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COAGULASE-NEGATIVE *Staphylococci* AND THEIR PATHOGENIC ROLE: A FOCUS ON NOSOCOMIAL INFECTIONS

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ABSTRACT

Staphylococci, involved in many human diseases, are present on human and animal body surfaces as well as in air, food and water. The general classification of staphylococci divides them in two major groups; coagulase positive and negative staphylococci. *Coagulase-negative staphylococci* (*CoNS*) are present as a part of normal skin and mucous membrane flora in humans. *CoNS* have long been considered as non-pathogenic organism but now they are considered pathogenic involved in infections related to prosthetic materials particularly in surgical wounds. Virulence factors in *CoNS* are not very clearly established. *Staphylococcus epidermidis* is evolving as a resistant and powerful microbe related to nosocomial infections. Characteristics like biofilm formation, drug resistance and evolution of genetic variables contribute to its pathogenicity. This review elaborates the evidence of involvement of *CoNS* in various clinically important infections from recent and past literature.

Keywords: Biofilm, coagulase-negative *staphylococci*; nosocomial infections; *S. epidermidis*

1. INTRODUCTION

Staphylococci are Gram-positive round (cocci) bacteria which appear as cellular clusters under a microscope. They are present on body membranes and skin of human and other animals (Becker *et al.*, 2014). Staphylococci have been isolated from a variety of environmental sources such as air, dust, water and food (Widerstrom *et al.*, 2012). *Staphylococcus aureus* (coagulase positive) is a recognized pathogen while *Coagulase-negative staphylococci* (*CoNS*), previously considered non-pathogenic, are usually responsible for various sub-clinical infections lacking typical signs and symptoms of the disease (Von Eiff *et al.*, 2002). *CoNS* are now considered important pathogens causing infections related to prosthetic materials particularly surgical wounds. Over forty species of *CoNS* have so far been identified. The main species involved in human infections is *S. epidermidis*, while *S. haemolyticus, S. warneri, S. hominis, S. capitis, S. intermedius, S. schleiferi* and *S. simulans* are the other *CoNS* which rarely cause human infections (Foster, 2009). Based on simple test scheme, *CoNS* have been categorized into five groups (De Paulis *et al.*, 2003) as under:

- *S. epidermidis* group: *S. epidermidis, S. caprae S. capitis* and subsp. *ureolyticus*
- *S. haemolyticus* group: *S. haemolyticus, S. casseolyticus* and *S. auricularis*
- *S. saprophyticus* group: *S. hominis* subsp. *novobiosepticus* and *S. saprophyticus* subsp. s*aprophyticus*
- *S. cohnii* group: *S. xylosus* and *S. cohnii* subsp. *urealyticum, S. lugdunensis, S. schleiferi* subsp. *schleiferi, S. capitis* subsp. *capitis, S. simulans* and *S. cohnii* subsp. *cohnii*
- *S. warneri* group: *S. warneri* and *S. hominis* subsp. *hominis*

The virulence factors in *CoNS* are not yet clearly established. *Staphylococcus epidermidis* is evolving as a resistant and powerful bacterium responsible for nosocomial infections. Characteristics like biofilm formation, drug resistance and evolution of genetic variables contribute to its pathogenicity. The present review elaborates the virulence, antibiotics resistance and clinical significance of *CoNS* and provides suggestions for managing the clinical challenges associated with these organisms.

2. VIRULENCE

S. aureus carry many effective virulence factors and is a potential threat for infections in various clinical settings. In contrast, *Coagulase-negative staphylococci (CoNS)* usually require some opportunity such as catheter-aided entry into the body for infection (Becker *et al.*, 2014). After breaching surface layer, *CoNS* can cause infections. In fact, for a long time *CoNS* were considered non-pathogenic to human but are now regarded a key pathogen involved in catheter-related diseases, hospital-acquired infections and bacteraemia (Pourmand *et al.*, 2011; Heilmann *et al.*, 2019). The infections caused by *CoNS* are usually chronic and not very severe because they possess a fewer virulence factors as compared to *S. aureus* (Foster, 2009). Many factors such as increased elderly population, premature births, chronic and co-morbid illnesses, immune-compromised patients and more frequent use of foreign indwelling devices etc., increase the incidence of *CoNS* related infections (Widerstrom *et al.*, 2012; Becker *et al.*, 2014; Heilmann *et al.*, 2019).

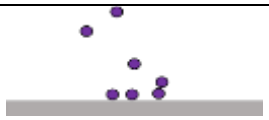
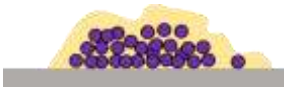
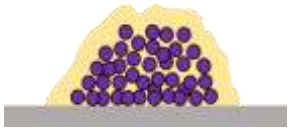
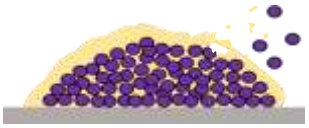
CoNS have the ability to attach and grow on the surface of medical devices and form a protective biofilm layer around them (Von Eiff *et al.*, 2002). The development of resistance tolerance to multiple drugs in *S. epidermidis* is linked to biofilm production and its growth pattern (Donlan, 2000). Moreover, they can avoid the host immune system and possess virulent factors which cause damage to the host tissues. Such characteristics impart pathogenicity to *CoNS* (Kloos and Bannerman, 1994).

The growth of microbes in biofilm mode is clinically very important (Hussain *et al.*, 2017). Biofilms consist of bacteria and their products which are collectively called extracellular polymeric substance (EPS). Bacteria are around 5-25% of a biofilm and rest of it is EPS (Chen and Wen, 2011; Hoiby *et al.*, 2011). Intercellular adhesion (*ica*) genes play a vital role in the pathogenicity of *S. epidermidis*. The gene group consists of *icaA*, *icaB*, *icaC* and *icaD* genes and a repressor gene, *icaR* gene, regulate *icaADBC* operon activity, thus regulating biofilm synthesis (Conlon *et al.*, 2002; Kozitskaya *et al.*, 2005; Rohde *et al.*, 2006). Biofilm formation can occur under the influence of genes (*ica* genes) or by other mechanisms which involve various proteins. The layers of biofilm act as a barrier against immune components as well as antibiotics thus enhance long-term survival of these microbes. For instance, removing biofilm from wound surface by using the methods like surgical debridement, enzymatic and chemical debridement is included in treating chronic wounds (Hussain *et al.*, 2017). The four main stages of biofilm formation and maturation are attachment, accumulation, maturation and detachment. The main process involved and the examples of virulence factors associated in each of these stages are given in Table 1. There are other factors which contribute to the virulence of these microbes. For instance, antibiotic resistance spread via plasmids in *CoNS.* and resistance to methicillin spreads from *S. epidermidis* to *S. aureus* through mobile genetic elements (Hanssen *et al.*, 2004; Malachowa and Deleo, 2010).

3. ANTIBIOTICS RESISTANCE

It is clinically significant that *S. epidermidis* strains have developed resistance to various antimicrobials (Donlan, 2000). Pourmand *et al.* (2011) have reported 34.3% positivity for slime production in *S. epidermidis* isolates from medical staff nasal mucosa samples. These slime producing isolates showed resistance to penicillin (94%), tetracycline (88%), erythromycin (79%) and clindamycin (77%) with overall 66.7% isolates resistant to more than one antibiotic at a same time. Mack *et al.* (2002) reported the presence of methicillin (oxacillin) resistance in 90% staphylococcal isolates obtained from the hospital environment. The presence of increased resistance in isolates from hospital settings is attributable to the regular use of antibacterial agents for treatment and disinfection

Table 1: The stages of biofilm formation and maturation

Biofilm stage	Example of virulence/ chemical factor	Process	
Attachment	AtlE, bacterial wall teichoic acid	Bacteria use various attachment factors to attach themselves to biotic and abiotic surfaces.	
Accumulation	AtlE, PIA, various proteins such as Bap	Bacteria settle and start growing and secrete various EPS.	
Maturation	AtlE, PIA, various proteins such as Bap	This stage involves proliferation and secretion of EPS to establish a mature and stable growth of bacteria in multilayer structure.	
Detachment	PSMs, enzymes such as proteases, nucleases and hydrolases	This stage involves breaking of parts of biofilm, which can disperse and spread such as via bloodstream to other parts of body.	

AtlE (autolysin); EPS (extracellular polymeric substance); PIA (polysaccharide intercellular adhesin); PSMs (phenol-soluble modulins); ▬ represents a body or an indwelling device surface; ● represents bacteria; ▲ depicts products of bacteria

(McCann *et al.*, 2008). Hellmark *et al.* (2009) studied the antimicrobial activities of 16 antibiotics against S. *epidermidis*, isolated from prosthetic joint infections (PJIs), and found 91% isolates resistant to multiple antibiotics (multi-resistance).

Biofilms layers play important role in evading toxic effects (Donlan, 2000). Antibiotic resistance is usually linked to the better survival of microbes in biofilms. In fact, it is not the resistance to antibiotics in majority cases; but the "tolerance" of these biofilm communities against antimicrobials. The biofilm layers provide protective cover to the bacteria and hinder deep penetration of these agents. Further, this tolerance helps them to survive against immune system, as immune system cells and other components cannot penetrate through the layers of biofilm (Hussain, 2020).

4. INFECTIONS RELATED TO *CoNS*

S. epidermidis, until recently, was regarded as non-pathogenic organism but research-based evidences have proved its pathogenic nature. *CoNS* are now considered as important pathogens involved particularly in nosocomial infections (Widerstrom *et al.*, 2012; Bizzarro *et al.*, 2015). In a statistical report from the National Nosocomial Infections Surveillance (NNIS) program, *CoNS* constituted 8% of all nosocomial infections (Banerjee *et al.*, 1991). *S. epidermidis* is evolving as a resistant and powerful microbe related to nosocomial infections because it has different properties which independently and in combination make it a successful infectious agent, especially in the hospital environment. Such characteristics include biofilm formation, drug resistance and the evolution of genetic variables (Ziebuhr *et al.*, 2006). Biofilm production is controlled by *ica* genes (intercellular adhesion) in *S. epidermidis*. Bacterial growth, survival and tolerance against antibiotics as well as immune system increase in biofilm mode of growth (Hussain *et al.*, 2017; Hussain, 2020). Bacterial tolerance against antimicrobials when they grow in form of biofilms has been associated to mechanisms such as slow growth, nutritional limitation, altered metabolism and reduced antibiotic penetration through layers of biofilm (Stewart, 2002; Hussain, 2020). Emergence of more resistant strains is also clinically important particularly in hospital settings. *S. epidermidis* is involved in nosocomial infections such as catheter-associated infections (Von Eiff *et al.*, 2002).

The clinical use of different types of catheters is increasing dramatically. In United States alone about 180 million peripheral and seven million central devices are annually used (Hanna and Raad, 2005). *Staphylococci* significantly cause indwelling device-related infections and are responsible for severe complications Infections related to foreign bodies could be divided into local and systemic infections. Foreign body-related infections (FBRIs) are clinically important (Becker *et al.*, 2014). Such infections are usually termed as device associated health care-associated infections [DA-HAIs] (Von Eiff *et al.*, 2005) or health care associated infections (Widerstrom *et al.*, 2012). These devices can even present clinically with systemic infections without local infection (Leroy *et al.*, 2012).

CoNS are also involved in causing septicaemia (Morad Asaad *et al.*, 2016; Hitzenbichler *et al.*, 2017; Pedroso *et al.*, 2018). About two hundred and fifty thousand catheter-related septicaemia cases are diagnosed annually in the United States, which results in 1-2% mortality, increased cost and longer hospital stay (Raad *et al.*, 2017). Mostly, the bacteraemia caused by *CoNS* is not life-threatening especially if prompt treatment is provided timely. Occasionally systemic sepsis syndrome can also occur particularly in patients with suppressed immune system (Otto, 2009). *CoNS* occur as the most frequent organisms involved in cerebrospinal shunt infections. Factors which increase the chances of central venous catheter infections include an age of less than six months, surgeon`s skills and duration of surgery. Once infection is suspected, cerebrospinal fluid (CSF) culturing or culturing of catheter surfaces after its removal is usually needed (Boyce *et al.*, 2004). Though, the removal of central venous catheters is a controversial topic, treating infection with antibiotics without removing the catheter can be attempted but successive positive cultures on three occasions is an indication for the removal of the device (Venkatesh *et al.*, 2006).

Both *S. aureus* and *CoNS* are recognized as most important organisms involved in surgical site infections (Rogers *et al.*, 2009). Two factors are important for *CoNS* infection of surgical sites which include the cut is not very deep and/or the procedure did not have contamination risk. In other words, it can be said that organs such as bowels are at less risk of contracting a *CoNS* infection compared to the surgeries performed on relatively cleaner areas such as skin (Boyce *et al.*, 2004). This is interesting and clinically important. Furthermore, *CoNS* can infect prosthetic vascular grafts, which usually occur within 30 days of graft insertion. Infection rates and complications depend on sites of insertion; for example, aortic graft infections have a higher mortality rate (Rogers *et al.*, 2009).

CoNS have been reported as one of the important bacterial group involved in orthopaedic infections, especially prosthetic joint infections (PJIs) and implant loosening (Peel *et al.*, 2012; De Vecchi *et al.*, 2018; Lourtet-Hascoët *et al.*, 2018; Heilmann *et al.*, 2019). For prosthetic joint infections, various procedures such as joint replacement, resectional-arthroplasty and simple debridement can be performed with different indications, merits and demerits (Rogers *et al.*, 2009). These organisms are also involved in neonatal intensive care unit infections (Klingenberg *et al.*, 2007). As *CoNS* are nosocomial pathogens, they have significance in intensive care unit infections (Marchant *et al.*, 2013). Factors such as preterm and low birth weight, Total parenteral nutrition (TPN), umbilical lines and risk carrying pregnancies are believed to be involved in more reported cases of neonatal infections caused by *CoNS*. Neonates might require antimicrobial therapy as well as device removal (Benzies, 2008).

CoNS are also involved in cardiac pacemaker infections, prosthetic vascular grafts, vascular catheters infections, cardiac devices and coronary stents related infections (Leroy *et al.*, 2012). Systemic antimicrobials and device replacement by surgical intervention is required to treat such patients successfully (Saleem *et al.*, 2010). *CoNS* have also been reported to cause skin and soft-tissue infection like abscesses and paronychia (Natsis and Cohen, 2018). *S. aureus* is regarded as an important infectious organism in mastitis**,** but recent studies have suggested the involvement of *CoNS* (especially *S. epidermidis*), which have a significant role in such infections (Mediano *et al.*, 2017). Delgado *et al.* (2009) compared the culture results of milk from 30 women with mastitis and on culturing 27 grew bacteria with *S. epidermidis* present in 26 and *S. aureus* in 8 samples. *CoNS* are also involved in ocular infections (Deguchi *et al.*, 2018). *S. epidermidis* is an important organism causing endophthalmitis. In fact, it is the most common cause for endophthalmitis after the

penetration of *S. epidermidis* to injuries in the eye and in such cases the risk increases up to 15% if foreign material remains at the injury site or if prosthetic material is used for treatment (Selton-Suty *et al.,* 2012). *CoNS* have also been reported to be involved in acute urethritis (Becker *et al.,* 2014) and urinary tract infections (Widerstrom *et al.,* 2012). This all clearly demonstrates that *CoNS* are clinically very important. Their involvement in infections related to multiple systems of body urge physicians to be clinically vigilant and consider *CoNS* as important pathogens particularly while dealing with patients with indwelling devices and foreign materials.

5. PREVENTION, PROBLEMS AND FUTURE STRATEGIES

S. epidermidis is a part of normal flora but research evidences reveal its role as opportunistic pathogen. This emphasizes to have detailed study about the life cycle of *S. epidermidis* both as a commensal and pathogen as well as to find out the relationship between these two life cycles (Otto, 2009). Since only in a few cases S. *epidermidis* can grow due to contamination, so from the diagnostic point of view it is vital to avoid contamination as a laboratory cannot differentiate growth source whether from bacteraemia or contamination (Persson *et al.,* 2006). The emergence of multi-resistant *S. epidermidis* and its associated virulence has also prompted further research in this area.

Studies have shown the transmission of *CoNS* from medical staff involved in care and treatment. Shittu *et al.* (2006) reported 18% isolates from the nasal samples from medical personnel/students to be *CoNS*. *S. epidermidis* colonizes human skin so can easily contaminate a medical device during insertion. Patient skin, contaminated devices, medical staff and air are potential sources of *S. epidermidis* related infections (McCann *et al.,* 2008). Paul *et al.* (2011) reported that the hands of health care workers become more contaminated after dealing with patients and found 90.9% health care workers contaminated after working with patients as compared to 59.1% before seeing the patients. Staphylococci are the main organisms involved in such contaminations. They have proposed that to avoid these hospital-acquired infections and there is a need to strictly follow hygiene and sterile procedures. Moreover, treating such infections using routine therapy is not effective because the organisms, introduced from clinical settings, are more resistant to routine antimicrobials (Paul *et al.,* 2011). As it is extremely difficult to eradicate *CoNS* thus it is necessary to follow good clinical practices to avoid their infections (Rogers *et al.,* 2009). For instance, it is essential to properly sterilize the equipment and use sterile techniques while inserting any prosthetic material or device. Rogers *et al.* (2009) have listed some measures to control the infections related to the use of indwelling materials. Such measures include the use of prophylactic antimicrobials (especially if surgery is performed), use of antiseptics, shaving of the surgical area, adhering to the proper surgical procedures and wound care after surgery.

The use of vaccine and eradication of *S. epidermidis* is not appropriate (Otto, 2009). One reason is that there is no proven vaccine available. Secondly, if we eradicate *S. epidermidis,* there are high chances of some other harmful organism colonizing the skin and mucous membranes which may pose therapeutic challenges. For example, *Propionibacterium acnes* is involved in causing acne vulgaris. *S. epidermidis* inhibit the overgrowth of this organism thereby prevents acne vulgaris (Wang *et al.,* 2014). Thus, prevention by sterilization of equipment and devices as well as adhering to the disinfection principles is the best way to avoid infections (Rogers *et al.,* 2009). There is still no success in the production of such devices, which can absolutely avoid bacterial colonization (Becker *et al.,* 2014). There are a lot of grey areas when we consider available information on *CoNS*. So further research in required to elaborate details related to these bugs. This will help to develop diagnostic and therapeutic tools (Becker *et al.,* 2014). Thus, it is very important to determine the exact source of *CoNS* involved in infections. If these sources can be identified accurately, prevention strategies to avoid the introduction of these organisms can be implemented (Table 2). It is clear from this review that the prevention of entry of these bacteria is most important as an effective way to avoid infections. Antibiotic therapy should not be used as the first option as more and more antibiotic resistance is developing in these bacteria. Therefore, excessive use of antibiotics should be discouraged, and other strategies should be applied to avoid contamination of medical devices and

Table 2: Clinical strategies to manage nosocomial infections

A. Measures before invasive procedure or device insertion	Relation[$]
➢ Proper sterilization of equipment used for invasive diagnostic or therapeutic procedure	*
➢ Adoption of sterilized techniques during insertion of indwelling devices (gloves, gown, mask, etc.)	*
➢ Use of sterilized products and fluids to be infused through devices	*
➢ Use of indwelling devices only in emergency when clinically specified after assessing their benefits and risks	*
➢ Use of good quality sterilized indwelling devices	**
➢ Use of antibiotics impregnated catheters and antiseptic impregnated catheters	**, ***
➢ Use of prophylactic antimicrobials especially prior to the surgical procedures	*, ****
B. Measures after a procedure or device insertion	
➢ Proper diagnosis of infection development and treatment	****
➢ Removal/replacement of devices when clinically indicated	****
➢ Treatment of established infection with appropriate antibiotics	****
➢ Use of quorum-sensing inhibiting chemicals to discourage bacterial growth & maturation	****
➢ Use of techniques to remove/disrupt the established biofilms (e.g, enzymatic removal by oxidoreductases & endopeptidases, physical removal like debridement of wounds)	****
➢ Use of antibiotic flush or antibiotic lock techniques	***,****
➢ Bacteriophage therapy	***

[$]Relationship of measures with the stages of infection/biofilm development
* = Minimize/ avoid the introduction of bacteria to the procedure site
** = Mainly avoid contamination of external devices such as catheters
*** = Minimize and control "attachment" of microbes to biotic and abiotic surfaces
**** = Detach bacteria and discourage their growth, maturation and spread to other sites of body

wounds by these bacteria. For the development of such strategies, the main source(s) of *CoNS* should be identified. There is a great need to investigate the characteristics of *CoNS* both at cellular and molecular levels. By acquiring such information, we will be able to develop the techniques which will enable us to accurately diagnose *CoNS.* This will help in choosing appropriate therapy and improve clinical outcome.

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