Review Article
Volume 7:1, 2023

Clinical Infectious Diseases: Open Access

ISSN: 2684-4559 Open Access

Small Colony Variants of *Staphylococcus aureus* and their Diagnostic Methods: A Narrative Review

Esayas Tekle[1*], Yonas Adisu[1], Sisay Teferi[2] and Gemechu Tiruneh[3]

[1]*Department of Medical Laboratory Sciences, Institute of Health Sciences, Wallaga University, Nekemte, Ethiopia*

[2]*Department of Medical Laboratory Sciences, College of Health Sciences, Mettu University, Mettu, Ethiopia*

[3]*Departments of Public Health, Institute of Health Sciences, Wallaga University, Nekemte, Ethiopia*

Abstract

One of the most well-known human pathogens, *Staphylococcus aureus*, causes infections ranging from life-threatening conditions including sepsis, necrotizing pneumonia and, endocarditis to benign superficial skin infections. It was formerly thought to be an extracellular bacterium. However, it has been demonstrated that this pathogen can live and dwell inside cells. These features allow the pathogen to survive periods of antibiotic treatment or pressure from the immune system of the host and further enable it to start the infection once again after the environmental stress declines. Such characteristics are considered properties of *Staphylococcus aureus* small colony variants. *Staphylococcus aureus* small colony variants are often difficult to recognize due to the fact that they are endowed with unusual metabolic, physiological, and morphological characteristics that will cause difficulties for the routine diagnostic laboratory. As a result, they are associated with persistent infections.

Keywords: Auxotrophs • *Staphylococcus aureus* • Chronic diseases • Small colony variant

Abbreviations: ATP: Adenosine Triphosphate; BHI: Brain Heart Infusion; Cl: Chloride Ion; Camp: Cyclic Adenosine Mono Phosphate; CF: Cystic Fibrosis; FADH2: Flavin adenine Dinucleotide; LVAD: Left Ventricular Assist Device; MRSA: Methicillin Resistant *S. aureus*; MSSA: Methicillin-Susceptible *S. aureus*; MIC: Minimum Inhibitory Concentration; MHA: Muller Hinton agar; NADH: Nicotinamide Adenine Dinucleotide; PCR: Polymerase Chain Reaction; PJIs: Prosthetic Joint Infection; SCVs: Small Colony Variants; TD: Thymidine-Dependent; TD-SCVs: Thymidine Dependent Small Colony Variants; TKA: Total Knee Arthroplasty; TMP-SXT: Trimethoprim Sulfamethoxazole; WT: Wild type

Introduction

Staphylococcus aureus

Staphylococcus aureus (S. aureus) is the most well-known human pathogen, associated with a wide variety of manifestations, ranging from benign superficial skin infections to life-threatening problems, such as necrotizing pneumonia, endocarditis, and sepsis [1]. It is known as a versatile human pathogen which causes many community and hospital acquired infections [2]. The special feature of *S. aureus* infections is their chronic and recurring nature in spite of appropriate treatment [3]. Heart valves and bone tissues are among the most affected parts of the organism [4].

The diversity and severity of staphylococcal infections, as well as the hardiness of treating clinical cases, indicate the versatility of *S. aureus* and the challenges hospitals face. The ways in which *S. aureus* escapes the host's defences and produces several virulence factors contribute to the diversity and risk of staphylococcal diseases [5]. It has been estimated that at least 25 to 30% of the global population are permanent carriers of *S. aureus*. In addition to emerging antibiotic resistance, relapse and persisting infections substantially add to morbidity and mortality [6].

Small Colony Variants (SCVs) of *Staphylococcus aureus*

Staphylococcus aureus has usually been considered an extracellular bacterium. However, it has been shown that this pathogen can reside and live intracellularly as a staphylococcal phenotype unlike the Wild Type (WT), and that looks to be related to cell invasion and clinical persistence. These characteristics are considered properties of *S. aureus* Small Colony Variants (SCVs) [7,8]. This is an interesting feature which allows the pathogen to

***Address for Correspondence:** Esayas Tekle, Department of Medical Laboratory Sciences, Institute of Health Sciences, Wallaga University, Nekemte, Ethiopia, Tel: 25110287294; E-mail: esagutu.20@gmail.com*

Received: 29 September, 2022, Manuscript No. JID-22-76226; **Editor assigned:** 03 October, 2022, PreQC No. JID-22-76226 (PQ); **Reviewed:** 14 October, 2022, QC No. JID-22-76226; **Revised:** 28 December, 2022, Manuscript No. JID-22-76226 (R); **Published:** 06 January, 2023, DOI: 10.37421/2684-4559.2023.7.186

survive periods of antibiotic treatment or pressure from the immune system of the host and further enables it to start the infection once again after the environmental stress declines [9].

Small Colony Variant (SCV) subpopulations of staphylococci result from alteration of electron transport [10]. A decrease in electron transport activity and biofilm formation account for their resistance to several antibiotics as well as provide a mechanism for persisting within host tissues [11]. Many of the SCVs are menadione or hemin auxotroph. On solid media, Staphylococcal SCVs are eight times smaller than the parent strain [12].

On Mueller-Hinton agar staphylococcal SCVs have small colonies due to auxotrophy for thiamine, menadione, or hemin, and if the medium is supplemented with 1 mg/mL of these compounds, SCVs grow as the parent strains in overnight incubation [13]. The large majority of clinical isolates of small colony variant *S. aureus* are auxotrophic for one of these three substances. These compounds (menadione, hemin, and thiamine) are essential for the biosynthesis of electron transport chain components.

Disruption of electron transport results in a decreased electrochemical gradient and reduced quantities of Adenosine Triphosphate (ATP). Because ATP is used in so many cellular reactions, the phenotype of menadione or hemin-auxotrophic *S. aureus* SCVs varies distinctly from that of the parental strain. At 18 hours, the colonies are very small, nonpigmented, and nonhemolytic [14-16]. Large amounts of ATP are required for cell wall biosynthesis; thus, the slow growth leads to small colonies, and electron transport is directly linked to the biosynthesis of carotenoid pigments, rendering the colonies non-pigmented. The limited hemolysis and slow coagulase reaction are in part related to decreased amino acid biosynthesis and uptake (both energy-dependent processes); consequently, the biosynthesis of nonessential proteins for survival appears to be reduced.

Biochemical reactions tested in the clinical microbiology laboratory show the use of glucose and fructose but not mannitol or other sugars. Thus, SCVs are often difficult to recognize as staphylococci, and they may be misidentified by clinical laboratory personnel [17]. Several studies have found that staphylococcal SCVs have decreased respiratory or dye-reducing activity [18,19]. Decreased electron transport marks multiple phenotypic deviations or changes.

Auxotrophs of small-colony variants of *Staphylococcus aureus*

Within the past three decades, several reports have described the association between recurrent infections and the existence of SCVs of *S. aureus*. A special phenotype with decreased virulence, thereby facilitating intracellular persistence and evasion of the immune system of the host. When specific substrates, such as hemin, menadione, or thymidine, support the growth of SCVs, it is said to be auxotrophism [20]. Antibiotics used to treat patients may cause particular genetic mutations to SCVs which may result in auxotrophs. Menadione or hemin dependent SCVs-is associated with the treatment of aminoglycoside for its emergence. Thymidine-Dependent (TD) SCVs are strongly associated with the treatment of trimethoprim-Sulfamethoxazole (SXT).

Menadione is iso-prenylated to form menaquinone, the acceptor of electrons from Nicotinamide Adenine Dinucleotide (NADH) or Flavin Adenine Dinucleotide (FADH2) in the electron transport chain.

Hemin is required for the biosynthesis of cytochromes, which accept electrons from menaquinone and complete the electron transport chain. Whereas thiamine is required for menadione biosynthesis. The incidence of a thymidine-dependent auxotrophy is most known in *S. aureus* SCVs that are identified from clinical samples. The SCVs' thymidine dependence is genetically based on random mutations in *thyA*, the gene encoding thymidylate synthase. The mutations can be positioned anywhere in the gene and can either be point mutations, insertions or deletions. The acquisition of such mutations has been revealed to be encouraged by the frequent use of the antibiotic Trimethoprim-Sulfamethoxazole (TMP-SXT) for the management of chronic lung infection in CF patients.

The impact of SXT on the emergence of thymidine dependent small colony *S. aureus* (TD-SCVs) was shown by experiments *in vitro* five years ago, by cultivating the laboratory *S. aureus* strain Newman with SXT (240 g/ml) in Brain Heart Infusion (BHI) broth for several days and after growing on Columbia blood agar. After several repetitions of these experiments, they were able to isolate one particular SCV in the strain. This identified SCV strain failed to grow on Muller Hinton Agar (MHA) plates and was confirmed to be thymidine dependent by auxotrophism. By sequencing of the *thyA* gene of this strain, a point mutation was detected. In a low-thymidine situation, treatment with SXT will induce the formation of SCV phenotypes in the whole *S. aureus* population by blocking thymidylate synthesis. Two types of functional inactivation of *thyA* are possible:

Point mutations, leading to single amino acid substitutions, and deletions, leading to frameshift or in-frame mutations (Figures 1 and 2).

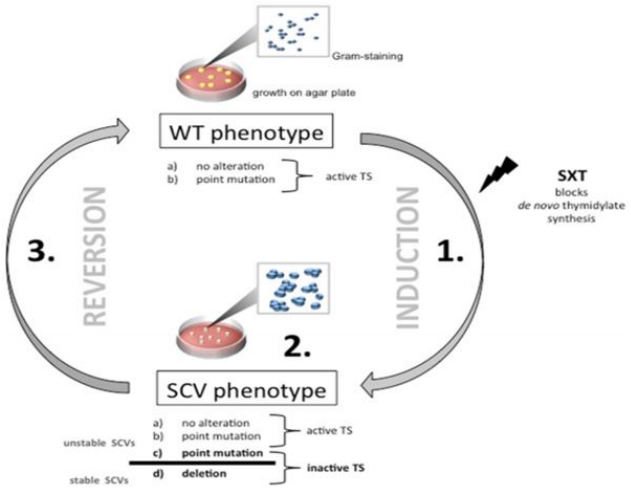

Figure 1. A model for the dynamics of TD-SCVs.

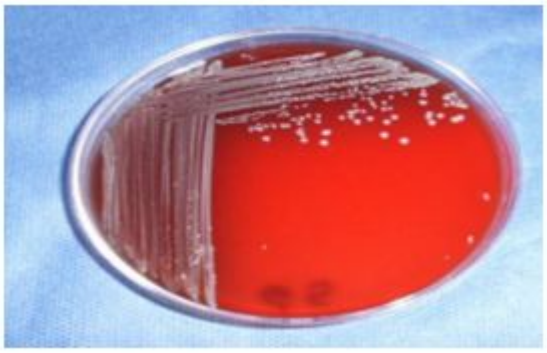

Figure 2. *Staphylococcus aureus* small colony variants in blood agar.

Point mutations of TD-SCVs can revert back to the WT phenotype, representing unstable SCVs, while TD-SCVs resulting from deletions are very improbable to return, hence representing a stable TD-SCV population. Once the strains are no longer exposed to SXT, induced SCVs and TD-SCVs with point mutations revert back to the WT phenotype.

Staphylococcus aureus small colony variants in chronic diseases

S. aureus SCV can cause a chronic type of disease with repeated infections in patients with osteomyelitis, device related infections, and airway infections. They can endure inside non-professional phagocytes, such as osteoblasts and endothelial cells. These SCVs have a reduced production of cytotoxins, which results in a down-regulated induction of apoptosis or cell lysis.

In contrast to the wild type, they do not destroy the cells since they produce very little α-toxin. Their diminished virulence generates a totally new place for these bacteria inside the host cell. The intracellular site may protect these SCVs from host immunity and antibiotic exposure, making clearance of SCVs from host tissues difficult. In addition, they can also be phagocytosed by macrophages and monocytes. Also, they can hide inside these host cells, then return/revert to the highly virulen, rapidly growing form and lyse the host cell when the host defense response has been thrown down and antibiotic treatment is complete.

Literature Review

Cystic Fibrosis (CF)

Cystic Fibrosis (CF) is an autosomal recessive genetic disorder, caused by a mutation in the cystic fibrosis gene affecting the cAMP regulated transmembrane Chloride ion (Cl-) channel, resulting in abnormal respiratory secretions. Due to changes caused by the mutated cystic fibrosis transmembrane regulator gene, which codes for an ion channel, the airways of CF patients are characterized by a highly viscous mucous layer which impairs muco-ciliary clearance, thereby facilitating colonization and infection by particular microorganisms.

Cystic fibrosis is characterized by severe bronchopulmonary infections and inflammation of the airways with a characteristic microflora such as *S. aureus* contributing to progressive and ultimately fatal lung disease. In the lungs of patients with cystic fibrosis, *S. aureus* has to deal with pressure from antibiotics, the immune system of the host, and hypoxia. One form of adaptation to these challenging environmental conditions is switching to a small colony variant (SCV). Frequently TD dependent SCVs are isolated from patients with cystic fibrosis infection.

Heart assist device infection

In the case of a 47 years old man who received a Left Ventricular Assist Device (LVAD) implantation, on day 89 after this implantation, he experienced bacteremia caused by both Methicillin-Susceptible *S. Aureus* (MSSA) and Methicillin Resistant *S. Aureus* (MRSA). This case was diagnosed as LVAD related endocarditis. In spite of long-term treatment with vancomycin and linezolid, the MRSA bacteremia persisted. After one year and four months after implantation of LVAD, two MRSA strains with different colony morphology on blood agar were recovered from blood culture.

Prosthetic Joint Infections (PJIs)

Persistent and relapsing Prosthetic Joint Infections (PJIs) caused by staphylococcal SCVs have been the focus of current studies. Two *S. aureus* clinical isolates were obtained from a patient who benefited from the implantation of a left Total Knee Arthroplasty (TKA). Nine years after the implantation, the patient developed PJIs. The bacteriological samples made at the time of the surgery were positive for *S. aureus* that demonstrated the characteristics of a stable small colony variant.

In a 4 years prospective study, during which they identified 5 of 83 cases (6%) of PJI associated with *S. aureus* SCVs, which were cultured from joint aspirations or intraoperative tissues. The histories of the patients included the number of surgical revisions, the presence of bacteremia, and antibiotic therapy in the months prior to the detection of SCVs. Staphylococcal infection relapsed or persisted in all cases until SCVs were detected. Using transmission electron microscopy, an image of the periprosthetic tissue of one patient revealed intracellular bacteria residing within fibroblasts. The intracellular bacteria recover, where they are saved from host immunity and treatments. Also, it is where they can escape and cause recurrent infection.

Subacute or chronic bone infections

Osteoarticular infections are difficult to treat and, in most cases, need a collective treatment of surgical involvement with long-term antibiotic regimens. A high phenotypic diversity of infecting pathogens, including normal, small, and SCV like phenotypes, is found on agar plates when tissue samples of chronic bone infections are plated. The tendency of staphylococci to cause chronic bone and joint infections is only slightly understood but might originate in the complex bacterial interaction with bone tissue. The exact location of bacteria during chronic osteomyelitis is still not precisely known, and several options are discussed here.

Adherence to the bone matrix: *S. aureus* is recognized to show a multitude of surface proteins with adhesive functions, called adhesions. These exhibit binding to components of the extracellular bone matrix, such as collagen, and many different adhesins reveal redundant functions. Consequently, tight *S. aureus* adhesion to

extracellular bone matrix was found to be a general characteristic of different *S. aureus* clinical isolates. Consequently, the bone milieu is a possible site that shelters bacteria in the acute stage of infection when bacteria reach the bone tissue and strongly adhere to initiate an infection.

Intracellular location: Even though *S. aureus* was typically considered an extracellular pathogen, it became more and more clear that this bacterium could efficiently attack different types of host cells, including fibroblasts and osteoblasts. A comparative analysis with other cell types revealed that osteoblasts take up many fewer bacteria than, for example, endothelial cells; nevertheless, bacteria within osteoblasts are able to persist for several days while increasingly forming SCVs. Although the bacterial intracellular position is very hard to verify within the *in vivo* situation, cell culture experiments strongly suggest that intracellular occurrence within osteoblasts may be a basis for chronic osteomyelitis.

Dead bone fragments: in chronic forms of osteomyelitis, diseased bone fragments deprived of blood can develop. Deceased bone fragments may be an optimal basis for bacterial biofilm development that encourages SCV development as well.

Diagnosis of small colony variants of *Staphylococcus aureus*

The unusual metabolic, physiological, and morphological characteristics of SCVs are problematic for the routine diagnostic laboratory. For patients with cystic fibrosis infection, sputum samples can be taken since the disease is related to advanced age. However, in cases where sputum is impossible to collect, deep throat swabs can be used. When the samples to be diagnosed for patients suspected of small colony variants of *S. aureus* and the infection is other than cystic fibrosis, tissue samples or tissue fluid are used. For example, it is important to gain material, such as joint aspirates, intraoperative tissue samples, biopsy specimens, or fluids in prosthetic joint infection.

Culture and presumptive identification

Since SCVs differ from the wild type phenotype in their growth requirements, colony morphology, generation time, and many metabolic and other physiological characteristics, they are difficult to detect, often overlooked, or misidentified. Because of a 6-9 fold reduction in generation time.

To become visible on solid media, SCVs often need more than 24 h (mostly 48 to 72 h). Their occurrence in mixed cultures with the parental strain and their instability and tendency to revert to the normal phenotype further hinder their recovery and identification.

Staphylococcal SCVs can be grown on Columbia blood agar incubated in either air or CO_2. They can also grow on other solid media and enrichment broths used for the cultivation of gram-positive cocci, such as chocolate agar (in 5% CO_2). Most SCV strains do not exhibit a change of color on selective solid agar media used for the cultivation of staphylococci, such as mannitol salt agar. Use of selective media should always be accompanied by the inoculation of blood agar plates. Bear in mind that SCVs are very quickly overgrown in mixed cultures, which are mainly with enrichment broth.

Colony morphology

Small colony variants of *S. aureus* colonies have a pinpoint size that is about 1/10–1/8 the size of the parental strain when cultivated on agar plates. In contrast to isogenic parental strains of a given species, SCVs are nonpigmented or show strongly reduced pigmentation and decreased hemolysis. In particular, thymidine dependent SCVs differ in some characteristics from the typical features of hemin and menadione dependent SCVs with tiny, non-pigmented, nonhemolytic colonies on Blood agar plates. In addition to this phenotype, TD-SCVs can exhibit various phenotypes, including pinpoint colonies and a "fried egg" appearance with translucent colonies on blood agar.

For example, the samples from foot ulcers of diabetic patients were inoculated on blood agar and mannitol salt agar. After incubation of 24 hours at aerobic conditions, forty *S. aureus* were identified from a total of 120 samples. This identification was made by using beta-hemolysis on blood agar and confirmed using gram stain and biochemical staining. After incubation for an additional 24 hours (a total of 48 hours), they observed that four samples had small, non-hemolytic, nonpigmented, and colonies around the typical *S. aureus* colonies that were originally identified. After further sub-culturing of these colonies on both Blood agar and Mannitol Salt Agar in aerobic conditions, they observed small, non-hemolytic, nonpigmented, and pinpoint sized colonies.

Species confirmation

Biochemical tests: Regardless of their auxotrophs, SCVs show a type of anaerobic metabolism and share metabolic features in central carbon metabolism characterized by a reduced carbon flux through the citric acid cycle. These metabolic variations and their decreased growth are the key reasons that SCVs typically display a deficiency or at least a drop in biochemical reactions, leading to misidentification in the conventional extensive scheme for fermentation, oxidation, hydrolysis, and degradation. Also, clumping factor, catalase, and coagulase reactions or other assays used for presumptive identification are also delayed as positive or even negative. Consequently, all diagnostic approaches based on biochemical procedures are unreliable in the case of SCVs.

PCR and sequencing: Molecular targets that have been proven for *S. aureus* identification (e.g., nuc, clfA, eap, coa, and sodM) work well for the identification of *S. aureus* SCVs. The use of lysostaphin is recommended during the extraction of staphylococcal nucleic acid.

Determination of auxotrophies

Determination of SCV auxotrophies supports us in knowing the causal mechanisms leading to the generation of this different phenotype. Nevertheless, phenotypic instability is a common phenomenon detected in clinical SCV. Independently of the use of solid or broth media, this phenotype reversion may occur after only overnight incubation or after days or weeks of incubation Mostly, only some SCV cells switch back to the normal phenotype, but the whole culture may revert. This instability hampers diagnostics and study of this phenotype. It can be tested by the application of hemin, menadione, and thymidine disks to the top of a solid agar plate inoculated with the test isolate, as done in principle for conventional agar disk diffusion assays. Positive if a zone of growth surrounds the

impregnated disks after an incubation period of 18 hours or longer. Standard disks are commercially available for some of these compounds. Combined auxotrophies are detectable by impregnating the disks with several compounds.

Susceptibility testing

Small colony variants of *S. aureus* are more resistant than their parent strain. Because an electrochemical gradient is required for the import of positively charged molecules, such as aminoglycosides (e.g., *S. aureus* SCVs are more resistant to gentamicin) into the bacterium. The antibiogram of SCV isolate should not be expected to be identical to that of its wild type parent strain. For example, aminoglycosides have no activity against SCV with hemin or menadione auxotrophy and antifolate drugs are inactive against thymidine-dependent SCV. But, wild type parental strains may be susceptible to these antibiotics. Antimicrobial Susceptibility Testing (AST) of SCV is therefore necessary but poses a significant challenge to clinical laboratories, since many of them will not cultivate in commercial, automated AST systems.

Similarly, many strains will not grow on Mueller Hinton agar (MHA), which is suggested by the Clinical and Laboratory Standards Institute (CLSI) for the testing of typical *S. aureus* isolates. Some of the research and clinical laboratories have evaluated AST performed on alternative test media, based on supplementation for the typical auxotrophies.

Discussion

Current and future treatment regimens of SCVS of *S. aureus*

Staphylococcal SCVs are problematic in several ways, in terms of treatment by antibiotic agents and susceptibility testing.

Mostly, a given SCV isolate reflects the genetically encoded resistance pattern of its parental strain. Thus, beta-lactamase-producing SCVs and mecA based MRSA-SCVs have been described frequently. But, in addition to and beyond these usual mechanisms of antibiotic resistance explained for staphylococci, the SCV phenotype is seen as a main example of the expression of phenotypic resistance. This means that regardless of an isolate testing as susceptible *in vitro*, a treatment regimen containing the respective antibiotic agents might fail clinically.

Furthermore, in addition to the SCV phenotype generated alterations in antibiotic susceptibilities, the implications of the intracellular lifestyle as well as biofilm caused functional resistance due to foreign body related infections should be considered. For example, SCVs in biofilms can become completely resistant to clinically achievable concentrations of antibiotics. In the context of antibiotic treatment of staphylococci overall, one had better keep in mind that the administration of antibiotics itself may lead to the development of SCVs.

Conclusion

During infections, *S. aureus* can produce SCVs. Since they are difficult to find in routine microbiological screenings and are linked to persistent infections, these have significant clinical issues. Microbiologists should consider the discovery of this SCV in samples from patients with chronic disorders such cystic fibrosis, osteomyelitis, and infections of prosthetic joints even if the culture is negative within the suggested time for the wild type of *S. aureus*.

References

1. Tong, Steven YC, Joshua S Davis, Emily Eichenberger, and Thomas L Holland, et al. "*Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and *management*." *Clin Microbiol Rev* 28 (2015): 603-661.
2. Lowy, Franklin D. "*Staphylococcus aureus* infections." *N Engl J Med* 339 (1998): 520-532.
3. Proctor, Richard A, James M Balwit, and Omar Vesga. "Variant subpopulations of *Staphylococcus aureus* as cause of persistent and recurrent infections." *Infect Agents Dis* 3 (1994): 302-312.
4. Cosgrove, Sara E, George Sakoulas, Eli N Perencevich, and Mitchell J Schwaber, et al. "Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis." *Clin Infect Dis* 3 (2003): 53-59.
5. Schechter-Perkins, Elissa M, Patricia M Mitchell, and Kate A Murray, et al. "Prevalence and predictors of nasal and extranasal staphylococcal colonization in patients presenting to the emergency department." *Ann Emerg Med* 57 (2011): 492-499.
6. Jackson, Kelly A, Michele K Bohm, John T Brooks, and Alice Asher, et al. "Invasive methicillin-resistant *Staphylococcus aureus infections* among persons who inject drugs six sites, 2005–2016." *MMWR Morb Mortal Wkly Rep* 67 (2018): 625.
7. Melter O, and Radojevic B. "Small colony variants of *Staphylococcus aureus*." *Folia Microbiol (Praha)* 55 (2010): 548-558.
8. Tuchscherr, Lorena, Vanessa Heitmann, Muzaffar Hussain, and Dorothee Viemann, et al. "*Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence." *J Infect Dis* 202 (2010): 1031-1040.
9. Kittinger, Clemens, Daniela Toplitsch, Bettina Folli, and Lilian Masoud Landgraf, et al. "Phenotypic stability of *Staphylococcus aureus* Small Colony Variants (SCV) isolates from cystic fibrosis (CF) patients." *Int J Environ Res Public Health* 16 (2019): 1940.
10. Proctor, Richard. "Respiration and small colony variants of *Staphylococcus aureus*." *Microbiol Spectrum* 7 (2019): 7-3.
11. Crabbe, Aurelie, Peter Ostrup Jensen, Thomas Bjarnsholt, and Tom Coenye, et al. "Antimicrobial tolerance and metabolic adaptations in microbial biofilms." *Trends in microbiol* 27 (2019): 850-863.
12. Loss, Guilherme, Patricia Martins Simoes, Florent Valour, and Marina Farrel Cortes, et al. "*Staphylococcus aureus* small colony variants (SCVs): news from a chronic prosthetic joint infection." *Front Cell Infect Microbiol* 9 (2019): 363.
13. Schleimer, Nina , Ursula Kaspar, Mike Drescher, and Jochen Seggewiß, et al. "The energy-coupling factor transporter module EcfAA'T, a novel candidate for the genetic basis of fatty acid-auxotrophic small-colony variants of *Staphylococcus aureus*." *Front Microbiol* 9 (2018): 1863.
14. Mitchell, Gabriel, Alexandre Fugere, Karine Pepin Gaudreau, and Eric Brouillette, et al. "SigB is a dominant regulator of virulence in *Staphylococcus aureus* small-colony variants." *PloS one* 8 (2013): 65018.
15. Watanabe, Yuji, Nozomi Oikawa, Maya Hariu, and Masafumi Seki, et al. "Evaluation of agar culture plates to efficiently identify small colony variants of methicillin-resistant *Staphylococcus aureus*." *Infect Drug Resist* 12 (2019): 1743.
16. Archer, Nathan K, Mark J Mazaitis, J William Costerton, and Jeff G Leid, et al. "*Staphylococcus aureus biofilms: properties, regulation, and roles in human disease*." *Virulence* 2 (2011): 445-459.

17. Yoshikawa, Thomas T, and Burke A Cunha. "Osteomyelitis in elderly patients." *Clin Infect Dis* 35 (2002): 287-293.
18. Green, Nicole, Jane L Burns, Nicole Mayer-Hamblett, and Margaret Kloster, et al. "Lack of association of small-colony-variant *Staphylococcus aureus strains with long-term use of azithromycin in patients with cystic fibrosis*." *J Clin Microbio* 49 (2011): 2772-2773.
19. Bogut, Agnieszka, Justyna Niedzwiadek, Maria Koziol-Montewka and Dagmara Strzelec-Nowak, et al. "Characterization of *Staphylococcus epidermidis* and *Staphyloccocus warneri* small-colony variants associated with prosthetic-joint infections." *J Med Microbiol* 63 (2014): 176-185.
20. Proctor, Richard A, Christof Von Eiff, Barbara C Kahl, and Karsten Becker, et al."Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections." *Nat Rev Microbiol* 4 (2006): 295-305.

How to cite this article: Tekle, Esayas, Yonas Adisu, Sisay Teferi and Gemechu Tiruneh. "Small Colony Variants of *Staphylococcus aureus* and their Diagnostic Methods: A Narrative Review." *Clin Infect Dis* 7 (2023): 186.