1 **Intestinal Carriage of Carbapenemase-Producing Organisms (CPO):**

2 **Current Status of Surveillance Methods**

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22

23 Running Head: Surveillance of Carbapenem Resistant Enterobacteriaceae

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59 **SUMMARY**

60 Carbapenemases have become a significant mechanism for broad-spectrum β-lactam
61 resistance in *Enterobacteriaceae* and other Gram-negative bacteria such as *Pseudomonas* and
62 *Acinetobacter* spp. Intestinal carriage of carbapenemase-producing organisms (CPO) is an
63 important source of transmission. Isolation of carriers is one strategy that can be used to limit
64 spread of these bacteria. In this review, we critically examine the clinical performance, advantages
65 and disadvantages of methods available for the detection of intestinal carriage of CPO. Culture-
66 based methods (CDC protocol, chromogenic media, specialized agars, double disk synergy tests)
67 for detecting carriage of CPO are convenient due to their ready availability and low cost, but their
68 limited sensitivity and long turn-around-time may not be always optimal for infection control
69 practices. Contemporary nucleic acid amplification techniques (NAAT) such as real-time PCR,
70 hybridization assays or loop-mediated isothermal amplification (LAMP), or a combined culture

71 and NAAT approach may provide faster results and/or added sensitivity and specificity compared
72 with culture-based methods. Infection control practitioners and clinical microbiologists should be
73 aware of the strengths and limitations of available methods to determine the most suitable
74 method for their medical facility to fit their infection control needs.

75 **OVERVIEW AND HISTORICAL PERSPECTIVE**

76 **A Thirty-Year Epidemic of Increasing Resistance**

77 At some point, almost all *Enterobacteriaceae* were susceptible to broad-spectrum β-lactam
78 antibiotics, including β-lactam/β-lactamase inhibitor combinations, oxyimino-cephalosporins
79 (e.g., ceftriaxone, ceftazidime, and cefotaxime), aztreonam, and carbapenems. Regrettably, two
80 seminal events occurred in the past thirty years that have had a major impact in the therapy of
81 infectious diseases. In a manner analogous to the HIV epidemic and its human toll, the evolution of
82 extended-spectrum β-lactamases (ESBLs) three decades ago significantly crippled the activity of
83 oxyimino-cephalosporins and aztreonam, followed by the more recent appearance of
84 carbapenemases in the clinic has limited the efficacy of all currently available β-lactams causing a
85 staggering economic and human burden (1). We have learned that increased colonization
86 pressure from CPO is linked to development of infection (2); and gastrointestinal carriage of ESBL-
87 producing *Enterobacteriaceae* leads to subsequent infection (3). Still today, after the initial report
88 in 1983 of SHV-2 (the first ESBL reported), and despite significant advances in infection control
89 and supportive care, infectious caused by ESBL-producing *Enterobacteriaceae* exact an
90 unacceptable mortality rate and add significantly to health care costs (4–6). The emergence of
91 carbapenemases in the past 15 years has only added to the crisis caused by ESBL producers (7).
92 The global impact of *Klebsiella pneumoniae* carbapenemase (KPC) and the New Delhi Metallo-β-
93 lactamase (NDM) created a worldwide fear that we are at the "*end of the antibiotic era*" (8, 9). The
94 World Health Organization (WHO) has classified carbapenemase-producing *Enterobacteriaceae*
95 (CPE) as one of the three greatest threats to human health (10). Surveys of the molecular
96 epidemiology of carbapenemases, including KPC, OXA-48, VIM, IMP, and NDM producers, reveal

97 that the dissemination of these carbapenemases is rapid and lasting. Authorities have advocated
98 for local and regional screening programs as available evidence shows that travelers are a major
99 source of spread (11, 12). Furthermore, in endemic settings, transmission of ESBL-producing
100 *Enterobacteriaceae* between healthcare facilities creates a significant challenge for controlling
101 spread of resistance (13). The rate of CPE cases in community hospitals in the Southwestern
102 United States (US) has increased five-fold in the last few years (14).

103 **Current Status of Carbapenemases**

104 Carbapenemases are present among all four classes of β-lactamases (Table 1) (15–17). A
105 rare class C β-lactamase, CMY-10, also demonstrates weak "carbapenemase activity", but its
106 clinical significance is unclear (17, 18). Additionally, CPO are commonly resistant to multiple drug
107 classes such as aminoglycosides, quinolones, tetracyclines and folate inhibitors due to additional
108 types of resistance genes carried by the organisms (19, 20). To provide the appropriate
109 background for evaluating the detection methods discussed herein, we review for the reader the
110 major carbapenemases that are threatening our β-lactam arsenal.

111 **Class A carbapenemases** One of the most common mechanisms of carbapenem resistance
112 among class A enzymes, is the production of KPC β-lactamases. KPCs were initially detected in a
113 clinical isolate in 1996 in North Carolina; since then, 19 variants have been discovered (21–24).
114 KPC has been found in a variety of *Enterobacteriaceae*, including *Klebsiella spp., E. coli,*
115 *Enterobacter spp., Citrobacter spp., Morganella spp., Serratia marcecens* (25–29), *Roultella spp.*
116 (30), *Kluyvera* (31), *Salmonella* (32), and non-*Enterobacteriaceae* such as *Aeromonas* (33)*,*
117 *Pseudomonas,* and *Acinetobacter baumannii* (34).

118 Attributable and crude mortality from infections caused by bacteria harboring KPCs are
119 higher than in those patients with non-KPC-producing isolates (35); the reason for this increased
120 mortality is still enigmatic. Epidemiological studies suggest that KPC-producing *K. pneumoniae*
121 belonging to Sequence type (ST) 258 are of 2 distinct clones and that the clinical behavior of
122 isolates bearing bla_{KPC-2} is different than bla_{KPC-3}. Molecular differences between the two clones
123 include aminoglycoside resistance and ability to form biofilms (36, 37). The molecular reason for
124 this difference in clinical behavior is not yet understood. The prevalence of KPC-producing
125 bacteria varies widely. In one surveillance study 37% of patients in an intensive care unit (ICU)
126 carried bla_{KPC} (38). Other studies place its prevalence between 0-5%, depending on the population
127 being surveyed (39, 40).

128 KPC is endemic in some areas of Europe (Greece, Italy, and Poland), South America
129 (Colombia and Argentina), the Middle East (Israel), and North America. Recently, cases and
130 localized outbreaks are linked to importation from endemic areas (22, 41). In addition, long-term
131 care facilities (LTCFs) are rapidly becoming reservoirs for KPC producers (41). Other class A
132 carbapenemases are important in some specific locales, such as GES-5 in Brazil where it
133 constitutes the main carbapenemase in *Enterobacteriaceae* (22). SME carbapenemases, also
134 belonging to class A and associated with *S. marcescens*, are quite rare.

135 **Class D carbapenemases** Another important carbapenemase in *Enterobacteriaceae* is a
136 class D β-lactamase, OXA-48. This β-lactamase, sometimes referred to as the "*phantom menace*",
137 was initially identified in a Turkish patient in 2001 (42–44). For the next 5 years, OXA-48 was not
138 isolated from any other country. In 2008 OXA-48 spread outside of Turkey and became prevalent
139 in clinical isolates from Continental Europe, the Middle East, and Northern Africa (45, 46). Since

140 then, outbreaks throughout Europe have been reported (45, 47). Most recently, OXA-48 was
141 detected in the US, Canada and South Africa (20, 48–50). Many of these reports involve patients
142 previously treated in Middle Eastern and North African countries (51). Nonetheless, an early
143 outbreak of OXA-48-producing *K. pneumoniae* in England was not linked to known endemic
144 regions (52). More concerning, however, was the retrospective analysis that uncovered an
145 outbreak of OXA-48-producing *Enterobacteriaceae* in a Dutch hospital that had been ongoing for
146 two years (53). OXA-48 has been disseminated to a wide variety of *Enterobacteriaceae* species,
147 including, *Klebsiella spp., E. coli, Citrobacter spp., Serratia marcescens* (54–56), *Enterobacter spp.,*
148 *Morganella morganii* (55), *Providencia stuartii* (57), *Raoultella planticola* (56), *and Salmonella*
149 *enterica* (51).
150 OXA-48 is contained in a 61.8 kb self-conjugating IncL plasmid which likely contributes to
151 its ability to spread in *Enterobacteriaceae* (22, 58, 59). Other OXA-48-like enzymes with
152 carbapenemase activity in *Enterobacteriaceae* that either have caused, or have the potential to
153 cause, outbreaks include OXA-181, OXA-204, OXA-232, and OXA-162 (22, 43, 60–62). Other class D
154 carbapenemases of clinical importance are OXA-23 and OXA-24/40; these carbapenemases are
155 found mainly in *Acinetobacter baumannii* (63). Recently, some OXA-type carbapenemases have
156 been re-classified based on their hydrolytic activity. To illustrate, once thought to be a
157 carbapenemase, the kinetic profile of OXA-163 resembles more an ESBL than a carbapenemase
158 (62).
159 **Class B carbapenemases** The Class B metallo-β-lactamases (MBLs) hydrolyze a broad
160 range of β-lactams including carbapenems (18). The most widespread MBLs include the NDM,
161 VIM, and IMP family enzymes. Of the MBLs, NDM-1 has emerged as a major cause of concern due

162 to its widespread dissemination (64). NDM-1 was initially reported in a patient of Indian origin in
163 Sweden in 2007 (65). NDM-1 was subsequently found to be widespread in the Indian
164 subcontinent, including in environmental samples (66) and has now been reported in more than
165 15 countries (67). In the United Kingdom (UK), 52% of 101 patients with NDM-producing isolates
166 collected from 2008 to 2013 reported healthcare exposure or travel to the Indian subcontinent
167 (68). NDM has spread between different bacterial species, including *Enterobacter cloacae*, *K.*
168 *pneumoniae*, and *Escherichia coli* (69).

169 Horizontal spread of NDM has also been described in the clinical setting; in a recent study
170 four neonates from India acquired an NDM-1-producing *E. coli* from the environment and
171 developed sepsis (70). Clonal spread of NDM-1-producing isolates has been documented in some
172 regions in India, while spread elsewhere, including to the UK, likely happened due to transfer of
173 plasmids (71).

174 Equally important, IMP- and VIM-producing bacteria have also been found in the US,
175 Europe (mostly Greece, Italy and Southern France), the Middle East, the Indian subcontinent,
176 Japan, and China (22, 72–74). Outbreaks have occurred throughout the world as these MBLs
177 spread as part of complicated integrons (42). To illustrate, a recent surveillance study performed
178 in Northeastern Ohio uncovered a clinical isolate of *Pseudomonas aeruginosa* with $bla_{VIM\text{-}2}$ in a
179 class I integron that was proximal to a *Salmonella* genomic island (SGI), suggesting a
180 recombination event between these two bacteria. Detailed analysis of this genetic locus showed
181 multiple resistance and transposing elements that likely resulted in the successful dissemination
182 of this isolate (75).

183 **MECHANISMS OF CARBAPENEM RESISTANCE**

184 Resistance to carbapenems can be mediated by different mechanisms; these include porin
185 mutations, upregulation of efflux pumps, changes in penicillin binding proteins (PBPs), and
186 production of carbapenemases (76–78). A significant subset of carbapenemase genes are encoded
187 in readily transmissible plasmids. These plasmids, in some circumstances, can be shared between
188 *Enterobacteriaceae* and non-*Enterobacteriaceae*. While the other mechanisms of resistance are
189 also genetically encoded, their transmission is not as frequently observed as for carbapenemase
190 genes and therefore are of a lesser concern.

191 In this treatise, we generally refer to carbapenem resistant organism (CRO) as bacteria that
192 are resistant to imipenem, meropenem, doripenem and ertapenem. We are particularly focused on
193 Gram-negative CROs. These can be divided in *Enterobacteriaceae* and non-*Enterobacteriaceae.*
194 Carbapenem-resistant *Enterobacteriaceae* are frequently referred to as CRE. Organisms that are
195 carbapenem-resistant due to production of a carbapenemase are referred as carbapenemase-
196 producing organisms (CPO); and when the bacteria are *Enterobacteriaceae*, we refer to them as
197 carbapenemase-producing *Enterobacteriaceae* (CPE). In addition, there are some bacteria that
198 produce carbapenemases though their MICs for carbapenems do not reach the resistance
199 breakpoint. Given that carbapenemase genes are usually transmissible via plasmids, we argue that
200 they should be targeted for screening and we include them as CPOs or CPEs. It must be noted that
201 some non-*Enterobacteriaceae* CPOs such as *Burkholderia spp.* and *Stenotrophomonas maltophilia*
202 carry chromosomally-encoded carbapenemases. As such, the chromosomally-encoded
203 carbapenemases are unlikely to be transmitted to other bacteria. When we refer to CPOs in this
204 review, we focus on all *Enterobacteriaceae* and the non-*Enterobacteriaceae* that are known to

205 carry carbapenemase-encoding plasmids, even when their MIC increase does not reach the
206 resistance breakpoint.

207 **INTESTINAL CARRIAGE OF CPO**

208 As noted above, CPO have emerged as significant healthcare-associated pathogens
209 worldwide (38, 79). While most studies refer to (CPE), we contend that similar conclusions can be
210 applied to CPO, encompassing both *Enterobacteriaceae* and non-fermenting Gram-negative
211 bacteria. Furthermore, since carbapenemases are transferred via plasmids, both
212 *Enterobacteriaceae* and non-*Enterobacteriaceae* are capable of serving as reservoirs and vectors.
213 Intestinal carriage serves as a reservoir of CPE and can promote cross-transmission in healthcare
214 settings (80). Thus, infection control programs directed at detecting intestinal carriage are
215 essential tools to limit the spread of these pathogens.

216 Several examples highlight the importance of detecting intestinal carriage for effective
217 control of CPO infections. A study in New York documented a significant decrease in carriage rate
218 1 year after an infection control program in an intensive care unit (ICU) was implemented (81).
219 The program involved screening for intestinal carriage of carbapenem-resistant *K. pneumoniae*
220 and *Acinetobacter baumannii* with culture of rectal swabs (BBL CultureSwab Plus, Becton-
221 Dickinson) and isolating patients while results were pending or if they were positive. Isolation
222 was carried out at the end of an ICU where rooms were divided only by curtains. The program also
223 involved extensive cleaning with isopropanol and a quaternary ammonium compound that
224 included closing the unit for 2 days. The lack of a quick screening test was a limiting factor for the
225 success of this program. Nonetheless, the investigators were able to reduce the mean number of

226 new KPC-producing *K. pneumoniae* cases from 9.7 to 3.7 per 1,000 patient–days. In another study,
227 Enfield *et al*. were successful in decreasing CPE incidence in a surgical ICU from 7.77 per 1000
228 patient days to 1.22 cases per 1000 patient days by using enhanced infection control measures
229 and increased surveillance by implementing a PCR-based assay (82). Two outbreaks of KPC-
230 producing organisms were successfully controlled using a "bundle approach", of which screening
231 for CPE carriage is an integral part (83, 84). Schwaber *et al*. reported on a country-wide
232 mandatory program that involved physical isolation and dedicated nursing staff in Israel that was
233 able to significantly decrease the incidence of KPC-producing isolates (85). Although screening for
234 asymptomatic carriage was not part of the program this effort involved a very broad isolation
235 policy that relied on careful tracking of known cases throughout the healthcare system. In addition
236 to these real-life examples, a mathematical model also validates the usefulness of screening
237 followed by patient isolation to control CPOs (86).
238

239 **Failure of carbapenem breakpoints to detect all CPO.**

240 Detection of CPO in the clinical microbiology laboratory is challenging because
241 interpretation of routine susceptibility testing may fail to flag an isolate as a potential CPO (14,
242 87–89). Although the presence of a carbapenemase confers some resistance, the increase in the
243 MICs due to the β-lactamase may not be enough to consider the isolate resistant to a carbapenem
244 given the defined cut-off values for interpretation of resistance (90). Despite the changes made by
245 the Clinical and Laboratory Standard Institute (CLSI) to the carbapenem interpretative criteria for
246 *Enterobacteriaceae* in June 2010, which lowered the MIC values that are interpreted as "non-
247 susceptible" (from ≤ 4μg/mL to ≤1 μg/mL for meropenem) to capture more CPE than under

248 previous guidelines, there are some isolates that still escape detection (90). Recently, both the
249 European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI have proposed
250 not testing for resistance mechanisms on clinical isolates, arguing that the lower breakpoints
251 should suffice for treatment purposes (Table 2) (91, 92).

252 We must emphasize that clinical breakpoints are for implementation in the care of patients
253 and not designed for epidemiological surveillance. In any case, uniform consensus on this issue
254 does not exist; the authors of this review agree with Livermore *et al.* and advocate testing for
255 carbapenemase resistance genes for infection control monitoring, as well as for routine
256 microbiological diagnosis (93). The rationale for this assertion is that although raised MICs against
257 carbapenems may suggest the presence of a CPO, clinical experience demonstrates that MICs will
258 not always reveal the presence of carbapenemases. EUCAST has proposed the use of
259 epidemiological breakpoints for this purpose followed by phenotypic confirmation by inhibition
260 disks or the Carba NP test (94). All of these changes reflect the notion that to prevent spread of
261 resistance, it is necessary to prevent transmission not only of isolates that are phenotypically
262 resistant, but also of those that carry transmissible elements that may spread to susceptible
263 bacteria confer resistance under the right conditions.

264 Carbapenemases, when not accompanied by other β-lactamases may confer a low-level of
265 resistance to carbapenems (even MICs ≤ 0.5 μg/mL) that does not become evident until it is
266 combined with another resistance mechanism such as production of an ESBL or acquired AmpC
267 (95, 96), porin mutations (97, 98), or changes in porin expression (99). Conversely, these changes
268 may raise carbapenem MICs in the absence of carbapenemases. OXA-48 is particularly known for

269 consistently failing detection if not accompanied by another broad-spectrum β-lactamase (100).
270 This was demonstrated in the unrecognized Dutch outbreak above described (53).

271 Reports show that automated susceptibility systems are also not reliable for detection of
272 carbapenemase-producing *K. pneumoniae* isolates (101). Using the interpretative criteria for
273 meropenem on the 2005 CLSI M-100 document S15 (which correspond to M100-S20 on Table 2),
274 non-susceptible rates in a panel of confirmed KPC-producers ranged from 93% with Microscan
275 (Beckman Coulter, Brea, California, USA) to 20% with Sensititre AutoReader (Thermo Scientific,
276 Waltham, MA, USA) compared with 100% for broth microdilution and disk diffusion (102). For
277 KPC-producing non-*Klebsiella* isolates, the rate of false-negatives may be higher; though there has
278 been some improvement using the revised CLSI breakpoints (88). The use of stricter criteria and
279 expert rules in automated systems have increased the sensitivity of CPE detection, but with
280 significant decline on specificity (103). A study comparing disk diffusion, Etest, and VITEK2
281 (bioMérieux, Marcy l'Etoile, France) using previous CLSI and EUCAST breakpoints for meropenem,
282 imipenem, or ertapenem found multiple discrepancies with KPC, ESBL, and MBL producers.
283 However, the VITEK2 system, when using meropenem as a reporter substrate successfully
284 detected all CPE producers (87).

285 On an operational basis ertapenem and meropenem are proposed as the most suitable
286 antibiotics for screening of carbapenemase producers (89). Anderson *et al*. found that, depending
287 on the method used, 0-6% of KPC-producing isolates were susceptible to ertapenem using the
288 former CLSI ertapenem resistance breakpoint of ≥8 μg/mL. After decreasing the breakpoint to ≤ 1
289 μg/mL (which is higher than the current breakpoint of 0.5 μg/mL) almost all methods tested were
290 able to detect 100% of the KPC producers. Interestingly, the VITEK2 platform when using

291 ertapenem as indicator still failed to detect 6% of those isolates (89). The EUCAST guidelines
292 reflect these issues, suggesting meropenem (with a cut-off of 0.125 μg/mL) as the antibiotic with
293 the best balance between sensitivity and specificity, while noting that ertapenem is the most
294 sensitive though lacking specificity (94). Faropenem, a penem antibiotic, has also been proposed
295 as an alternative to carbapenems for detection of carbapenemases (104, 105). Faropenem showed
296 99% sensitivity and 94% specificity when tested against a known panel of 166 PCR-confirmed
297 isolates of carbapenemase-producing *Enterobacteriaceae* (though OXA-48-producing isolates
298 were underrepresented) and 82 negative controls. Another study compared and a panel of 62
299 PCR-confirmed KPC-producing *Enterobacteriaceae* and 73 producers of other β-lactamases,
300 showing a non-overlapping inhibitory zone around a 5μg faropenem disk between KPC producers
301 and non-producers (104).

302 **Tests for carbapenemase activity in isolated cultures**

303 Tests that detect carbapenemase activity in isolated cultures within a short time period can
304 be used to rapidly determine if a clinical isolate is a CPO. These tests are generally not suited for
305 direct testing of non-sterile specimens without prior isolation or enrichment steps, so they would
306 not be used to screen fecal specimens or perirectal swabs directly. However, they can be employed
307 as confirmatory assays when using culture-based screening.

308 **The modified Hodge Test (mHT)** was the initial screening test recommended for
309 carbapenemase production (89). However, mHT lacks specificity and may produce false-positives
310 in bacteria with complex ESBL or AmpC (both plasmid and overexpressed chromosomal enzmes)
311 backgrounds combined with porin mutations/loss (106, 107). The mHT must be noted that it
312 should be performed with either meropenem or ertapenem as it is known to perform poorly when

313 using imipenem as a substrate (92). When focused on KPC enzymes, specificity of this test can be
314 increased by using an EDTA disk as described by Yan *et al*. (108); however, the most common
315 reason for false positive results (i.e., AmpC hyper-production) is not addressed with this
316 modification. The mHT is also unable to distinguish between carbapenemases and it lacks
317 sensitivity for some carbapenemases such as NDM (particularly low-level producers), some of the
318 OXA family, and SME (107, 109).

319 **Synergy testing with inhibitors** can be used to differentiate MBLs from other enzymes by
320 inhibiting class A, C, and D enzymes. The phenylboronic acid double-disk synergy test (PBA-DDST)
321 with either meropenem or ertapenem was able to successfully screen for KPC β-lactamase in a
322 collection of clinical specimens (110). Pournaras *et al*., evaluated PBA and EDTA with meropenem
323 in disks in a sample of bacterial colonies isolated from 189 rectal swabs where 97 were positive
324 for a carbapenemase (KPC and VIM) and showed excellent sensitivity and specificity (111). Doi *et*
325 *al.,* also showed that inhibition by 3-aminophenyl boronic acid could be used to differentiate KPC
326 from other β-lactamases (112).

327 Another proposed improvement to disk based testing that allows differentiating between
328 the different β-lactamase classes is use of avibactam (formerly NXL104) disks (113, 114). Class C
329 enzymes can also be identified with similar inhibition tests (115, 116). Further improvement on
330 these tests allow for identification of concurrent mechanisms, such as KPC with a MBL, such as the
331 one suggested by Miriagou *et al.* (117). In this study, PBA had improved MBL inhibition, allowing
332 for less misclassifications of VIM + KPC compared to using aminophenyl boronic acid. These disk
333 tests, however, require pure cultures, making them inappropriate for screening of the lower

334 gastrointestinal tract of patients. Nevertheless, these assays remain a low cost, low technology
335 option.

336 **The Carba NP test, the Blue-Carba, and the Rapid Carb Test** are based on the detection
337 of carbapenemase activity. The Carba NP test (bioMérieux, France) detects a change in pH that is
338 coupled to the hydrolysis of imipenem. The testing procedure consists of cell lysis followed by
339 incubation of the enzymatic lysate with imipenem and phenol red for up to 2 hours (118–120).
340 Testing of *Enterobacteriaceae* showed Carba NP was able to detect all tested isolates from a
341 worldwide collection of bacteria that produced class A (KPC, NMC, SME, GES), class B (IMI, NDM,
342 VIM, IMP), and class D (OXA-48 or OXA-181) enzymes (119). In this report false positive results
343 were not detected. The Carba NP test has also been used directly against blood culture bottles
344 spiked with an array of class A, B and D carbapenemases, where this assay demonstrated a
345 sensitivity and specificity of 97.9% and 100% respectively (121). However, in another study, the
346 Carba NP test produced non-interpretable results when testing isolates grown on MacConkey or
347 Drigalski agar (120) suggesting that these media may affect the performance of the test. Also, use
348 of the Carba NP test on *A. baumannii* may require some modifications (122). In any case, the
349 Carba NP test is one of the recommended tests for confirmation of carbapenemase production in
350 pure isolates by the CLSI and EUCAST (92).

351 The Carba NP can be performed in most microbiology laboratories with no additional
352 equipment. It can be used on any isolate with suspected carbapenemase activity. The main
353 advantage when compared to agar screening and molecular methods is the broad target range; as
354 the test will be positive as long as there is enough carbapenemase, regardless of its class. However,
355 there is some concern regarding the lack of sensitivity for certain class D carbapenemases (e.g.,

356 OXA-48, OXA-58, OXA-181) (123, 124). A possible drawback over molecular methods (and disk
357 inhibition testing) is its inability to differentiate between enzymes, although one report suggests
358 that this can be achieved with some modifications to the Carba NP test (125). The Carba NP test
359 has also been compared to an alternative colorimetric test for carbapenemase activity, the Rapid
360 CARB Screen (Rosco Diagnositics, Denmark), showing similar sensitivity (97 and 98%) but
361 superior specificity (100 and 83%) when tested against a panel of 66 *Enterobacteriaceae* carrying
362 class A, B, or D enzymes and 69 non-carbapenemase producers. (126). Another similar test to the
363 Carba NP, the Blue-Carba test (not commercially available) uses a different indicator and a
364 simplified protocol. The Blue-Carba test has reported better sensitivity, including for OXA-type
365 carbapenemases (127). An added advantage of the Blue-Carba test is a faster turn-around time
366 than the Carba NP test as there is no need to extract the β-lactamase. The clinical performance of
367 the Blue-Carba test still needs to be established.

368 **Spectrometry** has also been used to detect CPO. These tests include UV spectrophotometry
369 and mass spectrometry using matrix-assisted laser desorption ionization-time of flight mass
370 spectrometry (MALDI-TOF MS). UV spectrophotometry involves the detection of hydrolyzed
371 imipenem by a cell lysate (128). This method was found to be 100% sensitive and specific for
372 detecting a wide array of class A, B, and D enzymes in *Enterobacteriaceae* (129). The clinical
373 application of this method still is challenging due to the technical expertise and equipment
374 required to perform it.

375 MALDI-TOF MS can detect carbapenemases by comparing the proportion of hydrolyzed
376 and intact imipenem on a centrifuged cell sample (130). This approach was able to detect 72% of
377 carbapenemase-producing isolates directly from positive blood culture vials (131). Although still a

378 research application, this method may become attractive as MALDI-TOF MS becomes more
379 common in the microbiology laboratory, but this method is suboptimal for detection of small
380 molecules, such as carbapenems and its degradation products. Liquid chromatography tandem
381 mass spectrometry (LC-MS/MS) is more suitable for this task and has also been used successfully
382 to detect carbapenemase activity from cultures (132). These methods have the potential of
383 differentiating between classes of β-lactamases by using inhibitors such as EDTA (133). It must be
384 noted that imipenem undergoes spontaneous hydrolysis in basic buffers (134) and that a negative
385 control should always be included when using any of these methods. At the present time, a mass
386 spectrometry method that is as sensitive and easily implemented as agar-based or PCR-based
387 screens is not yet present.

388 **Screening methods to detect fecal carriage of CPO**

389 Screening tests to detect CPO in stool present three major challenges: rapid detection,
390 detection of isolates with low level carbapenem resistance, and detection of proportionally low
391 numbers of CPO. Infection control programs rely on contact isolation for patients who test
392 positive, which also must be in place while waiting for the result. A "good screening test" must
393 minimize turn-around time, maximize sensitivity, preserve reasonable specificity, detect multiple
394 types of carbapenemases, and be cost-effective. Detecting low-level resistance is important
395 because it may already signify the presence of a genetic trait (such as bla_{KPC}) (90) that may spread
396 to other bacteria through horizontal transfer where it could result in carbapenem resistance in
397 new bacterial strains (135). Finally, since the main reservoir is the intestinal tract, the bacteria of
398 concern may just represent a small proportion of the overall bacterial load. Therefore the
399 inoculum of CPO on a surveillance swab may be below the limit of detection (LOD). It is also worth

400 mentioning that non-*Enterobacteriaceae* Gram-negative bacteria may also harbor
401 carbapenemases, although screening protocols based on culture methods try to exclude them. For
402 instance the US Centers for Disease Control and Prevention (CDC) protocol, and the suggested
403 interpretation for the SUPERCARBA agar suggest that only lactose-fermenting colonies should be
404 reported. Manufacturers of chromogenic media also endorse reporting of colonies with certain
405 appearances that correspond to lactose fermenters. Bacteria other than lactose-fermenting
406 *Enterobacteriaceae* can be detected by appropriate culture methods, and molecular screening
407 tests will yield a positive result if a carbapenemase is present regardless of the host organism.
408 Inclusion of bacteria other than lactose fermenters in a screening program is important as they
409 can also transmit resistance elements to or within the *Enterobacteriaceae*, as it has been
410 previously suggested (75).
411 A summary of tests is provided in Table 3. As shown, the cost, labor intensity, and turn-
412 around time vary by assay. Mathers *et al*. reported that the annual cost of a surveillance program
413 for a hospital containing 708 acute care beds and 40 long-term beds with weekly screening and a
414 CPE prevalence of 2.7% was about $225,000 for a qPCR (quantitative real-time polymerase chain
415 reaction) assay and $23,000 for the CDC screening culture method (136). Although Mathers *et al.*
416 accounted for the cost of decreased specificity, the cost of decreased sensitivity is much more
417 difficult to calculate. For instance, a false positive (product of low specificity) would result in
418 further follow up testing; however, a false negative (product of low sensitivity) may result in
419 spread of the CPO potentially adding very significant costs for the hospital to care for infected
420 patients, while instead it would appear to decrease the cost of the screening program. The
421 apparent difference between costs of methods can translate into many thousands of dollars per

year for a hospital performing routine screening in large volume. Added to the cost of screening is the cost of isolation. A 2014 Canadian study estimated a cost of $925 (CAD) (approx. USD 740) per non-ICU patient when isolating for 3 days while awaiting results (137).

Culture-based methods. Culture-based testing is easier to implement as the necessary equipment and knowledge are already present in the routine microbiology laboratory. These tests also have the potential to detect reduced susceptibility to carbapenems caused by newly emerging mechanisms as long as the mechanism is able to achieve at least a moderate level of resistance.

The CDC screening method addresses, with significant limitations, the need for detection of "low-level" resistance (MIC< 2 mg/L) and the ability to detect low loads of resistant bacteria. This method consists of an enrichment phase where a rectal swab is inoculated into 5 mL of Trypticase-soy broth (TSB) in which a disk impregnated with 10 µg of ertapenem or meropenem has been immersed, and incubated for 24 hours. This broth is then sub-cultured onto MacConkey agar, where only lactose-fermenters are selected. The CDC notes that many laboratories add a meropenem or ertapenem disk to this agar. A limitation of this test is that further testing is needed to determine the species and antimicrobial susceptibility of isolates growing on the agar (138). Furthermore, bacteria other than lactose fermenters that can harbor carbapenemases are routinely missed. Given the increased length of time needed for detection when using methods such as the CDC method, selective agars (see below) have been developed to optimize detection while obtaining results in a shorter time span. More important is that the CDC method will fail to detect the presence of bacteria with low-level resistance unless these bacteria are present in a large inoculum and without competition of other CROs; conditions that are unlikely to happen. Furthermore, low inocula of fully resistant CPOs can be missed if there is a large inoculum of

444 bacteria that have "low-level" carbapenem resistance through mechanisms other than
445 carbapenemases. Although the CDC broth enrichment method was meant to increase sensitivity,
446 recent reports demonstrate that some of the selective agar methods have superior or at least
447 comparable performance to the CDC method, so the delay of an overnight enrichment is not
448 necessary (139–141).

449 **Specialized solid media** aim to simplify the detection of CPE. Chromogenic media
450 incorporate chromogenic enzyme substrates (mainly glycosides) that release a pigment when
451 hydrolyzed by bacterial enzymes (142). Antibiotics added to the media make them selective for a
452 particular resistance trait. Chromogenic media have been compared regarding their limit of
453 detection of CPE at different inocula when used for stool screening (80, 139, 143–146). At the
454 present time the currently available media are not cleared by the Food and Drug Administration
455 (FDA) in the US.

456 Available chromogenic media that may be used for detection of carbapenemases include
457 CHROMagar KPC (CHROMagar, France), HardyChrom (Hardy Diagnostics, CA, US), chromID Carba
458 (bioMérieux, France), chromID ESBL (bioMérieux, France), chromID OXA-48(bioMérieux, France),
459 Colorex KPC (Biomed Diagnostics, OR, US), RambaChrom KPC (Gibson Bioscience, US), SpectraCRE
460 (Thermo Diagnostics, US), and *Brilliance* CRE (Thermo Diagnostics, US). Colorex KPC media
461 consists of media commercially prepared from dry CHROMagar reagents.

462 Some of these media are designed to target KPC producers and have markedly decreased
463 sensitivity for mechanisms based on other enzymes, particularly OXA-48 (143, 147). This is
464 specifically addressed with a medium designed for detection of OXA-48 producers, the chromID-
465 OXA48.

466 Table 4 shows the performance characteristics of different chromogenic media when tested
467 with pure cultures. Specificity varies depending on the type of negative controls used (clinical
468 specimens or known non-carbapenemase-producing, but carbapenem resistant isolates). In
469 addition, although not shown in the table, all testing methods had slightly but consistently lower
470 sensitivity for VIM β-lactamases than for other class B β-lactamases (80, 139, 143–146). This may
471 be due to the inclusion of isolates that contained plasmids carrying at most another β-lactamase,
472 rather than isolates with more complex backgrounds that have now become prevalent (148).
473 However, this likely does not hold true for bacteria harboring VIM-containing plasmids that also
474 carry an ESBL or another carbapenemase.

475 SUPERCARBA agar is another specialized medium that incorporates the use of ertapenem
476 [0.5 mg/L] in addition to cloxacillin in a zinc-supplemented Drigalski Lactose agar (149).
477 Ertapenem will select for carbapenem resistance, cloxacillin is added to inhibit growth of AmpC
478 producers such as *Serratia* and *Enterobacter* species, while the zinc enhances the activity of MBLs
479 (149). Different studies have shown sensitivity around 96% with specificity of 60%. These
480 numbers are similar to those obtained for chromogenic media (143, 149). The authors
481 recommend selecting only lactose-fermenting bacteria, limiting its ability to detect
482 carbapenemases in bacteria other than lactose fermenters. Another disadvantage is that the shelf
483 life of the medium is limited to 7 days, a significant obstacle in any routine clinical laboratory
484 (143).

485 **Relative performance of culture methods.** Studies describing different methods for
486 screening of CPE are difficult to compare and each study has its own limitations and particular
487 variations. Some studies have addressed the detection limit of different commercial assays by

488 using previously characterized CPE isolates. Those isolates, however, may not be representative
489 of the population at a specific hospital and clinical performance in actual practice may vary due to
490 the prevalence of the different β-lactamases in different institutions. Meanwhile, other studies
491 have compared the performance of a test in a particular setting, such as hospitals where there is a
492 particular distribution of resistance mechanisms within the bacterial population. It is difficult to
493 extrapolate the performance of these tests into other clinical settings. In addition, some studies
494 use comparators that are known to perform poorly which may exaggerate the performance of
495 certain media.

496 Performance characteristics of the different media when used for screening rectal or
497 perirectal swabs are shown in Table 6. Of the thirteen studies mentioned, nine showed an almost
498 exclusive presence of KPC producers (80, 139, 141, 150–156), while two revealed the exclusive
499 presence of NDM producers (157, 158). Only two studies were done at institutions where KPC and
500 VIM producers were reported as coexisting (140, 159); and only one with OXA-48-producing
501 isolates (160). Furthermore, the prevalence of ESBL producers at these locations is not taken into
502 account and could impact the specificity of these screening methods. The different screening
503 systems performed variably on stool specimens compared with pure cultures (Table 4), mostly
504 showing a decline of both sensitivity and specificity with stool specimens.

505 We assert here that the sensitivity of a screening media corresponds to the sum of
506 sensitivities for each particular mechanism (e.g., OXA-48, KPN, NDM). If a particular medium is
507 tested where one mechanism is over-represented, it will have a greater contribution to the
508 calculated sensitivity for CPE. For instance, consider that medium A has a sensitivity of 90% for
509 KPC and 70% for OXA-48. If this medium is tested where 95% of CPE are KPC while 5% are OXA-

510 48 producers, the study will show an overall sensitivity for CPE of 89%. However, if 70% of CPE

511 are OXA-48 and 30% are KPC it will show an overall sensitivity of 76%.

512 To place our analysis in a clinical perspective, we performed a statistical analysis

513 comparing the sensitivity, specificity, and diagnostic odds ratio (DOR) for the different methods

514 used for screening pure cultures employing bivariate random-effects model (161) using the *mada*

515 package of the R programming language (162, 163). DOR is the ratio of the odds of the test

516 producing a true positive result to the odds of it producing a false positive result. The bivariate

517 random-effects model is a meta-analysis technique for pooling diagnostic performance measures

518 across studies and estimating covariate effects. Corresponding forest plots were generated with

519 ggplot2 (164). Methods that did not present data detecting all three carbapenemase classes were

520 excluded. Figures 2 and 3 illustrate the sensitivity, specificity, and DOR for the different media in

521 each study and in aggregate respectively. Table 5 shows the model estimated 95% confidence

522 intervals for these parameters. Given the proportion of Class B carbapenemase-producing isolates

523 included on these studies, their effect on the estimated pooled performance characteristics is

524 likely disproportionate. The same approach was used to analyze performance on rectal/perirectal

525 swabs (Table 6). Given the low number of specimens for these analyses we only included in the

526 analysis those studies done where KPC was the predominant enzyme (over 98%). We excluded

527 methodologies that were not available commercially, except for the CDC protocol. Model-

528 estimated sensitivity, specificity, and DOR with their corresponding 95% confidence intervals are

529 shown in Table 7. Forest plots for the individual and aggregate studies are shown in Figures 4 and

530 5.

531 Analysis of the results of the screening media on pure cultures shows that chromID ESBL,
532 chromID Carba, and SUPERCARBA have similar sensitivities. *Brilliance* CRE media and the CDC
533 method with ertapenem results overlap with the 95% confidence interval of chromID Carba and
534 chromID ESBL media. Large confidence intervals can be seen with the CDC method, Colorex KPC
535 and CHROMagar KPC reflecting low number of tested isolates and conflicting results. For instance
536 CHROMagar KPC performed well in some studies (151, 158, 159), but not all (149, 155, 156), with
537 sensitivities ranging from 40 to 98%.

538 Analysis of specificity is more homogeneous among the different methods. There is,
539 however, a tendency for superiority favoring chromID Carba, while the opposite holds for
540 chromID ESBL. This is expected as the growth of ESBL-producing *Enterobacteriaceae* is
541 considered a false positive when screening for CPE. SUPERCARBA had a wide range of specificity,
542 ranging from 35% to 82% depending on the details of the analysis; which is reflected on its large
543 confidence interval. On this analysis, chromID Carba and SUPERCARBA have a clear advantage in
544 the clinic when compared to the other methods. Given the large confidence intervals, these results
545 must be interpreted with caution. Not included in the above analysis is the study by Hirsch *et al.* as
546 it only involved 18 isolates of KPC-producing *Enterobacteriaceae* (105).

547 Analysis of media performance on rectal/perirectal swabs is limited to those studies where
548 KPC was the prevailing enzyme. Unfortunately, there is not enough data for a meaningful
549 comparison of these media under different conditions. Available data with note of the enzyme
550 distribution can be found in Table 6. Sensitivity for detecting KPC on rectal/perirectal swabs
551 shows overlapping confidence interval for all methods, except for the CDC protocol which is
552 clearly inferior. Specificities also show significant overlap. HardyChrom agar showed the worse

553 specificity though it had a very large confidence interval product of only being tested in one study
554 (141). MacConkey agar with imipenem also performed acceptably in some studies (80, 111, 139,
555 150) showing sensitivities and specificities as high as 92% and 100%, respectively. Analysis of
556 DOR shows homogeneity for most methods. The overall trend is for the CDC method to be inferior
557 to others. Improved performance is suggested for SpectraCRE, HardyChrom, and chromID Carba.
558 Confidence intervals for HardyChrom and SpectraCRE, are however, exceedingly large.
559 SpectraCRE was tested in a single study in a Chicago LTCF (156), which likely explains its broad
560 confidence interval.

561 Due to its limited scope the chromID OXA-48 was not included in this statistical analysis.
562 For Zarakolu et al. it shows 75% sensitivity when tested against clinical specimens containing
563 OXA-48, with 99.3% specificity. When used in conjunction with the chromID Carba, sensitivity
564 and specificity reached 90.9% and 98.5% respectively (165).

565 Overall differences in sensitivity between the media can be explained by the
566 carbapenemase being tested. Most media perform reasonably well with class A enzymes, while
567 performance with class B and D enzymes is more variable. The chromID Carba media performed
568 well in both pure culture and when tested against rectal/perirectal swabs. The SUPERCARBA
569 media did well on pure cultures. However, it was not tested with patient specimens. SpectraCRE
570 did well on rectal/perirectal swabs though one must be aware of its confidence interval. The CDC
571 method underperformed when tested against pure cultures and against clinical specimens. Other
572 methods that were tested, particularly those involving "house-grown" techniques, could not be
573 analyzed with the same rigor, and unless more studies are done, we would caution against their
574 use in clinical practice.

575 It must be emphasized that many of the studies of these selective plates are limited to KPC-
576 producing isolates. Furthermore, the various media evaluated in Tables 4, 6, and 8 are not
577 available in all countries. Therefore, the practical issues of cost and availability affect the choice
578 made by an individual laboratory that must decide if optimal sensitivity is desired, knowing that
579 additional work-up will be required to detect false positives if the method has low specificity.

580 Studies analyzing the LOD include bacteria with specific genetic backgrounds on pure
581 cultures that may not necessarily represent the backgrounds present in a specific clinical setting (
582 Table 8). The LOD will directly impact the sensitivity of the screening method. Given the
583 abundance of *Enterobacteriaceae* in stool, it is desirable to inhibit the growth of the carbapenem-
584 susceptible population. However, this inhibition comes at expense of sensitivity. A relatively low
585 inoculum of a CPE with borderline susceptibility will need to overcome this inhibitor and the
586 medium would have a higher LOD. On the other hand, adjusting growth inhibitors to obtain a
587 lower LOD would allow for growth of other bacteria and would decrease specificity.

588 High-resource settings where healthcare is already expensive may have a lesser impact on
589 isolating more patients and may want to err on the side of higher sensitivities. Furthermore, the
590 medical care provided in high-resource settings tends to be more invasive; therefore there is a
591 higher cost of missing a colonized patient. On the other hand, lower-resource settings may still
592 benefit from selecting a method with lower sensitivity that would decrease their isolation costs
593 while still have an impact on the local spread of CPO.

594 Table 8 summarizes the limit of detection of the different agar screening media. All of the
595 tested media and SUPERCARBA performed reasonably well when detecting class A enzymes
596 (KPC); achieving a LOD in the 1 X 10^1 to 1 X 10^2 CFU/mL range (80, 140, 143, 149, 166); except

597 for the chromID OXA-48 which, as expected, performs better with Class D enzymes (OXA-48)
598 (167). The LOD for class B enzymes on chromogenic media are approximately 1 log higher than
599 for class A (80, 140, 143, 149). The MacConkey agar with disks had a LOD about 1-2 log higher
600 than the other media for both class A and B enzymes (80, 140). The chromID OXA-48 showed
601 poor performance for both class A and B enzymes, with a LOD of 1×10^{7} CFU/mL (167). Class D
602 enzymes were not tested for all methods. LOD remained in the same 1×10^{1} CFU/mL range
603 consistently for SUPERCARBA. Other methods had a significant increase on their LOD for class D
604 enzymes. Remarkably, chromID KPC, CHROMagar KPC, the CDC method, and chromID Carba had a
605 LOD up to 6 log higher than more sensitive methods (140, 143, 149). As expected, chromID OXA-
606 48 performed exceptionally well with class D enzymes, with a LOD of 5×10^{1} CFU/mL (167).

607 **Nucleic acid amplification technology (NAAT)** detects the presence of a specific gene or
608 genes, in most cases limiting its usefulness to previously characterized determinants.
609 Furthermore, newly emergent variants of previously characterized genes may not be reliably
610 detected. Since various genes can encode different carbapenemases, a broad panel of tests is
611 needed to detect all targets. Because it is not practical to detect every enzyme, these tests have
612 been designed to cover the most common carbapenemases. A challenge for nucleic acid-based
613 testing is DNA extraction from stool. Feces contain PCR-inhibiting substances and poor results
614 may be obtained due to excessive shearing of DNA (168). Despite these concerns, very good
615 methods are available for extracting DNA from stool, and multiplex molecular assays are routinely
616 performed on stool specimens for gastrointestinal pathogens. It is critical to note that detection of
617 resistant determinants in pure cultures or in specimens where a single organism is expected (such
618 as blood or urine) is significantly easier than detecting the same genes on a more complex

619 specimen such as a stool swab. In addition, epidemiological data such as species information is
620 lost in most assays.

621 There are several NAAT based methodologies that may be employed to detect
622 carbapenemase genes in bacterial isolates (169). Theoretically, all of them can be used to screen
623 stool specimens. These methodologies include single and multiplex end-point PCR, loop-mediated
624 isothermal amplification (LAMP), single and multiplex quantitated real-time PCR (qPCR), and
625 microarrays. Next-generation sequencing (NGS) may be another option though it is not readily
626 available in most clinical laboratories at the present time (169). NGS remains prohibitively
627 expensive due to high equipment acquisition costs, need for significant computing processing
628 power, and data storage(170).

629 Regardless of the NAAT based methodology selected by a laboratory, there are complex
630 regulatory requirements that vary from region to region. Implementation of a laboratory-
631 developed assay involves determining the test's performance characteristics. The burden of an
632 involved development and validation may be partially relieved by the use of commercial assays.
633 FDA regulations within the United States are evolving at this time and will likely result in
634 increased regulatory burden on the lab in the future.

635 **End-point PCR** is useful when there is a large quantity of the target gene. Specificity
636 cannot be assured unless positive results are followed up with DNA sequencing or hybridization
637 with specific probes. With proper validation, a PCR method can be acceptable. The Hyplex Super
638 Bug ID system (Amplex Biosytems GmbH, Giessen, Germany) for the detection of carbapenemases
639 is based on a multiplex end-point PCR followed by ELISA hybridization (171). Although it has not
640 been tested on direct stool specimens, this NAAT showed a 98% sensitivity and specificity for

641 VIM-producing CPE when used on DNA extracted from clinical specimens, including blood, urine,
642 pus, and respiratory samples from Greece (172). Another multiplex end-point PCR was developed
643 by Voets *et al.* and allows for detection of a wide range of resistance genes (173). Some of these
644 multiplex assays were developed by independent laboratories and are not widely available to
645 most clinical laboratories. Yet, there is great value in demonstrating that these comprehensive
646 assays can be developed.

647 **Microarrays** consist of oligonucleotides bound to a solid surface. The target gene of the
648 pathogen is then labeled and hybridized to the immobilized probe. This reaction is then measured
649 with a scanner (169). Microarrays are difficult to standardize (169) and published studies
650 describing the use of microarrays to directly screen for β-lactamase genes from stool are not
651 available. Most assays, however, can be used to confirm and characterize the β-lactamase gene on
652 suspicious colonies of a screening culture. These tests have excellent sensitivity and specificity as
653 shown by a study with 149 previously characterized *Enterobacteriaceae* that were subjected to a
654 commercial Check-points microarray assay (Check-Points Health, Wageningen, Netherlands),
655 which was found to have 100% sensitivity and specificity (174). Direct testing from blood cultures
656 also showed 98% concordance between a microarray method and routine microbiological testing
657 (175). The Verigene BC-GN Test is a microarray-like detection system. It detects nine
658 genus/species targets and six resistance determinants including (KPC, NDM, OXA, VIM, and IMP)
659 without the need for prior PCR amplification (176). Future studies are needed to determine if
660 microarrays will be used to screen direct perirectal or stool specimens, though this may be
661 hampered by their high cost and the advent of next generation sequencing.

662 **Loop-mediated isothermal amplification (LAMP)** is a modification of conventional PCR
663 where several oligonucleotides that bind to the target gene are incubated at the same temperature
664 with the DNA polymerase. As DNA polymerizes there is release of pyrophosphate that can be
665 detected with a fluorescent dye or a compound that will increase the turbidity of the solution
666 (177). This method's advantages include increased sensitivity to lower DNA concentrations
667 compared to end-point PCR, no need for a thermo-cycler, and a simple way of visualizing the
668 result. LAMP assays can be particularly useful for low-resource settings (169). A LAMP assay for
669 detection of NDM-1 was successfully used in 336 clinical specimens, including rectal swabs (178).
670 The investigators found a limit of detection of 10.70 pg/μL of genomic DNA, which would roughly
671 correspond to 1 X 10^3 CFU, compared to 1070 pg/μL (or 1 X 10^5 CFU) for the end-point PCR assay
672 used as a comparator in the study. Solanki et al developed two LAMP assays for detection of KPC
673 and NDM-1 (179). These assays were able to detect all 48 tested isolates with either NDM or KPC
674 while end-point PCR detected only 44. Other studies have found improved performance of LAMP
675 vs end-point PCR for microbiological targets other than CPE, but not against real-time assays (180,
676 181). Therefore, LAMP assays may have a useful role in detecting CPO, but they are not the most
677 sensitive assay for clinical microbiology laboratories that have access to other types of NAAT.
678 **Real time or quantitative PCR (qPCR)** is based on coupling the PCR with detection of the
679 amplified target. Real Time PCR has been used for screening of CPO both using commercial and
680 an "in-house" kits with the advantage of more rapid results, increased sensitivity, and increased
681 specificity (152, 153, 182, 183). A recent seven-center study in the Netherlands found 100%
682 sensitivity and specificity with a multiplex assay detecting KPC, NDM, VIM, IMP, and OXA-48 on
683 twenty selected laboratory isolates (184).

684 Many laboratories have experience in using qPCR for direct screening of stool/rectal swab
685 specimens. Examples of stool/rectal swab testing with qPCR in routine clinical practice include
686 screening for vancomycin-resistant enterococci, Group B streptococcus, and *Clostridium difficile*
687 (185–187). The validity of using qPCR, including for quantification of KPC-carriage load was
688 evaluated by Lerner *et al.* (188). They determined a detection limit of 10 plasmid copies, which
689 the authors presume is close to 1 X 10^1 CFU/mL.

690 Another in-house qPCR for NDM-1 found a limit of detection of 1 X 10^1 to 3 X 10^1 CFU/mL
691 of stool, compared to 2 X 10^1 to 1 X 10^2 CFU/mL for chromID ESBL and 2 X 10^1 to 4 X 10^3 CFU/mL
692 for CHROMagar KPC(189). An additional study found a limit of detection with end-point PCR of 1
693 X 10^4- 1 X 10^5 CFU/mL for KPC and 1 x 10^3 CFU/mL for NDM(141). Naas *et al*. found a limit of
694 detection for OXA-48 using an in-house qPCR assay of 1 X 10^1- 1 X 10^2 CFU/mL in stool for qPCR,
695 compared to 1 X 10^1- 1 X 10^2 CFU/mL for SUPERCARBA and 2 X 10^1 - 3 X 10^2 CFU/mL for chromID
696 ESBL (182). A comparison between agar screening and qPCR for KPC showed a 100% sensitivity
697 for the qPCR assay compared to 77% with the culture method(155). Overall, limits of detection for
698 single genes assays (152, 155, 182, 190) tend to be lower than those for multiplex assays (141,
699 191).

700 **Commercial Assays** for molecular multiplex CPO detection include Check-Direct-CPE,
701 Check-MDR Real Time (Check-Points Health), Hyplex SuperBug ID (Amplex Biosystems), eazyplex
702 SuperBug CRE (Amplex Biosystems), and Xpert MDRO (Cepheid). Check-MDR Real Time consists
703 of an oligonucleotide probe that binds to the target sequence (VIM, NDM, KPC, and OXA-48), to a
704 pair of universal primers, and to a molecular beacon. Real-time PCR amplifies only the bound
705 target sequences at the same time that the molecular beacon emits fluorescence to measure the

706 amplification. The manufacturer has established a limit of detection of less than 5 copies per
707 reaction. Testing on pure cultures found a 100% sensitivity and specificity (192). The
708 manufacturer however, recommends using pure cultures, which is clearly not the method used to
709 screen perirectal swabs directly. The Check-Direct CPE is a real time assay using probe detection
710 chemistry. It has a limit of detection of 5 copies per reaction. Using "spiked" stool specimens,
711 Check-Direct CPE was able to detect a bacterial inoculum of 10^3-10^5 CFU/mL, with less sensitivity
712 for KPC (191). The NucliSENS EasyQ KPC (bioMérieux) is another real-time assay that uses
713 molecular probes. This was compared to chromID ESBL with ertapenem disks, using surveillance
714 specimens. Although a limit of detection was not determined, the assay performed with 93%
715 sensitivity (193). SuperBug CRE system is a multiplex LAMP system able to detect KPC, VIM, NDM,
716 OXA-48 (and some variants), in addition to ESBLs CTX-M-1 and CTX-M-9. On pure cultures
717 eazyplex SuperBug CRE was able to correctly identify all 139 *Enterobacteriaceae* isolates (194).
718 However, when used against a panel of 82 *Acinetobacter spp* isolates it produced 5 false positive
719 results (195). The Xpert MDRO assay has been used to detect CPO directly from rectal and
720 perirectal swabs. The assay was able to detect KPC, NDM, and VIM with 100% sensitivity and 99%
721 specificity on 328 discarded peri-rectal, rectal, or stool samples from two US and one Spanish
722 hospital (183).
723 Table 9 summarizes the molecular methods that have been used on clinical specimens.
724 Some of the reports show excellent sensitivity and specificity for molecular assays; however, other
725 studies show a broad range for the LOD of specific carbapenemases, with numbers comparable to
726 agar-based methods. Limitations include the cost and the inability to detect new or unanticipated
727 carbapenemases. Pooling of specimens for initial screen, followed by confirmatory testing of

728 positives, may be an option to contain costs especially in low prevalence settings, but
729 investigations are needed to see if the loss of sensitivity is too great. The cost-effectiveness of the
730 approach will also depend on the prevalence of CPO at an individual institution, which will
731 determine the number of specimens that would require follow-up when a positive pooled result is
732 obtained, because all individual specimens in the pool would need to be retested. The concern
733 about detecting new, not yet described, carbapenemases will need to be addressed by constant
734 vigilance in updating targets in a chosen assay; if a laboratory reports a new carbapenemase in the
735 local geographic region, or when a medical center treats a high volume of international patients,
736 adjustments will need to be made.

737 **SCREENING OPTIONS**

738 Based on the above discussion there are several screening options that may be easy to
739 implement in a clinical microbiology laboratory. Any of these options should be closely
740 coordinated with the infection control program of the institution. One must know the baseline
741 prevalence and type of the resistance enzymes in a specific setting, as the choice of method will be
742 dependent on these variables. At the present time we could not find data to suggest any advantage
743 of using stool specimens vs rectal or perianal swabs. Most studies have been done on rectal swabs
744 and it is likely that most institutions would tend to prefer this modality.

745 Whatever modality is chosen for screening, laboratories must be aware that local
746 validation will be required. When implementing a screening program it is important to determine
747 the factors listed in Table 10. Deciding who to screen will always be controversial as the balance of
748 cost to risk will be subject to different interpretations. Ideally screening should be universal, but

749 most institutions do not have the necessary resources for both the screening testing and the
750 isolation requirements while waiting for test results. Table 11 proposes a set of criteria that can
751 be used to screen certain patient populations.

752 **Cultured-based screening with molecular confirmation**

753 Culture-based screening includes the use of a chromogenic agar, SUPERCARBA, or the CDC
754 method to perform perirectal or rectal swab screening of patients. While universal screening has a
755 higher potential for detecting and preventing outbreaks, it comes at a significant financial cost.
756 Based on our analysis, we would favor the use of chromID Carba. However, if the hospital is
757 located in a geographic area with high incidence of OXA-48, the clinical microbiology laboratory
758 should strongly consider using SUPERCARBA or adding an OXA-48 specific media such as the
759 chromID OXA-48 media. A bi-plate containing chromID Carba and chromID OXA-48 is available
760 (chromID CARBA SMART, bioMérieux, France). Bacteria growing on the SUPERCARBA media
761 should be identified by conventional microbiological tests or MALDI-TOF MS. Similarly, isolates
762 on chromogenic media that cannot be readily classified as *Enterobacteriaceae* should also be
763 identified. While non-*Enterobacteriaceae* can carry plasmids encoding carbapenemases,
764 commonly carbapenem resistance in these organisms is mediated by other mechanisms. The
765 decision to isolate patients with carbapenem-resistant organisms other than *Enterobacteriaceae*
766 should be based on local epidemiology. Confirmation of positive specimens should ideally be
767 sought with molecular testing with either a broad panel of PCR reactions or with a microarray
768 method. Alternatively, a phenotypic test (such as CarbaNP, Blue-Carba, inhibitory disk synergy
769 testing, or mHT) can be used to confirm the presence of carbapenemases, reserving molecular
770 testing for a random sample of positive isolates. Random sampling will come at the cost of

771 decreased hospital epidemiology data. To track the prevalence and type of carbapenemases in an
772 institution, isolates from clinical specimens, not only surveillance specimens, demonstrating
773 decreased carbapenem susceptibility should also be subjected to an assay for detection of
774 carbapenemase activity or a PCR panel/microarray.
775 Figure 6 proposes an algorithm when using a culture-based approach. Note that depending on the
776 laboratory capabilities some tests may be referred to a research laboratory.

777 **Molecular-based approach**

778 The use of universal perirectal screening via molecular methods may not be desirable or
779 affordable due to low prevalence or due to increased costs. Screening of high-risk patients, such
780 as those coming from endemic areas, transferred from LTCFs, or who have had extensive exposure
781 to carbapenems (41), may be advisable. A multiplex real-time PCR assay that includes KPC, OXA-
782 48, NDM, and VIM should be used in most locales. However, specific areas where IMP or GES-5 is
783 common should either develop their own assays or have simultaneous routine culture testing.
784 Indeterminate results and a sample of negative specimens obtained through molecular testing
785 should be tested with a culture based-method with high sensitivity, such as SUPERCARBA or
786 chromID Carba. Suspect colonies should be subjected to antimicrobial susceptibility testing or to a
787 test for carbapenemase activity (e.g., CarbaNP or Blue-Carba). A test such as the double disk
788 synergy test with avibactam-ertapenem with follow up ertapenem-boronate or moxalactam-EDTA
789 depending on the result may enable a lab to distinguish between class A, B, and D enzymes (113);
790 making it particularly attractive for this scenario. Figure 7 suggests an algorithm for CPO
791 screening based on molecular methods.

792 **Combined approaches**

793 Some combined approaches can be useful in specific situations, such as during an outbreak
794 caused by a CPO with a known enzyme. We speculate that a LAMP or qPCR assay could be
795 implemented for universal screening of carbapenemase involved in an outbreak. Patients testing
796 positive could be quickly cohorted, while patients testing negative could be subjected to routine
797 culture-based screening. This strategy would maximize available hospital beds while attempting
798 to minimize patients on enhanced infection control precautions.

799 **CONCLUSIONS**

800 In this review we stress that screening for intestinal carriage of CPO is of significant
801 importance for the development of infection control strategies. However, the optimal screening
802 modality remains to be established for each location and for each specific purpose. Culture-based
803 screening methods have the advantage that they involve technologies that are readily available in
804 clinical microbiology laboratories. Some enhancements, such as the use of chromogenic media,
805 make culture-based screening more convenient; however, the turn-around time is long and
806 sensitivity of some culture methods is not as high as desired. In addition, culture-based methods
807 may not be optimal for detection of low-level carbapenemase production, which is important for
808 epidemiological purposes (93).

809 Agar-based procedures always require confirmatory testing to detect the type of *bla* gene
810 present after a potentially resistant isolate is detected. Clinical microbiology laboratories may
811 choose an agar-based screen with follow up molecular testing, or a molecular method with reflex
812 to culture if further investigation of the isolate is desired. On the other hand, NAAT offers a

813 promising approach for screening for carriage of CPO. These methods offer faster availability of
814 results and increased sensitivity, but with significantly increased expense and unclear specificity
815 on direct specimens at this time.

816 Our review indicates that the chromID Carba and the SUPERCARBA media have excellent
817 sensitivity for class A β-lactamases that rival that of real-time PCR. As KPC is becoming endemic in
818 more communities, the use of screening for this class of enzymes may become less useful because
819 the high prevalence could make empiric therapy and initial isolation procedures prior to
820 surveillance results default to the assumption of a KPC-positive isolate. It is still hoped however,
821 that communities in which KPC-positive organisms are not endemic may contain the spread of
822 resistant isolates for some time. We argue that screening should shift to those carbapenemases
823 that are threatening to become endemic and that have a high potential of causing outbreaks, such
824 as NDM and OXA-48. Real-time PCR appears to be ideally suited for this goal; however, qPCR
825 implementation is hampered by cost. In addition, there could be false positives for OXA-48 due to
826 amplification of similar chromosomally-encoded enzymes in species such as *A. baumannii*.
827 Furthermore, data on their performance on the clinical setting compared to that of culture-based
828 screening is not yet available. Nonetheless, improved turn-around time and improved accuracy of
829 NAAT and direct carbapenemase detection assays may result in limiting the unnecessary
830 prolonged isolation of new admissions, thus saving costs to the infection control program.
831 Nevertheless, the molecular tests with high sensitivity have a cost that is difficult to offset and can
832 be prohibitive for many clinical microbiology laboratories (136). We must choose wisely.

833 An urgent need exists to define the appropriate criteria and clinical circumstances to
834 conduct screening for gastrointestinal carriage of CPO and to determine the optimal methods to

835 use. The best method will differ from institution to institution depending upon the prevalence in
836 the community, the travel patterns and demographics of the population, level of care rendered by
837 the hospital, the age of patients, the technical capabilities of the microbiology laboratory, and the
838 resources allocated by the hospital administration for infection control monitoring.

839 The agar-based and colorimetric screens are usually more affordable and are able to detect
840 presence of "new and emerging" carbapenemases before they are characterized. In addition, most
841 agar based tests can be performed without the need for significant investments by the clinical
842 microbiology laboratory. However, they may lack sensitivity for carbapenemase producers that
843 confer low level of resistance. Furthermore, variability in performance according to the β-
844 lactamase class makes the selection of a particular method more difficult. For instance, it is easier
845 to justify the resources needed to perform a screening test that will reliably detect class A
846 carbapenemases in a KPC-endemic area. At the same time the detection of class D carbapenemase
847 producers may allow for the institution of a program that will prevent them from becoming
848 endemic. Laboratories may consider a combined approach of two independent assays to screen
849 for a broader spectrum of carbapenemases. This strategy however, comes with the disadvantage
850 of increased cost and labor.

851 At this time, clinical microbiology laboratories that choose to implement a CPO screening
852 methodology must have a reliable procedure for detection of the carbapenemases endemic to
853 their area. In most of the US, this would be KPC while laboratories in Europe and the Middle East
854 should likely screen for OXA-48. Once a *bla* gene is found they should choose which of the other
855 carbapenemases they want to include in their screening approach, knowing that they will miss
856 colonized patients carrying a CPO not included in their infection control algorithm.

857 Although this review has focused on carbapenemase producing *Enterobacteriaceae*, 858 plasmids encoding carbapenemase genes have been identified in non-*Enterobacteriaceae*, so 859 vigilant monitoring of both may be warranted in the future. We assert that until a carbapenem 860 resistant isolate is recovered in a clinical culture and detected by routine susceptibility testing, the 861 number of carbapenemase targets to include will be determined by cost, time available for labor, 862 and technical abilities of the laboratory. It is likely easier to adapt an in-house molecular assay 863 compared to a commercial assay to detect emerging carbapenemases by adding additional 864 primers and probes to an existing assay which provides information about both the presence of 865 enzymes and the type of enzyme. In contrast, commercially available assays have the advantage of 866 manufacturer validation, but they cannot be modified quickly and only when the manufacturer 867 chooses to update an assay. Continued surveillance is warranted to detect carbapenemases not 868 detected with the chosen assay. These considerations are most important to prevent and control 869 infections caused by carbapenemase producers and protect the public health. A proactive 870 approach trying to halt the spread of carbapenemase producers is desperately needed.

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1527 **Table 1: Carbapenemases and selected characteristics** (16, 17, 22)

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Molecular Class	Representative β-lactamase	Characteristics	Inhibitors	Enzymes currently Endemic	Areas where Endemic
A	**KPC** **GES** **SMC**	Serine β-Lactamase Plasmid encoded	Boronic acid derivatives	KPC	North America, Greece, Italy, Poland, Colombia, Argentina, Israel, China
				GES-5	Brazil
B	**NDM** **VIM** **IMP** **GIM-1** **SPM**	Metallo β-Lactamase Zinc requiring Plasmid encoded/ chromosomal	EDTA, dipicolinic acid	NDM	Indian Subcontinent, Kenya, China
				VIM	Indian Subcontinent, Greece, Italy, Southern France, Japan, Lebanon, Brazil, Portugal, Ireland, UK, Germany, Poland
				IMP	Indian Subcontinent, Greece, Japan, China
C	**CMY-10**	Serine β-lactamase Cephalosporinases Mobile or chromosomal Uncommon	Cloxacillin, boronic acid derivatives	AmpC	Worldwide
D	**OXA-48** **OXA-181** **OXA-204** **OXA-162** **OXA-23** **OXA-24**	Serine β-lactamases Weak activity of those that are carbapenemases Plasmid encoded	No specific inhibitors available	OXA-48	France, Belgium, Canada, South Africa, Middle East, Turkey, Northern Africa, Switzerland, Germany, Lebanon, Israel, Morocco

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1530 **Table 2: Clinical breakpoints for carbapenems according to the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility**
1531 **Testing (EUCAST)**

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Carbapenem	**Susceptible (µg/mL)**			
	CLSI M100-S20 2010	**CLSI M100-S21 2010**	**CLSI M100-S22 2012**	**EUCAST 2009-2014**
Doripenem	Not Defined	≤1	≤1	≤1
Ertapenem	≤2	≤0.25	<0.5	≤0.5
Meropenem	≤4	≤1	≤1	≤2
Imipenem	≤4	≤1	≤1	≤2
Intermediate is interpreted as one dilution higher and resistant is interpreted as ≥2 dilutions higher, except for EUCAST Ertapenem interpretation where >1 µg/mL is considered resistant.				

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CLSI M100-S23,24 (2013,2014) do not change the interpretative criteria for carbapenems
CLSI M100-S22(2012) only changed interpretative criteria for Ertapenem. Other carbapenems were not changed
Doripenem was not included in the 2009 and 2010 editions of the CLSI-M100

1534 **Table 3: Characteristics and approximate costs of screening methods to identify fecal carriage of CPE. Adapted from Mathers et al.**(136)

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Method	Description	Turn-around time for Positive or Preliminary Positive	Price (USD)
Centers for Disease Control(138)	Broth enrichment of rectal swab in ertapenem media followed by subculture on MacConkey with carbapenem disk, followed by identification of suspect isolates	48-72 hrs#	Negative Test: $1-2 Positive Test: $2-6
SUPERCARBA(149)	Direct plating of rectal swab in selective media	24-48 hrs#	$1*
Chromogenic Media	Direct plating of rectal swab in selective media with chromogenic molecule	24-48 hrs#	$4-7
Real-Time PCR** (in house methods)	DNA extraction followed by PCR and probe-based detection.	2-5 hrs	$10-30
Commercial PCR assay**	DNA extraction followed by PCR and probe-based detection.	2-3 hrs possible	$30-60

USD: US Dollars as of 2015

*SUPERCARBA media has been patented. Cost is that of raw materials; Not available to many laboratories.

** Cost of PCR assay may increase with increased number of targets

Confirmation testing might include single-plex PCR, multiplex PCR, CarbaNP, or identification and susceptibility testing. Cost may range from an additional $2 to $50 and turn-around time may range from an additional 2 -24 hr for confirmatory testing, depending on methods chosen by laboratory. Hospital epidemiology can act on negative results and preliminary positive results, pending confirmation. NAAT negative results likely do not require any further testing, but positive results may require confirmation, depending on false positive rate of assay.

1540 **Table 4: Use of known resistant isolates to test performance of CPE screening methods**

Method	Overall sensitivity	Sensitivity by β-lactamase class/No. Isolates Tested			Specificity/ No. Negative Isolates Tested	Type of Isolates	Reference
		Class A	Class B	Class D			
SUPERCARBA	95.6%	100%/18	90%/52	100%/44	82.2%/62	CLI	(149)
chromID ESBL	87.7%	100%/18	98%/52	70%/44	24.2 %/62		
CHROMagar KPC	40.3%	66.7%/18	55.8%/52	13.6%/44	85.5%/62		
SUPERCARBA	96.5%	100%/20	92%/51	100%/43	60.7%/28	CLI	(143)
CHROMagar KPC	43%	70%/20	58.8%/51	11.6%/43	67.8%/28		
Brilliance CRE	76.3%	85%/20	78.4%/51	69.8%/43	57.1%/28		
Brilliance CRE	86%	100%/17	72%/25	88%/58	40%/77	CLI	(147)
Colorex KPC	48%	100%/17	52%/25	31%/58	39%/77		
SUPERCARBA	97%	100%/17	88%/25	100%/58	35%/77		
Brilliance CRE	78%	83%/12	79%/103	67%/15	66%/70	CLI	(144)
chromID Carba	91%	100%/12	93%/103	67%/15	89%/70		
chromID ESBL	96%	100%/12	98%/103	80%/15	19%/70		
Colorex KPC	56%	83%/12	52%/103	60%/15	77%/70		
CDC ertapenem	78%	83%/12	80%/103	73%/15	69%/70		
CDC meropenem	47%	67%/12	46%/103	40%/15	79%/70		
Brilliance CRE	94%	100%/36	94%/34	84%/25	71%/160	CLI CCI	(146)
mHT	100%	100%/18	ND/0	ND/0	96.7%/32	CCI	(110)
RambaChrom KPC	95%	95%/18	ND/0	ND/0	77.1%/32		
Mero-PBA DDST	100%	100%/18	ND/0	ND/0	100%/32		
Erta-PBA-DDST	100%	100%/18	ND/0	ND/0	91.4%/32		

Types of Isolates:

CCI: Characterized clinical isolate: isolate originating from a clinical specimen and later characterized in the laboratory

CLI: Characterized Laboratory isolate: isolate retrieved from a laboratory source. It may have originated from a clinical specimen but may had been modified to express certain characteristics in the laboratory.

ND: Not determined.

mHT: Modified Hodge test

PBA DDST: phenylboronic acid double disk synergy test

Mero, meropenem; Erta, ertapenem

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1543 **Table 5: Comparison of model estimates of diagnostic performance for different screening methods on pure cultures**

Method (number of studies)	Sensitivity % (95% CI)	Specificity % (95% CI)	DOR (95%CI)	Aggregate number of isolates (positive isolates)
*Brilliance*CRE (4)	81.3 (77.1-84.88)	58.89 (39.31-76.02)	6.23 (2.6-14.91)	774 (439)
CDC Ertapenem (1)	79.23 (71.21-85.47)	68.57 (30.95-91.4)	8.32 (1.49-46.57)	200 (130)
CDC Meropenem (1)	46.92 (38.24-55.8)	78.57 (42.41-94.81)	3.24 (0.58-18.19)	200 (130)
CHROMagar KPC (2)	42.11 (35.69-48.81)	78.13 (52.18-92.12)	2.6 (0.73-9.27)	318 (228)
chromID Carba (1)	90.77 (84.36-94.72)	88.57 (59.31-97.63)	76.21 (11.97-485.11)	200 (130)
chromID ESBL (2)	91.01 (86.26-94.23)	21.27 (7.95-45.8)	2.74 (0.75-9.95)	376 (244)
Colorex KPC (2)	52.45 (45.78-59.04)	59.13 (32.06-81.6)	1.6 (0.48-5.35)	377 (230)
SUPERCARBA (3)	96.27 (93.53-97.87)	60.38 (37.33-79.59)	39.29 (12.52-123.33)	176 (114)

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CI: Confidence Interval

DOR: Diagnostic Odds Ratio

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1550 **Table 6: Performance of culture methods on rectal/perirectal swabs**

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Method	No of positives / No. of Swabs	Sensitivity	Specificity	Enzyme distribution	Reference Standards	Reference
MacConkey with IPM MacConkey with IPM/MEM/ETP disks CHROMagar KPC	33/ 139	84.9% 75.8% 84.9%	94.3% 89.6% 88.7%	KPC 100%	PCR for KPC	(80)
chromID Carba MacConkey with IPM CDC	86/ 177	96.5% 89.5% 98.8%	91.2% 31.9% 80.2%	KPC 98% VIM 1%	qPCR for KPC or VIM, mHT, aminophenyl-boronic acid/meropenem, and EDTA/meropenem testing	(139)
MacConkey with ETP disk. MacConkey + MEM alone, MEM+Phenyboronic Acid (PBA), MEM+EDTA, and MEM + PBA	97/189	96.9% 94.8%	98.9% 100%	KPC: 61.9% VIM: 9.3% KPC+ VIM: 26.8% OXA-48: 3%	Colony PCR for KPC, VIM, OXA-48, IMP, and NDM. Negative isolates were confirmed with a mHT.	(111)
MacConkey with MEM CDC Enriched BH culture with ertapenem re-plated on chromID ESBL chromID CARBA chromID ESBL	92/ 200	89.1% 89.1% 92.4% 92.4% 92.4%	85.2% 86.4% 93.3% 96.9% 84.7%	KPC: 68% VIM: 31%	PCR for KPC, IMP, NDM, VIM, and OXA-48 VITEK susceptibility testing and mHT.	(140)
chromID Carba *Brilliance* CRE	32/ 175*	100% 59%	98% 34%	NDM 100%	NDM PCR , disks with meropenem, boronic acid, cloxacillin, and dipicolinic acid. (Rosco KPC/MBL confirm Kit) Agar dilution MICs for carbapenems, negative Rosco KPC/MBL	(157)
CDC MacConkey with ETP disk with a 27 mm breakpoint	33/ 149	65.6% 97%	49.6% 90.5%	KPC 100%	KPC PCR. Broth microdilution susceptibilities for ertapenem, imipenem, and meropenem. mHT	(150)
MacConkey with IPM, MEM, and ETP disks CHROMagar KPC	41/ 122	92.7% 100%	95.9% 98.4%	KPC 100%	Direct KPC PCR from swab	(151)

MacConkey with MEM and ETP disks	54/ 187^	87%	100%	KPC 100%	qPCR on swab and isolates followed by gel electrophoresis and sequencing	(152)
MacConkey with IPM	64/ 755	87.5%	99.4%	KPC 100%	KPC PCR, KPC PCR, mHT, repeat culture, repeat PCR	(153)
chromID Carba (prototype) Colorex KPC	64/37 200*	100% 97%	93% 96%	NDM 100%	PCR for IMP, VIM, GIM, SPM, SIM, and NDM. mHT.	(158)
CHROMagar KPC MacConkey with IPM	46/ 126	97.8% 78.3%	98.7% 97.5%	KPC 72.5 % VIM 27.5%	Phoenix susceptibility. EDTA/IMI confirmatory disks. PCR for KPC and VIM. Negative not confirmed if negative by both methods.	(159)
HardyChrom CDC	46/126	76.1% 78.3%	100% ∫ 100% ∫	KPC 100%	qPCR$^{\alpha}$ for KPC and NDM, PCR for SME, VIM, IMP, GES, OXA-48, and AmpC	(141)
SUPERCARBA with enrichment step chromID ESBL with enrichment step *Brilliance* CRE w/enrichment step	10/ 77	80% 100% 90% 100% 80% 100%	98.5% ∫ 68.6% 86.6%	OXA-48 100%	Positives and negatives: PCR$^{\alpha}$ for KPC, NDM, VIM, IMP, NDM, OXA-48, and for ESBL panel.	(160)
CHROMagar KPC VAN/AMB/CAZ/CLI Plate	66/ 95	77.3% 77.3%	100% 100%	KPC 100%	Positives: confirmed with PCR$^{\alpha}$. Negatives had negative qPCR$^{\alpha}$ and PCR	(155)
CHROMagar KPC Spectra CRE MacConkey with ETP Disk	47/150**	76% 97.8% 83%	75.7% 86.4% 73.8%	KPC (presumed)	Confirmed with KPC qPCR and Microscan susceptibilities.	(156)
CDC chromID OXA-48 chromID Carba chromID Carba+ chromID OXA-48	33/302	57.6% 75.8% 57.6% 75.8%	95.2% 99.3% 98.9% 94.4%	OXA-48 100%	Initial screen with inhibition disk synergy testing followed by PCR and sequencing on positive results	(165)

∫ Non-fermenting bacteria excluded; * Stool specimens

** Peri-rectal swabs

mHT: Modified Hodge Test

α PCR done on directly from specimen. If not noted, PCR was performed on pure cultures derived from the sample.

Antibiotic abbreviations: MEM: Meropenem; IPM: Imipenem; ETP: Ertapenem; VAN: Vancomycin; CLI: Clindamycin; CAZ: ceftazidime; AMB; Amphotericin B

^ Includes both perianal and perirectal swabs

1559 **Table 7: Comparison of model estimates of diagnostic performance for screening methods on rectal/perirectal swabs for detection of KPC-producing *Enterobacteriaceae***

Method (number of studies)	Sensitivity % (95% CI)	Specificity % (95% CI)	DOR (95%CI)	Aggregate tested swabs (positive swabs)
CDC (3)	85.37 (58.05-96.09)	82.94 (41.85-97.05)	28.37 (0.67-1209.67)	452 (165)
CHROMagar KPC (4)	85.16 (61.39-95.4)	92.74 (70.56-98.55)	73.35 (1.96-2743.26)	506 (187)
chromID Carba (1)	95.98 (65.92-99.66)	90.76 (30.51-99.55)	234.36 (4.99-10996.66)	177 (86)
HardyChrom (1)	75.53 (22.42-97.06)	99.38 (72.54-99.99)	497 (4.9-50431.85)	126 (46)
SpectraCRE (1)	96.88 (65.46-99.8)	86.06 (22.15-99.26)	191.34 (3.45-10605.72)	150 (47)

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CI: Confidence Interval

DOR: Diagnostic Odds Ratio

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1571 **Table 8: Geometric mean limit of detection according to the culture method and β-lactamase class in pure cultures**

Method	Class A CFU/mL	Class B CFU/mL	Class D CFU/mL	Sp* per class A/B/D	Reference
SUPERCARBA	1.47×10^{1}	3.36×10^{1}	1.37×10^{1}	18/52/44	(149)
chromID ESBL	1×10^{1}	1.26×10^{1}	1.21×10^{3}	18/52/44	
chromID KPC	8.1×10^{1}	1.64×10^{3}	3.43×10^{6}	18/52/44	
SUPERCARBA	1.41×10^{1}	2.81×10^{1}	1.59×10^{1}	20/51/43	(143)
Brilliance CRE	1.12×10^{2}	2.86×10^{2}	1.45×10^{3}	20/51/43	
CHROMagar KPC	5.89×10^{1}	8.93×10^{2}	4.62×10^{6}	20/51/43	
MacConkey Agar with Imipenem	4.68×10^{2}	1.24×10^{3}	NT	8/2/0	(80)
MacConkey Agar with Meropenem/Ertapenem disks	2.62×10^{6}	3.32×10^{5}	NT	8/2/0	
CHROMagar KPC	2.02×10^{3}	1.24×10^{4}	NT	8/2/0	
CDC	6.87×10^{1}	8.66×10^{2}	5.2×10^{7}	5/2/1	(140)
chromID ESBL with prior enrichment on BHI + 10ug ertapenem	2.6×10^{1}	5.55×10^{1}	ND	5/2/1	
chromID ESBL	7.49×10^{1}	4.42×10^{2}	ND	5/2/1	
chromID Carba	2.11×10^{1}	4.42×10^{2}	5.5×10^{7}	5/2/1	
Brilliance CRE	2.67×10^{1}	3.41×10^{1}	3.77×10^{1}	12/14/5	(166)
chromID OXA-48	1×10^{7}	UD	3.36×10^{1}	10/10/57	(167)
chromID Carba	1×10^{1}	2.0×10^{1}	1.62×10^{4}	10/10/57	
SUPERCARBA	3.16×10^{1}	2.51×10^{2}	2.98×10^{1}	10/10/57	
CHROMagar KPC	ND	ND	1.26×10^{4}	0/0/9	(100)
CHROMagar ESBL	ND	ND	5.26×10^{3}	0/0/9	

1572 NT: Not tested

1573 ND: Not detected

1574 * Number of isolates

1575 **Table 9: PCR-based testing for CPO in clinical specimens**

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Target	Methodology	Specimen	Sensitivity	Specificity	Limit of detection (CFU/mL)	N	Reference
KPC	PCR	ES	92.2%	99.6%	Not Calculated	755	(153)
KPC	qPCR	ES	97%	96.6%	4×10^0-4×10^2FU/mL	95	(155)
KPC	qPCR	S, MC	100%	98%	5×10^0 CFU/mL	216	(190)
VIM, IMP, KPC, OXA-48, NDM-1	PCR (Hyplex SuperBug ID)	MC	98.0%	98.6%	Not calculated	236	(172)
OXA-48	qPCR	PC, SS	100%	100%	10^1-10^2 CFU/mL	35	(182)
KPC, NDM-1	qPCR	S, SS	100%	100%	KPC: 10^4-10^5 CFU/mL NDM: 10^3 CFU/mL	46/80*	(141)
KPC	qPCR(EasyQ KPC)	S	93.3%	99%	Not Calculated	806	(193)
VIM, OXA-48, NDM, KPC	qPCR(Check-MDR Carba)	SS	100%	100%	10^3-10^5 CFU/mL	25	(191)
KPC, NDM, VIM	qPCR(Xpert MDRO)	S, SS	100%	99%	$< 3 \times 10^2$ CFU/mL	328	(183)
KPC	qPCR	S	97.9-100%	95-96.4%	5×10^0 CFU/mL	187	(152)
NDM-1	qPCR	SS	100%	Not calculated	10^1-3×10^1 CFU/mL	32	(189)

* Specificity panel of 80 known negative specimens.

ES: Stool or peri-rectal swab with prior enrichment culture; S: Stool, stool swabs, or peri-rectal swabs; MC: mixed clinical specimens; SS: spiked stool or stool swabs; PC: pure cultures

1577 **Table 10: Factors to consider when implementing a screening program**

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- Epidemiology of CPO in the community
  - Prevalence rates of each carbapenemase
  - Ability to identify high-risk groups
- Availability and cost of isolation beds
- Existing logistics for collecting specimens. (e.g., an existing screening program for carriers of vancomycin-resistant *Enterococcus* spp.)
- Current clinical microbiology laboratory capabilities
  - Availability of molecular diagnostic tools
  - Available technologists and ability to accommodate testing
  - Experience developing and validating in-house assays
  - Experience/availability of other technology to detect carbapenemases
    - CarbaNP or Blue-Carba
    - Inhibition disk assays (double disk synergy test)
    - UV spectrometry or MALDI-TOF
    - Modified Hodge Test

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1596 **Table 11: Proposed criteria to screen patients for CPO**

Non-endemic areas	Endemic areas

• Patients with multiple hospital admissions
• ICU patients
• Patients who have received medical care in endemic areas over the last 12 months
• Patients who reside in healthcare settings
• Patients with prior history of CPO infections or colonization
• Patients with prior prolonged hospital stays
• Patients coming from endemic areas
• Patients who are , or who are expected to become incontinent or unable to take care of their personal hygiene
	• Everyone (as resources for testing, isolation, and cohorting allow)
	• Particular emphasis on
	  ○ Critically ill patients
	  ○ Patients unable to take care of their excreta
	  ○ Patients with an expected prolonged hospital stay

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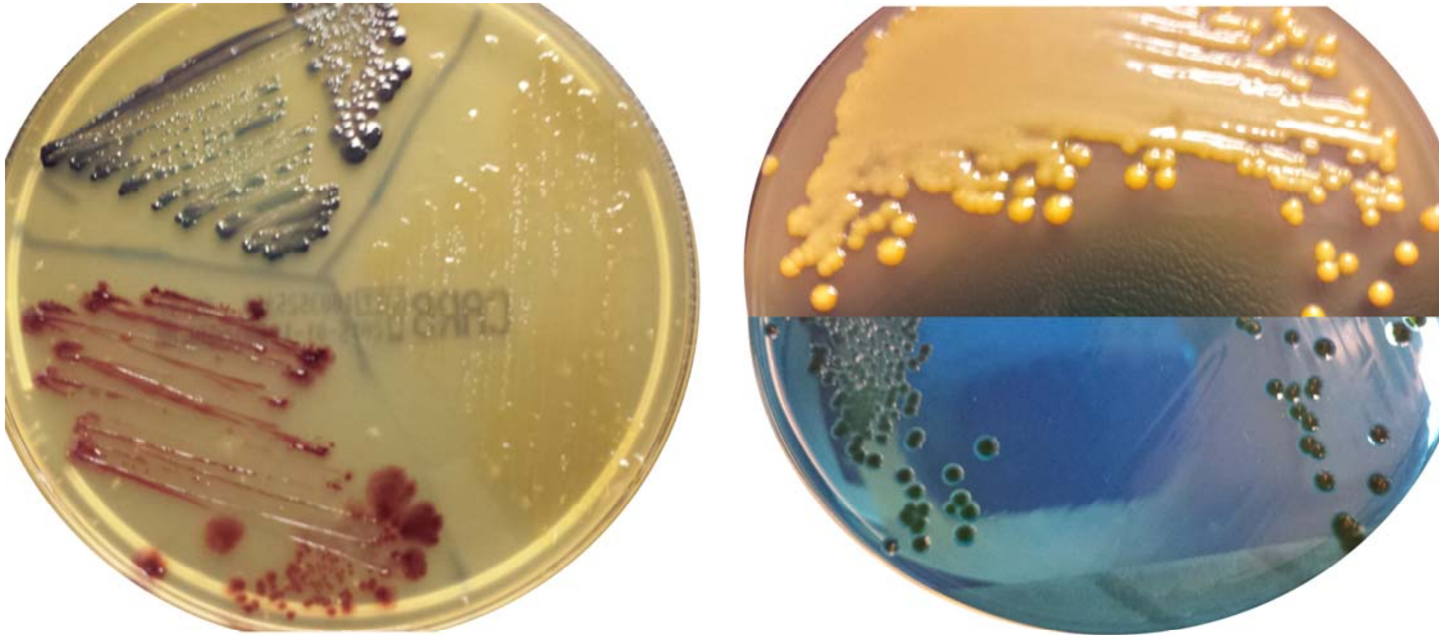
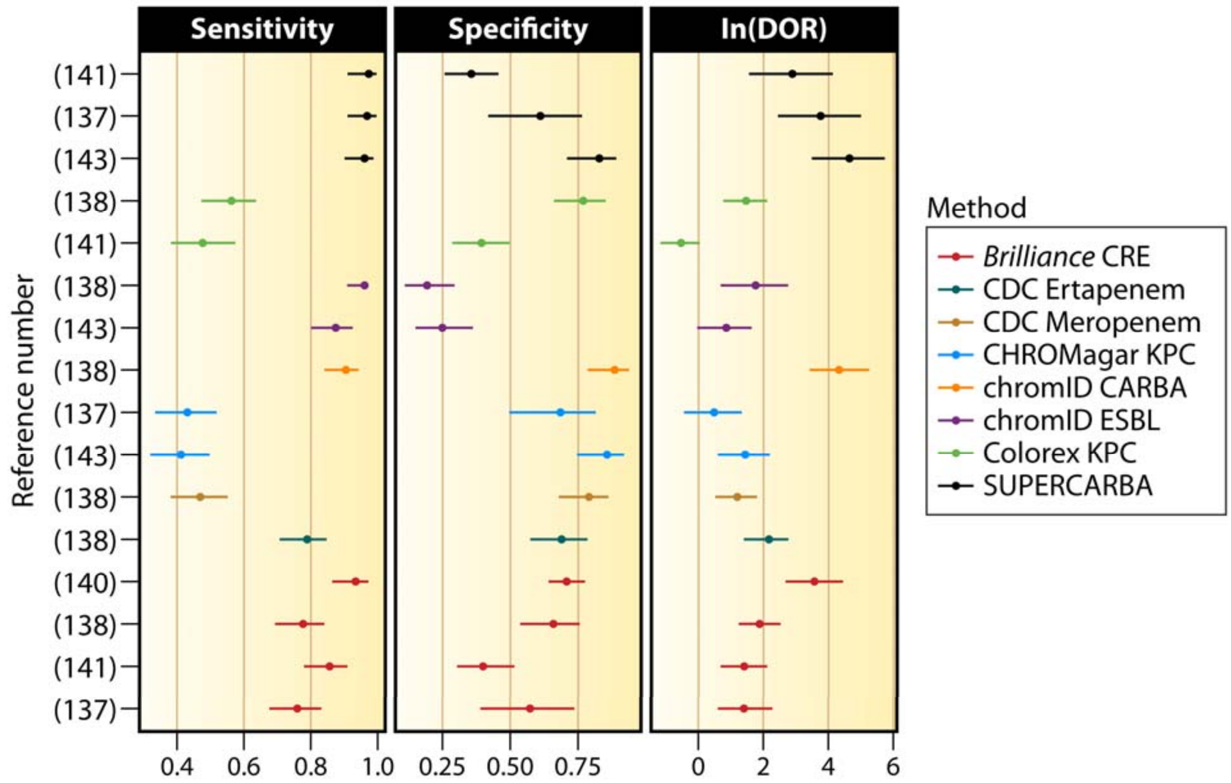


Figure 1: Appearances of different *Enterobacteriaceae* on chromID Carba and SUPERCARBA media. Left plate: chromID CARBA plate. Red colonies represent *K. pneumoniae;* blue colonies *E. coli;* and yellow colonies *Pseudomonas aeruginosa*. The right plate SUPERCARBA medium composite picture: the upper half *K. pneumoniae* (yellow colonies due to lactose fermentation)*.* Bottom half shows *Pseudomonas aeruginosa.* (black/dark green colonies with no lactose fermentation)



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1609 **Figure 2: Per observation estimates of sensitivity, specificity, and DOR for screening methods used on pure cultures included in statistical analysis.**

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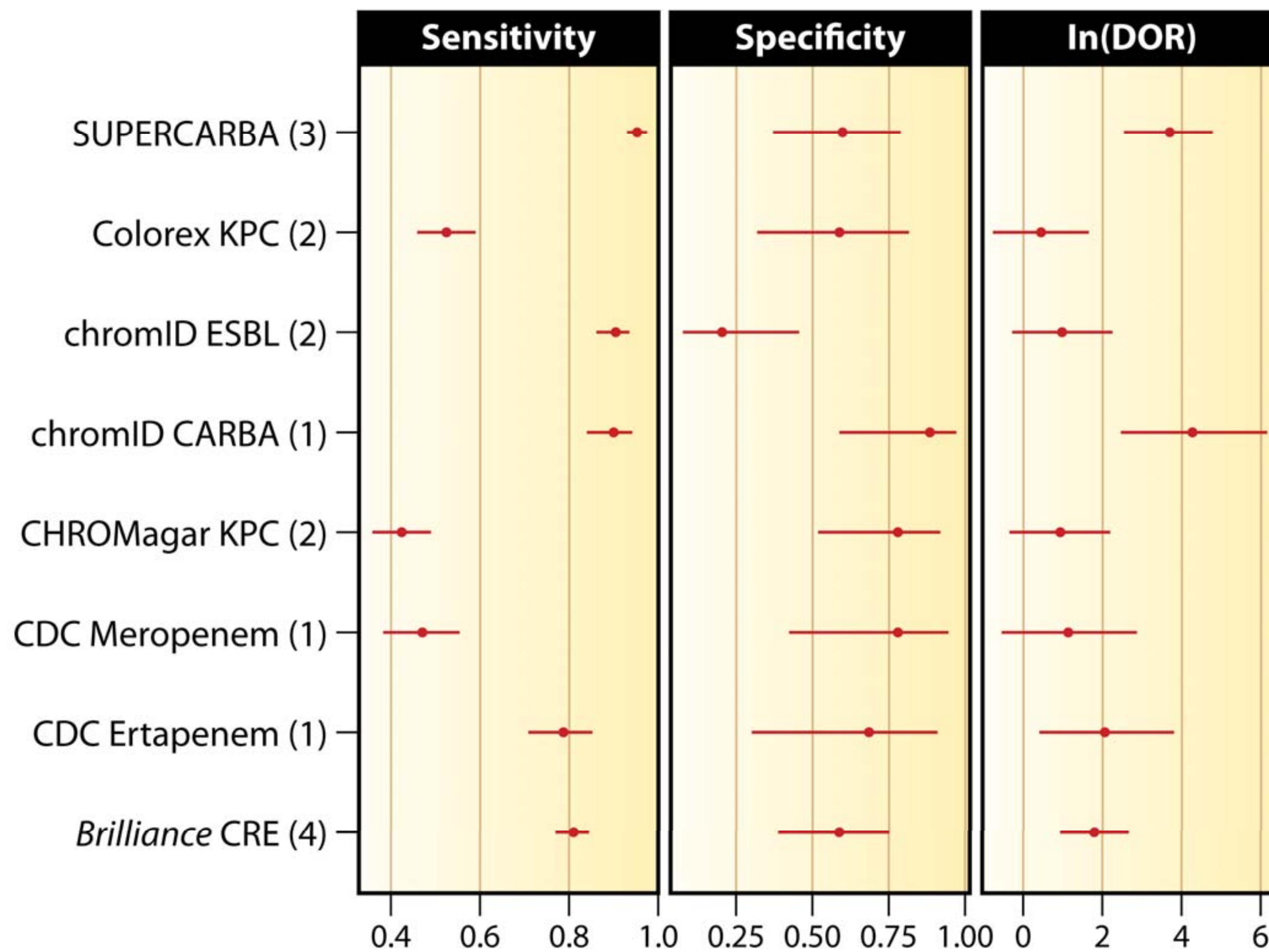

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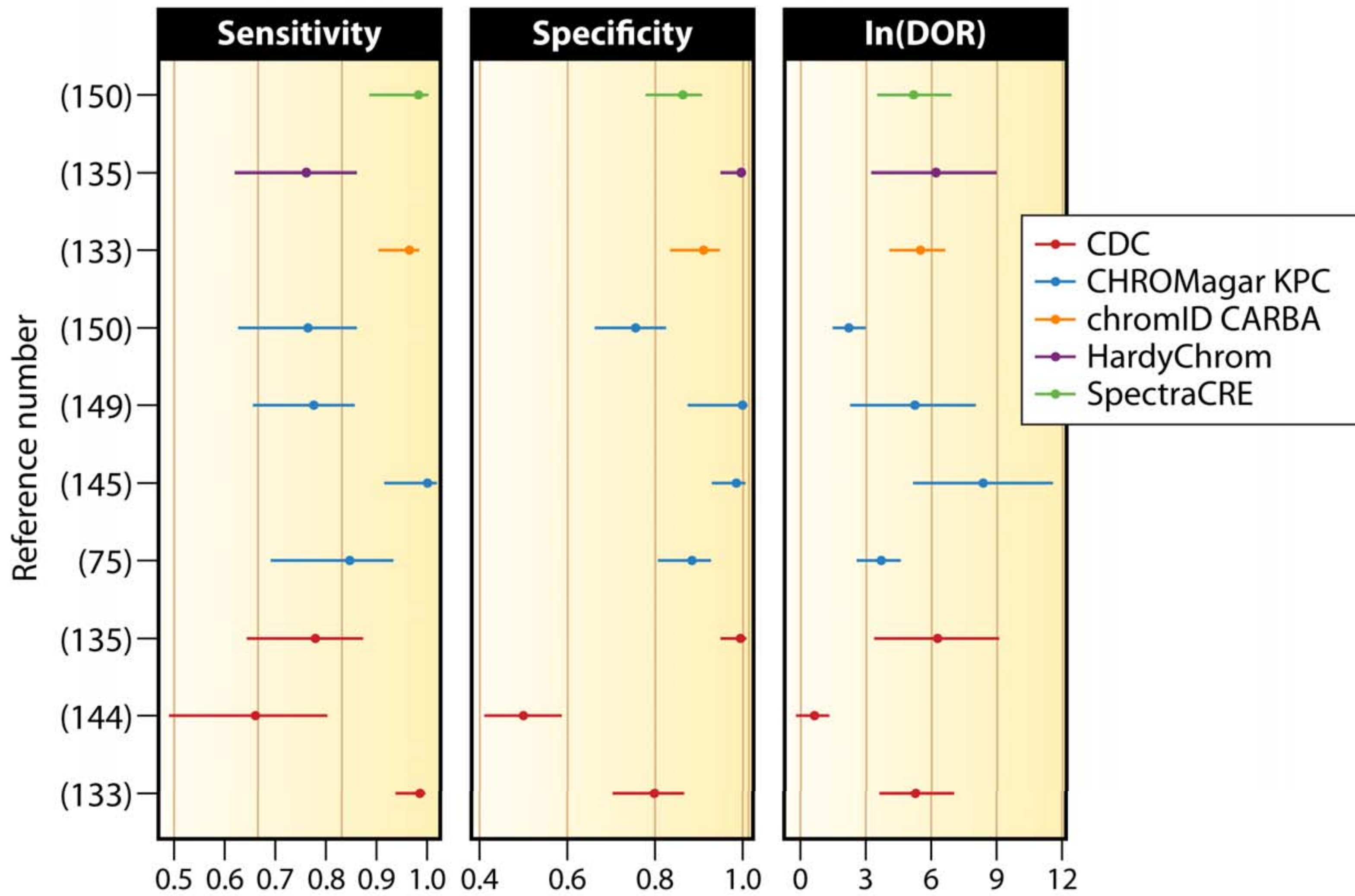
1612 **Figure 3: Aggregate estimates of sensitivity, specificity, and DOR for screening methods used on pure cultures.**



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1614 **Figure 4: Per observation estimates of sensitivity, specificity, and DOR for screening on rectal/perirectal swabs.**

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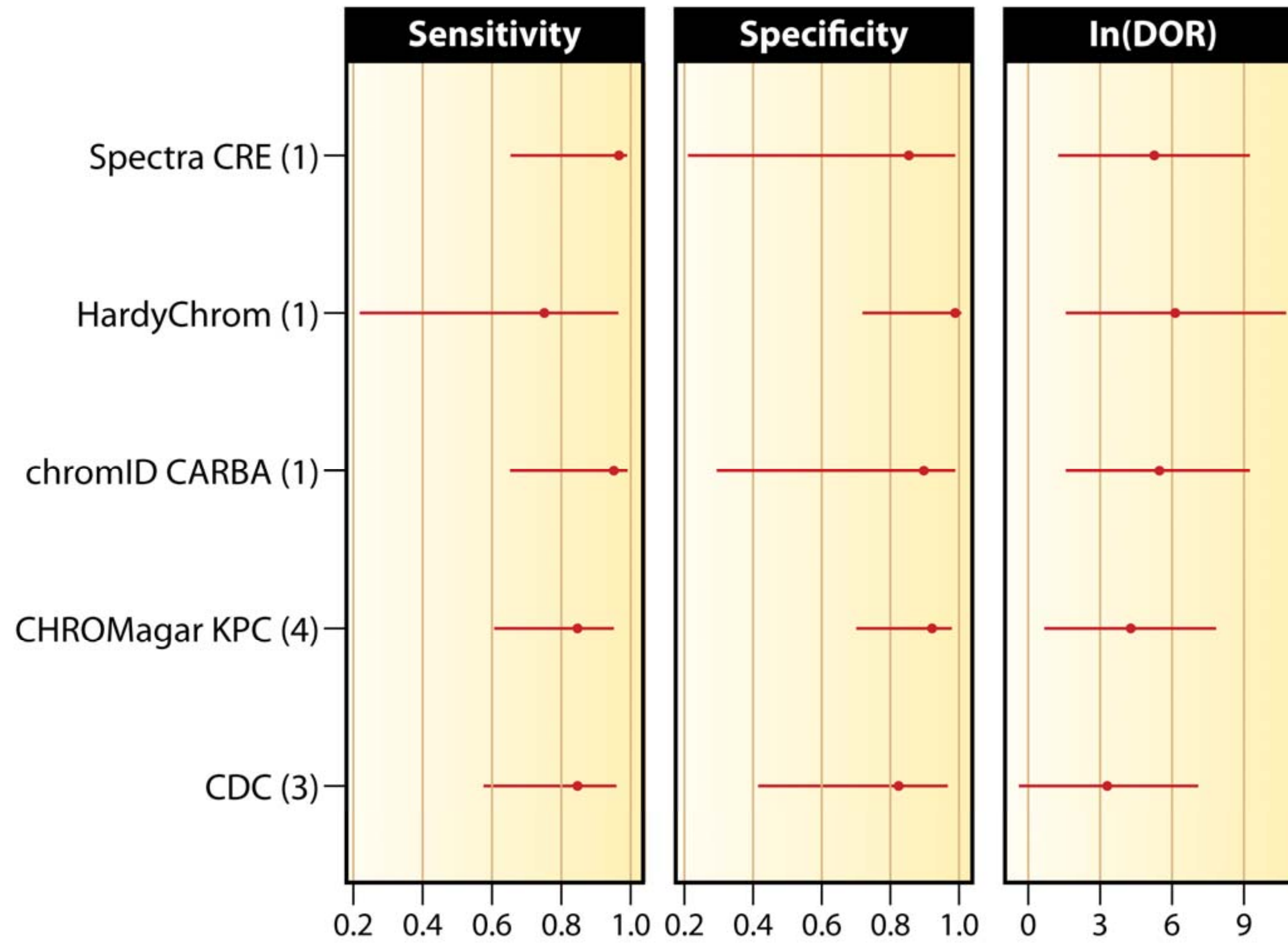

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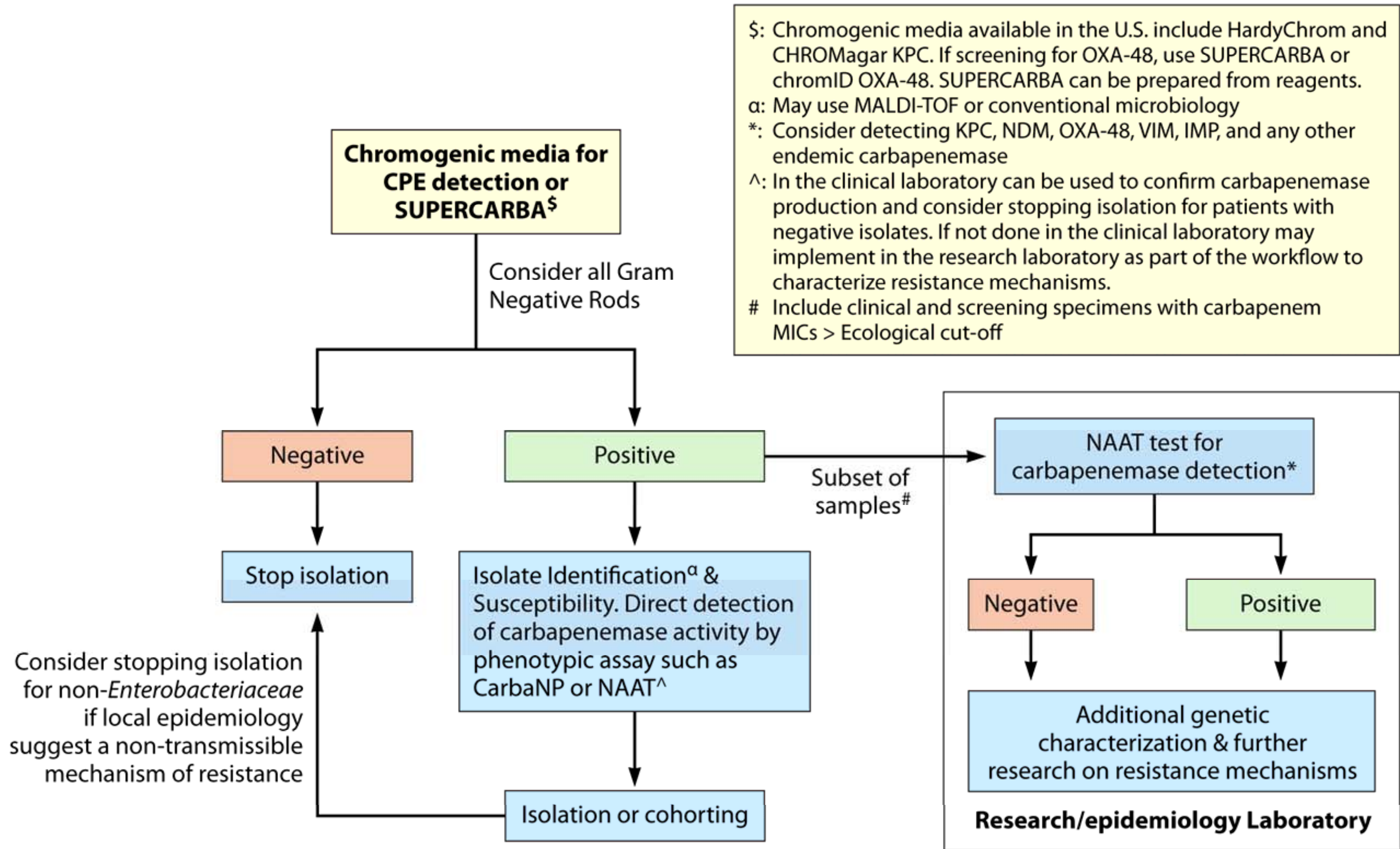
1617 **Figure 5: Aggregate estimates of sensitivity, specificity, and DOR for screening methods on rectal/perirectal swabs.**



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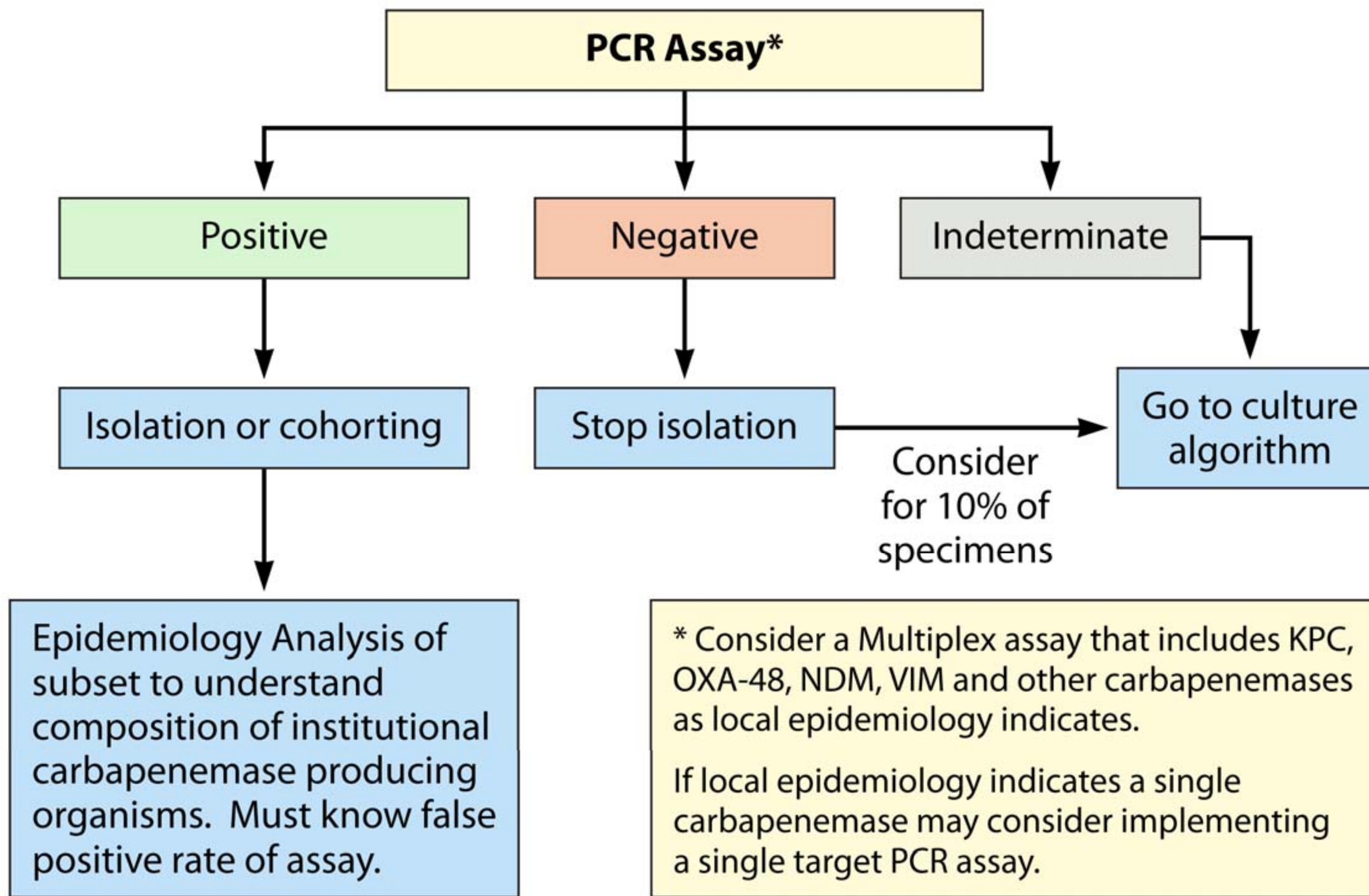
1620 **Figure 6: Screening with conventional microbiology**



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1623 **Figure 7: Molecular screening algorithm**

Author Biographies

Roberto Viau

Dr. Viau was born in Guatemala City, Guatemala. He obtained his medical degree from Universidad Francisco Marroquin where he graduated *cum laude* in 2008. He has been involved in gram negative resistance since then. Roberto completed an Internal Medicine Residency in Jacobi Medical Center in New York and is currently an Infectious Diseases Fellow at Case Western Reserve University. He is interested in molecular diagnosis and on the epidemiology of resistance mechanisms. He will be joining the faculty at Case Western Reserve University upon completion of his fellowship. He will continue working on gram negative resistance as well as on diagnostics microbiology.

Karen M. Frank

Dr. Frank is the Chief of Microbiology for the National Institutes of Health Clinical Center. She completed her M.D. and Ph.D. in Biochemistry at the University of Pennsylvania in 1994, studying magainins and discovering the antimicrobial molecule squalamine. She completed a Clinical Pathology Residency at the Brigham & Women's Hospital and conducted postdoctoral research on V(D)J recombination in a Howard Hughes Medical Institute laboratory in the Boston Children's Hospital. She was a Pathology faculty member at the University of Chicago for 12 years before relocating to NIH in 2012, investigating DNA repair pathways as well as Staphylococcal toxins. In collaboration with investigators in the National Human Genome Research Institute and the Clinical Center, she is using DNA sequencing to characterize carbapenem-resistant *Enterobacteriaceae*, tracking infections and the spread of the resistance genes on plasmids between bacteria, as well as conducting in vitro analyses of bacterial conjugation of several outbreak strains.

Michael R. Jacobs

Michael R. Jacobs, MD, PhD, received his medical degree and Medical Microbiology Doctorate from the University of the Witwatersrand in Johannesburg, South Africa, where he also did his Medical Microbiology Residency. He is a Diplomate of the American Board of Medical Microbiology, Member of the Royal College of Pathologists, and Professor of Pathology and Medicine at Case Western Reserve University and Director of Clinical Microbiology at University Hospitals Case Medical Center. Dr. Jacobs' research interests include epidemiology, antimicrobial susceptibility and mechanisms of resistance of antibiotic-resistant *Streptococcus pneumoniae* and carbapenem resistant *Enterobacteriaceae* and non-*Enterobacteriaceae*. He has served as an Examiner for the American Board of Medical Microbiology and as an Editorial Board member of Antimicrobial Agents and Chemotherapy, and is currently on the Editorial Board of Journal of Clinical Microbiology. Dr. Jacobs has presented numerous abstracts at national and international conferences as well as authored over 400 papers in peer-reviewed journals.

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38 **Brigid Wilson**

39 Brigid Wilson has a B.A. in Mathematics from Swarthmore College and a PhD in Statistics from UCLA. She
40 currently works at the Louis Stokes VA Medical Center in Cleveland under the Geriatrics Research,
41 Education, and Clinical Center and provides statistical support to VA-affiliated researchers. She has
42 previously provided statistical support to researchers while working for UCLA's Statistical Computing
43 Group and to pharmaceutical, bio-defense, and biomedical companies while working in private
44 biostatistics consulting.

45 **Keith Kaye**

46 Dr. Kaye is a Professor of Medicine in the Division of Infectious Diseases and Department of Medicine at
47 Wayne State University and Detroit Medical Center (DMC). He is Corporate Vice President of Quality
48 and Patient Safety, and the Corporate Medical Director of Hospital Epidemiology and Antimicrobial
49 Stewardship at DMC. Dr. Kaye's particular academic interests and skills are epidemiology of and
50 outcomes associated with multi-drug resistant bacteria; infections in the elderly; surgical site infection;
51 device-related infections and antimicrobial stewardship.

52 Dr. Kaye received his medical degree from the University of Pennsylvania and served his Internal
53 Medicine residency and was an Infectious Diseases fellow at Beth Israel Deaconess Medical Center in
54 Boston, MA. During fellowship, Dr. Kaye earned a Masters in Public Health from the Harvard School of
55 Public Health. Dr. Kaye is currently PI on a multi-center NIH-funded contract studying polymyxin-based
56 therapy for infections due to extremely-drug resistant (XDR)-Gram-negative bacilli.

57 **Curtis Donskey**

58 Dr. Donskey received his M.D. from the Medical College of Wisconsin in 1990. He completed an Internal
59 Medicine Residency and Chief Residency at Brown University and then completed an Infectious Diseases
60 Fellowship at University Hospitals of Cleveland. He is currently the Chairman of the Infection Control
61 Committee at the Cleveland Veterans' Affairs Medical Center and Associate Professor of Medicine at
62 Case Western Reserve University. His research focuses on infection control and the role of intestinal
63 colonization in the spread of resistant bacteria in hospital settings.

64 **Federico Perez**

65 Federico Perez is an Assistant Professor of Medicine at Case Western Reserve University School of
66 Medicine, and a member of the medical and research services at the Louis Stokes Cleveland Department
67 of Veterans Affairs Medical Center. He is currently a scholar at the Clinical and Translational Science
68 Collaborative of Cleveland. He first became interested in the molecular epidemiology and transmission
69 dynamics of multidrug resistant Gram negative bacteria in 2002, while working at the Centro
70 Internacional de Entrenamiento e Investigaciones Medicas in Cali, Colombia.

71

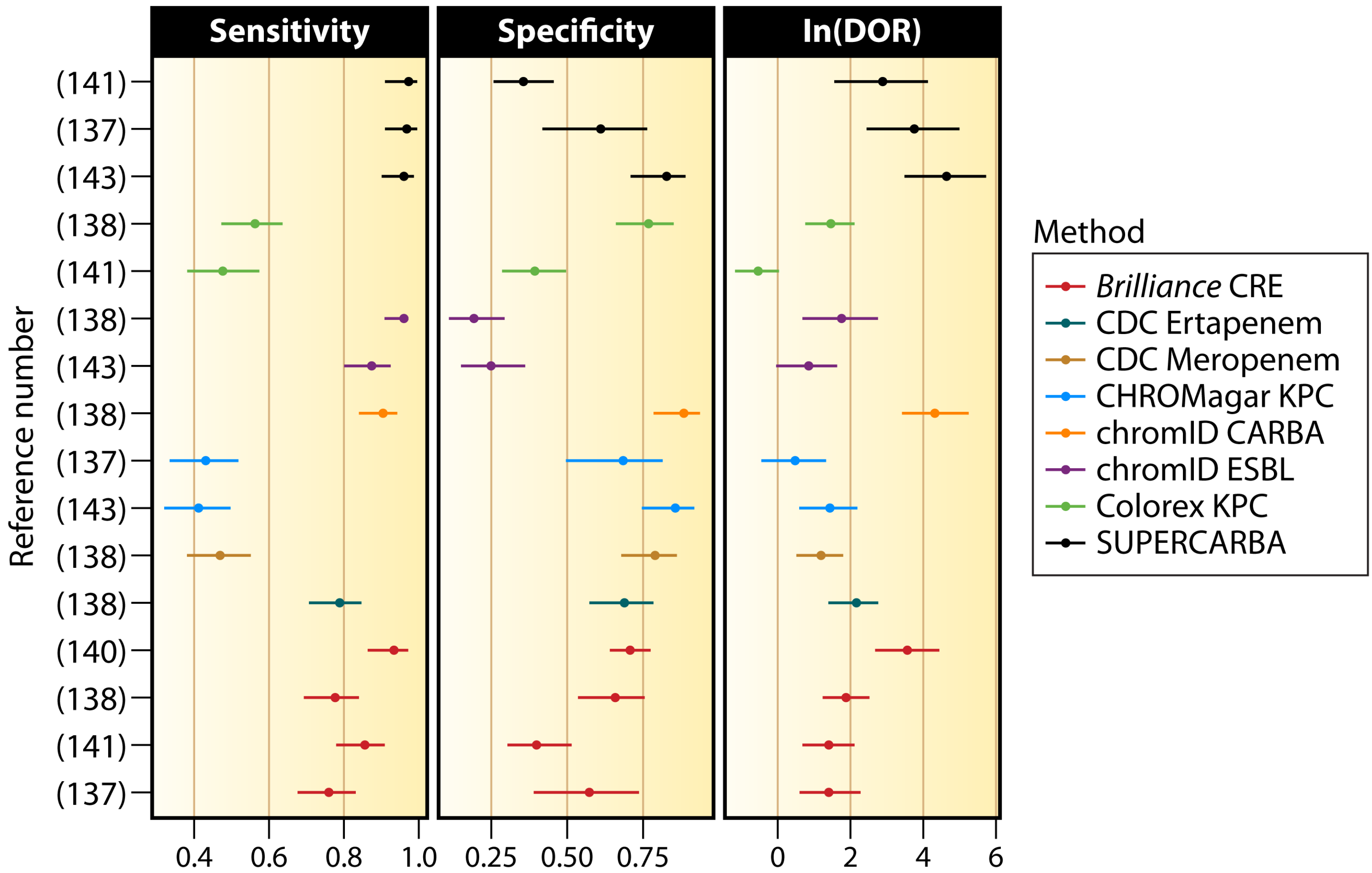
72 **Andrea Endimiani**

73 Dr. Andrea Endimiani got a Medical Degree (1999), the Board in Medical Microbiology and Virology
74 (2003), and a PhD in Immunopathology (2007) from the University of Insubria (Varese, Italy). Dr.
75 Endimiani has been trained in molecular microbiology at the Antibiotic Management Program of
76 University of Pittsburgh (2006-2007) and Case Western Reserve University School of Medicine in
77 Cleveland (2007-2010). Currently, he is a Researcher and Medical Microbiologist at the Institute for
78 Infectious Diseases of University of Bern (Switzerland). Endimiani's group focuses on the genetic
79 background of antibiotic resistant Gram-negative pathogens (particularly those producing ESBLs and/or
80 carbapenemases); they are also very interested in the development of new strategies (e.g., microarrays,
81 multiplex real-time PCRs) to speed up the diagnosis of infections due to multidrug-resistant bacteria and
82 sexually transmitted diseases (mainly those due to *Neisseria gonorrhoeae*). Dr Endimiani has been
83 recently (2014) qualified as full Professor of Microbiology and Medical Microbiology.

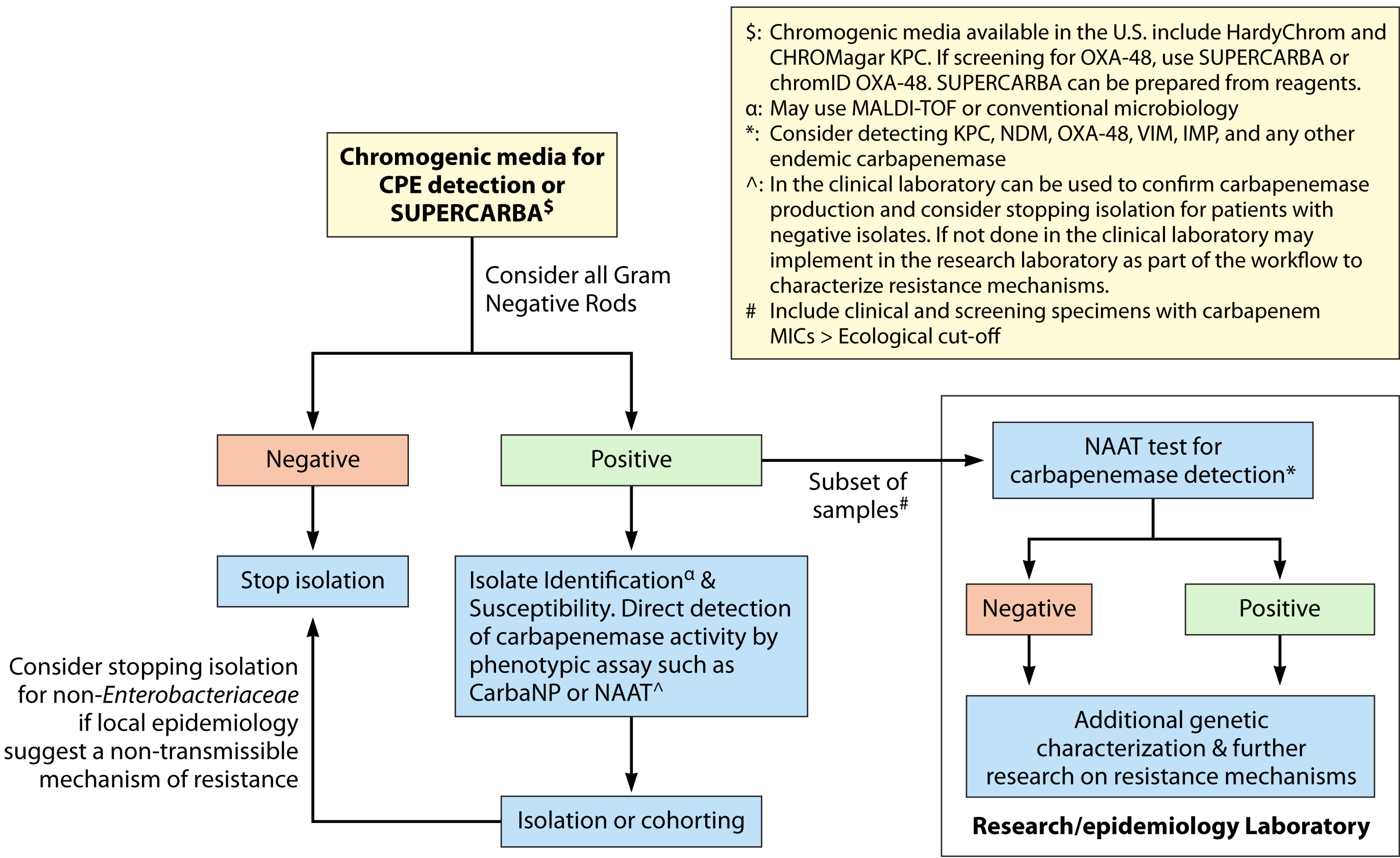
84

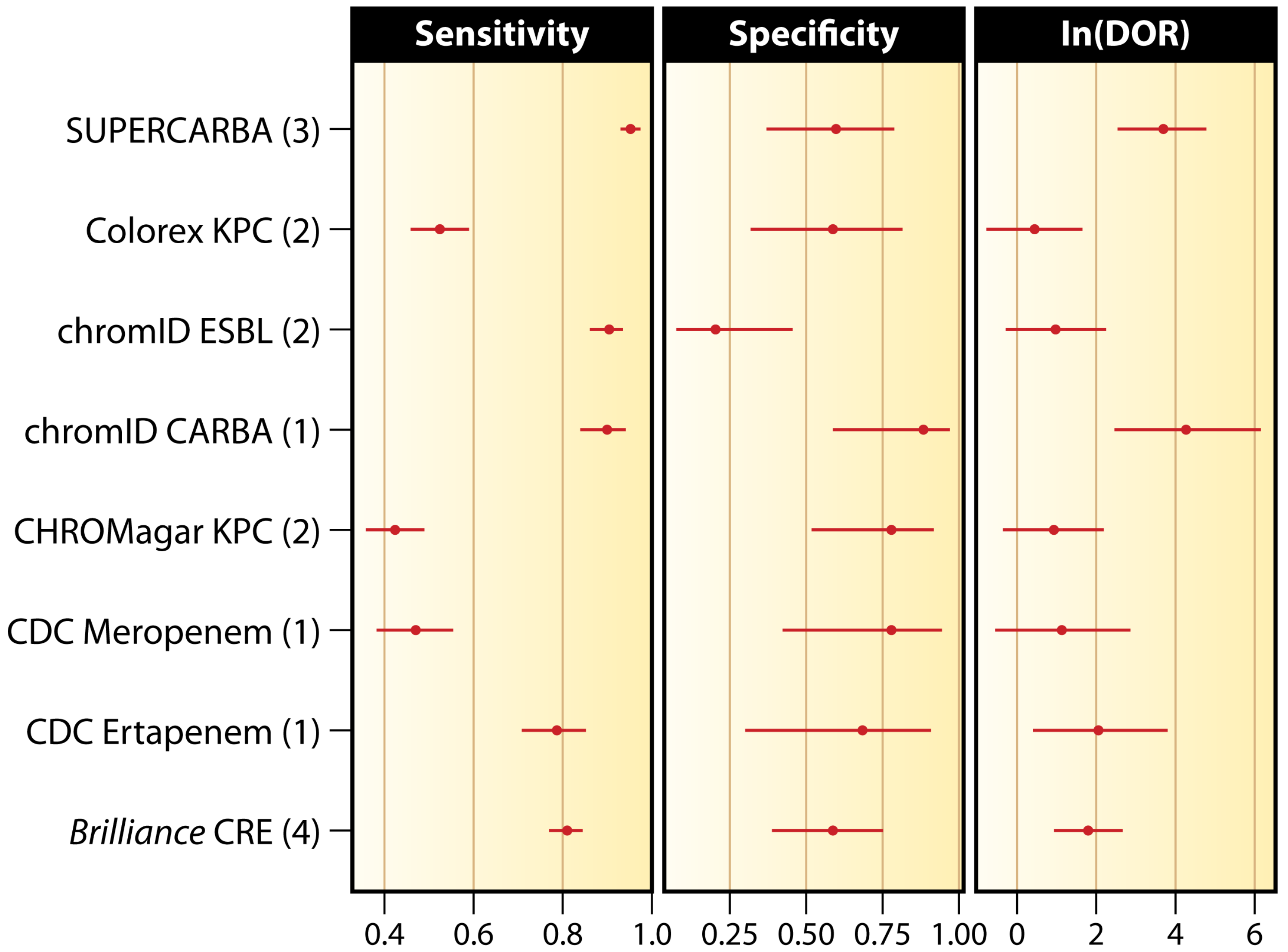
85 **Robert A. Bonomo**

86 Robert A. Bonomo received his M.D. from the Case Western Reserve University School of Medicine. He
87 trained in Internal Medicine and Infectious Diseases at University Hospitals of Cleveland and received a
88 Certificate of Added Qualification in Geriatrics. Dr. Bonomo served as section chief of the infectious
89 disease division at the Cleveland Veterans Affairs Medical Center before becoming Director of the
90 Veterans Integrated Service Network 10 Geriatric Research, Education, and Clinical Center (GRECC). He
91 also serves as Chief of Medicine. He is a Professor of Medicine at Case Western Reserve University
92 School of Medicine and also holds appointments in the Departments of Pharmacology and Molecular
93 Biology and Microbiology. Dr. Bonomo's research focuses on microbial resistance to antibiotics,
94 especially β-lactams.



Sensitivity
Specificity
ln(DOR)
Reference number
(141)
(137)
(143)
(138)
(141)
(138)
(143)
(138)
(137)
(143)
(138)
(138)
(140)
(138)
(141)
(137)
0.4
0.6
0.8
1.0
0.25
0.50
0.75
0
2
4
6
Method
Brilliance CRE
CDC Ertapenem
CDC Meropenem
CHROMagar KPC
chromID CARBA
chromID ESBL
Colorex KPC
SUPERCARBA


Chromogenic media for CPE detection or SUPERCARBA$
Consider all Gram Negative Rods
Negative
Positive
Stop isolation
Isolate Identificationα & Susceptibility. Direct detection of carbapenemase activity by phenotypic assay such as CarbaNP or NAAT^
Consider stopping isolation for non-Enterobacteriaceae if local epidemiology suggest a non-transmissible mechanism of resistance
Isolation or cohorting
Subset of samples#
NAAT test for carbapenemase detection*
Negative
Positive
Additional genetic characterization & further research on resistance mechanisms
Research/epidemiology Laboratory
$: Chromogenic media available in the U.S. include HardyChrom and CHROMagar KPC. If screening for OXA-48, use SUPERCARBA or chromID OXA-48. SUPERCARBA can be prepared from reagents.
α: May use MALDI-TOF or conventional microbiology
*: Consider detecting KPC, NDM, OXA-48, VIM, IMP, and any other endemic carbapenemase
^: In the clinical laboratory can be used to confirm carbapenemase production and consider stopping isolation for patients with negative isolates. If not done in the clinical laboratory may implement in the research laboratory as part of the workflow to characterize resistance mechanisms.
Include clinical and screening specimens with carbapenem MICs > Ecological cut-off



Sensitivity
Specificity
ln(DOR)
SUPERCARBA (3)
Colorex KPC (2)
chromID ESBL (2)
chromID CARBA (1)
CHROMagar KPC (2)
CDC Meropenem (1)
CDC Ertapenem (1)
Brilliance CRE (4)
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0.6
0.8
1.0
0.25
0.50
0.75
1.00
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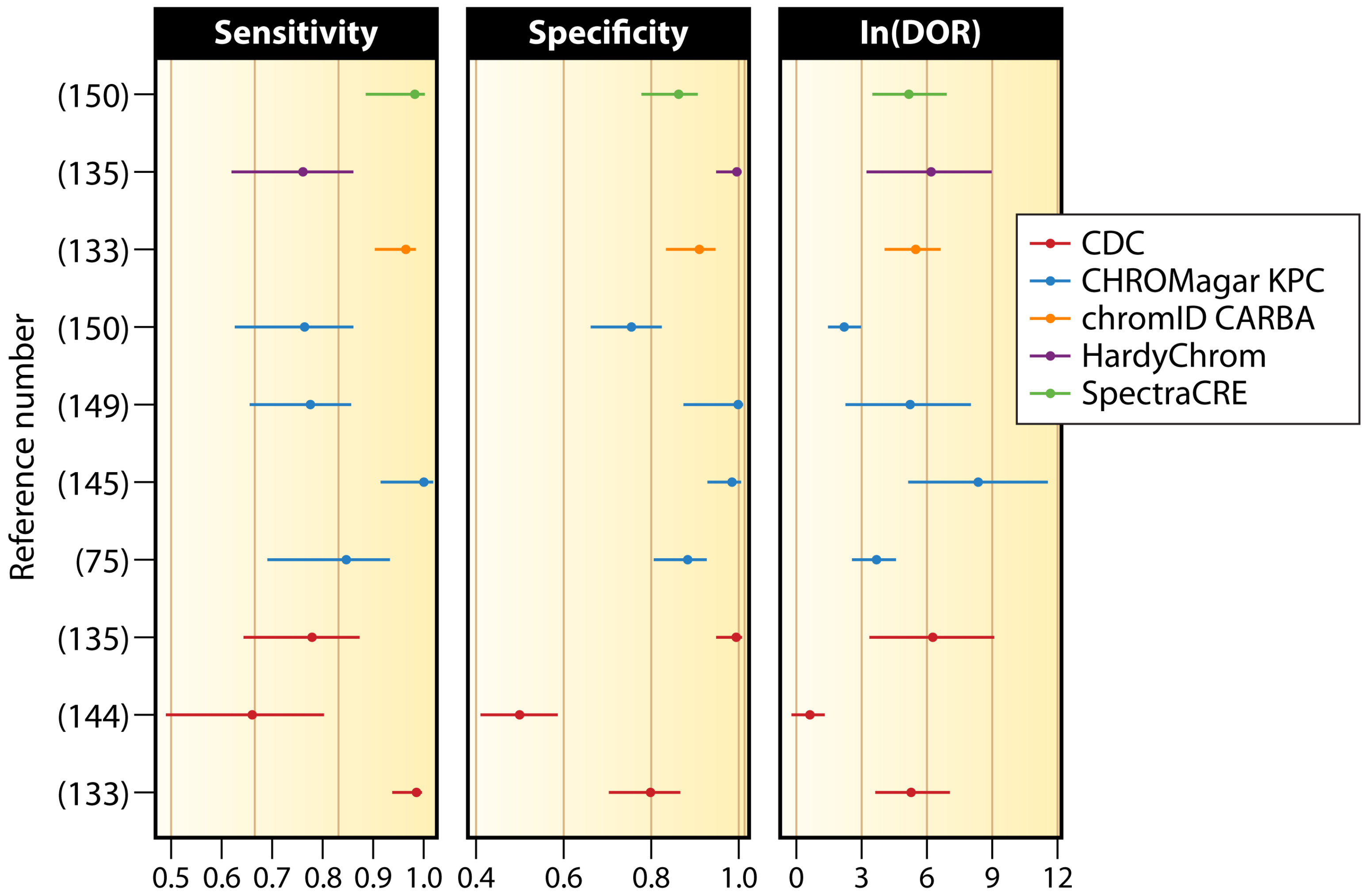

Sensitivity
Specificity
ln(DOR)
Reference number
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(133)
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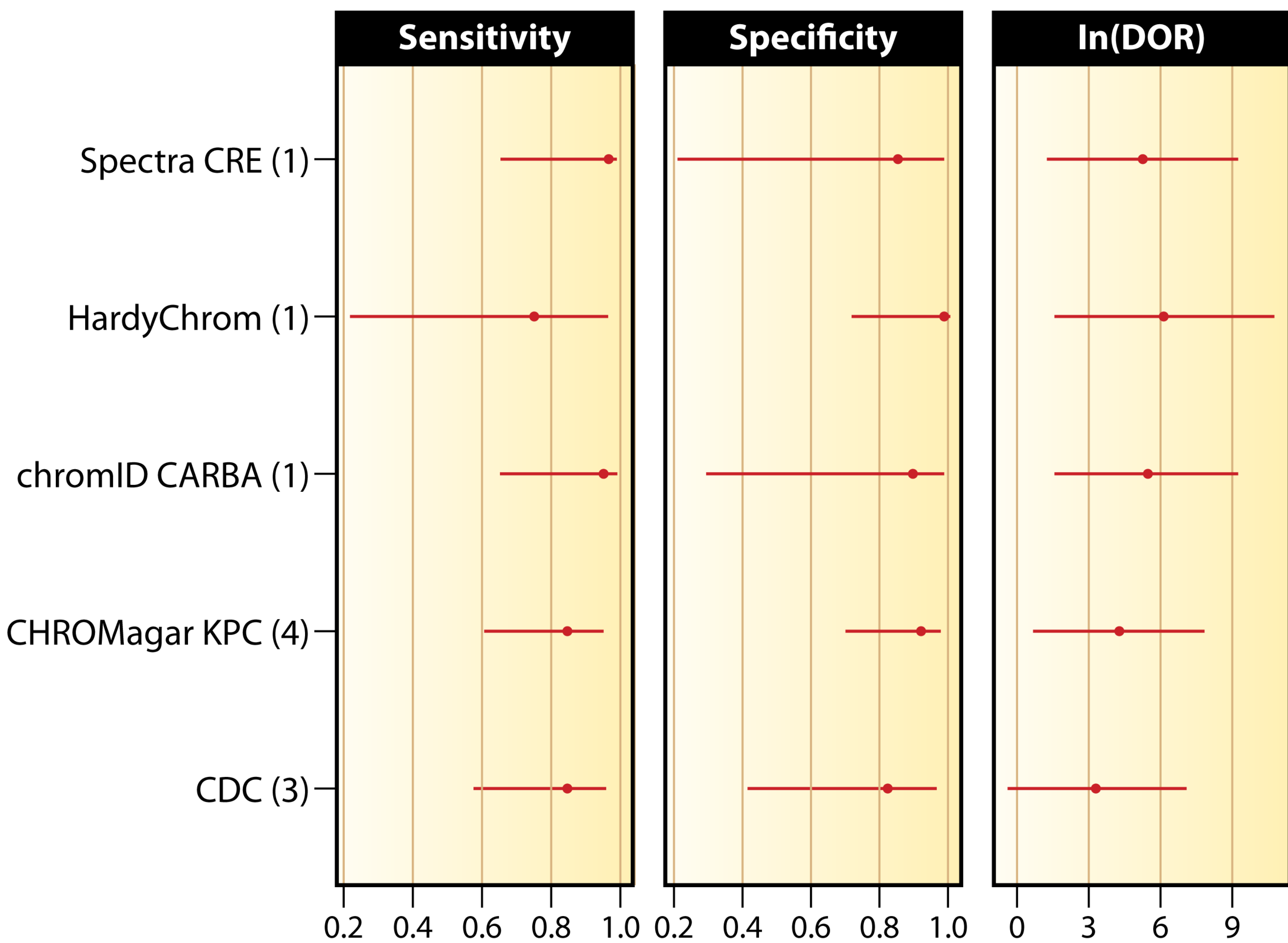
CDC
CHROMagar KPC
chromID CARBA
HardyChrom
SpectraCRE



Sensitivity
Specificity
ln(DOR)
Spectra CRE (1)
HardyChrom (1)
chromID CARBA (1)
CHROMagar KPC (4)
CDC (3)
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0.2
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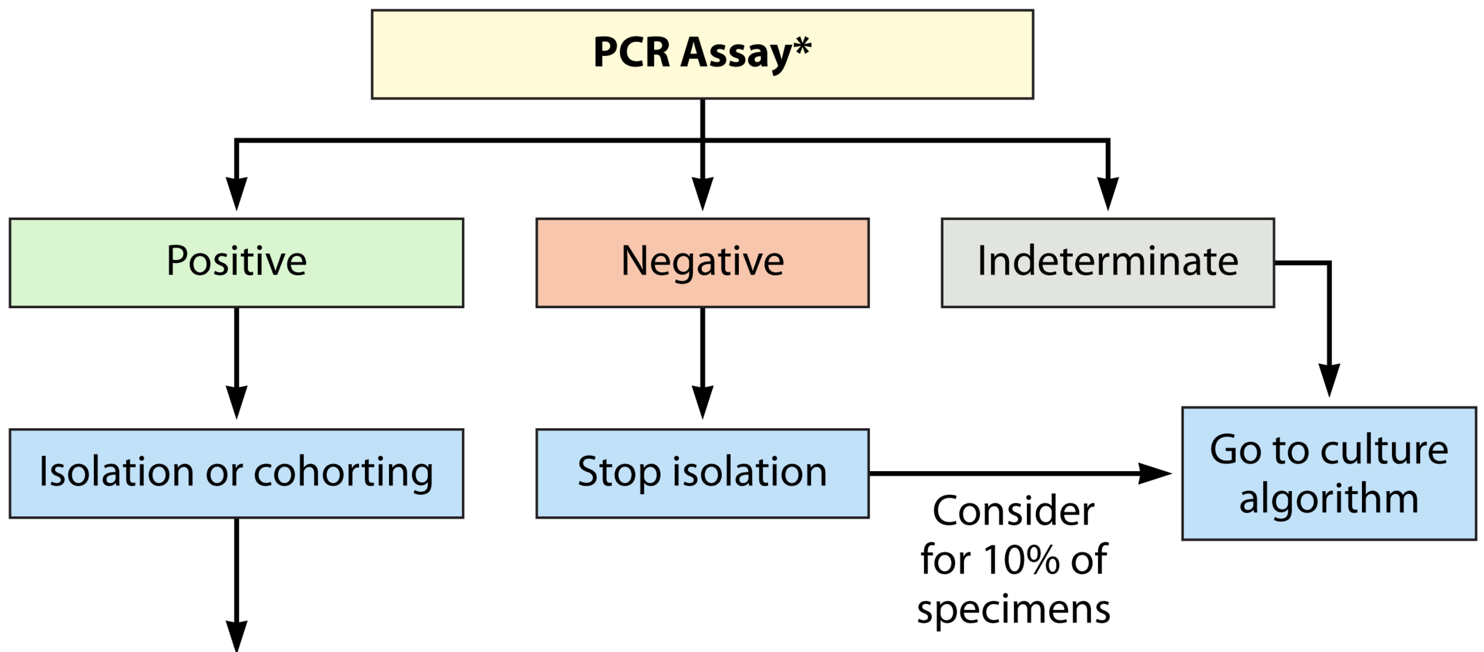

PCR Assay*
Positive
Negative
Indeterminate
Isolation or cohorting
Stop isolation
Go to culture algorithm
Consider for 10% of specimens
Epidemiology Analysis of subset to understand composition of institutional carbapenemase producing organisms. Must know false positive rate of assay.
* Consider a Multiplex assay that includes KPC, OXA-48, NDM, VIM and other carbapenemases as local epidemiology indicates.
If local epidemiology indicates a single carbapenemase may consider implementing a single target PCR assay.