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Staphylococcus Species and Emerging Traits in their Commensal Subgroup: A Call to Arms

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Staphylococcus **[Greek *Staphyle* (bunch of grape) and *kokkos* (granules): is a Gram positive coccus, catalase positive, aerobic and/or facultative anaerobes, non-motile, non-spore forming, occurring singly, in pairs or in irregular clusters. Though they have both beneficial roles or can act as infectious agents, yet the emergence of dynamic virulent traits among the commensal Staphylococci and their implication in serious life-threatening multidrug resistant infections qualifies them as true "grapes of wrath" like their pathogenic counterpart. Such traits might have arisen due to interplay of multiple factors like biofilm formation with the concomitant effect of its intrinsic factor *cap* operon and *ica* operons, mutation and/or horizontal gene transfer (HGT), genetic recombination or other interconnected intrinsic tendencies. There is an urgent need to keep in check the potential menace that emerging traits in commensal *Staphylococcus* may bring, not only to the immunocompromised to which many of them have become great threat recently than ever before but to the healthy individuals, farm animals and for public health concern.**

Key words: *Staphylococcus*; commensal; emerging traits, antibiotic resistance, resistance genes.

Staphylococci are common natural commensals that inhabit the body of humans and warm-blooded animals. Most of them are found on the skin on mucosal surfaces surrounding openings in the body surface [1,2]. They are Gram positive cocci, catalase positive, aerobic and/or facultative anaerobes, non motile, non-spore forming, occurring singly, in pair or in irregular clusters; having got its name from the Greek words " Staphyle" and "kokkos" which mean "bunch of grape" and "granules" respectively [3]. About forty species and 17 subspecies of *Staphylococci* are recognized [4,5] and they are broadly differentiated on the basis of coagulase production. Coagulase-positive *Staphylococci* (CPS) e.g. *Staphylococcus aureus* are the best known and have been frequently implicated as the etiology of infections and toxicity in animals and humans, as against many coagulase-negative *Staphylococci* (CNS), considered to be saprophytic, commensals and/or rarely pathogenic when present in their large numbers [6]. *S. hominis*, *S. warneri*, *S. capitis*, *S. simulans*, *S. cohnii*, *S. xylosus*, and *S. saccharolyticus* are examples of the CNS that may be referred to as commensals as they are mostly non-invasive [7], though may also be opportunistic pathogens of both human and animals preferentially affecting the immunocompromized, long-term hospitalized and critically ill patients [5,8].

Species within the *Staphylococcus* genus are known to ferment mannitol, but a few do not. So, reliance on cultural characteristics alone might not be enough to identify all the variants of *Staphylococcus* species in a natural environment[9]. Also, they exhibit variations in cell sizes which

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depend on the nutrient composition of the cultivating media. In some, this might be due to genetic reasons[10]. Meanwhile, recent times have seen a burgeoning literature on some characteristics that used to be the exclusive preserves of clinical staphylococcal isolates, but now in the commensal subgroups. Some of these attribute position commensal staphylococcal species as epidemiological threat to human and non-human animals, though as commensals, they can be beneficial. In this review, we attempt to present general overview of *Staphylococcus* species as well as some clinical traits in the commensal subgroup.

***Staphylococcus* species as Beneficial Bugs**

Many species of *Staphylococci* play beneficial roles in nature. Both CNS and CPS occupy specific niche in their ecosystem [11] and as such are important in maintenance of ecological balance. The presence of some commensals in a niche creates microbial antagonism against pathogens [12]; inhibits pathogen colonization of the niche; and diminishes infection in the host. Iwase *et al.* [13] demonstrated that the commensal *Staphylococcus epidermidis* occupies a niche in the nasal cavity and secretes serine protease Esp which inhibits the formation of *S. aureus* biofilms and reduces *S. aureus* nasal colonization. The commensal, in this case confers a non-specific immunity against *Staphylococcus aureus* colonization/infection. This characteristic is also being exploited to reduce contamination by pathogens on produce and meat products [12].

Beside the aforementioned, some commensal species of *Staphylococci* are also considered to be of biotechnological importance in food fermentation. *S. xylosus, S. carnosus,* and *S. equorum* are used as starters for the manufacture of fermented sausages [14,15]. These bacteria ensure colour stabilization during sausage ripening as well as contribute to fragrance formation [16]. *S. xylosus, S. pulvereri, S. succinus, S. pasteuri* and *S. equorum* are prevalently found in naturally fermented products and in the natural environment of traditional workshops manufacturing dry sausage without using starters [17].

The challenges pose by large chunks of waste water from various industrial activities coupled with the adverse effects of the chemical flocculants for waste treatment on human health and the aquatic ecosystem led to the search for biological flocculants [18]. A research by Zhang *et al.* [19] reported that *Staphylococcus* sp. strain BAFRT4 in consortium with other bacteria produced biofloculants with flocculating activity of 89.7% at pH 6 and cultivating temperature of 30°C. This further affirms the organism as of benefit into its natural environment and makes it one of the participants for natures' cleansing dynamics.

***Staphylococcus* species as Infectious Agents**

Despite their industrial usage, *Staphylococcus* species have been implicated as aetiologies of wide range of superficial and systemic infections of humans and animals. They have been cross-implicated in many superficial and systemic infections [2,20,21] and this has brought such serious concern that Holden *et al.* [22] referred to them as "grapes of wrath". The most virulent is *S. aureus* [23], the most common cause of hospital-acquired bacteremia, though CNS are, as a group, the most frequently encountered bacteria in Medical Microbiology laboratories [24--27]. CNSs are the most common cause of bacterial colonization of indwelling devices leading to bacteremia [27,28]. Specific examples are *S. epidermidis, S. saprophyticus* and *S. haemolyticus* which have repeatedly been associated with human infections [22, 29]. *S. epidermidis* has been consistent aetiology in nosocomial infections [30] while native valve endocarditis in neonates and other patients with internal prosthetic devices, peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) and urinary tract infection (UTI) in general have long been attributed to *S. saprophyticus* [31]. Also, Agvald-Ohman *et al.* [32] reported that 14/20 patients were involved in at least one and up to eight probable nosocomial CNS transmission events.

In a more severe trend, the scourge of CNS (especially *Staphylococcus epidemidis*) in immunocompromised individuals is becoming enormous [33,34], and evidence from literature suggests the need for public health expert to channel more research input to alleviate the suffering. Some instances include native valve endocarditis caused by *S. epidermidis*, myelodysplasia with severe neutropenia, recurrent infections and a Mediport [33], a case of persistent omphalitis in infant with severe congenital neutropenia [35], acute leukemia linked with *S.*

epidermidis [36] and *Staphylococcus* related community acquired pneumonia among HIV-infected patients [37]. Nevertheless, these do not suggest that the CPS has less impact. In fact, *Staphylococcus aureus* is the predominant pathogen in non limb-threatening foot infections of pretreated diabetic patients [38], osteomyelitis [39], the vast majority of skin and soft tissue infections (SSTIs) and localized pus-producing lesions like boils, abscesses, carbuncles and localized wound sepsis [40].

The pathogenic strains have a number of virulent factors being utilized for their pathogenesis and pathogenicity. These factors can be grouped into three: factors that mediate adhesion of bacteria to host cells [41]; those that produce tissue damage [42]; and those that protect the staph and/or other pathogen concomitantly present against the host's immune system [43,44] and antibiotics. Staphylococcal coagulase promotes adhesion and reacts with prothrombin in the blood to form staphylothrombin which enables serine, cysteine- and metalloprotease [45.46] to convert fibrinogen to fibrin and hence, clotting of the blood. Coagulase can coat *S. aureus* surface with fibrin upon contact with blood to resist phagocytosis, the primary host defense mechanism making the bacteria more virulent.

The polysaccharide capsule also facilitates resistance to phagocytosis against *S. aureus* [47]. Surface proteins mediate Staphylococcal attachment to selected host surfaces via tissue matrix molecules [48]. Enterotoxins produce a sepsis syndrome by functioning as superantigens. *S. aureus* in this case produces the superantigen that causes damage by stimulating a T-cell response [49], that can result in the development of toxic shock syndrome (TSS). The superantigen may also lead to the production of interleukins 4 and 10 which activate T helper 2 (TH2) cells leading to a reduced clearance of microbial pathogens [50]. Paradoxically, the *S. epidermidis* earlier noted to prevent *S. aureus* infection also plays a role as a significant opportunistic pathogen that disrupts skin integrity; weakens hosts defenses and permits bacteremia and internal tissues' invasion [51]. The aforementioned virulence factors among others bring about the clinical manifestation observed in animals.

Besides Koch's postulates, identification of virulence gene in *Staphylococcus* isolates from a specific clinical situation enable identification of

Table 1. Attributes in commensal *Staphylococcus* that position them as public health concern

Attributes	Illustration/Examples	References
Biofilm production	Present in both invasive and commensal *Staphylococcus* strains, though may be weakly present in some commensal strains	93;100
Antibiotic resistance	Highly reported among commensals globally	34; 86; 98; 101
Virulence genes	Bhp, atlE, fbe, embp and aap are prevalent in both commensal and invasive strains, while ica gene and IS256 are less (but also present) among commensals	93; 99

Table 2. Enzymatic activity of commensal and nosocomial *S. epidermidis* strains

Enzyme	Commensal strains (n=83)		Nosocomial strains (n=30)	
	Positive reaction	Negative reaction	Positive reaction	Negative reaction
Alkaline phosphatase(PHO)	40 (48.0%)	43 (52.0%)	15 (50.0%)	15 (50.0%)
Urease (URE)	77 (93.0%)	6 (7.0%)	23 (77.0%)	7 (23.0%)
Arginine dehydrolase(ARG)	80 (96.4%)	3 (3.6%)	30 (100%)	0 (0%)
Maltosidase (PAM)	18 (22.0%)	65 (78.0%)	6 (20.0%)	24 (80.0%)
N-acetylglycosaminehydrolase (FGA)	0 (0%))	83 (100%)	0 (0%)	30 (100%)
L-piroglutamic acid hydrolase (FPY)	0 (0%)	83 (100%)	0 (0%)	30 (100%)
β- glucosidase (LAC)	78 (94.0%)	5 (6.0%)	28 (93.0%)	2 (7.0%)

them as the disease aetiology. Akineden *et al*. [52] noted that severity of mastitis is related to virulence factors produced by *S. aureus*. This virulence varies in various species of organisms and influences their degree of pathogenicity [52]. Turkyilmaz and Kaya [53] reported their observation in bovine mastitis, dog's external ear infection and chicken infections that CNS are more virulent than CPS and have been known for rapid onset of infection. So, there is need to be cautious about the CNS as much as CPS since they have been implicated as aetiologies of skin infections, abscesses, septicemia/bacteremia, gastroenteritis, endocarditis, toxic shock syndrome (TSS) and certain food intoxications of both human and farm animals [40].

Appropriate diagnosis to detect these bacteria remains a baseline step in Staphylococcal control. Predictive treatment of animals in cases of mastitis and other relevant clinical or subclinical infection of farm animals may only yield highest of 67.8 % [54]. So, in a clinical situation in which *Staphylococci* have been suspected, the Centers for Disease Control (U.S.) recommends the collection of appropriate patient's sample such as in a purulent skin lesion or sepsis for culture and antibiotic susceptibility testing. This relatively will facilitate effective diagnosis and appropriate prescription of antibiotic therapy. Wrong diagnosis and arbitrary treatment that could lead to upsurge of antibiotic resistance would therefore be prevented. Misdiagnosis of some opportunistic infection from coagulase negative Staphylococcal might lead to recurrent infection, especially among immunodeficient subjects [55].

Staphylococcus spp. from infection and/ or from environment are diagnosed primarily in cultures [56]. Most but not all species within this genus ferment mannitol. So, absolute reliance on cultural characteristics and cell sizes might be insufficient in identifying all the variants of *Staphylococcus* spp. from a natural environment as these depend on the nutrient compositions of the cultivating media. Diagnostic kits include VITEK 2, the BD Phoenix system and the Analytical Profile Index (API) Staph identification kit. The use of API STAPH to identify to species level by comparing the biochemistry of the isolates with the existing database is a notable landmark in bacterial identification. However, this may place limitations on innovations, as new isolates different from those within the existing database [57] might be regarded as having unacceptable profile. In clinical laboratories unlike in research, due to clinical emergencies, the cultures are not usually employed for thorough confirmation, but to provide a medium to test for antibiotic susceptibility testing on the presumed aetiologies (monomicrobial or polymicrobial) and effect prompt treatment. The research laboratory however utilizes culture, morphology, biochemistry immunochemistry and genetics of organisms for their characterization and identification.

The genetic perspectives for identification include genus-specific identification and specie-specific identification and are more reliable. The polymerase Chain Reaction (PCR) is used for the identification and can identify isolates or the presence of species of interest from highly contaminated samples [58,59]. Generally, one remarkable achievement of PCR is revelation of many organisms that are non-culturable or difficult to culture or isolate [60].

Epidemiological investigation of *Staphylococci* employs other tecniques. In this regard, numerous molecular techniques have been employed over the past decade, though with some shortcomings [61]. These methods include Multi-Locus Sequence Typing (MLST), Pulse field gel electrophoresis (PFGE), phage typing, random amplified polymorphic DNA ribotyping, plasmid DNA restriction patterns and coagulase genotyping. Subtyping is an important investigative tool [61]: for example, Zang *et al*. [62] applied Real-time PCR to detect nuc gene as a specific marker for *S. aureus*, mecA gene encoding methicillin resistance and 5 other genes encoding Staphylococcal enterotoxins. Notable enough, these methods are not without their limitations [63]. There is the limitation of failure of some techniques during isolate typing, hence the need for more robust and simpler typing assays [64]. Phenotypic characterization thus maintains a vital role in the overall management of infectious organisms [65], and methodological review and improvement are pertinent steps for successful epidemiological tracking of *Staphylococcus* species [66]. While less consideration is given to the control of less virulent species, their ability to acquire virulent gene(s) Hacker *et al*., [67] should not be overlooked. Hence,

improved safety measures should concomitantly be incorporated with the methodological review to accommodate the potential for horizontal transfers of virulent gene(s) between clinical and commensal organisms.

Due to their tendencies to be pathogenic either by acquired or intrinsic potentials, *Staphylococcus* spp. should be controlled, irrespective of their role(s) in a niche. Their control may be prophylactic or therapeutic. The prophylactic measure includes general rules of hygiene that reduce the bacterial load on their (animal) host [68,69]. This sanitary prophylaxis should be given preference in Staphylococcal control arsenal as the use of antibiotics may predicate antibiotic resistance [70]. Adequate washing of hands (and the entire body in human), ensuring grazing of farm animal in controlled hygienic vegetation, proper disinfection of the skin with methylated spirit before administering injections or vaccines etc will prevent the opportunity for commensals to exhibit their pathogenic potentials *in vivo* [71]. Emphatically, arbitrary administration of antibiotics for prophylaxis should be discouraged as this encourages the emergence of resistance [70].

During the therapy of *Staphylococcus* infection, penicillins, macrolides, fusidic acid, vancomycin, and cephalosporins are antibiotics with activity against many species of staphylococci, but most strains of *S. aureus* (particularly the clinical strains) are resistant to penicillin due to the production of plasmid-coded â-lactamase [56]. For this infection therapy using antibiotics stable to â-lactamase are encouraged. Methicillin is a baseline recommended drug in this regard, although antibiotic susceptibility test should always precede the choice of best and cost effective antibiotic. Vancomycin was the recommended last line of staphylococcal control (especially against methicillin resistant *S. aureus* (MRSA), however, Vancomycin-resistance has been widely observed [72,73]

Emerging Clinical Traits among Commensal *Staphylococci*

Staphylococcus species are among the commensals with close proximity to human. However, many inherent attributes that connote virulence and resistance to control, like their pathogenic counterparts are increasingly being identified among some of these commensals in alarming proportion. One very important attribute in commensal *Staphylococcus* is biofilm formation [12], which is recognized as a driving force for virulence and resistance. The expression of serious multidrug resistant infection with seeming high level of virulence (an attribute of clinical isolates) expressed by some commensal *Staphylococcus* species might be adduced to the capacity to form biofilms by adhering to the surfaces of foreign bodies and to matrix proteins of the host [74,75]. It can also be as a result of simultaneous presence of any of the *cap* operon in *S. epidermidis* encoding the polyglutamate capsule which have been recognized as a major virulence factor in *Bacillus anthracis* [76] and the intrinsic factor like *ica* operon that produces the biofilm exopolysaccharide [77], which, as said, encourages the emerging virulence and resistance within the commensal subgroup.

Biofilm formation among commensals aggregates large population of organisms together and serves as easier traffic for interspecific and intraspecific genetic transfer, which can convert avirulent commensal to very virulent pathogen[78].

Irrespective of the degree of production, biofilms are produced by both invasive and commensal strain and as such makes them organisms of public health concern (See Table 1). The structure of biofilm gets stabilized by with appropriate transfer of certain genes, usually from pathogen to commensals, leading to the emergence of certain peculiar attributes of pathogenic strains among the resident commensals, including, but not limited to, the spread of antibiotic resistance [79]. On the interspecific level, therefore, a synergistic biofilm structure was observed in a study conducted for *Candida albican* coexisting with *Staphylococcus aureus*. Molecular analysis of such biofilm revealed the expression of unique protein aggregates different from conventional single specie biofilm [80], but enhancement of resistance to vancomycin among *S. aureus* has been observed by this biofilm formation [81].

Commensal therefore continues to show continued importance in public health and infection control/management[82]. A report on Erasme Hospital in 2002 showed that commensal microbes belonging to *Staphylococcus* spp., *Enterococcus* spp., *Klebsiella* spp., etc are responsible for hospital acquired infections [83]. Upadhyaya *et al*. [83] concluded following a research on a Gram positive

isolates that, at present, there are just 1% difference in biofilm production rate between the commensals and clinical isolates, and that the attribute is central to the emergence of nosocomial infection. This has also been proven to enhance commensal's ability to cause persistent prosthetic device-related infection [84] and to easily acquire mobile genetic elements. Factors like insertion sequence element, (IS) which is one of such mobile genetic elements known to be important in genome flexibility and in allowing heterogenous expression of gene [85-88] may be incorporated in an enhanced format. Wide range of virulent factors and antibiotic resistance get easily incorporated into their genetic makeup turning them to a global threat as found in *S. epidermidis* strain ST27/ ST2, which is currently recognized as an emerging nosocomial threat globally [34,81,86]. So, the biofilm ica operon and SCCmec gene cassettes, conferring methicillin resistance which are peculiar to typical clinical strains [67] are now being harboured by commensals from individuals without prior hospital visit and by non-human animals in communities around the globe [89-92]. Virulence genes like bhp, atlE, fbe, embp and aap (Table 1) are prevalent in commensal strains of *Staphylococcus* [93].

The 'commensals-turn-pathogens' [30] or accidental pathogens [75] bear within their genetic makeup many copies of the insertion sequence element IS256. This IS256 plays a significant role in both formation of biofilm and methicillin resistance among the commensal, conferring virulence trait and the antibiotic resistance. It is believed that identification of this genetic marker for resistance, biofilm formation with ica operon and IS element in commensal shows that clinical features reside in them [7]. This is because the detection of IS256 is linked with biofilm formation and the icaADBC operon with gentamicin and oxacillin resistance in the clinical strains Kozitskaya *et al*. [85]. Therefore, commensal *Staphylococci* on human carrier or udder of farm animal for milk production that possess these traits may be referred to as potential source of epidemics and this calls for great caution.

If the problems of antibiotic resistance genes and its accompanying phenotypic expressions must be overcome therefore, commensals harbouring these clinical traits must not be neglected, because of their role as reservoir and in the transmission. Epstein *et al*. [94] observed 17% prevalence of methicillin-resistant *Staphylococcus intermedius* (MRSI) which were commensals from dogs in Hong Kong which may be a source of epidemiological threat for the rearers, just as livestock-associated methicillin-resistant *Staphylococcus aureus* ST398 has been a threat and has been reported by Schijffelen *et al*. [95] in a clinical case involving human endocarditis. Following a molecular characterization of 22 commensal methicillin resistance MRSA from pet animals and veterinary staff, Zhang *et al*. [19] also observed the presence of *mecA*, e*rmB* and *linA* genes with the expression of phenotypic resistance to such genes by the bacteria *in vivo* suggested cross-transmission of these resistant bacteria among the MRSA may have occurred between pet animals and veterinary staff. This same trend applies to other resistance genes, in term of possible transfer from animal to man and vice versa, or in transfer from one commensal to pathogen and vice versa; both horizontally and vertically [96]. Enzyme production in enhancing survival continues to make the commensal *Staphylococcus* a force of relevance in its niche and to exhibit pathogenic potentials given the opportunity to do so. The results of the research conducted by Zilevica *et al*. [97] showed that enzyme production does not differ between commensal isolates and nosocomial isolates of *S. epidermidis* except in urea utilization as in Table 2. This support the possible shift between commensalism and parasitism among various species of *Staphylococcus*, and position them as potential threat irrespective of their current mode of life.

CONCLUSION

Staphylococcus species are no doubt useful and close commensal of human and non-human animals but a number of phenotypic and genotypic traits for virulence and antibiotic resistance have positioned them beyond being gentle bacteria. Their scourge as opportunistic pathogens on the immunocompromised individuals and as aetiologies of severe systemic infections in healthy individuals, coupled with their roles in the emergence and transfer of resistance gene and its phenotypic expressions like their clinical counterparts leave much to be desire. This

necessitates the need for more concerted effort toward reducing factors that enhance the emergence of resistance such as arbitrary use of antibiotics and its use in agricultural practices (meat production). Compartmentalizing the ecosystem will also reduce the transfer of virulence and antibiotic resistance genes while personal and general hygiene also known as sanitary prophylaxis is hereby re-advocated as it reduces the bacteria load on human carriers, environmental reservoir and hospital fomites.

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