Chapter

Staphylococcus aureus and the Veterinary Medicine

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Abstract

Staphylococcus aureus has vital importance in veterinary medicine. Within the ruminants, it is one of the major causes of mastitis, the problem that was and is, with no definite solution to date. Along with that, it also affects the health of animals, pets, and poultry in several ways as the tissue tropism for this organism in poultry is the bones and the joints. This review is focused on habitat, species differentiation, differential biochemical tests, pathogenesis, clinical infections, economic importance, public health significance, immune response, the regulation of virulence in the staphylococci, and cytokines response against *S. aureus*.

Keywords: cytokines, superantigens, tissue tropism, virulence, zinc

1. Introduction

Staphylococci are Gram-positive cocci bacteria of 1 pico-meter diameter. They are observed with gram staining under the microscope as a bunch of grapes. The word staphylococcus is originated from the Greek words staphyle and kokkos. Staphyle means the "bunch of grapes", while the word kokkos means "the berry". The normal habitat of staphylococci is skin and mucus membranes. There are approximately 30 species of staphylococcus. They act as commensals but some of them are opportunistic pathogens too. They a famous for their pyogenic infection-causing property. Most staphylococci are facultative anaerobes, non-motile, oxidase-negative, non-spore-forming, and catalase-positive. S. aureus subsp. aureus is the coagulase-positive that has very much importance concerning the disease status of animals. Production of coagulase is directly correlated with the pathogenicity of the staphylococcus i.e. coagulase-negative bacteria are usually non0-pathogenic to animals and humans [1]. They can be grown on non-enriched media. They are facultative anaerobes and non-motile. They are found as commensals on mucous membranes and skin. They are stable in the environment **Figure 1** [99].

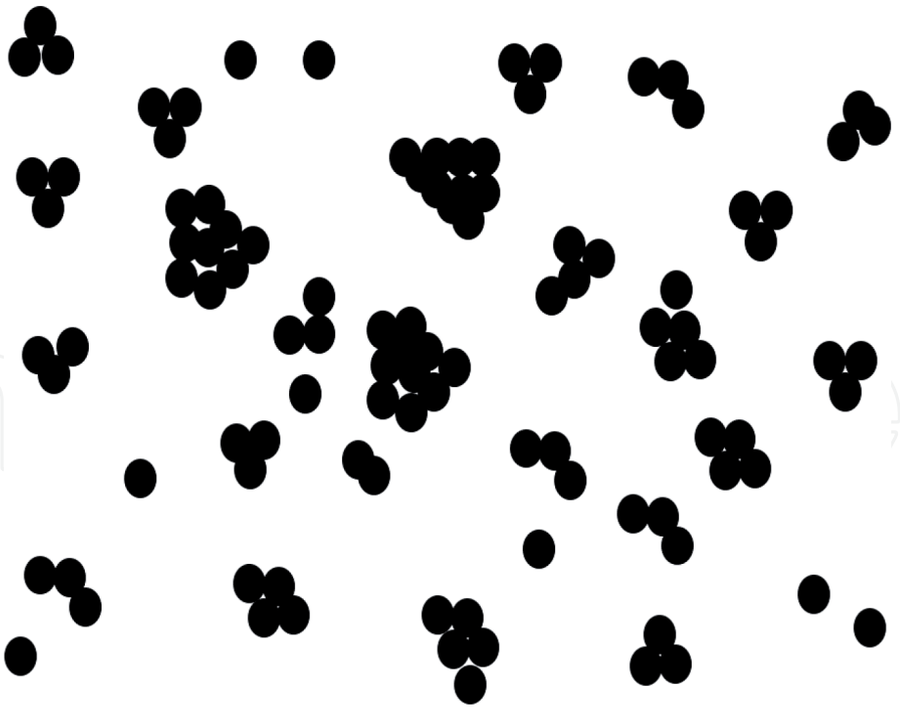

Figure 1.
'Bunches of grapes' appearance of Staphylococci. Modified from [1].

2. Habitat

Staphylococcal species occur on humans and animals on the skin, mucosa of the upper respiratory system, lower urinary, and genital tract, and as transients in the digestive tract. They are stable in the environment, have a selective affinity for particular species. They have limited zoonotic importance [1, 2].

3. Specie differentiation

While confirming a bacterial colony to be a staphylococcus or not, it is necessary to differential differentiate it from closest resembling bacteria named micrococcus

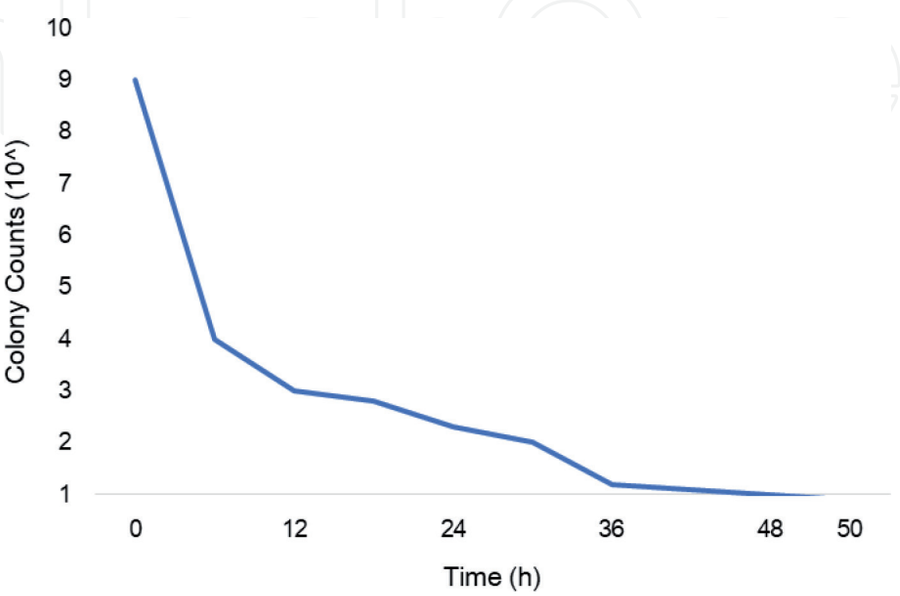

Figure 2.
Growth curve of Staphylococcus aureus within bovine aortic endothelial cells. Modified from [1].

and streptococcus species. The point that differentiates the Staphylococci from staphylococci is that staphylococci are mostly catalase-positive while the streptococci are mostly catalase-negative. Other tests of vital importance within the differentiation of the Staphylococcus species are hemolytic pattern, biochemical profiles, colonial appearance, and rRNA gene restriction patterns [2]. S. aureus and S. intermedius are often confused clinical cases of dogs and cats. Coagulase-negative staphylococci are ordinarily reserved for isolates from pure cultures. Their colonies are white, opaque and up to 4 mm in diameter, some are golden yellow and some have pigmented colonies. Sheep or ox blood agar presents alpha, beta, gamma, and delta hemolysis. Strains of the staphylococcus species are differentiated based on their capability of haemolysin production [1, 2].

The growth curve of *Staphylococcus aureus* within bovine aortic endothelial cells under optimal conditions is presented in **Figure 2**.

4. Biochemical tests for differentiating *Staphylococcus aureus* and Staphylococcus intermedius

A rapid test for the detection of acetoin has been developed [3]. Purple agar, containing bromocresol purple as a pH indicator and 1% maltose, is used to differentiate S. aureus from S. intermedius [4]. Purple is the color of most of the colonies of that bacteria. The energy source used by the *Staphylococcus aureus* in the culture medium is maltose which is utilized by that microbe and the resultant metabolic by-product is acid production. The by-product acid changes the color of the medium and colonies to yellow. Staphylococcus intermedius is a maltose fermenter so it means that it will not affect the color of the medium. There is also the commercial availability of the Biochemical tests which can be used for the confirmation of the staphylococcal species which can further be confirmed by molecular techniques like a polymerase chain reaction and multiplex PCR [5]. There are also studies on the molecular typing of the isolates of different regions of the world. The techniques that are and can be used in near future for the molecular epidemiology of the different isolates of Staphylococcus species can be but not limited to the Multilocus Sequence Typing (MLST) [6–9] and Multilocus variable number of Tandem Repeats (MLV) [10–12].

5. Pathogenesis and pathogenicity

Staphylococci are pyogenic and cause suppurative lesions. Virulent factors for this gram-positive bacterium are capsule, plasmid or phage-mediated, cell wall proteins, teichoic acids, and protein A **Figure 3** [2].

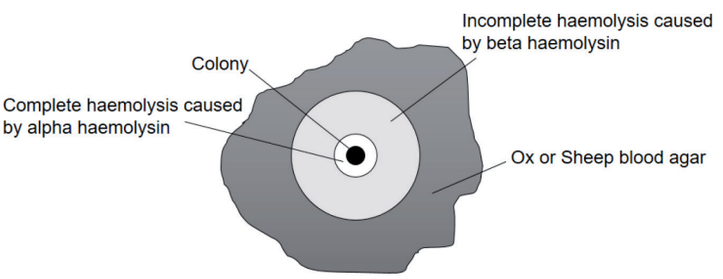

Figure 3.
Sheep or ox blood agar with Double haemolysis of S. aureus. Modified from [1].

6. Clinical infections

Staphylococci infections can be endogenous or exogenous in origin. Many infections are opportunistic and associated with other infections or the immune-compromised state of the host. Coagulase-positive staphylococci are mostly pathogenic. There are no effective vaccines against this malaise to date. Antibiotic sensitivity testing should have to be applied to check the efficacy of the drug against this bacterium. This is because many strains of this bacteria have developed resistance against many antibiotics. Common diseases of veterinary importance the Staphylococci are tick pyaemia, mastitis, botryomycosis, exudative epidermitis, and pyoderma [1].

6.1 Bovine mastitis

Staphylococcal mastitis is a common form of mastitis worldwide. Most infections are subclinical, but they can be acute or chronic, per acute and gangrenous. In gangrenous mastitis, the quarter is cold and blue-black and sloughing by the alpha-toxin causing necrosis of blood vessels and releases lysosomal enzymes [2–4].

6.2 Tick pyaemia

Tick pyaemia of lambs is a disease of hill-grazing regions having the tick Ixodes ricinus. Clinical signs include septicemia and rapid death, localized abscess formation, arthritis, posterior paresis, and ill-thrift. 30% of lambs between half a month old to up to three months of age can be affected. More infections are reported in spring and early summer [4–7].

6.2.1 Diagnosis, treatment and control

In young grazing lambs, clinical signs, microscopy of pus, and isolation and identification are required. Treatment is usually ineffective so control measures should have to be applied as tetracyclines injectables to the susceptible ones. Dipping to avoid tick-control measures should have to be practiced [2–5].

6.3 Exudative epidermitis (greasy-pig disease)

This is the disease pigs that are of up to 3 months old. It is contagious, with excessive sebaceous secretion, and exfoliation of the skin. Clinical signs include anorexia, depression, fever, dermatitis with an exudate. Death may be within 2–4 days with morbidity rate to be 20 to 100%, and mortality rates can be up to 90%. Isolation and identification of this bacteria can be from the vaginal mucosa and skin. Agalactia, weaning, and intercurrent infections are the predisposing factors for this disease [1].

6.3.1 Diagnosis, treatment and control

A high mortality rate in exudative, non-pruritic skin lesions. Along with the isolation and identification of the bacteria is required for confirmatory purposes. Antibiotic therapy with antiseptics is proven to be effective in many cases. Isolation of affected pigs, cleaning, and disinfection of surroundings. Antiseptic application before farrowing is also an effective way of prevention [1–3].

6.4 Botryomycosis

Botryomycosis is chronic, a suppurative granulomatous malaise of horses that is after castration infecting the stump of the scirrhous cord and mammary glands of sows [3–6].

6.5 Staphylococcal infections in dogs and cats

Otitis externa and Pyoderma, endometritis, mastitis, osteomyelitis, and cystitis are reported to be due to the S. aureus in many cases [2–6].

7. Staphylococcosis and poultry

Along with humans and animals, poultry is also susceptible to infections by staphylococcus [13–17]. There are no definitive signs of that bacteria in the poultry and it varies from case to case and the lesions are usually dependent upon the point of entry of the bacteria within the host. Unlike the animals where the staphylococcus mainly targets the skin and mucosa, the skin is less likely to be infected in the poultry and the organs that are more susceptible to the infection of staphylococcus species in poultry are bones, tendons, and joints [14, 16, 18–21]. The infections are characterized by the increased heterophil count and their accumulation into the affected regions [22]. It is also responsible for the acute deaths in layers [23] within the hot climates and is required to be differentially diagnosed with the fowl cholera. Staphylococcal infections in poultry are required to have in-depth studies by future researchers as there is less knowledge about the route of entry, immunity interaction, pathogenesis, and the possible prognosis of that organism. It impedes chronic infections mostly in poultry having poor antibiotic response. Immunization against that pathogen also requires more in-depth studies as the currently available vaccines are not as potent as the poultry business farmers and expecting [23–25].

7.1 Economic importance

Along with the studies that they present acute infection in hot climates, they can infect almost all types of climates and target both poultry and turkey. They have very much economic importance as they decrease the feed conversion ratio, weight gain, egg production, and septicemia. They target the bones resulting in lameness and osteomalacia. Their pathological lesion may lead to the condemnation of the carcass [24, 25]. There is a study correlating the green discoloration of the liver with the staphylococcal infections and it is concluded it these studies that there is a high correlation between the green discoloration of the liver and the staphylococcal infections, and they termed that condition as the “green-liver osteomyelitis complex” [26, 27] of the turkeys. It should have to be remembered that this pathogen is not the only etiological agent for that correlation, other isolates within these studies were Escherichia coli and many others [26–28].

7.2 Public health significance on poultry

Approximately 50% of the *Staphylococcus aureus* strains are responsible for human food poisoning through their enterotoxins [28–32] that are subjected to the condemnation of carcass upon their identification on food processing. Sources of the Staphylococcal infections may be the un-hygienic conditions of the processing plant and the poultry meat handling personals of the processing plant [33–36].

There is also a close associate of the Methicillin-resistant *Staphylococcus aureus* (MRSA) with the poultry meat [37–47]. MRSA has different strains each is resistant to a class of antibiotics as the commonly reported antibiotics against which the MRSA has evolved the disease tolerance includes the semi-synthetic penicillins [48, 49], Methicillin [50], fluoroquinolones [51], Vancomycin [52, 53], Sulphonamides and trimethoprim [54], tetracyclines [55–57], aminoglycosides [58–60], chloramphenicol [61], and clindamycin [62]. MecA gene is reported to be responsible for the methicillin resistance in the *Staphylococcus aureus*. This gene is also attributed to be transmitted from poultry to humans. The most common isolates of MRSA are CC398, ST9 [28–30].

7.3 History and transmission

Firstly reported cases of the isolates have reported the susceptibility of bones with this pathogen and the prominent clinical signs as synovitis and arthritis [63–66]. Navel of the day-old chicks, surgery as trimming, and vaccination in un-hygienic conditions can be the trigger for the infection. Diseases that involve the predilection site to be the immune organs as being directly involved can also the root cause of this infection as the infectious bursal disease [67] and chicken infectious anemia. This is usually fatal as it leads to septicemia. Aged turkeys can have this infection with exposure to the hemorrhagic enteritis virus (HEV) [68]. Genetics of the poultry as the major MHC is also the predisposing factor for the skeletal-related problems of the poultry [69]. The incubation period of 2–3 days is a thumb rule but it is dependent upon several factors as the immune status of the host, the potency, and route of infection of the bacteria as the aerosol and tracheal routes are reported not to be the potent routes of infection [70, 71]. Infections with less than 105 organisms/kg body weight are reported to be defeated by the immune system of a healthy bird [25, 72].

7.4 Clinical signs, morbidity, incubation period, and pathology

Clinical signs of this disease include lameness, depression, pyrexia, and gait abnormalities, and death. Survived animals have arthritis, osteomyelitis [73, 74] unable to stand and sit on the hock and keel [25, 75]. This makes the fragility of the bones, mostly the femur and the tibiotarsus. It also leads to the congestion of the spleen, liver, lungs, and kidneys [23], gangrenous dermatitis, and ultimately the "blue wing disease" that presents the infection to the tip of wings of the birds that are infected with the chicken infectious anemia virus. Other clinical signs include enlarged yolk sac, planter abscess, discolored liver [27, 76]. Usually, the bacteria are not subjected to enough titer that may be the cause of higher mortality rates as compared to another fatal disease as the New Castle disease, etc., under optimal environmental conditions with most of the birds. But this bacterium has also been reported to have very high mortality rates that were primarily due to the immune-compromised state of the birds and the poor management conditions, and this bacteria in these conditions too is not the primary cause of the losses. The common site for the isolation and identification of that agent is the joints [18, 76–78].

7.5 Immune response

There are no convincing reports of the facts the active immunity or passive immunity other than that of the anti-*Staphylococcus aureus* antibodies may have any effect on this bacterium [79, 80]. Immunized hens can have antibodies within their

egg yolk that can be used to prevent the bacteria in vitro. Toxoids are ineffective in other species [81, 82], and vaccines have not proven to be a very effective way of controlling the disease [83–86].

7.6 Diagnosis

Isolation and identification of the samples of yolk, joints, and internal organs from the infected bird should have to be practiced. Bacteria are harvested on the blood agar from the sheep or bovine and results are visible within a day of incubation. Selective media for this organism can be used as mannitol salt agar [87–89]. Serology testing includes microtiter plate agglutination assay and indirect immunofluorescent antibody titer assay. It can be differentially diagnosed from the diseases of the joints of the poultry [79, 83].

7.7 Management and control

Sharp objects should not have contact with the birds of the poultry farm, Sanitation and optimal environmental conditions are key to good farming practices that will minimize the chances of infection [22, 67, 90–91]. Nutritionists are also considering the point of adding herbs and plants as Moringa oliefera [92] to boost the immune system, they also claim to have the composition of these herbs that helps the birds to cope up with the pathogens. In ovo inoculation is also advocated to boost the immune system to cope-up with the infectious agents [93]. Passive immunity against this bacterium to the susceptible population is also a rational option to cope up with the disease outbreaks [99].

7.8 Vaccination

Staphylococcal bacterins [81, 94], strain 115 [95], aerosol vaccine S. epidermidis 115 [71, 95–98] and PNSG are available with an aim to prevent the Staphylococcal infections. The capsule of live or dead cells of the Smith diffuse strain of S. aureus is most antigenic and was proved and used as the earliest potent vaccinal candidate, as the antibodies produced against the capsule can deal with the strategy of this bacteria of dodging the phagocytosis [13–19]. The single intraperitoneal injection can protect from the challenge of a lethal dose of 108 CFU [26–27]. Anti-microcapsule vaccines are not proved to be as effective as capsular candidates. Bivalent vaccines are also been approved to be the effective ones. The capsule requires a monophosphoryl lipid A as adjuvant and a booster dose to show an optimal antibody response [98–102].

7.9 Treatment

It is recommended to have antibiotic sensitivity testing before deciding the application of the antibiotic. The commonly used antibiotics against this bacterium are penicillin, tetracycline, streptomycin, novobiocin, sulfonamides, lincomycin, and spectinomycin. Most bacteria to date, are resistant to penicillin and many are resistant to other antibiotics as methicillin too. Vancomycin is considered now to be the most effective antibiotic against this bacterium. It is good to know that the cure rate of Staphylococcal infection with antibiotics does not exceed far beyond thirty percent, so vaccines should be the priority in dairy herd management [99–102].

8. The regulation of virulence in the Staphylococci

Virulence factors are the substances that aid in the pathogenesis of an organism. Pathogenesis of *Staphylococcus aureus* does not depend on a single factor and there are a set of substances that collectively leads to the successful colonization of that bacteria into its host [98–99]. These virulence factors also diversify in their composition of proteins as exoproteins and surface proteins. To date, there are many reports of mutants, which behave differentially concerning the expression of different exoproteins in different environmental conditions [100–102]. Most of the exoproteins are secreted at the post exponential phase. The polysaccharide of the capsule of *Staphylococcus aureus* also acts as the virulent factor. This bacterium can also be classified based on the structure of the capsule into 11 different serotypes [99]. Serotypes 1 and 2 and mucoid, while the serotypes 3 to 11 are microcapsules as which are non-mucoid and have thin capsules [96–101]. Among these 11 serotypes, 5 and 8 are the most prevalent. The capsule is vital to this bacterium as it is responsible for evading the phagocytosis by masking the C3b that is placed on the surface of these bacteria by the host immune cells. The significance of microcapsules in pathogenesis is not well established as there are many controversial studies in this regard. The genes responsible for the formation of microcapsules are cap5H, cap8J, and cap5P. The cap8B and cap5B genes are homologous to each other in several proteins, and cap8B acts as the chain length regulator of the capsule [98–100]. The chemical composition of serotypes 1, 2, 5, and 8 are presented in **Figure 4**.

The agr and sar 16 loci have been extensively studied and believed to have vital importance in the virulence of this bacteria. Alpha toxin is also a virulence factor of Staphyloccocus aureus, which forms the pores to the cells resulting in cytolysis of the surrounding cells of invasion [97–100]. Not all the virulence factors are active throughout the life of the bacteria, but on the as-required basis, to overcome the metabolic burden [96–100]. Currently, the exact mechanism behind these virulence factors is not well elucidated. Staphylococcus is blessed with these virulence factors for its survival in diversified environmental conditions, and the primary purpose of these is not to cause the disease. Passaging the bacteria to nutritive media in vitro leads to the bacteria of less virulency and the passage of bacteria to the live animal or host leads to the bacteria with more virulency [99, 100].

Microbial surface components recognizing adhesive matrix molecules (MSCRAMMS), Sialoprotein, laminin, elastin, etc. are the proteins that are responsible for the adhesion of staphylococcus to its surrounding [98–100].

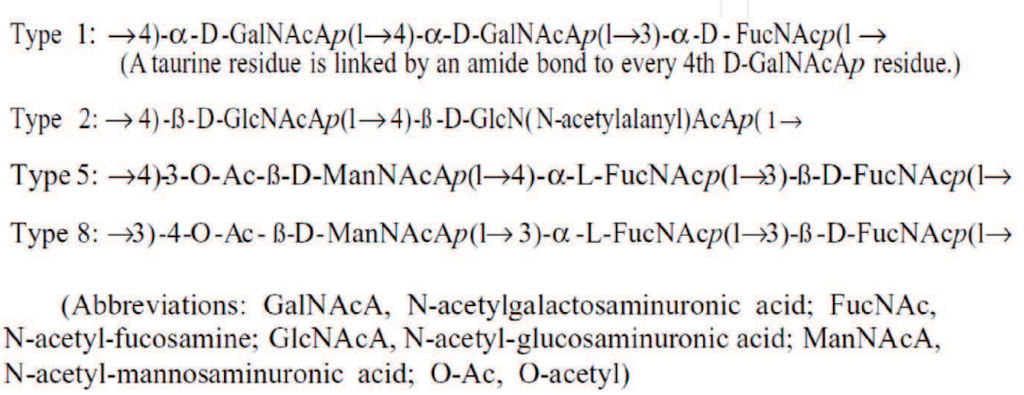

Figure 4.
The chemical compositions of serotypes 1, 2, 5, and 8. Modified from [99].

To dodge the host immune system is a requirement of the successful colonization of each pathogen. Staphylococcus is also blessed with these factors as protein A for binding the IgG antibodies [99–101].

This bacterium has a system of coordination with environmental conditions as temperature, pH, etc. This system of coordination is named the "two-component systems" having two proteins and a single operon and upon detection of the signal these proteins active certain genes for transcription. A small colony-sized SVC subpopulation is also a potent strategy of this bacteria against the immune system of the host and antibiotic therapy [97, 98].

The bacterial secretions having mitogen properties are also called superantigens. These superantigens are pathogenic and may cause an autoimmune response. They are also responsible to activate macrophages, zinc having a vital role in that, by initiating the IFN-gamma secretion from T cells. Superantigens can initiate an immune response without the increased concentration of IFN-gamma, whereas in mice it is necessary to have the increased concentration of IFN-gamma to initiate the immune response. It is not clear whether the response of MHC I and MHC II are synergistic or not, in the immunologic response against the pathogenesis of Staphylococcus **Figures 5** and **6** [97–101].

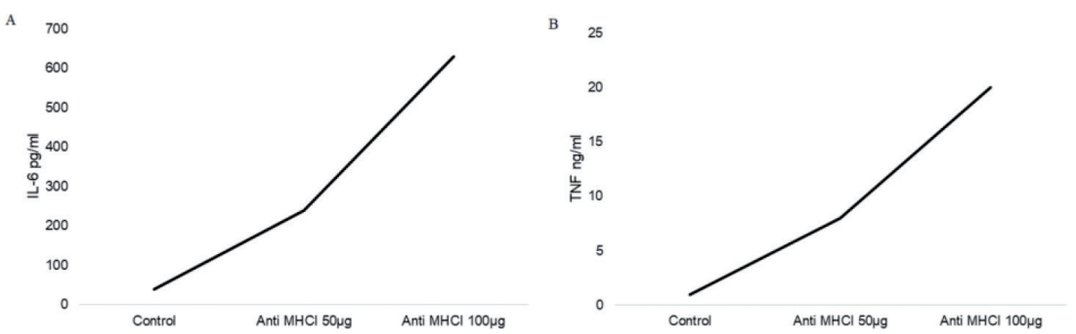

Figure 5.
(A) Response of IL-6 against anti-MHC-I 50 µg and MHC-II 100 µg antibodies incubated with C2D macrophages. (B) Response of TNF against anti-MHC-I 50 µg and MHC-II 100 µg antibodies incubated with C2D macrophages. Modified from [99–102].

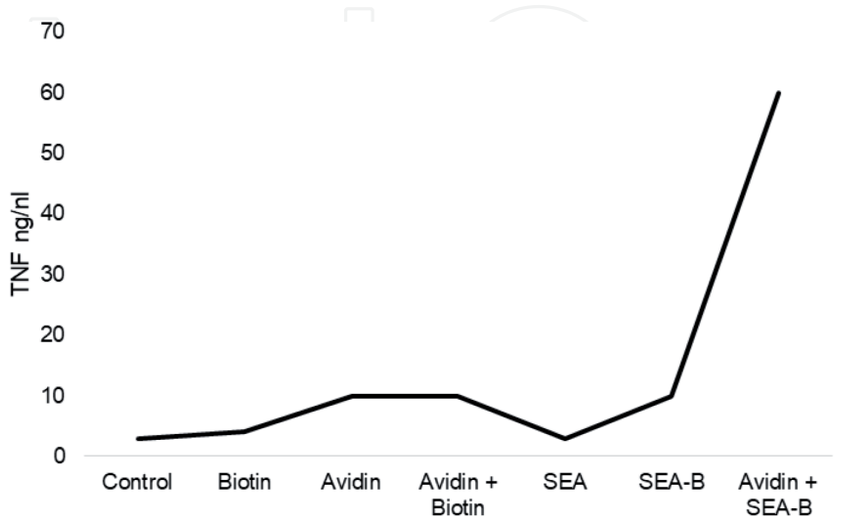

Figure 6.
Response of TNF against various stimuli. (TNF: Tumor Necrosis Factor, SEA: Staphylococcal enterotoxin A, SEA-B: Staphylococcal enterotoxin A). Modified from [99].

9. Endogenous IFN-gamma, TNF, and IL-6 in *Staphylococcus aureus* infection

Endogenous IFN-g plays a detrimental role in S. aureus infection. IFN-g, TNF, and IL-6 levels are elevated within 24 hours of infection even though whether the infection is lethal or non-lethal. In nonlethal cases, Bacteria is not present in the blood but in the kidneys and remains there for up to three weeks of infections. IFN-g peaks again in the spleens and kidneys. Among these three cytokines, the only cytokine that is detected in the serum is IL-6. In lethal infection, IFN-g and IL-6 in the sera and TNF in the kidneys peaked before death [98–102].

10. Conclusion

Staphylococcus aureus has vital importance in the ruminants, as it is one of the major causes of Mastitis. Along with that, it also affects the health of animals, Pets, and Poultry in several ways as the diseases of bones in poultry. The Regulation of Virulence in the Staphylococci mainly are the exoproteins and surface proteins, and capsule, agr, sar 16 loci, and Alpha toxin. Bacteria potentiates cytokines for host resistance [97–101]. IFN-g and TNF play a protective role against Listeria monocytogenes, Mycobacterium species, Salmonella typhimurium, and Francisella tularensis. IFN-g and TNF also mediate gram-negative septic shock and endotoxin shock. Staphylococci induce TNF, interleukin-1, IFN-g, IL-2, and IL-6 in humans and animals [101, 102].

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Conflict of interest

The authors declare no conflict of interest.

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