Making sense of resistance genes

New technology in defining phenotypic and molecular methods in detecting and understanding bacterial resistance

In recent years, a wide variety of new technology has been introduced in the laboratory for detecting resistance markers. This has helped the scientific and medical community in detecting and understanding antimicrobial resistance. One thing that we have learned from this new technology is that both phenotypic standardised methods and molecular techniques are needed to understand the complex evolution of resistance. Table 1 shows organisms with unusual bacterial resistances that need reference laboratory confirmation [1].

New resistance may emerge from the appearance of a new resistant marker in an opportunistic organism – pathogen or commensal. The appearance of new mutations in chromosomes or plasmids, the acquisition of the 'new' resistance mechanisms and transfer of 'old' gene into 'new' species can all impact on resistance. The resistant bacterial population requires reliable detection with standardised susceptibility testing methods such as CDS, CLSI or BSAC and some confirmation with validated molecular methods [2].

Apart from those unusual resistances shown on Table 1, resistance genes that may require further molecular confirmation in some routine laboratories include: methicillin/oxacillin resistance for the *mecA* gene in staphylococci *(Staphylococcus aureus* and coagulase-negative staphylococci); *vanA* and *vanB* genes in enterococci (in Australia the low level *vanB* gene detection has been a problem for many laboratories); quinolone resistance *gyrA* and *parC* genes in staphylococci and enterobacteria (ciprofloxacin resistance in *Salmonella typhi* and *Salmonella paratyphi* in enteric fevers); *mef* and *erm* genes with mediated macrolides (MLS) resistance in *S. pneumoniae* or staphylococci (especially community acquired MRSA); inducible beta-lactamases resistances in extended spectrum beta-lactamases (ESBLs) and

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carbapenemases resistance (metallo-beta-lactmases resistances) in Gram-negative bacteria (GNB). These genes and mechanisms of resistance have been dealt with in detail in the latest ASM Antimicrobial SIG textbook *Antimicrobial susceptibility testing: methods and practices with an Australian perspective* [3].

Standardised susceptibility methods in most cases provide >95% agreement for some organisms *in vitro* in detecting resistance. Population analysis reveals that there are other physiological barriers or constraints apart from a possible genetic marker for an organism carrying resistance within a population. In such cases, irrespective of clinical breakpoint criteria published, microbiologists and laboratories need to re-consider susceptible and resistant breakpoints set by different committees [4]. This was the focus of the ASM Antimicrobial SIG Workshop in Canberra 2005 [5].

Physiological bacterial resistant barriers in both Gram-negative and Gram-positive bacteria may include one or several factors such as modified penicillin-binding proteins (PBP) sites, exclusion by porin down-regulation, efflux systems and inner membrane transporters, membrane fusion proteins and efflux-associated outer membrane protein. Figures 1-5 illustrate some of these physiological resistance mechanisms [3, 6].

In GNB, the ability to regulate access to the cell through a hydrophobic outer membrane, coupled with the ability to acquire genetic material under selection of antibiotic pressure, may be one of the reasons why bacteria such as *Acinetobacter baumannii* and *P. aeruginosa* are becoming highly resistant and an emerging resistant threat in many hospitals [3]. This is one topic for discussion in a symposium titled *Gram-negatives rule the resistance roost!* for the ASM annual meeting in 2006.

In Gram-positive organisms, a similar problem is encountered. This is best exemplified in the detection of heterogeneous vancomycin-intermediate *S. aureus* (hVISA). Therapeutic failures have been documented worldwide in clearing MRSA and some MSSA infections [7]. Isolates from such infections contain subpopulations that are resistant to the glycopeptides vancomycin and/ or teicoplanin.

Phenotypic characteristic of hVISA are a thickened cell wall with alteration in the chemistry, structure and degrees of cross-linking of the peptidoglycan. An altered proportion of PBPs, increased production or decreased breakdown of cell wall, together with altered enzyme profiles and substrate requirements, result in altered cell wall charge and structure. Glycopeptide becomes trapped

MICRO-FACT

There is more life below the earth than above it.

Figures 1-5. Physiological mechanisms of resistance in bacteria [3,6].

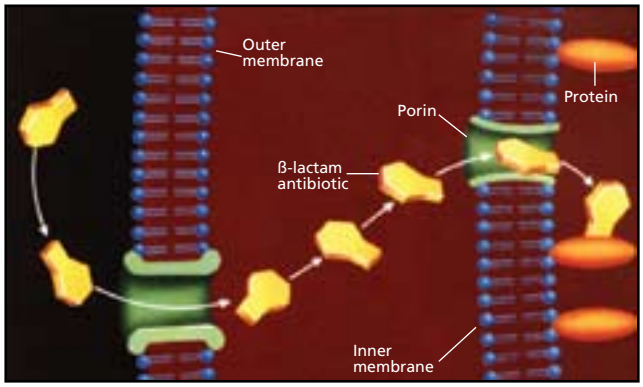

Figure 1. Permeability – the outer membrane of Gram-negative (GNB) bacteria contains porins that allow entry of antibiotic molecules.

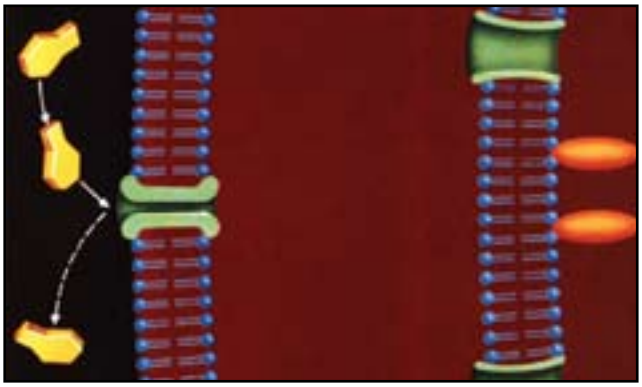

Figure 2. Permeability changes – the closing of the porins inhibits the passage of antibiotic molecules through the outer membrane, causing bacterial resistance.

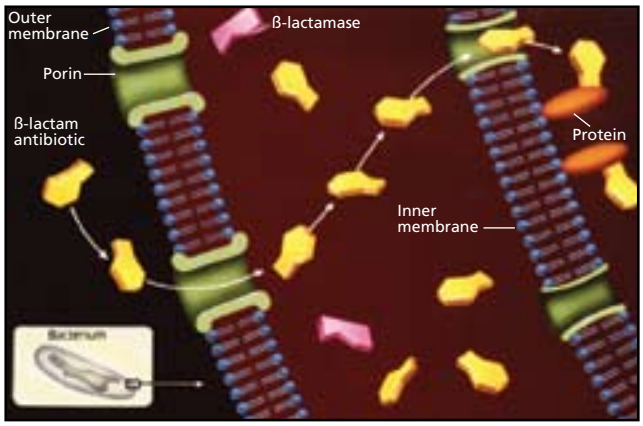

Figure 3. Beta-lactamases (uninduced state) – bacteria continuously produce a very small amount of beta-lactamase. Certain beta-lactam antibiotics can induce the production of high levels of these enzymes, which causes the bacteria to become resistant to many antibiotics.

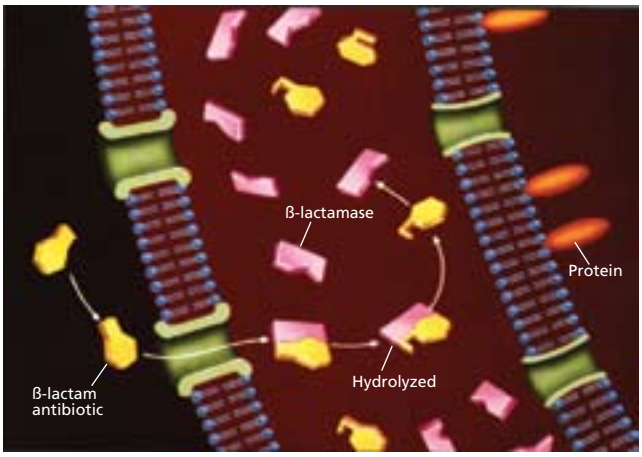

Figure 4. Beta-lactamase hydrolysis – when beta-lactamases interact with beta-lactams, the antibiotic is usually changed to a form that has no antibacterial effect.

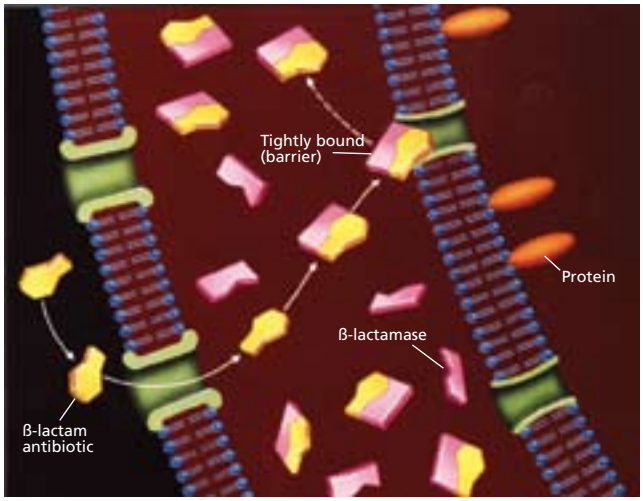

Figure 5. Trapping – Beta-lactamases and antibiotic molecules may interact to form a stable complex. This new structure is unable to bind to the target sites, and is prevented from having any bacterial effect. This mechanism involves a barrier effect, referred to as 'trapping'.
According to this concept, enzymes interact with beta-lactam molecules, forming a complex which is unable to bind to the target sites. The antibiotic is 'trapped' in an inactive form and even highly stable drugs may be slowly hydrolysed in this trapped form.

to the outer layers of the cell wall and the resulting permeability barrier prevents access of glycopeptides to the active site D-ala-D-ala terminus in the stem penta-peptide of pentaglycan.

The genetic basis of hVISA is yet to be determined. Confirmation of organisms with positive screens requires confirmation by population analysis profiling by a laboratory experienced in such a technique. hVISA is not detectable using routine susceptibility testing. Some automated susceptibility methods cannot distinguish such resistance. The resistance is mediated by a combination of factors, and not related to just one single genetic marker. Genome sequencing has shown that there are multiple differences between strains of *S. aureus* with and without glycopeptide resistance [7, 8]. Aspects of hVISA will be further discussed at the ASM Gold Coast meeting in a presentation titled *hVISA outbreak – bad or really bad?*

Inadequacies in the availability of qualitative reliable methods in detecting antimicrobial resistance in some multi-resistant and fastidious organisms – meaning that "laboratories must retain the versatility to apply several different approaches to detect resistance in both common and infrequently encountered bacterial pathogens" – has also been pointed out by Jorgensen & Ferraro[9]. Investigators will need to become more sophisticated to be able to identify multiple beta-lactamases with multiple combinations of amino acid substitutions in organisms with different levels of porin production under different growth conditions [10].

Antimicrobial susceptibility testing in microbiology is a challenging, continuing, educational science. It has significance for public health at the local, national and global level. As new technology is introduced in the near future, susceptibility testing procedures in detecting antimicrobial resistance will become more complex as organisms acquire new avenue of resistance and adapt to various environments and selective pressures for survival.

Table 1. Unusual resistances demanding reference laboratory confirmation [1].

Organism	Resistance
S. aureus	Glycopeptides, linezolid, synercid®
Coag-negative staphylococci	Vancomycin, linezolid, synercid®
JK coryneforms	Glycopeptides, linezolid, synercid®
S. pneumoniae	Meropenem, Glycopeptides, linezolid, synercid®
Group A, B, C, G Beta-haemolytic streptococci	Penicillin, Glycopeptides, linezolid, synercid®
Enterococci	Vancomycin, linezolid, both ampicillin, synercid®
Enterobacteria	Meropenem, imipenem
H. inflenzae	3rd-generation cephalosporins or carbapenems
M. catarrhalis	Ciprofloxacin, 3rd-generation cephalosporins
N. meningitidis	Penicillin (high-level), ciprofloxacin
N. gonorrhoeae	3rd-generation cephalosporins
Acinetobacter species; *P. aeruginosa*	Colistin
Anaerobes	Metronidazole, carbapenems

We need to understand and make sense of both the genetic and physiological basis of resistance mechanisms. The aim of the Antimicrobial SIG Workshop (2006) will be the detection of multi-resistant Gram-positive (MRSA and VRE resistances) and Gram-negative (MRAB, ESBLs and *ampC* resistances) organisms and the demystification of molecular methods, chaired by our invited overseas speaker Professor Nancy Hanson and a panel of local scientists.

Any new technology introduced in a laboratory in detecting antimicrobial resistance needs to be based on accurate susceptibility determinations, reliable detection of resistance mechanisms and resistant populations, with combined phenotypic/physiological and genotypic detection. Mechanisms of resistance may be apparent in susceptible bacterial populations. The identification and further research investigation with new technology in such resistance maybe the first step in the control of emergence and re-emergence of resistance in a variety of organisms.

References

1. Livermore DM. How can we identify resistance? Phenotypic methods. *Identifying Resistance International Newsletter.* Biomérieux, August 2001.
2. Courvalin P. How can we identify resistance? Genotypic methods: detection of resistance genes in the absence of growth. *Identifying Resistance International Newsletter.* Biomérieux, August 2001.
3. Iredell J, Valenzuela J & Thomas L. Beta-lactam resistance in Gram-negative bacteria (Chapter 5). *Antimicrobial Susceptibility Testing Methods and Practices with an Australian Perspective.* ASM Antimicrobial SIG. Sydney, Australia, 2004.
4. Canton R. Why identify resistance? Epidemiology needs. *Identifying Resistance International Newsletter.* Biomérieux, August 2001.
5. Merlino J, van Belkum A, Fernandes C, James G, Thomas L & Chin J. Resistance markers, nucleic acid technology, bacterial population analysis and application for the routine laboratories in detecting resistance. What does the future hold in detecting and screening resistance for our laboratories? Abstract. *Antimicrobial workshop Canberra.* ASM Annual Meeting 2005.
6. Lederle Laboratories. *Clinical Implications of Bacterial Resistance.* Lederle International, New Jersey, USA, 1987.
7. Ward PB, Charles P, Howden BP, Johnson PD, Mayall B, & Grayson ML. VISA and hVISA in Australia and New Zealand. *Australian Society for Antimicrobials Newsletter* 2003; 12 (March).
8. Merlino J. Gram-positive bacteria – resistance and multidrug resistance (Chapter 4). *Antimicrobial Susceptibility Testing Methods and Practices with an Australian Perspective.* ASM Antimicrobial SIG. Sydney, Australia, 2004.
9. Jorgensen JH & Ferraro MJ. Antimicrobial susceptibility testing: special needs for fastidious organisms and difficult to detect resistance mechanisms. *Clin Infect Dis* 2000; 30.
10. Bush K. New beta-latamases in Gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin Infec Dis* 2001; 32.