Susceptibility testing of Anaerobes

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FASTEST GROWING UK CAPITOL CITY...

MAJOR SPORTING EVENTS...170,000 CHAMPIONS LEAGUE FINAL

Susceptibility testing of Anaerobes
Public Health Wales encompasses a number of public health organisations including Microbiology Services (also Screening, Health Protection)

Wales wide network of Microbiology Laboratories with the primary aim of protecting the public from disease

A number of reference units sit within this structure including the UK Anaerobe Reference Unit (UKARU)
Anaerobe Reference Unit (UKARU)

- Serves the whole of the UK
- One of a select few world-wide
- Remit of ARU includes:

1. Identification & susceptibility testing of anaerobes including AMR surveillance (AD)
2. *C. difficile* ribotyping & surveillance for Wales
3. Clinical & technical advice
4. Maintenance of a collection of clinical isolates c.40,000
5. Training – P&CMAn – **Annual 2 day practical course**
6. R&D, product evaluations
Susceptibility testing

Sydney Finegold:

“Susceptibility testing of anaerobes should be done in 4 settings”:

1. Determine patterns of susceptibility to new agents
2. Monitor susceptibility patterns Nationally
3. Monitor susceptibility patterns locally
4. Assist in the management of individual patients
   - Persistence of infection/ failure of usual regimes/ difficulty making decisions based on precedent
   - Brain abscess/ endocarditis/ osteomyelitis/ prosthetic device infection/ septic arthritis
Why should I do what these people ask?

Finegold 1989

Finegold 2016

Morris 2017

Susceptibility testing of Anaerobes
Additional reasons!

- Resistance exists even to first-line agents e.g. Metronidazole
- Susceptibility patterns can be unpredictable
- Susceptibilities can differ by geographic region or even hospital
- Therapeutic guidance + results linked to outcome
- Resistance transferable and inducible
- Mortality in Bacteroides bacteremia can be as high as: 25-50%
Current situation:
‘Typical’ UK SOP for susceptibility testing ANO2...

- Agar: Any (selective or non-selective).
- Inoculum: Direct from sample, mixed, not standardised.
- Antimicrobial agents: metronidazole.
- Incubation: (Leave on bench till 5pm!?) Incubate AnO₂ 18-72hrs.
- Controls: None?
- Interpretation:
  - Any sized zone = mixed anaerobes, sens to MZ
  - Colonies within zone = must be aerobes!
  - No zone = no anaerobes isolated!
'Mixed anaerobes sensitive to MZ'

But what are those colonies in the MZ zone?

? Aerobes

? Actinomyces

? Microaerophiles

? MZ resistant anaerobes

Pus from cerebral abscess
Primary FAA plate
Incubated 5 days AnO₂

Susceptibility testing of Anaerobes
Would we report....

'Mixed aerobes sensitive to meropenem'

'Fungus present, try Athlete's Foot powder'

'Virus detected, have a hot whisky with lemon and go to bed with a hot water bottle'

Is this acceptable or ‘ISO accreditable’?
BSAC guidelines for anaerobes

Limited disk testing guidelines for *B. fragilis*, *B. thetaiotaomicron*, *C. perfringens* – including zone sizes

**But:** '...the disc diffusion technique, in which the size of the zone is related to a critical concentration of antimicrobial agent, a critical population of organisms and a critical time, makes it an unsuitable method for testing slow-growing organisms.'

**So:** '...recommendation for slow-growing anaerobic organisms is to test for susceptibility by MIC...'
EUCAST guidelines

Methods broadly similar to CLSI – in fact most European studies use CLSI methodology

For E-tests: Mueller-Hinton-F & horse blood (5%) & β-NAD (20mg/L)

Wild type distributions – but many gaps for anaerobes.
CLSI guidelines

Approved methods: agar dilution, microbroth dilution

Not approved: disc diffusion

Now also covered: commercial MIC systems e.g. Etest (Biomerieux), MICE (Oxoid)

Medium: Brucella agar & laked blood (haemin) & vitamin K₁
Replaces: Wilkins-Chalgren

Modifications - higher inoculum, longer incubation, supplements for fastidious organisms if necessary but must be adequately controlled
Methodologies

Dilution methods:

Agar Dilution – CLSI recommended gold standard

Broth microdilution (Bacteroides fragilis group)

Diffusion methods:

Gradient Strip Methods – now CLSI approved

Disc diffusion?
Dilution methods

Uses agar or broth - usually doubling dilutions of antibiotic i.e. 0.008 mg/l to 32mg/l

Minimum Inhibitory Concentration (MIC)

MIC = ‘lowest concentration of antimicrobial agent that prevents development of visible growth’
Agar dilution

Advantages:

Gold standard approved method for determining MIC

Used for monitoring trends over time at reference labs

Data comparable across institutions and countries

Disadvantages:

Labour intensive to perform & interpret (ask my staff!)

Only suitable for large studies...
Microbroth dilution

Commercial panels available e.g. Sensititre TREK plates

ARU has ‘verified’ for *Bacteroides spp.*

Good correlation with agar dilution and gradient MIC strip

**Advantages:** Convenient, long shelf life, bespoke available

**Disadvantages:** Only suitable for fast growing anaerobes
Diffusion methods: Gradient strips

- **Advantages:**
  - Accurate MICs (Quantitative)
  - Simple, rapid to do
  - Wide range available
  - Long-shelf life
  - Suits occasional use

- **Disadvantages:**
  - Expensive

Susceptibility testing of Anaerobes
….disc diffusion

Limited at present in terms of standardisation
What do we need & why?

Reliable, reproducible, cost effective, timely and relevant susceptibility testing methodology for anaerobes!

Due to increased resistance!

What is currently missing?

1) High profile support from leading authority on susceptibility testing – EUCAST!

2) Technical requirements – media, specific breakpoints, control ranges – currently only CLSI
Where to start?

Step 1 – hold a meeting of likeminded people!

First meeting held in Manchester 2016 during BSAC User Day – Derek Brown, Ulrik Justesen, Gunnar Kahlmeter & Trefor Morris

Step 2 – Decide what we are trying to achieve?

Goal - Must be realistic for clinical labs, accessible to all & reproducible!
Step 3 – Devise a pilot study.

3 different sites, same technician reading

Follow the EUCAST rules 15.15.15.

4 easily identifiable, common & fast growing anaerobes:

*Bacteroides fragilis*, *Clostridium difficile*, *Peptostreptococcus anaerobius* & *Fusobacterium necrophorum*

EUCAST support – Dr Herjan Hendrikus Jacobus Bavelaar
Preliminary Results...

C. difficile – not ‘difficult’ - easy to measure zones!

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More difficult to measure...

*Fusobacterium necrophorum*

*Peptostreptococcus anaerobius*
Next steps!

1) Concordance between 3 different sites with reading zones
2) Compare zone sizes to MICs by Gold Standard AD
3) Compare different depths of agar
4) Use different incubation methods (Gassing system, sachets)
5) Use different manufacturers ‘FAA’ (in house and pre poured)
6) Widen range of organisms...
What we do at UKARU:

Agar Dilution panel - MZ, CLI, MER, PEN, TAZ, AUG, CRO, VAN

Gradient MIC for specific therapeutic options (e.g. MDR)

Annual surveillance of clinically relevant UK isolates –

2016  *Bacteroides*

2017  *Clostridium*

2018  *Anaerobic cocci*

Detection of resistance genes in all significant isolates - WGS
Focus!

Isolates

From patients likely to undergo long term therapy

From therapy failures

From cases for which the therapeutic decisions will be influenced by the results

Sites:

Brain abscesses, endocarditis, osteomyelitis, joint infections, prosthetic device infections, recurrent bacteremia, & all anaerobes isolated in pure culture

Susceptibility testing of Anaerobes
Summary

Not all anaerobes are ‘MZ sensitive’!

*Bacteroides spp* can be MDR – particularly if MZ R

If in doubt – contact us and refer – ARU can perform gold standard Agar Dilution (+/- extended panel) in timely manner

Disc diffusion coming soon!
‘Anaerobes: thriving without the oxygen of publicity...’

Dr Mark Evans
Porton Down, PHE
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Thanks for listening!

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