Evaluation of different media for introduction of a CPE screening program at a UK hospital.

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1. Background

Carbapenems are effectively last-line antibiotics for the treatment of infections due to multidrug-resistant *Enterobacteriaceae*. During the last decade, carbapenem resistance has been increasingly reported and carbapenemase-producing *Enterobacteriaceae* (CPE) is emerging as a growing challenge in healthcare settings (Grundmann *et al*., 2010). The resistant organisms produce different types of β-lactamases capable of hydrolysing carbapenems. These enzymes include the class A carbapenemases (KPC and GES types), the class B or metallo-β-lactamases (MBLs) (VIM, IPM, and NDM types), and the class D oxacillinases (e.g., OXA-48-like enzymes) ,(Livermore, 2012). It is also noted that the carbapenemase genes harboured by CPE are mostly transposon- and/or integron-encoded determinants that can easily spread to other *Enterobacteriaceae* strains and species, which means cross infection is of great concern within the NHS Hospitals (Johnson and Woodford, 2013). There is, therefore, a need to implement adequate preventive measures, including active surveillance, in order to contain the spread of these pathogens. Since gastrointestinal carriers of CPE are thought to be the reservoir of cross-transmission in health care settings, surveillance is necessary. Public Health England (PHE) recommend risk factor-based screening on admission to all acute hospital Trusts. In order to manage the laboratory demand for a large screening program, a trial of different media and in-house testing protocols was performed. The chosen screening method was then introduced for the first 6 months of patient screening.

2. Material/methods

236 CPE screening rectal swabs (including positive controls confirmed by Polymerase Chain Reaction (PCR)) were emulsified in 0.5ml saline and plated onto 4 chromogenic media – Brilliance CRE (Oxoid), ChromID carba (Biomerieux), CXmSCARBA-1, CXmSCARBA-2 (E&O), and also onto MacConkey agar with ertapenem and meropenem discs (current method). Plates were incubated for 18 hrs and interpreted according to manufacturer's instructions (Figure 2). Presumptive colonies were identified by MALDI-TOF, and disc susceptibility with the inclusion of MAST CAT-ID discs (MAST group Ltd, UK) performed and interpreted using the EUCAST (European Committee on Antimicrobial Susceptibility Testing) guideline (EUCAST,2015). Xpert Carb-R-PCR (Xpert® Carba-R 1,2014) was performed on 29 ertapenem and / or meropenem resistant isolates, and the isolate sent to the reference laboratory for confirmation (figure1). A dilution series (to 10^{-8} from a 0.5 MacFarland suspension) following the method from Wilkinson *et al*. 2012 was performed using 20 known positive isolates with 5 different mechanisms and each dilution was plated onto the 4 selective media.

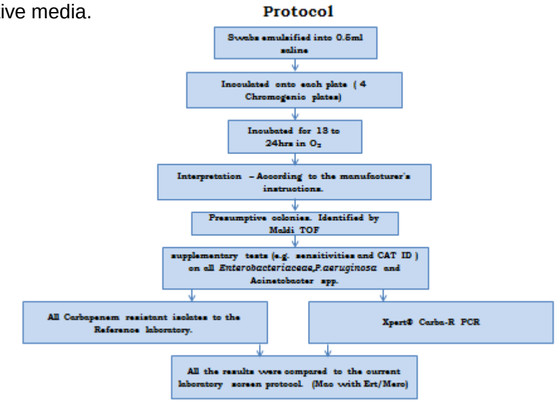

Figure 1: flowchart showing the method

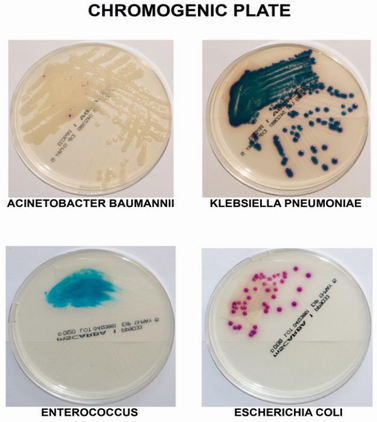

Figure 2:

	Mac with Mero/Ert	BCRE	Chrom ID Carba	CXmSCARBA-1	CXmSCARBA-2
NEG	213	204	204	206	206
POS (CPO)	23	32	32	30	30
Non-CPO	19	28	19	18	17

Table 1: Number of positive and negative CPE's isolated using different chromagenic media

Media	NDM	OXA48	KPC	VIM	IMP
BCRE	10^{-6}	10^{-6}	10^{-7}	10^{-6}	10^{-6}
Chrom ID CARBA	10^{-6}	0.5 Mac	10^{-5}	10^{-6}	10^{-6}
CXmSCARBA-1	10^{-6}	10^{-6}	10^{-7}	10^{-7}	10^{-6}
CXmSCARBA-2	10^{-6}	10^{-5}	10^{-5}	10^{-5}	10^{-5}

Table 2: Limit of detection of the chromagenic media

3. Results

49 different isolates were identified and sent to the reference lab for confirmation, yielding 33 confirmed CPEs (32 NDM, 1 OXA-48), and 19 false positive Enterobacteriacae (a combination of ESBL and AmpC hyperproducing strains with porin loss). None of the media detected all of the strains - Brilliance CRE and ChromID carba each missed 1 NDM, and CXmSCARBA-1 and CXmSCARBA-2 each missed 3 NDM isolates; MacConkey agar missed 10. The false positive detection rate was as follows: Brilliance CRE 28, ChromID Carba 19, CXmSCARBA-1 18, CXmSCARBA-2 17, MacConkey 19 (Table 1).

Out of the validation of four plates, CXmSCARBA-1 was selected as our screening method as it showed 91% specificity and sensitivity for the detection of carbapenames. It also excelled in displaying the colony morphology of all carbapenamse producing organisms. The study also concluded the limit of detection of CXmSCARBA-1(10^{-6}) was lower than the Brilliance CRE, Chrome ID CARBA and CXmSCARBA-2. Genotypic assay of Xpert® Carba-R PCR showed 100**%** specificity and sensitivity for the detection and differentiation of the *bla*KPC,*bla*NDM,*bla*VIM,*bla*OXA-48 and *bla* IMP-1 genes associated with carbapenem resistance. The MAST CAT-ID discs showed 100% sensitivity and 85% specificity and proved to be a good indicator for the presence of carbapemase enzyme in *Enterobacteriaceae* but its low specificity was due to the detection of false positives (i.e. isolates with porin loss).

In the dilution experiments, CXmSCARBA-1 was most sensitive (10^{-6} to 10^{-7} for all isolates). ChromID Carba was unable to detect the OXA48 isolates even from an undiluted 0.5 MacFarland suspension (Table 2).

CXmSCARBA-1 was the agar selected for the routine screening program and over the first 6 months 19,506 rectal screening swabs were received. 790 (4%) grew Gram negative colonies which required further work, and 283 were sent to the reference laboratory. 59 isolates were confirmed carbapenemase producers (giving a prevalence of 0.3%), including 51 confirmed as CPEs (predominantly NDM and OXA48). Xpert Carb-R-PCR gave 100% concordance with the reference lab PCR for the genes contained in the PCR, though some isolates were identified which were not included in the PCR (subsequently found to be GES-5 positive).

4. Conclusions

The sensitivity of all chromogenic agar tested was higher than using a reference method (MacConkey) agar. The Xpert PCR performed well for in-house confirmation, allowing earlier and more accurate information to be passed to infection control and clinicians, but was restricted to 5 genes. Laboratory detection and confirmation remains challenging as there is phenotypic overlap with ESBL/AMPC hyper production with permeability which led to a delay in the recognition of the GES-5 producing isolates. The protocol chosen was able to support a widespread screening program.

*PS and JD contributed equally to this work.

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