

Use of the Acuitas Resistome Test to detect and type carbapenemase producing organisms (CPOs): A pilot study

GL Vanstone¹, S Rattenbury¹, R Smith², D Mack² & I Balakrishnan²

¹ Health Services Laboratories
² Royal Free London NHS Foundation Trust

Royal Free London **NHS**
NHS Foundation Trust

BACKGROUND

Characterisation of Gram-negative multi-drug resistant organisms (MDRO) in the diagnostic laboratory can be difficult because the different mechanisms (carbapenemase, ESβL, AmpC etc.) are associated with many different genes and phenotypes.

Culture based methods based on antimicrobial susceptibility testing (AST) are often cost effective and easy to implement. However, at best, they only provide detail on the class of MDRO (e.g., ESβL, AmpC, MβL) rather than the specific genes that are present. The presence of multiple enzymes may not be detected, and methods can lack sensitivity and/or specificity (particularly for OXA-48 carbapenemase detection).

Molecular based detection methods are rapid, but assays currently available to diagnostic laboratories are unable to detect the large number of genotypes present worldwide; only those deemed to be the most relevant and/or prevalent in a particular setting are detected. Methods can be expensive and lack the ability to detect novel/newly emerging genotypes.

MDRO detection methods currently available to routine diagnostic laboratories do not provide any typing information. This service is currently provided by reference facilities and long turn around times can result in delayed identification of outbreaks.

The OpGen Acuitas® Resistome Test (OpGen Inc. Gaithersburg, MD) allows detection of genes associated with carbapenemase (25 genes/208 subtypes), ESβL (13 genes/557 subtypes) and AmpC (11 genes/147 subtypes) production.

Results can be used to produce 'Acuitas Lighthouse Profile', created from the positive and negative results of the resistance genes, the organism ID, AST profile, and the number of occurrences (present in a previously tested database) (Figure 1), therefore providing detailed typing of organisms that could allow early outbreak identification, and improved information for clinical management and infection control decision making.

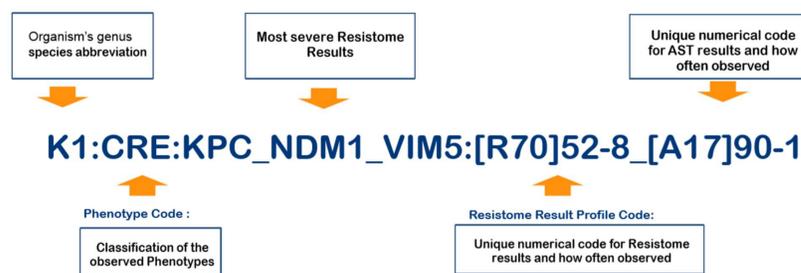


Figure 1 Example of Lighthouse Profile, together with information used to create the unique identification

METHODS

50 isolates were tested by the Acuitas Resistome Test, a microfluidic PCR array.

Isolates included; 44 CPO confirmed strains (14 *E. coli* [6 NDM, 8 OXA-48], 14 *Klebsiella* spp. [7 OXA-48, 3 NDM, 2 KPC and 2 NDM + OXA-48], 6 *P. aeruginosa* [5 VIM, 1 NDM], 5 *A. baumannii* [2 OXA-23, 2 NDM and 1 OXA-23 + NDM], 2 *Enterobacter* spp. [1 OXA-48 and 1 IMI], 2 *Providencia* spp. [2 VIM], 1 *Serratia* spp. [1 OXA-48]) isolated from clinical and screening samples between 25/01/16 to 01/11/16, and 6 control isolates.

All CPO isolates were obtained from either clinical or screening samples received at The Royal Free London NHS Foundation Trust during 25/01/16 – 01/11/16. The presence of carbapenemase genes was confirmed by the reference laboratory (AMRHAI, PHE).

RESULTS

- Results from the Resistome Test show that the CPO isolates harbour a wide range of other resistance genes (Fig. 2).
- 29/33 (87.9%) carbapenemase positive Enterobacteriaceae were shown to have other β-lactamases (ESβL and/or AmpC) present - these ESβL and AmpC genes were not detected by our routine culture methods, as in most cases the presence of the carbapenemase would have masked the presence of the other β-lactamases.
- The correct carbapenemase genotypes were identified in all isolates tested.

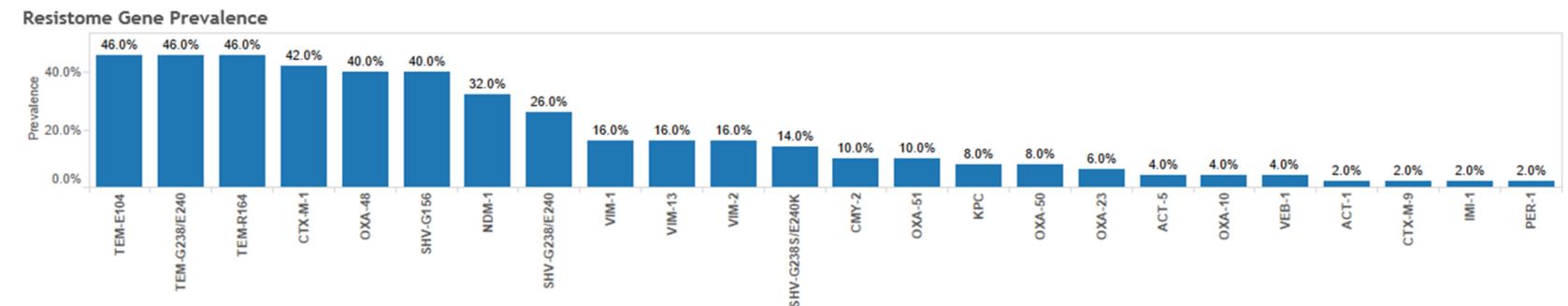


Figure 2 MDRO gene prevalence in clinical isolates detected using the Resistome Test

- From the 44 clinical isolates tested, 28 different lighthouse profiles were obtained, of which, 19 were unique.
- Identical lighthouse profiles were obtained for 9 groups of clinical isolates, including 5 *P. aeruginosa* VIM, 3 *E. coli* NDM, 3 *E. coli* OXA-48, 3 *K. pneumoniae* OXA-48, 3 *K. pneumoniae* KPC, 2 *E. coli* NDM, 2 *E. coli* OXA-48, 2 *K. pneumoniae* OXA-48 and 2 *P. stuartii* VIM isolates (Fig. 3).
- Within these, were 7 groups of isolates (2 *P. aeruginosa* VIM, 3 *E. coli* NDM, 2 *E. coli* NDM, 2 *E. coli* OXA-48, 2 *P. stuartii* VIM, 2, *K. pneumoniae* KPC and 2 *K. pneumoniae* OXA-48) obtained from 6 patients, either isolated over time, or from different sites.

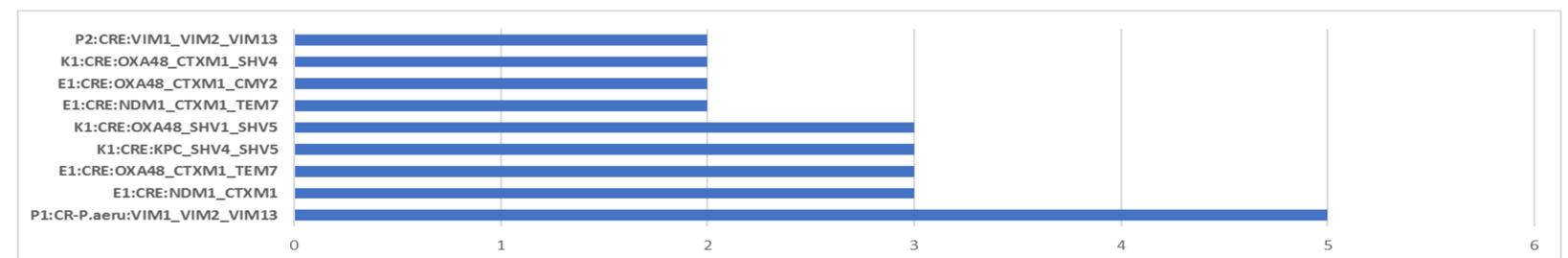


Figure 3 Lighthouse profiles obtained multiple times from clinical isolates

- Most diversity was seen amongst the Enterobacteriaceae (*E. coli* = 8 Lighthouse profiles from 14 isolates [10 patients], *K. pneumoniae* = 10 Lighthouse profiles from 14 isolates [12 Patients]). Less diversity was seen amongst the VIM producing *P. aeruginosa* isolates (1 lighthouse profile from 5 Isolates [4 Patients]). In general terms, this mirrors what we see from VNTR typing, that is performed on some CPOs sent to the Reference Laboratory.

CONCLUSIONS

- The Resistome Test is able to detect a large number of MDR genes, and is able to type isolates based on ID, the resistance genes the organism carries and the AST profile.
- Identical Lighthouse profiles were obtained from 7 groups of isolates obtained from 6 individual patients, either isolated over time, or from different sites – highlighting potential for this assay to be used as a typing tool to aid outbreak identification.
- Lighthouse profiles can be compared to a database of previously tested isolates, which can include information related to the patients, such as location within the hospital/community and other demographics; potentially allowing detection of outbreaks earlier than currently possible in the routine diagnostic laboratory.
- To fully evaluate the potential of this assay as a typing tool that can be used for outbreak management, we plan to compare results to whole genome sequencing and other more established typing methods, such as VNTR, particularly for isolates obtained from different patients that had identical lighthouse profiles.

CONTACT / ACKNOWLEDGEMENTS

Contact: gemma.vanstone@nhs.net, Resistome testing performed by OpGen