Biofilm production by *Pseudomonas aeruginosa* clinical isolates and its relationship with pseudomonas quinolone signal *(pqsA)* gene and antibiotic resistance

Thesis

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List of Abbreviations

ADP	Adenosine diphosphate
AHLs	Acyl-homoserine lactones
AI	Autoinducer
AQs	Alkyl-quinolones
BHL	butyryl homoserine lactone
BP	Base pair
cAMP	Cyclic adenosine monophosphate
CF	Cystic fibrosis
CFU	Colony forming unit
CLSI	Clinical and laboratory standards institute
CNS	Central nervous system
CSF	Cerebrospinal fluid
CVCs	Central venous catheters
DNA	Deoxyribonucleic acid
eDNA	Extracellular deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPS	Extracellular polymeric substances
Exo A	Exotoxin A
GIT	Gastrointestinal tract
GTPase	Guanosine triphosphatase
H2S	Hydrogen sulfide
HAI	Hospital-acquired infections
HCAP	Health care-acquired pneumonia
HHQ	2-heptyl-3-hydroxy-4-quinolone
ICU	Intensive care unit
Ig	Immunoglobulin
IL	Interleukin
IQS	Integrated quorum-sensing
Las	Elastas
LPS	Lipopolysaccharide
MBEC	Minimal biofilm eradication concentration
MDR	Multidrug resistant

MH	Muller-Hinton
MIC	Minimal inhibitory concentration
MTP	Microtiter plate
NFkB	Nuclear factor kappa B
OD	Optical density
ODc	Optical density cut off
OHL	Oxo-dodecanoyl homoserine lactone
Opr	Outer membrane protein
P value	Probability
P. aeruginosa	*Pseudomonas aeruginosa*
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PDT	Photodynamic therapy
PEL	Pellicle
PH	Potential of hydrogen
PQS	Pseudomonas quinolone signal
PSL	Polysaccharide synthesis locus
QS	Quorum sensing
QSIs	Quorum sensing inhibitors
RANTES	Regulated on Activation Normal T Cell Expressed and Secreted
RND	Resistance-Nodulation-Division
RPM	revolutions per minute
rRNA	Ribosomal ribonucleic acid
RTI	Respiratory Tract infections
SP.	Species
SPSS	Statistical Package for the Social Sciences
TLR	Toll-like Receptor
TNF	Tumor necrosis factor
UTI	Urinary tract infection
UV	Ultraviolet
Vs	Versus
$\chi 2$	Chi-square test

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Abstract

Background: Biofilms are complex microbial communities anchored to biotic or abiotic surfaces. They contribute to more than 80% of hospital-acquired infections. *Pseudomonas aeurginosa (P. aeurginosa)* is an important pathogen able to form biofilm which is regulated by quorum sensing molecules including pseudomonas quinolone signal (pqs).

Objectives: The present work aimed to study the ability of different *P. aeurginosa* clinical isolates to produce biofilm and their association with *Pqs A gene and* antibiotic resistance.

Methodology: This study was conducted on 30 isolates of *P. aeruginosa* obtained from different clinical samples. Detection of antibiotic susceptibility was done by disc diffusion method. Detection of biofilm formation was done using microtitre plate assay. Minimal inhibitory concentration (MIC) and minimal biofilm eradication concentration (MBEC) were done only for biofilm forming isolates. Detection of *Pqs A* gene was done using conventional PCR.

Results: Seventeen out of 30 (57%) isolates were biofilm producers. Antibiotic resistance was higher among biofilm producing than non biofilm producing isolates. There was statistically significant difference between MIC and MBEC of meropenem and amikacin. There was statistically significant association between biofilm production and *Pqs A* gene.

Conclusion: Biofilm producing strains have high resistance to antibiotics and *Pqs A* gene has a significant role in biofilm production. Thus, it is recommended to detect MBEC rather than MIC to antimicrobials for treatment of biofilm associated infections and to study the effect of pqs inhibition on biofilm control.

Keywords: *P. aeruginosa*, biofilm, antibiotic resistance, MBEC, MIC, *pqs A.*

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*),which is known as a non-fermentative gram negative opportunistic pathogen, is the leading cause of diverse infections including pneumonia, wound and urinary tract infection **(Maita and Boonbumrung, 2014)**. It has become an important cause of community - acquired and health- care associated infections, especially in immune compromised patients and those with indwelling medical devices **(Cole et al., 2014).**

Infections caused by *P. aeruginosa* are difficult to treat, as the majority of strains exhibit intrinsic resistance to several antibiotics **(Iregbu and Eze, 2015).** This is due to constitutive expression of β-lactamases, efflux pumps combined with low permeability of the outer-membrane **(Mesaro et al., 2007)**.

Bacterial β-lactamases are divided into four major groups (A-D) according to the Ambler classification scheme. Group A hydrolyze penicillin and cephalosporins. Group B are metallo-β-lactamases (MBLs) requiring Zn2+ ion(s) for activity, which hydrolyze all β-lactam antibiotics with the exception of monobactams. Group C include enzymes such as AmpC-type β-lactamases that degrade cephamycins and cephalosporins and not inhibited by

clavulanic acid. Group D have high hydrolysis ratethat degrade penicillin, cephalosporins, monobactams and carbapenems **(Jacoby and Munoz-Price, 2005 and Juan et al., 20005).**

Extended-spectrum β-lactamases (ESBLs) confer resistance to all β-lactams except for the carbapenem family and cephamycins **(Kumar et al., 2012)**. Coexistence of multiple β-lactamases in clinical isolates of *P. aeruginosa*is is common, causing resistance to almost all β-lactam antibiotics **(Upadhyay et al., 2010).**

One of the antibiotic resistant mechanisms that the bacteria employ is the formation of biofilm **(Høiby et al., 2010).** The biofilm is composed of sessile populations of microorganisms that are surrounded by a slime layer and can be attached on biotic or abiotic surfaces **(Karatuna and Yagci, 2010).** The capability of *P. aeruginosa* to form biofilms is a key requirement for its chronic colonization of human tissues **(Maita and Boonbumrung, 2014)**.

Biofilms not only provide a physical barrier to antimicrobial agents and host immune responses but also facilitate the exchange of antibiotic resistant genetic material between organisms and may also contain antibiotic degrading enzymes such as β-lactamases **(Hoiby et al., 2010 and Heydari and Eftekhar, 2015).** Thus, biofilms render pathogenic microorganisms difficult to eradicate and

contribute to localized or systemic inflammation **(Hu et al., 2011).**

The pathogens living inside the biofilm communicate with each other by quorum-sensing system mediated by the two chemically distinct classes of signal molecules, the ***N***-acylhomoserine lactones **(Davenport et al., 2015)** and the 4-alkyl-quinolones (AQs) **(Diggle et al., 2006).** The latter group consists of more than 50 compounds **(Deziel et al., 2004)**. 2-heptyl-3-hydroxy-4-quinolone, commonly referred to as pseudomonas quinolone signal (Pqs), is the most active signal molecule in this group **(Mashburn et al., 2009).**

Pqs is pleiotropic, regulating production of pyocyanin, elastases, rhamnolipids and Lectin, biofilm production and motility **(Dubern and Diggle, 2008 and Mashburn et al., 2009).** Synthesis of AQ depends on the *pqsABCDE* locus, which is responsible for generating multiple 4-quinolones **(Heeb et al., 2011).** The first step of the 4-quinolones synthesis pathway is the generation of the *pqsA* gene product **(Deziel et al., 2004).** A study suggested that the *pqsA* gene could be a candidate for screening bacteria that form biofilms **(Maita and Boonbumrung, 2014)**.

Aim of the Work

The aim of this work is to:

- Determine the ability of different *P. aeruginosa* clinical isolates to produce biofilm.
- Determine the association of biofilm production with the presence of the *pqsA* gene.
- Determine the association of biofilm production with antibiotic resistance.

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