الكشف الجزيئى عن جينات الفان (vanA and vanB genes) فى المكورات المعوية المضادة للفانكوميسين المعزولة من مرضى أمراض و أورام الدم

رسالة

للحصول على درجة الماجستير فى العلوم الطبية الأساسية
(علم الكائنات الدقيقة و المناعة)

مقدمة من

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Molecular Characterization of Van genes (VanA and VanB) in Vancomycin-Resistant *Enterococcus spp*. (VRE) Isolated from Hematology-Oncology Patients

Thesis

Submitted in the partial fulfillment of
The Master Degree in Basic Medical Science
(Microbiology and Immunology)

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CONTENTS

List of Abbreviations

AAC	Antibiotics Associated Colitis
AFLP	Amplified Fragment Length Polymorphism
ALL	Acute Lymphocytic Leukemia
AME	Aminoglycoside Modifying Enzymes
AML	Acute Myeloid Leukemia
ANC	Absolute Neutropenic Count
API	Analytical Profile Index
ARA	Arabinose
BHI	Brain Heart Infusion
C. difficile	*Clostridium difficile*
CDC	Centers For Disease Control and Prevention
CFU	Colony Forming Unit
CML	Chronic Myeloid Leukemia
CMV	Cytomegalo virus
CoNS	Coagulase Negative Staphylococcus
D-Ala-D-Ala	D-alanine-D-alanine
D-Ala-D-Lac	D-alanine-D-lactate
D-Ala-D-Ser	D-alanine-D-serine
DIC	Disseminated Intravascular Coagulopathy
DNA	Deoxy Ribonucleic Acid
dNTP	deoxy-nucleotide triphosphates
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
FUO	Fever of Unknown Origin

G+C	Guanine + Cytosine
GIT	Gastrointestinal tract
GRE	Glycopoetide Resistant Enterococci
HAIs	Hospital-Acquired Infections, Health Care Associated Infections
HCWs	Health Care Workers
HICPAC	Hospital Infection Control Practices Advisory Committee
HLAR	High Level Aminoglycoside Resistance
ICU	Intensive Care Unit
IV	Intravenous
LAP	Leucine Aminopeptidase
MAN	Mannitol
MDR	Multiple Drug Resistant
MGP	Methyl-A-D-Glucopyranoside
MIC	Minimum Inhibitory Concentration
MOT	Motility
MRSA	Methicilin Resistant *Staphylococcus aureus*
NaCl	Sodium Chloride
NCCLS	National Committee For Clinical Laboratory Standards
NHL	Non Hodgkin Lymphoma
NNISS	The National Nosocomial Infections Surveillance System
PBP	Penicillin Binding Protein
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electropheresis
PG	Peptidoglycan
PIG	Pigment
PYR	Pyrolidonyl-B-Naphthylamide
PYU	Pyruvate

RAF	Raffinose
RAPD	Randomly Amplified Polymorphic DNA
rRNA	Ribosomal RNA
S. aureus	*Staphylococcus aureus*
SBL	Sorbitol
SCT	Stem Cell Transplantation
SOR	Sorbose
SUC	Sucrose
TEL	Tellurite
UTI	Urinary Tract Infections
UV	Ultraviolet
VBNC	Viable But Non-Culturable Cells
VDE	Vancomycin Dependant Enterococci
VRE	Vancomycin Resistant Enterococci
VRSA	Vancomycin Resistant *Staphylococcus aureus*
VSE	Vancomycin Susceptible Enterococci

List of Tables

List of Figures

AIM OF THE STUDY

The aim of this study was to determine:

1- The occurrence of colonization and bacteremia with vancomycin resistant enterococci (VRE) among hematology-oncology patients at Ain Shams University Hospitals.
2- The different risk factors associated with VRE colonization or infection.
3- The enterococcal species of all isolates.
4- The antimicrobial susceptibility pattern of the isolated VRE strains.
5- The Molecular characterization of *vanA* and *vanB* genes in these isolates.

INTRODUCTION

Enterococcus species are part of the normal gastrointestinal flora, together with close to 100 other species of aerobic and anaerobic bacteria. Initially, the enterococci were considered to be only slightly virulent, however the rapid emergence and dissemination of vancomycin resistant enterococcus strains (VRE) has completely changed the clinical relevance of these pathogens **(*Caiaffa et al., 2003*).** Enterococci have increasingly become responsible for serious clinical and nosocomial infections, including bacteremia, endocarditis, and urinary tract infections **(*Appleman et al., 2004*).**

Enterococcus sepsis can have overall mortality of 30% or higher with significantly higher mortality in burn patients and other immunocompromised patients. The appearance of resistance to vancomycin has made the therapy for enterococcal bacteremia much more difficult ***(Sherwood et al., 1998*)**.

There is concern that resistance genes in VRE might be transferred to other Gram-positive microorganisms, making the situation even worse. In addition, VRE has caused outbreaks and became endemic in several hospitals, presenting a challenge for hospital infection control teams **(C*etinkaya et al., 2000*)**.

Vancomycin resistance in enterococci has coincided with the increasing incidence of high-level enterococcal resistance to penicillin and aminoglycosides, thus presenting a challenge for physicians who treat patients who have infections caused by these microorganisms. Treatment options are often limited to combining antimicrobials or experimental compounds that have unproven efficacy **(*Handwerger et al., 1993*)**.

The epidemiology of VRE has not been clarified; however, certain patient populations are at increased risk for VRE infection or colonization. These populations include critically ill patients or those with severe underlying disease or immunosuppression (e.g., patients in ICUs or in oncology or transplant wards); persons who have had an intraabdominal or cardio-thoracic surgical procedure or an indwelling urinary or central venous catheter; and persons who have had a prolonged hospital stay or received multiantimicrobial and/or vancomycin therapy ***(Husni et al., 2002).***

Because enterococci are part of the normal flora of the gastrointestinal and female genital tracts, most infections with these microorganisms have been attributed to the patient's endogenous flora **(*Vergis et al., 2001*).** However, recent studies have indicated that VRE and other enterococci can be transmitted directly by patient-to-patient contact or indirectly by transient carriage on the hands of