DETECTION OF HIGH-LEVEL AMINOGLYCOSIDE RESISTANCE AND REDUCED SUSCEPTIBILITY TO VANCOMYCIN AMONG ENTEROCOCCAL CLINICAL ISOLATES

Thesis

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Dedication

I would like to dedicate this work to ***my father****, for whom I owe a lot as he always used to say my greatest investment is my children. May he always rest in peace and be forever proud of me.*

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First of all greatest thanks to ***Allah***

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ABSTRACT

Enterococci are Gram-positive bacteria that have become an important clinical pathogen and are considered as the second leading cause of healthcare-associated bacteremia with *E. faecalis* and *E. faecium* representing the two most common enterococcal species comprising about 5 to 15% of cases of infectious endocarditis, as well as UTIs followed by intra-abdominal, pelvic and soft tissue infections. Enterococci have the capacity to acquire resistance to antibiotics including high-level resistance to aminoglycosides (HLAR) and glycopeptides including VRE. This evolution of anti-microbial resistance in enterococci poses enormous challenges for treatment especially when faced with patients with severe infections. Antibiotics such as linezolid, daptomycin and tigecycline may offer alternative solution for treatment of VRE infections. In the current study we aimed to identify the enterococcal species, determine their antibiotic sensitivity, including HLAR, low susceptibility to vancomycin and detect β-lactamase production. In addition we aimed to evaluate therapeutic options for MDR enterococci namely linezolid and tigecycline. Fifty enterococcal species were isolated, *E. faecalis* (38/50, 76%) was predominant over *E. faecium* (12/50, 24%). Among the 50 studied enterococcal isolates, HLSR and HLGR represented 56% and 46%, respectively, while vancomycin resistance was detected in (2/12, 16.7%) of isolated *E. faecium*. None were β-lactamase producer. Twenty five (50%) out of the 50 enterococcal isolates were MDR, which were all sensitive to both linezolid and tigecycline.

Key words: *E. faecalis*, *E. faecium*, HLAR and VRE.

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LIST OF ABBREVIATIONS

AACs	Acetyltransferases
Ace	Adhesin of collagen from enterococci
AMEs	Aminoglycoside-modifying enzymes
ANTs	Nucleotidyltransferases
APHs	Phosphotransferases
API	Analytical profile index
ARA	Arabinose
ARG	Arginine
AS	Aggregation substance
ATP	Adenosine triphosphate
BE-test	Bile-Esculin test
CDC	Centers for Disease Control and Prevention
CFR	Chloramphenicol-florfenicol resistance
CFU	Colony forming unit
ChromID	Chromogenic ID medium (BioMérieux)
CLED	Cysteine lactose electrolyte deficient
CLSI	Clinical and laboratory standard institute
D- Lac	D-Lactate
D-Ala	D-Alanine
DNA	Deoxyribonucleic acid
D-Ser	D-Serine
Esp	Enterococcal surface protein
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	United States food and drug administration
GelE	Gelatinase

gelE	Gelatinase gene
GI tract	Gastro-Intestinal tract
GRE	Glycopeptide resistant enterococci
HLAR	High level aminoglycoside resistance
HLG	High level gentamicin
HLGR	High level gentamicin resistance
HLS	High level streptomycin
HLSR	High level streptomycin resistance
Hyl	Hyaluronidase gene
ICU	Intensive Care Unit
IS	Insertion element
kb	kilobase
LAPase	Leucine amino-peptidase
MAN	Mannitol
MDR	Multi-drug resistance
MGP	Methyl α-D-glucopyranoside
MHA	Muller Hinton Agar
MIC	Minimum inhibitory concentration
MOT	Motility
MSCRAMM Ace	Microbial surface component recognizing adhesive matrix molecule adhesin of collagen from enterococci
MSCRAMM	Microbial surface components recognizing adhesive matrix molecules
MsrC	Macrolide–streptogramin resistance protein
NaCl	Sodium chloride
NCCLS	National committee for clinical laboratory standards
PAI	Pathogenicity islands

PBPs	Penicillin binding proteins
PIG	Pigment
PYR	L-pyrrolidonyl-beta-naphthylamide
PYRase	Pyrrolidonyl arylamidase
PYU	Pyruvate
RAF	Raffinose
rRNA	Ribosomal Ribonucleic acid
SBL	Sorbitol
SOR	Sorbose
sprE	Serine protease gene
SUC	Sucrose
TBRI	Theodor Bilharz Research Institute
TEL	Tellurite
Tn	Transposon
UTI	Urinary tract infection
Vat	Virginia-mycin acetyltransferase
Vgb	Virginia-mycin B lyase
VRE	Vancomycin resistant enterococci
VSE	Vancomycin-sensitive enterococci

INTRODUCTION

Enterococci are Gram-positive bacteria; members of the genus *Enterococcus* were classified as group D *Streptococcus* until 1984 when genomic DNA analysis placed them in their own genus ***(Schleifer and Kilpper-Balz, 1984; Dalal et al., 2008).*** They have long been regarded as harmless commensals of the gastrointestinal tract of a wide variety of hosts; humans and other mammals, birds, reptiles and insects. However, they have emerged in the 1970s as one of the leading causes of multidrug-resistant hospital-acquired infections and have gained increasing clinical importance through the 1990s due to anti-microbial misuse patterns ***(Murray, 1998; Donskey et al., 2000; Lebreton et al., 2014).***

The most common type of enterococcal infections occurs in the urinary tract; nevertheless enterococci have also been recovered from cultures of intra-abdominal, pelvic, and soft tissue infections and have become the second leading cause of healthcare-associated bacteremia ***(Helmi et al., 2008)***. The genus now includes over forty distinct species of enterococci ***(Lebreton et al., 2014)***. *Enterococcus faecalis* is the most common and causes 85-90% of enterococcal infections, while *Enterococcus faecium* causes 5-10% ***(Hidron et al., 2008; Fisher and Phillips, 2009).***

The ability of enterococci to colonize the gastrointestinal tract of hospitalized humans for long periods is a crucial factor that influences the development of drug resistance ***(Moellering, 1992)***. Such resistance has resulted in a major decrease in therapeutic options, because the majority of enterococcal isolates have a remarkable ability to adapt to exposure to antibiotics. They show intrinsic resistance to cephalosporins, trimethoprim-sulfamethoxazole and low-level aminoglycosides ***(Adhikari, 2010)***.