UNIVERSITI PUTRA MALAYSIA

RAPD ANALYSIS, PLASMID PROFIlES, ANTIBIOTIC RESISTANCE AND OCCURRENCE OF THE VAN GENES IN ENTEROCOCCUS SPECIES ISOLATED FROM HUMAN AND POULTRY

HARYANTI TOOSA

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By

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia

April 2001

Teristimewa untuk:

Yang tersayang…..

Mak

Abah

Adik-adik

Teman-teman

Atas sokongan kalian……

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

RAPD ANALYSIS, PLASMID PROFILES, ANTIBIOTIC RESISTANCE AND OCCURENCE OF THE *VAN* GENES IN *ENTEROCOCCUS* SPECIES ISOLATED FROM HUMAN AND POULTRY

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April 2001

Chairman: Assoc. Prof. Dr. Son Radu

Faculty: Food Science and Biotechnology

The purpose of this study was to evaluate the molecular relatedness of the *Enterococcus* spp. isolated among poultry and clinical samples. A total of 71 poultry isolates and 29 clinical isolates were examined in this study. The poultry samples obtained from market in Sri Serdang, Selangor and Makmal Kesihatan Awam Veterinar. Petaling Jaya, whereas the clinical samples were from Hospital Universiti. Kuala Lumpur. *E. faecalis* (41of 71, 58%) was the dominant species isolated from poultry samples. Besides that, *E. faecium* (3 of 71, 4%), *E. casseliflavus* (4 of 71, 6%), *E. durans* (18 of 71, 25%) and *E. hirae* (5 of 71. 7%) were also detected. Twenty-nine isolates from clinical samples were identified as *E. faecalis* (19 isolates,

66%), *E. faecium* (8 isolates, 28%), *E. mundtii* (1 isolate, 3%) and *E. raffinosus* (1 isolate, 3%). All isolates were resistant against ceftazidime, erythromycin. kanamycin, nalidixic acid and streptomycin (100%). Clinical isolates also demonstrated high resistance to cephalothin, gentamicin and norfloxacin (100%). Sixty-four of 71 poultry isolates, and 26 of 29 clinical isolates were resistant to vancomycin and this indicated high prevalence of vancomycin resistant enterococci detected among the isolates. All seventy-one isolates from poultry exhibited multiple resistance with Multiple Antibiotic Resistance (MAR) indices ranging between 0.53 to 1.0 while for clinical isolates the range were between 0.6 to 0.86. These high MAR index suggests that all the isolates originated from high risk sources. According to plasmid profile analysis. 29 plasmid patterns were observed among poultry isolates with the plasmid DNA bands ranging in sizes from 1.1 to 35.8 megadalton. The plasmid analysis among clinical isolates were grouped into 9 plasmid patterns ranging in sizes from 1.85 to 35.8 megadalton. RAPD-PCR has been used to generate polymorphic genomic fingerprints to discriminate the enterococci isolates. Two primers (GEN15008 and GEN15009) were chosen after screening a set of 10 primers. These two primers yield reproducible and typeable results in most isolates examined with the bands ranging in sizes from 0.25 kb to 5.0 kb. From the dendrogram generated to study the interspecific relatedness among the isolates. 2 main clusters were observed and further subdivided into several subclusters defining the genetic heterogeneity among the isolates. The

*van*A specific (732 bp) fragment was detected in 96 of 100 (96%) of the isolates. 29 (100%) of clinical isolates and 67 of 71 (94%) of poultry isolates were positive for *van*A gene. 4 of 71 (6%) of poultry were positive for *van*C2/C3 gene (439 bp). Isolates containing the *van*B or *van*C1 gene were not found.

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

ANALISIS RAPD, PROFIL PLASMID, KERINTANGAN ANTIBIOTIK DAN KEHADIRAN *VAN* GEN PADA SPESIES *ENTEROCOCCUS* DIPENCILKAN DARIPADA KLINIKAL DAN TERNAKAN

Oleh

HARYANTI TOOSA

April 2001

Pengerusi: Profesor Madya Dr. Son Radu

Fakulti: Fakulti Sains Makanan dan Bioteknologi

Tujuan kajian ini adalah untuk menilai perkaitan molekul *Enterococcus* spesies yang dipencilkan daripada sumber ternakan dan klinikal. Sejumlah 71 sumber ternakan dan 29 sumber klinikal dikaji. Sampel-sampel ternakan daripada pasar di Serdang, Selangor dan Makmal Kesihatan Awam Veterinar, Petaling Jaya manakala sumber klinikal diperolehi daripada Jabatan Mikrobiologi Perubatan, Hospital Universiti, Kuala Lumpur. *E. faecalis* (41 daripada 71, 58%) adalah spesies dominan yang dipencilkan daripada sumber ternakan. Selain itu, *E. faecium* (3 daripada 71, 4%). *E. casseliflavus* (4 daripada 71, 6%), *E. durans* (18 daripada 71, 25%) dan *E. hirae* (5 daripada 71, 7%) juga dikesan. 29 pencilan daripada sumber klinikal dikenalpasti sebagai *E. faecalis* (19 pencilan, 66%). *E. faecium* (8 pencilan, 28%), *E. mundtii* (1 pencilan, 3%)

dan *E. raffinosus* (1 pencilan, 3%). Kesemua pencilan rintang terhadap ceftazidime, erythromycin, kanamycin, nalidixic acid dan streptomycin (100%). Pencilan-pencilan klinikal juga menunjukkkan kerintangan yang tinggi terhadap cephalothin, gentamicin dan norfloxacin (100%). 64 daripada 71 pencilan ternakan, dan 26 daripada 29 pencilan klinikal rintang terhadap vancomycin dan ini menunjukkan kerintangan yang tinggi pada enterococci rintang vancomycin dikesan dikalangan pencilan. Kesemua 71 pencilan ternakan menunjukkan kerintangan terhadap pelbagai antibiotik dengan julat indeks kerintangan pelbagai terhadap antibiotik (MAR) di antara 0.53 hingga 1.0 manakala julat untuk pencilan klinikal antara 0.6 hingga 0.86. MAR indeks yang tinggi mencadangkan bahawa semua pencilan berasal dari sumber yang berisiko tinggi. Berdasarkan analisis plasmid profil, 29 corak plasmid diperolehi daripada pencilan ternakan dengan saiz plasmid di antara 1.1 ke 35.8 megadalton. Plasmid analisis dari pencilan klinikal dikumpulkan kepada 9 corak plasmid bersaiz di antara 1.85 ke 35.8 megadalton. RAPD-PCR telah digunakan untuk menghasilkan corak genomic polimorfik untuk membezakan pencilan-pencilan enterococci. Dua primer (GEN15008 dan GEN15009) dipilih selepas menyaring 10 primer. Dua primer tersebut menghasilkan keputusan yang pada keseluruhan pencilan yang dikaji dengan saiz jalur di antara 0.25 kb ke 5.0 kb. Dendrogram yang terhasil dikaji perkaitan interspesifik antara pencilan, dua kluster utama diperolehi dan dibahagi kepada beberapa subkluster menunjukkan kepelbagaian genetic di kalangan pencilan. Fragmen *van*A

spesifik (732 bp) dikesan pada 96 daripada 100 (96%) pencilan. 29 (100%) pencilan klinikal dan 67 daripada 71 (94%) pencilan ternakan adalah positif pada gen *van*A. 4 (6%) pencilan ternakan adalah positif pada *van*C2/C3 (439 bp). Pencilan-pencilan yang mengandungi gen *van*B atau *van*C1 tidak dikesan.

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Last but not least, also thanks to my housemates; Ja, Ju, Eima, Kuzah. Pura and P-nat who share much of my joy and sorrow.

I certify that an Examination Committee met on 16th April 2001 to conduct the final examination of Haryanti Toosa on her Master of Science thesis entitled "RAPD Analysis, Plasmid Profiles, Antibiotic Resistance and Occurrence of the *van* Genes in *Enterococcus* Species Isolated from Human and Poultry" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.

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Date: 12 JUL 2001

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

HARYANTI TOOSA

Date: 26.04.2001

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Abbreviations

A	adenine or adenosine
ATP	adenosine triphosphate
Am	ampicillin
B	Bacitracin
bp	base pair
C	chloramphenicol
Cf	cephalothin
Caz	ceftazidime
ccc	covalently closed circular
Da	dalton (unit of molecular mass)
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dH_2O	distilled water
DNA	deoxyribonucleic acid
dTTP	deoxythymidine triphosphate
E	erythromycin
EDTA	Ethylenediamine tetraacetic acid
EtBr	ethidium bromide
g	gram
HCl	hydrochloric acid
i.e.	that
ID	identification number
K	kanamycin
kb	kilobase pair (number of bases in thousand)
KDa	kilodalton
LB	Luria Bertani
M	Molar or molarity, moes of solute per liter of solution

MDa	megadalton
ml	milliliter
mm	millimeter
mM	millimolar
μg	microgram
μl	microliter
mol	mole
Na	nalidixic acid
NaCl	sodium chloride
NaOH	sodium hydroxide
P	penicillin
R	resistant
RAPD	Randomly Amplified Polymorphic DNA
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolution per minute
s	sensitive
S	streptomycin
sdH_2O	sterile distilled water
SDS	sodium dodecyl sulphate
Taq	Thermus aquaticus DNA (polymerase)
TBE	Tris-Borate EDTA electrophoresis buffer
Te	tetracycline
Tec	teicoplanin
Tris	tris (hydroxymethyl) methylamine
UV	ultraviolet
V	volts
Va	vancomycin

CHAPTER I

INTRODUCTION

Enterococcus species belong to the family Streptococcaceae. They are catalase-negative, gram-positive cocci, occur singly, in pair and in short chain organisms. These organisms were considered as a part of the normal flora of the bowel, genital tract and anterior urethrae of humans with some also being found on the skin, vaginal secretions and in the perineal area (Kaye, 1982). They are not only found in humans but also widely distributed in nature and animals.

Among the 17 currently recognized species, *Enterococcus faecalis* and *Enterococcus faecium* are the most frequently recovered species (Kaye, 1982; Facklam and Collins, 1989). *E. faecalis* accounts for about 90% of all encountered clinical isolates of *Enterococcus* and *E. faecium* accounts for 5-10% of the isolates (Low *et al.*, 1994). Besides the two species, they also found *E. durans* as clinical isolates.

Vancomycin resistant-enterococci (VRE) gained much attention nowadays. It means that these organisms are resistant to vancomycin. Besides that, it also means that the organism has acquired an incredible array of genes, which code for enzymes that permit this growth phenomenon to

occur. Prior to 1986, VRE were considered a clinical rarity. Since 1988, reports of VRE have been limited to a small number of outbreaks (Leclercq *et al.*, 1988; Uttley *et al.,* 1988). However, within the last 6 years, enterococci have become increasingly important nosocomial pathogens. Between 1989 and 1993 there was a 23-fold rise in VRE infections, from 0.3% to 7.9% of nosocomial enterococcal infections. They have occurred as the second most common cause of nosocomial infections and surgical wound infections (Schaberg *et al.*, 1991; Jarvis and Marton, 1992; Moellering, 1992; Mortensen and Larocco, 1992) and the third most frequently reported cause of bacteremia (Schaberg *et al.,* 1991, Lemmen and Daschner, 1996). They are becoming increasingly important agents of human disease, largely because of their resistance to antimicrobial agents to which other streptococcus are generally susceptible (Lee and Wetherall, 1987) and has been commonly characterized as multiple-drug-resistant gram-positive cocci (Mortensen and LaRocco, 1992). VRE outbreak have occurred around the world in the past 2 years and in some enterococci, low-level innate antibiotic resistance has mutated into high-level resistance to some agents example aminoglycosides and increased incidence of multiple drug resistant enterococci (Montecalvo *et al.*, 1994; Antalek *et al.*, 1995, Huycke *et al.*, 1998). Before the findings that these bacteria were resistant to multiple antimicrobial agents (Boyce *et al.,* 1992; Murray, 1995; Son *et al.,* 1999), aminoglycosides and vancomycin were considered the drug of choice for the treatment of serious enterococcal infections (Calia, 1996).