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Review article

Small colony variants phenotype and biofilm formation: Implication in persistent *Staphylococcus aureus* infection

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ABSTRACT

Staphylococcus aureus survival strategy for persistence can be attributed to their metabolic versatility under unfavorable conditions by switching to small colony variants (SCVs) phenotype and forming biofilm; these attributes contribute to their widespread dissemination, difficulty to detect in routine microbiological screening and challenging eradication particularly in clinical setting, resulting to persistent nature of their infections. The SCVs can survive intracellularly, misinterpreted as coagulase negative *Staphylococcus aureus* (CoNS) and down regulate some virulence factors. Biofilm formation provides protection against antibiotics and immune responses; the matrix serve as a physical barrier and transfer of resistant determinant via conjugation occur among the encased bacteria because of their proximity. Thus; SCVs and biofilm aid evasion of antibiotics and immune response resulting to persistent infection. Therefore, understanding the process associated with the ability of bacteria to persist could significantly aid in the development of a better therapeutic options that will ultimately reduce the risk of morbidity and mortality.

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram positive cocci shaped bacterium that commonly colonizes human epithelial surface like the skin and respiratory tract and is a pathogen of interest in nosocomial and community acquired infections [1]. Some strains of *S. aureus* are resistant to many antibiotics thereby posing serious threat to public healthcare due to their association with high rate of morbidity and mortality as well as economic cost worldwide [2]. An important risk factor for potential infection is the asymptomatic colonization [3]; several reports have shown the association between asymptomatic colonization with invasive infection [4-6]. Unlike many bacteria, *S. aureus* produces an array of virulence factors including plethora of toxins and immune evasion factors that enable host colonization during infection [6].

Staphylococcus aureus can rapidly alter their physiology and cellular activities by metabolic modifications which enhance their fitness under unfavorable conditions thus; enabling their persistence and dissemination and can also affect the nature of their pathogenesis [2]. One of such survival strategy is the switching to a distinct small colony variants (SCVs) phenotype which is responsible for its intracellular survival and difficulty in eradication. The iatrogenic nature of *S. aureus* infections is due to its ability to colonize the surface of indwelling medical devices and form

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biofilm by adhering to the surface molecules of the devices which serve as reservoir for infection [6]. A greater understanding of SCVs and biofilms and their impacts will remarkably improve the prognosis of chemotherapeutics of *S. aureus* infections.

This study aim to provide further understanding of the mechanisms that are involved in the recalcitrant nature of biofilm and SCVs of *S. aureus* toward antibiotics activity, which can ultimately lead to the development of an effective treatment strategy against wide range of persistent infections cause by *S. aureus* thus; the review describe the multi-factorial nature of biofilm and SCVs that enable their antibiotic resistance.

Persistent *Staphylococcus aureus* infection

Persistent infection refers to where bacteria remain in the host for prolonged periods of time and sometimes with recurrent infection nature despite active antibiotics therapy [7]. Antibiotic resistance and immune evasion ability is key to persistent and recurrent nature of many infections [8], and some of the strategies used by *S. aureus* include formation of biofilms and SCVs phenotype for their persistence [9]. Persistent *S. aureus* infection can occur despite appropriate antibiotic regimens and absence of antibiotics genetic resistance determinants. The treatment of such infections often requires prolonged or repeated course of antibiotic regimens which causes increase in selective pressure thereby leading to the emergence of resistant strains.

The adoption of the SCVs phenotype facilitate the bacterial intracellular survival and their ability to revert easily to the wild-type phenotype provides a mechanism for the relapsing of a virulent infection [10]. Biofilms provide a chronic infectious reservoir with a higher resistance to immune defenses and antibiotics action. Thus, biofilm associated infections present a harder challenge for eradication [11, 12].

Small colony variants phenotype of *Staphylococcus aureus*

The capacity of *S. aureus* to persist and remain clinically not active relies on alternative lifestyle adopted by the bacteria to protect itself from an unfavorable environment [13,14]. The SCVs of *S. aureus* serves as a survival strategy that enables their intracellular persistence of the otherwise extracellular growing *Staphylococcal* species [15]. The SCVs are potential cause of chronicity of infections and also enhances their resistance to the host immune system and to antibiotic therapy [13,15,16]. The association of SCVs with chronic and relapsing course of infections is based on their intracellular survival ability [7,15] and some of its morphological features including; small colony size which is usually one tenth of the normal colony size, slow growth, thick cell wall, non-hemolytic growth, reduced coagulase production and lack carotenoid pigmentation [13]. Some of the *S. aureus* SCVs are auxotrophic for menadione, hemin and thymidine which cause electron transport deficit and low ATPs production. The electron transport deficit is responsible for some of the biochemical characteristics of these variants [7, 15, 17].

Environmental factors induce the emergence of SCVs which represent a transient state that can revert to their wild-type under favorable conditions via regulatory mechanisms involving global regulators like *sigB*, *sarA*, and *agr* [7,13], others include permanent genetic changes which make them consequently stable and irreversible [10, 13,18]. The phenotypic reversion occurs rapidly and is thought to circumvent any lasting fitness cost. But permanent genetic alteration seems to depend largely on the nature of the environmental stress and is prevalent in SCVs that are auxotrophic for either hemin, menadione or thymidine [13, 18, 19].

The detection and identification of SCVs in routine laboratories and their accurate studies in research laboratories are difficult because of their slow growth rate and pinpoint colony on agar plate which can be overlooked or overgrown by the wild-type colonies [20]. *Staphylococcus aureus* SCVs are often misinterpreted as coagulase negative *Staphylococcal* due to the absence of pigmentation, weak hemolytic activity and decreased in coagulase production [21]. Thus, difficulty in detection and identification of SCVs might lead to diagnostic underestimations, which often cause therapeutic failures in the clinical settings [20].

Implication of *Staphylocoocus aureus* SCVs phenotype in persistent infection

Staphylococcus aureus SCVs have been associated with persistent nature of some infection like osteomyelitis, endocarditis, cystic fibrosis, sepsis, arthritis, brain abscess, sinusitis and some foreign body associated infection [22]. The SCVs can persist intracellular in the host for decades due to their decreased susceptibility to antibiotic and inability of routine medical laboratories test to detect them [20, 23]. Their intracellular persistence protects them from antibiotics and the host innate defense system. They do not stimulate immune response or activate compliment system due to their down regulated virulence factors (e.g. α- toxin and proteases) in SCVs, which normally contributes to inflammation and tissue destruction [13, 20].

Small colony variants have an innate tolerance to antibiotics which is not associated with

resistance genes [24] because some antibiotic have poor penetration ability into the mammalian cells where they persist [16]. The decrease in the electrochemical gradient in SCVs cell wall impairs with the transportation of cationic antibiotics across their cell wall [24]. The growth dormancy of SCVs also reduces the effectives of most antibiotics that target the metabolic processes of actively growing cells but nonetheless they have the tendency to frequently generate fast growing phenotype without sacrificing their antibiotic resistance feature thus, becoming more adaptable and problematic [24, 25].

The SCVs are only visible after 48-72h of incubation period, therefore correct identification and susceptibility testing in clinical laboratory become complicated which may result to diagnostic and therapeutic failure [20].

Staphylococcus aureus biofilm formation

Staphylococcus aureus have the ability to adhere to medical devices and host tissue surfaces to form biofilm. This virulence factor is an important trait in the etiology of life threatening infections because it serve as a cause of chronicity by enabling the bacteria to evade host immune system and also enhanced their resistance to antibiotics [1, 12, 26, 27]. Biofilm associated infections represent 80% of nosocomial infections, indwelling devices related infections [10, 28].

Biofilms are bacterial aggregations embedded in an extracellular matrix with specific gene expression or metabolic networks different from their planktonic counterparts [12]. Bacteria embedded in biofilm exhibit altered phenotype with regard to growth, gene expression and protein production [29]. The biofilm extracellular matrix composition varies among strains and growth conditions, but in general it can contain host factors, polysaccharide, protein and extracellular DNA (eDNA) [12]. In addition, *S. aureus* produces a non-ribosomal generated peptide aureusimine (phevalin) that plays an important role in maintenances of the biofilm structure and confer resistance to antibiotics [29,30]. Biofilm formation is a highly complex process, in which microorganism cells transform from planktonic to sessile mode of growth [26] and the process is dependent on the expression of specific genes that guide the establishment of the biofilm [29, 31]. The biofilm formation process can either be independent or dependent on the polysaccharide intercellular adhesin (PIA) or Poly-β(1-6)N-acetylglucosamine (PNAG) [32]. The PIA or PNAG is responsible for intercellular adhesion of bacterial cells and bacterial adhesion to external surfaces and its synthesis is controlled by the intercellular adhesion (ica) locus; *icaADBC* and *icaR* regulatory genes [33,34]. These series of events eventually leads to adaptation under diverse nutritional and environmental conditions [34].

The attachment of the *S. aureus* is mediated by surface proteins, referred to as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [6]. During infection, these proteins play major roles in attachment to host tissues such as fibrinogen, fibronectin, and collagen while attachment to an abiotic surface of an indwelling medical device is dependent on the physicochemical characteristics of the device and bacterial surfaces; the attachment is driven mostly by hydrophobic or electrostatic interactions [28]. Biofilm maturation occurs through cell division and the production of the extracellular polymeric matrix; following accumulation in the biofilm, a distinct three dimensional structure is formed with channels that are formed by the surfactant activity of phenol soluble modulin (PSMs) and degradative exo-enzymes such as proteases [6].

The primary biofilm dispersal strategy utilized by *S. aureus* is by production of various exo-enzymes such as proteases, nucleases and surfactants like substance called phenol soluble modulin (PSM) which is an amphipathic surfactant like peptides with important structuring and dispersal function [12]. They have the ability to degrade the extracellular polymeric matrix and the effectiveness of this mechanism is highly dependent on the composition of the matrix. The dispersion process is controlled by the accessory gene regulator (*agr*) quorum sensing system which is dependent on cell density and the accumulation of signaling molecule [12, 26]. The sessile cells transformed to planktonic form by expressing genes that are for motility [32]; and can re-attach at a remote site thus, allowing the persistent dissemination of an infection in the body [12].

Implication of biofilm formation in persistent infection

Biofilms confers protection to bacteria from stress by providing a thick layer of extracellular polymeric substance which act as a physical barrier against antibiotics action and immune responses [35]. Bacteria embedded in biofilm are more resistant to antibiotics than their corresponding planktonic counterpart [32]. And even a highly sensitive sessile bacterium without any inherent genetic makeup for antibiotic resistance develops 10-1000 folds resistance when embedded in a biofilm [36]. It also provides an ideal environmental condition for conjugation to occur between bacteria's within the biofilm, thereby allowing the exchange of genetic materials via

horizontal transfer of resistant determinants between them thereby enhancing the development and spread of antibiotics resistance [37].

The low availability of nutrients in the biofilm causes slow growth rate, decreased protein synthesis and other physiological activities; bacteria sequestered in biofilms are less susceptible to antibiotics by virtue of their reduced growth rates and the emergence of persister cells [31,34].The activity of many antibiotics like penicillin acts on actively growing cells and the persister cells are important factor in chronic diseases and resistant to antibiotics without undergoing genetic change. Therefore once the environment stress is lifted, they will revert to their normal phenotype [33].

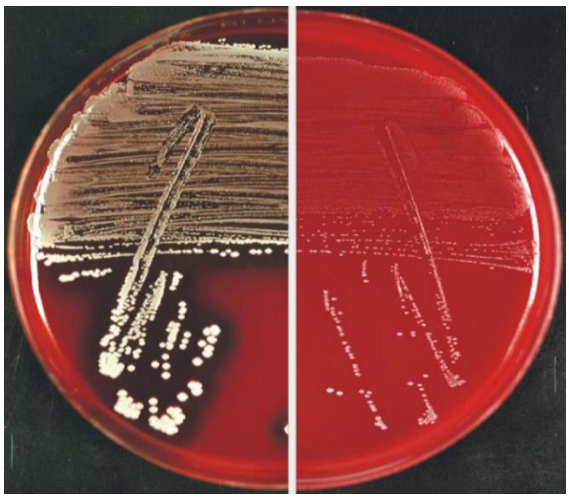
Staphylococcus aureus are able to accumulate high amount of beta lactamase within their biofilm matrix as a defense mechanism which enables their tolerance to beta-lactam antibiotics [38]. Efflux pumps play an important role in biofilm formation by excretion of quorum sensing molecules that coordinate the formation of biofilm, excretion of extracelluar matrix molecule and also the efflux of harmful molecule like antibiotic. The efflux pumps gene expression is up-regulated in *S. aureus* during biofilm growth, possession of an active resistance efflux pumps which can either be express constitutively or intermittently confer resistance to a wide range of antibiotics [39,40].

Conclusion

The persistent and chronic nature of *Staphylococcus aureus* infection is as a result of its ability to develop diverse strategies to overcome the host immune system and antibiotic therapy leading to high rate of morbidity and mortality. The SCVs produce higher levels of biofilm and are capable of intracellular persistence thereby contributing to the chronicity or recurrence of infections. Biofilm producing bacteria often causes persistent infection because of the difficulty in their eradication. The understanding of how bacteria respond and evolve in the presence of threat like antibiotic and immune response can be of importance in aiding the optimization of antibiotic use and development of novel antibiotic to combat the increasing menace of antibiotic resistance.

Further studies is needed to identify the pathways that are involved in the formation of SCVs phenotype and their reversion to the wild type which can serve as a potential therapeutic target. Efflux pump inhibitors significantly decrease biofilm formation therefore inhibiting efflux pumps activity is a promising approach to potentially inhibit the formation of biofilm and also reversing multidrug resistance.

Figure 1. *S. aureus* wild-type (Right) and SCVs (Left) phenotype on Columbia blood agar after 48h



Disclosure of potential conflict of interest

There is no conflict of interest

Authorship contributions

Haruna Adamu; contributed in the conception and design of the study, acquisition of data, drafting the manuscript article and approval of the final manuscript to be submitted.

Abdulrahman Kasim; contributed in the design of the study, acquisition of data, drafting the manuscript article and approval of the final manuscript to be submitted.

Shitu Abdullahi; contributed in the design of the study, acquisition of data, revising of the manuscript and approval of the final manuscript to be submitted.

Patrick Yila Shaibu; contributed in the design of the study, acquisition of data, revising of the manuscript and approval of the final manuscript to be submitted.

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