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Can We Use Imipenem and Meropenem Vitek 2 MICs for Detection of Suspected KPC and Other-Carbapenemase Producers among Species of *Enterobacteriaceae*?▽

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Imipenem and meropenem Vitek 2 MICs were evaluated for a panel of 104 *Enterobacteriaceae* for identification of carbapenemase producers. The sensitivity and specificity values for the new CLSI interpretative criteria (CLSI document M100-S20-U, 2010) were 98% and 83% for imipenem and 76% and 83% for meropenem, respectively. We propose an algorithm that is highly sensitive (98%) and specific (94%) for carbapenemase screening based on the combined use of imipenem and meropenem MICs.

Carbapenems are increasingly utilized as drugs of last resort against a variety of infections due to the emergence of extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae* (23). The emergence of carbapenem-resistant *Enterobacteriaceae* is therefore worrisome, as the antimicrobial armamentarium is consequently restricted (6, 17). The resistance of *Enterobacteriaceae* to carbapenems could be related to carbapenemases or to a dual mechanism associating an outer membrane permeability defect with β-lactamases such as AmpC cephalosporinase and ESBLs, particularly with the presence of CTX-M variants (9–11, 15, 16, 27, 29). The vast majority of acquired carbapenemases belong to three of the four known classes of β-lactamases, namely, Ambler class A (KPC, Sme, NMC-A, IMI, and some allelic variants of the GES/IBC enzymes), Ambler class B (metallo-β-lactamases [MBLs]), and Ambler class D (oxacillinases [OXAs]) (5). The locations of carbapenemase genes on highly mobile genetic elements have contributed to their rapid spread and the frequent cotransfer of multiple other antibiotic resistance factors (6, 18, 19). The ability to limit the spread of carbapenemase producers will require effective laboratory screening methods to rapidly identify patients infected with these organisms. Automated antimicrobial susceptibility testing systems, such as Vitek 2 (bioMérieux, Marcy L'Etoile, France), are commonly used in microbiology laboratories to decrease the laboratory turnaround time. However, several reports have questioned the ability of Vitek 2 to identify carbapenemase producers (1, 2, 17, 25, 28). Previous reports suggest an ertapenem MIC of ≥4.0 μg/ml (formerly, an ertapenem resistance result [7]) as the most accurate way to detect KPC carbapenemase (2, 17). In Argentina, among several other countries in which CTX-M is endemic, a large proportion of nosocomial *Enterobacteriaceae* display ertapenem resistance (about 5%, 15%, 20%, and 25% of the *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Enterobacter* species strains, respectively, from WHONET-Argentina Network [n = 55,351]), although most of them did not produce carbapenemases, as determined by molecular methods. The selection of CTX-M-2-producing mutants with porin loss was responsible for this ertapenem resistance. With ertapenem and meropenem as indicators (MICs of ≥4 μg/ml and ≥8 μg/ml, respectively), Vitek 2 was not able to differentiate true resistance mediated by carbapenemases from that mediated by AmpC cephalosporinases and ESBLs in combination with an outer membrane permeability defect, largely overestimating the number of carbapenemase producers (28). Therefore, in areas where these alternative mechanisms of carbapenem resistance are common, the use of indicators with such low specificity could significantly delay the identification of patients infected with producers of true carbapenemases, affecting the appropriate infection control policies. In addition, the need to confirm a larger number of strains could also cause an increase in lab costs. For these reasons, it is necessary to find a screening strategy based on the use of carbapenems other than ertapenem for a more accurate identification of suspicious isolates producing true carbapenemases. In addition, many Vitek 2 cards do not contain ertapenem; therefore, having an alternative strategy for carbapenemase screening also benefits users of these models.

The Clinical and Laboratory Standards Institute (CLSI) recently changed the susceptibility breakpoints for meropenem, imipenem, and doripenem to ≤1.0 μg/ml, and the ertapenem susceptibility cutoff was modified to ≤0.25 μg/ml (document M100-S20-U [8]). The resistant breakpoints were changed to ≥4 μg/ml for imipenem, meropenem, and doripenem and ≥1 μg/ml for ertapenem. Detection of carbapenemase producers by Vitek 2 based on these new susceptibility breakpoints has not yet been evaluated.

Herein, we evaluated the ability of Vitek 2 to screen carbapenemase-producing *Enterobacteriaceae* using imipenem and meropenem MICs and the updated CLSI breakpoints. We also assessed the ability of these carbapenems to distinguish the production of true carbapenemases from carbapenem re-

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sistance resulting from combinations of impermeability and AmpC or an ESBL. In addition, we propose an algorithm that will help clinical laboratories to detect true resistance mediated by carbapenemase producers in scenarios with high baseline resistance to ertapenem due to noncarbapenemase mechanisms.

(The findings of this study were partly presented at the 14th International Congress on Infectious Diseases, abstr. 1964, in 2010 [22a].)

A panel of genotypically characterized *Enterobacteriaceae* ($n = 104$) composed of diverse bacterial genera with distinct carbapenem susceptibility patterns were included in this study. The genotypes of the isolates were characterized previously (21, 22) by PCR for bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{KPC}, bla_{Sme}, bla_{OXAs} (subgroups I, II, and III as defined in reference 26), $bla_{\text{OXA-48}}$, $bla_{\text{IMI/NMC-A}}$, $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{PER}, bla_{SHV}, and bla_{AmpC}. In addition, to exclude the possible presence of other not-yet-described carbapenemases among the negative-control panel, the imipenemase activities of cell extracts from overnight broth culture were determined by spectrophotometric assays (21). Outer membrane proteins among ertapenem-resistant carbapenemase nonproducers were detected by SDS-PAGE (22). AmpC hyperproduction was initially screened by isoelectric focusing (IEF) using a substrate-based development method. Crude extracts with positive AmpC IEF bands and *in situ* inhibition with oxacillin (1 mM) were subjected to spectrophotometric analysis as described previously (22). The carbapenemases represented were KPC (23 *K. pneumoniae* isolates, 4 *Enterobacter cloacae* isolates, 2 *C. freundii* isolates, 1 *E. coli* isolate, 1 *Serratia marcescens* isolate, and 2 *Salmonella enterica* isolates), Sme (8 *S. marcescens* isolates), IMI/NMC-A (2 *E. cloacae* isolates), GES (2 *K. pneumoniae* isolates, 1 *Enterobacter agglomerans* isolate), OXA-163, a novel carbapenemase closely related to OXA-48 with an extended activity against expanded-spectrum cephalosporins (4 *K. pneumoniae* isolates, 1 *E. cloacae* isolate) (23), IMP-8 (1 *E. cloacae* isolate), and VIM-2 (2 *K. pneumoniae* isolates and 1 *Providencia rettgeri* isolate). The carbapenemase nonproducers ($n = 49$) were 10 *K. pneumoniae* isolates, 2 *Proteus mirabilis* isolates, 1 *E. coli* isolate, 1 *Klebsiella oxytoca* isolate, and 1 *Proteus penneri* isolate with diverse ESBLs (CTX-M-2, CTX-M-9, CTX-M-15, PER-2, SHV-2, or SHV-18); 4 *K. pneumoniae* isolates with CTX-M-2 and porin loss; 6 *E. cloacae* isolates with hyperproduction of AmpC plus porin loss; 2 *E. cloacae* isolates and 1 *C. freundii* isolate with hyperproduction of AmpC; 2 *S. marcescens* isolates with inducible AmpC and ESBLs (CTX-M-2) plus porin loss; 2 *E. cloacae* isolates, 2 *C. freundii* isolates, 1 *S. marcescens* isolate, 1 *Morganella morganii* isolate, and 1 *Providencia stuartii* isolate with inducible AmpC and inducible expression; 3 *K. pneumoniae* isolates, 1 *E. coli* isolate, 1 *P. mirabilis* isolate, and 1 *Shigella flexneri* isolate with diverse plasmidic AmpCs (CMY, AAC-1, DHA-1, or FOX-5); 1 *E. coli* isolate and 1 *Citrobacter koseri* isolate with penicillinases; and 2 *E. coli* quality control strains, 1 *E. cloacae* quality control strain, and 1 *Salmonella* sp. quality control strain. By using agar dilution, we determined that 98% and 56% of the carbapenemase producers and nonproducers, respectively, were nonsusceptible to ertapenem. Strains were subcultured twice before being tested. The isolates were from clinical sources (with the exception of the quality control strains and two *Salmonella* strains with KPC that were obtained by conjugation assays), and they were single isolates from each patient. All the isolates corresponded to different clonal types as revealed by pulsed-field gel electrophoresis (PFGE), with the exception of *K. pneumoniae* strains possessing KPCs (strains were divided into six pulse types). Isolates of the major PFGE pattern of *K. pneumoniae* possessing KPC (40% of the strains) corresponded to the molecular type ST258 (13).

The MICs of carbapenems were determined in duplicate by Vitek 2 using the AST-N082 card, which included imipenem (range, 1 to 16 μg/ml) and meropenem (range, 0.25 to 16 μg/ml), according to the manufacturer's instructions. Inoculums were adjusted to the required optical density using Vitek Densi-Check. Plate counts of representative samples were analyzed to corroborate the compliance with the required inoculum size.

We evaluated two criteria for carbapenemase screening using Vitek 2. (i) We assessed the ability of the Advanced Expert System (AES), a software that uses the antimicrobial susceptibility results to suggest a presumed mechanism of resistance of the tested isolate, and (ii) we analyzed the carbapenem susceptibility results to identify isolates suspected of carbapenemase production.

The Vitek 2 AES compares each MIC result for the tested isolate with the modal MIC distribution of bacteria with known resistance mechanisms included in the database. Based on this comparison, the system indicates the potential presence of carbapenemases. (It is required that at least all but one of the MICs obtained for the tested strains fit the modal distribution of the presumed resistance mechanism.) The AES software predicted the presence of carbapenemases in 42/55 carbapenemase producers (76%) (the highest efficiency was for the detection of OXA-163, with 100% of producers correctly detected; the next highest efficiencies were for KPCs, with 87%, Sme, with 77%, other class A carbapenemases, with 40%, and MBLs, with 25% of producers correctly detected). The AES offered no interpretation (isolates were erroneously reported to have an "inconsistent" result) for 9 carbapenemase producers (2 *S. marcescens* isolates with Sme, 2 *E. cloacae* isolates with IMI/NMC-A, 1 *E. agglomerans* strain with GES, 1 *K. pneumoniae* strain and 1 *P. rettgeri* strain with VIM, and 1 *E. coli* isolate and 1 *S. marcescens* isolate with KPC), even though all of them were resistant to imipenem (MICs ≥ 4 μg/ml). The 5 remaining carbapenemase producers (2 *K. pneumoniae* isolates with KPC, 1 *K. pneumoniae* isolate with KPC plus PER-2, 1 *S. marcescens* isolate with Sme plus CTX-M-2, and 1 *K. pneumoniae* isolate with VIM plus CTX-M-2) were inferred by the AES to have ESBL production alone or in combination with impermeability. Nine out of 49 isolates (18%) without true carbapenemases (all 9 isolates had permeability defects, 5 had CTX-M-2, and 4 had AmpC) were also inferred to produce a carbapenemase, giving 82% specificity.

To address the usefulness of the second criterion, the Vitek 2 defined the isolates as susceptible, intermediate, and resistant based on their MICs of imipenem and meropenem according to the updated 2010 CLSI breakpoints (8). Intermediate susceptibility or resistance (a carbapenem MIC of ≥ 2 μg/ml) was detected in 98% and 76% of the 55 carbapenemase producers with imipenem (Fig. 1a) and meropenem (Fig. 1b), respectively. Strains that remained susceptible to at least one

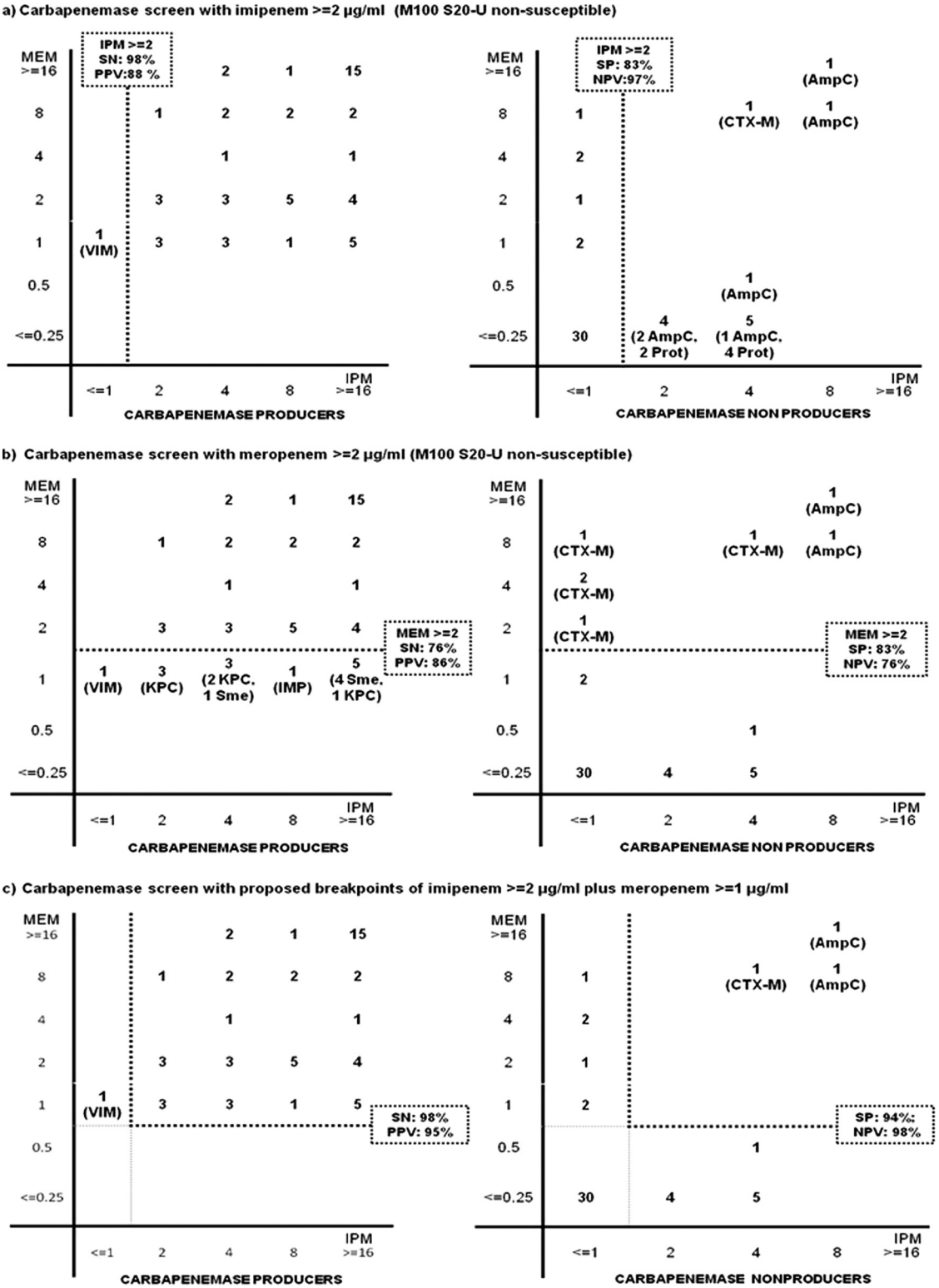

FIG. 1. Scattergrams showing numbers of isolates at the indicated carbapenem MICs (averages of duplicate values, in µg/ml, of imipenem [IPM] and meropenem [MEM]) obtained by Vitek 2 for carbapenemase producers and carbapenemase nonproducers. (a) The sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) were calculated for carbapenemase screening using the imipenem M100-S20-U CLSI nonsusceptibility breakpoint (a MIC of ≥2 µg/ml) (members of the *Proteeae* tribe were excluded from the group of carbapenemase nonproducers for the calculation of SP, PPV, and NPV). (b) Meropenem M100-S20-U CLSI nonsusceptibility breakpoint (a MIC of ≥2 µg/ml [dashed lines]). (c) Cutoff values recommended in this work for a combination of imipenem (MIC ≥ 2 µg/ml) and meropenem (MIC ≥ 1 µg/ml). The mechanisms of resistance are indicated for undetected carbapenemases or isolates with interfering mechanisms. Prot, *Proteeae*; CTX-M, production of the CTX-M-2 ESBL plus porin loss; AmpC, derepressed expression of chromosomal cephalosporinase plus porin loss.

of the carbapenems tested (MIC ≤ 1 μg/ml) were 1 *K. pneumoniae* isolate (VIM) with imipenem (Fig. 1a) and 5 *K. pneumoniae* isolates (4 KPC isolates and 1 VIM isolate), 5 *S. marcescens* isolates (Sme), 1 *E. coli* isolate (KPC), and 1 *E. cloacae* isolate (KPC) with meropenem (Fig. 1b). Only the *K. pneumoniae* isolate with VIM retained susceptibility to both carbapenems (Fig. 1a and b). In 12% and 18% of the carbapenemase nonproducers, we detected intermediate susceptibility or resistance (a carbapenem MIC of ≥2 μg/ml) to imipenem (*Proteeae* members were excluded because these isolates have naturally elevated imipenem MICs, as shown in Fig. 1a) and meropenem (Fig. 1b), respectively. Strains that showed MICs in the nonsusceptible categories were 4 *E. cloacae* isolates (with AmpC plus porin loss) with imipenem (Fig. 1a); 3 *K. pneumonia*e isolates (CTX-M-2 plus porin loss), 2 *S. marcescens* isolates (CTX-M-2 plus porin loss), and 1 *E. cloacae* isolate (AmpC) with meropenem (Fig. 1b); and 2 *E. cloacae* isolates (AmpC plus porin loss) and 1 *K. pneumoniae* isolate (CTX-M-2 plus porin loss) with both carbapenems (Fig. 1a and b). Thus, the recently approved CLSI breakpoints for meropenem and imipenem, which define nonsusceptibility by a MIC of ≥2 μg/ml, increased the capture of carbapenemase producers, especially with imipenem. However, these changes also enhanced the poor ability of the commercial system to distinguish carbapenemase producers from isolates with an ESBL and/or AmpC combined with porin loss, resulting in poor specificity (Fig. 1a and b). Thus, we explored other screening cutoff points for a more specific detection of suspected carbapenemases by Vitek 2, with retention of the highest possible sensitivity. As the overlap in the MICs for isolates with these contrasting resistance mechanisms involved mostly only one of the two carbapenems tested, we explored a screening strategy based on the combined use of imipenem and meropenem MICs. As shown in Fig. 1c, almost all of the carbapenemase producers (with the exception of 1 *K. pneumoniae* isolate with VIM-2) showed simultaneously an imipenem MIC of ≥2 μg/ml and a meropenem MIC of ≥1 μg/ml. Conversely, most of the strains with alternative carbapenem-resistant mechanisms, including the *Proteeae* members, had at least one carbapenem MIC below these cutoff values (an imipenem MIC of ≤1 μg/ml or a meropenem MIC of ≤0.5 μg/ml). Only 1 *K. pneumonia*e isolate (CTX-M-2 plus porin loss) and 2 *E. cloacae* isolates (AmpC plus porin loss) displayed MICs of ≥4 μg/ml for both carbapenems (Fig. 1c). Based on these findings, we propose an algorithm for carbapenemase screening by Vitek 2 (Fig. 2): when an isolate has both an imipenem MIC of ≥2 μg/ml and a meropenem MIC of ≥1 μg/ml (this means that both MICs have to be above their respective cutoff points), a carbapenemase producer should be suspected. Subsequently, this assumption should be confirmed by means of more-specific methods such as the doubly modified Hodge test (22) or by the use of meropenem disks supplemented with APB, DPA, and cloxacillin (12). In contrast, if the MIC of imipenem is ≤1 μg/ml (indicating susceptibility according to the updated CLSI interpretative criteria) or that of meropenem is ≤0.5 μg/ml, the presence of carbapenemases can be excluded. The sensitivity, specificity, positive predictive value, and negative predictive value for screening suspected carbapenemase producers (an imipenem MIC of ≥2 μg/ml and a meropenem MIC of ≥1 μg/ml) were 98%, 94%, 95%, and 98%, respectively (Fig. 1c). It should be mentioned that this algorithm is based on an epidemiological cutoff value for meropenem (MIC ≥ 1 μg/ml) within the redefined CLSI susceptibility category (8). It has recently been published that the Vitek 2 system tends to produce meropenem MICs that are significantly lower than the broth microdilution for the KPCs (4). This tendency of Vitek 2 to give lower meropenem MICs for carbapenemase producers could explain why we found that the meropenem epidemiological cutoff value was within the susceptibility category.

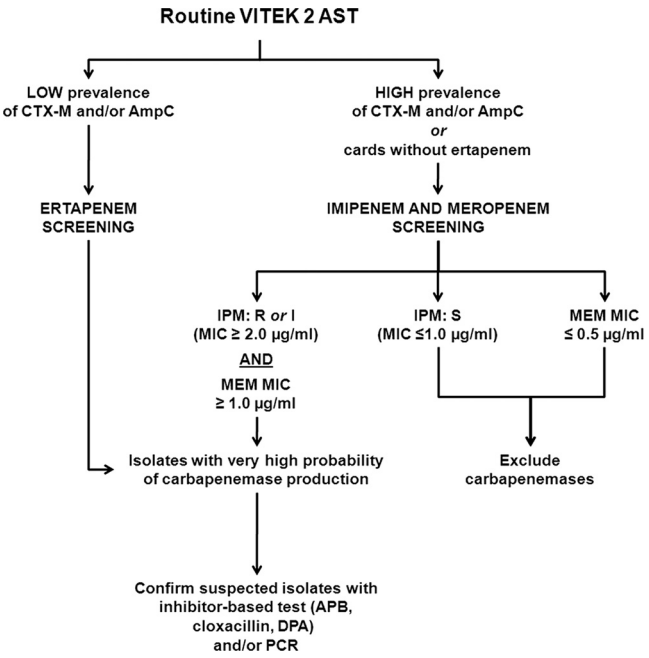

FIG. 2. Proposed flowchart for screening *Enterobacteriaceae* suspected of producing carbapenemases with Vitek 2. IPM, imipenem; MEM, meropenem; R, resistant; I, intermediate; S, susceptible (as defined by CLSI document M100-S20-U [8]); APB, aminophenylboronic acid; DPA, dipicolinic acid.

Based on our results, several recommendations can be offered to clinical microbiology laboratories to improve routine detection of carbapenemases by Vitek 2. (i) In consideration of the fact that the AES software failed to infer carbapenemase as the resistance mechanism in several isolates, including those with KPCs, laboratories should avoid using the AES recommendations to detect carbapenemase production. A similar problem with the lack of sensitivity of the AES was recently reported (28), although in that report, several OXA-48 isolates (but not KPC isolates) were incorrectly detected. The differences observed between both studies (we observed that several class A enzymes, including KPCs and MBLs, were the most undetected carbapenemases by the AES) could be due to the different carbapenems used in the cards. The discrepancy in detection of OXA-163 and OXA-48 could also be due to differences in the hydrolytic profiles of these enzymes (24). (ii) Ertapenem (nonsusceptibility is reported to be indicated by a MIC of ≥4 μg/ml, as was defined with previous CLSI breakpoints [7]) has been proposed as the carbapenem that most accurately detects the presence of KPC by several methods, including Vitek (2, 3, 17); however, for those areas or institutions where *Enterobacteriaceae* that are ertapenem resistant by dual mechanisms have become more prevalent, routine laboratories using the Vitek

2 commercial system can identify strains with true carbapenemases using the imipenem and meropenem MICs (21). Isolates suspected of producing carbapenemases will have an imipenem MIC of ≥2 μg/ml (a result indicating nonsusceptibility) and, at the same time, a meropenem MIC of ≥1 μg/ml (Fig. 2) by Vitek 2. Our data indicate that laboratories using this Vitek 2 algorithm will detect >95% of *Enterobacteriaceae* with true carbapenemase production. (iii) The shortcomings of using a screening strategy that is different from current CLSI interpretative criteria might be overcome by customizable rules that can be added to the Vitek 2 system by routine laboratories. (iv) It is important to mention that the algorithm proposed here is not intended to replace the use of ertapenem in those areas where ertapenem has already demonstrated optimal performance as a screening test for KPCs (2, 17). However, key ertapenem issues could arise in the near future for two main reasons: (a) the recently approved CLSI breakpoints, which define ertapenem nonsusceptibility by a MIC of ≥0.5 μg/ml (8), could be associated with a large number of interfering isolates and may even affect those areas with a low prevalence of dual mechanisms, and (b) the worldwide emergence of *E. coli* ST131-producing $bla_{\text{CTX-M-15}}$ as a major cause of serious multidrug-resistant infections could produce a dramatic epidemiological change (14, 20), as this ESBL has a high potential to contribute to the selection of ertapenem-resistant mutants by binding with high affinity to this molecule (10, 11). Thus, in our opinion, laboratories should be prepared for a new evolutionary scenario with rising levels of resistance to ertapenem due to the mobilization of $bla_{\text{CTX-M-15}}$ by this dominant strain. (v) Finally, laboratories that cannot test ertapenem on their current Vitek 2 systems, as it is not available in many cards, may consider using the approach proposed in this study.

In conclusion, we reviewed the strategies for identification of KPCs and other carbapenemases by Vitek 2 for scenarios with high baseline ertapenem resistance. The use of a strategy based on the combined use of imipenem and meropenem MICs (cutoff values of ≥2 μg/ml and ≥1 μg/ml, respectively) will enable routine labs to identify, with high confidence levels, those isolates suspected of producing carbapenemases. This includes one of the most important epidemiological challenges of recent times, the KPCs.

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