SUSCEPTIBILITY TESTING OF ANAEROBIC BACTERIA

Hannah M. Wexler, Ph.D.

GLAVAHCS and UCLA School of Medicine
FACULTY DISCLOSURE

The Clinical and Laboratory Standards Institute and the Association of Public Health Laboratories adheres to established standards regarding industry support of continuing education for healthcare professionals. The following disclosures of personal financial relationships with commercial interests within the last 12 months as relative to this presentation have been made by the speaker(s):

Hannah M. Wexler

“Nothing to disclose”.
Estimated cost of antimicrobial resistance:

5-30 billion dollars annually

90,000 deaths in U.S. alone

Bad Bugs, No Drugs

As Antibiotic Discovery Stagnates . . .

A Public Health Crisis Brews
ScienceDaily (Mar. 22, 2010) — While prevention methods appear to be helping to lower hospital infection rates from MRSA, a deadly antibiotic-resistant bacterium, a new superbug is on the rise, according to research from the Duke Infection Control Outreach Network.
WHY TEST ANAEROBES?

Anaerobes can have unpredictable susceptibilities to antimicrobial agents

We now see resistance even to first-line agents

Susceptibilities may differ by geographic region or even hospital

In vitro susceptibility testing can help guide therapeutic decisions
MULTIPLE, TRANSFERABLE RESISTANCE DETERMINANTS

HEAVY USE OF SOME ANTIBIOTICS MAY SELECT FOR RESISTANCE

ANTIBIOTIC USE MAY INCREASE RESISTANCE TRANSFER
Bacteroides fragilis: WHO CARES?

Common component of infections in VA patients –

• 61 % of 195 infections of the buttock, hip or groin

• 41 % of 179 foot infections

Mortality in Bacteroides bacteremia is high: 25-50%

Mortality is even higher in patients with liver disease
The Clinical Laboratory Standards Institute (CLSI) (Wayne, PA) standardized many clinical procedures, including anaerobic susceptibility testing and has published documents for anaerobic susceptibility testing (commonly called M11) (6).
ARE THERE OTHER STANDARDS?

EUCAST: breakpoints; are not always equivalent to those of CLSI

However, EUCAST does not actually specify a testing method for anaerobes; most European studies use CLSI methodology

Argentina: Published method closely based on CLSI

Japan: Until 2007, had their own standard. Since 2007, adopted CLSI methodology

Germany: A recent study referred to a specific-non CLSI-method
IS THE METHODOLOGY IMPORTANT?

Differences may seem trivial

However, in cases where MICs cluster around breakpoint values, small changes in MICs (due to differences in media, inocula or endpoint reading method) may lead to perceived significant differences in resistance rates.

Therefore, when trying to evaluate or compare published studies, the published method should be taken into account.
WHAT ISOLATES SHOULD BE TESTED?

Susceptibility testing may not be necessary for many routine patient isolates

Isolates from blood, brain abscess, endocarditis, osteomyelitis, joint infection, infection of prosthetic devices or vascular grafts

Isolates from normally sterile body sites
Isolates from patients likely to undergo long term therapy

Isolates from a therapy failure

Isolates in cases for which the therapeutic decisions will be influenced by the results
WHAT AGENTS SHOULD BE TESTED?

Selection of the most appropriate antimicrobial agents to test and report routinely is a decision made best by each clinical microbiology laboratory in consultation with the infectious disease practitioners and the hospital pharmacy.
Annual surveillance tests recommended to elucidate local patterns.

The numbers and choice of species of strains tested should reflect the frequency with which they are isolated.

At least 50~100 strains should be tested in order to get an accurate picture of the pattern of local isolates.
If isolates from different body sites are available, they should be included.

At least 20 isolates of *Bacteroides* spp. and ten isolates from other frequently isolated genera should be tested.

If the expertise is not available in the hospital clinical laboratory they should be sent to a reference laboratory.
OTHER ANAEROBIC SPECIES WITH KNOWN RESISTANCE CHARACTERISTICS

*Prevotella* spp.
*Peptostreptococcus* spp.
*Clostridium* spp.
*Fusobacterium* spp.

**Intrinsic resistance:**
*Sutterella wadsworthensis*
*Bilophila wadsworthia*
Agar Dilution

Broth microdilution (*Bacteroides fragilis* group)

Gradient Strip Methods

Disc diffusion---NOT recommended for anaerobes
STOCK CONCENTRATIONS

\[ \text{Weight (mg)} = \frac{\text{Volume (mL)} \cdot \text{Concentration (\(\mu\text{g/mL}\))}}{\text{Potency (\(\mu\text{g/mg}\))}} \]

or

\[ \text{Volume (mL)} = \frac{\text{Weight (mg)} \cdot \text{Potency (\(\mu\text{g/mg}\))}}{\text{Concentration (\(\mu\text{g/mL}\))}} \]
AGAR DILUTION TESTING

In advance:

- Read procedure
- Order or prepare the following as necessary:
  - Antimicrobial agents
  - Solvents and diluents
  - Plastic petri dishes, tubes, pipettes
  - Media ingredients
AGAR DILUTION-IN ADVANCE 2:

- Order or prepare agar and broth media.
- Order sheep blood.
- Prepare and freeze antimicrobial stock solutions.
- Subculture test isolates and quality control (QC) organisms from a $\leq-60^\circ\text{C}$ freezer to supplemented Brucella blood agar. Check purity of isolates.
- (Subculture onto test agar to check for adequate growth).
Doubling dilutions made to end in 10X the final desired concentration
Add dilution to molten agar and pour into petri dish
After plates solidify, inoculate with using a mechanical replicator such as a Steers replicator.
Each antimicrobial will have a series of dilution plates to examine.
Plates are read after 48 hours of growth by comparing the series.
Agar Dilution Testing: Steers Replicator
The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

The most prevalent cause of variability in MIC reports is the variation in interpreting what the MIC is in cases where endpoints are not very clear.

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READING ENDPOINTS

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READING ENDPOINTS

0.5 μg/mL

1 μg/mL

2 μg/mL

4 μg/mL
IN HOUSE or COMMERCIAL?

Commercial plates can be bought frozen or lyophilized.

If made in-house, trays can be tailored to the particular needs of the laboratory, using the drugs and concentration ranges needed.
BROTH MICRODILUTION TESTS

In advance:
- Read procedure
- Order or prepare the following as necessary:
  - Antimicrobial agents
  - Solvents and diluents
  - Plastic microdilution plates, tubes, pipettes
  - Media ingredients
- Prepare and freeze antimicrobial stock solutions
- Order or prepare broth media
Drugs can be diluted at the final required concentration if an inoculator is used to deliver the bacterial inocula, or at twice the desired final concentration if a manual pipettor is used to deliver the bacterial inocula.
• Subculture isolates and QC organisms from freezer or subculture clinical isolates. Check purity of isolates.
• Defrost plates if necessary
• Prepare inocula
• Inoculate strains with inoculator or manual pipette
BROTH MICRODILUTION TESTING
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DETERMINING ENDPOINTS

Control

0.5 µg/mL

1 µg/mL

2 µg/mL

4 µg/mL

0 µg/mL

16 µg/mL
ETEST

Isolate suspended in broth

Swabbed on Brucella agar plate

Etest strip placed on plate

48 hours incubation

MIC read where the elliptical zone of inhibition intersects the strip

Etest®, Etest® and the Etest gradient strip are registered trademarks of AB BIODISK.©AB BIODISK, 2007. Photo courtesy of bioMerieux
PROBLEMS READING ETHER TEST STRIPS
OTHER GRADIENT STRIP METHODS

Liofilmchem MIC strips (Liofilmchem, Italy)

MICE MIC strips (Oxoid)
M.I.C.E. TEST STRIPS
Comparison of the M.I.C.Evaluator™ (Oxoid M.I.C.E.) and ETest® (AB Biodisk – bioMérieux) for antimicrobial susceptibility testing of anaerobic bacterial species.

L. Turnbull, C. Bronskoff, R.P. Rennie
Medical Microbiology, University of Alberta Hospital, Edmonton, Alberta, CANADA

Materials and Methods

Objective: Historically only one gradient end point product has been available for the routine antimicrobial susceptibility testing of common aerobic and anaerobic bacteria. This study was conducted to compare a new agar gradient end point system (MIC-Evaluator™ Thermo Fisher Scientific). For routine testing, such devices are important for anaerobes that cannot be tested on automated systems. Methods: A total of 102 recently isolated strains comprising of 28 species of anaerobes were tested. Four agents were tested: amoxicillin-clavulanate (AMC), imipenem (IPM), meropenem (MTZ) and penicillin (P). High and low concentrations were used. The agar dilution test were also performed on brucella agar supplemented with 2% lysed sheep blood, 0.5% hemin, and vitamin K according to CLSI guidelines. ETest™ was also tested. Strips for each agent were added to the plates, according to manufacturers’ instructions, and the plates were incubated for 24-72 h at 35°C in an anaerobic atmosphere. Results: Microbiological results for all methods were compared. All quality control tests for all methods were performed. Clinical strains of anaerobes were prepared in previous experiments. Clinical strains were compared to the agar dilution test method. The consistency of MIC-Evaluator™ and ETest results for the clinical strains of anaerobes was used for overall agreement.

Discussion and Conclusions

- M.I.C.E and ETest results for 102 clinical strains of anaerobes were similar and an overall agreement between different devices was achieved (M.I.C.E 98.3%, ETest 98.1%).
- Quality control results with the gradient end point tests were in range, but there were differences between clinical isolates when the agar dilution method was used.
- The consistency between MIC-Evaluator™ and ETest results for these clinical strains suggests that the gradient end point methodology provides an easy, rapid and reproducible means of determining antimicrobial susceptibility for most anaerobic bacterial species.

Results

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Performance Criteria:
- 90% Baseline and Categorical Agreement
- 90% Major Error Rate
- 95% Test Reproducibility
- 95% Control within acceptable range
- 10% Growth Failure

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Results

Qualitative results: The qualitative results obtained were in range for all anaerobic agents tested by agar dilution, MIC-Evaluator™ and ETest. All strains were tested a total of three times for each method.

Summary

The MIC-Evaluator™ and ETest methods provide an easy, rapid and reproducible means of determining antimicrobial susceptibility for most anaerobic bacterial species.

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- M.I.C.E and ETest results for 102 clinical strains of anaerobes were similar and an overall agreement between different devices was achieved (M.I.C.E 98.3%, ETest 98.1%).
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COMPARISON OF M.I.C.E. AND ETEST

EA and CA compared to agar dilution were 90% for AMOX/C and IPM and ~83% for MTZ and PEN

_Fusobacterium, Eggerthella_ and some _Clostridium_ showed very major error for MTZ and PEN (susceptible by M.I.C.E. and ETest; high MICs by agar dilution)

M.I.C.E. and ETest easier to perform than agar dilution
SPIRAL GRADIENT ENDPOINT: AUTO PLATER
PATTERN OF ANTIMICROBIAL DEPOSITION ON SGE PLATE
“TAIL” ON SGE PLATE
READING RESULTS ON SGE PLATE
CURRENT USES OF SGE TECHNOLOGY

Expensive equipment
Not generally used in clinical lab
Excellent to study mutation frequency
Used for screening strains in molecular biology studies
β-LACTAMASE ENZYMES

β-lactam

PBP
Anaerobic organisms may be tested for the presence of the \( \beta \)-lactamase enzyme using a chromogenic cephalosporin test such as nitrocefin disks.
Look to the future!

If resistance is due to the presence of a specific gene......
RAPID TESTS: PCR???

MCM now lists about four pages of PCR assays that are potentially available to screen clinical samples for "a whole host" of resistance genes.

"the practicality of that has never been realized, partly, I believe, because bacteria are always generating new mechanisms of resistance."

(Dr. Fred Tenover)
RESISTANCE MECHANISMS FOR WHICH NO RAPID TEST IS YET AVAILABLE

Quinolone resistance due to gyrA or parC mutations

Resistance due to other mutations in drug target (e.g. PBP’s)

Resistance due to increased efflux pump activity
IDSA 2010

Bad Bugs Need Drugs

10x '20
Ten new ANTIBIOTICS by 2020