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Improving the processing time for the detection of carbapenemase-producing enterobacterales using an evolving algorithm

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Carbapenemase-producing enterobacterales (CPE) have disseminated worldwide, with the enzyme OXA-48 (so-named by its hydrolysis of the antibiotic oxacillin) emerging as the most prevalent carbapenemase, whilst there is also a place for *Klebsiella pneumoniae* carbapenemase [1,2]. Recognition of CPE in the routine laboratory is challenging: CPE are not universally resistant to carbapenems and not all carbapenem-resistant enterobacterales produce carbapenemases [3]. Recovery of CPE from surveillance specimens is dependent on utilizing screening media. Detecting CPE among clinical isolates is initially reliant on minimum inhibitory concentration values from automated antimicrobial susceptibility testing. Expert rules are activated where carbapenem minimum inhibitory concentrations meet alert criteria to prompt subsequent confirmation or exclusion of CPE. Recognition of OXA-48 enzymes is difficult, as these enzymes weakly hydrolyse carbapenems. High level temocillin resistance (>128 mg/L) is recommended as a phenotypic marker for OXA-48 production, but lacks specificity, so that the use of confirmatory tests for CPE has been recommended [3–5]. However, as a standardized detection algorithm has only recently been defined [6], individual laboratories have been compelled to develop in-house strategies for CPE recognition and confirmation, either via a molecular genetic approach or externally by national reference facilities, with associated delays. In 2016, when chromogenic screening media and phenotypic and molecular genetic detection methodologies became available, we developed a local algorithm to enhance recognition and reduce reporting turnaround times. We present the evolution of methods to detect and confirm CPE over eight years and the development of an algorithm to improve processing times.

Our accredited microbiology laboratory processes approximately 140,000 patient specimens annually, utilizing the latest European Committee for Antimicrobial Susceptibility Testing (EUCAST) recommendations [4]. Processing of rectal swabs for CPE was introduced in February 2011. Between February 2011 and October 2016, CPE detection methodology from rectal swabs initially employed pre-enrichment in tryptone soya broth, which was sub-cultured onto MacConkey agar with a carbapenem disc. This method later evolved to direct culture onto MacConkey agar with an ertapenem disc (MacE). Enterobacterales isolates recovered from within a defined zone diameter of the carbapenem disc on the MacConkey screening agar were considered suspect CPE. For suspect CPE from clinical specimens, alert criteria of ≥0.5 mg/L for meropenem and ertapenem were employed for Enterobacterales other than *Enterobacter* species on the BD Phoenix™ Antimicrobial Susceptibility Testing analysis software system (BD Diagnostics Systems, Sparks, MD, USA); *Enterobacter* species were subjected to an alert criterion of ≥8 mg/L for ertapenem. The modified Hodge test was performed on suspect isolates as an additional phenotypic test. Presumptive CPEs were referred to the national CPE reference laboratory service for confirmation. In 2011, a further phenotypic-based assay, the *Klebsiella pneumoniae* carbapenemase, MBL and OXA-Confirm Kit (Rosco Diagnostica, Taastrup, Denmark) was incorporated.

As technologies evolved [7], the algorithm was revised. In October 2016 (Figure 1), the chromID® Carba Smart (bioMerieux, Marcy L'Etoile, France) screening agar replaced the MacE. The RESIST-3 O.K.N K-SeT (Coris BioConcept, Gembloux, Belgium) was introduced and positive results allowed preliminary reporting of suspect CPE. An assay based on molecular genetics, the Xpert® Carba-R (Cepheid, Sunnyvale, CA, USA), was also introduced. Alert criteria of ≥0.5 mg/L for ertapenem and ≥0.125 mg/L for meropenem were employed for antimicrobial susceptibility testing analysis. A final confirmatory test, the carbapenemase inactivation method [8], was introduced to further substantiate a CPE negative result. In March 2017, reporting procedures were amended to allow final reporting of CPE based on the Xpert® Carba-R findings with antimicrobial susceptibility testing confirmation. Due to the reliability of the Xpert® Carba-R positive result, referral to the reference laboratory was no longer deemed essential for confirmation, but continued for national epidemiological purposes.

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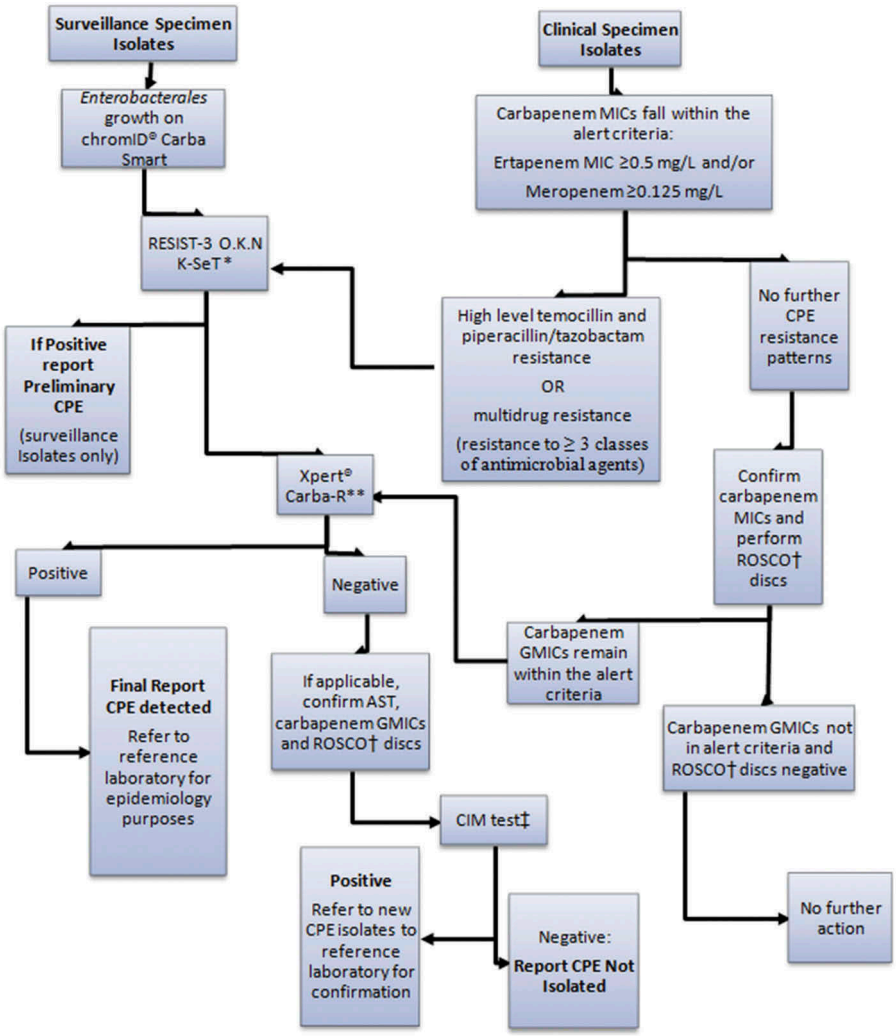

Figure 1. CPE Detection Algorithm.

A retrospective review was performed from the initial detection of CPE in January 2011 to the end of December 2018. Samples yielding the first CPE of each species isolated from an individual patient were included. The impact of the evolving methodology was assessed by calculating the processing time for each specimen. The processing time was defined as time of inoculation of the specimen to preliminary or final reporting of a CPE, as confirmed on the hospital's IT system. Statistical analysis was performed by means of Mann-Whitney U test using IBM SPSS software, version 25 (IBM Corp, Armonk, NY, USA).

Between January 2011 and December 2018, 123 patient specimens yielded 143 non-duplicate CPE isolates; two or more CPE isolates were recovered from 18 specimens. Rectal surveillance swabs accounted for 97 positive specimens (79%), with the remainder clinical specimens (n = 26; 21%). The predominant carbapenemase was OXA-48 (n = 127), followed by *Klebsiella pneumoniae* carbapenemase (n = 8), NDM (n = 5), VIM (n = 2) and IMI (n = 1). Two CPE were identified in 2011, eight in both 2012 and 2013, three in 2014, eight in 2016, 49 in 2017 and 65 in 2018; CPE was not detected in 2015.

In total, processing times were evaluated on 138 of the 143 eligible CPE isolates; sufficient information to allow processing time calculation could not be retrieved for two clinical and three surveillance specimens. Before March 2017, the median processing time for final reporting of all eligible CPE isolates was 20.0 days (n = 25, range: 9.0–36.0) which fell to 2.2 days (n = 113, range: 1.1–6.1) between March 2017 to December 2018 ($p < 0.001$). For clinical isolates, the median processing time for final reporting was 17.2 days (n = 10, range: 9–24), decreasing to 3.0 days (n = 14, range: 1.9–5.1) after reporting based on CPE detection algorithm results ($p < 0.001$) (Figure 2). From the first isolation of CPE in October 2013 to March 2017, the median processing time of preliminary and final CPE reporting in surveillance specimens was 4.4 days (n = 7, range:3.-0–7.1) and 21.3 days (n = 15, range: 11.1–36.0), respectively. From March 2017 to the end of 2018, the median processing time for preliminary and final CPE reporting decreased to 1.1 days (n = 98, range: 0.9–4.1) ($p < 0.001$) and 2.1 days (n = 99, range: 1.1–6.1) ($p < 0.001$), respectively.

Early detection of CPE is vital to inform treatment decisions in the setting of suspected infection, to allow timely infection prevention and control measures and to enable

the effective use of limited isolation facilities. The introduction of the algorithm in March 2017 led to an 89% reduction in the average processing time for final reporting of eligible CPE isolates. Final reporting processing time of CPE from clinical samples decreased by 14.2 days (83%). Preliminary and final reporting processing time for surveillance specimens fell by 3.3 days (75%) and 19.2 days (90%), respectively. A large proportion of CPE was detected from clinical specimens (21%). Increased numbers of CPE surveillance cultures were processed in 2017 and 2018 in response to increased incidence of CPE and associated outbreaks. The proportion of CPE detected from clinical specimens fell to 15% during this period (n = 16). As our practice moves towards full implementation of CPE national screening guidelines [6], with an increase in surveillance specimens, the proportion of CPE detected via clinical specimens is expected to decrease.

There were substantial limitations in the CPE detection methodology initially employed in 2011. An evaluation study demonstrated poor sensitivity and specificity for the detection of CPE using MacE and concluded that detection was reliable only for overtly carbapenem resistant CPE (Ertapenem minimum inhibitory concentration ≥32 mg/L) [9]. Hence, the potential for non-detection of OXA-48 producing Enterobacterales was considerable, as OXA-48 producers frequently exhibit ertapenem minimum inhibitory concentrations below this detection limit [10]. In addition, the modified Hodge test is highly susceptible to error where outer membrane porin loss is coupled with ESBL/AmpC production [11,12]. Sub-optimal detection of OXA-48 producers using the modified Hodge test has also been reported [12].

Due to the absence of a convincing phenotypic assay to resolve carbapenemase production before October 2016, referral of isolates to the reference laboratory depended on the carbapenem minimum inhibitory concentration screening breakpoints in use at that time. Consequently, false positives were common, leading to potentially avoidable enhanced infection prevention and control measures being utilized in the period between isolate referral and receipt of the reference laboratory result confirming that carbapenemase was not detected.

Molecular genetic confirmation and characterization of carbapenemase genes using the Cepheid Xpert® Carba-R real-time PCR assay is the cornerstone of the current algorithm. This assay provides sufficient evidence for final reporting of CPE, removing the requirement for reference laboratory confirmation, and hence is the greatest contributor towards reduction in processing time. However, the limitation of molecular methods for detecting novel variants is acknowledged and consequently other methods are used to complement molecular tests to confirm CPE. The carbapenem inactivation method test [8] was incorporated into the detection algorithm to confirm the absence of carbapenemase production detected by the Xpert® Carba-R assay. The value of the carbapenem inactivation method confirmatory test was also proven when it demonstrated carbapenemase activity in an IMI-producing *Enterobacter cloacae* isolated from a surveillance specimen; the Xpert® Carba-R and RESIST-3 O.K.N K-SeT had both returned negative results.

Alert criteria were amended on the BD Phoenix™ antimicrobial susceptibility testing analysis software in October 2016. The meropenem expert rule alert criterion, ≥0.125 mg/L, is aligned with the epidemiological cut-off value published by EUCAST to prompt CPE investigation [4]. The ertapenem cut-off in use, ≥0.5 mg/L, enhances specificity for carbapenemase production [5]. The upper limit of temocillin minimum inhibitory concentration detectable on the BD Phoenix™ UNMIC-409 panel is >32 mg/L, which is considerably lower than the cut-off of >128 mg/L recommended by EUCAST for OXA-48 production [4]. To mitigate this limitation in the minimum inhibitory concentration range, a combination of high level temocillin resistance (>32 mg/L) and high-level piperacillin/tazobactam resistance (>16/4 mg/L), with ertapenem and/or meropenem minimum inhibitory concentrations which fall within the alert criteria, is employed as a phenotypic indication of

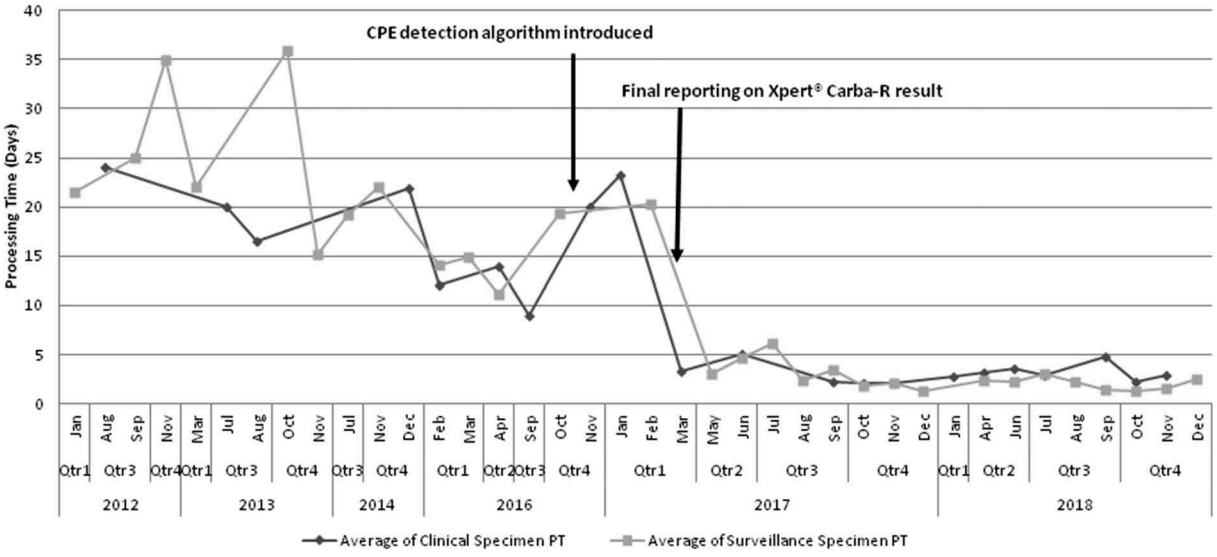

Figure 2. Average processing times for non-duplicate final CPE reporting from clinical and surveillance specimens, 2011–2018.

OXA-48 production. This resistance signature is based on recommendations for OXA-48 detection by Huang *et al* and Finlay *et al* [13,14].

Our CPE detection algorithm reflects others suggesting an immunochromatographic assay to confirm carbapenemase production, with a modified carbapenem inactivation method test validating negative results, with a combined sensitivity of 99.3% [15]. A limitation of our study was the inability to calculate median processing time for preliminary reporting of CPE from clinical isolates. This information could not be adequately defined due to inconsistencies in preliminary CPE reporting in the specimen work card. The value of our algorithm in reducing processing time has been demonstrated with increasing numbers of isolates. The proven capability of the carbapenem inactivation method test in recognizing a rare carbapenemase type is reassuring. However, the system remains to be fully assessed for novel and uncommon variants and should be evaluated elsewhere.

This work represents an advance in biomedical science as it demonstrates the benefit in microbiology laboratory efficiency where newly available technologies are introduced into routine use, and suggests how different methodologies can be used and adapted to meet local needs and expertise.

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Approval

No ethical approval was sought as CPE detection is a function of the routine laboratory process.

Disclosure statement

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