UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF ANTIBIOTIC SUSCEPTIBILITY AND HIGH LEVEL AMINOGLYCOSIDE RESISTANT GENES IN Enterococcus faecalis AND Enterococcus faecium CLINICAL ISOLATES

AYAN ADEN MOUSSA

FPSK(M) 2018 37

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By

AYAN ADEN MOUSSA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the degree of Master of Science

May 2018

DEDICATION

To my parents

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CHARACTERIZATION OF ANTIBIOTIC SUSCEPTIBILITY AND HIGH LEVEL AMINOGLYCOSIDE RESISTANT GENES IN *Enterococcus faecalis* AND *Enterococcus faecium* CLINICAL ISOLATES

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May 2018

Chairman : Azmiza Syawani Jasni, PhD
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Enterococcus faecium and *Enterococcus faecalis* are among the predominant species causing hospital-acquired infections, with high mortality and morbidity rates. Enterococci which exhibit high level aminoglycoside (HLAR) and vancomycin resistance (VRE) possess a significant problem in therapeutic treatment. These resistance strains are becoming more widespread in Europe, USA and Asia. Studies have shown that the different distributions of aminoglycoside modifying enzymes (AMEs) among HLAR are based on the geographical regions. In Malaysia, however, data on the prevalence of HLAR and the distribution of AMEs are still limited. Hence, the aims of this study were to determine the antibiotic susceptibility patterns of the clinical isolates *E. faecalis* and *E. faecium* and the distribution of HLAR genes among the resistant isolates. Seventy-five clinical enterococci isolates used in this study were originally obtained from a tertiary centre, in the year 2009 and 2010. These isolates were isolated from different sources including pus (50%), blood (32%), urine (11%) and other sources such as CSF and HVS (7%). Re-identification of these isolates was carried out using several methods including sub-culturing on selective medium and Gram staining, followed by confirmatory tests using a commercial biochemical profiling (API20 strep) and 16s rRNA PCR amplification. Antimicrobial susceptibility tests were performed using disc diffusion, E-test and broth microdilution methods. The antibiotics used were aminoglycosides (gentamicin, streptomycin, kanamycin, amikacin, tobramycin) and other antibiotics which are commonly

used in the hospital to treat enterococcal infections such as ampicillin, vancomycin, erythromycin, tetracycline, chloramphenicol and linezolid. Detection of HLAR genes was performed on resistance isolates using single PCR amplifications. Out of 25 (33.3%) isolates of *E. faecium,* 84% and 68% showed high level gentamicin resistance (HLGR) and high level streptomycin resistance (HLSR), respectively. Resistance against erythromycin and tetracycline were 100%, while ampicillin and chloramphenicol showed 84% and 32% resistance rates, respectively. None of the *E. faecium* isolates showed resistance towards vancomycin and linezolid. On the other hand, all 50 (66.7%) *E. faecalis* isolates were resistance to all aminoglycosides tested with the following percentages; amikacin (100%), kanamycin (90%), tobramycin (70%), HLGR (36%) and HLSR (46%). These isolates were also resistance to tetracycline (98%), erythromycin (96%), chloramphenicol (46%), ampicillin (24%), linezolid (8%), and vancomycin (4%). Interestingly, the *E. faecalis* isolates that were resistant to vancomycin were previously reported as susceptible. In this study, *E. faecium* exhibited higher resistance rates to all antibiotics except vancomycin and linezolid compared to *E. faecalis*. Additionally, 46% of *E. faecalis* showed MIC of streptomycin up to 1042 µg/mL while another 36% showed MIC of gentamicin equal to 512 µg/mL. The MIC of aminoglycosides determined in this study showed similar level as observed in other countries, despite the variation of the methods used. The presence of aminoglycoside modifying enzyme (AME) genes [*aac(6)-Ie-aph(2)-Ia, aph(2)-Ib, aph(2)-Ic, aph(2)-Id, aph(3)-IIIa*] were detected using PCR amplification. The results demonstrated that the HLGR gene, *aac(6)-Ie-aph(2)-Ia* and the HLSR gene, *aph(3)-IIIa* were detected in 32% and 40% of *E. faecalis* and *E. faecium* resistance isolates, respectively. The spread of these genes were responsible for high level resistance to gentamicin and streptomycin among enterococci isolated in this study. As HLAR genes are highly transferable to not only among the enterococci species but also among various bacterial species, continuous antibiotic surveillance in Malaysian hospitals is warranted in future and preventive measures can be implemented accordingly.
Keywords: High level aminoglycosides resistance, Enterococci

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMPROFILAN KERENTANAN ANTIBIOTIK DAN PENYEBARAN GEN KETAHANAN AMINOGLIKOSIDA TAHAP TINGGI DI KALANGAN *Enterococcus faecalis* DAN *Enterococcus faecium*

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Enterococcus faecium dan *Enterococcus faecalis* merupakan antara spesis utama yang menyebabkan infeksi dapatan hospital, dengan kadar kematian dan morbiditi yang tinggi. Enterococci yang rintang terhadap aminoglikosida (HLAR) dan vankomisin (VRE) tahap tinggi merupakan masalah yang signifikan di dalam rawatan terapeutik. Strain tersebut semakin meluas di Eropah, USA dan Asia. Kajian telah menunjukkan bahawa perbezaan penyebaran bagi enzim pengubahsuaian aminoglikosida (AMEs) di kalangan HLAR adalah berdasarkan kawasan geografi. Di Malaysia, walau bagaimanapun, data mengenai kelaziman HLAR dan penyebaran AME masih lagi terhad. Oleh itu, kajian ini bertujuan untuk menentukan pola kerintangan antibiotik bagi pencilan klinikal *E. faecalis* dan *E. faecium* dan penyebaran gen HLAR di kalangan pencilan yang rintang ini. Tujuh puluh lima pencilan enterococci klinikal yang digunakan di dalam kajian ini telah diperoleh daripada pusat tertier, pada tahun 2009 dan 2010. Pencilan tersebut telah dipencilkan daripada sumber yang berbeza termasuk nanah (50%), darah (32%), air kencing (11%) dan sumber lain seperti CSF dan HVS (7%). Pengenalpastian semula pencilan tersebut telah dijalankan menggunakan pelbagai kaedah termasuk sub-kultur ke atas medium selektif dan pewarnaan Gram, diikuti dengan ujian pengesahan menggunakan pemprofilan biokimia komersial (API20 strep) dan amplifikasi 16s RNA PCR. Ujian kerintangan antimikrobial telah dijalankan menggunakan difusi disk cakram kertas, Ujian-E dan kaedah mikrodilusi pencairan kaldu. Antibiotik yang digunakan ialah

aminoglikosida (gentamisin, streptomisin, kanamisin, amikasin, tobramisin) dan antibiotik lain yang biasanya digunakan di hospital bagi merawat infeksi enterococcal, seperti ampisilin, vankomisin, eritromisin, tetrasiklina, kloramfenikol dan linezolid. Pengesanan gen HLAR telah dijalankan ke atas pencilan yang rintang dengan menggunakan kaedah amplifikasi PCR tunggalDaripada 25 (33.3%) pencilan *E. faecium,* 84% dan 68% masing-masing menunjukkan tahap ketahanan gentamisin (HLGR) dan ketahanan streptomisin (HLSR) tahap tinggi. Kerintangan terhadap eritromisin dan tetrasiklina ialah 100%, manakala ampisilin dan kloramfenikol, masing-masing menunjukkan 84% dan 32% kadar kerintangan. Tiada pencilan *E. faecium* menunjukkan kerintangan terhadap vankomisin dan linezolid. Di samping itu, kesemua 50 (66.7%) pencilan *E. faecalis* adalah rintang terhadap semua aminoglikosida yang diuji dengan peratusan berikut; amikasin (100%), kanamisin (90%), tobramisin (70%), HLGR (36%) dan HLSR (46%). Pencilan-pencilan tersebut juga rintang terhadap tetrasiklina (98%), eritromisin (96%), kloramfenikol (46%), ampisilin (24%), linezolid (8%), dan vankomisin (4%). Yang menariknya, pencilan *E. faecalis* yang rintang pada vankomisin sebelum ini telah dilaporkan sebagai rentan. Dalam kajian ini, *E. faecium* memperlihatkan kadar kerintangan yang tinggi terhadap semua antibiotik kecuali vankomisin dan linezolid berbanding dengan *E. faecalis*. Tambahan lagi, 46% *E. faecalis* menunjukkan MIC streptomisin sehingga 1042 µg/mL manakala selainnya 36% menunjukkan MIC gentamisin bersamaan 512 µg/mL. MIC aminoglikosida yang ditentukan dalam kajian ini menunjukkan tahap yang sama seperti yang didapati di negara lain, meskipun kepelbagaian kaedah yang digunakan. Kewujudan gen enzim pengubahsuai aminoglikosida (AME) [*aac(6)-Ie-aph(2)-Ia, aph(2)-Ib, aph(2)-Ic, aph(2)-Id, aph(3)-IIIa*] telah dikesan dengan menggunakan amplifikasi PCR. Keputusan menunjukkan bahawa gen HLGR, *aac(6)-Ie-aph(2)-Ia* dan gen HLSR, *aph(3)-IIIa* telah dikesan, masing-masing di dalam 32% dan 40% pencilan *E. faecalis* dan *E. faecium* yang rintang. Penyebaran gen tersebut bertanggungjawab terhadap kerintangan tahap tinggi pada gentamisin dan streptomisin di kalangan pencilan enterococci di dalam kajian ini. Disebabkan gen HLAR berkeupayaan untuk pindah bukan sahaja di kalangan spesis enterococci yang sama tetapi juga pelbagai spesis bakteria yang berlainan, pengawasan antibiotik yang berterusan di hospital di Malaysia adalah perlu agar langkah-langkah pencegahan dapat dilaksanakan dengan sewajarnya.

Kata kunci: Tahap kerintangan, aminoglikosida tahap tinggi, Enterococci

ACKNOWLEDGEMENTS

First and foremost, all praise is due to Allah, I am thankful to Allah who gives me the courage, the guidance, compassion and mercifulness to allow me to complete these research objectives.

I would like to express my profound appreciation and gratitude to my supervisor Dr. Azmiza Syawani Jasni and my co-supervisor, Associate Professor Dr. Rukman Awang Hamat, for their invaluable and critical suggestions, patience and understanding in reading, correcting and tirelessly guiding me throughout the period of writing the thesis.

I would like to express my deep appreciation to all my family members especially my beloved mother Hawa Moh'ed Ahmed and dear sister Warda also other members of my family who always encourage and support me financially.

My sincerest appreciation goes to members of Medical Microbiology and Parasitology laboratories for their invaluable support and guidance during my study.

Not forgetting my beloved friend Khalild Abdullahi Ali who helped me directly and indirectly during my study journey.

I certify that a Thesis Examination Committee has met on 16 May 2018 to conduct the final examination of Ayan Adan Moussa on his thesis entitled "Characterization of Antibiotic Susceptibility and High Level Aminoglycoside Resistant Genes in *Enterococcus faecalis* and *Enterococcus faecium* Clinical Isolates" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
IMR	Institute for Medical Research
AAC	Aminoglycoside N-acetyltransferase se
AME	Aminoglycosides modifying enzyme
ANT	Aminoglycoside O- adenyltransferase
APH	Aminoglycoside O-Phosphotransferase
AST	Antibiotic susceptibility testing
ATCC	American type culture collection
BHI	Brain heart infusion agar
Blast	Basic local alignment search tool
CFU	Colony forming unit
CLSI	Clinical Laboratory Standard Institute
DNA	Deoxyribonucleic acid
HLAR	High level aminoglycoside resistance
ICU	Intensive care unit
MDR	Multi drug resistance
MHA	Muller Hinton agar
MIC	Minimal inhibitory concentration
NCBI	National Centre for Biology Information
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
SPSS	Statistical Package for the Social Sciences
TBE	Mixture of tris base, boric acid, EDTA and water
VRE	Vancomycin resistant enterococci

CHAPTER 1

INTRODUCTION

1.1 Background

Enterococcal infections are significant hospital and community acquired infections due to multidrug resistance as widely reported (Arias & Murray, 2012). They have potential capacity to persist under various environmental conditions such as extreme temperature and osmolality (Garrido et al., 2014). These organisms are able to persist on various surfaces in hospitals and on hands of healthcare workers (Kuch et al., 2011). The Centre for Disease Control and Prevention (CDC) and prevention have estimated about 4 million patients in USA have hospital acquired infections (HAIs) annually (Weng et al., 2013). Recent report indicated that enterococci were placed as a second most common cause of hospital acquired infection after *Staphylococcus aureus* (Arias et al., 2010). In Malaysia between 2006 and 2007 about 2263 and 2647 patients respectively, were reported with HAIs which were due to enterococcal infections (Weng et al., 2013).

Enterococci are Gram positive bacteria which are found mostly in the gastrointestinal tract as normal flora (McBride et al., 2007). Presently, 54 various species have been described (O'Driscoll & Crank, 2015). The enterococci infection which include *Enterococcus faecium* and *Enterococcus faecalis* are predominantly associated with human diseases such as urinary tract infection, bacteremia, endocarditis, wound infections such as surgical burns, intra-abdominal abscess, septicemia, pelvic infection, meningitis as well as skin and soft tissue infections that result in high morbidity and mortality (Wang et al., 2013;Vu & Carvalho 2011). It is estimated that about 60% of enterococcal infections are associated with *E. faecalis.* However, infections due to *E. faecium* have risen over the last few decades in Europe, USA as well as Asia. This leads to a growing burden of enterococcal infections in general, and to a decreased ratio of *E. faecalis to E. faecium* infections (down to 60:40). Although *E. faecalis* remains generally less resistant to antibiotics than *E. faecium*, antimicrobial resistance in the former poses an increasing challenge to therapy as earlier reported (Kuch et al., 2011).

Enterococci are able to withstand harsh environmental conditions and acquire antibiotic resistant determinants. Enterococci are subject to horizontal gene transfer (HGT) elements, a characteristic which empowers bacterial organisms to develop rapidly through rapid exchange and distribution of beneficial trait-

encoding components, including antibiotic resistance genes (Fisher & Phillips, 2009).

Recently, several resistance determinants were reported to lead to the development of resistance to the seven groups of antibiotics by enterococci. Other determinants are encoded genes substances such as insertion sequence, intergrons, transposon and plasmids (Li et al., 2015). Horizontal gene transfer has been reported to be the main route through which 25% of genomic determinants are acquired by the enterococcus species (García-Solache et al., 2016). According to Patel et al (2008), enterococci are a normal microbial flora of the gut which is responsible for the spread of antibiotic resistant determinants. However, management of infection due to *E. faecium* today is highly challenging to the clinician due to multidrug resistance (MDR) which is currently on the increase in the population (Miller et al., 2014). Presently, enterococci are one of the leading etiologies of HAIs which are reported by National Healthcare Safety Network from CDC.

Management of enterococcal infections involves the actual synergistic effect of aminoglycosides and cell wall active antibiotics such as glycopeptides (vancomycin) or beta-lactam (ampicillin). To date, enterococci have exhibited multidrug-resistance (MDR) with high level of aminoglycoside resistance (HLAR) and vancomycin resistance (VRE). This has caused significant problem in clinical anti-infective therapy (Lebreton et al., 2016; Li et al., 2015). The strains of enterococci are becoming more widely spread in Europe, USA and Asia (Lebreton et al., 2016). Several studies have documented different distribution of aminoglycoside modifying enzymes (AMEs) and encoding genes differently based on the geographical regions (Zarrilli et al., 2005; Padmasini et al. 2014; Udo et al., 2004). In Malaysia, the data regarding the prevalence of HLAR and the distribution of AMEs are still limited. Hence, this study is aimed to determine the distribution of AMEs genes among enterococci isolates from clinical sample.

1.2 Problem statement

The Centre for Disease Control and Prevention (CDC, 2014) has reported that approximately 4 million hospitalized patients had nosocomial infections which are caused by antimicrobial resistance organisms. Among the pathogens, enterococci have been reported as the second most common cause of hospital acquired infections due to their wide-range of antimicrobial resistance and synergistic effects with beta-lactams, glycopeptides and aminoglycosides, which are currently being used in clinical practices.

Enterococci are inherently resistant to aminoglycosides due to aminoglycoside modified enzymes. Nevertheless, the types and distribution of resistance genes are different and geographically dependent. In Malaysia, data on the prevalence of HLAR and distributions of the resistance genes are still limited.

1.3 Objectives

General Objective

The aims of this study are to determine the antibiotic susceptibility patterns of the clinical isolates *Enterococcus faecalis* and *Enterococcus faecium* and the distribution of HLAR genes among the resistant enterococci isolates.

Specific Objectives

1. To re-identify *E. faecalis* and *E. faecium* using phenotypic and genotypic approaches.
2. To determine the antibiotic susceptibility profiles of *E. faecalis* and *E. faecium* by using disc diffusion.
3. To determine the minimum inhibitory concentrations of the resistant enterococci isolates using microdilution method.
4. To determine the distribution of aminoglycoside resistance encoding genes in the resistance enterococci isolates using molecular biology approaches.

REFERENCES

Adhikari, L. (2010). High-level aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial enterococci. *Journal of global infectious diseases*, *2*(3), 231.

Abdulhakim, A., Wondafrash, B., & Abdissa, A. (2015). Antimicrobial resistance profile of Enterococcus species isolated from intestinal tracts of hospitalized patients in Jimma, Ethiopia. *BMC research notes*, *8*(1), 213.

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*, *128*(6), 1037-1050.

Arias, C. A., & Murray, B. E. (2008). Emergence and management of drug-resistant enterococcal infections. *Expert review of anti-infective therapy*, *6*(5), 637-655.

Arias, C. A., & Murray, B. E. (2012). The rise of the Enterococcus: beyond vancomycin resistance. *Nature Reviews Microbiology*, *10*(4), 266-278.

Arias, C. A., Contreras, G. A., & Murray, B. E. (2010). Management of multidrug-resistant enterococcal infections. *Clinical microbiology and infection*, *16*(6), 555-562.

Auckland, C., Teare, L., Cooke, F., Kaufmann, M. E., Warner, M., Jones, G., ... & Johnson, A. P. (2002). Linezolid-resistant enterococci: report of the first isolates in the United Kingdom. *Journal of Antimicrobial Chemotherapy*, *50*(5), 743-746.

Barros, M., Martinelli, R., & Rocha, H. (2009). Enterococcal urinary tract infections in a university hospital: clinical studies. *Brazilian Journal of Infectious Diseases*, *13*(4), 294-296.

Billington, E. O., Phang, S. H., Gregson, D. B., Pitout, J. D. D., Ross, T., Church, D. L., ... & Parkins, M. D. (2014). Incidence, risk factors, and outcomes for Enterococcus spp. blood stream infections: a population-based study. *International Journal of Infectious Diseases*, *26*, 76-82.

Boost, M., Lai, L., & O'Donoghue, M. (2004). Drug resistance in fecal enterococci in Hong Kong. *Journal of infection and chemotherapy*, *10*(6), 326-330.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R., & Harwood, V. J. (2012). Enterococci in the environment. *Microbiology and Molecular Biology Reviews*, *76*(4), 685-706.

Cattoir, V., & Giard, J. C. (2014). Antibiotic resistance in Enterococcus faecium clinical isolates. *Expert review of anti-infective therapy*, *12*(2), 239-248.

CDC. (2014). Prevention (CDC).(2014). *Violence prevention at CDC*.

Cetinkaya, Y., Falk, P., & Mayhall, C. G. (2000). Vancomycin-resistant enterococci. *Clinical microbiology reviews*, *13*(4), 686-707.

Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, *284*(5418), 1318-1322.

Dan, M., Poch, F., Leibson, L., Smetana, S., & Priel, I. (1999). Rectal colonization with vancomycin-resistant enterococci among high-risk patients in an Israeli hospital. *Journal of Hospital Infection*, *43*(3), 231-238.

Daniel, D. S., Lee, S. M., Gan, H. M., Dykes, G. A., & Rahman, S. (2017). Genetic diversity of Enterococcus faecalis isolated from environmental, animal and clinical sources in Malaysia. *Journal of Infection and Public Health*.

De Kraker, M. E. A., Jarlier, V., Monen, J. C. M., Heuer, O. E., Van De Sande, N., & Grundmann, H. (2013). The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clinical Microbiology and Infection*, *19*(9), 860-868.

de Niederhäusern, S., Bondi, M., Messi, P., Iseppi, R., Sabia, C., Manicardi, G., & Anacarso, I. (2011). Vancomycin-resistance transferability from VanA enterococci to Staphylococcus aureus. *Current microbiology*, *62*(5), 1363-1367.

Domig, K. J., Mayer, H. K., & Kneifel, W. (2003). Methods used for the isolation, enumeration, characterisation and identification of Enterococcus spp.: 2. Pheno-and genotypic criteria. *International journal of food microbiology*, *88*(2), 165-188.

Domig, K. J., Mayer, H. K., & Kneifel, W. (2003). Methods used for the isolation, enumeration, characterisation and identification of Enterococcus spp.: 2. Pheno-and genotypic criteria. *International journal of food microbiology*, *88*(2), 165-188.

Dunny, G. M., Hancock, L. E., & Shankar, N. (2014). Enterococcal biofilm structure and role in colonization and disease.

El-Ghazawy, I. F., Okasha, H. A., & Mazloum, S. M. (2016). A study of high level aminoglycoside resistant enterococci. *African Journal of Microbiology Research*, *10*(16), 572-577.

Fisher, K., & Phillips, C. (2009). The ecology, epidemiology and virulence of Enterococcus. *Microbiology*, *155*(6), 1749-1757.

Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: the agents of open source evolution. *Nature Reviews Microbiology*, *3*(9), 722-732.

Galimand, M., Schmitt, E., Panvert, M., Desmolaize, B., Douthwaite, S., Mechulam, Y., & Courvalin, P. (2011). Intrinsic resistance to aminoglycosides in Enterococcus faecium is conferred by the 16S rRNA m5C1404-specific methyltransferase EfmM. *RNA*, *17*(2), 251-262.

García-Solache, M., Lebreton, F., McLaughlin, R. E., Whiteaker, J. D., Gilmore, M. S., & Rice, L. B. (2016). Homologous recombination within large chromosomal regions facilitates acquisition of β-lactam and vancomycin resistance in Enterococcus faecium. *Antimicrobial agents and chemotherapy*, *60*(10), 5777-5786.

Garsin, D. A., Frank, K. L., Silanpää, J., Ausubel, F. M., Hartke, A., Shankar, N., & Murray, B. E. (2014). Pathogenesis and models of enterococcal infection.

Gilmore, M. S., Lebreton, F., & van Schaik, W. (2013). Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. *Current opinion in microbiology*, *16*(1), 10-16.

Gyles, C., & Boerlin, P. (2014). Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. *Veterinary pathology*, *51*(2), 328-340.

Heintz, B. H., Halilovic, J., & Christensen, C. L. (2010). Vancomycin-Resistant Enterococcal Urinary Tract Infections. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *30*(11), 1136-1149.

Hendrickx, A. P., van Luit-Asbroek, M., Schapendonk, C. M., van Wamel, W. J., Braat, J. C., Wijnands, L. M., ... & Willems, R. J. (2009). SgrA, a nidogen-binding LPXTG surface adhesin implicated in biofilm formation, and EcbA, a collagen binding MSCRAMM, are two novel

adhesins of hospital-acquired Enterococcus faecium. *Infection and immunity*, *77*(11), 5097-5106.

Hidron, A. I., Edwards, J. R., Patel, J., Horan, T. C., Sievert, D. M., Pollock, D. A., & Fridkin, S. K. (2008). Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infection Control & Hospital Epidemiology*, *29*(11), 996-1011.

Higuita, N. A., & Huycke, M. M. (2014). Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet].

Hijazi, N., Elmanama, A. A., & Al-Hindi, A. (2009). Vancomycin-resistant enterococci in fecal samples from hospitalized patients and non-hospitalized individuals in Gaza City. *Journal of Public Health*, *17*(4), 243-249.

Hollenbeck, B. L., & Rice, L. B. (2012). Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence*, *3*(5), 421-569.

Hucker, G. J., & Conn, H. J. (1923). Methods of Gram staining.

Huycke, M. M., Sahm, D. F., & Gilmore, M. S. (1998). Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. *Emerging infectious diseases*, *4*(2), 239.

Ishmael, F. T., & Stellato, C. (2008). Principles and applications of polymerase chain reaction: basic science for the practicing physician. *Annals of Allergy, Asthma & Immunology*, *101*(4), 437-443.

Jabalameli, F., Emaneini, M., Shahsavan, S., Sedaghat, H., Abdolmaliki, Z., & Aligholi, M. (2009). Evaluation of antimicrobial susceptibility patterns of Enterococci isolated from patients in Tehran University of Medical Sciences Teaching Hospitals. *Acta Medica Iranica*, *47*(4), 325-328.

Jain, S., Kumar, A., Kashyap, B., & Kaur, I. R. (2011). Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. *International Journal of Applied and Basic Medical Research*, *1*(2), 80.

Johnson, A., Tysall, L., Stockdale, M., Woodford, N., Kaufmann, M., Warner, M., ... & Allerberger, F. (2002). Emerging linezolid-resistant Enterococcus faecalis and Enterococcus faecium isolated from two Austrian patients in the same intensive care unit. *European Journal of Clinical Microbiology and Infectious Diseases*, *21*(10), 751-754.

Kaçmaz, B., & Aksoy, A. (2005). Antimicrobial resistance of enterococci in Turkey. *International journal of antimicrobial agents*, *25*(6), 535-538.

KAFIL, H. S., & ASGHARZADEH, M. (2014). Vancomycin-resistant enteroccus faecium and enterococcus faecalis isolated from education hospital of iran. *Maedica*, *9*(4), 323.

Karmarkar, M. G., Gershom, E. S., & Mehta, P. R. (2004). Enterococcal infections with special reference to phenotypic characterization & drug resistance. *Indian Journal of Medical Research*, *119*, 22.

Kao, S. J., You, I. L., Clewell, D. B., Donabedian, S. M., Zervos, M. J., Petrin, J., ... & Chow, J. W. (2000). Detection of the high-level aminoglycoside resistance gene aph (2")-Ib in Enterococcus faecium. *Antimicrobial agents and chemotherapy*, *44*(10), 2876-2879.

Kobayakawa, S., Jett, B. D., & Gilmore, M. S. (2005). Biofilm formation by Enterococcus faecalis on intraocular lens material. *Current eye research*, *30*(9), 741-745.

Kobayashi, N., Alam, M. M., Nishimoto, Y., Urasawa, S., Uehara, N., & Watanabe, N. (2001). Distribution of aminoglycoside resistance genes in recent clinical isolates of Enterococcus faecalis, Enterococcus faecium and Enterococcus avium. *Epidemiology & Infection*, *126*(2), 197-204.

Kristich, C. J., Rice, L. B., & Arias, C. A. (2014). Enterococcal infection—treatment and antibiotic resistance.

Kuch, A., Willems, R. J., Werner, G., Coque, T. M., Hammerum, A. M., Sundsfjord, A., ... & Hryniewicz, W. (2011). Insight into antimicrobial susceptibility and population structure of contemporary human Enterococcus faecalis isolates from Europe. *Journal of antimicrobial chemotherapy*, *67*(3), 551-558.

Lasa, I., & Penadés, J. R. (2006). Bap: a family of surface proteins involved in biofilm formation. *Research in microbiology*, *157*(2), 99-107.

Lavery, A., Rossney, A. S., Morrison, D., Power, A., & Keane, C. T. (1997). Incidence and detection of multi-drug-resistant enterococci in Dublin hospitals. *Journal of medical microbiology*, *46*(2), 150-156.

Lebreton, F., Willems, R. J., & Gilmore, M. S. (2014). Enterococcus diversity, origins in nature, and gut colonization.

Li, W., Li, J., Wei, Q., Hu, Q., Lin, X., Chen, M., ... & Lv, H. (2015). Characterization of aminoglycoside resistance and virulence genes

among Enterococcus spp. isolated from a hospital in China. *International journal of environmental research and public health*, *12*(3), 3014-3025.

Lins, R. X., de Oliveira Andrade, A., Junior, R. H., Wilson, M. J., Lewis, M. A., Williams, D. W., & Fidel, R. A. S. (2013). Antimicrobial resistance and virulence traits of Enterococcus faecalis from primary endodontic infections. *Journal of dentistry*, *41*(9), 779-786.

Ma, X., Kudo, M., Takahashi, A., Tanimoto, K., & Ike, Y. (1998). Evidence of nosocomial infection in Japan caused by high-level gentamicin-resistant Enterococcus faecalis and identification of the pheromone-responsive conjugative plasmid encoding gentamicin resistance. *Journal of clinical microbiology*, *36*(9), 2460-2464.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Paterson, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.

Mathur, P., Kapil, A., Chandra, R., Sharma, P., & Das, B. (2003). Antimicrobial resistance in Enterococcus faecalis at a tertiary care centre of northern India. *Indian Journal of Medical Research*, *118*, 25.

McBride, S. M., Fischetti, V. A., LeBlanc, D. J., Moellering Jr, R. C., & Gilmore, M. S. (2007). Genetic diversity among Enterococcus faecalis. *PloS one*, *2*(7), e582.

Miller, W. R., Munita, J. M., & Arias, C. A. (2014). Mechanisms of antibiotic resistance in enterococci. *Expert review of anti-infective therapy*, *12*(10), 1221-1236.

Mishra, N. N., Bayer, A. S., Tran, T. T., Shamoo, Y., Mileykovskaya, E., Dowhan, W., ... & Arias, C. A. (2012). Daptomycin resistance in enterococci is associated with distinct alterations of cell membrane phospholipid content. *PLoS One*, *7*(8), e43958.

Moaddab, S. R., & Rafi, A. (2003). Prevalence of vancomycin and high level aminoglycoside resistant enterococci among high-risk patients.

Mohamed, J. A., & Huang, D. B. (2007). Biofilm formation by enterococci. *Journal of medical microbiology*, *56*(12), 1581-1588.

Montealegre, M. C., Singh, K. V., Somarajan, S. R., Yadav, P., Chang, C., Spencer, R., ... & Murray, B. E. (2016). Role of the Emp pilus subunits of

Enterococcus faecium in biofilm formation, adherence to host extracellular matrix components, and experimental infection. *Infection and immunity*, *84*(5), 1491-1500.

Mullis, K. B. (1989, December). Target amplification for DNA analysis by the polymerase chain reaction. In *Annales de biologie clinique* (Vol. 48, No. 8, pp. 579-582).

Mundy, L. M., Sahm, D. F., & Gilmore, M. (2000). Relationships between enterococcal virulence and antimicrobial resistance. *Clinical microbiology reviews*, *13*(4), 513-522.

Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology spectrum*, *4*(2).

Naser, S. M., Thompson, F. L., Hoste, B., Gevers, D., Dawyndt, P., Vancanneyt, M., & Swings, J. (2005). Application of multilocus sequence analysis (MLSA) for rapid identification of Enterococcus species based on rpoA and pheS genes. *Microbiology*, *151*(7), 2141-2150.

Niu, H., Yu, H., Hu, T., Tian, G., Zhang, L., Guo, X., ... & Wang, Z. (2016). The prevalence of aminoglycoside-modifying enzyme and virulence genes among enterococci with high-level aminoglycoside resistance in Inner Mongolia, China. *brazilian journal of microbiology*, *47*(3), 691-696.

O'Driscoll, T., & Crank, C. W. (2015). Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. *Infection and drug resistance*, *8*, 217.

Ogier, J. C., & Serror, P. (2008). Safety assessment of dairy microorganisms: The Enterococcus genus. *International journal of food microbiology*, *126*(3), 291-301.

Padmasini, E., Padmaraj, R., & Ramesh, S. S. (2014). High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of Enterococcus species in Chennai, India. *The Scientific World Journal*, *2014*.

Padmasini, E., Padmaraj, R., & Ramesh, S. S. (2014). High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of Enterococcus species in Chennai, India. *The Scientific World Journal*, *2014*.

Pesce, A., Debbia, E. A., Toni, M., & Schito, G. C. (1992). Antibiotic resistance of clinical isolates of Enterococcus in Italy. *Clinical infectious diseases*, *15*(3), 490-494.

Pesce, A., Debbia, E. A., Toni, M., & Schito, G. C. (1992). Antibiotic resistance of clinical isolates of Enterococcus in Italy. *Clinical infectious diseases*, *15*(3), 490-494.

Prieto, A. M. G., van Schaik, W., Rogers, M. R., Coque, T. M., Baquero, F., Corander, J., & Willems, R. J. (2016). Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? *Frontiers in microbiology*, *7*.

Pultz, N. J., Shankar, N., Baghdayan, A. S., & Donskey, C. J. (2005). Enterococcal surface protein Esp does not facilitate intestinal colonization or translocation of Enterococcus faecalis in clindamycin-treated mice. *FEMS microbiology letters*, *242*(2), 217-219.

Ramin, B., Asadpour, L., Forouhesh Tehrani, H., & Amirmozafari, N. (2017). Detection and Distribution of various HLAR Gene in Enterococcus faecalis and Enterococcus faecium by Multiplex-PCR. Modern Medical Laboratory Journal, 2(1), 76-81.

Rice, L. B., Carias, L. L., Rudin, S., Laktičová, V., Wood, A., & Hutton-Thomas, R. (2005). Enterococcus faecium low-affinity pbp5 is a transferable determinant. *Antimicrobial agents and chemotherapy*, *49*(12), 5007-5012.

Rosa, R. G., Schwarzbold, A. V., Santos, R. P. D., Turra, E. E., Machado, D. P., & Goldani, L. Z. (2014). Vancomycin-resistant Enterococcus faecium bacteremia in a tertiary care hospital: Epidemiology, antimicrobial susceptibility, and outcome. *BioMed research international*, *2014*.

Saifi M, Pourshafie M, Eshraghian M, Dallal M. Anti-microbial resistance of enterococci isolated from urinary tract infections in Iran. Iran Biomed J. 2008; 12:185–190. [PubMed]

Salem-Bekhit, M. M., Moussa, I. M. I., Muharram, M. M., Alanazy, F. K., & Hefni, H. M. (2012). Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. *Indian journal of medical microbiology*, *30*(1), 44.

Santagati, M., Campanile, F., & Stefani, S. (2012). Genomic diversification of enterococci in hosts: the role of the mobilome. *Frontiers in microbiology*, *3*.

Sarti, M., Campanile, F., Sabia, C., Santagati, M., Gargiulo, R., & Stefani, S. (2012). Polyclonal diffusion of beta-lactamase-producing Enterococcus faecium. *Journal of clinical microbiology*, *50*(1), 169-172.

Savini, V., Gherardi, G., Astolfi, D., Polilli, E., Dicuonzo, G., D'amario, C., ... & D'antonio, D. (2012). Insights into airway infections by enterococci: a review. *Recent patents on anti-infective drug discovery*, *7*(1), 36-44.

Schleifer, K. H., & Kilpper-Bälz, R. (1984). Transfer of Streptococcus faecalis and Streptococcus faecium to the Genus Enterococcus nom. rev. as Enterococcus faecalis comb. nov. and Enterococcus faecium comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *34*(1), 31-34.

Shankar, V., Baghdayan, A. S., Huycke, M. M., Lindahl, G., & Gilmore, M. S. (1999). Infection-derived Enterococcus faecalisstrains are enriched in esp, a gene encoding a novel surface protein. *Infection and immunity*, *67*(1), 193-200.

Sharifi, Y., Hasani, A., Ghotaslou, R., Naghili, B., Aghazadeh, M., Milani, M., & Bazmany, A. (2013). Virulence and antimicrobial resistance in enterococci isolated from urinary tract infections. *Advanced pharmaceutical bulletin*, *3*(1), 197.

Shete, V., Grover, N., & Kumar, M. (2017). Analysis of Aminoglycoside Modifying Enzyme Genes Responsible for High-Level Aminoglycoside Resistance among Enterococcal Isolates. *Journal of Pathogens*, *2017*.

Shukla, B. S., Shelburne, S., Reyes, K., Kamboj, M., Lewis, J. D., Rincon, S. L., ... & Zervos, M. J. (2016). Influence of minimum inhibitory concentration in clinical outcomes of Enterococcus faecium bacteremia treated with daptomycin: is it time to change the breakpoint?. *Clinical Infectious Diseases*, *62*(12), 1514-1520.

Sievert, D. M., Ricks, P., Edwards, J. R., Schneider, A., Patel, J., Srinivasan, A., ... & Fridkin, S. (2013). Antimicrobial-resistant pathogens associated with healthcare-associated infections summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. *Infection Control & Hospital Epidemiology*, *34*(1), 1-14.

Simonsen, G. S., Småbrekke, L., Monnet, D. L., Sørensen, T. L., Møller, J. K., Kristinsson, K. G., ... & Sundsfjord, A. (2003). Prevalence of resistance to ampicillin, gentamicin and vancomycin in Enterococcus faecalis and Enterococcus faecium isolates from clinical specimens and use of antimicrobials in five Nordic hospitals. *Journal of Antimicrobial chemotherapy*, *51*(2), 323-331.

Soheili, S., Ghafourian, S., Sekawi, Z., Neela, V., Sadeghifard, N., Ramli, R., & Hamat, R. A. (2014). Wide distribution of virulence genes among Enterococcus faecium and Enterococcus faecalis clinical isolates. *The Scientific World Journal*, *2014*.

Sood, S., Malhotra, M., Das, B. K., & Kapil, A. (2008). Enterococcal infections & antimicrobial resistance. *Indian Journal of Medical Research*, *128*(2), 111.

Spolidorio, D., L. Spolidorio, et al. (2005). "Técnicas básicas de biologia molecular." ESTRELA, C. Metodologia científica-Ciência-Ensino-Pesquisa. São Paulo: Artes Médicas.

Sreeja, S., Sreenivasa Babu, P. R., & Prathab, A. G. (2012). The prevalence and the characterization of the enterococcus species from various clinical samples in a tertiary care hospital. *Journal of clinical and diagnostic research: JCDR*, *6*(9), 1486.

Strateva, T., Atanasova, D., Savov, E., Petrova, G., & Mitov, I. (2016). Incidence of virulence determinants in clinical Enterococcus faecalis and Enterococcus faecium isolates collected in Bulgaria. *Brazilian Journal of Infectious Diseases*, *20*(2), 127-133.

Sundaram, M., Kavita, Y., & Mohiddin, S. K. (2016). Antibiogram of enterococcal species isolated from clinical specimens in a tertiary care teaching hospital. *Blood*, *14*, 13-08.

Suppli, M., Aabenhus, R., Harboe, Z. B., Andersen, L. P., Tvede, M., & Jensen, J. U. (2011). Mortality in enterococcal bloodstream infections increases with inappropriate antimicrobial therapy. *Clinical Microbiology and Infection*, *17*(7), 1078-1083.

Swaminathan, S., & Alangaden, G. J. (2010). Treatment of resistant enterococcal urinary tract infections. *Current infectious disease reports*, *12*(6), 455-464.

Szakacs, T. A., Kalan, L., McConnell, M. J., Eshaghi, A., Shahinas, D., McGeer, A., ... & Patel, S. N. (2014). Outbreak of vancomycin-susceptible Enterococcus faecium containing the wild-type vanA gene. *Journal of clinical microbiology*, *52*(5), 1682-1686.

test Pharyngitis, C. A. M. P., & Streptolysin, O. (2014). In the last two decades, DNA homology and DNA. *Textbook of Diagnostic Microbiology-E-Book*, 330.

Titze-de-Almeida, R., Rollo Filho, M., Nogueira, C. A., Rodrigues, I. P., Eudes Filho, J., Nascimento, R. S. D., ... & Van Belkum, A. (2004). Molecular

epidemiology and antimicrobial susceptibility of Enterococci recovered from Brazilian intensive care units. *Brazilian Journal of Infectious Diseases*, *8*(3), 197-205.

Tripodi, M. F., Rambaldi, A., Utili, R., Rosario, P., Attanasio, V., Locatelli, A., ... & Ruggiero, G. (1995). Resistance to aminoglycosides and other antibiotics among clinical isolates of Enterococcus spp. *The new microbiologica*, *18*(3), 319-323.

Tsai, J. C., Hsueh, P. R., Lin, H. M., Chang, H. J., Ho, S. W., & Teng, L. J. (2005). Identification of clinically relevant Enterococcus species by direct sequencing of groES and spacer region. *Journal of clinical microbiology*, *43*(1), 235-241.

Tula, M. Y., Iyoha, O., & Iruolaje, F. O. (2015). Antibiotic Resistance: Challenges and Prospect for Therapy in Developing Countries. *British Journal of Pharmaceutical Research*, *8*(3).

Udo, E. E., Al-Sweih, N., John, P., & Chugh, T. D. (2002). Antibiotic resistance of enterococci isolated at a teaching hospital in Kuwait. *Diagnostic microbiology and infectious disease*, *43*(3), 233-238.

Udo, E. E., Al-Sweih, N., John, P., Jacob, L. E., & Mohanakrishnan, S. (2004). Characterization of high-level aminoglycoside-resistant enterococci in Kuwait hospitals. *Microbial Drug Resistance*, *10*(2), 139-145.

Upadhyaya, P. G., Ravikumar, K. L., & Umapathy, B. L. (2009). Review of virulence factors of enterococcus: an emerging nosocomial pathogen. *Indian Journal of Medical Microbiology*, *27*(4), 301.Vakulenko, S. B., Donabedian, S. M., Voskresenskiy, A. M., Zervos, M. J., Lerner, S. A., & Chow, J. W. (2003). Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. *Antimicrobial agents and chemotherapy*, *47*(4), 1423-1426.

Valones, M. A. A., Guimarães, R. L., Brandão, L. A. C., Souza, P. R. E. D., Carvalho, A. D. A. T., & Crovela, S. (2009). Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. *Brazilian Journal of Microbiology*, *40*(1), 1-11.

van Harten, R. M., Willems, R. J., Martin, N. I., & Hendrickx, A. P. (2017). Multidrug-Resistant Enterococcal Infections: New Compounds, Novel Antimicrobial Therapies? *Trends in Microbiology*.

van Harten, R. M., Willems, R. J., Martin, N. I., & Hendrickx, A. P. (2017). Multidrug-Resistant Enterococcal Infections: New Compounds, Novel Antimicrobial Therapies? *Trends in Microbiology*.

Van Schaik, W., & Willems, R. J. (2010). Genome-based insights into the evolution of enterococci. *Clinical Microbiology and Infection*, *16*(6), 527-532.

Vu, J., & Carvalho, J. (2011). Enterococcus: review of its physiology, pathogenesis, diseases and the challenges it poses for clinical microbiology. *Frontiers in Biology*, *6*(5), 357.

Wang, J. T., Chang, S. C., Wang, H. Y., Chen, P. C., Shiau, Y. R., & Lauderdale, T. L. (2013). High rates of multidrug resistance in Enterococcus faecalis and E. faecium isolated from inpatients and outpatients in Taiwan. *Diagnostic microbiology and infectious disease*, *75*(4), 406-411.

Weng, P. L., Ramli, R., Shamsudin, M. N., Cheah, Y. K., & Hamat, R. A. (2013). High genetic diversity of Enterococcus faecium and Enterococcus faecalis clinical isolates by pulsed-field gel electrophoresis and multilocus sequence typing from a hospital in Malaysia. *BioMed research international*, *2013*.

Werner, G., Coque, T. M., Franz, C. M., Grohmann, E., Hegstad, K., Jensen, L., ... & Weaver, K. (2013). Antibiotic resistant enterococci—tales of a drug resistance gene trafficker. *International Journal of Medical Microbiology*, *303*(6), 360-379.

Willems, R. J., & Van Schaik, W. (2009). Transition of Enterococcus faecium from commensal organism to nosocomial pathogen. *Future microbiology*, *4*(9), 1125-1135.

Winston, L. G., Pang, S., Haller, B. L., Wong, M., Chambers, H. F., & Perdreau-Remington, F. (2004). API 20 strep identification system may incorrectly speciate enterococci with low level resistance to vancomycin. *Diagnostic microbiology and infectious disease*, *48*(4), 287-288.

Woegerbauer, M., Jenni, B., Thalhammer, F., Graninger, W., & Burgmann, H. (2002). Natural genetic transformation of clinical isolates of Escherichia coli in urine and water. *Applied and environmental microbiology*, *68*(1), 440-443.

Wolska, K. I. (2003). Horizontal DNA transfer between bacteria in the environment. *Acta Microbiologica Polonica*, *52*(3), 233-243.

Yean, C. Y. (2015). Genotypic variations of virulent genes in Enterococcus faecium and Enterococcus faecalis isolated from three hospitals in Malaysia.

Zarrilli, R., Tripodi, M. F., Di Popolo, A., Fortunato, R., Bagattini, M., Crispino, M., ... & Utili, R. (2005). Molecular epidemiology of high-level aminoglycoside-resistant enterococci isolated from patients in a university hospital in southern Italy. *Journal of Antimicrobial Chemotherapy*, *56*(5), 827-835.

Zhang, X., Paganelli, F. L., Bierschenk, D., Kuipers, A., Bonten, M. J., Willems, R. J., & Van Schaik, W. (2012). Genome-wide identification of ampicillin resistance determinants in Enterococcus faecium. *PLoS genetics*, *8*(6), e1002804.