An Introduction Of An External Quality Assessment Scheme For The Identification Of Carbapenemase Producing Organisms

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Schematic in the development of a new EQA scheme

Justify EQA is relevant to clinical needs → Background research including sending questionnaire to labs → Design specimens → Perform stability, homogeneity and viability studies → Carry out pilot studies with interest registered labs → Collate and analyse results for evidence to support the delivery of the EQA → Proceed to a live scheme

Introduction

Carbapenemase-producing organisms (CPOs) are multi-drug resistant bacteria which have emerged through increased and often inappropriate usage of carbapenems. Carbapenems are a broad-spectrum β-lactam antimicrobial frequently used as a 'last resort' for treating life-threatening nosocomial infections. CPOs hydrolyse carbapenems via the production of the enzyme carbapenemase, causing drug treatment to become ineffective[1].

The increase in prevalence and clinical impact of nosocomial infections caused by CPOs is a global health concern[2]. In 2017, the World Health Organization published their first ever list of 'priority pathogens' which considered carbapenem resistant *Acinetobacter baumannii, Pseudomonas aeruginosa* and, *Enterobacterales* priority 1 critical organisms for urgent need of new antimicrobial agents[3].

Due to their ability to readily spread and colonise patients in healthcare environments, preventing the transmission of these organisms is a major public health initiative. Therefore, there is a warranted interest for an external quality assessment (EQA) scheme to assess the performance of laboratories providing a service in the detection of the genes that cause the 'big five' CPOs – VIM, NDM, KPC, OXA-48, and IMP, which are the most prevalent in the UK[4].

Aim

To ascertain the most suitable format to prepare simulated specimens for distribution within an EQA scheme targeted at CPO screening.

Methods

A study was carried out preparing simulated specimens in both lyophilised and swab format, each containing organisms possessing one of the five dominant carbapenemases (NDM, OXA, IMP, VIM, KPC). The vials and swabs were stored at different conditions to examine the viability and stability of the target organisms over an eight week period.

Choice of sample medium

- To compare the viability, homogeneity and stability of lyophilised specimens (our gold standard) compared to swabs (liquid amies, dry swab inoculated with brain heart infusion broth, and fecal transwab).
- Swabs were chosen for evaluation, as this is the most sensitive medium for CPO screening[4].

Stability testing

- Swabs (liquid amies, dry swab and fecal transwab) were inoculated with a prepared bacterial suspension, containing the CPO.
- The simulated specimens were then stored at ambient temperature (22°C) and cold storage (4°C).
- Specimens were tested by inoculation onto both MacConkey and mSuperCARBA chromogenic agar (in duplicate).
- Results of colony morphology and growth were recorded from the lyophilised vials and swabs.
- A lateral flow test was performed using a CARBA-5 assay to identify the carbapenemase present.
- This was carried out at 10 time points, which included 24 and 48 hours post preparation, followed by weekly for a total of 8 weeks.

Note: Testing on the fecal transwab was carried out for a week due to unavailability of this swab type.

Pilot study

Two pilot distributions including both a lyophilized sample and a liquid amies swab containing the same target CPO was sent to 63 participants, all which expressed interest in participating in the pilot distributions.

Results

- All sample types provided expected and consistent results throughout the 8 week period of stability testing. However, the swabs stored at 4°C demonstrated reduced organism yield of viable growth of the test organism.
- The CARBA-5 assay correctly identified the CPO up to 8 weeks post preparation, with the dry swab demonstrating the least favorable results (faint bands).
- Swabs stored at ambient temperature demonstrated greater organism stability after 8 weeks in the growth of the pathogen compared to swabs stored at +4°C.

Table 1: Intended results for the pilot distributions 5150 and 5157 distributed to participants.

Distribution No.	Specimen No.	Organism	Carbapenemase Detected
5150	7064	*Klebsiella pneumoniae*	KPC
	7065	*Enterobacter cloacae*	NDM
5157	7092	*Pseudomonas aeruginosa*	IMP
	7093	*Klebsiella pneumoniae*	OXA-48

- The pilot distributions included CPO's that were selected for the stability testing study.
- Participant results were submitted online via the UK NEQAS for Microbiology website.
- Results from the pilot distributions are illustrated in Table 2.

Table 2: Results from the pilot distributions 5150 and 5157 for the lyophilized and liquid amies swab respectively.

Specimen Number	Reported correct results (%)		Reported incorrect results (%)		Not examined (%)		Reported correct CPO (%)
	Vial	Swab	Vial	Swab	Vial	Swab	Vial and Swab
7064	88	82	0	0	12	18	90
7065	96	78	0	4	4	18	69
7092	96	80	4	4	0	16	74
7093	80	70	14	12	8	20	84

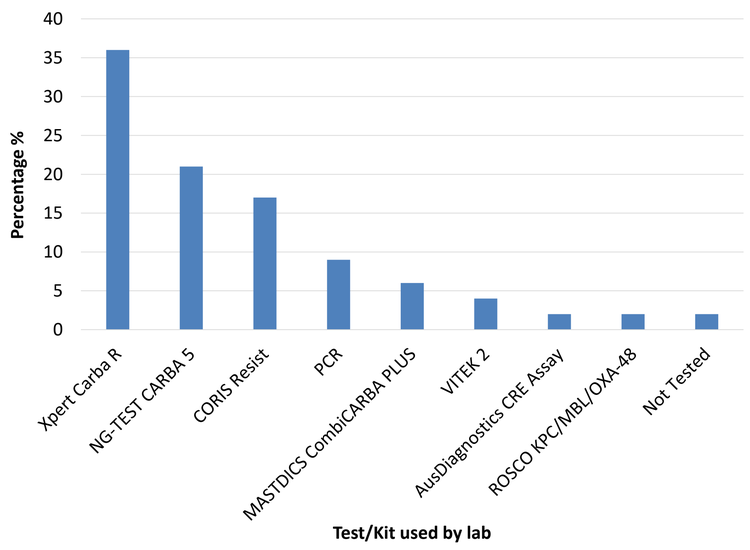

Figure 1: The type of test/kit used for CPO detection in the first pilot distribution 5150.

- The most common test identified was the Xpert Carba R method, with 36% of participants using this method.
- The NG-TEST CARBA 5 assay was used by 21% of participants. This kit was also used during the stability testing study.

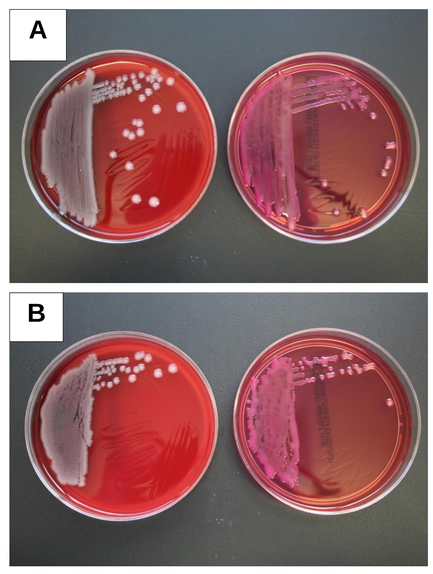

Figure 2: Returns testing plates (CBA and MACs) grown at 37°C for 24 hours for specimen 7093 of the pilot distribution 5157. **A**) Returns testing from a liquid amies sample. **B**) Returns testing from a lyophilized specimen.

- Returns testing is routinely performed by UK NEQAS. The process involves distributing specimens to selected laboratories in the UK. On return to UK NEQAS, the specimens are quality control checked for stability, homogeneity and viability. Thus ensuring the integrity of specimens are unaffected during transportation.
- Returns testing on specimen 7093 (Figure 2), illustrates growth from both the liquid amies swab and the lyophilised specimen.
- There are comparable results for both sample types, with the liquid amies samples producing a slightly higher yield.

Discussion

- The stability study confirmed CPO viability and homogeneity was maintained over the 8 week period.
- The most appropriate swab type was identified to be the liquid amies swab as it showed the most consistent results after 8 weeks.
- The liquid amies swabs were able to produce similar results to those of the lyophilised vials, containing the same organism. However, there were a greater number of participants who did not report results for the liquid amies swab, as some participants were unsure on how to process the sample.
- The correct CPO was reported by 69-90% of participants across the four specimens in both pilot distributions, as illustrated in Table 2.
- For specimen 7093, 12% of participants reported no growth of the CPO (Figure 2), however it was observed that all of these laboratories used the Brilliance CRE agar to culture the sample.
- There is currently further stability testing being performed to determine the viability of specimens to include commensal organisms. This is demonstrating promising results, therefore future distributions could include the use of selected commensals.

Conclusion

- Analysis of participant results has demonstrated swab format is an appropriate simulated specimen for distribution in this new EQA. This provides a more authentic sample type for clinical diagnostic laboratories.
- Participation in an EQA is a valuable tool in the quality assurance of CPO testing in the diagnostic laboratory and demonstrates the validity of comparing collated data between laboratories.
- EQA is an important tool in providing evidence of competence.

Acknowledgements

Thank you to the team at UK NEQAS for Microbiology at UKHSA Colindale for their excellent work in the development of the EQA in the detection of CPO's in clinical specimens. Thank you also to the laboratories that participated in the pilot distributions.

References

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