Active screening for multiresistant Enterobacteriaceae

The control of multiresistant organisms (MROs) is made difficult by a large reservoir of unrecognised, asymptomatic colonised patients. Hence, active screening is generally used as part of a multifaceted infection control intervention. Active screening for multiresistant Gram-negative bacteria (MRGNB) involves collection of screening specimens from patients with relevant risk factors. Positive results may result in institution of contact precautions, cohorting of patients and enhanced cleaning and surveillance. Active decolonisation is used for some Gram-positive bacteria (e.g. *Staphylococcus aureus*) but is not thought effective for Gram-negative bacteria, especially those in which resistance is highly transmissible (e.g. plasmid-borne). Accurate and rapid identification of positive specimens allows prompt intervention to interrupt the transmission cycle, and exclusion of MRO colonisation reduces the adverse impact on patient care and hospital workflow. Knowledge of MRO status may also improve the appropriateness of empiric antibiotic prescribing.

For MRGNB the ratio of colonised to infected patients has been estimated at ~8:1 for extended spectrum beta-lactamases and 9:1 for carbapenem-resistant Enterobacteriaceae (CRE). In addition to this, the very mechanism by which antibiotic resistance is transferred between common species like *Escherichia coli* and *Klebsiella pneumoniae* is such that resistance acquisition can be highly variable in phenotype and often relatively ‘silent’. Antibiotic susceptible organisms can acquire resistance mechanisms very quickly, so that the potential for acquisition by gene transfer is itself an important risk to understand.

The most appropriate specimen type for screening of MRGNBs depends on the organism’s likely habitat on the human body or clinical environment. Thus, the most sensitive specimens for detecting Enterobacteriaceae may be rectal swab, peri-rectal swab or faeces, but wounds and respiratory samples and even environmental sampling (sinks, drains, surfaces) may be more important for the control of outbreaks due to organisms such as *Pseudomonas* or *Acinetobacter*. Increased specimen volume and a higher MRO faecal density both increase yield of screening. For this reason faecal samples may perform better than rectal swabs, although the latter is more practical. Using more than one screening specimen or specimen type may increase sensitivity, but again for practical purposes one specimen is usual. Concurrent antibiotic use has a variable impact on MRO faecal density but logic dictates that detection of a reservoir of resistance is more likely in the presence of relevant selection pressure.

Active screening specimens may be processed in the laboratory using phenotypic (culture based) or direct genotypic (amplification and detection of bacterial DNA) methods. There is no consensus as to the optimal laboratory processing method but factors that should be considered are local molecular epidemiology (including the likely minimum inhibitory concentration (MIC) of relevant antibiotics against target isolates), availability of expertise and equipment, cost, capacity for integration into laboratory workflow and test performance including sensitivity, specificity, limit of detection and turnaround time. Currently, phenotypic methods are more widely used, although we anticipate that with advances in automation, data...
processing and reductions in cost these will be replaced by genotypic methods in the future. The main advantages of genotypic methods are shorter achievable turnaround times (and potential for point-of-care testing), improved sensitivity and reduced need for human resources. Potential disadvantages of genotypic methods include higher costs, a need for expertise/complex equipment and the lack of an isolate for susceptibility testing or typing if using direct screening without culture.

Regardless of which screening method (phenotypic or genotypic) is used for detecting transmissible antibiotic resistance, one must accept a trade-off between (a) the risk of overlap between the highest naturally occurring MIC of wild-type organisms (that is, those without the targeted resistance) and those with a low MIC in the presence of a relevant mechanism, especially one that is readily transmissible to an organism in which it facilitates high levels of resistance and (b) the failure to identify a new or variant mechanism. This is illustrated by the overlapping ceftazidime MICs of local E. coli populations with common transmissible ESBL genes (Figure 1).

We will now use the example of CRE to examine the spectrum of active screening options. CRE are currently infrequent in Australia; in the 2011 AGAR study of hospital-acquired Enterobacteriaceae, eight of 2635 isolates possessed a carbapenemase.11 However, recent importation of blaNDM12 and blaOXA-4813 and the development of endemic CRE in Australia (primarily blasmf-l14 prompted the Australian Commission on Safety and Quality in Health Care to release national guidelines recommending active screening for CRE15. This document suggests screening high-risk patients using a rectal swab, peri-rectal swab or faeces with or without a swab from open wounds or urinary catheter specimens.

These specimens may be processed using a variety of phenotypic methods that have a turnaround time of 24–48 h. Inhouse methods include a MacConkey plate impregnated with meropenem (1 ug/mL) or the ‘CDC Method’16, which involves inoculation of TSB broth containing a 10μg meropenem disc, then subculture the next day onto MacConkey agar. Although cheap and readily available, these methods do not perform as well as commercial chromagar17. At the time of writing, only three commercial chromagar designed specifically for CRE detection were available in Australia, all of which contain proprietary ingredients and cost approximately $3–4 per plate. Comparative studies are hampered by a lack of an agreed gold standard but commercial chromagar appear to be highly specific and have reasonable sensitivity17. A new agar medium not yet widely available, known as SuperCarba, may be a promising alternative18. Sensitivity of screening agars is less for isolates with a lower carbapenem MIC19. Phenotypic screens may be made more sensitive by deploying low-level screening cut-offs, although the latter needs understanding of other contributive factors that are more important in some organisms than others (Figure 2). For example, it would be most appropriate to screen with a low antibiotic concentration for a highly transmissible resistance plasmid with commonly low MICs in an organism like E. coli (e.g. typical plasmids carrying blasmf-blasmf) but a resistance gene with limited capacity to move beyond the host bacterial species (e.g. blaoxa23 in Acinetobacter baumannii) might be more efficiently screened using a much higher antibiotic concentration. Screening with meropenem 1 μg/mL is almost certainly too high in E. coli; a cut-off of meropenem or ertapenem ≥0.5 μg/mL has been recommended20.

The ceftazidime epidemiological cut-off (ECOFF) of 0.25 μg/mL appears to be reliable for metallo-betalactamases and blakePC and can also be expected to additionally identify plasmid AmpC and ESBL gene carriage. For emerging blaoxa-48-121-containing strains
reported cefotaxime MICs are lower\textsuperscript{21}. Although ESBLs are not uncommon accompaniments, there is no strong genetic link to \textit{bla}_{OXA-48}\textsuperscript{22} so that active screening with cefotaxime only may not suffice. An alternative approach with piperacillin/tazobactam and temocillin discs for screening of suspected isolates appeared effective in a large recent European study\textsuperscript{23}. Regardless of the culture-based method being used, laboratories should evaluate test performance in their local setting using clinical specimens and/or stored genotypically confirmed CRE isolates.

Genotypic screening may be conducted by manual or semiautomated nucleic acid test methods such as PCR, gene probe, or some combination of variation of these, either direct or following an antibiotic-containing growth step, as above. Several direct methods are practical for laboratories with basic molecular biological expertise (Table 1), but rely on either a known target or at least an understanding of the likely mechanism. These can be better focused by the judicious use of phenotypic screens, including specific detection of relevant (e.g. carbapenem hydrolysis) activity such as the NP Carba test\textsuperscript{24}.

Genotypic screens (even direct PCR) can have high predictive value if informed by local epidemiology. The negative predictive value of genetic testing for resistance to aminoglycosides and/or major beta-lactams can be greater than 99.5% in a country like Australia with relatively low pre-test probability\textsuperscript{25}, but the optimal frequency with which this must be surveyed and the applicability of this approach in other countries, including high-prevalence settings, remains to be tested.

In summary, when used alongside other infection control measures, active screening is a useful tool that may assist with limiting the spread of MRGNBs. There is no consensus as to the optimal laboratory processing method, although genotypic methods are likely to become the most frequently used in the future. An understanding of local molecular epidemiology and MIGs will assist laboratories in selecting the most appropriate screening assay for their setting.

### References


### Table 1. Carbapenemase detection molecular assays for use direct on specimens.

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<th>Specimen</th>
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</table>

ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.
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In Focus


Biographies

Dr Paul Ingram graduated from the University of Western Australia in 1999. He is both an Infectious Diseases Physician and Clinical Microbiologist working at Royal Perth Hospital, Western Australia. He completed an Infectious Diseases Fellowship at the National University of Singapore and is currently Clinical Senior Lecturer at the University of Western Australia. His interests include beta-lactamases in gram-negative bacteria, the epidemiology and infection control responses to antibiotic resistance and antimicrobial stewardship.

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