



## Antibiotic Susceptibility Testing

Two important roles of the microbiology laboratory are identifying organisms that cause disease and providing information regarding antimicrobial sensitivity. In bacteriology, antimicrobial susceptibility testing is a complex, ever evolving field. Fortunately, the National Committee for Clinical Laboratory Standards (NCCLS), provides documents addressing organism identification/drug testing based on clinical efficacy, prevalence of antimicrobial resistance, minimizing emergence of resistant strains, cost, FDA indication, and current consensus for first choice and alternative drugs.

### Which organisms are tested?

Generally, organisms with an unpredictable antibiogram are tested for antimicrobial susceptibility. Bacteria with predictable patterns of sensitivity are not routinely tested. For example, *Neisseria gonorrhoea* is generally treated with a third generation cephalosporin without need for susceptibility testing. Other organisms, such as *Staphylococcus aureus*, are routinely tested for antibiotic sensitivity because of varying resistance patterns in clinical isolates.

Some organisms are not tested when isolated in culture because they are considered contaminants or normal flora. Examples of this include the isolation of coagulase-negative *Staphylococcus* from a wound swab or in only one blood culture.

Other bacteria are excluded from routine testing because "studies are not yet adequate to develop reproducible, definitive standards to interpret results." (NCCLS M100-S13 2003) This includes *Campylobacter* spp., *Corynebacterium* spp., and *Bacillus* spp.



### Which antibiotics are chosen for testing?

NCCLS provides tables of antibiotics to test for different bacterial groups. For example, when appropriate, *Staphylococcus* spp. are to be routinely tested against oxacillin and penicillin (NCCLS Group A, routinely tested). A second category of antibiotics including erythromycin, clindamycin, linezolid, trimethoprim/sulfamethoxazole, and vancomycin are options for testing *Staphylococcal* isolates (NCCLS group B, report selectively). A third group (group C by NCCLS designation) includes alternative or supplemental

antimicrobial agents that may require testing in difficult to treat cases. For *Staphylococcus* spp., these include chloramphenicol, fluoroquinolones including ciprofloxacin, gentamycin, and quinupristin-dalfopristin.

The last NCCLS category is supplemental drugs that are only reported for urinary tract infections. For the example of *Staphylococcus* spp., these include norfloxacin and nitrofurantoin.

Other major groups of organisms that are routinely tested include the *Enterobacteriaceae* (e.g. *E.coli*), *Pseudomonas*, and other gram negative non-

*enterobacteriaceae*, *Enterococcus* spp., and *Streptococcus pneumoniae*.

### What testing is available at Rex?

At Rex Hospital, the microbiology laboratory provides routine testing on the most prevalent aerobic bacteria. Susceptibility testing of anaerobes and mycobacterium is sent out to reference laboratories. These include the North Carolina State Public Health Laboratory, the Mayo Clinic, and rarely the CDC .

(continued on page 2)

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### at Rex?

The majority of antimicrobial susceptibility testing at Rex is performed on an automated instrument (Vitek 2 from bioMérieux). This testing yields minimal inhibitory concentration (MIC) results which are divided into susceptible (S), intermediate (I), or resistant (R) categories. This testing is performed on prefabricated cards with up to twenty antibiotics. Different cards are used in different situations (gram positive versus gram negative, inpatient versus outpatient). In some cases, organisms cannot be tested by an MIC method, and disk diffusion is used. In this case a circle of inhibition forms around the antibiotic disk, and the size of the circle yields an interpretation of S, I, or R.

### How are sensitivity reports interpreted?

Clinicians who have received sensitivity reports will realize that although twenty antibiotics are typically tested against a given organism, a smaller number are actually reported. This stems from the fact that not all antibiotics are appropriate for reporting on a given organism. The Vitek 2 automated system routinely tests an array of antibiotics and only reports those that are considered appropriate by NCCLS standards. Many drugs listed for testing are redundant (eg. listing all of the fluoroquinolones against a single organism) and others have been removed from the hospital formulary for safety or efficacy reasons (eg. cefotetan) and are therefore not reported.

In some cases, sensitivity/resistance can be inferred based by testing of surrogate antibiotics. For example, all beta-lactams are determined for *Staphylococci* based on testing of penicillin and oxacillin. If both are susceptible, then the organism is considered susceptible to all beta-lactams (penicillins and cephalosporins). If both test as resistant, then the organism is reported as a methicillin-resistant *Staphylococcus aureus* (MRSA) and is considered resistant to all beta-lactams. If the *Staphylococcal* isolate is penicillin resistant and oxacillin sensitive, then the organism is considered resistant to penicillinase-labile penicillins (eg. penicillin, ampicillin, amoxicillin) but susceptible to other penicillinase-stable penicillins (eg. methicillin, nafcillin), beta-lactam/beta-lactamase inhibitor combinations (e.g. amoxicillin-clavulanic acid, ampicillin-sulbactam) and cephalosporins. These inferences are considered more accurate than testing individual beta-lactams other than oxacillin and penicillin.

### What do the MIC numbers mean?

When multiple drugs test as sensitive for an organism, it is important to note that one cannot compare MICs between drugs to determine which drug is more efficacious. Antibiotics often have different MIC cutoffs between S, I, and R. For example the break point between S and I for *Streptococcus pneumoniae* when tested against penicillin is 0.06 micrograms/ml while for tetracycline it is 2 micrograms/ml. An MIC result of 0.06 micrograms/ml to penicillin is S, but only one dilution from intermediate, while an MIC of 0.5 micrograms/ml to tetracycline is S and several dilutions from the S/I breakpoint. It is best to use the S, I, and R results only unless consulting with an infectious disease practitioner or comparing antibiotics with similar MIC break points.

### Conclusions:

The majority of antimicrobial sensitivity testing reports provide a straightforward answer to the treating clinician. However, cases will arise where organisms show resistance to a broad array of antibiotics. Other difficulties include antibiotic allergies in patients to the drug of choice. In these cases, the microbiology laboratory can be contacted regarding additional testing. The laboratory keeps additional antibiotics in stock that can often be used in these situations. For example, linezolid is not routinely tested or reported against *Staphylococcus* species but this testing can be performed at the clinician's request (usually through an infectious disease practitioner). An example of a drug allergy that can be crucial for testing is penicillin allergy in a pregnant female with *group B Streptococcus*. Without information about a serious penicillin allergy, the microbiology department will not conduct testing since there is no reported penicillin resistance. If this allergy is noted on the request or order, erythromycin and clindamycin will be tested to aid in the treatment of the mother at the time of labor.

While the field of antimicrobial sensitivity testing has become a specialty in itself, the majority of cases can be appropriately treated by ensuring a good clinical specimen for culture, avoiding contamination, and providing any relevant history to the microbiology laboratory (919) 784-3051, including drug allergies and any special requests.

Vincent C. Smith M.D.

### References:

Performance Standards for Antimicrobial Susceptibility Tests; Approved Standards. NCCLS M100-S14. January 2004.

Hindler, JF. Important Considerations for Detecting and Reporting of Antibacterial Resistance. CDC Presentation. October 2003.



## Gut Check #4: Dysplasia in Barrett's Esophagus

Barrett's esophagus refers to benign **metaplastic** glandular epithelium lining the tubular esophagus. Over time, the definition has been revised to include only intestinal-type (goblet cell) glandular metaplasia (cf. gastric) as only the former is believed to confer an increased risk of adenocarcinoma. An earlier issue of the *Bulletin* addressed the challenges of diagnosing Barrett's esophagus (BE) in distal esophageal biopsies.<sup>1</sup> Recognition and classification of glandular **dysplasia** (a putative precursor to esophageal adenocarcinoma) in these biopsies pose additional challenges for general surgical pathologists and expert gastrointestinal pathologists alike.

In 1988, GI pathologists from 4 institutions (U. Washington, Seattle WA; UCLA, Los Angeles CA; Harvard/Beth Israel, Boston MA and U British Columbia, Vancouver BC) published a collaborative effort to classify dysplasia in BE.<sup>2</sup> They adopted the same 5-tiered classification scheme proposed for reporting dysplasia in ulcerative colitis: negative for dysplasia, indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia, and intramucosal carcinoma. In the initial phase of the study, 71 slides from 40 patients with BE were reviewed by 3 GI pathologists simultaneously using a multi-headed scope. Each slide was assigned to one of the above 5 categories. *Of note was the fact that no consensus could be reached in 9 cases despite joint review and discussion.* Subsequently, specific criteria for classification and a study set of 20 cases were created. This material was then used to instruct 8 additional GI pathologists from the 4 participating institutions to recognize and classify glandular dysplasia in BE. A challenge set of 70 slides from 32 patients was prepared and each of the 8 GI pathologists individually reviewed each slide and placed it into one of the five categories. After a several month hiatus, the same 70 slides were randomized again and the 8 GI pathologists were asked to review the study set, then repeat the new 70-slide challenge. The results of the study were not reported for the five diagnostic categories, presumably

because the level of agreement was poor. The study did report the level of agreement for two broad categories (high-grade dysplasia/intramucosal carcinoma vs. other diagnosis AND negative for dysplasia vs. other) and a three tiered comparison (negative vs. indefinite for dysplasia/low-grade dysplasia vs. high grade dysplasia/intramucosal carcinoma). The level of agreement was considered good for the combined category of high-grade dysplasia/intramucosal carcinoma vs. other. For other categories, the performance was not as good either for interobserver or intraobserver agreement (Table 1). The authors concluded, "...further refinement of histologic criteria or alternate diagnostic methods will be needed to achieve the reproducible diagnosis of indefinite changes or low-grade dysplasia."<sup>2</sup>

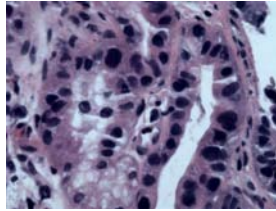


Figure 1: Low-grade dysplasia in Barrett's esophagus.

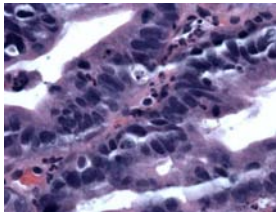


Figure 2: High-grade dysplasia in Barrett's esophagus.

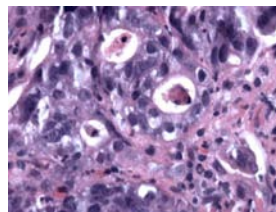


Figure 3: Intramucosal adenocarcinoma in Barrett's esophagus.

Over 10 years later a similar exercise was performed by 12 GI pathologists representing 12 U.S. university medical centers.<sup>3</sup> Esophageal mucosal biopsy specimens from 250 cases of BE classified in one of the 5 categories described above were submitted. The cases were divided into two groups of 125 slides each, with an effort to have a similar distribution of the 5 categories in both. The first group was reviewed, without knowledge of the submitting diagnosis, on two separate occasions by all 12 GI pathologists, using the criteria developed in the original study.<sup>1</sup> Following this all 12 pathologists attended a consensus conference to review the results and revise the original criteria in an attempt to improve reproducibility. Consensus diagnosis was achieved in only 70% of the cases. The revised criteria were then applied to the second group of 125 slides. The findings are reported using kappa statistics rather than percent agreement, but the bottom line is essentially the same. When broad categories are used (negative/indefinite/low-grade dysplasia vs. high-grade dysplasia/intramucosal carcinoma) there is very good agreement. There is also substantial agreement in cases, which were judged negative for dysplasia vs. all other diagnoses. However agreement was only fair for low-grade dysplasia and slight for indefinite for dysplasia.

**Table 1**  
**Dysplasia in Barrett's Esophagus (70 Cases)**  
**GI Pathologist Interobserver/Intraobserver Agreement<sup>2</sup>**

Category	Interobserver Round 1	Interobserver Round 2	Intraobserver (Average)	Intraobserver (Range)
High-grade dysplasia/intramucosal carcinoma	87%	85%	88%	80-96%
Negative for dysplasia vs. other	71%	72%	83%	70-91%
Negative vs. Indefinite/low-grade vs. other	58%	61%	74%	67-80%

An interesting companion study by the same authors attempted to correlate patient's clinical course on follow-up with the original interpretations of the endoscopic biopsies by the contributing pathologists as well as those offered by the majority of the GI pathologists (where a majority opinion could be achieved).<sup>4</sup> Follow-up data could include subsequent mucosal biopsy, esophagectomy, or adequate documentation of metastasis. From the original 250 cases, follow-up was available for 138 patients. A majority diagnosis was defined as one in which at least 13 "votes" were cast for one of the five categories (negative for dysplasia, indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia, or intramucosal carcinoma). Each case was reviewed independently on two occasions by 12 pathologists, thus a total of 24 "votes" was possible. *Of interest, no majority diagnosis was achieved for 39/138 cases (28%).* There was no significant survival difference between the two groups (Table 2). The authors found that the follow-up information correlated more closely with the contributing pathologists' interpretation than the majority panel interpretation. They concluded (correctly in my opinion) that often this was the result of the contributing pathologist having access to the pertinent clinical information (e.g. knowledge of a mass lesion or endoscopic photograph) not made available to the review panel. This study validates the presence of dysplasia as an important marker for identifying BE patients at risk for development of adenocarcinoma. The study further suggests that management of patients judged to be "indefinite for dysplasia" should be similar to that applied to those with low-grade dysplasia, in part because of the difficulty in separating the two. Finally, the authors note that ulceration in the setting of BE presents difficulties in interpretation and requires close follow-up (15/21 or 71% of patients with ulcers developed invasive carcinoma).

The studies discussed above indicate that even in the "best of hands", evaluation for glandular dysplasia in BE is somewhat subjective. It's not clear that referral to an "expert" pathologist leads one closer to the truth, but this has

been suggested by some to confirm cases diagnosed as "high grade dysplasia".<sup>5</sup> More objective means of assessing risk for neoplastic progression in BE have been sought including flow cytometry, cell proliferation markers, oncogene expression, and other chromosomal abnormalities. At the present time, review of routine H&E stained sections is still regarded as the gold standard for evaluation of dysplasia in BE.<sup>6,7</sup>

At Rex, cases of BE thought to be suspicious for any degree of dysplasia are generally reviewed by at least two pathologists. Problematic cases are referred for expert consultation at the discretion of the attending pathologist or at the request of the contributing endoscopist.

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(Photographs courtesy of John P. Sorge, MD)

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**Table 2  
Kaplan-Meier Survival Statistics  
Esophageal Biopsy Interpretation in Barrett's Esophagus<sup>4</sup>**

<b>Interpretation</b>	<b>Median Progression Free Survival (Contributing pathologist Interpretation)</b>	<b>Median Follow-up of Progression-Free Patients</b>	<b>Median Progression Free Survival (GI Panel Majority Interpretation)</b>	<b>Median Follow-up of Progression-Free Patients</b>
Negative	No progression	38.5 mo	No progression	48 mo
Indefinite for dysplasia	62 mo	36	86% survival at 2 mo	30
Low-grade dysplasia	60	24	60	21
High-grade dysplasia	8	13	7	23.5
Intramucosal carcinoma	< 1	Not applicable	< 1	Not applicable

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