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Susceptibility Profiles of *Enterococcus faecalis* to Selected Antibiotics

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ABSTRACT

This study was conducted in order to determine the susceptibility of *Enterococcus faecalis* to the antibiotics penicillin, erythromycin, tetracycline, chloramphenicol and gentamicin through measuring the zone of inhibition. The susceptible, intermediate and resistant categories were assigned on the basis of the critical points recommended by the Clinical and Laboratory Standards Institute. *E. faecalis* was susceptible to tetracycline as low as 20 µg/20 µl. Starting at a dose of 60 µg/20 µl and 200 µg/20 µl, the bacterium was susceptible to penicillin and erythromycin, respectively. The bacterium was resistant to chloramphenicol even at the highest dosage of 400 µg/20 µl. Meanwhile, from 5 to 100 µg/20 µl, the bacterium was resistant to gentamicin and the classification was changed into intermediate starting at 200 µg/20 µl.

Keywords: *Enterococcus faecalis*, antibiotics, susceptibility

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INTRODUCTION

Enterococci are Gram-positive, catalase-negative, non-spore forming and facultative anaerobic bacteria that can occur either as single cocci or in chains[1]. Enterococci are considered commensals of the gastrointestinal tract of a variety of organisms, including humans. They are found in a number of environments, due to dissemination in animal excrement and environmental persistence[2]. Enterococci are originally defined by Shernan[3] as hardy microorganisms that can withstand harsh conditions such as growth at 10 °C up to 45 °C, at pH 9.6, in 6.5 % NaCl broth and 40 % bile salts, and survive at 60 °C for 30 minutes. Starving *Enterococcus faecalis* maintain their viability for extended periods and become resistant to UV irradiation, heat, sodium hypochlorite, hydrogen peroxide, ethanol and acid[4].

The origins of *Enterococcus* spp. vary from environmental to animal and human resources[1]. Enterococci are essential part of the microflora of both humans and animals. The numbers of *E. faecalis* in human feces range from 10^5 to 10^7 per gram, and 10^4 to 10^5 for *E. faecium*. The isolation of *E. faecium* and *E. faecalis* is less prevalent from livestock than from human feces[5].

Many factors are attributed to the virulence of *Enterococcus* spp. such as (1) ability to colonize the gastrointestinal tract, which is the normal habitat; (2) ability to adhere to a range of extracellular matrix proteins, including thrombospondin, lactoferrin and vitronectin; and (3) ability to adhere to urinary tract epithelia, oral cavity epithelia and human embryo kidney cells[5]. Most infection is thought to be endogenous, by translocation of the bacteria through the epithelial cells of the intestine, which then cause infection via lymph nodes and thus spread to other cells within the body[5].

The ability of enterococci to acquire new resistance genes imported on plasmids, transposons and conjugative transposons is alarming, especially if these resistances are associated with the pathogenicity. The antibiotic resistance of *Enterococcus* is well documented[6]. *Enterococcus* spp. may show resistance to glycopeptides such as vancomycin, teipclopanin and aminoglycosides[6]. It has been reported that if glycopeptide resistant enterococci are present in an infected animal rather than an antibiotic-susceptible strain, clinical treatment failure is increased by 20% and mortality is increased by 27 to 57%[7]. When assessing the studies on enterococcal antibiotic resistance, the pattern that is emerging is the possible occurrence of multidrug resistant strains[8].

MATERIALS AND METHODS

Source and Maintenance of *E. faecalis*

Pure culture of *E. faecalis* was obtained from the Fish Pathology Laboratory of the College of Fisheries in Central Luzon State University, Philippines. The identity of the isolate was confirmed by 16s rRNA sequencing. The isolate was maintained in Trypticase Soy Agar (TSA) with mineral oil under room temperature.

Preparation of Filter Paper Discs

Approximately 6 mm holes were made in Whatman filter paper No. 3 using a puncher. The filter paper discs were autoclaved at 15 lbs pressure for 30 minutes.

Preparation of Antibiotic Stock Solution

Powdered form of ampicillin was purchased in drugstores. In order to obtain a stock solution of 20 µg/µl, a known weight of the antibiotics was dissolved in sterile distilled water. The stock solution was diluted at the time of disc preparation to obtain the working solution of 10 mL. The concentrations of antibiotics solutions that were evaluated are presented in Table 1. Using a micropipette, a fixed volume of 20 µl was loaded on each disc one by one.

Drying and Impregnation of Discs

The antibiotic discs were allowed to dry in a clean incubator at 37 °C for 4 hours. Meanwhile, about 2 to 3 colonies of 18 to 24-hour bacterium were suspended in Trypticase Soy Broth (TSB). The tube was incubated at 37 °C for 1 to 2 hours. The bacterial suspension was adjusted to 0.5 McFarland turbidity standards and was evenly spread in TSA plates using a sterile cotton swab. After the inoculum has dried, the prepared antibiotic discs were placed on the surface of the inoculated plate using sterile forceps. The plates with discs were incubated at 37 °C and were observed after 18, 24 and 48 hours of incubation. The diameter of the zone of inhibition was measured in millimeters using ruler. The susceptible, intermediate and resistant categories were assigned on the basis of the critical points recommended by the Clinical and Laboratory Standards Institute[9].

Table 1: Computed volume of stock solution in each concentration of working solution

Concentration of Stock Solution (µg/µl)	Volume of Stock Solution (mL)	Concentration of Working Solution (µg/20 µl)	Volume of Working Solution (mL)
20	0.00	0	10
20	0.13	5	10
20	0.25	10	10
20	0.50	20	10
20	1.00	40	10
20	1.50	60	10
20	2.00	80	10
20	2.50	100	10
20	5.00	200	10
20	10.00	400	10

RESULTS AND DISCUSSION

Susceptibility Profiles of *E. faecalis*

The susceptibility profiles expressed as zone of inhibition (ZOI) of *E. faealis* on the various dosages of antibiotics are presented in Table 2. As a general trend, the susceptibility of the bacterium increases along with the dosages of the antibiotics. From a dose of 5 µg/20 µL until the highest dose of 400 µg/20 µL, the ZOIs of tetracycline were significantly higher as compared to penicillin, erythromycin, chloramphenicol and gentamicin. The best antibiotic for the treatment of *E. faecalis* in Nile tilapia was tetracycline based on the result of this present study. Except for tetracycline, the ZOIs of penicillin at 10, 60, 80 and 100 µg/20 µL were significantly higher as compared to erythromycin, chloramphenicol and gentamicin, thus, these antibiotics were the second choices for treating tilapia infected by *E. faecalis*. The antibiotic that ranked third based on the diameter of ZOIs was erythromycin, with ZOIs that were significantly higher to chloramphenicol from 20 to 400 µg/20 µL and to gentamicin at 10 µg/20 µL and from 40 to 400 µg/20 µL. The last two antibiotics that had the smallest recorded ZOIs were chloramphenicol and gentamicin.

Table 2: Susceptibility profiles of *E. faecalis* on the various dosages of antibiotics

Dosages (µg/20 µL)	Zone of Inhibition (mm)				
	Penicillin	Erythromycin	Tetracycline	Chloramphenicol	Gentamicin
0	6.00±0.00[a]	6.00±0.00[a]	6.00±0.00[a]	6.00±0.00[a]	6.00±0.00[a]
5	7.67±0.52[b]	8.00±0.00[b]	22.92±2.54[a]	7.17±0.41[b]	8.83±0.26[b]
10	9.83±0.75[b]	7.17±0.41[c]	23.83±1.83[a]	7.25±0.61[c]	11.50±0.45[b]
20	15.17±1.47[b]	14.25±1.04[b]	24.92±0.20[a]	7.00±0.00[c]	12.58±0.38[b]
40	17.08±1.80[b]	18.08±1.43[b]	26.75±1.33[a]	7.17±0.41[d]	12.50±0.45[c]
60	20.25±0.52[b]	16.33±0.88[c]	26.33±0.98[a]	6.17±0.26[e]	12.50±0.45[d]
80	20.75±0.42[b]	17.33±1.72[c]	30.92±0.58[a]	7.25±0.61[e]	13.67±0.82[d]
100	20.75±0.42[b]	17.67±0.88[c]	31.25±2.34[a]	6.67±0.52[e]	14.50±0.45[d]
200	22.92±0.92[b]	20.08±1.20[b]	30.25±0.27[a]	7.00±0.00[d]	16.00±0.55[c]
400	22.42±1.88[b]	20.58±0.92[b]	30.25±1.04[a]	6.33±0.52[d]	16.92±0.49[c]

Means (±SD) not sharing a common superscript between columns are significantly different ($p \leq 0.05$)

Based on CLSI[9], the bacterium *E. faecalis* was susceptible to tetracycline as low as 20 µg/20 µl. Starting at a dose of 60 µg/20 µl and 200 µg/20 µl, the bacterium was susceptible to penicillin and erythromycin, respectively. The bacterium was resistant to chloramphenicol even at the highest dosage of 400 µg/20 µl. Meanwhile, from 5 to 100 µg/20 µl, the bacterium was resistant to gentamicin and the classification was changed into intermediate starting at 200 µg/20 µl (Table 3).

Table 3: CLSI classifications of *E. faecalis* on the various dosages of antibiotics

Dosages (µg/20 µL)	CLSI Classification				
	Penicillin	Erythromycin	Tetracycline	Chloramphenicol	Gentamicin
0	--	--	--	--	--
5	R	R	S	R	R
10	R	R	S	R	R
20	I	R	S	R	R
40	I	I	S	R	R
60	S	I	S	R	R
80	S	I	S	R	R
100	S	I	S	R	R
200	S	S	S	R	I
400	S	S	S	R	I

Note: Resistant (R) = < 14 mm; Intermediate (I) = 15 to 19 mm; Susceptible (S) = > 20 mm

Tetracycline is a broad spectrum antibiotic that can inhibits almost all Gram-negative and Gram-positive bacteria. Tetracycline binds with the 30S subunit of ribosomes which inhibits the protein synthesis of the bacterial cells[10,11]. This leads to the susceptibility of *E. faecalis* isolate to tetracycline.

Penicillin or benzylpenicillin belongs to the β-lactams group of antibiotics in which it targets the cell wall synthesis. Penicillin-resistant bacteria resist the actions of β-lactams antibiotics by producing an enzyme called β-lactamase and penicillin-binding proteins[12]. Penicillin G or benzylpenicillin is active primarily against Gram-positive bacteria because the Gram-negative groups of bacteria are impermeable to β-lactams group of antibiotics[13]. This could explain why *E. faecalis* was susceptible to β-lactams group of antibiotics specially at higher concentration.

Erythromycin is a broad-spectrum antibiotic that targets the 50S subunits of the bacterial ribosome that partially inhibits the protein synthesis. The partial inhibition of protein synthesis leads to the preferential translation of some proteins and restricts the translation of others that results to the imbalance of proteome which may consequently resulted to the disruption of metabolic process[13].

Chloramphenicol acts by inhibiting the 30S protein synthesis by disrupting the translation with the interactions of ribosomes often involving to ribosomal RNA (rRNA)[13]. Similar to the study of Franz et al.[14], in which the study

reported that *E. faecalis* strains were mostly resistant to chloramphenicol.

The aminoglycosides groups are composed of gentamicin, streptomycin and its derivatives, kanamycin and neomycin. This group of antibiotic target the 20S subunit of ribosomes, inhibiting the protein synthesis and are notably useful for the treatment of the Gram-negative group bacteria[13]. Natural resistance of anaerobic bacteria including *Enterococcus* spp. to aminoglycosides is due to the lack of oxidative metabolism to drive uptake of the antibiotics[15,16]. Low level of intrinsic resistance to aminoglycosides is mediated by the ability of the enterococcal cell wall to limit the uptake of the drug[17]. Another mechanism of high-level resistance to aminoglycosides is through the production of aminoglycosides-modifying enzymes which is common among enterococci. High-resistance to gentamicin is mediated by the functional enzyme 6'-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase [AAC(6')-Ie-APH(2″)-Ia][18].

Wide spread use of antibiotics in aquaculture as prophylactic and therapeutic agents to bacterial diseases has been associated with the emergence of antibiotic resistance in bacterial pathogen and the alteration of the microbiota of aquaculture environment[19,20]. According to Kuhn et al.[21], *Enterococcus* spp. is a good indicator of antimicrobial resistance in animals, human, and the environment including soil, manure, and water samples. They are known for the capability to acquire resistance determinants by rapid adaptation to environmental conditions. Resistance to antimicrobial drugs can arise either from new mutations in the bacterial genome or through the acquisition of genes encoding antibiotic resistance. These genetic changes consequently alter the defensive function of the bacteria by changing the target of the drugs, by detoxifying or ejecting the antimicrobial, or by routing metabolic pathways around the disrupted point[21]

CONCLUSION

The bacterium *E. faecalis* was susceptible to tetracycline as low as 20 µg/20 µl, and to penicillin and erythromycin starting at 60 µg/20 µl and 200 µg/20 µl, respectively. The bacterium was resistant to chloramphenicol even at the highest dosage of 400 µg/20 µl. Meanwhile, the bacterium was resistant to intermediate to gentamicin.

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