Phenotypic Detection of Biofilm Formation among Clinical Isolates of *Staphylococci*

Thesis

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By

Basma Sherif Fahmy Mahmoud

MB Bch
Faculty of Medicine, Ain Shams University

Under Supervision of

Professor/ Nevine Nabil Kassem

Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Doctor/ Rania Mohamed Abd Al-Halim

Lecturer of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
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Candidate

Basma Sherif Fahmy

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List of Abbreviations

Abbr.	Full-term
Aap	: Accumulation-associated protein
***Agr* locus**	: Accessory gene regulator locus
AHLs	: Acylated homoserine lactones
AIP	: Autoinducing peptide
AIs	: Autoinducers
AMPs	: Antimicrobial peptides
ATP	: Adenosine triphosphate
BTA	: BioTimer assay
CA-MRSA	: Community acquired Methicillin resistant *Staphylococcus aureus*
CAUTI	: Catheter acquired urinary tract infections
CDC	: Center for Disease Control and Prevention
CFU	: Colony Forming Units
CLSI	: Clinical and laboratory standards institute
CLSM	: Confocal Laser Scanning Microscopy
CONS	: Coagulase negative Staphylococci
CRA	: Congo red agar
CV	: Crystal violet
CVC	: Central venous catheter
DNA	: Deoxyribonucleic acid
DNase	: Deoxyribonuclease
DRIs	: Device-related infections
eDNA	: Extracellular Deoxyribonuclease
EM	: Electron microscopy
Embp	: Extracellular matrix-binding protein
EMRSA	: Epidemic Methicillin resistant *Staphylococcus aureus*
EPS	: Exopolysaccharide
FDA	: Fluorescein-di-acetate
FnBPs	: Fibronectin binding proteins
GISA	: Glycopeptide intermediately susceptible *Staphylococcus aureus*

HCAI	: Healthcare-associated infections
***ica* locus**	: Intercellular adhesion locus
ICU	: Intensive care unit
IVC	: Intravascular catheter
MHA	: Mueller-Hinton agar
MIC	: Minimum inhibitory concentration
MRCONS	: Methicillin resistant Coagulase negative Staphylococci
MRSA	: Methicillin resistant *Staphylococcus aureus*
MSCRAMMs	: Microbial surface components recognizing adhesive matrix molecules
NADPH	: Nicotinamide adenine dinucleotide phosphate (reduced form)
NIR laser	: Near-infrared laser
PBP- 2a	: Penicillin-binding protein 2a
PCR	: Polymerase chain reaction
PGA	: Poly-gamma-glutamic acid
PIA	: Polysaccharide intercellular adhesin
PVL	: Panton-Valentine leukocidin
qRT-PCR	: Quantitative-Reverse Transcription-polymerase chain reaction
RNA	: Ribonucleic acid
RT- PCR	: Real-time polymerase chain reaction
S. aureus	: *Staphylococcus aureus*
SCC	: Staphylococcal cassette chromosome
SEM	: Scanning electron microscopy
TCP	: Tissue culture plate
TEM	: Transmission electron microscopy
TM	: Tube method
TNase	: Thermonuclease
TSB	: Tryptone soya broth
TSBglu	: Tryptone soya broth containing 1% glucose
VISA	: Vancomycin Intermediate *Staphylococcus aureus*
VRE	: Vancomycin-resistant enterococci
VRSA	: Vancomycin resistant *Staphylococcus aureus*
XM	: X-ray microscopy

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Introduction

Staphylococci are recognized as important cause of disease around the world. Staphylococcal infections are of particular concern because the causative agent offers resistance to a wide range of antibiotics. Infections due to multiple drug resistant strains are becoming more critical due to their capacity to produce biofilm which is one of the known virulence factors of staphylococci ***(Sharvari and Chitra, 2012)*** where Coagulase negative staphylococci (CONS) species are more common biofilm producers than *Staphylococcus aureus (S. aureus)* and also higher percentage of CONS are highly biofilm producers than *S. aureus* ***(Ramakrishna et al., 2014).***

Coagulase negative staphylococci especially *staphylococcus epidermidis (S. epidermidis)* is the most frequent cause of hospital-acquired infections. Most *S. epidermidis* infections are subacute or chronic and occur mainly in immunocompromised individuals or in patients with indwelling medical devices. Biofilm formation on the surface of indwelling devices is often involved in the pathogenesis. Mechanisms to evade the host immune response are important in the pathogenesis of *S. epidermidis* infections. The two main virulence factors that contribute to *S. epidermidis* immune evasion are biofilm formation and production of a poly-γ-dl-glutamic acid (PGA) capsule. The PGA capsule protects *S. epidermidis* from high

osmolarity and may cause resistance to polymorphonuclear (PMN) phagocytosis. While biofilm formation and Polysaccharide intercellular adhesion (PIA) protect *S. epidermidis* from PMN phagocytosis, antimicrobial effect and deposition of both antibodies and complement ***(Macintosh et al., 2009).***

Staphylococcus aureus is a virulent organism resistant to most of the conventionally prescribed antibiotics. It is difficult to treat long-term *S. aureus* infections such as endocarditis, osteomyelitis and especially those infections associated with implanted medical devices. One reason these organisms are capable of defending themselves from host immune systems is their capability to form biofilms. The interior of the bacterial biofilms presents greater resistance to the opsonization by antibodies and to phagocytosis, which explains the chronic character of these infections (***Yasmeen et al., 2012).***

Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide, which facilitate the adherence of microorganism ***(Agarwal and Jain, 2012).***

Biofilm helps Staphylococci to form stable communities of protection rather than live as free planktonic cells. It also impedes delivery of antibiotics. Biofilms can resist antibiotic concentration 10 – 10,000 folds higher than those required to

inhibit the growth of free floating Staphylococci. These infections are generally associated with the use of catheters and other medical devices. Biofilm producing staphylococci have also been isolated from various clinical samples like blood, urine, pus, skin surface etc. The differentiation of staphylococci with respect to its biofilm phenotype might help to elucidate the impact of staphylococci in diagnosis and these observations may have utility in the prevention of infections ***(Sharvari and Chitra, 2012).***

Biofilm is an increasing cause of morbidity and mortality associated with chronic and nosocomial infections, so a greater understanding of the nature of intracellular bacterial communities in infections, their early detection and management will aid in the development of new and more effective treatments ***(Sarita et al., 2014).***

A number of tests are available to detect slime production by staphylococci; which include qualitative methods such as tube method (TM) ***(Afreenish et al., 2011)*** and Congo red agar (CRA) ***(Kaiser et al., 2013)***, and quantitative methods such as tissue culture plate (TCP), which is considered as the gold-standard method for biofilm detection ***(Sarita et al.,2014).***

Aim of the Work

1. To detect biofilm formation by Staphylococci isolated from different clinical specimens using different phenotypic methods.
2. To compare between different phenotypic methods for detection of biofilm production among Staphylococci.
3. To assess the relation of biofilm formation with Methicillin resistance and antimicrobial resistance.

Chapter (1): Staphylococci

Taxonomy

Staphylococcus (*S.*) comes from Greek "staphyle" meaning a bunch of grapes, and "Kokkos" meaning granule. It is a genus of Gram-positive bacteria ***(Ajai, 2011).*** Staphylococci, Micrococci and *Stomatococcus* belong to the family Micrococcaceae ***(Surinder, 2012).***

Staphylococci are gram positive cocci that occur in grape-like clusters**.** Staphylococci are non-motile, non-spore forming, usually non-encapsulated, catalase positive, and facultative anaerobes, except for *S. saccharolyticus* which are anerobic and catalase negative, oxidase negative, except for *S.lentus*, *S. scuiri*, and *S. vitulus*, Bacitracin resistant and Lysostaphin sensitive ***(Janet and Paul, 2007).***

Micrococcus species are strictly aerobic Gram positive cocci arranged in tetrads or irregular clusters. They are seldom motile and are non-sporing. They are also catalase positive, oxidase positive giving blue color as they possess Cytochrome C enzyme. Their colonies are usually pigmented in shades of yellow or red and grow on simple media. The optimum growth temperature is 25-37°C. They have a respiratory metabolism, often producing little or no acid from carbohydrates and are usually halotolerant, growing in 5% NaCl. They are Lysostaphin resistant. They are generally considered harmless saprophytes that inhabit or contaminate the skin, mucosa, and

also the oropharynx; however they can be opportunistic pathogens in certain immunocompromised patients ***(UK Standards for Microbiology Investigations, 2014).***

Stomatococcus species are capsulated Gram-positive cocci arranged in pairs or clusters. On culture, they produce white mucoid colonies ***(Parija, 2009).***

Classification of staphylococcal species:

The genus *Staphylococcus* contains 33 defined species which are initially classified by the coagulase test into two groups: Coagulase-positive (*Staphylococcus aureus*) and coagulase-negative (CONS), *S. epidermidis* and *S. saprophyticus* are the most clinically significant in this group, characteristics distinguishing the three species of the genus *Staphylococcus* are mentioned in table (1) ***(Surinder, 2012).***

- **Characateristics of *Staphylococcus aureus* strains*:***

Colonies produce golden yellow pigment, Beta hemolysis on blood agar, Coagulase positive, ferment mannite, liquefy gelatin, produce phosphatase, produce black colonies on tellurite blood agar and produce Deoxyribonuclease (DNAase) ***(UK Standards for Microbiology Investigations, 2014).***

- **Characateristics of Coagulase-negative Staphylococci:**

Commonly present as normal skin flora, they are opportunistic pathogens that cause infection in immunocompromised patients. *Staphylococcus epidermidis* accounts for 75% of all clinical isolates, probably reflecting

its preponderance in the normal skin flora. Other clinically important species include *S. saprophyticus*, *S. hemolyticus*, *S. hominis* ***(Surinder, 2012).***

- ***Staphylococcus epidermidis:***

Has a distinct predilection for foreign bodies, such as artificial heart valves, indwelling intravascular catheters, central nervous system shunts, and hip prosthesis. Many strains are able to produce large amount of polysaccharide glycocalyx known as (slime) ***(Gladwin and Trattler, 2014).***

- ***Staphylococcus Saprophyticus:***

Occur as normal skin flora and among the periurethral and urethral flora ***(Parija, 2009).***

Table (1): Characteristics distinguishing three species of the genus *Staphylococcus* ***(Surinder, 2012).***

Characteristic	***S. aureus***	***S.epidermidis***	***S. saprophyticus***
1.Anaerobic growth & fermentation of glucose	+	+	–
2.Coagulase production	+	–	–
3.DNAase production	+	–	–
4.Phosphatase	+	_ / weak	–
5.α toxin	+	–	–
6. Protein A in cell wall	+	–	–
7.Novobiocin sensitivity	sensitive	sensitive	resistant