دراسة حساسية الفيلم الحيوي للمكورات العنقودية للمضادات الحيوية بطرق مختلفة

رسالة
توطئة للحصول على درجة الدكتورة
في الميكروبيولوجيا الطبية و المناعة

مقدمة من
الطبيبة/نيرمين محمود احمد عبد الله
ماجستير الميكروبيولوجيا الطبية والمناعة
كلية الطب جامعة عين**شمس**

تحت اشراف

ا.د فاتن مصطفى علي
استاذ الميكروبيولوجيا الطبية و المناعة
كلية الطب جامعة عين شمس

ا.د ايمان عبد المنعم ابراهيم الجوهري
استاذ الباثولوجيا الاكلينيكية
كلية الطب جامعة عين شمس

ا.د شيرين بنداري السيد
استاذ الميكروبيولوجيا الطبية و المناعة
كلية الطب جامعة عين شمس

د.لمياء فؤاد فتحي
استاذ مساعد الميكروبيولوجيا الطبية و المناعة
كلية الطب جامعة عين شمس

كلية الطب
جامعة عين شمس
٢٠١٤

Study of antimicrobial susceptibility of Staphylococcal biofilm using different methods

Thesis
Submitted for partial fulfillment of M.D. degree in Medical Microbiology and Immunology

Submitted By
Nermeen Mahmoud Ahmed Abdallah
Master degree of Medical Microbiology and Immunology

Under supervision of

Prof. Faten Mostafa Ali
Professor of Medical Microbiology and Immunology
Faculty of Medicine Ain Shams University

Prof. Iman Abdelmonaem Ibraheem Elgohary
Professor of clinical pathology
Faculty of Medicine Ain Shams University

Prof .Shereen Bendary Elsayed
Professor of Medical Microbiology and Immunology
Faculty of Medicine Ain Shams University

Dr. Lamia Fouad Fathi
Assistant Professor of Medical Microbiology and Immunology
Faculty of Medicine Ain Shams University

Faculty of Medicine
Ain Shams University
2014

Acknowledgment

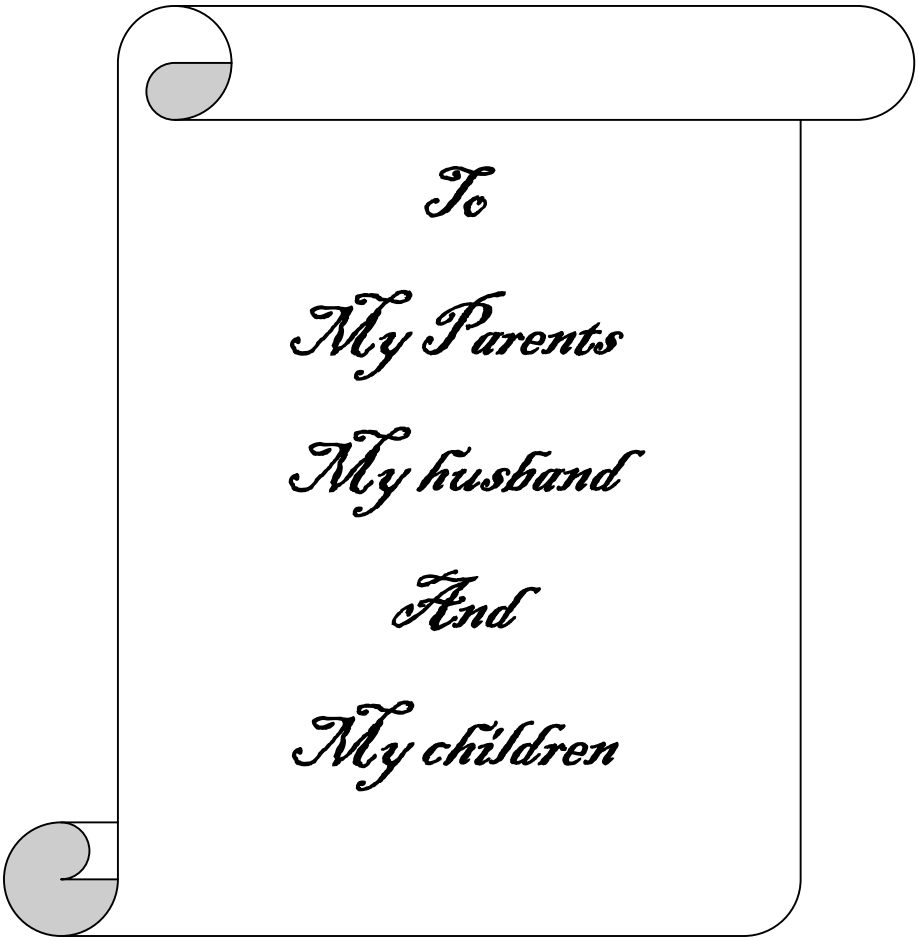
First and foremost thanks to “ALLAH” who is the most beneficial and the most merciful.

I would like to express my deep sincere appreciation and thanks to **Dr. Faten Mostafa Ali ,** Professor of Medical Microbiology and Immunology Faculty of Medicine Ain Shams University, for her kind supervision, tremendous assistance, valuable criticism, and encouragement throughout the work.

My grateful appreciation to **Dr Iman Abdelmonaem Elgohary** Professor of Clinical Pathology Faculty of Medicine Ain Shams University for her kind supervision.

I wish to thank **Dr .Shereen Bendary Elsayed** Professor of Medical Microbiology and Immunology Faculty of Medicine Ain Shams University for her active participation , effective help ,and careful comments.

I wish also to thank **Dr. lamiaa Fouad Fathi** Assistant Professor of Medical Microbiology and Immunology Faculty of Medicine Ain Shams University Faculty of Medicine Ain Shams University for her continuous help and kind cooperation in this work.


To
My Parents
My husband
And
My children

Contents

List of abbreviations

AHLs	Acyl homoserine lactones
Aap	Accumulation associated protein
AB	Antibiotics
AFM	Atomic force microscopy
A_{HW}	absorbance at higher wavelength minus the media blank
AI-2	Autoinducer 2
AIP	Autoinducing peptides
AK	amikacin
A_{LW}	absorbance at lower wavelength minus the media blank
AO_{HW}	Absorbance of resazurin in media – Absorbance of media only at higher wavelength
AO_{LW}	Absorbance of resazurin in media – Absorbance of media only at lower wavelength
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
Bap	Biofilm associated protein
BTA	BioTimer assay
C.albicans	*Candida albicans*
C.elegans	Caenorhabditis elegans
CABSI	Catheter associated bloodstream infections
CDC	Center for disease control and prevention
CDFF	Constant depth film fermenter
CFU	Colony forming unit
CLSM	Confocal Laser Scanning Microscopy
CoNS	Coagulase negative Staphylococci
CRM	Confocal Raman Microscopy
CV	Crystal violet
CVCs	Central venous catheters
DESI	Desorption-Electro-Spray-Ionization
DMMB	1.9 dimethyl methylene blue
DNases	Deoxyribonecleases
DspB	Dispersin B
eDNA	Extraccellular DNA
EDTA	Ethylenediaminetetraacetic acid
EM	Electron microscopy
EPS	Extracellular polymeric substances
FAO	Food and agriculture organaization
FDA	Fluorescein di acetate

FISH	Fluorescence in situ Hybridization
FnBPs	Fibronectin binding proteins
GNB	Gram negative bacilli
H&E	Hematoxylin and Eosin
HS	Highly significant
HSL	Homoserine lactones
I	Intermediate
ica	Intracellular adhesion
LM	Light microscopy
MBC	Minimal bactericidal concentration
MBEC	Minimal biofilm eradication concentration
MBIC	Minimal biofilm inhibitory concentration
MHB	Mueller Hinton broth
MIC	Minimal inhibitory concentration
MRC	Minimal regrowth concentration
MRD	Modified Robbins device
MRSA	Methicillin-resistant Staphylococcus aureus
MS	Mass spectrometry
MSCRAMMs	Microbial surface component recognizing adhesive matrix molecules
MTP	Microtiter plate
No	Number
OD	Optical density
P.aeruginosa	*Psudomonas.aeruginosa*
PCR	Polymerase chain reaction
PIA	Polysaccharide intercellular adhesin
PLUNC	Palate,lung,nasal epithelium clone
PNAG	Poly N acetylglucosamine
PQS	Pseudomonas quinolone signal
PSMs	Phenol soluble modulins
qPCR	Quantitative Polymerase chain reaction
qRT-PCR	Quantitative Reverse Transcription PCR
QS	Quorum sensing
QSI	Quorum sensing inhibitor
R	Resistant
$\mathbf{R_0}$	AO_{LW}/AO_{HW}
rRNA	Ribosomal ribonucleic acid
S	Sensitive
S.aureus	*Staphylococcus aureus*
SD	Standard deviation

SEM	Scanning Electron microscopy
SPM	Scanning probe microscoby
Spp	Species
STXM	Scanning transmission X ray microscopy
TEM	Transmission Electron microscopy
TSB	Tryptic soy broth
UPEC	Uropathogenic *E. coli*
VAN	Vancomycin
WHO	World health organization
X^2	Chi square test
XM	X ray microscopy
XTT	(2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilide)

List of tables

List of figures

Introduction

Biofilm are microbial communities of surface-attached cells embedded in a self-produced extracellular polymeric matrix. They can cause significant problems in many areas, both in medical settings (e.g. persistent and recurrent infections, device-related infections) and in industrial settings (e.g. biofouling in drinking water distribution systems and food processing environments) ***(López et al., 2010).***

A feature of biofilm-based infections is represented by the higher resistance of bacterial and fungal cells growing as biofilm to antibiotics and as well as resisting phagocytosis and other components of the body's defense system, when compared to planktonic cells ***(Høiby et al .,2010).***

Microbiologists have evaluated the efficacy of antibiotics by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). In all diagnostic laboratories, these measurements are made on freely floating, planktonic, laboratory phenotypes. These assays measure only the concentration of chemotherapeutic agent required to inhibit growth or kill planktonic bacteria ***(Ceri et al.,2006***).

For some antibiotics, the concentration required to kill sessile bacteria, may be greater than a thousand times that required to kill planktonic bacteria of exactly the same strain. Thus, even well-chosen treatment based upon laboratory results often suppresses an infection until biofilm-associated organisms are reactivated and cause another clinical infection ***(Pratten and Ready, 2010).***

Staphylococcal infections are most associated with their colonization on implanted medical devices such as central venous catheter. The increased use of indwelling medical devices has a

considerable impact on the role of Staphylococci in clinical medicine. The crucial step in the pathogenesis of these infections is the biofilm formation on the surface of the implanted biomedical device **(*Fitzpatrick et al., 2005).***

The high rate of resistance of nosocomial Staphylococci to β-lactams, make empiric therapy based on glycopeptides including vancomycin is the drug of choice for treatment. The determination of biofilm susceptibility of vancomycin is, therefore, a key factor for treating infections caused by Staphylococcus spp. growing on indwelling devices ***(Antunes et al.,2010).***

Biofilm have been found to be involved in a wide variety of microbial infections in the body including urinary tract infections, middle-ear infections, formation of dental plaque, gingivitis, endocarditis, infections in cystic fibrosis and chronic sinusitis (***Burmølle et al., 2010***).

There are many laboratory biofilm models available which can be used to assess the susceptibility of these distinctive resistant phenotypes. The complexities of these models vary considerably and the antimicrobial susceptibility of biofilms grown in these different models are also not standardized. Models are often chosen based on either their simplicity or those that reflect growth and environmental survival conditions of the bacterial species of interest ***(Pratten and Ready, 2010).***

The criteria for a successful biofilm assay will include the ability to form biofilms of organisms associated with biofilm infection, the formation of multiple equivalent biofilms for susceptibility testing with characteristics consistent to those observed in vivo, the ability to carry out assays as simply as in the MIC assay, the possibility of automation, and obtaining the data

in a time frame mandated by the need to initiate therapy (***Ceri et al., 2006& Antunes et al., 2010).***

The use of polystyrene microtitre multi-well plates offers the advantage of producing a high number of replicates and therefore make high throughput testing possible ***(Pratten and Ready, 2010).***

There are many models assessing the viability of biofilm after challenging with antibiotics the most commonly investigated include : a simple method with no dyes based on refilling the wells after antibiotic challenge with broth and inability to grow after incubation denotes loss of biofilm viability. The use of calorimetric indicator resazurin is another method. Metabolically active cells reduce this resazurin to a pink product resorufin. The use of a simple method based on crystal violet for susceptibility determination is the best approach, as it would decrease the duration of the method and warrants better reading and interpretation of results ***(Cernohorska and Votava, 2004 , Antunes et al., 2010 and Punithavathy et al ., 2012)*** .

There is no gold standard test to evaluate biofilm susceptibility in the laboratorial routine therefore, there is a clear need to compare different methods for testing in vitro susceptibility of cells in the biofilm mode of growth, which should be of low-cost, efficient and time-effective.

.

Aim of the work

Aim of this work was to:

1-Study vancomycin & amikacin susceptibility of Staphylococcal biofilm compared to vancomycin &amikacin MIC of planktonic Staphylococci.

2- Study the biofilm susceptibility of vancomycin and amikacin by applying different methods for detection to select the most applicable.