Detecting carbapenemase genes 24 hours earlier:
verification and implementation of the Cepheid Xpert Carba-R assay for bacterial isolates grown on alternative agar plates

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INTRODUCTION

Background

Increasing carbapenem resistance among Gram-negative bacteria is a major public health concern worldwide [1].

As part of Public Health England's (PHE) national enhanced surveillance programme for carbapenemase producing enterobacteriaceae (CPE), the PHE Public Health Laboratory Birmingham provides a specialist regional service for molecular detection and confirmation of the most common carbapenemase genes.

At inception (January 2015), this service utilised an in-house PCR assay developed by the PHE antimicrobial resistance and healthcare associated infections (AMRHAI) reference unit. However, since then, version 2 of the Xpert Carba-R assay from Cepheid has become available.

The Xpert Carba-R assay is a random access PCR which rapidly detects and differentiates the five most common carbapenemase genes (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{IMP}$). However, all previous validation/verification studies have used the Xpert Carba-R assay with isolates taken from the manufacturer's recommended solid media i.e. blood agar or MacConkey agar [2-6]. In many clinical diagnostic laboratories these are not commonly used media for the culture or referral of enterobacteriaceae. So, to use the Xpert Carba-R according to the manufacturer's validated recommendations requires sub-culture prior to testing, thereby introducing a delay of up to 24 hours in carrying out confirmatory testing for suspected CPEs.

Aims

- To verify the Cepheid Xpert Carba-R assay for bacterial isolates grown on alternative agar plates: CLED, chromID CARBA SMART and nutrient agar.
- To evaluate whether implementation of the Cepheid Xpert Carba-R assay improved turnaround times (TATs) for molecular detection and confirmation of the most common carbapenemase genes.

METHODS

Verification of carbapenemase gene detection from isolates grown on blood agar and alternative agar media

65 bacterial isolates were used including 6 IMP, 12 KPC, 14 NDM, 13 OXA, 14 VIM positive and 6 negative controls.

All resistance gene profiles were well characterised by National Collection of Type Cultures (NCTC) or previous confirmation by the AMRHAI reference unit.

All isolates were sub-cultured to Columbia blood agar (CBA) and a sub-collection of 19 isolates (table 1) were also sub-cultured to Cystine-Lactose-Electrolyte-Deficient agar (CLED); chromID CARBA SMART agar (bioMérieux) and nutrient agar slopes and incubated in air overnight at 35-37ºC to obtain single colonies for testing.

Suspensions of bacterial strains grown on the different media were prepared and tested using the Xpert Carba-R Assay v2 (Cepheid) run on a GeneXpert IV instrument as per the manufacturer's instructions. Results of the assay were automatically interpreted by the GeneXpert software using embedded calculation algorithms.

Carba-R assay cartridge

Evaluating laboratory turnaround times

The Xpert Carba-R Assay v2 was implemented in the laboratory on 10/04/2017.

Data were downloaded from the laboratory information system (LIMS) for every bacterial isolate tested in the laboratory for molecular CPE detection and confirmation from 14/11/2016 to 28/06/17.

TAT was calculated in days for each tested isolate by subtracting the date of receipt of the isolate from the date of result authorisation.

RESULTS

Verification of carbapenemase gene detection from isolates grown on blood agar and alternative agar media

For all 65 isolates tested, the Xpert Carba-R assay result from CBA plates was completely concordant with the known gene profiles.

The 19 isolates (table 1) that were tested from CLED, chromID CARBA SMART agar and nutrient agar slopes showed no differences in the gene profiles obtained by the Xpert Carba-R Assay on any of the media tested when compared with the expected profile and that obtained from CBA.

Study no. or type collection no.	Species	Known carbapenemase gene profile
C1	*Enterobacter cloacae* complex	IMP
C2	*Klebsiella pneumoniae*	IMP
C3	*Escherichia coli*	IMP
C25	Kluyvera sp.	KPC
C44	*Citrobacter freundii*	KPC
C54	*Raoultella planticola*	KPC
NCTC 13438	*Klebsiella pneumoniae*	KPC
C17	*Citrobacter freundii*	NDM
C18	*Klebsiella pneumoniae*	NDM
C22	*Escherichia coli*	NDM
NCTC 13443	*Klebsiella pneumoniae*	NDM
NCTC 13442	*Klebsiella pneumoniae*	OXA-48
C35	*Escherichia coli*	OXA-48
C36	*Klebsiella pneumoniae*	OXA-48
C43	*Enterobacter aerogenes*	OXA-48
C10	*Klebsiella oxytoca*	VIM
C11	*Citrobacter amalonaticus*	VIM
C9	*Enterobacter cloacae* complex	VIM
NCTC 13437	*Pseudomonas aeruginosa*	VIM

Table 1. Sub-collection of 19 isolates tested with Xpert Carba-R from CBA, CLED, chromID CARBA SMART and nutrient agar slopes

Laboratory turnaround times before and after implementing Xpert Carba-R Assay v2

- No of samples in data set for service using in-house PCR =145
- No of samples in data set for service using Xpert Carba-R assay = 112

Figure 1 shows that implementation of the Xpert Carba-R assay has improved TAT by about one day. Using the Xpert Carba-R assay, 38% of isolates submitted for testing had results authorised on the same day and 74% within two days.

On investigation of the TATs over three days (no service at weekends), we found that most were due to samples that had been received in the laboratory for culture, and then cultured isolates had been sent for molecular detection and confirmation of carbapenemase genes a few days after initial sample receipt, thus making the time of receipt earlier than it was actually received for the carbapenemase gene assay.

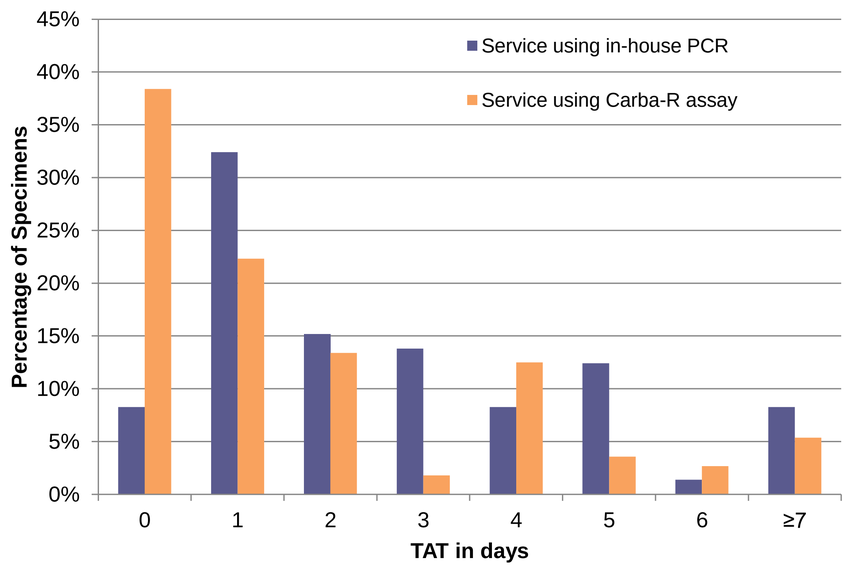

Figure 1. Turnaround times for molecular detection and confirmation of carbapenemase genes before and after Xpert Carba-R assay implementation

A SIDE STORY

Isolate C22 (an NDM positive *E. coli*) tested positive for the NDM gene when taken from CBA and CLED (at this point not yet tested from chromID CARBA SMART).
However, when sub-cultured to a nutrient agar slope, the growth on the slope tested negative for all carbapenemase genes and was also negative on repeat testing from the same slope.
Fresh chromID CARBA SMART, CBA and CLED purity plates were made from the original sub-culture from the freezer and from a sweep of culture from the nutrient agar slope culture. Ertapenem disks (10 µg) were added to the CBA and CLED plates.
Following overnight incubation, the colony picked from the original sub-culture from the freezer was observed growing up to the ertapenem disks and on the chromID CARBA SMART agar (phenotypically carbapenem resistant).
The growth taken from the nutrient agar slope was morphologically identical to the one from the original sub-culture but showed a clear zone of growth inhibition around the ertapenem disk and was not growing on the chromID CARBA SMART agar (phenotypically carbapenem sensitive).
The genotypic results from the Xpert Carba-R assay from this slope were therefore consistent with the apparent phenotype.

CONCLUSIONS

- By verifying the Xpert Carba-R assay for testing isolates directly from media more likely to be used for CPE culture or submission by post, we have removed the need for pre-PCR sub-culture and a consequent overnight delay.
- The apparent discrepant results described for isolate C22 (see side story) indicate that isolates can lose carbapenemase genes on subculture and therefore sub-culturing of suspected CPEs to non-selective media should be minimised where possible.
- Because the Xpert Carba-R is random access, and hands-on time is minimal, isolates can be tested as they arrive and turnaround times from receipt of suspected CPE isolates to reporting after implementation are on average one day shorter since the implementation of the new assay.
- Issuing results of confirmation of carbapenemase genes to users in a more timely fashion will allow implementation of appropriate infection prevention and control measures as soon as possible.

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