 8/10 ESBL- and AmpC-producing strains, 11/14 AmpC-producing strains, and 35/128 ESBL- and AmpC-negative strains. Phoenix thus misclassified nearly half of the isolates as being ESBL positive, requiring manual testing for confirmation. The inclusion of aztreonam with or without clavulanic acid (CA) and cefpodoxime with or without CA in the testing algorithm increased the ESBL detection rate by 6%. Boronic acid-based screening identified 24 isolates as being AmpC positive, but in a subset of genotypically characterized strains, it appeared to have a high false-positivity rate. These data confirm the high sensitivity (93%) but questionable specificity (68%) reported for Phoenix ESBL detection (259, 285). However, the use of phenotypic rather than genotypic methods to confirm enzyme production in the test library again raises caveats regarding the validity of the conclusions.

Robberts et al. (209) stated that the emergence of ESBL and plasmid-mediated AmpC (pAmpC) enzymes in *E. coli* raises concerns regarding the accurate laboratory detection and interpretation of susceptibility testing results. Twenty-six cefpodoxime ESBL screen-positive, cefoxitin-resistant *E. coli* isolates were subjected to clavulanate ESBL confirmatory testing employing disc inhibition zone augmentation, Etest, and the BD Phoenix NMC/ID-132 panel. Phenotypic pAmpC production was assessed by boronic acid disc augmentation. ESBL and pAmpC genes were detected by amplification and sequencing. ESBL genes (bla_{SHV} and/or $bla_{CTX\text{-}M}$) were detected in only 7/26 ESBL screen-positive isolates. Of 23 aminophenylboronic-acid-screen-positive isolates, pAmpC structural genes were detected in 20 of them ($bla_{CMY\text{-}2}$ or $bla_{FOX\text{-}5}$). A high incidence of false-positive ESBL confirmatory results was observed for both clavulanate disc augmentation (9/19 isolates) and Phoenix (5/19). All were associated with the presence of pAmpC with or without TEM-1. The Etest performed poorly, as the majority of interpretations were nondeterminable. In addition, false-negative ESBL confirmatory results were observed for isolates possessing concomitant ESBL- and pAmpC-specifying genes for Etest (4/5 isolates), Phoenix (3/5), and disc augmentation (1/5). The results indicate a poor performance of currently employed ESBL confirmatory methods in the setting of concomitant pAmpC. Some isolates with pAmpC and ESBL genes fell within the susceptible category for extended-spectrum cephalosporins, raising concern over currently employed breakpoints.

MicroScan. Moland et al. (177) studied 75 strains of species producing well-characterized β-lactamases using two MicroScan conventional microdilution panels, Gram Negative Urine MIC 7 (NU7) and Gram Negative MIC Plus 2 (N+2), to determine if results could be utilized to provide an accurate indication of β-lactamase production in the absence of frank resistance to extended-spectrum cephalosporins and aztreonam. The enzymes studied included Bush groups 1 (AmpC), 2b (TEM-1, TEM-2, and SHV-1), 2be (ESBLs and K1), and 2br, alone and in various combinations. In tests with *E. coli* and *K. pneumoniae* and the NU7 panel, cefpodoxime MICs of ≥2 μg/ml were obtained only for isolates producing ESBLs or AmpC β-lactamases. Cefoxitin MICs of >16 μg/ml were obtained for all strains producing AmpC β-lactamase and only 1 of 33 strains producing ESBLs. For the N+2 panel, ceftazidime MICs of ≥4 μg/ml correctly identified 90% of ESBL producers and 100% of AmpC producers among *E. coli* and *K. pneumoniae* strains. Cefotetan MICs of ≥8 μg/ml were obtained for seven of eight producers of AmpC β-lactamase and no ESBL producers. For tests performed with either panel and *K. oxytoca* strains, MICs of ceftazidime, cefotaxime, and ceftizoxime were elevated for strains producing ESBLs, while ceftriaxone and aztreonam MICs separated low-level K1 from high-level K1 producers. These results suggest that microdilution panels can be used by clinical laboratories as an indicator of certain β-lactamases that may produce hidden but clinically significant resistance among *E. coli*, *K. pneumoniae*, and *K. oxytoca* strains. Although it may not always be possible to differentiate between strains that produce ESBLs and those

that produce AmpC, this differentiation is not critical, since therapeutic options for patients infected with such organisms are similarly limited.

Komatsu et al. (144) assessed use of the MicroScan ESBL confirmation panel for the detection of eight ESBL-producing strains of the *Enterobacteriaceae*. Of 137 bacterial strains isolated from patients in 32 hospitals in Japan, 91 produced ESBLs and comprised 60 bacteria (*E. coli*, *K. oxytoca*, and *K. pneumoniae*) targeted by the CLSI ESBL test and 31 nontarget bacteria, such as chromosomal AmpC-producing bacteria (e.g., *S. marcescens* and *Enterobacter* spp.). ESBL production was judged to have occurred when three 2-fold concentration decreases in an MIC for either cefotaxime or ceftazidime tested in combination with clavulanic acid versus its MIC when tested alone was observed. The sensitivity and specificity of the MicroScan panel for the target bacteria were 92% and 93%, respectively; the sensitivity and specificity for nontarget bacteria were 52% and 100%, respectively. There were 20 ESBL-positive strains that were not inhibited by clavulanic acid in the MicroScan panel (3 of 32 *E. coli*, 1 of 24 *K. pneumoniae*, 1 of 4 *K. oxytoca*, 8 of 13 *E. cloacae*, and 7 of 14 *S. marcescens* strains), and most of them were bacteria not targeted by the CLSI test. For 19 of the 20 strains, the synergy effect of clavulanic acid was observed in the modified double-disc synergy test (DDST) using only the cefepime disc. Because these strains had high MICs of $\geq$16 μg/ml of cephamycins such as cefoxitin and cefmetazole, these strains might produce high levels of AmpC in addition to ESBLs. The MicroScan ESBL confirmation panel showed an excellent performance in detection of target but not other bacteria. The addition of cefepime and clavulanic acid to the MicroScan panel may significantly improve the detection of nontarget bacteria. In this study, the ability to detect ESBL-producing bacteria was remarkably increased for the nontarget bacteria by the use of "fourth-generation" cephalosporins (broad-spectrum antibiotics that have enhanced activity against Gram-positive bacteria and β-lactamase stability, e.g., cefepime or cefpirome) in combination with clavulanic acid and a modified DDST.

Sturenburg et al. (244) aimed to assess the performance of the MicroScan ESBL Plus confirmation panel (WalkAway 96 SI) by using a series of 87 oxyiminocephalosporin-resistant Gram-negative bacilli of various species. Organisms tested included 57 ESBL strains comprised of *E. aerogenes* (3 strains), *E. cloacae* (10), *E. coli* (11), *K. pneumoniae* (26), *K. oxytoca* (3), and *P. mirabilis* (4). Also included were 30 strains resistant to oxyiminocephalosporins but lacking ESBLs, which were characterized by other mechanisms, such as the inherent clavulanate susceptibility of *Acinetobacter* spp. (4); the hyperproduction of AmpC in *C. freundii* (2), *E. aerogenes* (3), *E. cloacae* (3), *E. coli* (4), *H. alvei* (1), and *M. morganii* (1); the production of plasmid-mediated AmpC in *K. pneumoniae* (3) and *E. coli* (3); or the hyperproduction of the K1 enzyme in *K. oxytoca* (6). The MicroScan MIC-based clavulanate synergy test correctly classified 50 of 57 ESBL strains as being ESBL positive and 23 of 30 non-ESBL-producing strains as being ESBL negative (yielding a sensitivity of 88% and a specificity of 77%). Rates of false-negative results among ESBL producers were highest for *Enterobacter* spp. due to the masking of interactions between ESBL and AmpC β-lactamases. False-positive classification occurred for two *Acinetobacter* sp. isolates, one *E. coli* isolate producing plasmid-mediated AmpC, and two *K. oxytoca* isolates hyperproducing chromosomal K1 β-lactamase. The MicroScan clavulanate synergy test proved to be a valuable tool for ESBL confirmation. However, this test has limitations in the detection of ESBLs in *Enterobacter* spp. and in the discrimination of ESBL-related resistance from the K1 enzyme and from inherent clavulanate susceptibility in *Acinetobacter* spp. As an indicator for ESBL screening, the susceptibilities of the organisms to the five extended-spectrum β-lactams available on the MicroScan ESBL Plus panel were 98% for cefotaxime, 98% for cefpodoxime, 100% for ceftriaxone, 94% for aztreonam, and 96% for ceftazidime when interpreted according to CLSI criteria. Leaving aside ceftriaxone, these data add support to the CLSI recommendation that 100% sensitivity of ESBL screening can be achieved only by the testing of more than one agent. Importantly, no single drug at any one concentration accurately differentiated between strains producing ESBL and non-ESBL phenotypes. If a lowering of the MIC of either ceftazidime or cefotaxime by more than three 2-fold dilutions was taken as the criterion for ESBL confirmation, the MIC difference test was able to detect 50 ESBL strains out of the total of 57 identified by molecular characterization (yielding 88% sensitivity). False-negative results among ESBL producers were highest for tests with *Enterobacter* spp. The addition of clavulanic acid to ceftazidime (or cefotaxime) failed to lower MICs at least 8-fold in tests with 9 (or 7) out of 13 ESBL-producing *Enterobacter* strains. This poor performance was due partly to the ability of clavulanate to induce the chromosomal AmpC β-lactamase, which often resulted in MICs of the combinations being higher than that of the drug alone. The remaining ESBL-producing isolates demonstrated a significant clavulanic acid effect with both cefotaxime and ceftazidime. Only in tests with the CTX-M producers was cefotaxime clearly the single best antibiotic in its ability to confirm ESBL status, since clavulanate synergy was much more pronounced with cefotaxime (mean $\log_2$ reduction in MIC, 8.7) than with ceftazidime (mean $\log_2$ reduction in MIC, 4.8). In fact, when used alone, MIC difference testing with ceftazidime would have failed to recognize 4/10 CTX-M-producing ESBL strains. Among the 30 isolates resistant to oxyiminocephalosporins but lacking ESBLs, apparently positive results in MIC difference testing (3- to 8-fold increase in the MIC) were observed with cefotaxime and ceftazidime in 7 and 5 cases, respectively. Since the CLSI requires only one of the ESBL tests to be positive for an organism to confirm ESBL production, this resulted in an overall specificity of only 77% (23/30 strains). Accordingly, in the present study, clavulanate-based synergy testing failed to detect reliably ESBL production in tests with 54% to 69% ESBL-producing *Enterobacter* strains. The overall ability of clavulanic acid to lower the MICs of either cefotaxime or ceftazidime, due to these constraints, was diminished to 50/57 strains (88% sensitivity) and 46/57 strains (81% sensitivity), respectively. Approaches to overcome these difficulties include the use of tazobactam or sulbactam, which are much less likely to induce AmpC β-lactamases and are therefore preferable inhibitors for ESBL detection tests with these organisms, or the testing of cefepime as an ESBL detection agent. Cefepime is more reliable for ESBLs in the presence of an AmpC β-lactamase, as this drug is stable for AmpC but labile for ESBLs.

In a previous study employing the Etest ESBL system, cefepime with and without clavulanic acid had promising utility in identifying ESBLs in isolates that also carried an AmpC β-lactamase.

Lee et al. (155) evaluated the accuracy of cefotetan susceptibility determinations by using the MicroScan WalkAway Neg Combo panel type 32 system for AmpC-producing *K. pneumoniae* isolates. In total, 57 *K. pneumoniae* isolates that showed a D-shape flattening in a double-disc synergy test were studied. Cefotetan MICs were determined by the agar dilution method. The bla_{DHA} gene was detected in all 57 isolates, 1 of which coharbored bla_{CMY-1}. According to the MicroScan system, 28 isolates were susceptible, 18 were intermediate, and 11 were resistant to cefotetan. Compared with agar dilution, rates of very major, minor, and major errors were 28% (16/57 isolates), 47% (27/57), and 2% (1/57), respectively.

Ko et al. (142) evaluated the performance of the MicroScan Neg Combo type 44 panel, which was developed to confirm ESBL-producing *Enterobacteriaceae* using ceftazidime-clavulanate and cefotaxime-clavulanate. Nonduplicate strains (206), including 106 *E. coli*, 81 *K. pneumoniae*, 11 *K. oxytoca*, and 8 *P. mirabilis* strains, were tested with type 32 and type 44 panels. The results were compared with those of the CLSI phenotypic confirmatory test (CLSI-PCT) and disc approximation test (DAT). Isolates not susceptible to cefotetan or flagged as "possible ESBL, unable to interpret confirm test (possible ESBL)" on type 44 panels were tested with boronic acid discs to confirm AmpC production. Of the 206 isolates tested, 44 (21%) produced ESBLs by CLSI-PCT or DAT, including 27 *E. coli* isolates, 14 *K. pneumoniae* isolates 2 *K. oxytoca* isolates, and 1 *P. mirabilis* isolate. Thirty-eight isolates flagged as "confirmed ESBL" on type 44 panels were all confirmed as being ESBL producers. Of 14 *K. pneumoniae* isolates flagged as "possible ESBL," 6 were confirmed as coproducers of ESBL and AmpC, and 8 were confirmed as being AmpC producers. Type 44 panels showed an excellent performance in the detection of ESBL-producing *E. coli*, *Klebsiella* sp., and *P. mirabilis* isolates. When isolates were flagged as "confirmed ESBL," no other confirmatory test was necessary to report isolates as ESBL producers; however, a result of "possible ESBL" required a differential test for AmpC production.

Comparative studies. In a study by Hope et al. (109), 16 laboratories in South East England submitted 1,195 consecutive isolates of the *Enterobacteriaceae* found to be resistant, by their routine methods, to any or all of the drugs cefpodoxime, ceftazidime, and cefotaxime. These isolates were retested centrally with various cephalosporin-clavulanate combinations and with multiplex PCR for the bla_{CTX-M} and bla_{ampC} alleles. ESBLs were confirmed by reference investigation for 97 (77%) isolates out of 125 inferred to have ESBLs by the Phoenix system; the corresponding proportions increased to 102/111 (91%) for the Vitek 2 system ($P < 0.05$). The site using a Vitek system sent just eight isolates as ESBL producers, and ESBL production was confirmed for six of these isolates. The automated systems incorrectly inferred ESBL production in a few (11) AmpC hyperproducers and in isolates that proved susceptible upon reference testing; worryingly, all these systems failed to detect ESBLs in a few (14) cephalosporin-resistant strains (mostly *K. pneumoniae* or *E. cloacae*) that were found to have these enzymes by reference testing.

Wiegand et al. (285) compared three commercially available microbiology identification and susceptibility testing systems with regard to their abilities to detect ESBL production in isolates of the *Enterobacteriaceae*, i.e., the Phoenix, Vitek 2 (AST N020 [no clavulanate test], software 3.02), and MicroScan WalkAway-96 systems, using routine testing panels. One hundred fifty putative ESBL producers were distributed blindly to three participating laboratories. Conventional phenotypic confirmatory tests such as the disc approximation method, the CLSI double-disc synergy test, and the Etest ESBL were also evaluated. Biochemical and molecular characterization of β-lactamases performed at an independent laboratory was used as the reference method. One hundred forty-seven *E. coli*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *E. aerogenes*, *C. freundii*, *S. marcescens*, *P. mirabilis*, *P. vulgaris*, and *M. morganii* isolates were investigated. Of these isolates, 85 were identified as being ESBL producers by the reference method. The remaining isolates were either hyperproducers of chromosomal AmpC, K1, or SHV or lacked any detectable β-lactamase activity. The system with the highest sensitivity for the detection of ESBLs was Phoenix (99%), followed by Vitek 2 (86%) and MicroScan (84%); however, the specificity was more variable, ranging from 52% (Phoenix) to 78% (Vitek 2). Vitek 2 data reflected the reliance of detection on expert systems in the absence of a confirmatory test. The detection of ESBL-positive isolates of the *Enterobacteriaceae* was variable, particularly with organisms such as K1-hyperproducing *K. oxytoca* and AmpC-producing *Enterobacter* and *Citrobacter* sp. isolates. The system with the highest sensitivity was Phoenix (99%), with a specificity of 52% (version 4.05W; GN Combo Panels 448541). The Phoenix ESBL test incorporated into the panel uses growth in the presence of cefpodoxime, ceftazidime, ceftriaxone, and cefotaxime, with or without clavulanic acid (CA), to detect the production of ESBL. The BDXpert system (version 3.81C) provides a series of rules, which are triggered by various conditions given by the bacterial species identification, the result of the ESBL test, and MIC data. The BDXpert rules associated with ESBL identification in *E. coli*, *K. pneumoniae*, and *K. oxytoca* strains include rules 1502 and 1505, "isolate is confirmed positive for ESBL," and rule 106, "screening tests suggest a possible ESBL producer, confirmatory testing is recommended." Interpretation rules for *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, and *Serratia* spp. include rule 1405, "isolate exhibits ESBL resistance"; rule 1430, "this isolate may exhibit resistance to extended-spectrum β-lactam antibiotics"; and rule 1433 (for *Enterobacter* spp. only), "isolate exhibits unusual resistance to third-generation cephalosporins, additional confirmatory testing for possible ESBL or AmpC hyperproduction is recommended." If the ESBL test is negative, then no rule is supplied. A printed report of each test indicates the actual MIC, the breakpoint-based interpretation, the expert system's interpretation, therapeutic advice at times, and the rule applied. Reports were considered a positive ESBL screening result for the purposes of the study if any of the rules listed above were triggered. The performances of the semiautomated systems differed widely with the species investigated. The sensitivities of the conventional test methods ranged from 93 to 94%. The double-disc synergy test showed the highest specificity and positive predictive value among all test meth-

ods, i.e., 97% and 98%, respectively. Considering the rather low specificity observed by that study, those authors recommended the use of a manual test for confirmation once an organism is reported to be positive for ESBL production by any of the semiautomated systems. Alternatively, one can use one of the test panels developed by the manufacturers of the semiautomated systems specifically for the confirmation of ESBL production. The integration of an ESBL confirmation test into the routine test panels of the semiautomated systems would considerably reduce the time to accurate ESBL detection in the laboratory and might contribute to an earlier institution of optimal antibiotic therapy and adequate infection control procedures. MicroScan (Neg/BP/Combo 30-B1017-306E combination panels) had a sensitivity of 84%. The integrated Lab-Pro system, version 1.12, which includes the Alert Expert System, uses growth in the presence of cefpodoxime (4 μg/ml) and ceftazidime (1 μg/ml), i.e., at concentrations recommended by the CLSI for ESBL screening, as primary indicators for possible ESBL production. MICs obtained for ceftriaxone, cefotaxime, and aztreonam are interpreted according to CLSI breakpoints, and results may also trigger rules that alert users to possible ESBL production. These results were considered a positive ESBL screening. Screening with this system is limited to *E. coli*, *K. pneumoniae*, and *K. oxytoca*, i.e., those species that are primarily dealt with in CLSI guidelines. Other *Enterobacteriaceae* which commonly harbor AmpC enzymes but additionally may produce ESBLs, such as *Citrobacter* spp., *Enterobacter* spp., *Serratia* spp., and members of the *Proteus* group, may also produce a positive screening result. However, the expert system does not support the detection of derepressed AmpC β-lactamases and ESBL production in these organisms and does not alert the user to the possibility of ESBL production. The MicroScan expert system discriminates between ESBL producers and K1 hyperproducers on the basis of the ceftazidime susceptibility of the latter. The MicroScan panel used in this study correctly identified all ESBL-producing *K. oxytoca* isolates but misclassified 7/8 K1 hyperproducers as being ESBL positive and did not point to the possibility of K1 production.

Farber et al. (81) tested 114 strains using the Etest as the standard, various available panels for both automated systems (for BD Phoenix, the NMIC/ID-50 and NMIC/ID-70 GN Combo panels for the combined identification and susceptibility testing of Gram-negative bacilli; for Vitek 2, the ID-GNB panel for the identification of Gram-negative bacilli and the AST-N020, AST-N041, and AST-N062 panels for susceptibility testing), and a chromogenic agar medium (bioMérieux). PCR for common ESBL gene families (TEM, SHV, OXA, and CTX-M) and for chromosomal or plasmid-borne AmpC genes was conducted to complete the study design. For the tested specimens overall, the chromID ESBL agar showed the highest sensitivity (96%) but the lowest specificity (10.5%) compared to those of the reference Etest for the detection of ESBL-producing strains. The Phoenix system showed sensitivities of 77% and 84% and specificities of 61.5% and 75% for the NMIC/ID-50 and NMIC/ID-70 panels, respectively. The sensitivity of the Vitek 2 system ranged from 79% (AST-N020) to 81% (AST-N062) and up to 84% (AST-N041). The specificities of the respective panels were 50% (AST-N041 and AST-N062) and 56% (AST-N020). In conclusion, the sensitivities and specificities of ESBL detection by the different methods differ depending on the microorganisms studied. Interestingly, all AmpC-positive *Enterobacter* sp. isolates were correctly reported as being non-ESBL-producing strains by the automated system. The integration of an ESBL screen with the panels for the Vitek 2 system, which is missing on the AST-N020 panel, improved the sensitivity, but not the specificity, of ESBL detection for all species and subspecies. Both the AST-N041 and AST-N062 panels were comparable in sensitivity and specificity. The Vitek panel included cefotaxime, ceftazidime, and cefepime, and the use of cefepime might explain the good results of Vitek 2 for the AmpC-producing group, although a low number of these isolates was included. All panels of the Vitek 2 system and the chromID ESBL agar had a sensitivity of 100%, whereas the specificities were relatively low, at just 33%. Concerning species other than *E. coli*, the AST-N041 and AST-N062 panels of Vitek 2 and the chromID ESBL agar all showed a sensitivity of 100%. The Vitek 2 panel without an ESBL screen achieved a sensitivity value of 92%. Farber et al. (81) contrasted their performance values for the Vitek 2 AES, successfully detecting ESBL-producing organisms in AmpC-producing organisms such as *Enterobacter* spp., *Citrobacter* spp., and *Proteus* spp., with more impressive values reported by other workers (159, 259), who tested only *E. coli*, *K. pneumoniae*, and *K. oxytoca* strains.

Vitek 2 and *P. aeruginosa*

The method of determining resistance mechanisms in organisms used as test panels to evaluate automated instruments is even more critical for *P. aeruginosa* than for the *Enterobacteriaceae*, as many different resistance mechanisms (porin loss, efflux, and enzyme production, etc.) compound antibiograms, and many of these cannot reliably be discerned by using phenotypic methods. Some of the mechanisms of resistance of *P. aeruginosa* to antimicrobial agents may preferentially affect β-lactam compounds, and some automated systems may not correct for this in the interpretation of the results (124, 137, 242). Efflux pumps may differentially have an impact on carbapenems, especially meropenem versus imipenem. Similarly, the loss of porins (OprD) is not always expressed in resistance to antibiotics at clinical breakpoints. Variations in inoculum concentrations and incubation times may also affect the detection of β-lactam resistance in *P. aeruginosa* strains. Thus, rapid automated susceptibility testing systems may perform poorly in detecting resistance to some β-lactam compounds for technical reasons (including the methodologies used by the test system and software calculations) because of the underlying resistance mechanisms of the organism (21, 22, 70). The presence of spontaneous β-lactam-resistant mutants, which may appear as isolated colonies on the surface of the agar plate and may be selected during incubation with the antibiotic, may not be detected by a microdilution or automated method.

Mazzariol et al. (170) used a total of 78 *P. aeruginosa* isolates grouped according to the ceftazidime and imipenem phenotype to assess the accuracy of the Vitek 2 system. Comparisons were made with an MIC gradient test for piperacillin-tazobactam, ceftazidime, aztreonam, imipenem, meropenem, gentamicin, and ciprofloxacin. For the total of 546 isolate-antimicrobial combinations tested, the category agreement was 83%,

with 2%, 1.6%, and 13% very major, major, and minor errors, respectively. The accuracy of the Vitek 2 system was influenced differently by the resistance mechanisms, and interpretations of the results in relation to the phenotype could improve the performance of the system; e.g., altered permeability seemed to play an important role in decreasing the accuracy not only against carbapenems but also against ceftazidime, aztreonam, and gentamicin. Piperacillin-tazobactam and ciprofloxacin were less affected by permeability defects. False susceptibility to meropenem was detected only in this group. Those authors noted that the simultaneous testing of both carbapenems should help the microbiologist and the clinician identify possible problems with carbapenem susceptibility. High cephalosporinase and metallo-β-lactamase (MBL) levels were consistently associated with a significant bias toward false susceptibility to piperacillin-tazobactam. This result underlines the need to insert a new rule in the AES that excludes this result in the interpretation of the AST results of isolates with these phenotypes.

Torres et al. (265) selected clinical isolates of *P. aeruginosa* to assess the quantitative (MIC) and qualitative (clinical category) agreement between the microdilution broth reference method and disc diffusion, Etest, and Vitek 2 for determinations of susceptibility of to piperacillin, piperacillin-tazobactam, ceftazidime, aztreonam, cefepime, and imipenem. The results obtained by the reference method were compared with those obtained by the other methods. As a result of this study, the AES was modified with new interpretation rules. Overall, Vitek 2 showed the lowest MIC_{90} values for the six antibiotics. The reference method categorical testing (susceptibility and resistance) rates with *P. aeruginosa* were 12% and 88% for piperacillin, 23% and 77% for piperacillin-tazobactam, 15% and 78% for ceftazidime, 13% and 54% for aztreonam, 17% and 75% for cefepime, and 8% and 90% for imipenem, respectively. Very major errors (falsely susceptible) were detected only for aztreonam and cefepime with disc diffusion and for imipenem with three methods. Major errors (falsely resistant) were generally acceptable for all antibiotics except piperacillin-tazobactam. Vitek 2 yielded a high level of minor errors (trends toward false susceptibility), mainly with ceftazidime and cefepime. A good agreement was obtained for all antibiotics/methods assayed, thus highlighting the importance of the AES for the categorization of β-lactam susceptibility in *P. aeruginosa*. Although Vitek 2 yielded lower MIC values for some of the antibiotics, susceptibility was correctly assigned by the AES.

Carbapenem Resistance

Clinical microbiology laboratories have often found it difficult to achieve accurate susceptibility testing results for carbapenems. For example, early studies documented false resistance to imipenem due to the degradation of the drug in Sensititre panels (190, 284); this was apparently remedied by changes of desiccants in the packages (59).

Vitek. Studies with the Vitek system demonstrated false resistance, specifically with *P. mirabilis* (70). Several recent proficiency testing studies have shown problems of both false resistance and false susceptibility with imipenem and meropenem among a variety of enteric species (242, 243). Even quality control measures failed to detect all false-resistance problems (43). Yigit and colleagues described the KPC-1 β-lactamase in an imipenem-resistant isolate of *K. pneumoniae* from the United States in 2001 (302). Bratu and colleagues reported false-susceptible results for *K. pneumoniae* with the MicroScan WalkAway system, which were attributed in part to a low inoculum size (33). Similar problems with false-susceptible results were noted for the Vitek system (32).

Tsakris et al. (269) reported that the introduction of Vitek GNS-506 susceptibility testing cards resulted in an apparently high prevalence of imipenem-resistant *A. baumannii* isolates. When 35 of these isolates were further tested by disc diffusion, broth microdilution, and agar dilution, 32 were imipenem susceptible by all tests, and 3 were susceptible or intermediate, depending on the method. The pseudoresistant *Acinetobacter* strains did not form a genetically homogeneous group. Those authors suggested that the detection of imipenem-resistant *A. baumannii* strains by Vitek should be confirmed by an additional test.

Sanders et al. (222) studied the impact of the Vitek 2 system (T01.01.0038) and the Advanced Expert System (AES X01.00P) on the clinical laboratory of a university-based hospital. A total of 259 consecutive isolates, including 170 nonduplicate strains of *Enterobacteriaceae* (AST-N009 card), were studied. Of concern was the false resistance to imipenem among 7 of the 13 isolates of *P. mirabilis*. Although the AES indicated to the user that this result was probably incorrect, this problem with the Vitek 2 system should ultimately be resolved in the algorithm used to determine susceptibility results. Since imipenem resistance, although rare among the *Enterobacteriaceae*, can occur in *P. mirabilis*, it is imperative to be able to ascertain when resistance is real and when it is due to a problem with the test system.

Nakamura and Takahashi (181) investigated whether or not the AES correctly categorized the β-lactamases derived from the Vitek 2 susceptibility result using the AST-N025 test card and VT2-R03.02 software with strains of the *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii*. They used 131 strains genotypically studied. The AES result matched the phenotype testing result for 120 (92%) of the 131 strains. However, 3 *Serratia* sp. strains with impermeability with or without β-lactamase were classified as being carbapenemase producers, and 2 carbapenemase-producing *C. freundii* strains were classified as being ESBL producers (no phenotype in database). In addition, an ESBL-producing *P. rettgeri* strain with an elevated imipenem MIC (>16 μg/ml) could not be identified; carbapenemases in *M. morganii* (1 isolate) and *P. rettgeri* (2 isolates) were not identified (no phenotype in the database).

Thomson et al. (259) noted that even though a significantly elevated imipenem MIC of 16 μg/ml was detected for a KPC-2-producing *K. pneumoniae* isolate, the Vitek 2 AES did not suggest the production of carbapenemase. Upon retesting, the imipenem MIC remained at 16 μg/ml, but the AES changed it to 2 μg/ml.

BD Phoenix. Thomson et al. (259) studied 102 well-characterized strains of ESBL-positive or -negative *E. coli*, *K. pneumoniae*, and *K. oxytoca*. At least 38 distinct ESBLs were included. The strains were chosen to include some strains known to cause false-positive and false-negative CLSI ESBL confirmatory test results. Therefore, enzyme characterization, rather

than the CLSI test, was the reference method for the evaluation. The currently available Phoenix expert system recognized unusually elevated carbapenem MICs for two of three KPC-2-producing *K. pneumoniae* strains and for a KPC-3-producing *E. coli* strain, while the Vitek 2 expert system recognized reduced carbapenem susceptibility in only two of the four strains. Neither system suggested that an unusually elevated carbapenem MIC was consistent with possible carbapenemase production.

Fisher et al. (86) noted that the phenotypic identification of KPC among members of the *Enterobacteriaceae* remains challenging. That study compared the Phoenix system (instrument version 5.15A, software version 5.10A/V4.31A) with the CLSI confirmatory method to identify ESBL production among 200 *E. coli* and *K. pneumoniae* isolates. PCR screening revealed eight KPC-positive isolates, all of which tested as ESBL positive or ESBL positive plus AmpC positive by phenotypic methods, but half were reported as being carbapenem susceptible by the Phoenix system. Overall, these results indicate that laboratories should use the Phoenix ESBL results only as an initial screen, followed by confirmation with an alternative method. Many automated instruments fail to detect carbapenemases, which is a serious concern for the laboratory (253).

Ogunc et al. (188) evaluated imipenem and meropenem susceptibilities by disc diffusion, Etest, and broth microdilution of *P. aeruginosa* and *A. baumannii* isolates found to be resistant or intermediate to imipenem-meropenem by BD Phoenix: 85 nonduplicate *A. baumannii* and 51 nonduplicate *P. aeruginosa* strains were tested by Etest, disk diffusion, and the reference broth microdilution (BMD) method according to CLSI recommendations. All 51 *P. aeruginosa* strains determined to be imipenem and/or meropenem resistant or intermediate by BD Phoenix were found to be imipenem and/or meropenem resistant or intermediate by the reference BMD method. Minor-error rates were the same for all testing systems (2%), except for the meropenem results of the BD Phoenix system (6%). No major errors were produced by any system. For *A. baumannii*, only one very major error was detected for meropenem by Phoenix. The number of minor errors determined for meropenem by all testing systems compared to the reference test ranged from 2 (2.4%) to 3 (3.5%). It was concluded that carbapenem susceptibility results obtained by Phoenix for *P. aeruginosa* and *A. baumannii* isolates could be reported without an additional susceptibility testing method unless indicated on a per-case basis.

MicroScan. Previous proficiency testing surveys have documented carbapenem testing problems with MicroScan (70, 107, 162, 243). Fernandez et al. (84) evaluated the reliability of MIC values of imipenem for Gram-negative rods obtained with the MicroScan WalkAway-98 system. One hundred seventy-three consecutive clinical isolates of Gram-negative rods for which the MICs of imipenem were ≥4 μg/ml (Urine-Combo 6I [U6I] panels) or ≥8 μg/ml (Neg-Combo 6I [N6I] panels) were evaluated, including 104 nonfermenting Gram-negative rods (NFGNR) and 69 isolates of the *Enterobacteriaceae*. Microdilution, according to CLSI guidelines, was used as the reference. MICs of imipenem determined by WalkAway-96 and microdilution differing by ≥2 dilution steps from those obtained with microdilution were considered discrepant results. The percentages of discrepancies in the MICs of imipenem determined with U6I panels were 74% and 84% for NFGNR and *Enterobacteriaceae*, respectively. No very major errors were detected. Major errors were observed for 6% and 12% of the strains with U6I panels for NFGNR and *Enterobacteriaceae*, respectively, and for 12% (NFGNR) and 50% (*Enterobacteriaceae*) of the strains with N61 panels. With U61 panels, minor errors were observed for 11% and 25% of isolates of NFGNR and *Enterobacteriaceae*, respectively, while with N61 panels, minor errors were observed for 39% and 45% of these groups, respectively. The MIC of imipenem of ≥4 μg/ml obtained with the WalkAway-96 system for Gram-negative rods, particularly in the case of strains of the *Enterobacteriaceae*, should be confirmed with a reference method.

Gordon and Wareham (101) reported the failure of the automated MicroScan WalkAway system to detect carbapenem heteroresistance in *E. aerogenes*. Carbapenem resistance has become an increasing concern in recent years, and robust surveillance is required to prevent the dissemination of resistant strains. Reliance on automated systems may delay the detection of emerging resistance. When colonies of strain EA2 taken from a primary plate subcultured directly from positive blood culture bottles were used, a resistant subpopulation of colonies with an MIC of >32 μg/ml could repeatedly be seen growing within the zones of inhibition surrounding ertapenem and imipenem Etest strips. The mechanisms of carbapenem resistance have not been fully characterized. A modified Hodge test did not indicate carbapenemase production, and PCR screening using published primers did not detect any known class A (bla_{KPC}, bla_{IMI}, bla_{NMC}, bla_{SME}, bla_{GES}, and bla_{SFC}), class B (bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{SPM}, bla_{SFH}, bla_{AIM}, and bla_{KHM}), or class D ($bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}48\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, and $bla_{OXA\text{-}58\text{-}like}$) carbapenemase genes, suggesting that impermeability and AmpC overexpression may be important, as has been described for other isolates recently recovered in the United Kingdom. Further identification and susceptibility testing of this isolate were carried out with the MicroScan WalkAway system using the Negative Combo 42 panel, which reported the *E. aerogenes* strain (EA2) to be resistant to all cephalosporins, due to the production of an extended-spectrum β-lactamase, yet susceptible to carbapenems.

Problems with the detection of imipenem resistance in *P. aeruginosa* (130) and in *A. baumannii* isolates using MicroScan have also been reported. For the *A. baumannii* evaluation, there was no difference in detection when a heavier inoculum was used (147).

Rodriguez et al. (212) described the characterization of 9 new clonally related multiresistant *P. aeruginosa* isolates from Northern Spain possessing the $bla_{VIM\text{-}2}$ gene. Identification and preliminary susceptibility studies were performed with the MicroScan WalkAway system, and results were verified by a microdilution reference method. MICs of imipenem and meropenem for the 9 isolates ranged from 32 to 128 and 16 to 64 μg/ml, respectively. Nine isolates had a single repetitive extragenic palindromic (Rep)-PCR pattern and were intermediate or resistant to ceftazidime, cefepime, gentamicin, tobramycin, amikacin, and ciprofloxacin. Eight of the nine isolates were susceptible to aztreonam. The hydrolysis activity of imipenem in metallo-β-lactamase-positive isolates ranged from 162 ± 18 to 235 ± 28 pmol/min/μg protein and was abolished in the

presence of 5 mM EDTA. All isolates possessed an integron with the bla_{VIM-2} gene. In the clinical isolates studied, the presence of VIM-2 sufficed to explain their resistance to carbapenems.

Comparative studies. Steward et al. (242) investigated the overdetection of imipenem resistance by testing 204 selected isolates from the Project ICARE collection plus 5 imipenem-resistant challenge strains against imipenem and meropenem by agar dilution, disc diffusion, Etest, two MicroScan WalkAway conventional panels (Neg MIC Plus 3 and Neg Urine Combo 3), and two Vitek cards (GNS-116 containing meropenem and GNS-F7 containing imipenem). The results of each test method were compared to the results of BMD testing using in-house-prepared panels. Seven imipenem-resistant and five meropenem-resistant isolates of *Enterobacteriaceae* and 43 imipenem-resistant and 21 meropenem-resistant isolates of *P. aeruginosa* were identified by BMD. For the *Enterobacteriaceae*, the imipenem and meropenem test methods produced low numbers of very major and major errors. All systems produced low numbers of very major and major errors when *P. aeruginosa* was tested against imipenem and meropenem, except for Vitek (major error rate for imipenem, 20%). With MicroScan and 114 *P. aeruginosa* isolates, those authors recorded a total of 3 very major, 1 major, and 62 minor errors using two systems and two carbapenems. With 95 isolates of the *Enterobacteriaceae*, there were 3 very major, 2 major, and 21 minor errors. Further testing conducted in 11 of the participating ICARE hospital laboratories failed to pinpoint the factors responsible for the initial overdetection of imipenem resistance. However, that study demonstrated that carbapenem testing difficulties do exist and that laboratories should consider using a second, independent, susceptibility testing method to validate carbapenem-intermediate and -resistant results.

Giakkoupi et al. (97) determined the susceptibilities of five VIM-1-producing *K. pneumoniae* isolates to β-lactams by broth microdilution, Etest, disc diffusion, Vitek 2 using AST-N017 and AST-EXN2 susceptibility cards, Phoenix with the NMIC/ID-12 panel, and MicroScan (autoScan-4) with the Neg MIC type 30 panel. Significant discrepancies were observed for determinations of susceptibility to imipenem and meropenem with Vitek 2 and Phoenix. With the BMD reference method, as well as disc diffusion, all isolates were classified as being susceptible to imipenem and meropenem. The Etest MICs of carbapenems were the same as those determined by BMD, except for one strain, which was classified as being intermediate to imipenem (the Etest MIC was 8 μg/ml, while the BMD MIC was 2 μg/ml). Carbapenem susceptibility data produced by the MicroScan system were in agreement with those of the BMD method. Readings performed either by visual inspection of the panels or with the instrument consistently indicated that the imipenem and meropenem MICs were ≤4 μg/ml. By Vitek 2, four isolates were consistently characterized as being resistant to imipenem, with the MICs for them being ≥16 μg/ml, and the remaining isolate was characterized as being intermediate (MIC = 8 μg/ml). On the other hand, the Vitek 2 MICs of meropenem corresponded to those of the BMD method, ranging from 1 to 2 μg/ml. The Advanced Expert System (AES) of Vitek 2 (software version VTK-R01.02) interpreted the AST-N017 data (including imipenem but not meropenem and aztreonam) as being fully consistent with the organism identification and did not suggest any corrections. However, considering the AST-EXN2 data (including meropenem and aztreonam but not imipenem), either alone or combined with AST-N017, the AES suggested retesting or changing the MIC of meropenem (from 2 to 0.5 μg/ml) and the interpretation of the MIC of aztreonam (from susceptible to intermediate). With Phoenix, all five isolates were found to be resistant to both imipenem and meropenem (MICs ≥ 16 μg/ml). With the Phoenix system, the relevant BDXpert-triggered rules (codes) were rule 1513 (suggesting confirmation of resistance to carbapenems and, if confirmed, consideration of the isolate as being resistant to all β-lactams) and rule 106 (recommending testing for ESBLs); i.e., the resistance phenotype could not be interpreted. Extended-spectrum β-lactamase production was also indicated by MicroScan; the VIM-1 phenotype could not be interpreted. The overdetection of carbapenem resistance by automated systems has been attributed to errors such as high inocula, improper interpretation of the results, and antibiotic degradation.

Tenover et al. (253) noted that the detection of β-lactamase-mediated carbapenem resistance among *K. pneumoniae* isolates and other isolates of the *Enterobacteriaceae* is an emerging problem. In that study, 15 bla_{KPC}-positive *K. pneumoniae* isolates that showed discrepant results for imipenem and meropenem from 4 New York City hospitals were characterized by isoelectric focusing, BMD, disc diffusion (DD), and the MicroScan, Phoenix, Sensititre, Vitek, and Vitek 2 automated systems. All 15 isolates were either intermediate or resistant to imipenem and meropenem by BMD; 1 was susceptible to imipenem by DD. MicroScan and Phoenix reported 1 (7%) and 2 (13%) isolates, respectively, as being imipenem susceptible. Vitek and Vitek 2 reported 10 (67%) and 5 (33%) isolates, respectively, as being imipenem susceptible. By Sensititre, 13 (87%) isolates were susceptible to imipenem, and 12 (80%) were susceptible to meropenem. The Vitek 2 Advanced Expert System changed 2 imipenem MIC results from >16 μg/ml to <2 μg/ml but kept the interpretation of resistant. The problem of the Vitek 2 AES reporting imipenem-resistant results as <2 μg/ml has apparently been corrected in software version R04.02. Although the MicroScan and Phoenix systems produced results that were more consistent with those of the reference testing systems than those of Vitek and Sensititre AutoReader, problems in the detection of carbapenem resistance were still evident with the former systems. These problems may be partially attributable to differences in the inocula, although those authors also reported variations in susceptibility dependent upon the reference method used. This suggests variable expressions of resistance determinants, which could go undetected by some automated methods. The recognition of carbapenem-resistant *K. pneumoniae* strains continues to challenge automated susceptibility systems.

In Australia, several members of the *Enterobacteriaceae* with decreased susceptibility to carbapenems have recently emerged, associated with a carbapenem-hydrolyzing metallo-β-lactamase (MBL) encoded by bla_{IMP-4}. Where this MBL is present, elevated MICs of carbapenems can be modified by specific inhibitors *in vitro*. The inhibition of MBL-mediated resistance to extended-spectrum cephalosporins requires that no other cause of resistance coexists in the strain. Recognition is there-

fore complicated by the highly efficient transmission of the MBL structural gene to strains in which the carbapenem-resistant phenotypes differ and in which a variety of other antibiotic resistance mechanisms may occur, including ESBLs. Espedido et al. (78) showed that susceptibility profiles generated by diagnostic algorithms in commonly used automated bacterial identification systems such as the Phoenix NMIC/ID-101 panel and Vitek Legacy GNS-424, Vitek 2 AST-N044, and Vitek 2 AST-N019 Gram-negative cards failed to reliably identify a metallo-β-lactamase in strains of the *Enterobacteriaceae*. The misidentification of an ESBL may result in an inappropriate dismissal of drugs such as aztreonam in favor of carbapenems, which may in turn select for carbapenem resistance.

Anderson et al. (5) determined meropenem, imipenem, and ertapenem susceptibilities by BMD using cation-adjusted Mueller-Hinton broth in panels that were prepared in-house, disc diffusion, Etest, MicroScan autoScan using the NM32 panel, and Vitek 2 using the AST GN14 card. The testing of susceptibility to meropenem and imipenem was performed by Phoenix with the NEG MIC 30 or NEG MIC 112 panel, Vitek Legacy with the GNS-122 and GNS-127 panels, and Sensititre AutoReader with the GN2F panel. Using the criterion of a carbapenem MIC of >1 μg/ml, the sensitivity of detection of KPC-mediated resistance increased for ertapenem, meropenem, and imipenem, with only small changes in specificity. Meropenem susceptibility testing demonstrated greater than 90% sensitivity by BMD and MicroScan, whereas imipenem testing was at least 90% sensitive by BMD, Etest, Vitek 2, and MicroScan.

Horii et al. (110) showed that of 19 isolates of mucoid *P. aeruginosa*, 2 showed imipenem resistance conferred by reduced OprD production. Imipenem resistance was detected by MicroScan broth microdilution and Etest, but MICs could not be determined by Vitek for one isolate. Those authors concluded that in cases where susceptibility cannot be determined by broth microdilution, Etest results would be valuable for effective treatment.

Bulik et al. (36) reported the level of agreement between broth microdilution, Etest, Vitek 2, Sensititre, and MicroScan to accurately determine meropenem MICs and clinical characterization of KPC carbapenemase-producing *K. pneumoniae* isolates. A total of 46 *K. pneumoniae* isolates harboring bla_{KPC} by a modified Hodge test, collected from two hospitals in New York, were included. Results obtained with each method were compared with results of broth microdilution, and agreement was assessed based on MIC and CLSI interpretative criteria using 2010 susceptibility breakpoints. Based on broth microdilution, 0%, 2.2%, and 98% of the KPC isolates were defined as being susceptible, intermediate, and resistant to meropenem, respectively. MicroScan demonstrated the highest agreement, 96%, based on MICs, with 2.2% minor errors and no major or very major errors. The Etest demonstrated 83% agreement with broth microdilution MICs, a very-major-error rate of 2.2%, and a minor-error rate of 2.2%. The Vitek 2 MIC agreement was 30%, with a 24% very-major-error rate and a 39% minor-error rate. Sensititre demonstrated MIC agreement for 26% of isolates, with 3% very-major-error and 26% minor-error rates. The application of FDA breakpoints had little effect on minor-error rates but increased very-major-error rates to 59% for Vitek 2 and Sensititre. Meropenem MICs and clinical categorization for KPC-producing *K. pneumoniae* isolates differ depending on the methodologies. Confirmation of testing results is encouraged when an accurate MIC is required for antibiotic dosing optimization.

Woodford et al. (291) assessed the abilities of three commercial systems to infer carbapenem resistance mechanisms in 39 carbapenemase-producing isolates and 16 other carbapenem-resistant isolates of the *Enterobacteriaceae*. Sensitivity and specificity values for "flagging" a likely carbapenemase were 100% and 0%, respectively (Phoenix panel 41 NMIC/id-76, which tests ertapenem at 0.25 to 1 μg/ml and imipenem and meropenem both at 1 to 8 μg/ml); 82 to 85% and 6 to 19%, respectively (MicroScan Neg MIC Panel type 36 [NM36], which includes ertapenem at 0.5 to 4 μg/ml and imipenem and meropenem both at 1 to 8 μg/ml, and Neg BP Combo Panel type 39 [NBC39], which tests ertapenem at 2 to 4 μg/ml and imipenem and meropenem both at 2 to 8 μg/ml); and 74% and 38%, respectively (Vitek 2 AST N-054 card, which incorporates an ertapenem range of 0.5 to 8 μg/ml and a meropenem range of 0.25 to 16 μg/ml). OXA-48 producers were poorly detected, but all systems reliably detected isolates producing KPC and most with metallo-carbapenemases. Data indicated that laboratories using any of the commercial systems examined will detect >90% of isolates of the *Enterobacteriaceae* that are resistant or have reduced susceptibility to one or more carbapenems, with performance in the rank order Phoenix > Vitek 2 = MicroScan NMC36 > MicroScan NBC39. The systems differed, however, in their abilities to infer carbapenemase production accurately and in the degree to which they even attempted to do so. By this criterion, the rank order was Phoenix > MicroScan NM36 > MicroScan NBC39 > Vitek 2.

COMMENTARY

The approval by the authorized/notified bodies or self-declaration by the manufacturers of the devices/instruments for *in vitro* diagnostic use includes analytical and diagnostic performance analyses at the premarketing stage. However, it is strongly recommended that postmarket evaluations of the technologies from the aspects of clinical utility and various laboratory-related outcomes should be made before the technology acquisition decision is made and implementation is started. Flaws in these evaluation studies have been outlined by I. Cagatay Acuner (personal communication). It is the responsibility of the individual laboratory director to assess, in an evidence-based manner, both the results of a well-designed in-house evaluation study and relevant postmarket evaluation studies reported in the literature for a critical assessment of the available health care technologies (10, 183). Therefore, studies of performance analysis are much needed and well received in the literature, and there have already been numerous studies published. In this context, it is worth noting that the performance analysis scheme recommended by the *Sta*ndard for the *R*eporting of *D*iagnostic Accuracy Studies (STARD) initiative is not easy to comply with, and studies within the framework of the STARD initiative are lacking in the literature (26). In the domain of performance analysis of AST methods, most of the studies in the literature, if not all, are designed according to the FDA performance analysis scheme in a simplified form (88). Many published studies have deficiencies that cast doubt on the validity of the reported performance results. First, most

of the studies lacked an initial, well-designed, and true reproducibility testing step (among the important aspects are not including just QC strains, sample size, and number of replicates, etc.) required by the FDA procedure, and therefore, the validity of these studies inevitably remains controversial. Moreover, it is well known that the prevalence (prior probability) of the response variables affects the performance of a test method (posterior probability). Hence, although the SIR prevalence is necessary to include in study reports, most often it is not. This deficiency causes an inability to determine the setting for which the study results are valid. Another frequently encountered weakness of published studies is obviously insufficient sample sizes tested for a unique and standardized combination of "a microorganism group against an antimicrobial." Usually, a composite approach by merging different microorganism groups with insufficient numbers into one mixed group to reach a significant sample size is employed in performance analyses. Indeed, such a calculated performance result should not be claimed to be valid for each unique and standardized combination included. Other main considerations for an adequate study design are the choice of an appropriate reference method (a standardized MIC-based method should be preferred), the inclusion of well-characterized challenge strains, the inclusion of a mix of fresh and stock isolates, reporting of the version of the integrated software used, and the use of current AST and performance analysis standards in testing, interpretation, and validation.

CONCLUSIONS

The published performance data for commercial instruments appear to be related to several factors: the hardware, the version of software, the effectiveness of the expert system (125, 223), the number of antibiotics tested (174), the presence or absence of a specific ESBL confirmatory test (79), the use of key antibiotics (such as cefpodoxime and cefepime) (41), and the makeup of the strains being tested. One certain method of "sabotaging" expert system performance is to deliberately alter the composition of the card or panel, and there are competing demands: what is useful for interpretive readings, antibiotics that clinicians wish to use, antibiotics that companies wish to sell, and the fact that bacteria also alter with time. There are examples of systems failing to detect ESBLs because cefpodoxime has been replaced with "something more useful" and where systems cannot differentiate between ESBLs and AmpCs because cefoxitin has been replaced with another antibiotic. Clearly, any new card formulation should be tested thoroughly, and it is entirely inappropriate for manufacturers to quote data from studies with card "A" as evidence of the effectiveness of systems using card "B." Where stated in the original papers, software versions and card numbers are always cited in this review. There is a clear need for a simple interpretation of susceptibility patterns rather than complex interpretations that need intervention from the user. Even highly competent clinical microbiologists often ignore expert system comments because a number of interpretations are offered, and the microbiologists can be confused (K. Thomson, personal communication). One cannot help but feel that simple software changes would improve the performance of expert systems and help to differentiate between ESBLs and hyperproduced K1, for example, the strategy described by Derbyshire et al. (67). Furthermore, the introduction of specific enzyme inhibitors should facilitate the differentiation of ESBLs and AmpC enzymes (257). Changes to card or panel formulations are often met with a considerable time delay.

Antibiotic susceptibility tests are limited by their failure to detect low-level resistance to cephalosporins, ureidopenicillins, and carbapenems, etc., and by the fact that most tests detect bacteriostatic activity only. Expert systems are limited by the fact that knowledge has to be translated into a usable form; by the constant need to update systems (obviated by the use of neural nets); by the fact that new resistance mechanisms constantly arise, many giving phenotypes identical to existing ones; by the fact that bacteria can produce unexpectedly large or small amounts of enzyme; and by the differences between biochemical and clinical resistances. Furthermore, some bacteria have multiple resistance mechanisms, including enzymes, increased efflux, and porin loss: Shaw et al. (227) detected 70% multiple determinants in 4,088 aminoglycoside-resistant strains of the *Enterobacteriaceae*, and Essack et al. (80) detected 84 TEM and SHV *bla* genes in 25 *K. pneumoniae* isolates. Despite the highlighted shortfalls of many published studies, it is clear that the advantages of expert systems outweigh the disadvantages. Expert systems permit continuous quality assurance and ensure consistency, they detect weakly expressed resistance, they can deduce results for nontested antibiotics, they can improve the interpretation of results, they contribute toward local and global surveillance, they overcome breakpoint issues, they are educational, and they can be universal. Finally, they reduce the pool for nosocomial infection, improve antibiotic use, reduce associated costs, and stabilize the emergence of antibiotic-resistant pathogens.

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