Optimal detection of carbapenemase-producing Enterobacteriaceae from rectal samples: a role for enrichment ?

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Running title: CPE rectal screening OXA-48

25 **Summary**

26 Background: Successful laboratory detection of carbapenemase-producing Enterobacteriaceae (CPE)
27 in patient surveillance samples is a diagnostic challenge. In the absence of a gold standard to use for
28 screening rectal swabs for CPE, many phenotypic, genotypic, culture and non-culture based assays
29 have been proposed for identifying these bacteria.

30 Aim: To develop and optimise a CPE screening protocol capable of identifying all commonly
31 encountered CPE, including those producing OXA-48-like carbapenemases

32 Methods: Faropenem (CAT-ID) susceptibility was performed on 507 presumptive CPE isolated from
33 diagnostic samples and CPE rectal screens between March and August 2016. Results from this CPE
34 screening method were compared to those from direct culture on mSuperCARBA^{TM}, temocillin
35 enrichment culture and use of an antibiotic resistance algorithm, to determine the optimal method
36 to employ in the detection of CPE.

37 Findings: Faropenem was a poor predictor of carbapenemase production (58% true positives)The
38 combination of a temocillin enrichment stage and interpretitve reading of antibiotic resistance
39 phenotypes improved the recovery and identification of CPE significantly (91% true-positives),
40 particularly for OXA-48 producers (*P* = 0.03).

41 Conclusion: The combination of temocillin enrichment,a selective chromogenic media and an
42 antibiotic resistance-based algorithm significantly improved the detection of all CPE recovered from
43 routine and targeted surveillance samples.

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45 **Keywords:** carbapenemase, screening, faropenem, enrichment, OXA-48, algorithm

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51 **Introduction**

52 Acquired resistance to carbapenems in Enterobacteriaceae is a global problem facilitated by
53 plasmid-mediated spread of class A (KPC), B (IMP, VIM, NDM) and D (OXA-48-like) β-lactamases
54 among multiple species (*E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter* species). In the UK, there are
55 reports of hospital outbreaks which are increasingly difficult to control and also overlap with spread
56 into and within the community.[1-4]

57

58 Accurate detection of carbapenemase-producing Enterobacteriaceae (CPE) is fundamental to any
59 strategy aimed at addressing this problem. Many methods have been used to identify CPE including
60 phenotypic susceptibility tests, selective culture media, immunochromatographic assays, specific
61 PCRs and sequenced based molecular tests; all with varying degrees of sensitivity and specificity.[5, 6, 8-
62 10] Furthermore, identification of CPE from rectal swabs, commonly used in surveillance and outbreak
63 screening, is hindered by the lack of a gold standard test. Resistance to carbapenems as the sole
64 marker is complicated by the wide MIC distribution among the wild type population of CPE.
65 Thisspans current EUCAST and CLSI breakpoints (0.25 – 8mg/L), and may lead to many CPEs being
66 'missed', especially those that just produce OXA-48.[5, 6] With such diversity in the prevalence and
67 epidemiology of carbapenem resistant strains in the UK, there is an urgent need for a robust
68 laboratory screening protocol that is capable of detecting all CPEs.[1]

69

70 Public Health England (PHE) guidance advocates the use of a chromogenic media and resistance to
71 an indicator carbapenem for the primary isolation of CPE from rectal screening samples.[7] Using this
72 guidance as a basis, we assessed the utility of temocillin enrichment cultures together with
73 interpretitive reading of the antibiotic susceptibility profile, as a reliable screening algorithm for
74 enhanced accurate identification of CPE, particularly for OXA-48.[10]

75 **Methods**

76 This was a single-centre study performed as part of our continuous service development but in the
77 presence of an on-going OXA-48 outbreak (June 2016 – current).
78
79 Susceptibility to faropenem
80 *Bacterial culture*
81 All screening swabs were inoculated on mSuperCARBA™ chromogenic agar (E&O Laboratories,
82 Bonnybridge, UK) and incubated for 18-24 hours at 37°C. Pink (*E. coli*) and blue (other
83 Enterobacteriaceae) colonies were identified by MALDI-TOF mass spectrometry (Bruker Daltonik
84 GmbH, Bremen, Germany).
85 Urinary isolates underwent faropenem susceptibility testing if they were resistant to cefpodoxime
86 (BSAC disc diffusion) and all other isolates from other diagnostic specimens underwent faropenem
87 susceptibility testing if they were reported resistant to ≥1 carbapenem using the Microscan NM44
88 ?(MicroScan, Beckman Coulter, High Wycombe, UK).
89
90 *Antibiotic susceptibility testing (AST)*
91 AST (disc diffusion) was performed with meropenem (10µg), ertapenem (10µg), temocillin (30µg)
92 and piperacillin-tazobactam (75/10µg), according to BSAC guidelines (v14, 2015). Discs were
93 supplied by Thermo Scientific™ Oxoid™ (Basingstoke, UK).
94
95 *Faropenem susceptibility testing*
96 Faropenem (CAT-ID, Mast Group Ltd, Bootle, UK) susceptibility testing was performed according to
97 the manufacturer's instructions. Faropenem results were reported as either CPE positive (no zone
98 present/double zone/single colonies within zone of inhibition) or CPE negative (zone of inhibition
99 observed). All CPE positives were referred for reference testing by PCR (AMRHL..), which was
100 considered the gold standard or detection of carbapenemase genes.
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105 A retrospective evaluation of faropenem (CAT-ID) susceptibility results, for all suspected CPE isolated 106 from 507 specimens, was performed, between March and August 2016. These included 25 rectal 107 swabs taken for CPE screening and 482 diagnostic specimens (455 urines, 9 blood cultures, 7 wound 108 swabs, 6 fluids, 3 respiratory specimens, 1 tip and 1 tissue). Susceptibility results were also reviewed 109 in conjunction with reference molecular test results (Figure I).

110

111 Temocillin enrichment culture

112 Due to an OXA-48 outbreak in a surgical ward (June 2016 – current), direct culture was compared to 113 temocillin enrichment culture. Enrichment culture was specifically designed to recover OXA-48- 114 producers, in addition to all other CPE.

115 Rectal swabs (n = 95) were inoculated into 3 ml nutrient broth (Thermo Scientific™ Oxoid™) 116 containing a temocillin (30µg) disc. After overnight incubation at 37°C turbid broths were sub-117 cultured to mSuperCARBA™. Broths were set up in tandem with direct culture to assess the 118 advantages of enrichment culture and compared using a paired t-test, with a *p*-value ≤0.05 119 considered significant. Enterobacteriaceae were identified and underwent AST, as described above.

120

121 *CPE algorithm*

122 Identification of CPE was reported using an algorithm-based approach and the following interpretive 123 rules: 1) Enterobacteriaceae resistant to meropenem and ertapenem, irrespective of other AST 124 results, were reported as presumptive CPE; 2) Enterobacteriaceae resistant to temocillin and 125 piperacillin-tazobactam, but susceptible to meropenem, were reported as presumptive OXA-48.

126 All presumptive CPE/OXA-48 isolates were referred for reference testing PCR. The recovery of CPE by

127 direct plating and temocillin enrichment was compared using a two-tailed t-test, with a *p*-value

128 ≤0.05 considered significant.

129

130 A second retrospective evaluation was performed for all suspected CPE, isolated between

131 September 2016 and June 2017, from 215 CPE rectal swabs.

132 At this stage, all swabs (n = 156/215) associated with the OXA-48 outbreak ward underwent

133 temocillin enrichment culture, while swabs (n = 59/215) from all other wards underwent direct

134 culture on mSuperCARBA^{TM}. All Enterobacteriaceae recovered were identified by MALDI-TOF and

135 AST performed (Figure II).

136

137 **Results**

138 Performance of faropenem (CAT-ID) in detecting CPE

139 Retrospective evaluation of faropenem disc testing of 25 CPE rectal screens and 482 diagnostic
140 isolates identified 63/507 (12.4%) faropenem resistant Enterobacteriaceae as presumptive CPE.
141 However, the reference laboratory PCR identified carbapenemases in only 14/63 (22%) of these – 3
142 with NDM and 11 with OXA-48-like carbapenemases(Figure I). A further 16/444 'CPE negative'
143 isolates sent for reference PCR, due to resistance to ≥1 of temocillin, piperacillin-tazobactam,
144 ertapenem and meropenem, revealed that 10/16 (62 %) produced an OXA-48-like carbapenemase.
145 Together these results suggest a true-positive rate of 58%, a false-positive rate of 77% and an
146 approximate false-negative rate of 2% for faropenem disc testing for he detection of CPE.

147

148 As faropenem susceptibility was deemed a poor predictor of carbapenemase production, AST results
149 were reviewed to identify possible indicator antibiotics (Table I). All three NDM isolates were
150 resistant to meropenem, ertapenem and piperacillin-tazobactam and 2/3 were temocillin-resistant.
151 All twenty-one OXA-48 producers were temocillin-resistant and 20/21 (95%) were also resistant to
152 piperacillin-tazobactam. Only 2/21 (10%) OXA-48 producers were meropenem-resistant and only
153 11/21 (52%) were ertapenem-resistant. These findings informed a resistance algorithm with two
154 phenotypic groups for isolates recovered on mSuperCARBA^{TM}. Group 1: isolates with resistance to
155 meropenem and ertapenem were reported as presumptive CPE; group 2: isolates with resistance to
156 temocillin and piperacillin-tazobactam were reported as presumptive OXA-48 producers.
157 Using this algorithm 23/24 (96%) CPE recovered on selective media would have been correctly
158 identified with only 1/24 (4%) falsely identified as negative.

159

160 Temocillin enrichment culture

161 During the investigation of the CPE outbreak, enrichment with temocillin increased the number of
162 CPE recovered from 3% (3/95) to 12% (11/95), all of which were subsequently shown to produce

163 OXA-48 by reference PCR. As the increase in recovery was found to be significant (P = 0.03),
164 temocillin enrichment was added to our optimised CPE screening protocol, along with the existing
165 antibiotic resistance-based algorithm.
166
167 After the combination of temocillin enrichment and AST algorithm was introduced into the CPE
168 screening protocol, a further evaluation was performed to determine the performance of the new
169 method (Figure II). Presumptive CPE were then identified in 94% (203/215) of CPE rectal screens, of
170 which 185/203 were confirmed by reference PCR (18 NDM and 167 OXA-48). Together these results
171 demonstrated a true positive rate of 91% and a false-positive rate of 8% for the new protocol.
172 Statistical analysis revealed that temocillin enrichment significantly increased the recovery of all CPE
173 compared to direct culture (P = 0.01). Antibiotic resistances are detailed in table I.
174

175

176 Discussion

177 In our experience, accurate detection of OXA-48, the predominant carbapenemase in our institution,
178 has been a significant problem. The initial approach combining selective chromogenic media
179 (mSuperCARBA^{TM}) with faropenem resistance (CAT-ID discs) appeared sub-optimal. Despite reports
180 of faropenem performing well, we found it to be a poor predictor of OXA-48 producers (58% true-
181 positives).[8, 11] Similarly Koroska *et al* (2017) demonstrated a sensitivity of only 57.1% when using
182 CAT-ID to explicitly identify OXA-48.[6] In addition, our OXA-48 producers were typically meropenem
183 and ertapenem susceptible (table I) in contrast to previous studies with only carbapenem-resistant
184 isolates, demonstrating that resistance to faropenem alone may miss many OXA-48 producers.[8, 11]
185
186 Furthermore, AST results between March and August 2016 revealed that meropenem and
187 ertapenem resistance (10% and 52%, respectively) were also unreliable indicators of OXA-48
188 production, in contrast to temocillin resistance (100%). Therefore, faropenem resistance was
189 replaced with interpretive reading of the antibiogram to ensure optimal recovery of all OXA-48
190 producers.
191
192 CPE enrichment using ertapenem or imipenem is well documented, but temocillin enrichment to
193 improve recovery of OXA-48 producers is not.[5, 9, 10, 12] We found temocillin enrichment significantly
194 increased the recovery of OXA-48 CPE, both during the validation period and once it was introduced
195 into our CPE screening protocol (Figure II). Four temocillin-susceptible NDM-producing isolates,
196 recovered from diagnostic samples, may have been missed using the enrichment protocol alone, but
197 were recovered by direct culture. This demonstrates the importance of a multi-faceted approach to
198 CPE screening (temocillin enrichment for OXA-48 producers and direct culture for all other CPE),
199 which allows all possible susceptibility profiles. By introducing temocillin enrichment and
200 interpretive reading of AST results, we reduced the rate of isolates falsely reported as CPE from 77%

201 (faropenem method) to 8% demonstrating that a one-fits-all approach is unrealistic for precise CPE
202 detection. Accurate reporting of CPE is important in prioritising limited infection control resources,
203 but comes with a compromise, as temocillin enrichment adds 24 hours to the laboratory processing
204 times and increases costs.

205

206 Other studies have proposed diagnostic algorithms for CPE screening; employing faropenem and
207 temocillin susceptibility to differentiate non-CPE, CPE and OXA-48 producers.[6, 11] While they show
208 that a combination of methods increases the sensitivity of CPE detection, there are also
209 disadvantages. One relied on reduced carbapenem susceptibility to trigger a confirmatory CPE test
210 (CAT-ID), which isunlikely to be practical if the majority of OXA-48 producers are carbapenem
211 susceptible.[11] Another study detected OXA-48 using a lateral flow device (LFD) to compensate for
212 the poor performance of faropenem.[6] While simple and rapid, LFD devices capable of detecting
213 carbapenemases also increase CPE screening costs and may limit widespread use in high-throughput
214 diagnostic laboratories.

215

216 We have developed a robust and affordable protocol for identifying CPE from rectal swabs, which
217 has the flexibility of identifying carbapenemases in isolates with variable susceptibility to β-lactams,
218 including OXA-48. The inclusion of a temocillin enrichment step combined with an algorithm-based
219 interpretation of AST results enabled detection of all CPE from rectal screens with a high degree of
220 accuracy. The algorithm enabled us to harmonise differing methodologies used in carbapenem
221 susceptibility testing and CPE screening into a single protocol able to inform both treatment and
222 infection prevention and control strategies.

223

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227

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