Investigating The Influence of Polymicrobial Environments on Antimicrobial Susceptibility Outcomes

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Abstract

Treatment of chronic wound infections is often complicated by polymicrobial infection. Currently the gold standard method of investigating proper therapy involves isolating each individual organism, before antimicrobial sensitivity testing (AST). Bacteria, when in a polymicrobial setting, exhibit different growth and resistance characteristics as compared to monomicrobial isolates. Thus, the standard method does not accurately capture the environment in which they originate and cause harm. This study aims to investigate whether similar methods of bacterial culture and automated AST can accurately reflect the polymicrobial status of wounds. We compared the minimum inhibitory concentration (MIC) of three common wound pathogens, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* in monomicrobial culture, to their results when combined in a polymicrobial community. In select drugs, there was an increase in MIC in the polymicrobial mix, compared to individual controls, indicating bacterial synergism. Whereas decrease in MIC for the polymicrobial mix in certain drugs, compared to at least one of the individual organisms, pointed to possible enhanced efficacy.

Introduction

- Polymicrobial communities introduce variability between patients
- Mimicking the clinical environment could result in more personalized treatments.
- Previous research has identified that bacterial groups are capable of synergism, altering antimicrobial susceptibility of the group compared to the individual species.
- The environmental and nutrient conditions present at different infection sites can influence antimicrobial susceptibility.

Methods

- Three common wound pathogens: *P.aeruginosa* (ATCC 27853) , *S. aureus* (ATCC 27853), *E. faecalis* (ATCC 29212)
- Each organism was individually cultured, and MIC of numerous antimicrobial drugs is determined using the Beckman-Coulter MicroScan autoSCAN4.
- Spectrophotometric data is measured and interpreted into growth or no growth, giving a semi-quantitative MIC threshold
- To mimic a polymicrobial community, the bacteria were combined in a 1:1:1 ratio and measured using the same method as the monomicrobial
- MIC data was observed for changes in resistance and susceptibility between mono and polymicrobial

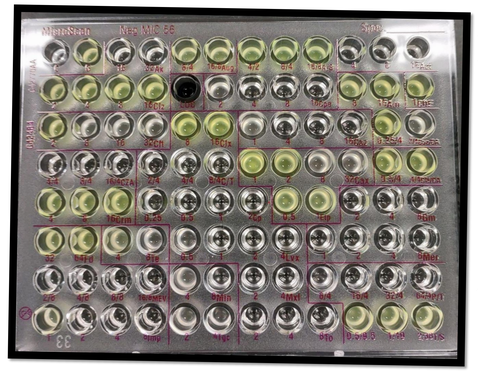


Image 1: MicroScan Negative MIC panel inoculated with all 3 organisms in a 1:1:1 ratio. Turbidity in well indicates growth, MicroScan (see below) quantifies relative growth and provides MIC information for each drug (Table1)

Image 2: Beckman-Coulter MicroScan autoSCAN4 plate reader system.



Drug	S. aureus	E. faecalis	P. aeruginosa	Polymicrobial
A/S	≤4/2	≤4/2	>16/8	>16/8
AK	≤16	>32	≤16	>32
AM	≤8	≤8	>16	>16
AUG	≤8/4	≤8/4	>16/8	>16/8
AZT	>16	>16	≤4	>16
C/T	>8	>8	≤2	>8
CAX	≤1	8	32	8
CAZ	4	16	≤1	>16
CAZ/CA	2	>2	>2	>2
CFT	≤2	≤2	8	16
CFT/CA	≤0.5	>4	>4	>4
CFTE	≤1	≤1	>1	>1
CFX	≤8	>16	>16	>16
CFZ	≤2	4	>16	>16
CP	≤0.25	0.5	≤0.25	0.5
CPE	≤2	8	4	16
CRM	≤4	8	>16	>16
CZA	≤4	>16	≤4	>16
ETP	≤0.5	>1	>1	>1
FD	≤32	≤32	>64	>64
GM	≤2	8	4	4
IMP	≤1	≤1	2	2
LVX	≤0.5	1	1	1
LZD	≤4?	NA	NA	NA
MER	<1	4	≤1	2
MEV	≤2	≤2	≤2	≤2
MIN	≤4	≤4	>8	>8
MXF	≤2	≤2	≤2	≤2
P/T	≤8	≤8	≤8	≤8
T/S	≤0.5/9.5	≤0.5/9.5	>2/38	>2/38
TE	≤4	>8	>8	>8
TGC	≤2	≤2	>4	>4
TO	≤2	>8	≤2	≤2

Table 1: MIC results for 33 antimicrobials and monomicrobial isolates compared to the polymicrobial community; Red highlight indicates the polymicrobial community was less sensitive to the drug; Blue highlight indicated greater sensitivity in the polymicrobial community compared to at least one of the individual species

Results

- With CFT* and CPE*, bacterial synergism is observed in the polymicrobial setting as there was an increase in MIC compared to individual controls
- A decrease in MIC for the polymicrobial mix with GM*, MER*, MEV*, and TO*, compared to at least one of the individual organisms, points to increased efficacy of the antimicrobial.

*Drug Abbreviations: Ceftazidime (CFT); Cefepime (CPE); Gentamicin (GM); Meropenem (MER); Meropenem-Vaborbactam (MEV); Tobramycin (TO)

Discussion

- Both increased and decreased resistance outcomes were observed indicating complex microorganism interaction when cultured in polymicrobial community
- Personalized treatment in complex infections lead to improved wound outcomes

Future Studies:

- Modifying the inoculating media of the MicroScan panels by replacing the recommended Prompt rehydration media with Lubbock Chronic Wound Media to better mimic the physiological environment experienced by pathogens in vivo.

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Acknowledgements:

We would like to thank the Department of Laboratory Science and Primary Care at Texas Tech University Health Sciences Center, as well as the Honors College at Texas Tech University for their support and resources provided for this research.