Antimicrobial Susceptibility Testing of Human Bacterial Pathogens to Antibiotics Used as Pesticides

Purpose: To evaluate the risk of antibiotic pesticide use for co-selecting antibiotic resistance in bacterial pathogens causing human infections by testing for resistance to the pesticide drugs (streptomycin and tetracycline), co-resistance to related antibiotics, and the potential for co-selection of resistance (i.e., antibiotic use that can select for multi-drug resistant bacteria).

Methods: Reference antimicrobial susceptibility testing of bacterial isolates from human specimens. Antimicrobial susceptibility testing was performed using frozen broth microdilution panels prepared by CDC in accordance with the Clinical and Laboratory Standards Institute (CLSI) Standard M7. Testing was performed and interpreted in accordance with CLSI Standards M7 and M100. Bacterial isolates tested were identified from the CDC's repository of bacterial isolates collected in national antibiotic resistance surveillance programs. A brief description of surveillance collection strategies as follows:

- *Enterobacteriaceae* isolates were collected as part of a surveillance program that targets collection of carbapenem-resistant *Enterobacteriaceae* (CRE) from hospital microbiology laboratories.
- *Staphylococcus* and *Enterococcus* isolates were collected as part of a sentinel surveillance program that captures representative isolates from hospital microbiology laboratories.

Enterobacteriaceae, *Staphylococcus*, and *Enterococcus* isolates were chosen for study because they represent some of the most common antimicrobial resistant (AR) infections in humans. These bacteria are also causes of AR infection or colonization in food-producing animals.

All isolates tested were independent (i.e., isolates came from different patients). Isolates were selected because they represent diversity in susceptibility to the pesticide drug. Also, isolates where chosen to represent a diversity of resistance mechanisms, when resistance to the pesticide drug is present.

Tables 1, 2 and 3 show the minimum inhibitory concentrations (MICs) for each drug and the interpretation. The interpretative categories are "susceptible", "intermediate", or "resistant". These are categories that correspond to therapeutic decisions for humans. A "susceptible" result suggests that the drug could be used for treatment, "resistant" indicates that therapy with the drug is likely to fail, and an "intermediate" result suggests that the drug may be active in the body site where the drug is concentrated. For more information, please see CLSI Standard M100. For some drugs, interpretive criteria (i.e., susceptible, intermediate or resistant determinations) have not be established. In these cases epidemiological cutoff values (ECV) are applied to determine when the MIC is in the normal range (i.e., ≤ ECV) or elevated (> ECV). ECVs applied in Table 1 were in either CLSI M100 or the EUCAST recommendations (http://www.eucast.org/mic_distributions_and_ecoffs/). An elevated MIC

suggests the strain has acquired a resistance mechanism to the drug. In the table normal range MICs are indicated by "N" and elevated MIC are indicated by "E".

Summary of Results

Susceptibility and genotyping results can be found in tables 1-3. These results demonstrate that (1) resistance to antibiotics used as pesticides can be found in bacteria causing human disease, (2) resistance is often conferred by a mechanisms that can be transferred from one bacteria to another, (3) mechanisms of resistance to the pesticide can confer resistance to related antibiotics including antibiotics commonly used in human medicine (i.e., cross-resistance) and (4) resistance often occurs in isolates that are resistant to unrelated resistance (i.e., use of the pesticide could select for resistance to the pesticide and to unrelated antibiotics including antibiotics commonly used in human medicine).

Below is a description of results for each pesticide:

1. Tetracycline is a member of the tetracycline drug class. Other tetracyclines include doxycycline and minocycline. These are commonly used antibiotics. Doxycycline is recommended for treatment of skin and soft tissue infections caused by *Staphylococcus aureus*. Minocycline and tigecycline are second-line treatment options for serious gram-negative infections.
    a. *Enterobacteriaceae* – Tetracycline resistance is common among *Enterobacteriaceae* that are resistant to other drugs including isolates that are carbapenem resistant and isolates that are resistant to aminoglycosides. Most isolates that are resistant to tetracycline are also resistant to doxycycline and minocycline. The mechanism of resistance could not be identified in all isolates, but most resistant isolates harbored an acquired mechanism of resistance (i.e., *tet*A, *tet*B or *tet*D) (see Tables 1A and 1B).
    b. *Staphylococcus aureus* - Tetracycline resistance occurs in *S. aureus* but resistance is not necessarily linked to other types of resistance, like oxacillin/cefoxitin resistance (i.e., indicator drugs for defining methicillin-resistant *S. aureus* or MRSA). Resistance to tetracycline resulted in elevated doxycycline MICs (i.e., reduced susceptibility to this drug). Isolates that are resistant harbor acquired resistance mechanisms *tet*M and *tet*K (see Table 2).
    c. *Enterococcus* spp. – Tetracycline resistance is a common finding in *Enterococcus* isolates, including isolates that are resistant to multiple other antibiotics like vancomycin and aminoglycosides. Tetracycline resistance demonstrates cross-resistance to doxycycline. Isolates that are resistant harbor acquired resistance mechanisms *tet*O, *tet*U, and *tet*L (see Table 3).

2. Streptomycin is an aminoglycoside drug. Aminoglycosides are drugs commonly used in combination with other drugs to treat serious infections caused by serious gram-negative and gram-positive bacteria. Other aminoglycosides include amikacin, gentamicin, and tobramycin.

a. *Enterobacteriaceae* – Aminoglycoside resistance, including resistance to streptomycin is common in *Enterobacteriaceae* that are resistant to other drugs including isolates that are carbapenem resistant (CRE). There are many different acquired resistance mechanisms and these can confer resistance to one or more aminoglycoside drugs. Most concerning are the resistance mechanisms that encode ribosomal methylases (*arm*A, *rmt*F, and *rmt*C are the methylase mechanisms found in this collection of isolates). These mechanisms confer resistance to all aminoglycosides including a new drug, plazomycin, which is in development for treating CRE (see Table 1A and 1B).

b. *Staphylococcus aureus* – Elevated MICs to streptomycin can occur, but resistance to gentamicin is more common and not necessarily linked to streptomycin resistance. Acquired resistance mechanisms are responsible for reduced susceptibility to streptomycin (see Table 2).

c. *Enterococcus* spp. – Resistance to streptomycin is found among *Enterococcus* that are resistant to other drugs. Streptomycin resistance is commonly found in isolates that are resistant to other aminoglycosides, although this is usually the result of bacteria acquiring multiple aminoglycoside resistant mechanisms rather than a single determinant conferring resistance to all aminoglycosides (see Table 3).

Discussion

The use of antibiotics as pesticides has the potential to select for antimicrobial resistant bacteria present in the environment. This is of particular concern if the bacteria can cause human infection and/or confers transferable resistance mechanisms to antibiotics commonly used to treat infections in humans. Resistance to tetracycline and streptomycin is usually conferred by acquired resistant mechanisms (e.g., genes that encode resistance mechanisms and can move from one bacteria to another).

Human bacterial pathogens may be exposed to antibiotics used as pesticides: if the crop or crop soil is fertilized or contaminated with human or animal waste, if the pesticide comes into contact with water sources contaminated with human or animal waste, or if humans or animals are exposed to the antibiotic when it is used as a pesticide. This study looks for the ability of antimicrobials licensed as pesticides to select for clinically relevant antimicrobial resistance in bacterial pathogens collected in surveillance programs. General conclusions from this study are:

1. Resistance to the pesticides is found in bacteria causing human disease

2. Resistance to the pesticides is often conferred by acquired resistance mechanisms that are known to be transferable from one bacteria to another
3. Pesticides can select for resistance to related antibiotics (i.e., cross-resistance)
4. Pesticides can select for bacteria that are resistant to one or more unrelated antibiotics used to treat infections (i.e., co-selection of resistance). This includes selection for CRE bacteria that have been identified as an urgent AR threat, as well as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* which have been identified as serious AR threats (https://www.cdc.gov/drugresistance/biggest_threats.html)

Table 1A. Antimicrobial Susceptibility Results – *Enterobacteriaceae* Isolates 1-10

Drug Class	Drug	*Escherichia coli* ATR-01		*Klebsiella pneumoniae* ATR-02		*Escherichia coli* ATR-03		*Enterobacter cloacae* ATR-04		*Enterobacter cloacae* ATR-05		*Escherichia coli* ATR-06		*Enterobacter aerogenes* ATR-07		*Klebsiella pneumoniae* ATR-08		*Escherichia coli* ATR-09		*Klebsiella pneumoniae ATR-10*	
		MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT
β-Lactam Drugs	Ampicillin	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Ampicillin-sulbactam	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Piperacillin-tazobactam	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R
	Aztreonam	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R
	Cefoxitin	8	S	>16	R-	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R
	Cefazolin	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R
	Ceftriaxone	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Ceftazidime	128	R	>128	R	128	R	>128	R	32	R	128	R	>128	R	128	R	>128	R	>128	R
	Cefepime	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Ceftazidime-avibactam	≤0.5	S	2	S	2	S	2	S	≤0.5	S	1	S	8	S	1	S	2	S	4	S
	Ertapenem	2	R	>8	R	>8	R	>8	R	2	R	>8	R	>8	R	>8	R	>8	R	>8	R
	Imipenem	4	R	2	I	1	S	16	R	2	I	8	R	16	R	8	R	2	I	>64	R
	Meropenem	2	I	2	I	4	R	8	R	0.5	S	8	R	8	R	>8	R	8	R	>8	R
	Doripenem	2	I	2	I	2	I	8	R	1	S	8	R	4	R	4	R	4	R	>8	R
Tetracyclines	Tetracycline	>32	R	16	R	>32	R	16	R	32	R	>32	R	32	R	8	I	>32	R	8	I
	Doxycycline	16	R	16	R	16	R	16	R	4	S	32	R	32	R	16	R	64	R	4	S
	Minocycline	≤4	S	16	R	8	I	16	R	≤4	S	16	R	>16	R	>16	R	>16	R	≤4	S
	Tigecycline	≤0.5	S	2	S	≤0.5	S	2	S	2	S	≤0.5	S	4	I	4	I	≤0.5	S	1	S
Aminoglycosides	Amikacin	>64	R	>64	R	2	S	2	S	4	S	2	S	≤1	S	≤1	S	16	S	≤1	S
	Gentamicin	4	S	>16	R	16	R	4	S	>16	R	>16	R	0.5	S	≤0.25	S	1	S	>16	R
	Tobramycin	>16	R	>16	R	>16	R	16	R	8	I	16	R	≤0.5	S	≤0.5	S	>16	R	>16	R

	Streptomycin	16	N	>64	E	64	E	>64	E	>64	E	>64	E	4	N	>64	E	>64	E	32	E
Polymyxins	Polymyxin B	0.5	N	>8	E	0.5	N	1	N	1	N	0.5	N	0.5	N	1	N	0.5	N	2	E
	Colistin	0.5	N	>8	N	0.5	N	0.5	N	0.5	N	0.5	N	0.5	N	0.5	N	≤0.25	N	0.5	N
Fluoroquinolones	Ciprofloxacin	>8	R	>8	R	>8	R	>8	R	2	I	>8	R	0.5	S	>8	R	>8	R	>8	R
	Levofloxacin	>8	R	>8	R	>8	R	>8	R	4	I	>8	R	0.5	S	>8	R	>8	R	>8	R
Other	Trimethoprim-sulfamethoxazole	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R
	Chloramphenicol	8	S	>16	R	>16	R	>16	R	>16	R	8	S	>16	R	>16	R	>16	R	>16	R
All resistance genes		aadA5,TEM-1B,CTX-M-15,KPC-3,OXA-1,mph(A),sul1,tet(A),dfrA17		aadA2,aac(6')-Ib,oqxB,oqxA,mph(A),catA1,sul1,dfrA12		aadB,TEM-1B,CTX-M-15,sul1,sul2,tet(A)		strB,aadA1,strA,aac(6')-Ib,OXA-9,TEM-1A,KPC-3,SHV-12,sul2,dfrA14		strB,strA,aac(3)-IIa,aac(6')Ib-cr,TEM-1B,ACT-16,CTX-M-15,KPC-2,OXA-1,sul2,dfrA14		strB,strA,aac(3)-IId,TEM-1B,KPC-3,sul2,tet(B),dfrA17		strB,sul2,dfrA14		strB,strA,TEM-1B,CTX-M-15,KPC-3,oqxA,QnrS1,fosA,sul2,dfrA14		strB,aadA2,strA,aadA5,aac(6')Ib-cr,CTX-M-15,OXA-1,mph(A),sul1,sul2,tet(A),tet(B),dfrA12,dfrA17		aadB,aadA2,KPC-3,oqxB,oqxA,QnrS1,mph(A),catA1,sul1,dfrA12	
Aminoglycoside resistance genes		aadA5		aadA2,aac(6')-Ib		aadB		strB,aadA1,strA,aac(6')-Ib		strB,strA,aac(3)-IIa,aac(6')Ib-cr		strB,strA,aac(3)-IId		strB		strB,strA		strB,aadA2,strA,aadA5,aac(6')Ib-cr		aadB,aadA2	
Tetracycline resistance genes		tet(A)				tet(A)						tet(B)						tet(A), tet(B)			

Note – Interpretive criteria (i.e., S, I R determinations) have not be established for some drugs. In these cases, epidemiological cutoff values (ECV) are applied to determine when and MIC is in the normal range (i.e., ≤ ECV) or elevated (> ECV). An elevated MIC suggests the strain has acquired a resistance mechanism to the drug.

Table 1B. Antimicrobial Susceptibility Results – *Enterobacteriaceae* Isolates 11-20

Drug Class	Drug	*Escherichia coli* ATR-11		*Klebsiella pneumoniae* ATR-12		*Klebsiella pneumoniae* ATR-13		*Klebsiella pneumoniae* ATR-14		*Klebsiella pneumoniae* ATR-15		*Klebsiella pneumoniae* ATR-16		*Klebsiella pneumoniae* ATR-17		*Klebsiella pneumoniae* ATR-18		*Escherichia coli* ATR-19		*Klebsiella pneumoniae* ATR-20	
		MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT
β-Lactam Drugs	Ampicillin	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Ampicillin-sulbactam	>32	R	>32	R	32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Piperacillin-tazobactam	64	I	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R	>128	R
	Aztreonam	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R	>64	R	32	R	>64	R
	Cefoxitin	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R
	Cefazolin	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>16	R
	Ceftriaxone	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Ceftazidime	128	R	>128	R	>128	R	128	R	>128	R	>128	R	>128	R	>128	R	>32	R	>128	R
	Cefepime	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R

	Ceftazidime-avibactam	≤0.5	S	1	S	1	S	2	S	>16	R	2	S	>16	R	2	S	>16	R	>16	R
	Ertapenem	≤0.12	S	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	8	R	>8	R	>8	R
	Imipenem	≤0.5	S	64	R	4	R	8	R	32	R	8	R	32	R	2	I	8	R	64	R
	Meropenem	≤0.12	S	>8	R	4	R	>8	R	>8	R	>8	R	>8	R	2	I	>8	R	>8	R
	Doripenem	≤0.12	S	>8	R	4	R	>8	R	>8	R	>8	R	>8	R	4	R	>8	R	>8	R
Tetracyclines	Tetracycline	>32	R	>32	R	≤2	S	8	I	8	I	>32	R	>32	R	>32	R	>32	R	8	I
	Doxycycline	>64	R	32	R	2	S	16	R	16	R	64	R	64	R	16	R	16	R	16	R
	Minocycline	>16	R	>16	R	≤4	S	16	R	16	R	>16	R	>16	R	8	I	≤4	S	16	R
	Tigecycline	≤0.5	S	1	S	≤0.5	S	4	I	4	I	≤0.5	S	2	S	1	S	≤0.5	S	2	S
Aminoglycosides	Amikacin	4	S	16	S	>64	R	>64	R	>64	R	8	S	>64	R	>64	R	>64	R	>64	R
	Gentamicin	>16	R	1	S	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R
	Tobramycin	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R
	Streptomycin*	>64	E	>64	E	8	N	>64	R	16	N	16	N	8	N	16	N	32	E	32	E
Polymyxins	Polymyxin B	1	N	1	N	0.5	N	1	N	1	N	0.5	N	0.5	N	2	N	1	N	1	N
	Colistin	0.5	N	0.5	N	0.5	N	0.5	N	1	N	0.5	N	0.5	N	0.5	N	1	N	0.5	N
Fluoroquinolones	Ciprofloxacin	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R
	Levofloxacin	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	>8	R	8	R	>8	R	>8	R
Other	Trimethoprim-sulfamethoxazole	>8	R	>8	R	>8	R	>8	R	>8	R	≤0.5	S	>8	R	>8	R	>8	R	>8	R
	Chloramphenicol	>16	R	>16	R	16	R	>16	R	>16	R	16	R	>16	R	>16	R	8	S	>16	R
All resistance genes		strB,strA,aac(3)-IId,aadA5,aac(6')Ib-cr,TEM-1B,CTX-M-15,OXA-1,catA1,catB3,sul2,tet(B),dfrA17		strB,strA,aadA5,aac(6')-Ib,TEM-1B,KPC-2,SHV-12,oqxB,oqxA,mph(A),sul1,sul2,tet(A),dfrA17		OXA-181,SHV-26,CTX-M-15,aadA2,armA,sul1,sul2,dfrA12,dfrA14,fosA		OXA-181,SHV-11,TEM-1B,CTX-M-15,rmtF,strA,strB,oqxA,oqxB,sul2,dfrA12,dfrA14,mph(A),ARR-3		NDM-5,OXA-232,SHV-12,TEM-1A,CTX-M-15,aadA1,rmtF,qnrS1,sul1,dfrA14,mphA,fosA,aph(3')-Ia,ARR-3		OXA-48,OXA-9,SHV-1,TEM-1A,CTX-M-15,aac(6')-Ib,oqxA,oqxB,tet(D)		OXA-232,OXA-1,SHV-28,CTX-M-15,aac(6')-Ib-cr,dfrA1,dfrA12,fosA		OXA-181,SHV-26,CTX-M-15,aadA2,armA,aac(6')-Ib-cr,sul1,sul2,dfrA12,dfrA14,tet(A),fosA,ARR-3		mph(A),CMY-6,dfrA17,sul1,tet(A),rmtC,aac(3)-IIa,OXA-1,aadA5		armA,aac(3)-IId,OXA-9,TEM-1A,CMY-4,CTX-M-15,OXA-1,oqxA,fosA,mph(E),msr(E),catA1,cmlA1,ARR-3,dfrA1	
Aminoglycoside resistance genes		strB,strA,aac(3)-IId,aadA5,aac(6')Ib-cr		strB,strA,aadA5,aac(6')-Ib		aadA2,armA		rmtF,strA,strB		aadA1,rmtF,aph(3')-Ia		aac(6')-Ib		aac(6')-Ib-cr		aadA2,armA,aac(6')-Ib-cr		rmtC,aac(3)-IIa,aadA5		armA,aac(3)-IId	
Tetracycline resistance genes		tet(B)		tet(A)								tet(D)				tet(A)		tet(A)			

Note – Interpretive criteria (i.e., S, I R determinations) have not be established for some drugs. In these cases, epidemiological cutoff values (ECV) are applied to determine when and MIC is in the normal range (i.e., ≤ ECV) or elevated (> ECV). An elevated MIC suggests the strain has acquired a resistance mechanism to the drug.

Table 2. Antimicrobial Susceptibility Results - *Staphylococcus* Isolates

Drug Class	Drug	*Staphylococcus aureus* ATR-21		*Staphylococcus aureus* ATR-22		*Staphylococcus aureus* ATR-23		*Staphylococcus aureus* ATR-24		*Staphylococcus aureus* ATR-25		*Staphylococcus aureus* ATR-26		*Staphylococcus aureus* ATR-27		*Staphylococcus aureus* ATR-28		*Staphylococcus aureus* ATR-29		*Staphylococcus aureus* ATR-30	
		MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT
β-Lactam Drugs	Penicillin	>2	R	>2	R	>2	R	>2	R	>2	R	2	R	>2	R	2	R	1	R	>2	R
	Oxacillin	16	R	>16	R	8	R	>16	R	>16	R	>16	R	8	R	≤0.25	S	0.5	S	>16	R
	Cefoxitin	16	R	>16	R	16	R	>16	R	>16	R	16	R	16	R	4	S	≤2	S	>16	R
Tetracyclines	Tetracycline	64	R	64	R	0.25	S	1	S	>64	R	0.25	S	≤1	S	>64	R	32	R	64	R
	Doxycycline	8	I	8	I	0.12	S	0.5	S	16	R	0.12	S	0.25	S	8	I	8	I	8	I
Aminoglycosides	Gentamicin	0.5	S	1	S	32	R	>64	R	>64	R	64	R	32	R	0.5	S	0.06	S	64	R
	Streptomycin	16	N	8	N	16	N	16	N	>64	E	16	N	16	N	8	N	4	N	8	N
Macrolides & Lincosamides	Erythromycin	1	I	>8	R	>8	R	>8	R	>8	R	0.5	S	>8	R	>8	R	0.5	S	>8	R
	Clindamycin	≤0.25	S	>16	R	≤0.25	S	>16	R	>16	R	≤0.25	S	>16	R	≤0.25	R*	≤0.25	S	>16	R
Fluoroquinolones	Levofloxacin	8	R	>16	R	≤0.5	S	>16	R	16	R	4	R	16	R	>16	R	≤0.5	S	>16	R
Other	Chloramphenicol	8	S	32	R	8	S	16	I	8	S	8	S	8	S	16	I	8	S	2	S
	Daptomycin	2	NS	1	S	≤0.5	S	4	NS	1	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	1	S
	Linezolid	2	S	2	S	2	S	2	S	≤1	S	2	S	2	S	4	S	2	S	2	S
	Vancomycin	1	S	2	S	1	S	2	S	2	S	≤0.5	S	1	S	≤0.5	S	≤0.5	S	1	S
	Rifampin	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S
	Mupirocin	>256	R	8	N	>256	R	>256	R	≤4	N	≤4	N	>256	R	≤4	N	≤4	N	>256	R
All resistance genes		tet(M)		aadD,spc		aadD,aac(6')-aph(2'')		aadD,aph(3')-III,spc,mph(C),aac(6')-aph(2''),msr(A)		mecA,aph(3')-III,spc,dfrG,tet(K),aac(6')-aph(2'')		aac(6')-aph(2'')		aph(3')-III,aac(6')-aph(2''),mecA,mph(C),msr(A)		aph(3')-III,spc,tet(K)		Not found		aadD,aph(3')-III,spc,mph(C),tet(K),aac(6')-aph(2''),msr(A)	
Aminoglycoside resistance genes				aadD,spc		aadD,aac(6')-aph(2'')		aadD,aph(3')-III,spc,aac(6')-aph(2'')		aph(3')-III,spc,aac(6')-aph(2'')		aac(6')-aph(2'')		aph(3')-III,aac(6')-aph(2'')		aph(3')-III,spc				aadD,aph(3')-III,spc,aac(6')-aph(2'')	
Tetracycline resistance genes		tet(M)														tet(K)				tet(K)	

Table 3. Antimicrobial Susceptibility Results – *Enterococcus* Isolates

Drug Class	Drug	*Enterococcus avium* ATR-31		*Enterococcus faecium* ATR-32		*Enterococcus faecalis* ATR-33		*Enterococcus faecium* ATR-34		*Enterococcus faecium* ATR-35		*Enterococcus faecium* ATR-36		*Enterococcus faecalis* ATR-37		*Enterococcus faecium* ATR-38		*Enterococcus faecium* ATR-39		*Enterococcus faecalis* ATR-40	
		MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT	MIC	INT
β-Lactam Drugs	Ampicillin	1	S	>128	R	1	S	128	R	>128	R	>128	R	1	S	>128	R	>128	R	2	S
Tetracyclines	Tetracycline	16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R	>16	R
	Doxycycline	8	I	16	R	16	R	16	R	8	I	16	R	16	R	16	R	16	R	8	I
Aminoglycosides	High Level-Gentamicin	>500	R	≤500	S	>500	R	≤500	S	≤500	S	≤500	S	>500	R	>500	R	>500	R	≤500	S
	High Level - Streptomycin	≤1000	S	≤1000	S	>1000	R	>1000	R	≤1000	S	≤1000	S	>1000	R	≤1000	S	>1000	R	>1000	R
Macrolides	Erythromycin	>8	R	>8	R	>8	R	>8	R	4	I	4	I	>8	R	>8	R	>8	R	>8	R
Fluoroquinolones	Levofloxacin	8	R	>8	R	1	S	>8	R	>8	R	>8	R	>8	R	>8	R	2	S	1	S
Other	Chloramphenicol	8	S	16	I	16	I	8	S	16	I	16	I	32	R	16	I	8	S	>32	R
	Daptomycin	1	S	4	S	2	S	4	S	4	S	4	S	2	S	8	NS	4	S	2	S
	Linezolid	2	S	4	I	2	S	2	S	4	I	4	I	2	S	2	S	2	S	2	S
	Vancomycin	256	R	512	R	1	S	0.5	S	512	R	1	S	1	S	0.5	S	0.5	S	2	S
	Rifampin	1	S	>4	R	2	I	>4	R	>4	R	>4	R	1	S	>4	R	>4	R	1	S
All resistance genes		VanS-A,VanA,VanR-Pt2,VanZ-A,VanH-A,tet(O),VanX-A,erm(B),aac(6')-aph(2'')		VanY-A,VanS-A,VanA,VanR-Pt2,aph(3')-III,VanZ-A,VanH-A,VanX-A,dfrG		VanS-A,VanA,VanR-Pt2,VanZ-A,VanH-A,tet(O),VanX-A,erm(B),aac(6')-aph(2'')		ant(6)-Ia,aph(3')-III,erm(T)		VanS-A,VanA,VanR-Pt2,VanZ-A,VanH-A,tet(U),VanX-A		dfrG		aph(3')-III,cat,dfrG,tet(L),aac(6')-aph(2'')		erm(T),dfrG,aac(6')-aph(2'')		ant(6)-Ia,aph(3')-III,aph(2'')-Id,tet(U),lnu(B)		ant(6)-Ia,lsa(A),aph(3')-III	
Aminoglycoside resistance genes		aac(6')-aph(2'')		aph(3')-III		A,aac(6')-aph(2'')		ant(6)-Ia,aph(3')-III						aph(3')-III,aac(6')-aph(2'')		aac(6')-aph(2'')		ant(6)-Ia,aph(3')-III,aph(2'')-Id		ant(6)-Ia,aph(3')-III	
Tetracycline resistance genes		tet(O)				tet(O)				tet(U)				tet(L)				tet(U)			