Extended-Spectrum Beta-Lactamase Detection with Different Panels for Automated Susceptibility Testing and with a Chromogenic Medium†

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Infections caused by extended-spectrum beta-lactamase (ESBL)- and ampC beta-lactamase-producing gram-negative bacteria complicate therapy and limit treatment options. Several different panels for ESBL detection with automated systems exist. In addition, a chromogenic agar medium is available for ESBL screening. We compared two automated identification and susceptibility testing systems with regard to their effectiveness in detecting ESBL production in Enterobacteriaceae: the BD Phoenix system (BD Diagnostic Systems, Sparks, MD) and the Vitek 2 system (bioMérieux, Marcy l’Etoile, France). We 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 combined identification and susceptibility testing of gram-negative bacilli, and for Vitek 2, the ID-GNB panel for identification of gram-negative bacilli and the AST-N020, AST-N041, and AST-N062 panels for susceptibility testing), and a chromogenic agar medium (bioMérieux, Marcy l’Etoile, France). PCR for common ESBL gene families (encoding TEM, SHV, OXA, and CTX-M) and for chromosomal or plasmid-mediated ampC beta-lactamase genes was conducted to complete the study design. For the tested specimens overall, the chromID ESBL agar showed the highest sensitivity (95.8%) but the lowest specificity (10.5%) compared to the sensitivity and specificity of the Etest (chosen as reference by the authors) for the detection of ESBL-producing strains. The BD Phoenix system showed sensitivities of 77.1% and 84.2% and specificities of 61.5% and 75.0%, respectively, for the NMIC/ID-50 and NMIC/ID-70 panels. The sensitivity of the Vitek 2 system ranged from 78.8% (AST-N020) to 80.6% (AST-N062) and up to 84.2% (AST-N041). The specificities of the respective panels were 50.0% (AST-N041 and AST-N062) and 55.6% (AST-N020). In conclusion, the sensitivities and specificities of ESBL detection by the different methods differ depending on the microorganisms under study.

In the Enterobacteriaceae, resistance to beta-lactam antibiotics is mainly due to beta-lactamases that hydrolytically destroy the beta-lactam ring, which inactivates the antibiotic. Widespread among beta-lactamases are the TEM-1 and SHV-1 enzymes. Due to alterations at the active site caused by specific point mutations, these extended-spectrum beta-lactamases (ESBLs) are also able to hydrolyze oxyimino-beta-cephalosporins (e.g., cefotaxime, cefpodoxime, and ceftazidime) and the monobactam aztreonam. Microbial resistance through ESBLs was reported first in the early 1980s in Europe and subsequently in the United States, soon after the introduction of third-generation cephalosporins in clinical practice (8). Various reports describe worldwide outbreaks of infection with ESBL-producing Enterobacteriaceae. The plasmid-encoded derivatives of ESBLs enable horizontal transmission, a fact which should result in strict infection control measures (22).

The failure to detect ESBL-mediated resistance has led to treatment failure (17, 22) and contributed to uncontrolled spread of ESBL-producing organisms (18). On the other hand, the use of surveillance cultures for laboratory-based detection of infection or colonization of patients by ESBL-producing organisms has proven useful in controlling and terminating outbreaks (13, 16, 19). Screening for ESBLs is needed to sort out patients with these infections to perform medically effective treatments and cost-effective isolation (25).

The abilities of different screening and confirmation tests to detect Escherichia coli and Klebsiella spp. have been validated. Problems in detecting ESBL production occur particularly with Enterobacter spp., Serratia spp., and Citrobacter spp. These produce a beta-lactamase encoded by the ampC gene which leads to an overlap of different resistance phenotypes. AmpC production is typically associated with in vitro resistance to all beta-lactam antibiotics except for carbapenems and cefepime. In contrast to ESBLs, the ampC-derived enzymes are not affected by available beta-lactamase inhibitors. Cefepime testing was also performed with and without clavulanate (ESBL Etest and AB Biodisk) in order to detect ESBL production in strains not validated by the Etest with ceftazidime and cefotaxime (27). Isolates are considered ESBL producers when clavulanate causes a decrease in the MIC of $\geq 3$ twofold concentrations.

Automated systems are increasingly used for routine species identification and susceptibility testing in clinical laboratories to decrease the in-laboratory turnaround time and to improve cost effectiveness. Numerous studies have reported on the accuracies and limitations of various automated systems (1–4, 6, 10, 15, 23, 26), forcing manufacturers to periodically update...
their product software. However, errors by any test system can have severe repercussions for the clinical outcome for patients.

The purpose of this study was to evaluate and compare the performances of two microbiological identification and susceptibility testing systems with regard to the correct detection of ESBL production in routinely found Enterobacteriaceae strains. Additionally, we checked the practicability of an ESBL-detecting chromogenic agar medium which has been available since 2006. A conventional phenotypic confirmatory test was conducted with the Etest, which was chosen as the reference method for this study.

In European surveys of ESBLs, the dominating enzyme variants until the late 1990s were TEM, SHV, and OXA. CTX-M ESBLs were rarely recorded (11, 12). However, this ESBL variant replaced TEM and SHV mutants as the predominant ESBL in many European countries. The major hosts at that time were E. coli and Klebsiella pneumoniae. Additionally, these CTX-M producers were increasingly isolated from patients with community-acquired infections (11). CTX-M ESBLs seem to have originated in Klyvera spp. by mobilizing insertion elements out of the chromosomes of this species (21). Thus, we screened for these important gene families by PCR. Furthermore, PCR for chromosomal and plasmid-mediated ampC beta-lactamase genes was performed in questionable cases.

**MATERIALS AND METHODS**

**Study design.** Two microbiological antimicrobial susceptibility testing systems were evaluated and compared with regard to their ability to detect ESBL production in Enterobacteriaceae. ESBL production has to be distinguished from chromosomally and plasmid-mediated ampC beta-lactamase production. The methods tested were the BD Phoenix automated microbiology system (Becton Dickinson Diagnostic Systems, Sparks, MD) and the Vitek 2 system (bioMérieux, Marcy l’Etoile, France). The manufacturers’ routine identification and susceptibility testing panels for gram-negative bacteria were used. The configurations and the differences of the two automated systems are shown in Table 1.

In addition, we analyzed the practicability of an ESBL-detecting chromogenic agar medium (chromID ESBL; bioMérieux, Marcy l’Etoile, France) that was designed especially for the screening of ESBL production. The Etest (AB Biodisk, Sweden) was chosen as the reference method by the authors, as it still is routinely employed for detecting ESBL production in clinical laboratories. PCR was employed to screen for the common ESBL gene families (encoding TEM, SHV, OXA, and CTX-M) for the dual purpose of completing the study design and validating the results. The different primers detect the original progenitors of extended-spectrum beta-lactamase genes. In the case of bacterial strains which showed conspicuous resistance patterns in the results from the automated systems together with a negative Etest, we included screening for chromosomal and plasmid-mediated ampC beta-lactamase genes.

**Bacterial isolates.** The Enterobacteriaceae isolates, 114 in total (72 Escherichia coli, 21 Klebsiella pneumoniae, 4 Klebsiella oxytoca, 11 Enterobacter cloacae, 1 Enterobacter aerogenes, 1 Serratia marcescens, 1 Citrobacter koseri, 1 Proteus mirabilis, 1 Proteus vulgaris, and 1 Morganella morganii), were obtained from clinical specimens collected at the University Hospital Magdeburg, Germany, between 2006 and 2007 and from the Centers for Disease Control and Prevention (CDC; United States), the Antibiogram Committee of the French Microbiology Society (CA-SEM; France), the collection of George A. Jacoby (Lahey Clinic, Burlington, MA), and W. Witte (Robert Koch-Institute, Wernigerode, Germany). The bacterial strains were divided into the following groups: E. coli; E. coli and Klebsiella spp.; the KESC group (Klebsiella spp., Enterobacter spp., Serratia marcescens, and Citrobacter spp.); and the PMP group (Proteus spp., Morganella morganii, and Providencia rettgeri) (7). We completely excluded copy strains. The isolates, collected from clinical specimens, were all from different patients and units. No epidemic outbreak was observed. In each assay, K pneumoniae ATCC 700603 and E. coli ATCC 25922 were used as the positive and negative control for ESBL production, respectively. The criterion to include a strain in this study was either an alert from the automated systems (BD Phoenix and Vitek 2) about ESBL detection or a noticeable pattern of resistance against cephalosporins in the antimicrobial susceptibility testing. Isolates were cultured from frozen stock or from lyophilized material onto Columbia agar with 5% sheep red blood cells for 16 to 24 h at 37°C, subcultured, and grown again for 16 to 24 h at 37°C just before testing.

**Determination of MICs by Etest.** MICs were determined by Etest (episometer assay; AB Biodisk, Sweden) on Mueller-Hinton agar (bioMérieux, Nürtingen, Germany). A sterile swab was dipped into a bacterial suspension at a McFarland standard of 0.5. After the entire plate surface was swabbed with the inoculum, Etest strips impregnated with the different antibiotics were placed on the agar surface. After an incubation period of 24 h (37°C), the MICs were determined.

The synergistic activity of clavulanic acid with ceftazidime, cefepime, and cefotaxime was confirmed by means of three different Etest strips containing ceftazidime, cefepime with clavulanate (MIC, 0.25 to 16 or 0.064 to 4 µg/ml), cefotaxime (MIC, 0.25 to 16 or 0.016 to 1 µg/ml) with or without clavulanate (respectively). Isolates of E. coli and Klebsiella spp. were considered ESBL producers when clavulanate caused a decrease of ≥3 twofold dilutions (ratio for antibiotics without clavulanate to antibiotics with clavulanate, ≥8) in the MICs of ceftazidime and cefotaxime in combination with a ceftazidime MIC of ≤1 µg/ml or a ceftazidime MIC of ≤0.5 µg/ml, respectively. The remaining isolates, like Citrobacter spp., Enterobacter spp., Morganella spp., and Serratia marcescens, were considered ESBL producers when clavulanate caused a decrease of ≥3 twofold dilutions (ratio for antibiotics without clavulanate to anti-biotics with clavulanate, ≥8) in the MIC of cefepime in combination with a cefepime MIC of ≥0.5 µg/ml. The four Proteus spp. strains were tested with all three Etest strips using the same interpretation criteria as explained above. Furthermore, a strain was considered ESBL positive if a phantom zone, which is an additional zone of inhibition between the ellipses of the antibiotic and the antibiotic plus clavulanic acid, or a deformation of the zone of the tested cephalosporins could be observed, irrespective of the ratios or MICs. A result was considered indeterminate when the MICs were outside the test range of the respective Etest device. This phenomenon may suggest the presence of inhibitor-resistant ampC or TEM enzymes. In addition, the outcome was considered indeterminate when the result of one strip was indeterminate and the results of the other strips were ESBL negative.
BD Phoenix system. For the evaluation of the BD Phoenix system (Becton Dickinson Diagnostic Systems, Sparks, MD), the strains were tested, both in terms of identification and antimicrobial susceptibility, with the NMIC/ID-50 and NMIC/ID-70 BD Phoenix GN Combo panels (Table 1). With regard to ESBL detection, the panels differ in their cephalosporin profiles and the ranges of their MICs. Because of these differences, diverse rules of the BDXpert system were applied. The panels were inoculated and incubated according to the manufacturer’s recommendations. The BD Phoenix ESBL screening test, included in both panels, utilizes the growth response to selected cephalosporins (cefotaxime, ceftazidime, and ceftriaxone), with or without clavulanic acid, to detect the production of ESBLs. The results are analyzed with the integrated BDXpert system (version 5.10ASR1).

Vitek 2 system. For the evaluation of the Vitek 2 system (bioMérieux, Marcy l’Etoile, France), all isolates were identified using the card for gram-negative strains. The Vitek 2 cards AST-N20, AST-N041, and AST-N062 were used for susceptibility testing (Table 1). The cards were inoculated and incubated in the system according to the manufacturer’s instructions. The antimicrobial susceptibility test cards AST-N20, AST-N041, and AST-N062 differ in their compositions of beta-lactam antibiotics. The ESBL confirmatory test is incorporated on cards AST-N041 and AST-N062. This ESBL screening test utilizes the growth response to cefazidime, cefepime, and ceftaxime in combination with or without clavulanic acid on both cards. All results were interpreted by using the Advanced Expert System (AES) (software version VT2-R04.03).

chromID ESBL. The chromogenic chromID ESBL agar (bioMérieux, Marcy l’Etoile, France) was obtained from the manufacturer as a prepared plate medium. Each bacterial strain was cultured on chromID ESBL agar (7), and the results were recorded and documented. The agar plates were incubated aerobically at 37°C for 18 to 24 h or, if necessary, for 48 h. Colonies of ESBL producers develop species-specific colors (E. coli shows pink to burgundy coloration of beta-lactamase enzymes; Klebsiella spp., Enterobacter spp., Serratia spp., and Citrobacter spp. show green and/or blue coloration of beta-glucosidase-producing colonies; and Proteus spp., Providencia spp., and Morganella spp. show orange to brown coloration of deacaminase-expressing colonies) on chromID ESBL agar. Non-ESBL producers grow with colorless colonies or not at all on chromID ESBL agar.

Isolation of genomic DNA. DNA from the bacterial colonies was extracted by using a QIAmp DNA mini kit (Qiagen).

PCR amplification. PCR for the common ESBL gene families (encoding TEM, SHV, OXA, and CTX-M) was done according to the method of Schwaber et al. (24). Detection of the plasmid-mediated ampC beta-lactamases was done as described by Pérez-Pérez and Hanson (20). Finally, PCR for the chromosomal ampC beta-lactamase was done according to the protocol of Bret et al. (5).

Analysis of results. The sensitivities, specificities, and positive and negative predictive values (PPV and NPV, respectively) of all panels of the automated systems, of the chromogenic ESBL agar medium, and for each species/group of species were calculated. We used the Etest results as the reference.

RESULTS

For 114 strains, the MICs of ceftriaxone, ceftazidime, and/or aztreonam were >1 μg/ml. All 114 isolates had matching identifications in both systems. Out of these 114, 93 isolates could be confirmed as ESBL positive according to Etest results. All phenotypically positive ESBL strains (n = 93) were also positive in the molecular screening for common ESBL gene families (Table 2). In this regard, the distribution of the beta-lactamase genes was as follows: 18 strains were positive for the TEM gene, 22 for the SHV gene, 46 for the TEM and SHV genes, and 7 for the CTX-M gene. No OXA gene family enzymes were detected. Thus, the SHV-type ESBLs are the predominant ESBLs. With regard to the 21 Etest-negative strains, the results of the three types of tests (the two automated systems and chromID ESBL agar) did not agree. The different results, including those of the various ESBL screening tests, are shown in Table 3. Because of the limitations of the Etest method, which was used in this study as a standard confirmatory test, we evaluated the strains with mismatches regarding the occurrence of beta-lactamase genes by PCR. In 6 of the 21 strains that were ESBL negative by Etest (E. coli, n = 3; K. oxytoca, n = 1; Enterobacter spp., n = 3; and S. marcescens, n = 1) (Table 3), no ESBL gene families, chromosomal ampC, or plasmid-mediated ampC beta-lactamase genes were found. In nine of the strains that were negative for ESBLs by Etest (Enterobacter spp., n = 5; E. coli, n = 2; and M. morganii, n = 1), the chromosomal (n = 8) or the plasmid-mediated (n = 1) ampC beta-lactamase gene was detected. However, six strains were shown to possess the TEM and SHV genes; the TEM, SHV, and OXA genes; or the CTX-M gene. It is evident from the results presented in Table 3 that the highest number of mismatches (n = 12) was seen when using
the chromID ESBL agar. Among these, seven mismatches belonged to AmpC beta-lactamase producers. Eight mismatches occurred with the AST-N020 panel in the Vitek 2 system. In all, we detected four false-positive and four false-negative results. Among the four isolates with false-positive results, three strains carried an ampC gene and one specimen lacked any of the gene loci tested. False-negative results were reported for the one strain that carried blaTIM SHV OXA genes. Vitek 2 panels with integrated ESBL screens produced more-consistent results (AST-N041, 3 mismatches, and AST-N062, 1 mismatch), and so did both BD Phoenix panels, NMIC/ID-50 and NMIC/ID-70 (1 and 2 mismatches, respectively). The Etest produced six mismatches and was not able to detect two isolates with blaCTX-M, two strains with blaTIM SHV, or the two blaTEM SHV OXA carriers. In summary, the presence of the ESBL screen in the Vitek 2 system resulted in a smaller number of mismatches. The Vitek 2 panels reported more false-positive results, whereas the BD Phoenix system showed only false-negative findings (Table 3).

Results for all strains. The results for all organisms tested (n = 114) are shown in Table 4. The chromID ESBL test showed the highest sensitivity (95.8%) but the lowest specificity (10.5%) for all strains. The BD Phoenix system showed sensitivities of 77.1% for the NMIC/ID-50 panel and 84.2% for the NMIC/ID-70 panel. The specificities for the panels were 61.5% (NMIC/ID-50) and 75.0% (NMIC/ID-70). The sensitivities of the Vitek 2 system for all strains ranged from 78.8% with the AST-N020 panel to 84.2% with the AST-N041 panel. The AST-N062 panel demonstrated a sensitivity of 80.6%. The Vitek 2 system’s specificity showed values of 50.0% (AST-N041 and AST-N062) and 55.6% (AST-N020). In summary, the BD Phoenix system with the NMIC/ID-70 panel presented the highest PPV (88.5%) and NPV (62.1%), respectively.

Results for E. coli. The results for all E. coli strains (n = 72) are shown in Table 4. With regard to the E. coli strains, the chromID ESBL test had a sensitivity of 94.2% and a specificity of 42.9%. The sensitivity and specificity of the BD Phoenix system were 78.7% and 66.7% with the NMIC/ID-50 panel and 88.7% and 100% with the NMIC/ID-70 panel. The sensitivities and specificities for the Vitek 2 system ranged from 74.0% and 42.9% with the AST-N020 panel to 78.7% and 66.7% with the AST-N041 panel and 74.0% and 66.7% with the AST-N062 panel. In summary, the NMIC/ID-70 panel in the BD Phoenix system and the chromID ESBL test showed the highest PPVs (94.0 and 94.2%). The highest NPV was from the NMIC/ID-70 (55.6%).

Results for Klebsiella pneumoniae. The results for K. pneumoniae strains (n = 97) are presented in Table 4. The sensitivity, specificity, PPV, and NPV values for all Klebsiella pneumoniae isolates were 100% for all tested panels with both automated systems and for the chromogenic medium.

Results for E. coli and Klebsiella spp. The results for E. coli and Klebsiella spp. (n = 97) are presented in Table 4. The highest sensitivity (95.6%) combined with the lowest specificity (23.1%) was obtained with chromID ESBL. The BD Phoenix system with the NMIC/ID-70 panel showed the highest specificity (45.5%), with a sensitivity of 91.4%, just behind chromID ESBL. The AST-N020 panel in the Vitek 2 system had the lowest sensitivity (78.0%). All Vitek 2 panels and the NMIC/ID-50 panel showed identical specificities of 33.3%. The highest PPVs and NPVs were obtained with the BD Phoenix system in combination with the NMIC/ID-70 panel (92.4% and
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ESBL DETECTION WITH DIFFERENT METHODS

The Etest was used as the reference method, and PCR was performed for molecular validation of results for all tested isolates.

<table>
<thead>
<tr>
<th>Method</th>
<th>K. pneumoniae (PMP)</th>
<th>K. pneumoniae (KESC)</th>
<th>E. coli (PMP)</th>
<th>E. coli (KESC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Etest</td>
<td>97% (95% CI: 87-100)</td>
<td>83% (95% CI: 73-93)</td>
<td>78.8%</td>
<td>78.6%</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>74% (95% CI: 64-84)</td>
<td>64.7% (95% CI: 55-74)</td>
<td>55.6%</td>
<td>58.8%</td>
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<tr>
<td>BD Phoenix</td>
<td>91.4% (95% CI: 81-100)</td>
<td>92.6% (95% CI: 83-100)</td>
<td>92.6%</td>
<td>95.6%</td>
</tr>
</tbody>
</table>

**TABLE 4.** Sensitivity, specificity, PPV, and NPV results for detection of ESBL production.

**DISCUSSION**

The exact and rapid identification of ESBL-producing Enterobacteriaceae has great importance for early infection control measures and correct treatment procedures. In many laboratories, automated systems are used for identification and antimicrobial susceptibility testing. Furthermore, our results are based on the consistency of the results generated by different panels in the same automated systems. Finally, we evaluated the performance of the chromID ESBL agar. The number of strains we evaluated was limited. However, the results obtained with chromID ESBL agar were similar to those obtained with the Vitek 2 and BD Phoenix systems. The chromID ESBL agar showed good agreement with these systems. The chromID ESBL agar was also shown to be a useful tool for the rapid detection of ESBL-producing Enterobacteriaceae. The chromID ESBL agar showed a sensitivity and specificity of 100% for the detection of ESBL-producing Enterobacteriaceae. The chromID ESBL agar was also shown to be a useful tool for the rapid detection of ESBL-producing Enterobacteriaceae.
ated in our study reflects the actual prevalence and distribution of potentially ESBL-producing Enterobacteriaceae strains at the university hospital in Magdeburg.

Because of the great impact of ESBL-producing Enterobacteriaceae, the correct identification of this resistance mechanism in isolates is a prerequisite for the proper treatment of patients. In the last few years, only a small number of published studies have investigated the automated systems regarding their effectiveness in reliably identifying ESBL-producing gram-negative bacilli. All of the studies reported different results for the sensitivities and specificities of the automated Vitek 2 and BD Phoenix systems, which is caused by the species involved. Leverstein et al. (9), Menozzi et al. (14), and Thomson et al. (28) tested only E. coli, K. pneumoniae, and K. oxytoca isolates. The levels of sensitivity and specificity reported were considerably higher than the ones we calculated. However, Enterobacter spp., Citrobacter spp., Proteus spp., and others are able to produce ESBLs. Only Wiegand et al. (29) chose an extended spectrum of Enterobacteriaceae with a species distribution similar to ours. The authors (29) found sensitivities with the BD Phoenix and Vitek 2 systems of 98.8% versus 85.9% for all tested isolates. The Vitek 2 system had a specificity of 78.0% for all tested strains. The specificity of the BD Phoenix system was 52.2%. Wiegand et al. (29) used a BD Phoenix panel which is not available anymore. In the Vitek 2 panel used, no ESBL screening tests were integrated (AST-N020). Thus, Wiegand et al. (29) proposed the integration of ESBL screening with the routine panels. In our study, the comparison of three Vitek 2 panels, one without (AST-N020) and two with integrated ESBL screening (AST-N041, AST-N062), resulted in an enhancement of the sensitivity (from 78.8% to 84.2%); the respective specificities were 55.6%, 50.0%, and 50.0%.

In most studies, different tests were used as reference methods to confirm a presumptive ESBL producer. For example, Thompson et al. (28) used enzyme characterization as the reference method, Menozzi et al. (14) confirmed their findings with a standard broth microdilution panel according to the CLSI guidelines, and Wiegand and coworkers (29) used biochemical and molecular characterizations. Genotypic determination of the bla gene families 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 microbiological laboratories, the ESBL Etest has become the most commonly employed confirmatory test. Our results also resemble the ones reported by Leverstein et al. (9). The ESBL Etest has an accuracy of about 94% compared with that of molecular identification of ESBL production (29). In our study, the ESBL Etest had false-positive results for 6% of the genotypically confirmed ESBL-positive specimens. Mismatches were found for two CTX-M-positive K. oxytoca isolates and for strains carrying two or more bla genes (TEM and SHV or TEM, SHV, and OXA).

The fast and accurate detection of ESBL producers is indispensable for the prevention of treatment failure. Among all the systems tested, the chromID ESBL agar detects ESBL-producing Enterobacteriaceae isolates with the highest sensitivities and the lowest specificities. The main advantage of the chromID ESBL agar is its sensitivity, which enables the recovery and identification of most ESBL-producing organisms within 24 h. A previous study by Glupczynski et al. reported a sensitivity of 97.7% and a specificity of 89.0% for the chromogenic agar (7). In our evaluation, the chromogenic agar showed a comparable high sensitivity of 95.8% but a specificity of only 10.5%. In their study, 97% of the ESBL-producing isolates were recovered by chromID ESBL from clinical specimens after 18 to 24 h of incubation. The prolongation of incubation for 48 h did not increase the recovery rates of ESBL-positive organisms. Often, it made the recovery more complex because of the growth of associated flora. So Glupczynski et al. (7) decided to record only the definitive results after 18 to 24 h. In our study, the agar delivered false-positive results for the Enterobacter spp. and E. coli isolates carrying chromosomal or plasmid-located ampC, which confirms the results of Glupczynski et al. (7). Interestingly, all ampC-positive Enterobacter spp. were correctly reported as non-ESBL producers by both of the automated systems. The high sensitivity leads to the conclusion that chromID ESBL agar is a convenient method for directly screening clinical samples for ESBL-producing organisms. Furthermore, the agar is useful in ESBL outbreak situations, for screening patients who will be transferred to other hospitals or admitted from clinics with a generally high incidence of ESBL-producing isolates. Additionally, this chromogenic medium is appropriate for monitoring the ESBL carrier status of critically ill patients. Concerning the low specificity, all suspected ESBL-producing strains should be verified with additional tests (e.g., the ESBL Etest).

The detection of potential ESBL production in organisms like Enterobacter spp., Citrobacter spp., and Serratia spp. can be complicated by the additional expression of a chromosomal AmpC beta-lactamase. Schwaber et al. (24) demonstrated that the Advanced Expert System (AES) of the Vitek 2 system reached a sensitivity of 62.5% in testing ESBL-producing Enterobacter spp. (n = 40). The reliability of the results depends on the creation of the ESBL screen and the expert systems, on the setting of the antibiotic substances of the panels, on the MIC ranges, and finally, on the resistance mechanisms of the bacteria species.

The ESBL screen from Becton Dickinson includes cefotaxime, ceftazidime, cefpodoxime, and ceftriaxone with or without clavulanate, whereas the panels from bioMérieux include cefotaxime, ceftazidime, and cefepime. The use of cefepime might explain the better results of the Vitek 2 system for the PMP group, although a low number of these isolates was obtained. 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 33.3%. Concerning the remaining species, like those in the KESC group, in our study the AST-N041 and AST-N062 panels of the Vitek 2 system and the chromID ESBL agar all showed a sensitivity of 100%. The Vitek 2 panel without an ESBL screen established a value of 92.3%. The lowest sensitivities were produced by both BD Phoenix panels (78.6%). Obviously the AST-N020 panel presented the highest sensitivity, specificity, PPV, and NPV for the KESC group (92.3%, 64.7%, 85.7%, and 73.3%). However, the K. pneumoniae strains skew the data. Therefore, we excluded these specific isolates in further calculations. The resulting changes in the sensitivity, specificity, PPV, and NPV values are shown in Table 4.
Among all panels tested, the NMIC/ID-70 panel (BD Phoenix) showed the highest sensitivities and specificities for E. coli and Klebsiella spp., which are the most frequently identified bacteria in clinical laboratories. The results for all K. pneumoniae isolates (n = 21) are also worth mentioning. All panels of both systems and the chromID ESBL agar had values of 100% for sensitivity, specificity, PPV, and NPV for these isolates. These results could imply that, for K. pneumoniae isolates, the automated systems perform especially reliably and that further time-consuming and cost-expending confirmatory tests are not necessary.

Conclusion. The customer should expect reproducible, stable results independent of the choice of panels of the different automated systems. In our study, we demonstrated that the updated panels of the two systems tested (BD Phoenix and Vetek 2) led to various values for specificity and sensitivity. The integration of an ESBL screen with the panels for the Vetek 2 system, which is missing on the AST-N020 panel, improved the sensitivity, but not specificity, of ESBL detection in all species and subspecies. Both panels, AST-N041 and AST-N062, are comparable in sensitivity and specificity. The BD Phoenix panel NMIC/ID-70 showed better results than its predecessor NMIC/ID-50 in almost every category. Furthermore, in comparison to the results for the Vetek 2 system, the sensitivity and specificity were higher for almost every group of bacteria tested. In conclusion, neither automated system should be used for further time-consuming and cost-expending confirmatory procedures. J. Clin. Microbiol. 44:4085–4094.


ERRATUM

Extended-Spectrum Beta-Lactamase Detection with Different Panels for Automated Susceptibility Testing and with a Chromogenic Medium

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Volume 46, no. 11, p. 3721–3727, 2008. Page 3724: The body of Table 3 should appear as follows.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates (n = 21)</th>
<th>Result(s) obtained using:</th>
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<td>E. coli</td>
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<td>Chromosomal ampC</td>
<td>+</td>
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<tr>
<td></td>
<td>1</td>
<td>TEM + SHV</td>
<td>+</td>
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<td>AmpC hyperproducer</td>
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<td>K. oxytoca</td>
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<td>+</td>
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<td>TEM + SHV</td>
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<td>Plasmid-encoded ampC</td>
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<tr>
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