Transition from microbiological culture to Cepheid CARBA-R nucleic acid amplification technique for the detection of carbapenemase producing Enterobacteriaceae in screening samples in a high-incidence setting

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INTRODUCTION

Carbapenems are broad-spectrum β-lactam antimicrobials that are typically used as a last resort to treat severe infections, such as those caused by resistant Gram negative organisms, including extended spectrum β-lactamases. These organisms are typically resistant to all β-lactams and may also be resistant to other families of antimicrobial. Infections caused by CPE have been associated with poorer clinical outcomes.[1-5] These organisms and/or their resistance-conferring mobile genetic elements are transmissible between individuals. Enterobacteriaceae inhabit the gut and the acquisition of such an organism can result in a resistant organism or organisms being a normal part of a patient's bowel flora. Therefore it is important in a healthcare setting to who carry such organisms to prevent onward transmission. This is important to prevent infections with multi-resistant organisms as identify those individuals well as limiting the reservoir of individuals who have these organisms in the bowel flora, who may subsequently transmit this to others. Outbreaks of CPE infections and acquisitions have occurred,[6-8] and various routes and reservoirs have been implicated, including endoscopes[9] and hand-wash basins.[10] Rapid identification, isolation and implementation of infection control procedures are required to prevent onward transmission. The detection of CPE in the bowel flora of individuals may be performed by various methods, such as microbiological culture using liquid media supplemented with imipenem[11] or chromogenic media[12] followed by confirmation by various methods such as modified Hodge Test (MHT) or incubation on solid media with molecules that inhibit the enzymes in question.[13] Nucleic acid amplification techniques (NAAT) are increasingly being used due to their speed, increased sensitivity and specificity.[14,15]

Here we describe the transition from a culture-based to NAAT screening technique in a high-incidence CPE setting. The rate of positivity and turnaround time were analysed during the periods when each method was in place.

METHODS

Culture-based detection of CPE

From December 2012 to March 2014, a total of 19,714 CPE screening specimens were obtained (rectal swabs and some stool samples) from patients within a large tertiary centre for the purposes of identifying CPE gastrointestinal colonisation. Samples were plated on to ChromID ESBL agar (bioMérieux SA) with a 10µg ertapenem disc placed on the plate between the initial inoculum and the first streak, and incubated overnight at 37˚ C in air. Colonies growing within 28 mm of the ertapenem disc, were considered to be potential carbapenemase producers, and were tested by modified Hodge Test (MHT). Antimicrobial susceptibility testing (AST) was performed on MHT-positive isolates using the Vitek 2 system (bioMérieux SA). MHT-positive isolates, for which the Vitek Advanced Expert System (AES) inferred KPC or MBL (metallo-β-lactamase), or AST determined a meropenem MIC of >4 mg/L or an ertapenem MIC of >1 mg/L, were considered to be CPE.

Gene Xpert CARBA-R

From April 2014 to January 2015, a total of 7,337 double-headed rectal swabs were tested for the presence of the five most common carbapenemase-encoding genes (Table 1) using the CARBA-R cartridge assay (Cepheid, USA), in accordance with the manufacturer's instructions.

Table 1. Carbapenemase enzymes classification

Class A	KPC (*Klebsiella pneumoniae* carbapenemase)
Class B (metallo-β-lactamases, MBL)	IMP (imipenemase) VIM (Verona integron-encoded MBL) NDM-1 (New Delhi MBL)
Class D	OXA-type carbapenenmases

RESULTS

- Turnaround times were greatly reduced when using the CARBA-R assay when compared to culture (Table 2.)
- An invalid result was obtained in 67/7338 samples (0.9%). This was largely due to a failure to amplify the internal control, indicating inhibition.
- Approximately two thirds of suspected CPE colonies cultured in the laboratory were subsequently confirmed not to be CPE.
- A sub-set of this data shows that approximately 10% of CARBA-R positive samples are culture negative.

Table 2. Summary of Results

	Culture *Dec 12 – Mar 14*	**CARBA-R** *Apr 14 – Dec 14*
Samples tested	19535	7338
Positive results (%)	783 (4.0%)	247 (3.4%)
Mean laboratory turnaround time	35 hrs 42 mins	6 hrs 03 mins

Figure 1. CARBA-R test cartridge

Figure 2. ESBL selective media

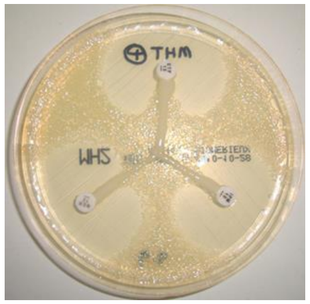

Figure 3. Positive Modified Hodge Test

Table 3. Changes implemented to CARBA-R processing to prevent contamination.

CARBA-R Procedural Changes
Samples processed in batches of 12 including a negative control swab
Work space cleaned with Tristel Duo (Tristel UK) and DNA Zap (Thermofisher, UK) before every batch
Swabs snapped over an absorbent piece of laboratory paper to enhance visualisation of splashes
Gloves changed after the snapping of every swab into buffer
All sample reagent vials inoculated first
Molecular grade tips and a pipette used to inoculate cartridges

DISCUSSION

The implementation of the molecular assay undoubtedly reduced the turnaround time for the detection of CPE in screening samples, which allowed faster isolation of colonised patients and allowed faster movement of negative patients. All positive CARBA-R samples were subsequently cultured to obtain the organism for speciation and full antimicrobial susceptibility testing. Due to the increased sensitivity of NAAT, not all positives could be confirmed by culture. This creates some issues in giving empirical antimicrobial advice in unwell, CPE-colonised patients.

Bacterial culture took considerably longer and resulted in the unnecessary characterisation of non-CPE isolates, which has staff time and cost implications.

Procedural changes were required (Table 3) for CARBA-R processing after false positive results were identified after the end of this study period. CARBA-R is marketed as a simple and easy to perform test. However, it is a sensitive molecular test, that requires meticulous sample processing to prevent false-positive results due to sample-to-sample contamination particularly during batch processing.

Positivity rates are comparable between the two methods. However, the patient populations that were screened in these two time periods differed due to operational requirements and risk assessments, so direct comparisons are not possible. Likewise, one might expect a higher numbers of positive results using a NAAT, but changes to the screened populations may account for this.

The CARBA-R molecular assay provided rapid, sensitive results for CPE screening that allowed rapid isolation of colonised individuals. However, consideration is required regarding whether a costly test is appropriate in every setting.

Further work is required to establish whether the provision of rapid results, followed by isolation of colonised patients results in decreased rates of transmission in our setting.

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