Occurrence of Staphylococcus Lugdunensis Among Coagulase Negative Staphylococci Isolated From Different Clinical Samples

Thesis

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7AMC	7-amino-4-methyl coumarin
Aap	Accumulation-associated protein
ADH	Arginine dihydrolase
Aae	Adhesins
AtlE	Autolysin
Bap	Biofilm-associated protein
BE	Bile esculine
BSIs	Bloodstream infections
CAPD	Continuous ambulatory peritoneal dialysis
CF	Clumping factor
ClfA	Clumping factor A
CLSI	Clinical Laboratory and Standards Institute
COA	Cellophaneover-agar
CoNS	Coagulase-negative Staphylococci
CRA	Congo red agar
CV	Crystal violet
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
FBRIs	Foreign body-related infections
GPI Card	Gram-positive identification test
ica	Intracellular adhesin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MIC	Minimal inhibitory concentraion
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules.

NaCl …… Sodium chloride
NAG …….. N-acetyl-glucosamine
NEC …… Necrotizing enterocolitis
NVE …… Native valve endocarditis
OatA …… O-acetyltransferase
PBP2a …… Penicillin binding protein 2a
PCR …… Polymerase chain reaction
PGE2 …… Prostaglandin E2
PIA …… Polysaccharide intercellular adhesin
PNAG …… Poly-N-acetylglucosamine
PS/A …… Polysaccharide adhesin
PVE …… Prosthetic valve endocarditis
PYR …… Pyrrolidonyl peptidase
RFLP …… Restriction fragment length polymorphism
rpoB gene …… RNA polymerase β-subunit gene
SCC …… Staphylococcal cassette chromosome
sIgA …… Secretory immunoglobulin A
SSIs …… Surgical site infections
tanA …… Tannase gene of Staphylococcus lugdunensis
TSI …… Triple sugar iron agar
TSST-1 …… Toxic shock syndrome toxin 1
tuf gene …… Gene encoding the elongation factor Tu (EF-Tu)
UTIs …… Urinary tract infections
VLBW …… Very-low birth weight
VP1, VP2 …… Voges-Proskauer test
vWbl …… von Willebrand factor-binding protein

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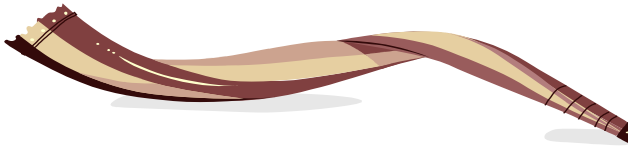

Radwa Mahmoud Mahmoud Shaker

INTRODUCTION

Staphylococcus (Staph.) lugdunensis is a coagulase-negative species of the genus Staphylococcus (CoNS) named for Lyon (Latin adjective of Lugdunum), the French city where the organism was first isolated **(Herchline and Ayers, 1991).**

Similar to other CoNS strains, *Staph. lugdunensis* is considered part of the resident flora of the human skin and mucous membranes. The organism preferentially colonizes the perineal region and has been rarely found in the anterior nares or nasal cavities of healthy subjects **(van der Mee-Marquet et al., 2003).**

However, during the past decade, *Staph. lugdunensis* has emerged as an important pathogen implicated in both community-acquired and nosocomial infections **(Patel et al., 2000 and Ebright et al., 2004)**. Clinical manifestations of infections with these organisms include spondylodiscitis **(Guttmann et al., 2000),** prosthetic joint infection **(Sampathkumar et al., 2000),** ventriculo-peritoneal shunt infection **(Elliott et al., 2001),** abscesses **(Bellamy and Barkham, 2002)**, meningitis **(Kaabia et al., 2002)**, and catheter-related bacteremia **(Ebright et al., 2004).**

Furthermore, *Staph. lugdunensis* can act as an etiologic agent of infective endocarditis. It may infect both prosthetic and native valves **(Fervenza et al., 1999). Patel and his coworkers** in **(2000)** found that *Staph. lugdunensis* accounted for 18% of CoNS strains causing infective endocarditis and 44% of CoNS strains causing native valve

endocarditis. The mortality rate as a result of endocarditis caused by *Staph. lugdunensis* is high **(van der Mee-Marquet et al., 2003)**.

Unlike other coagulase-negative Staphylococci, infections with *Staph. lugdunensis* tend to have a more fulminant course, with an outcome resembling that of *Staph. aureus* infections **(Seenivasan and Yu, 2003)** since both species seem to share virulence determinants such as the production of delta like toxin **(Mateo et al., 2005)**. In addition, these organisms are frequently misidentified as *Staph. aureus* because of their morphologic appearance with yellow pigmentation and complete hemolysis when cultured on blood agar **(Seifert et al., 2005)**. Moreover, although *Staph. lugdunensis* does not possess secreted coagulase, some isolates produce a membrane-bound form of the enzyme (clumping factor) that yields a positive result in slide coagulase and/or rapid latex agglutination tests. However, all isolates typically give negative results in the tube test for free coagulase **(Frank et al., 2008)**.

With respect to identification, the reference method of **Kloos and Schleifer (1975);** that used an array of morphological, physiological, biochemical antibiotic susceptibility patterns and cell wall characters is considered too complicated and too lengthy to be used in routine practice. On the other hand, commercial systems, designed to identify all coagulase-negative species (clinical, veterinary and alimentary), are not very specific, lack sufficient information in their database or show variable results when compared with other systems **(De Paulis et al., 2003)**. Ornithine decarboxylase (ODC), pyrrolidonyl peptidase (PYR) and the generation of acid from D-mannose are crucial in the identification of *Staph. lugdunensis* **(Sanchez et al., 2001 and De Paulis et al., 2003)**.

Staph. lugdunensis generally has been characterized as being susceptible in vitro to most antibiotics **(Frank et al., 2008)**. Early studies reported penicillin resistance rate 4% while more recent stusies reported more increasing penicillin resistance with the rates being 12% to 15% **(Mateo et al., 2005 and Hellbacher et al., 2006).** Moreover, **Tan and his colleagues (2008)** detected methicillin resistance in 5% of their studied isolates. Other than that for tetracycline, resistance to non ß-lactam antibiotics was found to be low.

Therefore, *Staph. lugdunensis* cannot be considred a “typical” species of coagulase-negative Staphylococci, and its successful position as an unusually virulent pathogen deserves attention. Correct identification and determination of the susceptibility profile of this Gram positive coccus is important since frank sepsis syndrome and fatal outcome may occur if this sepsis is involved **(Frank et al., 2008).**

AIM OF THE WORK

The aim of this work is to identify *Staph. lugdunensis* among coagulase-negative Staphylococci isolated from different clinical samples in Ain Shams University hospitals. Also, this work will throw light on its association with different clinical situations in order to reach a definite diagnosis to avoid its fatal outcome.

CHAPTER I

COAGULASE NEGATIVE STAPHYLOCOCCI

A) Historical Aspect:

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* (*Staph*.) *aureus* (golden yellow colony), and *Staph. albus* (grayish white colony). Later on, the lemon colored colony, *Staph. citrus*, was added by **Passett** in **1885 (Thorberg, 2008).**

Due to their ubiquitous nature and relatively low virulence, CoNS have been dismissed as culture contaminants, even in type 1 samples (samples obtained from a normally sterile site by needle aspiration or surgery) **(Christensen et al., 1982; Christensen et al., 1983 and Gill et al., 1983)**. In **(1958), Smith and his colleagues** published the first report on the potential pathogenicity of CoNS in patients with septicemia. Since then, CoNS have become increasingly recognized as important agents of nosocomial infection. Their role as significant pathogens following ophthalmologic, neurologic, and cardiothoracic surgery, in immunocompromised patients, and in patients with prosthetic devices has been well-established **(Lallemand et al., 2002).**

B) Taxonomy:

The name Staphylococcus was derived from the Greek word (staphylē) which means "bunch of grapes" and (kókkos) which means "granule". Under the microscope, Staphylococci appear as Gram positive cocci arranged in clumps linked together as bunches of grape. Staphylococci, Micrococci, Planococci and Stomatococci belong to the family Micrococcacae. The members of this family can be differentiated from members of the family Streptococcacae by the catalase test. Members of the family Micrococcacae are catalase positive, whereas members of the family Streptococcacae are catalase negative **(Bannermna, 2003).**

C) Classification:

More than 40 different Staphylococcal species have been described plus a number of subspecies **(Erkan et al., 2008).**

The main classification of Staphylococci is based on their ability to produce coagulase, an enzyme that causes blood clot formation **(Ryan, 2004). Daranyi** in **1925** discovered the correlation between the coagulase reaction and the pathogenicity of Staphylococci. Accordingly, two groups were formed coagulase positive pathogenic and coagulase negative non-pathogenic Staphylococci (CoNS) as shown in table (1). The coagulase positive Staphylocooci constitute the most pathogenic species *Staph. aureus*. The CoNS are common commensals of skin, although some species can cause infections **(Thorberg, 2008).**

Although, the division of Staphylococci into coagulase positive and negative was found to be artificial and indeed misleading in some cases as there is no direct evidence that it is a virulence factor. Also, some natural isolates of *Staph.aureus* are defective in slide coagulase **(Foster and Mcdevitt, 1994),** and some strains of CoNS, such as *Staph*. *hyicus*, produce tube coagulase but it is rarely pathogenic to human. Moreover, the coagulase negative *Staph. lugdunensis* and *Staph. schleiferi* produce slide coagulase. Nevertheless, the term is still in widespread use among the clinical microbiologists **(Bannerman, 2003).**

Another classification for Staphylocooci is based on their DNA relatedness using DNA-DNA hybridization **(Kloos and Ballard, 1997).**

Nearly 17 Staphylocoocal species may be found in human clinical specimens. The most commonly associated with human infections are *Staph. aureus* (most virulent), *Staph. epidermidis*, *Staph. haemolyticus*, *Staph. saprophyticus*, *S*taph. simulans, *Staph. cohnii* and *S*taph. warneri. In last years, several other Staphylococcal species have been implicated in human infections, notably *Staph. lugdunensis*, *Staph. schleiferi* and *Staph. caprae* **(Ryan, 2004).**

Table (1): Classification of Staphylococcal species according to the tube coagulase test:

Coagulase positive Staphylococci (tube coagulase positive):

- *Staph. aureus*
- *Staph. intermedius*
- *Staph. pseudointermedius*
- *Staph. hyicus*
- *Staph. schleiferi* subspecies coagulans

Coagulase negative Staphylococci (tube coagulase negative) :

- **The most commonly encountered:**
  - *Staph. epidermidis*
  - *Staph. haemolyticus*
  - *Staph. saprophyticus*
  - *Staph. lugdunensis*
  - *Staph. schleiferi* subspecies schleiferi
- **The less commonly encountered:**
  - *Staph.capitis*
  - *Staph.caprae*
  - *Staph.warneri*
  - *Staph.hominis*
  - *Staph.auricularis*
  - *Staph.cohnii*
  - *Staph.xylosus*
  - *Staph.simulans*
  - *Staph.saccharolyticus*
  - *Staph.pasteuri*
  - *Staph.kloosii*
  - *Staph.equorum*
  - *Staph.chromogenes*

(Bannerman, 2003)