Accuracy of the BIOMIC Image Analysis Interpretation of Disk Diffusion Susceptibility Tests on Morphotypes of *Pseudomonas aeruginosa* From Cystic Fibrosis Patients

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Revised Abstract

Background: Disk diffusion is the recommended method for susceptibility testing of *P. aeruginosa* morphotypes from cystic fibrosis (CF) patients. The BIOMIC Image Analyzer (Giles Scientific, Santa Barbara, CA) for reading and interpretation of disk diffusion tests is a time and labor saving method over other susceptibility test systems. We compared the interpretation of disk diffusion results using the BIOMIC Image Analysis reader with manual measurement of zone sized to determine if this device is a reliable method for interpreting the antibiotic susceptibility of these isolates that are often difficult to manually interpret. **Methods:** One-hundred-thirty-five isolates of *P. aeruginosa* morphotypes were evaluated (36 classic, 53 mucoid, 46 rough) for a total of 1620 antibiotic/organism combinations. Disk diffusion susceptibility tests were read on the BIOMIC Image Analysis System and compared to a manual interpretation of zone size diameters. **Results:** There were 1572 concordant and 48 discordant results. Discordant results were: 28 (1.7%) minor, 8 (0.5%) major, and 12 (0.7%) very major errors. Seven of the 8 major discrepancies occurred with one poorly growing mucoid strains of *P. aeruginosa*; the very major discrepancies occurred with 7 isolates of mucoid and rough strains. No single antibiotic was consistently associated with the major or very major discordant results; 64% of the minor errors occurred with chloramphenicol and ciprofloxacin. Overall agreement was 97%. **Conclusions:** The BIOMIC Image Analysis System is a reliable alternative to manual disk diffusion interpretation as well as a time and labor saving device for reading and interpretation of susceptibility testing of *P. aeruginosa* morphotypes from CF patients.

Introduction

Disk diffusion is a long used, economical, and well validated method of determining bacterial susceptibilities to several antibiotics at one time. Using a template a laboratory technician can read and interpret susceptibilities rapidly.

The BIOMIC Image Analysis System is a commercially available automatic disk diffusion susceptibility reader. Software includes internal checks for consistency based on CLSI (previously NCCLS) guidelines, provides downloadable results, and flags unlikely results for further action. The machine has a history of good agreement with visual laboratory technician readings. The current study examined technician and machine agreement in reading disk diffusion susceptibilities on *P. aeruginosa* strains isolated from cystic fibrosis patients.

Methods and Materials

Pseudomonas aeruginosa strains isolated from cystic fibrosis patients were tested using disk diffusion methodology against 12 antibiotics: aztreonam, cefepime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, gatifloxacin, meropenem, piperacillin/tazobactam, ticarcillin/clavulanate, tobramycin, and trimethoprim/sulfamethoxazole. Isolates are inoculated into brain-heart infusion broth and incubated in ambient air at 37°C and diluted, as needed, to a 0.5 McFarland standard. The bacterial suspension was inoculated onto a 150 mm Mueller Hinton agar plate by CLSI (NCCLS) standard methodology[1] and the antibiotic disks applied to the surface. Plates were incubated at 35°C in ambient air for 24 to 48 hours. Zones of inhibition were measured by hand using callipers. The same plate was also read in the BIOMIC machine. Data were analysed for agreement, using FDA standards for error measurement[2] as shown in the following table:

	Reference method results			
"New" method results	Susceptible	Intermediate	Resistant	Totals
Susceptible	SS	IS	RS	
Intermediate	SI	II	RI	
Resistant	SR	IR	RR	
Totals				Overall N

Agreement is calculated as a percentage of the totals as follows. SS, II, and RR are full agreement. Major error (ME) is defined as erroneous categorization of a susceptible organism as resistant, and is calculated as SR/N. Minor error (mE) is erroneous categorization of the true result (either S or R) as intermediate and vice versa; it is calculated using the cells (SI + RI + IS + IR)/N. Very major error (VME) is categorization of a resistant organism as susceptible. It is calculated as RS/N. All proportions are presented as percentages and should total 100. Acceptable agreement of a new method with a reference method is as follows:

- Overall agreement >90%
- Major error rate of =<3%
- Low very major error rate based on number of resistant isolates

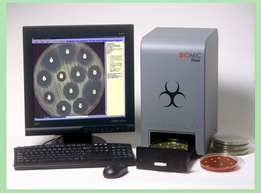
Results

A total of 135 *P. aeruginosa* strains were tested from the three typical CF-associated morphotypes: 36 classic, 53 mucoid, and 46 rough. These 135 were tested against 12 drugs for a total of 1,620 organism/antibiotic combinations.

There were 1,572 concordant results (97%) and 48 (<3%) discrepant results. Discrepant results were: 28 (1.7%) minor errors, 8 (0.5%) major errors, and 12 (0.7%) very major errors. These error rates were well within error rates allowed by the FDA for approval of a new system.

Overall, 7 of the 8 major errors occurred with one very poorly growing strain of mucoid *P. aeruginosa*. All 12 very major errors occurred with 7 isolates of mucoid and rough strains.

No single antibiotic was consistently associated with major or very major errors. Chloramphenicol (11) and ciprofloxacin (7) were more frequently associated with minor errors than any other agents. These two agents accounted for 64% of all minor errors.



BIOMIC Image Analysis System

The plate is placed in the BIOMIC tray and scanned. Zones of inhibition are measured and compared to standards for interpretation. Discordant results are flagged for technician review. This study was done on the system shown to the left. On the right is the V3, a more recent addition to the BIOMIC inventory, which is also capable of reading biochemical identification strips.

Photos provided courtesy of Giles Scientific

	N	Agent/Isolate combination	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)
Classic	36	432	427 (26.36)	4 (0.25)	0 (0)	1 (0.06)
Mucoid	53	636	608 (37.53)	14 (0.86)	7 (0.43)	7 (0.43)
Rough	46	552	537 (33.15)	10 (0.62)	1 (0.06)	4 (0.25)
Totals	135	1620	1572 (97.04)	28 (1.73)	8 (0.49)	12 (0.74)

Discussion

The BIOMIC method provides good agreement with standard methods of reading disk diffusion plates by hand that are well within FDA requirements. Test results are downloadable, providing protection from transcription errors, and greater speed of entry. Technicians involved with testing found it a simple, user-friendly method particularly for reading large numbers of tests.

References

1. CLSI (formerly NCCLS). Performance Standards for Antimicrobial Susceptibility Testing; 15th Informational Supplement, M100-S15. NCCLS, Villanova, PA.
2. US Department of Health and Human Services Food and Drug Administration Center for Devices and Radiological Health Division of Microbiology Devices Office of In Vitro Diagnostic Device Evaluation and Safety. Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and the FDA. February 5, 2003. Washington DC