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

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Running title: CPE rectal screening OXA-48
Summary

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

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

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

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

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

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

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

Accurate detection of carbapenemase-producing Enterobacteriaceae (CPE) is fundamental to any strategy aimed at addressing this problem. Many methods have been used to identify CPE including phenotypic susceptibility tests, selective culture media, immunochromatographic assays, specific PCRs and sequenced based molecular tests; all with varying degrees of sensitivity and specificity.⁵, ⁶, ⁸⁻¹⁰ Furthermore, identification of CPE from rectal swabs, commonly used in surveillance and outbreak screening, is hindered by the lack of a gold standard test. Resistance to carbapenems as the sole marker is complicated by the wide MIC distribution among the wild type population of CPE. This spans current EUCAST and CLSI breakpoints (0.25 – 8mg/L), and may lead to many CPEs being ‘missed’, especially those that just produce OXA-48.⁵, ⁶ With such diversity in the prevalence and epidemiology of carbapenem resistant strains in the UK, there is an urgent need for a robust laboratory screening protocol that is capable of detecting all CPEs.¹

Public Health England (PHE) guidance advocates the use of a chromogenic media and resistance to an indicator carbapenem for the primary isolation of CPE from rectal screening samples.⁷ Using this guidance as a basis, we assessed the utility of temocillin enrichment cultures together with interpretive reading of the antibiotic susceptibility profile, as a reliable screening algorithm for enhanced accurate identification of CPE, particularly for OXA-48.¹⁰

**Methods**
This was a single-centre study performed as part of our continuous service development but in the presence of an on-going OXA-48 outbreak (June 2016 – current).

Susceptibility to faropenem

Bacterial culture

All screening swabs were inoculated on mSuperCARBA™ chromogenic agar (E&O Laboratories, Bonnybridge, UK) and incubated for 18-24 hours at 37°C. Pink (E. coli) and blue (other Enterobacteriaceae) colonies were identified by MALDI-TOF mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany).

Urinary isolates underwent faropenem susceptibility testing if they were resistant to cefpodoxime (BSAC disc diffusion) and all other isolates from other diagnostic specimens underwent faropenem susceptibility testing if they were reported resistant to ≥1 carbapenem using the Microscan NM44 (MicroScan, Beckman Coulter, High Wycombe, UK).

Antibiotic susceptibility testing (AST)

AST (disc diffusion) was performed with meropenem (10μg), ertapenem (10μg), temocillin (30μg) and piperacillin-tazobactam (75/10μg), according to BSAC guidelines (v14, 2015). Discs were supplied by Thermo Scientific™ Oxoid™ (Basingstoke, UK).

Faropenem susceptibility testing

Faropenem (CAT-ID, Mast Group Ltd, Bootle, UK) susceptibility testing was performed according to the manufacturer’s instructions. Faropenem results were reported as either CPE positive (no zone present/double zone/single colonies within zone of inhibition) or CPE negative (zone of inhibition observed). All CPE positives were referred for reference testing by PCR (AMRHL..), which was considered the gold standard or detection of carbapenemase genes.
A retrospective evaluation of faropenem (CAT-ID) susceptibility results, for all suspected CPE isolated from 507 specimens, was performed, between March and August 2016. These included 25 rectal swabs taken for CPE screening and 482 diagnostic specimens (455 urines, 9 blood cultures, 7 wound swabs, 6 fluids, 3 respiratory specimens, 1 tip and 1 tissue). Susceptibility results were also reviewed in conjunction with reference molecular test results (Figure 1).

Temocillin enrichment culture

Due to an OXA-48 outbreak in a surgical ward (June 2016 – current), direct culture was compared to temocillin enrichment culture. Enrichment culture was specifically designed to recover OXA-48 producers, in addition to all other CPE. Rectal swabs (n = 95) were inoculated into 3 ml nutrient broth (Thermo Scientific™ Oxoid™) containing a temocillin (30µg) disc. After overnight incubation at 37°C turbid broths were sub-cultured to mSuperCARBA™. Broths were set up in tandem with direct culture to assess the advantages of enrichment culture and compared using a paired t-test, with a p-value ≤0.05 considered significant. Enterobacteriaceae were identified and underwent AST, as described above.

CPE algorithm

Identification of CPE was reported using an algorithm-based approach and the following interpretive rules: 1) Enterobacteriaceae resistant to meropenem and ertapenem, irrespective of other AST results, were reported as presumptive CPE; 2) Enterobacteriaceae resistant to temocillin and piperacillin-tazobactam, but susceptible to meropenem, were reported as presumptive OXA-48.
All presumptive CPE/OXA-48 isolates were referred for reference testing PCR. The recovery of CPE by direct plating and temocillin enrichment was compared using a two-tailed t-test, with a p-value ≤0.05 considered significant.

A second retrospective evaluation was performed for all suspected CPE, isolated between September 2016 and June 2017, from 215 CPE rectal swabs. At this stage, all swabs (n = 156/215) associated with the OXA-48 outbreak ward underwent temocillin enrichment culture, while swabs (n = 59/215) from all other wards underwent direct culture on mSuperCARBA™. All Enterobacteriaceae recovered were identified by MALDI-TOF and AST performed (Figure II).
Results

Performance of faropenem (CAT-ID) in detecting CPE

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

As faropenem susceptibility was deemed a poor predictor of carbapenemase production, AST results were reviewed to identify possible indicator antibiotics (Table I). All three NDM isolates were resistant to meropenem, ertapenem and piperacillin-tazobactam and 2/3 were temocillin-resistant. All twenty-one OXA-48 producers were temocillin-resistant and 20/21 (95%) were also resistant to piperacillin-tazobactam. Only 2/21 (10%) OXA-48 producers were meropenem-resistant and only 11/21 (52%) were ertapenem-resistant. These findings informed a resistance algorithm with two phenotypic groups for isolates recovered on mSuperCARBA™. Group 1: isolates with resistance to meropenem and ertapenem were reported as presumptive CPE; group 2: isolates with resistance to temocillin and piperacillin-tazobactam were reported as presumptive OXA-48 producers.

Using this algorithm 23/24 (96%) CPE recovered on selective media would have been correctly identified with only 1/24 (4%) falsely identified as negative.

Temocillin enrichment culture

During the investigation of the CPE outbreak, enrichment with temocillin increased the number of CPE recovered from 3% (3/95) to 12% (11/95), all of which were subsequently shown to produce
OXA-48 by reference PCR. As the increase in recovery was found to be significant ($P = 0.03$), temocillin enrichment was added to our optimised CPE screening protocol, along with the existing antibiotic resistance-based algorithm.

After the combination of temocillin enrichment and AST algorithm was introduced into the CPE screening protocol, a further evaluation was performed to determine the performance of the new method (Figure II). Presumptive CPE were then identified in 94% (203/215) of CPE rectal screens, of which 185/203 were confirmed by reference PCR (18 NDM and 167 OXA-48). Together these results demonstrated a true positive rate of 91% and a false-positive rate of 8% for the new protocol.

Statistical analysis revealed that temocillin enrichment significantly increased the recovery of all CPE compared to direct culture ($P = 0.01$). Antibiotic resistances are detailed in table I.
In our experience, accurate detection of OXA-48, the predominant carbapenemase in our institution, has been a significant problem. The initial approach combining selective chromogenic media (mSuperCARBA™) with faropenem resistance (CAT-ID discs) appeared sub-optimal. Despite reports of faropenem performing well, we found it to be a poor predictor of OXA-48 producers (58% true-positives).\textsuperscript{8, 11} Similarly Koroska et al (2017) demonstrated a sensitivity of only 57.1% when using CAT-ID to explicitly identify OXA-48.\textsuperscript{6} In addition, our OXA-48 producers were typically meropenem and ertapenem susceptible (table I) in contrast to previous studies with only carbapenem-resistant isolates, demonstrating that resistance to faropenem alone may miss many OXA-48 producers.\textsuperscript{8, 11}

Furthermore, AST results between March and August 2016 revealed that meropenem and ertapenem resistance (10% and 52%, respectively) were also unreliable indicators of OXA-48 production, in contrast to temocillin resistance (100%). Therefore, faropenem resistance was replaced with interpretive reading of the antibiogram to ensure optimal recovery of all OXA-48 producers.

CPE enrichment using ertapenem or imipenem is well documented, but temocillin enrichment to improve recovery of OXA-48 producers is not.\textsuperscript{5, 9, 10, 12} We found temocillin enrichment significantly increased the recovery of OXA-48 CPE, both during the validation period and once it was introduced into our CPE screening protocol (Figure II). Four temocillin-susceptible NDM-producing isolates, recovered from diagnostic samples, may have been missed using the enrichment protocol alone, but were recovered by direct culture. This demonstrates the importance of a multi-faceted approach to CPE screening (temocillin enrichment for OXA-48 producers and direct culture for all other CPE), which allows all possible susceptibility profiles. By introducing temocillin enrichment and interpretive reading of AST results, we reduced the rate of isolates falsely reported as CPE from 77%
(faropenem method) to 8% demonstrating that a one-fits-all approach is unrealistic for precise CPE detection. Accurate reporting of CPE is important in prioritising limited infection control resources, but comes with a compromise, as temocillin enrichment adds 24 hours to the laboratory processing times and increases costs.

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

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

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References


