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Comparison of BD Phoenix, Vitek 2, and MicroScan Automated Systems for Detection and Inference of Mechanisms Responsible for Carbapenem Resistance in *Enterobacteriaceae*[▽]

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We assessed the ability of three commercial systems to infer carbapenem resistance mechanisms in 39 carbapenemase-producing and 16 other carbapenem-resistant *Enterobacteriaceae*. The sensitivity/specificity values for "flagging" a likely carbapenemase were 100%/0% (BD Phoenix), 82 to 85%/6 to 19% (MicroScan), and 74%/38% (Vitek 2), respectively. OXA-48 producers were poorly detected, but all systems reliably detected isolates with KPC and most with metallo-carbapenemases.

Enterobacteriaceae with acquired carbapenemases are a growing global public health concern (5, 23, 24, 33). The β-lactamases are diverse, and the producers are geographically scattered. However, the prevalences of particular types differ significantly between countries. KPC variants are prevalent in the United States, Greece, and Israel (10, 16, 18, 21, 34); VIM metalloenzymes in Greece and the Süd Tirol region of Italy (2, 9, 14); and OXA-48 in Turkey (4, 12, 22). IMP enzymes are more scattered, but with foci in the Far East (13, 17, 27, 37–39). In the United Kingdom and many other countries, enterobacteria with these different enzymes are often linked to repatriation of patients from the countries mentioned (6, 26, 36) as well as to limited domestic spread. Recently, isolates with NDM-1 metallo-carbapenemase have been identified as an emerging problem in Europe, often associated with patients who have a history of travel to and/or hospitalization in India or Pakistan (40) (N. Woodford and D. M. Livermore, unpublished data).

Infection control measures for limiting the spread of carbapenemase-producing *Enterobacteriaceae* demand prompt recognition of these organisms in the clinical laboratory (3, 19). We therefore evaluated the abilities of the three most widely used commercial susceptibility testing systems to detect carbapenemase-producing *Enterobacteriaceae* and to distinguish them from those with diverse carbapenem resistance contingent on combinations of impermeability and AmpC or an extended-spectrum β-lactamase (ESBL).

Genotypically characterized carbapenem-resistant isolates ($n = 55$) were from the collection held by the Health Protection Agency's Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL). The five carbapenemases represented were KPC (7 *Klebsiella pneumoniae* isolates and 1 *Enterobacter cloacae* isolate) and OXA-48 (10 *K. pneumoniae* isolates and 1 *Escherichia coli* isolate), which are nonmetalloenzymes, and IMP (7 *K. pneumoniae* isolates, 2 *E. cloacae* isolates, and 1 *E. coli* isolates), VIM (3 *K. pneumoniae* isolates), and NDM-1 (3 *E. coli* isolates, 2 *Citrobacter freundii* isolates, 1 *E. cloacae* isolate, and 1 *K. pneumoniae* isolate), which are metalloenzymes. The isolates without a carbapenemase ($n = 16$) included 9 *K. pneumoniae* isolates with combined ESBLs (CTX-M-15, CTX-M-33, or SHV-11) and porin loss, 6 *Enterobacter* isolates with AmpC and/or ESBL (1 isolate with SHV-12) plus porin loss, and 1 *E. coli* isolate with CTX-M-15 and CMY-23 enzymes plus porin loss (8, 35). The isolates had been referred from many different laboratories, mostly in the United Kingdom, and belonged to multiple pulsed-field gel electrophoresis (PFGE)-defined strains. Inclusion of multiple representatives of some strains was in some instances unavoidable, e.g., many KPC producers belonged to the internationally disseminated ST258 clone. Susceptibilities had been determined previously by the British Society for Antimicrobial Chemotherapy (BSAC) agar dilution methodology, and carbapenemase genes had been detected by PCR. The commercial systems and antibiotic panels/cards tested were those with the most-widespread use within the United Kingdom, namely, (i) Vitek 2 (bioMérieux, Basingstoke, United Kingdom), AST-N054card, which incorporates ertapenem (range, 0.5 to 8 μg/ml) and meropenem (0.25 to 16 μg/ml), (ii) Phoenix (BD Diagnostics, Oxford, United Kingdom), panel NMIC/id-76, which tests ertapenem (0.25 to 1 μg/ml) and imipenem and meropenem (both 1 to 8 μg/ml), (iii) MicroScan (Siemens Healthcare Diagnostics Limited, Camberley, United Kingdom), "Neg MIC panel type 36" (NM36), which includes ertapenem (0.5 to 4 μg/ml) and imipenem and meropenem (both 1 to 8 μg/ml), and (iv) MicroScan (Siemens), "Neg BP combo panel type 39" (NBC39), which tests ertapenem (2 to 4 μg/ml) and imipenem and meropenem (both 2 to 8 μg/ml). The 55 test isolates were distributed "blind" to three collaborating laboratories and

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TABLE 1. Performance of commercial systems: inferring carbapenemase production in carbapenem-resistant *Enterobacteriaceae*

System and panel type	Carbapenemase warning given	No. of isolates with carbapenem resistance mechanism[a]		Sensitivity (%)	Specificity (%)
		Carbapenemase (n = 39)	Noncarbapenemase (n = 16)		
Phoenix NMIC/id-76	Yes	39	16	100	0
	No	0	0		
MicroScan NM36	Yes	33	15	85	6
	No	6	1		
MicroScan NBC39	Yes	32	13	82	19
	No	7	3		
Vitek 2 AST-N054	Yes	29	10	74	38
	No	10	6		

[a] Mechanisms were defined by PCR and sequencing.

were tested (one system in each laboratory) in accordance with the manufacturers' recommendations. All of the commercial systems interpreted susceptibilities using the CLSI breakpoints current in 2009; the CLSI has since proposed lower breakpoints, but cards calibrated against these are not presently available. Results were returned to ARMRL for collation and analysis.

Intermediate susceptibility or resistance to at least one carbapenem was detected in 100% (Phoenix), 95% (Vitek 2 and MicroScan NM36), and 91% (MicroScan NBC39) of the 55 test isolates: Vitek 2 failed to detect nonsusceptibility for one *K. pneumoniae* isolate and two *Enterobacter* isolates with ESBL/AmpC in combination with porin loss; the NM36 panel failed for two *K. pneumoniae* isolates with OXA-48 or an IMP enzyme and an *E. cloacae* isolate with AmpC/porin loss; and the NBC39 panel failed for three *K. pneumoniae* isolates with OXA-48 and one isolate each of *K. pneumoniae* and *E. cloacae* with ESBL/AmpC in combination with porin loss. Only one of these isolates, an *Enterobacter* sp. isolate with SHV-12 ESBL, AmpC activity, and reduced permeability, was consistently missed by all systems except the Phoenix.

The systems were more variable in their ability to predict carbapenemase production as the underlying mechanism of carbapenem resistance (Table 1). The Phoenix gave only a broad inference; it identified all 55 isolates as showing elevated carbapenem MICs, consistently triggering a rule to indicate the potential presence of a "metallo-β-lactamase" (interpreted by us more generally as a warning of any carbapenemase). Hence, all carbapenemase producers were correctly inferred (100% sensitivity), but all non-carbapenemase producers, with resistance contingent on combinations of porin loss and ESBL or AmpC, were "flagged" incorrectly, giving 0% specificity. The Vitek 2's advanced expert system (AES) attempted a more thorough interpretation, which resulted in improved, if unspectacular, specificity. Thus, 6/16 (38%) isolates with carbapenem resistance contingent upon porin loss in combination with an ESBL or AmpC enzyme were correctly inferred not to produce a carbapenemase. This advantage was, however, accompanied by reduced (74%) sensitivity. The MicroScan LabPro alert system "flagged" isolates as potential producers of a "KPC enzyme" (likely reflecting the dominance of and emphasis on this carbapenemase in, e.g., the United States), but again, this was interpreted more broadly by us to indicate the presence of any carbapenemase. Its performance with each panel fell between the performance of the other systems, with sensitivities/specificities of 85%/6% (NM36) and 82%/19% (NBC39), respectively (Table 1).

Like the Phoenix, the Vitek 2 and MicroScan systems correctly identified as carbapenemase producers all isolates with KPC (n = 8), VIM (n = 3), or NDM (n = 7) enzymes (Table 2). All producers of IMP metallo-carbapenemases (n = 10) were detected by the MicroScan NBC39 panel, but the Vitek 2 failed to infer a carbapenemase as the resistance mechanism in 4/10 isolates with IMP enzymes, even though two were intermediate (MIC, 4 μg/ml) and two resistant (MIC, ≥8 μg/ml) to ertapenem, with one also resistant to meropenem (MIC, ≥16

TABLE 2. Ability of commercial systems to infer carbapenemase production in isolates of *Enterobacteriaceae* with defined carbapenem resistance mechanisms[a]

Mechanism	No. of isolates							
	Phoenix		MicroScan NM36		MicroScan NBC39		Vitek 2	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
KPC (n = 8)	8	0	8	0	8	0	8	0
MBL (IMP, VIM, or NDM; n = 20)	20	0	19	1[b]	20	0	16	4[b]
OXA-48 (n = 11)	11	0	6	5	4	7	5	6
E. coli/*Klebsiella* spp., ESBL, porin loss (n = 10)	10	0	10	0	8	2	8	2
Enterobacter spp., AmpC/ESBL, porin loss (n = 6)	6	0	5	1	5	1	2	4

[a] There were 55 test isolates. Phoenix flagged likely carbapenemase producers as potentially having an MBL, whereas the MicroScan flagged them as potentially having KPC enzymes. These inferences were taken to mean carbapenemase positive.
[b] All IMP types.

μg/ml). The Vitek 2 AES offered no interpretation for three of these isolates, while the fourth was inferred to have a combination of ESBL production and impermeability. One of these anomalous IMP-positive isolates was also missed by the MicroScan NM36 panel, where it appeared susceptible (on CLSI criteria) to ertapenem (MIC, 2 μg/ml) and imipenem and meropenem (MICs for both, ≤1 μg/ml); its ertapenem MIC was 4 μg/ml (intermediate) on the NBC39 panel, and it was correctly inferred as a likely carbapenemase producer, though the imipenem and meropenem MICs were ≤2 μg/ml (susceptible).

The most challenging mechanism to detect was OXA-48 carbapenemase; 5 of 11 isolates with this enzyme were missed by the MicroScan NM36 and NBC39 panels and Vitek 2, 1 by the MicroScan NBC39 panel and Vitek 2, and 1 by MicroScan NBC39 only. The four OXA-48 carbapenemase producers that were "flagged" unequivocally were resistant to carbapenems (reference agar dilution MICs: for ertapenem, >16 μg/ml; for meropenem, 8 to >32 μg/ml; and for imipenem, 16 to >128 μg/ml) and also to cefotaxime (agar dilution MICs, >256 μg/ml) and ceftazidime (agar dilution MICs, 32 to 256 μg/ml), whereas, with one exception, those missed were found susceptible to these cephalosporins by the corresponding system and, in most instances, also by agar dilution (ceftazidime MICs, 0.5 to 4 μg/ml; cefotaxime MICs, 1 to 64 μg/ml); the anomalous isolate was resistant to both cefotaxime (agar dilution MIC, >256 μg/ml) and ceftazidime (agar dilution MIC, 32 μg/ml) but tested susceptible to carbapenems (MICs, ≤2 μg/ml) on the MicroScan NBC39 panel (it was intermediate to ertapenem [MIC, 4 μg/ml] on the NM36 panel, and by agar dilution, the MICs of all three carbapenems were only 8 μg/ml). It is well known that producers of the OXA-48 enzyme remain susceptible to oxyimino-cephalosporins if they lack ESBLs or AmpC enzymes (6, 22), but resistance to either or both of cefotaxime and ceftazidime in addition to a carbapenem seems to be an essential criterion for triggering the carbapenemase rules for Vitek 2 and MicroScan. Improved sensitivity for isolates with the OXA-48 enzyme may be achieved by updating the interpretation software to "flag" as potential carbapenemase producers those isolates that are resistant to carbapenems but remain susceptible to oxyimino-cephalosporins, although this would still not overcome the problem for any isolates that retain susceptibility to carbapenems.

The poor ability of the commercial systems to distinguish carbapenemase producers from isolates with an ESBL and/or AmpC combined with porin loss, and consequent poor specificity, is not surprising, since (i) there is considerable overlap in the MICs for isolates with these contrasting resistance mechanisms and (ii) human experts often fail to distinguish between the two groups of isolates unless provided with data from supplementary tests. Modification of the antibiotic testing panels for inclusion of synergy tests between carbapenems and β-lactamase inhibitors, such as boronic acid for KPC enzymes (7, 31, 32), cloxacillin for AmpC (29, 35), and dipicolinic acid (15, 20, 25, 28) or EDTA for metallo-β-lactamases, would permit more-stringent interpretive criteria and would be expected to improve specificity.

Accurate detection of carbapenem-resistant *Enterobacteriaceae* should be a global resistance priority, being essential for appropriate patient management—though, all too often, few good antibiotics remain active against producer strains—and for the prompt implementation of infection control procedures (3, 5, 19). The present recommendations from both CLSI and EUCAST are that MICs should be sufficient for patient management and that resistance mechanisms need be determined only if required for infection control purposes or for epidemiological surveillance; nevertheless, it seems to us that it is critical that a laboratory should know whether occasional carbapenem resistance reflects a few isolates—unlikely to spread—with combinations of impermeability and ESBL or whether it has imported strains with carbapenemases, which, based on experience in the United States with the ST258 *K. pneumoniae* clone with KPC, seem likely to pose a much greater threat. Previous studies have highlighted that carbapenem-resistant *Enterobacteriaceae*, including those that produce a carbapenemase, are not all detected reliably by automated systems (1, 11, 30). Reassuringly, our data indicated that laboratories using any of the commercial systems examined will detect >90% of *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 (data not shown). The systems differed, however, in their abilities to infer carbapenemase production accurately and in the degrees to which they even attempted to do so. By this criterion, the rank order was Phoenix > MicroScan NM36 > MicroScan NBC39 > Vitek 2 (Table 2). Arguably, it is preferable for the interpretation to retain the highest possible sensitivity even if, with existing testing panels, this must be at the expense of specificity in distinguishing isolates with a carbapenemase from those without. It should be noted that only two carbapenems were included on the Vitek 2 AST-N054 cards, compared with three on the other systems. The Vitek 2 system attempted the most complex interpretations and lost sensitivity, while only achieving 38% specificity, whereas the other two systems used generic warnings of potential carbapenemase production, with even poorer specificity, thus overestimating the number of true carbapenemase producers. These shortcomings might be overcome for systems with customizable rules if the individual laboratory adds a new rule for those organisms that show increased MICs of carbapenems but remain susceptible to cephalosporins or, alternatively, if synergy testing was undertaken for all isolates found nonsusceptible to one or more carbapenems.

As carbapenem-resistant *Enterobacteriaceae* become more prevalent, it is crucial that they can be detected reliably in the clinical laboratory, and we have shown that commercial systems can aid this. We would advise that all isolates "flagged" as potential carbapenemase producers should be submitted to a regional or national reference laboratory for confirmation and for molecular epidemiological investigation until prevalence is judged to have reached a level that makes this investigation impractical.

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Scotland. A.L. has received speaker's fees, has been an invited delegate to international conferences, and has acted in an advisory capacity for pharmaceutical companies. The other authors (M.F., C.K., R.M.Q., J.A.S., and J.Z.) have no conflicts of interest to declare.

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