

Daptomycin Non-Susceptible VRE: Problematic Pathogen or Misclassified Microbe?

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INTRODUCTION

- Vancomycin-resistant Enterococci (VRE) are common causes of hospital-acquired infections
 - Few therapeutic treatment options currently available
 - Mechanisms of vancomycin resistance: *VanA* and *VanB* acquired genes
- Daptomycin is a front-line treatment agent
 - Susceptibility is dependent upon testing method
 - Bryant *et al.* reported 20% (n=30) were confirmed daptomycin non-susceptible (DNS) when tested by the gold-standard method of broth microdilution (BMD)
- Role of new antimicrobial agents have yet to be established
 - Oritavancin has retained *in vitro* activity despite the presence of *VanA* or *VanB* unlike telavancin
 - Previous *in vitro* studies of the susceptibility profile of oritavancin against VRE isolates have shown low minimum inhibitory concentrations

OBJECTIVE

- Determine the rate of daptomycin non-susceptibility across susceptibility testing methods among VRE isolates at UPMC
- Define the role for new agents

METHODS

- Minimum inhibitory concentration (MIC)
 - Tested in duplicate by BMD method according to the Clinical and Laboratory Standards Institute (CLSI) M100-S26 including supplementation of medium with calcium(50mg/L) for daptomycin testing
 - Quality control (QC) strain: *E. faecalis* ATCC 29212
 - Antimicrobial concentration ranges tested (µg/mL) and non-susceptible (NS) definitions (µg/mL):

Vancomycin	Daptomycin	Tigecycline	Oritavancin	Minocycline
(0.5 – 512)	(0.06 – 64)	(0.015 – 16)	(0.015 – 16)	(0.06 – 64)
NS: MIC >4	NS: MIC >4	NS: MIC >0.5	Not defined	NS: MIC >4
Teicoplanin	Linezolid	Quinupristin/dalfopristin	Telavancin	
(0.06 – 64)	(0.06 – 64)	(0.015 – 16)	(0.015 – 16)	
NS: MIC >8	NS: MIC >2	NS: MIC >1	Not defined	

- Polymerase chain reaction (PCR)
 - VanA* and *VanB* PCR primers based upon prior studies
 - Quality control (QC) strains: *E. faecium* ATCC 700221 (*VanA* +) and *E. faecalis* ATCC 51299 (*VanB* +)
- Oritavancin specific handling methods per manufacturer
 - Addition of polysorbate-80 to media- minimize drug loss to surfaces (CLSI)
 - Powder not exposed to air for >5 minutes, final concentrations <16-32µg/mL, prepared solutions freshly, store on ice, and protected from light

Data was analyzed using GraphPad Prism software (version 7.0)

RESULTS

- Study Isolates:**
 - 40 Vancomycin-Resistant *Enterococci* (VRE) unique isolates
 - E. faecium* (n=37) and *E. faecalis* (n=3)
 - 92.5% (37/40) isolates *VanA* positive (+)

Table 1. MIC of Antimicrobial Agents with VRE Activity- BMD Methods

Antimicrobial Agent	Median MIC (µg/mL)	MIC90 (µg/mL)	Range (µg/mL)	Non-susceptible (NS) % (n) ¹
Vancomycin	512	>512	128 - >512	100 (40/40)
Teicoplanin	64	>64	0.5 - >64	97.5 (39/40)
Daptomycin	2	4	0.25 - 16	5 (2/40)
Linezolid	2	2	1 - 8	7.5 (3/40)
Minocycline	16	32	0.125 - 32	82 (31/38)
Tigecycline	0.06	0.25	0.03 - 16	10 (4/40)
Quinupristin/dalfopristin	0.5	1	0.25 - 8	2.5 (1/40)
Oritavancin ²	0.5	2	<0.015 - 8	-
Telavancin ²	8	16	0.12 - >16	-

¹Susceptibility percentages based upon CLSI standards M100-S26 (2016)

²Currently no defined breakpoint criteria for oritavancin or telavancin

Table 2 and Figure 1. Daptomycin MICs by Susceptibility Testing Method

Testing Method	Median MIC ³ (µg/mL)	MIC90 (µg/mL)	Range (µg/mL)	NS % (n)
BMD	2	4	0.25 - 16	5 (2/40)
Microscan	4	>4	0.5 - >4	26 (10/39) ¹
Etest	4	12	0.75 - >256	45 (18/40) ²

¹P= 0.01 [BMD vs Microscan]

²P= <0.001 [BMD vs Etest]

³Median MIC indicated by red line on graph

⁴Blue line indicates DNS breakpoint

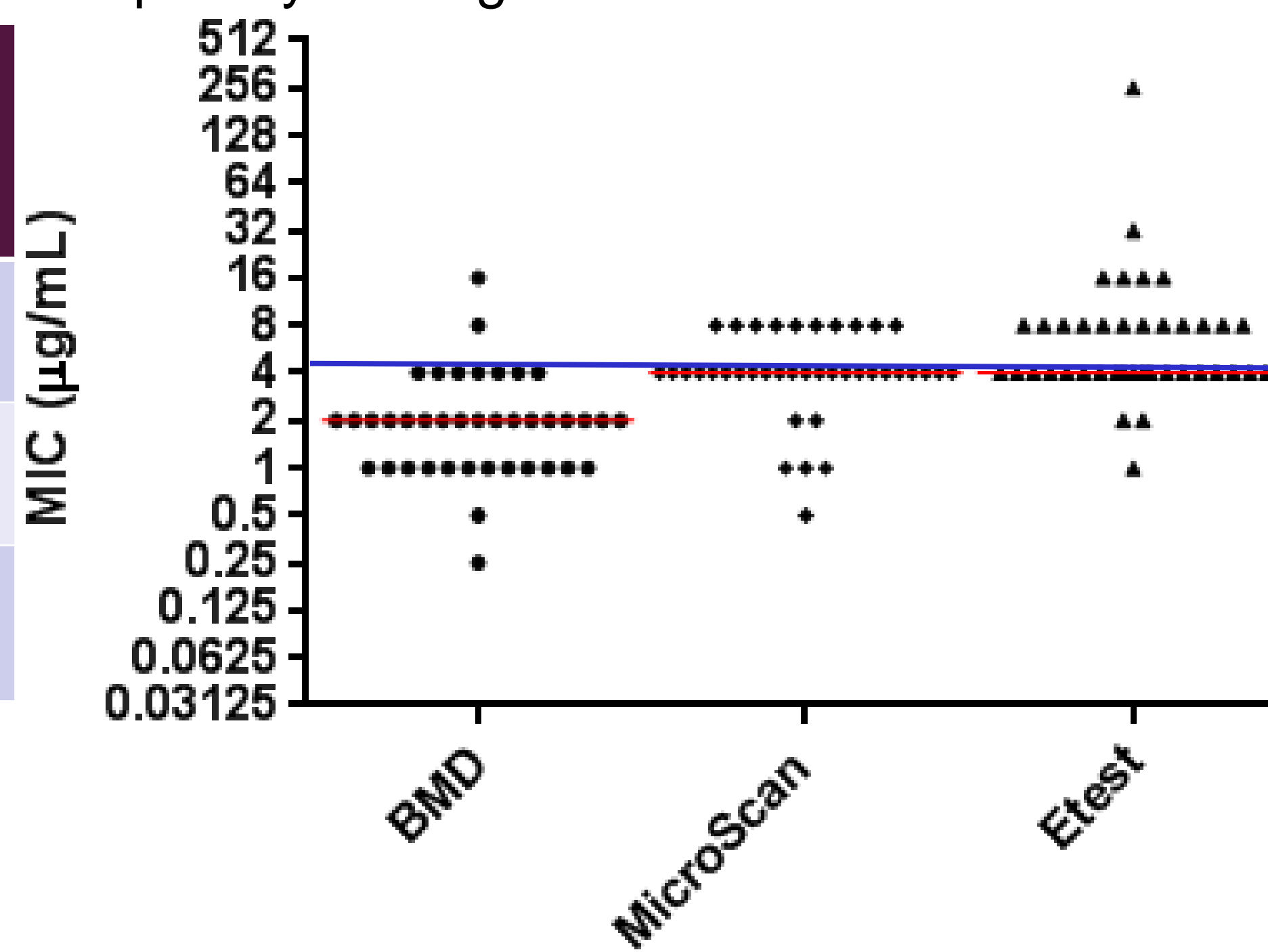
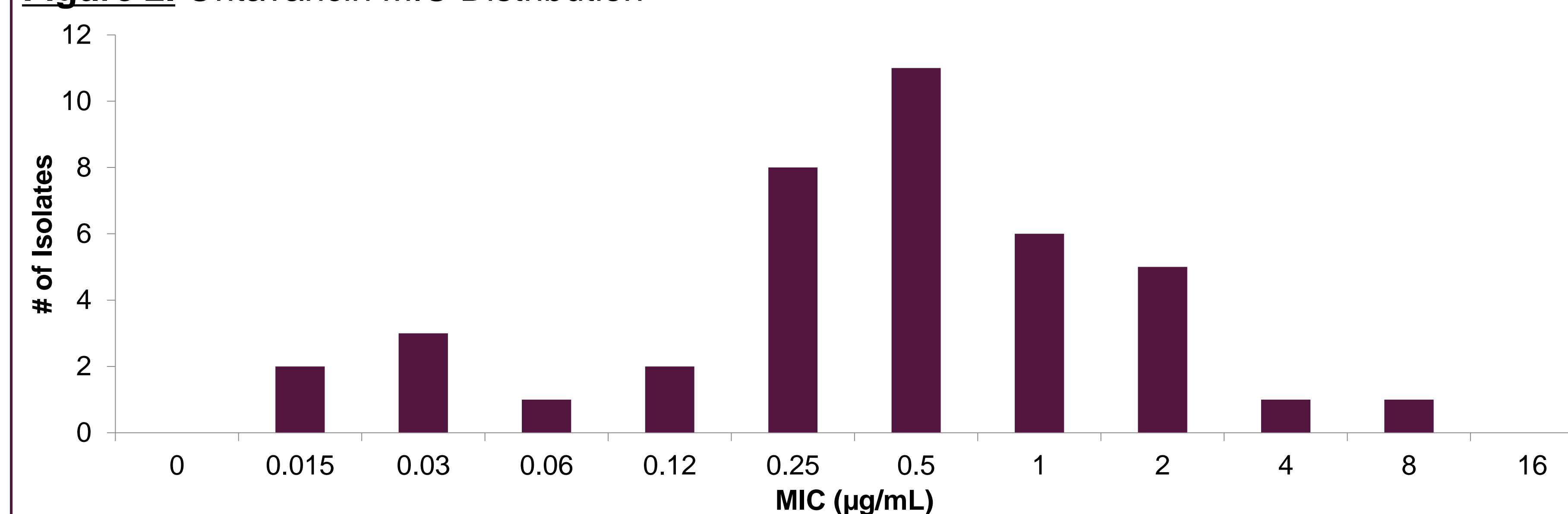


Figure 2. Oritavancin MIC Distribution



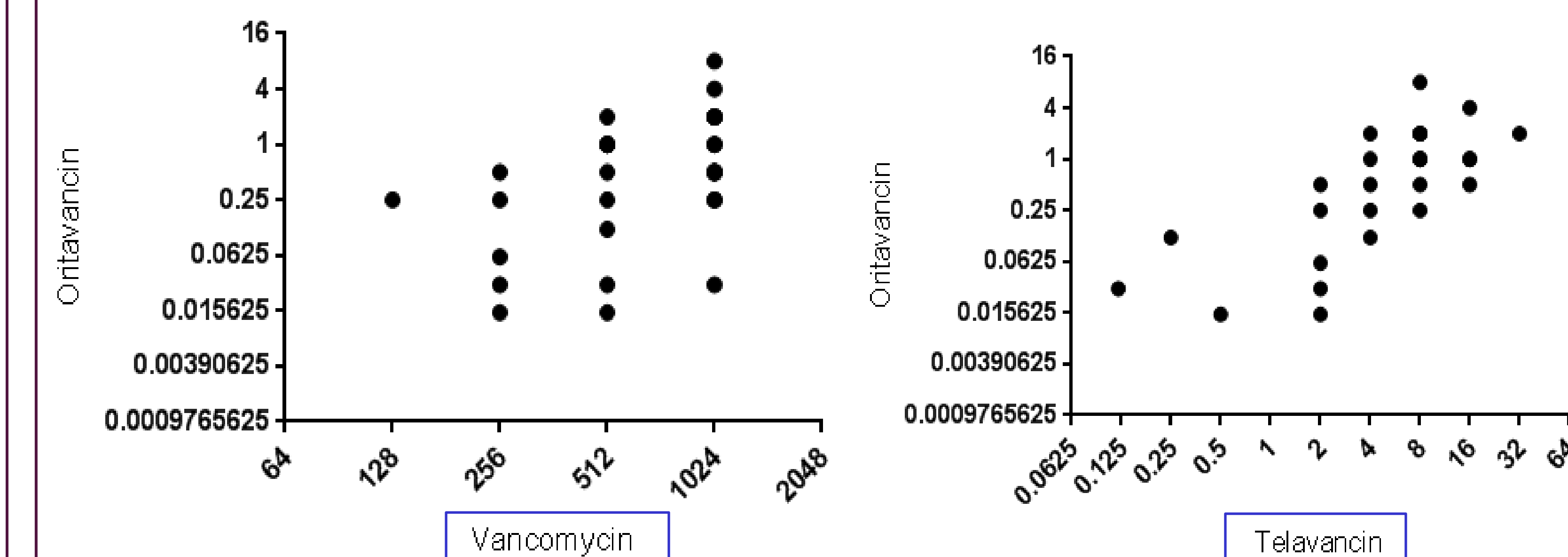
RESULTS (continued)

Table 3. Correlations Between Oritavancin and Other Antimicrobial Agents

Antimicrobial Agent	Correlation ¹ (r)	Correlation p-value	Mann-Whitney p-value
Vancomycin	0.4949	0.0012	0.0075
Teicoplanin	0.5942	<0.0001	0.0014
Daptomycin	0.1979	0.2210	0.7530
Linezolid	0.2417	0.1330	0.0554
Minocycline	-0.2815	0.0869	0.7582
Tigecycline	-0.203	0.2090	0.0272
Quinupristin/dalfopristin	-0.08818	0.5885	0.0726
Telavancin	0.685	<0.0001	<0.0001

¹Correlations between agents determined using Spearman's correlation coefficient

Figure 3. Correlation between Oritavancin MICs and Other Antimicrobial Agent MICs



CONCLUSIONS

- Compared to standard broth microdilution methods, rates of daptomycin non-susceptibility are over-estimated by MicroScan and Etest
 - Detecting daptomycin non-susceptibility appropriately is a critical issue for clinicians and microbiology laboratories
 - Standard broth microdilution methods should be employed
- At UPMC, the primary determinant of resistance among VRE is *VanA*
 - Against these isolates, rates of non-susceptibility against daptomycin and linezolid are low
 - Oritavancin susceptibility testing yielded a range of MICs
 - Standard susceptibility testing methods and clinical breakpoints are needed to determine the usefulness of this agent against VRE