Novel Therapeutics

Lecture 1: Methods for the Detection of Pathogenic Bacteria

- Antimicrobial treatment → traditional advice is to complete the course of antibiotics, but this does not always represent optimal use
  - » Should be specific to the patient and the infection they have (including severity)
  - » To improve antibiotic use, diagnostic tests could be utilised allowing prescribers to provide directive rather than empirical therapy and to advise patients whether or not they need to complete a course of antibiotics (reducing resistance)
- The problem:
  - » Global increase in the number of multidrug-resistant superbug infections
  - » Over 200,000 cases of healthcare-associated infections (HAIs), including multi-resistant organisms (MRO), in Aus acute healthcare facilities each year
    - i.e. patient goes in for a procedure and ends up with a bacterial infection
  - » Increasing clinical problem, with a particular concern being the transmission of resistance between these bacteria, which could ultimately lead to strains which have limited or no susceptibility to antibacterial agents

β-lactamases

- Enzymes produced by bacteria that confer resistance to β-lactam antibiotics (cleave the β-lactam ring, leading to inactivation of the antibiotics)
- Over 300 different forms present in the periplasmic space
- Different β-lactamases have different substrates e.g. AmpC (cephalosporins)
- Some e.g. extended spectrum β-lactamases (ESBL) hydrolyse even 3rd gen cephalosporins but not carbapenems

Carbapenamses

- Enzyme that breaks down carbapenem antibiotics
- Increase in carbapenem resistance genes (esp. KPC, OXA and metallo-β-lactamases)
- NDM-1 metallo-β-lactamase (*bla*$_{NDM-1}$ gene) now found throughout the world
  - Isolates found in Australia
- *bla*$_{NDM-1}$ gene also carries resistance to macrolides, aminoglycosides, rifampicin, sulfamethoxazole, tigecycline and aztreonam

Transmission of resistance

- In US, more than 20% of enterococcal isolates are vancomycin resistant
- In-vivo transmission of vancomycin resistance from GRE to MRSA reported in 2003
  - * MRSA infection is most likely to be treated with vancomycin
- MRSA is susceptible to very few agents, including glycopeptides (vancomycin and teicoplanin), quinuprustin-dalfoprisitn and linezolid
  - * Cases of methicillin and quinuprustin-dalfoprisitn resistant S. aureus reported in Eur

Antimicrobial stewardship

- Systematic approach to optimising the use of antimicrobials in hospitals
- Includes:
  1. Implementing clinical guidelines consistent with the latest version of the TG
  2. Establishing formulary restriction and approval systems that include restricting broad-spectrum and later generation antimicrobials
     - Broad spectrum usually used in empirical therapy
     - Later generation for most of them resistance still hasn't been built up so should be saved as a last resort for treatment
  3. Educating prescribers, pharmacists and nurses about good antimicrobial prescribing practice and antimicrobial resistance + educating the public
- What's needed?
  - » Rapid surveillance techniques (tests) for pathogenic bacteria

    - » Results used to identify colonised/infected patients and inform their directed (rather than empirical) treatment
    - » Should form the basis for an organism-specific approach to transmission-based precautions
    - » Effective infection prevention: contact precautions inc. isolation in a single-patient room or cohorting patients with the same strain of MROs in designated patient-care areas
- Universal surveillance of antimicrobial use has been shown to be effective

Healthcare associated infections in Australia

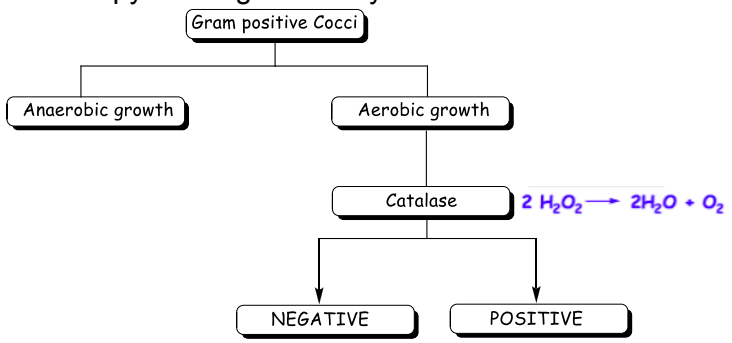
- C. difficile
- MRSA
- VRE

Requirements of bacterial surveillance method

1. Sensitive: correctly identifies microorganism = no false negatives
2. Specific: identifies *only* microorganism of interest = no false positives
3. Rapid - so it can be conveniently done in a health care setting before prescribing treatment
4. Reliable
5. Cost-effective
6. Simple to perform
7. Does not require specialist interpretation

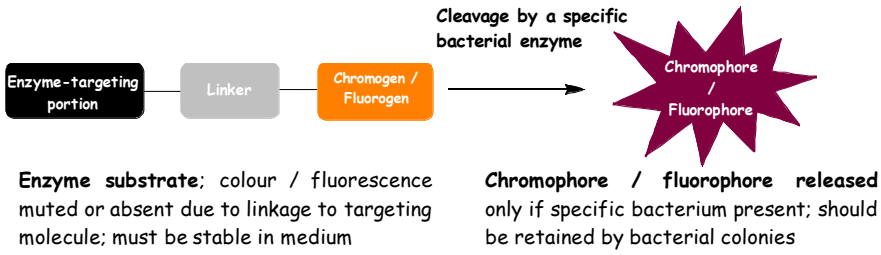
Detection methods

- Many detection methods including: microscopy, staining techniques, gene probes, PCR → laborious, time-consuming and/or expensive
    - » Microscopy identifies bacterial class (cocci, bacilli, spirillum)
    - » Staining identifies gram negative vs gram positive bacteria
- Recent advances use fluorescence or colour to visualise results → cheaper, easier, economically and practically viable and does not require specialist interpretation
- Microscopy/staining with enzymes:



    - » For this, we need to culture the bacteria, perform a gram stain then the catalase test

- Chromophore/flurophore detection



- Chromagen/fluragen: molecules which are colorless/non-fluroscent until they are released from the enzyme targeting portion (bonds broken), then they generate color
- Requirements for a chromagen
    - » Free chromogenic molecule must have strong color
    - » Color must be muted or absent when attached to targeting molecule