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Detection of Colistin and Polymyxin B Resistance in *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis (CF) Patients

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Abstract

Background: CLSI interpretative guidelines for colistin and polymyxin B are available for disk diffusion (DD) and microbroth dilution (MIC) testing of *Pseudomonas aeruginosa (PA)*. Many reports have suggested that DD tests for these antimicrobials may be inaccurate; at this time, there are no commercial MIC systems FDA cleared to test these agents. Physicians at our hospital require colistin for *PA* from CF patients. We compared DD, E-test (ET) and Sensititre® panels (Part No. GNXF) (TREK Diagnostic Systems, Cleveland, OH), a research use only product, to frozen reference panels for CF clinical isolates of PA from our hospital.

Methods: Fifty isolates of *PA,* 22 mucoid (*PAM*) and 28 non-mucoid (*PA*) were tested for susceptibility to colistin (CT) and polymyxin B (PB) by each of three methods: DD, ET (bioMerieux, Durham, NC) and Sensititre dried MIC panel GNXF. All results were compared to reference MIC panels prepared TREK. Frozen and dry MIC panels were read on the TREK Vizion™ System. Results of the four methods were analyzed and frozen reference panel was considered the "gold standard".

Results: Using CLSI interpretative guidelines, all 22 *PAM* isolates were susceptible by DD, GNXF and ET for both CT and PB.

For *PA* isolates, 27/28 isolates were susceptible to CT and PB by DD and 25 by ET and GNXF. Three isolates tested resistant by one or more methods for both CT and PB (1 DD, 3 ET, and 3 GNXF). For CT and PB there were 2 very major errors each by DD and no minor errors.

Conclusion: All three methods compared favorably to the reference method for susceptibility testing of *PAM* isolates to colistin and polymyxin B. For *PA* isolates, Sensititre dry MIC plates (GNXF) and E-test compared best to the reference method. Resistance may be missed if using DD. Testing of additional isolates may clarify this issue.
isolates. MIC90's for metronidazole and meropenem were 2.0 ug/ml and 0.5 µg/ml respectively..

Introduction

CLSI interpretive guidelines for colistin and polymyxin B are available for disk diffusion (DD) and microbroth dilution (MIC) testing of *Pseudomonas aeruginosa (PA).* Many reports have suggested that DD testing may be an unreliable method for evaluating the susceptibility to polymyxins. Currently there are no automated systems that are FDA cleared to test these agents, and Etest is available for investigational use only. TREK Diagnostic Systems has recently released a Research Use Only panel, Sensititre® GNXF, that includes colistin and polymyxin B. In our hospital, there is an increasing use of polymyxins to treat multidrug resistant organisms, particularly *PA* from cystic fibrosis (CF) patients. We compared our current disk diffusion method (DD), E-test (ET) and Sensititre® GNXF panels, to frozen reference panels for CF clinical isolates of *PA*.

Methods

Disk Diffusion
Inoculum: 0.5 McFarland
Media: Mueller Hinton agar (BBL)
Incubation: 35ºC, ambient air, 18-24 hours
Disks: 10 ug Colistin
300 units Polymyxin B
Interpretation: (CLSI M100-S19)
Colistin: ≥11 S ≤10 R
Polymyxin: ≥12 S ≤11 R

Sensititre® Panels (GNXF and Frozen Reference)
TREK Diagnostic Systems, Cleveland, OH
Inoculum: 0.5 McFarland
Media: Mueller Hinton broth (BBL) for dosing both panel types
Incubation of Panels: 35ºC, ambient air, 24 hours
Interpretation: Read using the TREK Vizion™ System

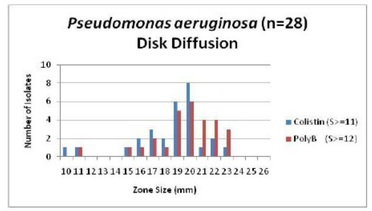
E-Test (bioMerieux, Durham, NC)
Inoculum: 0.5 McFarland
Media: Mueller Hinton agar (BBL)
Incubation: 35ºC, ambient air, 18-24 hours
Interpretation: (CLSI M100-S19)
Colistin: ≤2 S, 4 I, ≥8 R
Polymyxin: ≤2 S, 4 I, ≥8 R

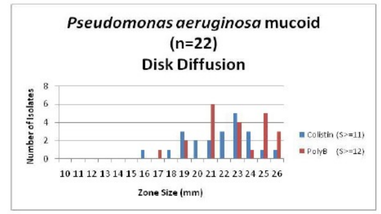
Results

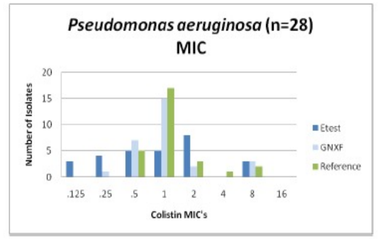
Using CLSI interpretative guidelines, all 22 *PAM* isolates were susceptible by DD, GNXF and ET for both CT and PB. For *PA* isolates, 27/28 isolates were susceptible to CT and PB by DD and 25 by ET and GNXF. Three isolates tested resistant by one or more methods for both CT and PB (1 DD, 3 ET, and 3 GNXF). For CT and PB there were 2 very major errors each by DD and no minor errors.

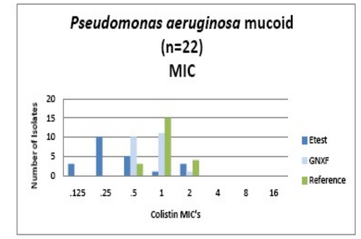
Very Major Errors
Pseudomonas aeruginosa

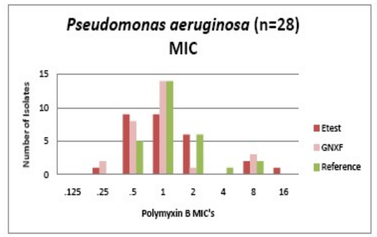
Isolate #	Antibiotic	Reference	ET	GNXF	DD
9	CT and PB	R	R	R	**S**
13	CT and PB	R	R	R	**S**

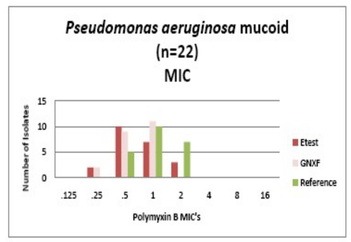












Conclusions

P. aeruginosa
► All methods compared favorably to the reference method

***P. aeruginosa* (mucoid)**
►E test and GNXF compared favorably to the reference method
►DD did not reliably detect resistance

Acknowledgement: Reagents, equipment and supplies were supplied by bioMerieux and TREK Diagnostics