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1 **Evaluation of PCR assays for direct screening of Carbapenemase-Producing**
2 ***Enterobacteriaceae* (CPE) from rectal swabs: a diagnostic meta-analysis:**
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19

20 **Running title:** PCR assays for direct screening of CPE

21 **Summary**

22

23

24 **Background.** Recently, molecular assays have been demonstrated to be reliable for rapid
25 detection of CPE directly from positive blood cultures, reducing significantly the time for
26 identification. Few studies have tested their performance on rectal swabs and no
27 comprehensive conclusion have been reached regarding their utility for infection control
28 management.

29 **Aim.** To review and assess the overall diagnostic test accuracy of PCR for the detection of
30 CPE in rectal swabs.

31 **Methods.** We searched the electronic database PubMed, up to October 1, 2019, without
32 language restriction or publication date restrictions. We began by defining the concepts of the
33 research questions: “Carbapenemase-producing *Enterobacteriaceae*”, “molecular testing”,
34 “test detection” and “rectal screening”. Two reviewers independently screened studies,
35 extracted data, and assessed quality using the QUADAS-2 instrument. Statistical analyses
36 were carried out in STATA using the bivariate model.

37 **Findings.** One hundred forty-three articles were screened and 16 studies were included. Five
38 (31%) of the studies were conducted in a context of a CPE outbreak, 1 study (6%) included
39 patients pre-identified with CPE in clinical samples (blood or tracheal secretions), while the
40 rest (63%) collected rectal swabs from patients considered at high-risk of colonization. The
41 molecular assays evaluated have a relatively good sensitivity of 0.95 (IC: 0.902-0.989), and
42 an excellent specificity of 0.994 (IC: 0.965-1).

43 **Conclusions.** Molecular techniques seem to be a useful accurate diagnostic tool in screening
44 for carriage of CPE in contact patients around a fortuitous discovery of a non-isolated
45 hospitalized carrier patient.

46

47 **Keywords.** Real-time PCR, Carbapenemase-Producing-*Enterobacteriaceae*, infection control,
48 outbreak, rectal screening, diagnostic meta-analysis.

49 **Text:**

50

51

52 **Introduction:**

53 Antibiotic resistance is a worldwide problem and multidrug-resistant organisms are
54 recognized global threats. In the past decades, new resistance mechanisms have aroused and
55 spread globally, increasing the cost of care and the occurrence of undesirable outcomes. New
56 forms of antibiotic resistance can cross international boundaries and spread between
57 continents with ease.¹⁻⁵ Many forms of resistance spread with remarkable speed, facilitated
58 by human mobility and the high prevalence of asymptomatic carriers.^{4,6,7} Carbapenem-
59 resistant *Enterobacteriaceae* (CRE), as a result of various mechanisms, the most common
60 being carbapenemase enzymes (carbapenemase producing *Enterobacteriaceae* or CPE), is
61 among the most frightening form of resistance, becoming a major health problem worldwide.
62 ⁸ CPE transmission may occur in both healthcare and community settings, although,
63 hospitalized patients may be particularly susceptible to infections. The major concern about
64 CPE, is their high potential to cause outbreaks in healthcare settings.⁸

65 It has become clear that in order to prevent their spread, detection of infected patients and
66 carriers with CPE is mandatory. Containment of this spread, currently recommended in North
67 American and European infection control guidelines, is based on the search and isolate
68 policy, considered the main infection prevention and control strategy to minimize
69 transmission of CPE.^{9,10} This policy aims to promote awareness of CPE and enable early
70 identification of intestinal CPE colonization, screening and isolation of high risk patients.¹¹

71 Therefore, its application requires a rapid and accurate microbiological technique for the
72 identification of colonization with CPE.

73 The current gold standard for intestinal CPE carriage detection is the agar-based culture on
74 selective media, with or without selective enrichment in broths containing carbapems to
75 increase the sensitivity, followed by confirmatory phenotypic and/or molecular tests
76 performed on suspected resistant bacterial isolates.¹² Phenotypic tests for carbapenemase
77 activity include: (i) The modified Hodge test, based upon *in vivo* production of

78 carbapenemase. This is a time-consuming test that may lack specificity (high- level AmpC
79 producers) and sensitivity (weak detection of NDM producers); (ii) Inhibitor based tests
80 (combined disk test, double disk synergy test, E-test) based upon *in vitro* observation of an
81 increase in inhibition zone diameter (or reduction of the MIC) of the tested isolate in the
82 presence of a carbapenem combined with a carbapenemase inhibitor compared to the same
83 carbapenem alone. These tests are also considered time-consuming; they are difficult to read
84 if many types of carbapenemases are combined and may lack of specificity; (iii) biochemical
85 detection methods, based upon hydrolysis of the β -lactam ring of imipenem by the tested
86 strain, followed by color change of a pH indicator. The specificity and sensitivity of the tests
87 are reported 100% in a survey of *Enterobacteriaceae*.^{10,13} The tests are cheap, easy to use
88 and requires less than 2h. Not only do the tests detect all known carbapenemases but they
89 also identify virtually any newly emerging carbapenemase.^{9,14} Moreover, biochemical
90 methods may be able to discriminate between the different types of carbapenemases (classes
91 A, B, and D) in *Enterobacteriaceae* and *Pseudomonas* spp. Carba NP test II, for example,
92 combines excellent sensitivity (100%), specificity (100%), cost-effectiveness, and rapidity
93 with the discrimination properties of tazobactam and EDTA as inhibitors of Ambler class A
94 and class B β -lactamases, respectively.¹⁵ (iv) Lateral flow immunoassays (LFIAs), which
95 detect carbapenemase enzymes through the use of specific antibodies, are antibody-based
96 methods to identify the presence of carbapenemases. A number of LFIAs have been recently
97 developed but generally enable the detection of one or a few of the most epidemiologically
98 important carbapenemases. Available data suggest that LFIAs are easy to perform and
99 produce accurate results from cultured isolates within 15 min. Recently, an LFIA targeting
100 the five main carbapenemase families was evaluated. The reported specificity of these tests is
101 100% and the specificity $\geq 95\%$.¹⁶ Few studies have evaluated the performance of LFIAs
102 directly from clinical specimens. When used on rectal swabs samples LFIAs appeared highly
103 specific but with reduced sensitivity compared with standard procedure encompassing a
104 culture step.¹⁷

105 Nevertheless, confirmatory tests for carbapenemases production on suspected bacterial
106 isolates is based on the use of molecular techniques. Different molecular assays have been
107 developed and have shown their effectiveness in identifying the carbapenem resistance genes
108 form bacterial colonies, within 2–6 hours with excellent sensitivity (71-100% depending on
109 the carbapenemase gene type) and specificity (100%).^{13,18}

110 The main concern about this conventional method of carriage screening is that it requires up
111 to 72h for obtaining a conclusive result.¹² Also, culture-based methods are limited by
112 sensitivity and specificity issues depending on the composition of the medium and the
113 targeted carbapenemases (e.g., KPC, VIM, NDM, IMP, or OXA-48 enzymes).^{9,10,19,20}

114 Recently, molecular assays as real-time PCR were demonstrated to be a reliable technique for
115 rapid detection of CPE producers directly from positive blood cultures, reducing significantly
116 the time for identification.^{21,22} Since identification of CPE carriers has also an important
117 clinical implication, which is the implementation of infection control measures, the rapid
118 cohorting of carriers is crucial. This justifies the need to use a rapid and accurate technique
119 for detection of CPE directly in rectal swabs. Several studies have evaluated the performance
120 of molecular assays and shown their effectiveness in identifying the resistance genes from
121 bacterial colonies, having lower limits of detection than conventional agar-based culture
122 methodologies.^{12,23–25} However, few studies tested their performance when used directly on
123 rectal swabs, and most importantly, no comprehensive conclusion have been reached
124 regarding their utility for infection control management.

125 As there is an urgent need to identify a sensitive and specific test for detection of CPE
126 carriers, as soon as possible after their hospitalization, the aim of the present study was to
127 review and assess the overall diagnostic test accuracy of PCR for the detection of CPE in
128 rectal swabs.

129 **Materials/methods:**

130 Search strategy and selection criteria

131 This meta-analysis was performed in accordance with the Preferred Reporting Items for
132 Systematic reviews and Meta-Analyses (PRISMA) guidelines.²⁶ Studies were systematically
133 searched from PubMed, up to October 1, 2019, without language restriction or publication
134 date restrictions. We began by defining the concepts of the research questions:
135 “Carbapenemase-producing *Enterobacteriaceae*”, “molecular testing”, “test detection” and
136 “rectal screening”. Then, for each concept, we selected both indexing terms and keywords
137 and we compiled the terms relating to the same concept by using OR as Boolean operator, in
138 order to make searches larger. To narrow our research, we combined the four sets of concepts
139 using AND logic.

140 A step-by-step search was carried out, including the following terms: Carbapenemase-
141 producing *Enterobacteriaceae* AND (molecular testing or test detection or screening
142 or polymerase chain reaction). At each step, all titles were evaluated by two of the authors
143 (Saliba R. and Zahar JR.) and abstracts, were extracted for review. Abstracts containing the
144 following keywords: “test detection” or “screening” or “molecular testing” or “multiplex
145 polymerase chain reaction”, were selected and articles read in details.
146 In addition, references of all articles in these eligible studies were also read to identify
147 additional relevant literature. Also, to be included, articles had to meet the following criteria:
148 (1) studies appraising PCR assays for the detection of CPE; (2) studies performing PCR
149 assays directly on rectal swabs; (3) sufficient information provided or can be used to estimate
150 true positive-TP, false positive-FP, false negative-FN and true negative-TN. Exclusion
151 criteria were: (1) duplications or overlapping studies; (2) studies without adequate data to
152 construct the 2x2 table.

153

154 Data extraction and quality assessment

155 Data extraction from eligible studies was performed independently by 3 investigators, and
156 disagreements were resolved by deliberation. From each study, the following study
157 characteristics were extracted: (1) basic information of articles (first author, year of
158 publication, country of publication); (2) research object's general features (sample size,
159 molecular technique); (3) data used for the final meta-analysis (detection method, TP, FP,
160 FN, and TN).

161 The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) instrument,²⁷ was
162 used to assess the methodological quality of individual studies. QUADAS-2 tool consisted of
163 four domains: patient selection, index test, reference standard, and flow and timing. Each of
164 the assessment has seven questions, which should be answered with yes, no, or unclear. An
165 answer of yes gets 1 score, while others get 0, the highest score is seven.²⁷

166 Statistical analysis

167 All the analyses of diagnostic accuracy were performed using STATA 15.0 software²⁸ with
168 function metandi²⁹ or R 3.5³⁰ with package meta4diag.³¹ The bivariate meta-analysis model
169³² was used to estimate the pooled results of sensitivity, specificity, positive and negative
170 likelihood ratios (PLR and NLR), diagnostic odds ratio (DOR) with their corresponding 95%
171 confidence intervals (CIs).

172 Bivariate random-effects regression model assumes that the logit transformed sensitivities
173 and specificities of the included studies follow a bivariate normal distribution around a
174 common mean of logit-transformed sensitivity and specificity, incorporating any correlation
175 that might exist between logit sensitivity and logit specificity.³² The number of patients
176 testing positive among the diseased patients in a particular study is assumed to follow a
177 binomial distribution, as is the number of patients testing negative among the non-diseased
178 patients.

179 The summary receiver operator characteristic (SROC) curve and the area under the SROC
180 curve (AUC) were generated to evaluate the accuracy of CPE carriage diagnosis. An AUC of
181 1.0 indicates perfect diagnostic accuracy whereas an AUC of 0.5 indicates poor diagnostic
182 accuracy.³³ The statistical heterogeneity from non-threshold was assessed by the Q value and
183 I² statistic. A probability value of $P < 0.05$ and $I^2 > 50\%$ indicated the existence of
184 significant heterogeneity among individual studies.³⁴ Meta-regression was performed to
185 explore the potential sources of heterogeneity in sensitivity and specificity. The following
186 covariates were included: the year of publication (2 groups: 2008-2015 and 2016-2018), the
187 country (2 groups: Europe and other countries), the inclusion criteria (2 groups: outbreak and
188 other inclusion criteria) and the PCR technique (2 groups: laboratory developed and
189 commercial kits).

190 Sensitivity analyses were performed and outliers were detected by influence analysis. Cook's
191 distance was calculated to assess for influential studies.

192 The potential publication bias was estimated by Deeks' funnel plot and a probability value of
193 $P < 0.05$ indicates significant publication bias.³⁵ Finally, the Fagan's nomogram was graphed
194 to estimate the post-test probabilities.

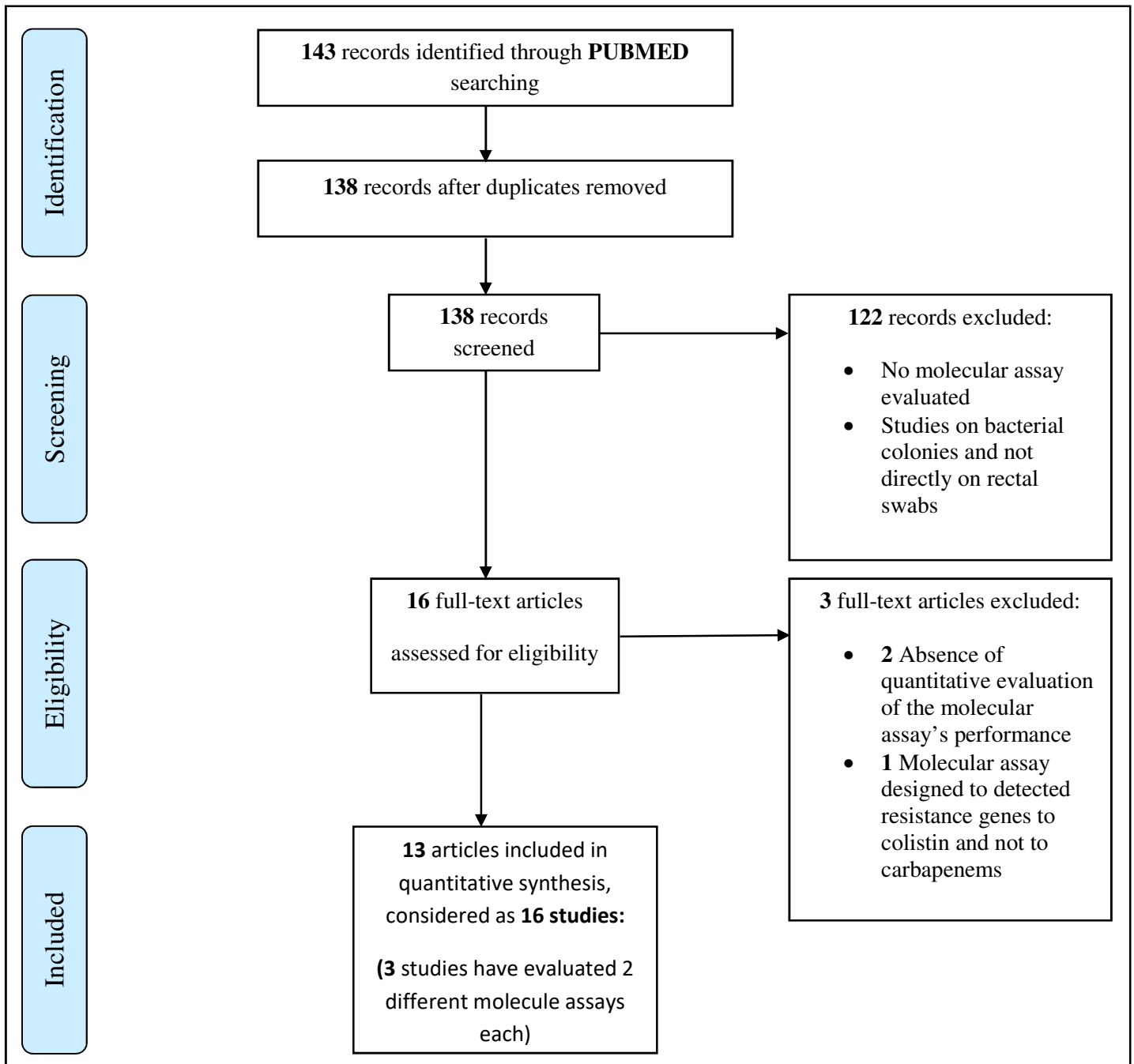
195 Bivariate model (and also hierarchical summary receiver operating characteristic (HSROC))
196 is challenging to fit in presence of few studies or sparse data (for example zero cells in
197 contingency tables due to studies reporting 100% sensitivity or specificity). In this case, the
198 model may give unreliable parameter estimates.³⁶ Therefore, we used Bayesian analysis to
199 check the estimates.

200

201 **Results:**

202 Included studies

203 After screening abstracts of 143 articles and reviewed full texts of 16 studies, 13 fulfilled the
204 inclusion criteria (figure 1). These 13 studies were carried out from 2008 through 2018. They
205 were conducted in Asia (n= 1), in Europe (n= 7), in Middle East (n= 1), in America (n= 3),
206 and in Africa (n= 1).



207 Figure 1. Flowchart diagram of the study selection process. Adapted from Moher et al., 2009.

208 Study characteristics and quality assessment

209 The main characteristics of the included studies^{11,12,25,37-46} are summarized and listed in
210 Table I, by order of publication year. Among the 13 included articles, 3 evaluated 2 different
211 molecular assays each. So, in total, we considered a total of 16 included studies in our meta-
212 analysis. Five studies over 16 (31%) were conducted in a context of a CPE outbreak, one
213 study (6%) included patients pre-identified with CPE in clinical samples (blood or tracheal
214 secretions), while the rest (63%) collected rectal swabs from patients considered at high risk
215 of colonization with CPE (patients admitted to ICU or geriatric unit, having received
216 hematopoietic stem cells transplant, patients directly admitted from abroad to any ward of the
217 hospital, or previously hospitalized in areas with high prevalence of CPEs, and finally contact
218 patients of known carriers). In addition to the clinical experiments directly on rectal swabs, 5
219 studies have evaluated analytically the performance of molecular assays on bacterial strains
220 (3 studies) and/or spiked clinical samples, such as blood or stool (4 studies).
221 Various different assays were evaluated, however, in only 11 studies (69%), the molecular
222 assays targeted all the most common carbapenemase genes (bla_{NDM} , bla_{KPC} , bla_{VIM} , bla_{IMP} ,
223 bla_{OXA48}). In fact, in the other 5 studies (31%), the evaluated technique targeted only one or 2
224 of the following carbapenemase genes: bla_{KPC} , bla_{NDM} , bla_{VIM} , bla_{OXA48} -like. The isolated CPE
225 bacteria from all the included studies were by decreased order of prevalence, *Klebsiella*.
226 *pneumoniae*, *Escherichia coli*, *Enterobacter* spp., *K. oxytoca*, *Citrobacter freundii* and
227 *Providencia. stuartii*.

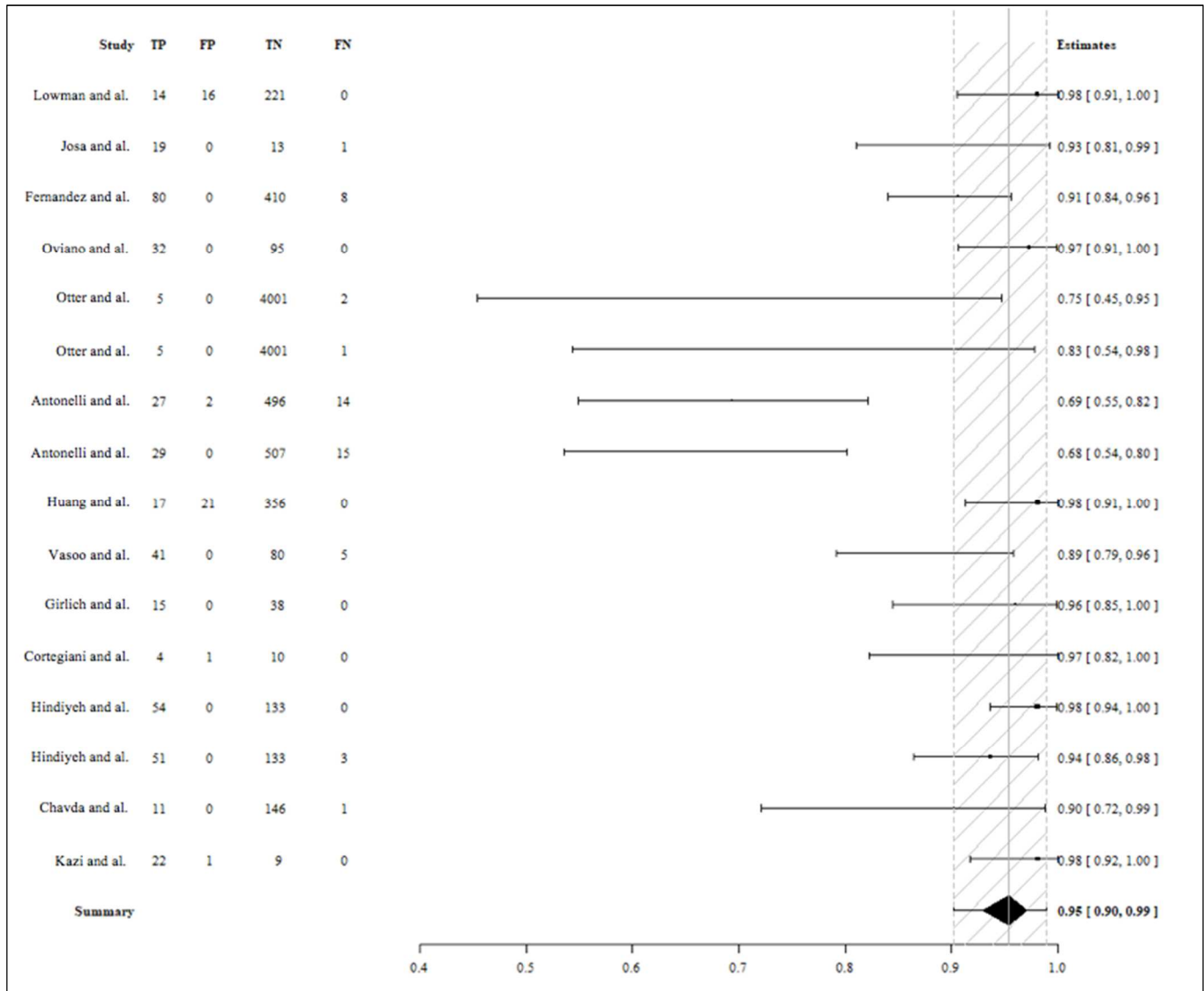
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229 Study-specific test performance estimates (Bayesian estimates)

230 The molecular assays used to detect CPE directly in rectal swabs evaluated in this meta-
231 analysis, had a relatively good sensitivity of 0.95 (IC: 0.902-0.989), and an excellent
232 specificity of 0.994 (IC: 0.965-1), estimated using Bayesian statistical inference. Bayesian
233 methods do not rely on asymptomatics, a property that can be a hindrance with their use.
234 Frequentist methods in small sample contexts, they are considered better equipped to model
235 data with small sample sizes. However, we also applied the Frequentist inference to our
236 included studies, that showed results almost similar to Bayesian for the estimated sensitivity
237 and specificity of the molecular assays (Table II).

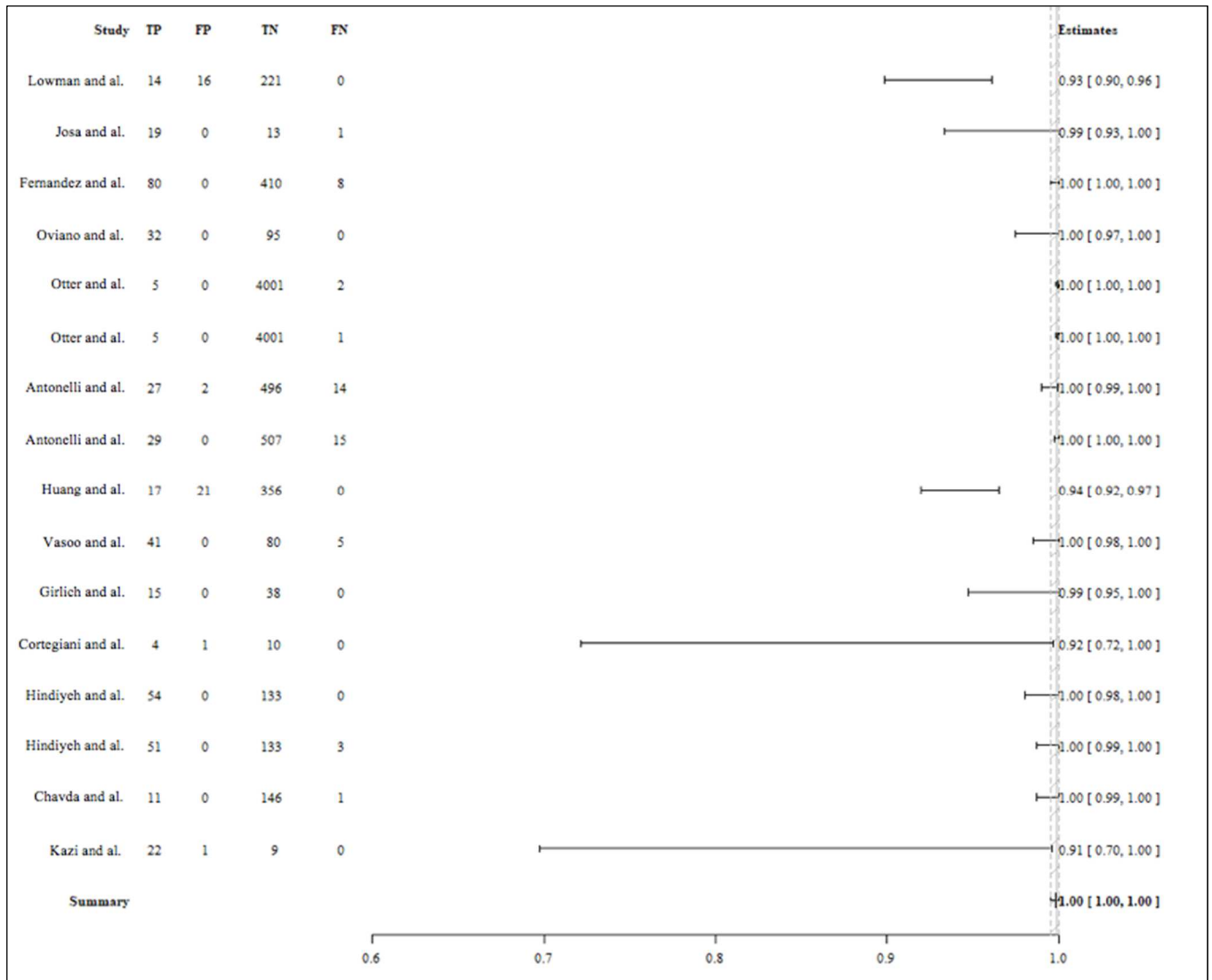
238 Study-specific test performance estimates of diagnostic scores are shown in the Forest plots
239 (Figure 2 and 3).

240



242

243 Figure 2. Forest plot for true positive rate (sensitivity). TP: true positive, FP: false positive,
 244 TN: true negative, FN: false negative.



245

246 Figure 3: Forest plot for true negative rate (specificity). TP: true positive, FP: false positive,
 247 TN: true negative, FN: false negative.

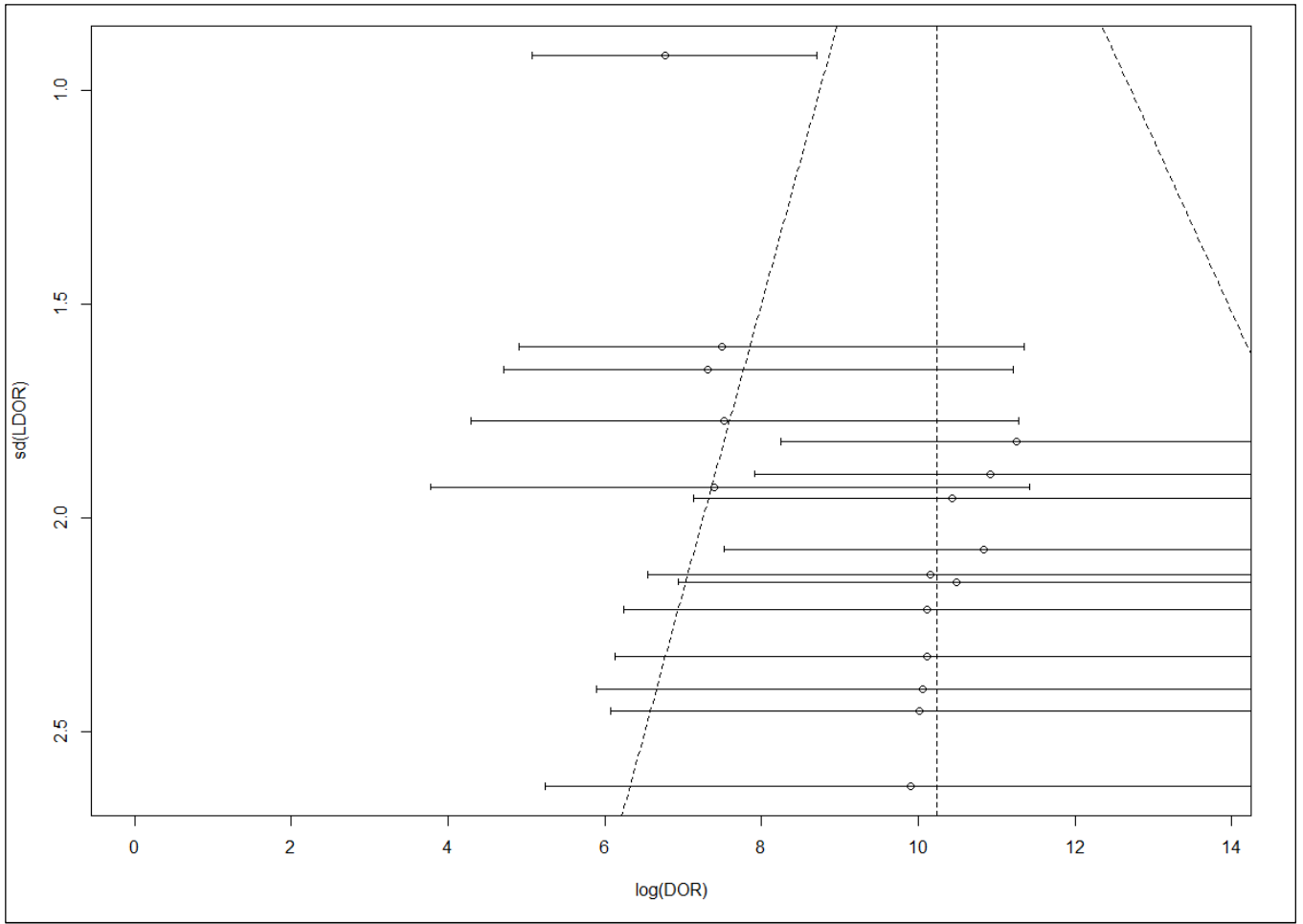
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249 We also noted a remarkable heterogeneity when it comes to the sensitivity of the molecular
 250 assays, with $I^2 = 90.64$, 95% CI = [87.17 - 94.11] and to their specificity, with $I^2 = 98.30$,
 251 95% CI = [97.93 - 98.67].

252

253 Publication bias

254 Deeks' funnel plot asymmetry test for publication bias found no significant publication bias in
 255 our study (Figure 4).



256

257 Figure 4. Deeks' funnel plot asymmetry test for publication bias (Bayesian).

258 **Discussion and Conclusion:**

259 To our best knowledge, this is the first systematic review focusing on evaluating the
260 performance of molecular assays detecting CPE directly in rectal swabs. Our meta-analysis
261 found out a good sensitivity of 0.95 and an excellent specificity of 0.994 for molecular assays
262 applied in this context, with no significant publication bias. Knowledge of the sensitivity and
263 specificity is essential for decision making by the infection control team in respect to the
264 value of a positive and a negative test result.

265 The remarkable heterogeneity that we noticed could be explained in a technical and/or
266 clinical aspect. In fact, the inclusion criteria of patients are different in the included studies,
267 as well as the molecular assay technique evaluated (Table I). However, when included in a
268 meta-regression model to explore the potential sources of heterogeneity in sensitivity and
269 specificity, none of the following covariates: year of publication, country, inclusion criteria
270 and PCR technique, were able to explain this heterogeneity.

271 Among the limited number of authors who have been interested in evaluating the
272 performance of molecular assays performed directly on rectal swabs, compared to the routine
273 culture techniques, the majority (63%) have studied its effectiveness at the admission of
274 patients considered as ‘high-risk’ for CPE carriage. Only 5 studies (31%) were conducted in a
275 context of a CPE outbreak. Hence, there are few data on the effectiveness of real-time PCR in
276 the specific situation of the discovery of a CPE carrier. Moreover, only one study evaluated
277 the clinical utility of PCR-based assays as a screening tool, demonstrating their potential
278 added value for infection control management in an epidemiological setting. The authors
279 suggested that real-time PCR, carried out at Day 0 is efficient in identifying among contact
280 patients those who acquired CPE, with a NPV of 98%, shortening the turnaround time by 5 to
281 27 days. Therefore, in an outbreak context, if the screening real-time PCR test for a patient at
282 Day 0 is negative, the risk that it will be discovered later colonized remains weak. Thereby,
283 contact patient’s isolation will only be limited to those who have their screening real-time
284 PCR positive.⁴⁷

285 Knowing that the main disadvantages of molecular-based technologies for detection of CPE
286 is the absence of detection of any novel carbapenemase gene and their cost,¹³ the ultimate
287 utility of these techniques would be more in the screening for carriage of CPE in contact
288 patients around a fortuitous discovery of a non-isolated carrier patient, than at the admission
289 of a patient suspected of being colonized. In this context, where we already know the CPE
290 bacteria and the carbapenemase gene it expresses, according to the 2013 French National

291 Guidelines recommendations ⁴⁸ and the international recommendations ⁴⁹ an extensive
292 screening policy for all isolated contact patients must be applied. This strategy seems to be
293 costly as it is associated with bed closures and reduction of medical activity, for a relatively
294 long duration of time. Whereas, by including the molecular-based technologies for detection
295 of CPE in a clinical algorithm, the cost behind the infection control measures implementation
296 can be significantly reduced. ⁵⁰ In addition to the cost reduction, the problem of isolated
297 patients with their exposure to a higher risk of complications as they are possibly receiving
298 less optimal management for their medical condition, compared to non-isolated patients with
299 the same medical condition, is also reduced. ⁴⁷

300 The use of molecular techniques for the detection of CPE carriers in contact patients may also
301 have some limitations. In fact, they can detect the presence of a carbapenemase gene not
302 carried by a CPE and thereby not transmittable. As for example, false-positive CPE detection
303 following a transiently carriage of *Shewanella* spp. which naturally produce OXA-48 variants
304 and is not transmittable, has been described previously. ²³ Moreover, knowing that molecular
305 methods may be more sensitive than the conventional culture method and that, heavily
306 colonized patients are more likely to yield opportunities for horizontal transmission, it raises
307 the issue of whether the excellent sensitivity of PCR and detection of very low levels of
308 colonization is significant, regarding the consequent costly infection control measures. ¹¹

309 In conclusion, our meta-analysis shows that molecular techniques seem to have excellent
310 specificity and very good sensitivity for detection of CPE directly in rectal swabs. However,
311 since only a limited number of authors have been interested in evaluating the performance of
312 molecular assays performed directly on rectal swabs, more studies must be conducted and
313 another meta-analysis performed then on a larger number of studies in order to confirm this
314 finding. Although culture methodologies remain necessary even though molecular techniques
315 are used, these latter may be a useful accurate diagnostic tool in the particular indication of
316 screening for carriage of CPE in contact patients around a fortuitous discovery of a non-
317 isolated hospitalized carrier patient.

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319

320

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323

324 **Conflicts of Interest**

325

326

327 None to report.

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329

330

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488 **Tables**

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490

491 Table I. Characteristic of included studies.

Author	Year	Country	Technique	N	TP	FP	FN	TN
Hindiye and al.	2008	Israel	RT-PCR TaqMan for detection of KPC: EasyMag extraction	187	54	0	0	133
			Roche extraction	187	51	0	3	133
Vasoo and al.	2013	USA	Duplex RT-PCR for KPC and NDM	126	41	0	5	80
Lowman and al.	2014	South Africa	Multiplex PCR	251	14	16	0	221
Huang and al.	2015	Belgium	Check-Direct PCR	394	17	21	0	356
Oviano and al.	2016	Spain	LightMix® modular multiplex PCR carbapenemase kits + Xpert Carba-R	127	32	0	0	95
Otter and al.	2016	UK	Check-Direct PCR	4006	5	0	2	4001
			Xpert Carba-R	4006	5	0	1	4001
Antonelli and al.	2016	Italy	Check-Direct PCR: BDMix	557	27	2	14	496
			CPMix	557	29	0	15	507
Cortegiani and al.	2016	Italy	Xpert Carba-R	15	4	1	0	10
Chavda and al.	2016	USA	Multiplex RT-PCR for detection of KPC	158	11	0	1	146

Kazi and al.	2016	India	Xpert MDRO (NDM, VIM, KPC)	32	22	1	0	9
Fernandez and al.	2017	Spain	Laboratory developed qualitative duplex real-time PCR for rapid detection of OXA-48-like	498	80	0	8	410
Girlich and al.	2018	France	ELIte InGenius RT-PCR	53	15	0	0	38
Josa and al.	2018	Colombia	Xpert Carba-R	33	19	0	1	13

492 N: number of included patients, TP: true positive, FP: false positive, TN: true negative, FN:
493 false negative.

494

495 Table II. Synthesis of sensitivity and specificity.

	Se	CI Se	Sp	CI Sp
Frequentist MA (bivariate)	0.96	0.98-0.99	1	0.99-1
Bayesian MA (INLA)	0.95	0.902-0.989	0.999	0.995-1
Bayesian MA (MCMC*, via jags)	<u>0.950</u>	<u>0.872-0.991</u>	<u>0.994</u>	<u>0.965-1</u>

496 *3 chains and 20000 iterations

497 MA: meta-analysis, Se: sensitivity, CI Se: sensitivity's confidence interval, Sp: specificity, CI
 498 Sp: specificity's confidence interval.

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501 **Figures**

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504 Figure 1. Flowchart diagram of the study selection process. Adapted from Moher et al., 2009.

505 Figure 2. Forest plot for true positive rate (sensitivity).

506 Figure 3. Forest plot for true negative rate (specificity).

507 Figure 4. Deeks' funnel plot asymmetry test for publication bias (Bayesian).