Role Of icaA and icaD Genes in Determining the Pathogenicity of Bacteremic Isolates of Coagulase -Negative Staphylococci

Thesis

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Youstina Samir

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List of Abbreviations

Abb.	Full term
AAP	Accumulation-associated protein.
AGR	Accessory gene regulator.
ASA score	American Society of Anasthesilogy scoring system.
Autl E	Autolysin E .
Autl S	Autolysin S .
BAP	Biofilm associated protein .
BHI	Brain heart infusion .
BLa	Beta Lactamase.
CoNS	Coagulase Negative Staphylococci.
CRA	Congo red agar.
CRF	Coagulase reacting factor.
CT	Cycle threshold.
CVC	Central Venous Catheter.
DHFR	Dihydro folate reductase.
EMBP	Extra cellular matrix binding protein.
FAME	Fatty acid modifying enzyme.
GPI	Gram positive identification.
Gyr A	Gyrase subunit A.
HPLC	High Performance Liquid Chromatography.
Ica	Intercellular adhesin .
Ica A	Intercellular adhesin A.
Ica D	Intercellular adhesin D.
ICU	Intensive Care Unit.
IQR	Inter quartile range.
MRSE	Methicillin Resistant Staphylococcus Epidermidis.
MSCRAMMS	Microbial surface components recognizing adhesive matrix molecules.
N HCL	Normal hydro chloric acid.
NEC	Necrotizing enterocolitis.
NNIS	National Nosocomial Infections Surveillance System.

List of Abbreviations *(Cont...)*

Abb.	Full term
NVE	Native valve endocarditis.
PCR	Polymerase Chain Reaction.
PFGE	Pulsed field gel electrophoresis.
PGE2	Prostaglandin E2.
PIA	Polysaccharide Intercellular Adhesin.
PSMs	Phenol-Soluble-Modulins .
PVE	Prothetic valve endocarditis.
S	Staphylococci .
Sig B	Sigma factor B.
TNase	Thermonuclease.
UTI	Urinary Tract Infection.
VLBW	Very –low –birth weight.

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Introduction

Coagulase-Negative Staphylococci (CoNS) are widely distributed over the surface of the human body, where they constitute the majority of the commensal bacterial microflora **(O'Gara and Humphreys, 2001).** Although they were formerly regarded as contaminants of clinical specimens, they have been increasingly recognized as nosocomial pathogens in patients whose defenses are compromised by an implanted foreign body or by the administration of immunosuppressive drug **(Rogers et al., 2009)**.

Infections caused by CoNS are often persistent and relapsing. CoNS are responsible for the majority of device-related infections. They are responsible for 50-70% of catheter-related blood stream infections, 40-50% of prosthetic cardiac valve infections, and 20-50% of joint replacement infections **(O'Gara and Humphreys, 2001).** In addition, CoNS have been isolated with increasing frequency as the causative pathogens of nosocomial sepsis, and account for approximately 30% of all nosocomial blood stream infections **(Piette and Verschraegen, 2009)**. The prevalence of methicillin resistance among CoNS and the emergence of vancomycin resistance among these isolates constitute a major threat to the clinician for the management of these infections **(Perez and d'Azevedo, 2008).**

The differentiation between pathogenic and commensal isolates of CoNS has practical importance because of its therapeutic implications in terms of unnecessary use of antibiotics and emergence of resistance. Also, failure to treat true bacteremia can lead to higher rates of morbidity and mortality especially in the critically ill or immunocompromised patients **(Kassis et al., 2009).**

Several indicators have been investigated in order to differentiate true bacteremia from contamination, including the number of positive blood cultures, the species of CoNS and biotype, quantitative antimicrobial susceptibility testing, similarity in colony morphology and clonality. However, these variables have the following two problems: none has a high positive predictive value and they are useful only when two or more blood cultures in one series of such cultures are positive. Nevertheless, publications from the last decade indicate that about 34 % of patients with nosocomial bacteremia had only one positive blood culture **(Garcia et al., 2004).**

In earlier studies, CoNS strains associated with disease were found to produce a wider range of extracellular toxins and enzymes as compared to commensal strains. More importantly, many CoNS produce an extracellular polysaccharide (slime) and have the ability to form biofilm on polymeric surfaces to which it

adheres and colonizes. This is believed to facilitate the establishment of infection on the surfaces of implanted foreign bodies. Slime production appears to depend on the species of CoNS and is associated with multiple antibiotic resistances **(Gad et al., 2009; Otto, 2012; Kitao et al., 2010)**.

Synthesis of the extracellular polysaccharide is mediated by the *ica* operon. Upon activation of this operon, a polysaccharide intercellular adhesin (PIA) is synthesized. This supports cell-to-cell bacterial contacts by means of a multilayered biofilm. The synthesis of the PIA is encoded by the intercellular adhesion (*ica*) locus, in particular by the *icaA* gene **(Rohde et al., 2010)**. However, the sole expression of *icaA* induces only low enzymatic activity, but co-expression of *icaA* with *icaD* leads to a significant increase in activity and is related to phenotypic expression of the capsular polysaccharide **(Arciola et al., 2001; Zhou et al., 2013)**.

Aim of the Work

The aim of the present work is to evaluate the hypothesis that isolates of pathogenic CoNS associated with bacteremia are more likely to be positive for *icaA* and *icaD* genes as compared to non-pathogenic (commensal) CoNS. Also, to determine the relationship between the presence of the *icaA* and *icaD* genes, in bacteremic isolates of CoNS, and the ability of these isolates to produce slime when subcultured on Congo red agar.

Coagulase Negative Staphylococci

A) Historical Aspect:

The name *Staphylococcus* was derived from the Greek word (staphylē) which means "bunch of grapes" and (kókkos) which means "granule"**(Bannerman, 2003).**

Staphylococci were first recovered from pus by **Koch (1878)** and **Pasteur (1880)** who cultivated them in liquid media. In **1881, Rosenbach** obtained a pure culture of *Staphylococci* on solid media and he classified them according to their colony appearance into two species: *Staphylococcus (S.) aureus* (golden yellow colony), and *S. albus* (grayish white colony). Later on, the lemon colored colony, *S. citrus* was added by **Passett** in **1885 (Thorberg, 2008).**

Von Dranyi in 1925, was the first medical bacteriologist to draw attention to the practical value of the coagulating principle (coagulase test) in the differentiation of *S.aureus* which is coagulase positive from *S.epidermidis* which is coagulase negative.

B) Definition of the Genus:

Members of the genus *Staphylococcus* are gram-positive cocci (0.5 to 1.5 µm in diameter).They are usually arranged in irregular grape-like clusters and, less often singly ,in pairs, tetrads ,and short chains (three or four cells).They are non-motile, non-

spore forming ,and usually catalase positive **(Konstantinidis et al., 2005)**.

C) Taxonomy:

Members of the genera *Staphylococcus* and *Micrococcus* are gram-positive, catalase –positive cocci that were placed together with *Stomatococcus* and *Planococcus* in the family *Micrococcaceae.* However, the results of DNA base composition testing DNA-rRNA hybridization and comparative oligonucleotide cataloguing of 16S rRNA indicate that the genera *Staphylococcus* and *Micrococcus* are not closely related. The genus *Staphylococcus* is most closely related to the newly described genus *Macrococcus,* but it also has a relatively close relationship to the genera *Bacillus, Brochothrix, Gemella, Listeria* and *Planococcus* **(Kwok et al., 2003).** These genera have been arranged together with *Staphylococci* and several other genera in the family *Bacillaceae* of broad *Bacillus-Lactobacillus-Streptococcus* cluster **(Murray et al., 2007).**

D) Species:

The genus of *Staphylococcus* consists of at least 40 different species. *Staphylococci* species are classified on the basis of DNA-DNA hybridization as determined by the relative binding of DNAs in reassociatin reactions conducted at optimal or restrictive conditions or both**.** Certain other biochemical

properties of *Staphylococci* are useful for the differentiation of species including *the ability to clot plasma which continues to be widely used for the identification of pathogenic Staphylococci associated with acute infections* ***(Bannerman, 2003).*** Colony morphology and pigment production can also serve as secondary characters for the classification of species **(Borriello et al., 2005).**

The currently recognized species of the genus *Staphylococcus* are shown in table (1).Ten of these species contain subdivision with subspecies designations. Among the Coagulase-Negative-*Staphylococci* (CoNS) subset, 18 species have been isolated from humans, but until now, only five species of CoNS have repeatedly been implicated in nosocomial spread **(Nováková et al., 2010).**