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Review Article

Staphylococcus aureus a Gram-positive Coccid Bacterium Causing Microbial Infections, and Toxins Symptoms Including Food Poisoning

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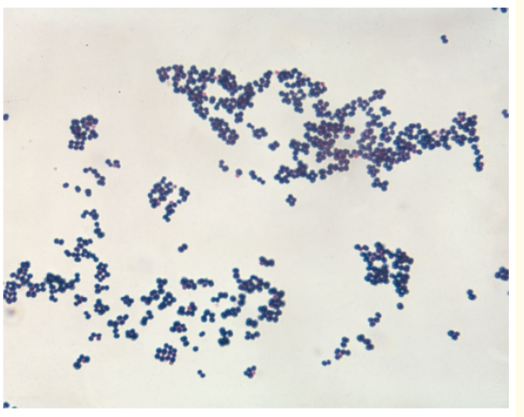
Abstract

Staphylococcus aureus is the most dangerous of all of the many common Staphylococcal species. These gram-positive, sphere-shaped bacteria causing to human skin infections, pneumonia, heart valve infections and bone infections. In addition, it is a food-borne pathogen contaminates foods and secrete heat-stable enterotoxins causing food poisoning and illness. *Staphylococcus* food poisoning is characterized by a sudden start of nausea, vomiting, stomach cramps, and most people have diarrhea. Symptoms usually develop within 30 minutes to 8 hours after eating or drinking an item containing the bacteria toxins. Symptoms usually last no longer than one day, and severe illness is very rare. The incidence of *S. aureus* bacteremia (SAB) has increased recently significantly and became a leading cause of bloodstream infections due to the emerging methicillin resistant *S. aureus* (MSRA). This MSRA poses a clinical challenge as antibiotics resistant strain infections with a worse patient outcome compared to the infection by methicillin sensitive *S. aureus* (MSSA). The antibiotic vancomycin is the only efficient antibiotic for the treatment of MSRA infections. The increasing incidence of emerging antibiotic vancomycin resistance *S. aureus* became very serious microbial pathogen and increase the need for developing new antibiotics or immunotherapy against the infection by the emerging *S. aureus* multidrug-resistant (superbug SA) for patient's treatment, and also increase the need in developing vaccines to protect human from these serous *S. aureus* infections.

Keywords: *S. aureus; Protein A; Hemolysin; Leukocidins (leukotoxins); Exfoliative; Staphylokinase; Enterotoxins; Toxic Shock Syndrome Toxin (TSST-1); staphylococcal Scalded Skin Syndrome (SSSS); Endocarditis; Osteomyelitis; Methicillin-sensitive S aureus (MSSA); Methicillin-resistant S aureus (MRSA); Multidrug-Resistant S. aureus (Superbug SA); Community-associated MSRA (CA-MRSA); Healthcare-associated MSRA (HA-MSRA)*

Introduction

Staphylococcus aureus belong to the bacteria family *Staphylococcaceae.* It is a Gram-positive, cluster-coccid (Figure 1), non-spore former, nonmotile, facultative anaerobe, catalase positive, coagulase positive and form golden yellow colony on agar media plates. This bacteria ferment mannitol which is the main laboratory test used for the differentiation between S *aureus* and *S. epidermidis*. In addition, *S. aureus* ferments glucose to lactic acid, grow at optimum temperature in the range of 15 to 45^{0}C, heat resistant, tolerate high sodium chloride (NaCl) concentrations as high as 15% and tolerate dryness. From these properties *S. aureus* is very hard to eliminate from human environment causing wide range of infections, and its salt tolerance property making *S. aureus* capable to grow and secret toxins in foods including salty foods.



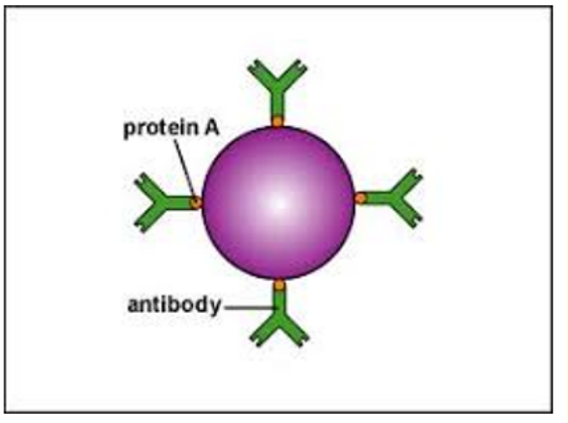
S. aureus cells morphology

Figure 1: *Gram-positive coccus, which appears as grape-like clusters under the microscope and is usually golden-yellow color colonies on agar plates.*

S. aureus is a normal microflora of humans found on skin, nasal passages, and mucous membranes, plus it is pathogenic to humans causes a wide range of suppurative infections, toxic shock syndrome, and food poisoning. The wide range of diseases to human reflects the diversity of virulence factors produced by S. *aureus* such as toxins, enzymes, adhesins, and other surface proteins that allow this pathogen the ability to spread through infected tissues and survive under extreme conditions. In addition, the wide variety of *S. aureus* virulence factors playing important rules in this bacteria mechanism of infection and interferes with host immune defense systems. These virulence factors are the following structural and soluble elements of *S. aureus* bacteria.

Microcapsules: *S. aureus* isolated from infections express surface polysaccharides that are only visible by electron microscopy. These surfaces polysaccharides are not expressed when *S. aureus* cultured on agar media, indicating that these microcapsules are virulence factors with function in infection that is not yet very clear. But it is clear that in the case of bacteremia (blood infection), these microcapsules of polysaccharides on bacteria surface damage heart valves of infected person [1].

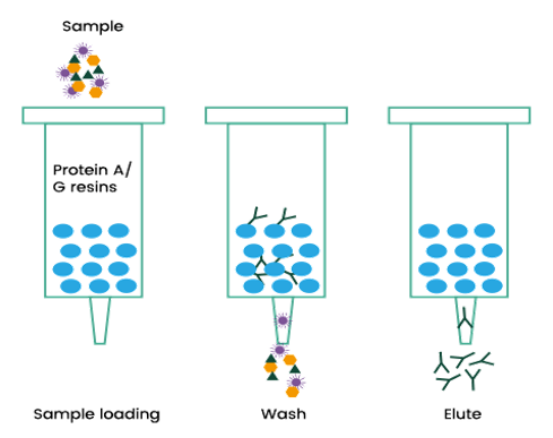
Protein A: Is a 42 kDa surface *protein on S. aureus* cell wall, with a function to disrupt host immune system mechanisms of opsonization and phagocytosis after the infection. The bacteria protein A (SPA) binds to FC region of the host antibody IgG in a wrong orientation (Figure 2) destructing host defense mechanism of opsonization and phagocytosis systems and cripple the host antibody-mediated immunity. In addition, this protein A of *S. aureus* bind to tumor necrosis factor receptor (TNFR-1) causing lung tissue inflammation and staphylococcal pneumonia after the infection [2].



S. aureus surface protein A (SPA) mechanism

Figure 2: *S. aureus protein A (SPA) is located on the bacteria cell wall surface with mechanism to bind to the 'Fc region' of IgG antibody and protect the bacteria from opsonophagocytic clearance by the host immune system.*

It is important to highlight that extracted and purified Protein A from harvested *S. aureus* cells play important roles in laboratory test methods specially, for immunoassay test methods and for the separation and purification of bio-manufactured antibodies from serum or cell cultures. In the case of immunoassay test methods extracted purified Protein A from *S. aureus* is often attached to FC region to antibody and coupled to other molecules such enzymes or fluorescent dye as assay marker without effecting the antibody binding site [3]. In the case of separation and purification of antibodies manufacturing [4]. Protein A is often immobilized onto a solid support and used as reliable method for separation and purifying total IgG from crude protein mixtures in cell culture or serum (Figure 3).



Antibody separation purification using protein A (SPA) affinity chromatography

Figure 3: *Due to the ability of protein A (SPA) to bind to the constant (Fc) region of IgG antibody, polyclonal antibody in serum protein can be separated in pure form from serum or cell culture using Protein A affinity chromatography in three steps process; loading, washing, and eluting.*

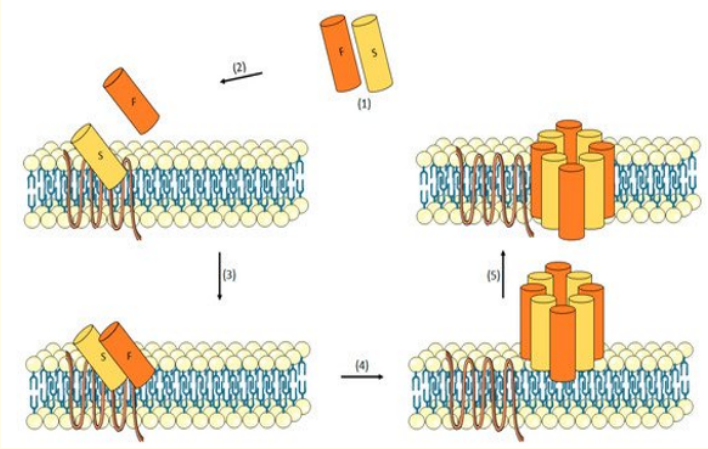
Enzymes

S. aureus produce extracellular enzymes [5] such as coagulase enzyme that convert fibrinogen to fibrin causing plasma clot that coat the bacteria cell surface after the infection as a protection from the host immune defense via phagocytosis. Hyaluronidase enzyme that break down hyaluronic acid in the host tissue to enhance bacterial infection spreading. Lipase enzyme that digest host lipids and impair the function of different host cell types involved in the human immune response like macrophages or platelets. Staphylokinase enzyme that dissolve the host fibrin to aid the infection spreading, deoxyribonuclease (nuclease) enzyme that breaks down the host cell's DNA, and help bacteria to escape from the host neutrophil extracellular traps (NETs). NETs are secreted by the host neutrophils to trap and kill bacterial infection. Catalase enzyme that convert hydrogen peroxide to water oxygen and may help counteract the neutrophil's ability to kill bacterial infection via the production of oxygen free radicals, and Beta-lactamase (β- lactamase) enzymes that break down ß-lactam antibiotics of penicillin and penicillin derivatives by hydrolyzing the antibiotics peptide bond of β-lactam ring rendering the antibiotic effectiveness resulting in antibiotic resistance infection. The gene for ß-lactamase enzyme is carried on plasmids that can transfers among *Staphylococci* bacteria accounting for rapid spreading of antibiotics resistance *S. aureus* mutant strains.

Leukocidins

Virulence factors bi-component proteins produced by *S. aureus* and by other *Staphylococcus* species. It is extracellular toxin also known by the name leukotoxins [6]. This virulence factor is pore-forming toxin (PFT) lyse the host immune cells leukocytes. The host immune

system leukocytes phagocytes bacterial cells and are responsible for the containment of *S. aureus* infection. *S. aureus* leukocidins, also target host natural killer (NC) cells, dendritic cells, and T-lymphocytes cells indicating that this virulence factor is capable to target both innate and adaptive host immune responses. It is important to highlight that *S. aureus* secrete leukocidins first as inactive monomeric subunits, and upon binding to the target host immune cells membrane it multimerize, resulting in the formation of a pore that spans the cell phospholipid bilayer inducing the host immune cells death (Figure 4).

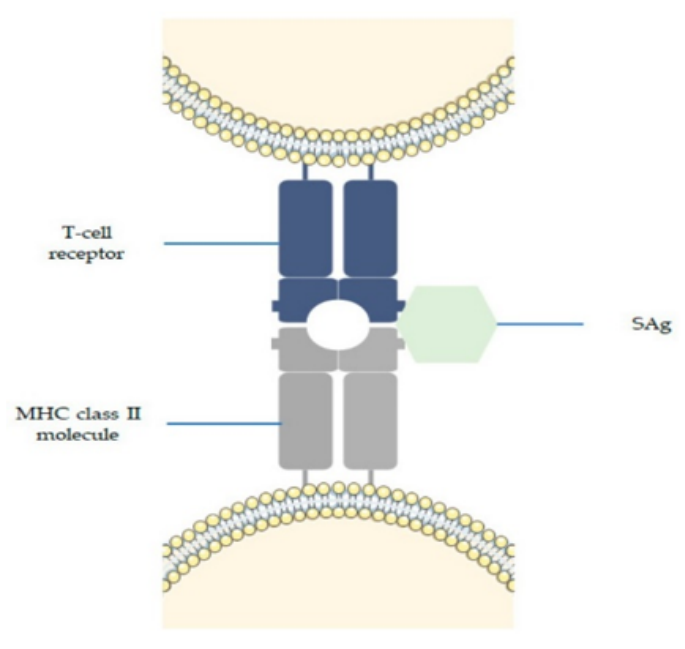


Leukosidines (Leukotoxins) mechanism

Figure 4: *The mechanisms is in five steps: (1) leukosidines are two different proteins, S- protein and F-protein. (2) S-protein binds first to the host cell surface receptor. (3) F-protein is recruited, and dimerization occurs. (4) S/ F dimers oligomerize on the host cell plasma membrane and a pre-pore appears. (5) formation of the transmembrane channel occurs on host cell membrane forming the barrel pore.*

Other extracellular toxins

S. aureus secretes other toxins of hemolysin, exfoliative, toxic-shock syndrome toxin (TSST-1), and enterotoxin. Toxin hemolysins are lipoproteins cause lysis to red blood cells (RBC) by the disruption RBC cell membrane, and there are many classes of hemolysins, including α, β and γ-hemolysins. The enzymatic ß- hemolysin is the primary virulence factor secreted by *S. aureus* and play a major role in the pathogenic progress specialty in pneumonia symptoms [7]. Hemolysins also, may contribute to the availability of iron for pathogenic bacterial growth by lysing red blood cells (RBC). Toxin exfoliative has protease activity to destroy the host epidermal barrier functions causing staphylococcal scalded skin syndrome (SSSS), that is mostly occurred in infants and young children [8]. Toxic shock syndrome toxin (TSST-1) act on the host vascular system causing inflammatory cascade including fever and shock by trigging the production of massive quantities of Tumor necrosis factor (TNF), Interleukins (IL), and other cytokines. This toxic shock syndrome toxin (TSST-1) occurred in infected surgical, in skin wounds, and in menstruating women [9]. Finally, enterotoxins are food poisoning that are heat-stable, not degraded by cooking, causing diarrhea and vomiting when ingested from contaminated foods. There are more than 20 identified staphylococcal enterotoxins (SE), and the most common ones are nine types named SE-A, SE-B, SE-C, SE-D, SE-E, SE-G, SE-H, SE-I, and SE-J [10]. It is important to highlights that both toxic shock syndrome toxin (TSST-1) and staphylococcal food poisoning (SEP) enterotoxins are Superantigens (SAgs). Superantigens [11] interferes with T-cells receptor by cross-link T-cell receptor TCR Vβ domains with the conserved structures of Major Histocompatibility Complex (MHC) class II, resulting in T-cells activation and proliferation without the need of antigen processing (Figure 5).



Super antigens (SAgs) mechanism of action

Figure 5: *Super antigens (SAgs) bind to the host Histocompatibility Complex (MHC) class II molecules to a variable region of T-cell receptor, leading to the stimulation (activations and proliferation) of host T-cells bypassing the conventional antigen recognition process.*

Spread and multiplication of S. aureus in infected host

Expression and secretion of these multi virulence factors by *S. aureus* bacteria are tightly regulated by a number of genetically regulatory systems [12] to allow this pathogenic bacterium to survive under extreme conditions with the ability to invade and spread through the host tissues causing range of diseases including secondary infection such as endocarditis (IE), and septic arthritis and osteomyelitis diseases. In addition, *S. aureus* has the ability to genetically mutate and became resistant to the currently available antibiotics. The emerging of various Methicillin-Resistant *S. aureus* (MRSA), and multidrug-resistant *S. aureus* (superbug SA) limited therapeutic options against the infection by this dangerous microbial pathogen. In addition, *S. aureus* exhibits resistance to antiseptics and disinfectants used in hospitals and food industries for sanitation such as quarter ammonia and others which aid its survival in hospitals and food process environments [13] causing wide range of infections and food poisoning.

Antibiotics resistance

Currently, there are two types of *S. aureus.* These types are methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). Both types carried on human skin, noses, and became invasive when inter the body through skin wound, surgery, or via intravenous (IV) injection needle causing wide range of infections from minor skin conditions to life threating infections. Minor skin *S. aureus* infection appears as a bump that might became red or swollen on the infected skin area. Sometime infected skin is full with pus or other drainage [14]. Life threating *S. aureus* infections occurred when the bacteria enter the person blood stream causing bacteremia and sepsis that could lead into the patient death if is not treated [15].

The difference between MSSA and MRSA is based on the susceptibility to the antibiotic methicillin. Methicillin antibiotic also known by the name Staphcillin is a semisynthetic penicillin-related antibiotic, that was introduced in the year 1959 to treat *Staphylococcal* infection. Lately, some S. *aureus* strains genetically mutates and became methicillin resistance. This methicillin resistance mutant is named methicillin-resistant *S aureus* (MRSA), as opposed to methicillin-susceptible *S. aureus* (MSSA). MRSA is more serious than MSSA because its ability to evolve causing serious infection, but the good thing this MRSA mutant is sensitive to the antibiotic vancomycin. This antibiotic vancomycin was introduced in the year 1954 and was recommended to be used intravenously for the treatment of complicated skin infections, bloodstream infections, endocarditis, bone infection, joint infections, and meningitis that are caused by this mutant of methicillin-resistant *S. aureus* (MRSA) [16]. Recently few strains of MRSA are genetically mutated and became resistant to this last remaining antibiotic vancomycin. This recently genetically mutated *S. aureus* is named multidrug-resistant *S. aureus* or superbug SA. This multidrug-resistant *S. aureus* is more life threating to patients due to the lack of susceptible antibiotics for infection treatments [17].

Epidemiologists differentiate between methicillin-resistant *S. aureus* (MRSA) according the origin of the infection into community associated-resistant *S. aureus* (CA-MRSA) and healthcare methicillin-associated *S. aureus* (HA-MRSA). CA-MRSA defined as an infection in a healthy patient with no risk factors and have not been hospitalized. This CA-MRSA is mainly skin infection caused by skin to-skin contact between people in the community such as team athlete, military recruit, prison inmates, and children in daycare. The infection with community associate methicillin-resistant *S. aureus* (CA-MRSA) is more likely to occur from a person with skin symptoms such as red, swollen look like boil with pus or fluids drain from the infected area [18]. Healthcare associated- resistant *S. aureus* (HA-MRSA) is genetically distinct from community associated-resistant S *aureus* (CA-MRSA) and is more severe infection occurred in hospitals or in other health setting areas such as nursing home and dialysis centers. HA-MRSA infect bloodstream, heart, lungs, other organs, urine tract, and areas of a recent surgery. Some symptoms from these severe infections by HA-MRSA include, chest pain, cough or shortness of breath, fatigue, fever or chills, headache, rash, wounds do not heal, and general ill feeling [19].

S. aureus infection diseases

These *S. aureus* strain MSSA, and mutants of MRSA and Superbug SA cause a range of infections. The most common infections are skin and subcutaneous tissues infection. Skin infection lead to the collection of pus called abscess and abscesses (boils). The development of this abscess is a complex process involves both the bacteria infection and host defense factors. Skin abscesses initiated after the infection and is due to the acute inflammatory reaction with the participation of the host neutrophils. Once the bacterial infection overcomes the host epithelial barrier, the infection either remains locally or spreads in the dermis causing subcutaneous soft tissue infection (SSTIs). SSTIs infection includes cellulitis, necrotizing fasciitis, and diabetic foot ulcers [19]. This SSTIs infection might increase second infection with other non-related multidrug-resistant microorganisms that are more invasive with high mortality rate. In addition, these common local skin and soft tissue infections (SSTIs) could lead to *S. aureus* invasive infections such bacteremia, pneumonia, endocarditis, urinary tract and osteomyelitis.

- **Bacteremia:** Is a blood stream infection with *S aureus* that is commonly seen in hospitals intensive care unit (ICU) with high mortality rate that could reach up to 60% due to the common infection by antibiotics resistant *S. aureus* such as MRSA that is sensitive to the antibiotic vancomycin or by superbug SA that is resistant to the antibiotic vancomycin [20].

- **Pneumonia:** *S. aureus* pneumonia, historically known, as post-influenza virus infection. MRSA pneumonia is more life-threating with higher mortality rate than MSSA pneumonia. Staphylococcal pneumonia is characterized by severe respiratory symptoms, associated with high fever hypotension that can rapidly progress into sepsis and septic shock [21].

- **Endocarditis**: MRSA endocarditis is commonly associated with catheters, and intravenous medical drugs used in hospitals. The balloon catheters are commonly used in a wide range of minimally invasive diagnostic and therapeutic procedures including dilating blood vessels, opening blockages, delivering stents, and more. These medical processes frequently cause complications

due to *S. aureus* infection. The medical process of balloon angioplasty is used to open the artery and is the best approach during a heart attack. Common signs and symptoms of endocarditis includes: flu-like symptoms, heart murmur, fatigue, joints and muscles aching, night sweats, breath shortness, and chest pain [22].

- **Urinary tract infection**: *S. aureus* is not the main common infection for urinary tract infection but it is often occurred as a secondary infection to Staphylococcal bacteremia. Urinary tract instrumentation and the presence of an indwelling catheter increase the risk of Staphylococcal urinary tract infection. Symptoms includes: urinary frequency, urinary urgency, painful urination, and increase white blood cells counts in the urine. In general, the more frequent Staphylococcal bacteria causing urinary tract infection is mainly *S. saprophyticus* not *S. aureus* [23].

- **Osteomyelitis:** The most common disease in adults caused by injury that expose bone to local infection, specially by S. *aureus* bacteria located on the patient skin, also is the extension of local infection such as a wound or hematogenous infection. MRSA is a serious infection that can cause osteomyelitis of spine, long bones of upper and lower extremities and cause septic arthritis for both native and prosthetic joints [24].

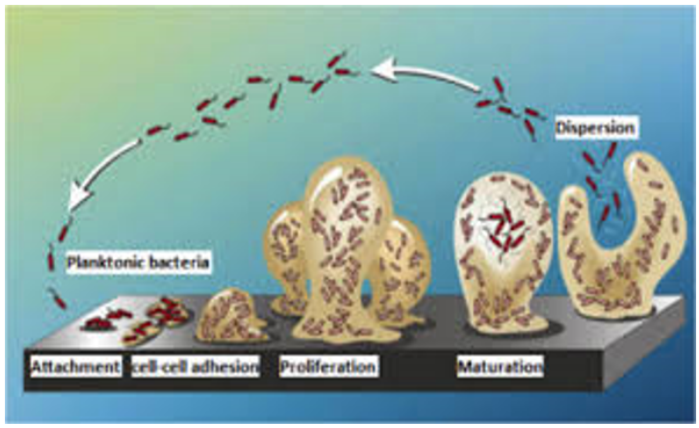
S. aureus food poisoning

Enterotoxins secreted by *S. aureus* bacteria are short chain proteins, that ae soluble in water and heat resistant. Food poisoning symptoms after the ingestion of sufficient amounts of enterotoxins in food or drink are nausea, vomiting and stomach cramps. Most patients also have fever and diarrhea. These symptoms are developed within 30 minutes to 8 hours after eating or drinking an item containing Staph enterotoxins [25]. Foods can be contaminated by *S. aureus* during the preparation by people carried *S. aureus* bacteria on their own skin, did not wash their hands and did not follow the standard food safety protocols before handling or process foods. This *S. aureus* bacteria is capable to multiply in food or drink under harsh environmental conditions of temperature, high salt content, and produce suffusion amounts of enterotoxins. The *S. aureus* bacteria can be killed by heat processing but its secreted enterotoxins in food or drink are heat stable, not deactivated by cooking and make people sick after food or drink ingestion [26]. Foods such as poultry, egg products, egg salads, tuna, chicken, potato, macaroni, cream filled pastries or cakes, cream pies, and chocolate eclairs, sandwich fillings, milk, dairy products, sliced meats, soups, cheeses, etc. are risky foods to be contaminated by *S. aureus* bacterium and enterotoxins secretion if these food products did not prepared properly under sanatory conditions. It is important to highlight that contaminated food or drink with Staph enterotoxins (SE) may not smell bad or looked spoiled, and sill causing food poisoning. The best way for the consumer to avoid food poising from these enterotoxins is to avoid holding raw or prepared foods at unsafe temperature ranging from 40^0F to 140^0F for more than two hours. These unsafe temperatures are known by the name dangerous zone. *S. aureus* bacteria is capable to grow at this dangerous zone with multiplication rate about 20 minutes doubling time (DT) and secrete enterotoxins specially in the growth rate of logarithmic (exponential) phase or during the transition from the logarithmic phase growth to the stationary phase growth. It is estimated that 1000 CFU (colony forming units) of *S. aureus* in contaminated food is enough to produce sufficient enterotoxins concentration enough to cause food poisoning [25].

Bacterial biofilm formation

Some bacteria genus and species are capable to grow and form biofilms on food matrix or on food processing equipment's surface. Biofilm consist of bacterial cells that settle on food contaminated surfaces and begin to aggregate into large clumps surrounded by a protective coating of polysaccharides (slime). Some human pathogens include *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Salmonella enterica*, and *Staphylococcus aureus* are known to form these types of biofilms. *S. aureus* is the most frequent causes of biofilm-associated with enterotoxins food poisoning [27]. It is important to highlight that biofilm formation is not limited to food processing but it is also, expanding to medical sector as well causing nosocomial infections to patients in hospitals from poorly sterile or contaminated

medical devices in process used such as heart valves, pacemakers, and catheters [28]. Bacteria within its biofilm develop different molecular strategies to protect itself from hostile conditions such as the interaction of biofilm matrix with antibiotics, or disinfectants. National Institutes of Health (NIH) estimated that about 65% to 80% of *S. aureus* infections and food poisoning are associated with biofilm formation. Biofilm formation initiated by the attachment of *S. aureus* bacteria to a surface of food production line or medical device and grow (multiply) forming biofilm. Molecules in the biofilm connect to S*. aureus* cells is extracellular polysaccharides secreted by the bacteria cells. Biofilm formation is occurred in four stages [29], these four stages are: bacterial attachment to a surface, microcolony formation, biofilm maturation, and finally detachment of free bacteria cells from the biofilm which may colonize in a new surface area to form new biofilm or causing microbial infection or food contamination and food poisoning (Figure 6).



S. aureus biofilm formation

Figure 6: *S. aureus cells attached to a solid surface and began to produce extracellular polysaccharides to adhere and colonize on the solid surface, bacteria proliferate (multiply) on the solid surface forming biofilm, finally mature biofilm is explode releasing free S. aureus bacteria cells capable to cause host infection or contaminate food causing enterotoxin food poisoning or reattached itself to other solid a surface location for new biofilm cycle formation.*

Currently biofilm formation prevention and disruption strategies are available in the market [30] includes steel surface modifications strategy such as (nanoparticles with different metal oxides, nanocomposites, or antimicrobial polymers), cell-signaling inhibition strategy such as (lactic or citric acids), chemical treatments strategy such as (ozone, quaternary ammonium compounds, Sodium hypochlorite or other sanitizers), enzymatic disruption strategy such as (cellulases, proteases, glycosidases, or nuclease), bacteriocins strategy such as (nisin), biosurfactants strategy, plant essential oils strategy such as (citral-or carvacrol-containing oils), and non-thermal plasma treatments strategy.

S. aureus microbial and toxins laboratory testing

Microbial detection methods

Gram staining is the first test to perform on isolated bacteria from the patient infection or from food sample to demonstrate that the isolated bacteria is a Gram-positive bacterium, cocci in cluster which is a typical for *S. aureus* morphology. Cultured isolated bacteria on blood agar medium plates form colonies frequently surrounded by ß-hemolysis clear zones (Figure 7) which is typical to *S. aureus*. The second laboratory test is culturing the bacteria isolate on a selective medium of mannitol salt agar. On this selective medium *S, aureus* is capable to grow at 7 - 9% sodium chloride and ferment the sugar mannitol dropping the medium PH and producing yellow colored colo-

nies. For the differentiation between *S. aureus* and other staphylococcal species catalase enzyme test is performed on bacteria colonies. *S. aureus* is the only staphylococcal species that is positive for catalase test (bubbles formation upon adding H_2O_2 on the bacteria colony). *S aureus* is also, positive for coagulase test (fibrin clot formation after adding human or rabbit serum), positive for nuclease test (zone of clearance on nuclease agar medium), positive for lipase test (a yellow color and rancid odor smell when the isolate grow on agar medium contain tributyrin oil), and positive for alkaline phosphatase test (convert the blue color of indolyl phosphate to clear color of indigo).

S. aureus ß-hemolysis test

Figure 7: *Beta-hemolytic (β-hemolysis), of Staphylococcus aureus colonies on blood agar with 5% of defibrinated sheep blood cultivated for 24 hours, at 37°C. Colonies of S. aureus showed surrounded by ß-hemolysis clear zones. β-hemolysis, complete lyse red blood cells in the media around and under the colonies.*

In addition, to these conventional biochemical test methods [31]. Molecular biology methods such sequencing the 16S ribosomal RNA (rRNA) genes, is emerged as a rapid *S. aureus* identification test method [32]. The genetic variation within 16S rRNA gene found among prokaryotes is adequate to be used in the phylogenetic analysis for the broad taxonomic ranges, plus 16S gene has multiple conserved regions that can be used as the priming sites for polymerase chain reaction (PCR). This 16S rRNA gene sequencing is currently used for prokaryotic bacteria identification at the species level and also assist in the differentiation between closely related bacterial species. This advanced method is currently used for *S*. *aureus* identifications and showed a sensitivity and specificity for the identification of *S. aureus* bacteria in infected wounds, contaminated blood (bacteremia), infected organs, and contaminated foods. The methodology is a polymerase chain reaction (PCR) technique for the amplification of bacterial 16S rRNA DNA fragment, using specific oligonucleotides as a primer. Amplified DNA fragment of 16S RNA for *S. aureus* by PCR can be identified by using 1% agarose for gel electrophoresis techniques and using ethidium bromide for amplified DNA bands staining [33].

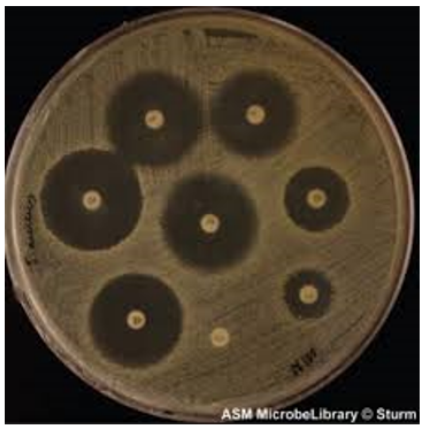
Toxins detection methods

Conventional Staphylococcal enterotoxins (SEs) assay methods such as animal test, serological test, and chromatographic test methods [34] are time consuming and labor-intensive methods that does not meet real time toxins detection. Polymerase chains reaction (PCR) is the faster and reliable test method. PCR is based on amplifying and detection of SEs corresponding DNA gene presence in *S. aureus* isolate. The detection of SEs by PCR was first reported by using two sets of primers to amplify the two SEB and SEC enterotoxin genes (*entB* and *entC1*) [35]. Other molecular biology test method is nucleic acid hybridization. This hybridization method is to identify *S. aureus* strain producing enterotoxins by targeting enterotoxins genes using colony blot hybridization method [36]. Immunoassay techniques is other

method that used to recognize the specific antibodies that bind to secreted *Staphylococcus* enterotoxins (SEs). This specific antibody recognition has appropriate biosensors called immunosensors [37]. Immunosensors play an important role in SEs qualitative or quantitate detection process in food samples. Immunosensors test methods are classified into three types: Optical detection that can be colorimetric by fluorescent, or chemiluminescent, Electrochemical Immunoassays, and Mass-based immunoassay [38].

Antibiotics susceptibility testing

Antibiotic susceptibility test (sensitivity test) is important test to assist physicians in the selection the most effective antibiotic that can be used with higher efficacy against the *S. aureus* isolated from the host infected location [39]. This test method is not limited to *S. aureus* infection but is also applicable for all microbial infections. Antibiotics susceptibility test is a simple method and is based on a small paper discs containing antibiotics placed on agar plate inoculated with isolated target microorganism from infected patient. If the bacteria are sensitive to antibiotic paper disc a clear ring or zone of inhibition is showed around the paper disc. if the bacteria are resistant to antibiotic paper disc a heavy bacterial growth is shown with no inhibition zone (Figure 8). Mueller-Hinton agar medium is frequently used for this antibiotic susceptibility test. Currently commercial antibiotics disk diffusion test kits and minimum inhibitory concentration (MIC) test kits are available across health centers and hospital facilities for be use as a rapid method for antibiotics susceptibility test. The MIC test kit is used to identify the minimum inhibition concertation of susceptible antibiotic against *S. aureus* infection.



Antibiotics susceptibility (sensitivity) test

Figure 8: *Thin paper discs containing an antibiotic placed on agar plate inoculated with isolated S. aureus bacteria. Results after incubation showed clear inhibition zone (no bacterial growth) around a disc indicating the bacteria is sensitive to the antibiotic diffused on the disc paper. The larger the inhibition zone the higher the sensitivity to this antibiotic. If test showed bacterial growth under and around the antibiotic disc (no inhibition zone) indicating the bacteria is resistant to the antibiotic defused on the disc paper. Minimums inhibition concentration (MIC) test is to identify the lowest sensitive antibiotic concentration with inhibition activity on the isolated S. aureus.*

S. aureus infection treatments

S. aureus bacteria cause a wide variety of infections, the infection can be surface such as skin infection, or internal such as blood, bones, joints, heart or lungs infections. Treatment from these infections are varied depends on the type of infection, the location in the body, and

the severity of the infection. Skin infection caused by *S. aureus* can be minor and healed without treatment or can be persistent with skin redness, swelling or sore and needs treatment with β-lactam antibiotics (penicillin or penicillin derivatives), the same treatment as in the case of internal infections. These ß-lactam antibiotics have the mechanism of inhibiting the bacteria cell wall biosynthesis of all Gram-positive bacteria including *S. aureus* and is the treatment of choice for this methicillin sensitive *S. aureus* (MSSA). *S. aureus* is notorious in developing resistance mutants against these antibiotics of choice by mutating and producing the enzyme β-lactamase (penicillinase), this extracellular enzyme break down the β-lactam ring of these ß-lactam antibiotics, rendering their effectiveness [40]. *S. aureus* worldwide have developed resistance strains against penicillin and its derivatives due to the immerging of *S. aureus* mutants that is named methicillin resistant *S. aureus* (MRSA) carrying the gene for β-lactamase (penicillinase) enzyme, this MRSA mutant expressing and secrete the enzyme β- lactamase (penicillinase) capable to inactivate β-lactam antibiotics of penicillin, and its derivatives of methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, flucloxacillin, ampicillin amoxicillin. Carbenicillin ticarcillin, azlocillin, mezlocillin and piperacillin. Patients infected by these MRSA mutants are usually treated by non β-lactam antibiotic such as vancomycin. Vancomycin is a glycopeptide antibiotic that inhibits cell wall biosynthesis of all Gram-positive bacteria including this *S. aureus* mutant (MRSA). In recent years some strains of *S. aureus* (MRSA) became resistant or less sensitive to the antibiotic vancomycin as well [41]. These MRSA mutants that became resistant to the antibiotic vancomycin is named multi drugs resistant *S. aureus* or superbug SA. Treatment from the infection by multi drugs resistant *S. aureus* is most likely by surgery [42] and patients with certain devices, such as artificial grafts, pacemakers, heart valves, or prosthetics, a surgery might be necessary to be removed and replaced with new one if the patient developed *S. aureus* (superbug) infection from these devices. It is important to highlight that patients suffering from food poisoning resulted from *S. aureus* enterotoxins, enterotoxins are not affected by antibiotics, and the most common treatment is drinking plenty of fluids to wash out these *S. aureus* enterotoxins from patient digestive system.

Vaccine against *S. aureus* invasive infections

S. aureus is invasive infection causing high rate of morbidity and mortality especially with the emerging methicillin-resistant (MRSA) and multi-drug resistant (superbug SA) mutant strains. Increasing the infection by these antibiotics resistant strains of *S. aureus* does not need current available antibiotics treatment but needs non-antibiotic treatments such as immune-based approaches that can be helpful in both infection treatment and protection from these antibiotics' resistant strains. Vaccination concept aimed to generate high titers of opsonic antibodies against *S. aureus* surface antigens to facilitate antibody-mediated bacterial infection clearance could be the solution. This vaccine concept has been investigated for future developing effective immune response and immunotherapies against *S. aureus* invasive infections. Currently, all attempts aimed to develop such vaccine was aimed to generate high titers of opsonic antibodies against *S. aureus* surface antigens have been failed in clinical trials [43]. The failure of developed such vaccine in clinical trials could be due to the limited information known about the specific immune responses that protect humans from *S. aureus* invasive infections. Some publications [44] on the subject suggested that for future developing effective and successful vaccine aimed to prevent people from invasive infections by these antibiotics' resistant mutant strains of *S. aureus* need first primary investigations of the following:

- Understand the immunity mechanism against the invasive *S. aureus* infections, and also understand immune defects that lead to increase the susceptibility or reduce clearance of *S. aureus* invasive infections.
- More understanding humeral antibodies, cytokines, and immune cell profiles during *S. aureus* invasive infections.
- Identify specific immune responses and genetic makeups that play important roles in reducing the severity of *S. aureus* invasive infections.

In the meantime, investigating the role of anti-toxin antibodies [45] for modulating the severity of *S. aureus* infections showed preliminary promising results indicating that *S. aureus* anti-toxin antibodies aimed to neutralize the activity of *S. aureus* toxins are more likely

to provide some therapeutic benefit in humans than targeting opsonophagocytic by eliciting antigenic antibodies bind to the bacterial surface to promote bacterial killing.

Other alternative molecular approaches that are currently investigating as theory and may be successful in the treatment of *S*. *aureus* invasive infections, are developing anti-virulence therapy to interfere with S. *aureus* virulence factors that regulate *S. aureus* invasive infections [46], and developing anti-toxins therapy to interfere with *S. aureus* pathway that regulate toxins secretion [47]. All these alternative approaches against *S. aureus* infection and protection still a theory and needs further laboratory investigations.

Discussion

Staphylococcus is a genus of Gram-positive bacteria that is belong to the family *Staphylococcaceae*. Under microscopic examination, bacteria cells appear spherical (cocci), forming grape-like clusters. *Staphylococcus* species are facultative anaerobic organisms capable to growth under both aerobic and anaerobic conditions. There are five species of staphylococci commonly associated with clinical infections. These five species are *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. saprophyticus.* From these five medical microbiology *Staphylococcus* species, *S. aureus* is the most important and serous one as microbial pathogen. *S. aureus which is also,* known by the name "staph" is found on human skin, in the nose, throat, vaginal wall, and GI tract without causing any harm. In some cases, it could cause surface infection on skin and cause soft tissue infection such as abscess, boils, furuncles, and cellulitis (red, swollen, painful, warm skin). S. *aureus* also can cause serious invasive infections such as bloodstream infections, endocarditis, pneumonia, and bone, joint infections. Certain groups of people are susceptible to these invasive staph infections than others, include patients with conditions such as diabetes, cancer, vascular disease, eczema, lung disease and people with drug addiction injecting illegal drugs with dirty needles. In addition, hospitalized patients in intensive care units (ICUs), patients undergone certain types of surgeries, or patients with medical devices inserted in their bodies, nursing home residents, and healthcare facilities common visitors are at great risk from these *S. aureus* infections.

Invasive strains of *S. aureus* are regulated with certain virulence factors such as adhesions, microcapsules, and protein A. These are surface proteins on the bacteria cell wall allow the bacteria to adhere to host cell surface, invade infected host blood or organs, and avoid the host immune system. *S. aureus* strains secrete toxins as virulence factors such as hemolysin, leukotoxin, exfoliative, enterotoxin and toxic-shock syndrome toxin (TSST-1). Aside expressing surface virulence proteins, and secreting toxins, staphylococcal virulence factors also include extra cellular enzymes such as nucleases, proteases, catalase, and other unique enzymes with enzymatic activities aiming to cleave, invade and inactivate various host immune defense such as coagulase, hyaluronidase, staphylokinase, catalase, etc.

The skin is the most important barrier that protect the body from pathogenic microbes including from *S. aureus* strains that are encountered in the environment. *S. aureus* colonized on human skin and can be harmless, but for unknown reasons these virulence factors of *S*. *aureus* are triggered and the colonized *S. aureus* on the skin became virulence breaches the skin epidermal barrier, and enter subcutaneous tissues through dry skin, wound or by other means causing blood stream infection (bacteremia) and other serious invasive staph infections. It is most likely that the combination of pathogen virulence mechanisms and the host susceptibility are factors that contribute in triggering the *S. aureus* bacteria virulence factors genes expression leading to invasive mechanisms of bacteremia and other serous organs infection.

S. aureus is notorious microbial pathogen for its ability to acquire resistant genes against antibiotics such as β-lactamase enzyme genes that the bacteria can acquired through horizontal gene transfer lead to the emerging of resistant *S. aureus* mutants against commonly known ß-lactam antibiotics of penicillin and penicillin derivatives. This antibiotic resistant mutant of *S. aureus* is known by the name methicillin resistant *S. aureus* or MRSA. This MRSA strain is susceptible to the only known non β-lactam antibiotic vancomycin.

MRSA was first emerged in the 1960's and became the major cause of nosocomial infection in hospitals. Epidemiologist use clear definition for MRSA according to the origin of infection into two groups. These two groups are the Community Associated Methicillin

Resistant *S. aureus* (CA-MRSA) occurs in individuals living in community, who are generally healthy and not received healthcare in a hospital or on any ongoing outpatient basis. These communities are such as team athlete, military recruit, prison inmates, and children in daycare. The second group is Health Care Associated Methicillin Resistant *S. aureus* (HA-MRSA) occurs in hospitals specially in Intensive Care Units (ICU), in dialysis centers, and in nursing homes. HA-MRSA strains are genetically distinct from CA-MRSA and is more severe infection. Some symptoms from these severe infections of HA-MRSA include, chest pain, cough or shortness of breath, fatigue, fever or chills, headache, rash, wounds that do not heal, and general ill feeling. A third group of MRSA is lately added by epidemiologists that infect animals and is named Livestock-Associated Methicillin Resistant *S. aureus* (LA-MRSA).

The treatment from MRSAs infection depend on the type and location of the infection, the severity of the symptoms, and the antibiotics to which the infected strain of MRSA responds (suspectable). MRSA strains are resistant to penicillin and penicillin derivative antibiotics, but it can be treated by non ß-lactam antibiotics vancomycin. Patient infected with MRSA must take the whole course of vancomycin antibiotic exactly as the physician prescribed.

Currently, from MRSA strain resistant mutants to the antibiotic vancomycin is emerged. This vancomycin-resistant *S. aureus* mutant is named multidrug *S, aureus* or superbug *S*. *aureus* (superbug SA) and are classified into three groups based on the rate of vancomycin resistance. These three emerged groups are vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), and heterogeneous vancomycin-intermediate *S. aureus* (hVISA).

Vancomycin-intermediate *S. aureus* (VISA) is also known by the name glycopeptide-intermediate *S aureus* due to its resistance to all glycopeptide antibiotics. This VISA bacterial strain has a thick cell wall and synthesis excess amounts of D-ala-D-ala peptide residues, which believe to reduce the ability to vancomycin to diffuse into bacteria cell wall for vancomycin effectiveness. Vancomycin-resistant *S. aureus* (VRSA) is the highest resistant level to vancomycin and has been rarely reported. This VRSA believed to be acquired vancomycin resistant gene from *Enterococcus faecalis* by gene transfer mechanism. Finally, heterogeneous vancomycin-intermediate *S. aureus* (hVISA) do not have resistant genes and its mechanism of resistance is the same as vancomycin-intermediate *S. aureus* (VISA) strain include the thicker cell wall that reduce the ability to vancomycin to diffuse into bacteria cell wall for effectiveness.

Continue emerging antibiotics resistant mutants of *S. aureus* became health concern that threating communities with non-treatable *S aureus* infections and accelerate the urgent need for novel therapeutic approaches that is not exert genetic mutations such as developing anti-virulence therapies to interfere with regulatory factors mediating pathways for virulence factors expression or toxins secretion. Such approaches are currently investigated with no success in clinical trials. Such current approach that has been evaluated is based on inducing opsonophagocytic of *S. aureus* by eliciting antibodies that bind to the bacterial surface and promote bacterial killing. Unfortunately, all clinical trials on this approach have been failed due to multiple factors including the lack of complete understanding the host immunity mechanism, and immune defects that lead to the increase host susceptibility to the invasive S. *aureus* infections.

S. aureus is also, foodborne pathogen produces enterotoxins that are the most common causes of food poisoning. Several Staphylococcal enterotoxins (SEs) have been identified and demonstrated to be highly heat-stable short chain proteins. The nine major serological types of heat stable enterotoxins are SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI and SEJ. These enterotoxins are belonging to the large family of pyrogenic toxin superantigens. Pyrogenic toxins cause super antigenic activity such as immunosuppression and nonspecific T-cell proliferation. *S. aureus* contaminate and multiply in food matrix and *s*ecrete enterotoxins that imposes potential health hazard to consumers upon the ingestion of contaminated food with secreted *S. aureus* enterotoxins causing human illness and economic loss to food industries. S. *aureus* bacteria in food products is inactivated by heat treatment process but its secreted enterotoxin maintains its activity and efficacy. Symptoms of SEs after the ingestion of contaminated food include nausea, vomiting, and abdominal cramps with or without diarrhea. Although Staphylococcal enterotoxins (SEs) symptoms are generally self-limited and resolved within 24 - 48 hours of onset but, it can be severe, especially for infants, elderly, and for immune-compromised patients. Antibiotics treatment are not used as a therapy and the most important treatment for patient with enterotoxins symptoms is drinking plenty of fluids.

The main preventive measures of food products from *S. aureus* contamination and the secretion of enterotoxins required proper food handling, food safety practice, maintaining cold chain, adequate cleaning and disinfection of equipment used in manufacturing plants or kitchen, the prevention of cross-contamination in food processing or kitchen and finally, the application of food safety practices from farm to fork.

Conclusion

S. aureus is the leading cause of potentially dangerous skin and invasive infections. *S. aureus* resistant antibiotics mutants are the most feared pathogen due to its resistant to all available antibiotics and became a serious threat to public health. Currently, all attempts to develop a vaccine against methicillin-resistant *Staphylococcus aureus* (MRSA) and against superbug SA have been failed.

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