Pathogenesis of *Staphylococcus aureus* Pneumonia

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**ABSTRACT:** Varying types and severities of diseases caused by *Staphylococcus aureus* have been reported in horses. The pathogenesis of infection and development of antimicrobial resistance pertaining to *S. aureus* are discussed in this article. The role of methicillin-resistant *S. aureus* (MRSA) in veterinary medicine is not well understood, and MRSA is an emerging problem with zoonotic potential. An 18-year-old Arabian gelding and a 2½-month-old Oldenburg filly were evaluated for persistently high fevers and thrombophlebitis at The University of Georgia Veterinary Teaching Hospital. Evaluation of each horse included physical examination, complete blood cell count, serum chemistry profile, thoracic radiography, and transtracheal aspiration. In both cases, the results of transtracheal aspiration revealed *S. aureus*, which is an uncommon primary pathogen of the equine lower respiratory tract and was considered to be secondary to jugular thrombophlebitis.

*Staphylococcus aureus* is an uncommon cause of pneumonia in horses. The results of one study documented isolates of *S. aureus* from only 1.7% of horses with bacterial pneumonia or pleuropneumonia. When staphylococcal pneumonia does occur, it can be difficult to treat. Adaptation of *S. aureus* to the modern hospital environment has been marked by the acquisition of drug resistance genes soon after antibiotics were introduced, beginning 2 years after the introduction of penicillin in 1944. The prevalence of methicillin-resistant *S. aureus* (MRSA) in nosocomial outbreaks has initiated worldwide concern. Likewise, community-acquired MRSA is emerging as a significant cause of skin infections among athletes and in state prisons. In contrast, there is limited published information regarding *S. aureus* infections in horses. Nosocomial MRSA infection or colonization may be a serious emerging condition in equine hospitals, as it is in human hospitals. Nosocomial transmission of MRSA in human hospitals is thought to occur primarily via the hands of hospital personnel contaminated by contact with infected or colonized patients. In horses, the nasal passages appear to be the most common site of colonization. Infections in horses have resulted in a range of clinical manifestations, including wound infections, surgical site infections, pneumonia, arthritis, catheter site infections, osteomyelitis, metritis, endocarditis, and dermatitis. In a 1999 study of a MRSA outbreak at a veterinary teaching hospital, concerns were raised regarding human-to-horse transmission of the organism. In that study, MRSA organisms were cultured from three of...
five staff members tested, and the isolates were identical to those obtained from the horses with MRSA infections. On the basis of the pattern associated with the infections in that outbreak, it was speculated that the hospital staff members were the primary source of the infections.

The purpose of this article is to increase awareness of *S. aureus* and the signs that may result from infection.

**THE ORGANISM AND PATHOGENESIS**

*S. aureus* is a gram-positive spherical coccal member of the Micrococcaceae family of bacteria. Although enterococci and bacilli are considered to be close relatives of *S. aureus*, staphylococcal organisms are phylogenetically unrelated to other genera in that bacterial family. Facultative anaerobes such as *S. aureus* grow by aerobic respiration or fermentation that yields lactic acid. All staphylococci are catalase positive, releasing oxygen in the presence of hydrogen peroxide. Many but not all species of staphylococci are coagulase positive, which refers to their ability to clot plasma. All *S. aureus* are coagulase positive; however, this is not considered a virulence factor. Staphylococci multiply by division in two planes, forming a cluster of cocci that morphologically distinguishes them from the chains of cocci that are typical of streptococci. The pathogenesis of *S. aureus* infection is multifactorial, involving surface proteins, factors that inhibit phagocytosis, and membrane-damaging toxins. The surface proteins laminin and fibronectin, which are found on epithelial and endothelial cells, form a part of the extracellular matrix and promote attachment of the cocci to host cells. *S. aureus*, aided by laminin and fibronectin, can adhere to endothelial cells and become internalized in a phagocytosis-like process, making it difficult to achieve effective antimicrobial concentrations because of the intracellular location of the organism. Fibronectin is also a component of blood clots. Most staphylococcal strains express a fibrinogen/fibrin-binding protein, which promotes attachment to blood clots and traumatized tissue. Other components of *S. aureus* that are purported to interfere with phagocytosis by the host are the capsular polysaccharide and protein A. Although it is hypothesized that the capsular polysaccharide (a microcapsule visualized only by electron microscopy) interferes with phagocytosis, its function is not entirely clear. Protein A is a surface protein that binds the Fc region of IgG, thereby interfering with opsonization and phagocytosis of the organism.

*S. aureus* produces a wide variety of exoproteins that contribute to its ability to colonize and cause disease in mammalian hosts. Nearly all strains of *S. aureus* secrete enzymes and cytotoxins, including four hemolysins (i.e., α, β, γ, δ), lipases, hyaluronidase, nucleases, proteases, and collagenase. Purportedly, the main function of these proteins is to convert local host tissues into nutrients required for bacterial growth. Some staphylococcal strains produce additional exoproteins, including toxic shock syndrome toxin-1 (TSST-1), the enterotoxins (types A, B, C, D, E, G, H, I, J, SEIR), the exfoliative toxins (ETs; i.e., ETA and ETB), and leukocidin. TSST-1 and the enterotoxins are known as *pyrogenic toxin superantigens*. Superantigens bind directly to the class II major histocompatibility complex of antigen-presenting cells outside the normal antigen-presenting groove, resulting in nonspecific stimulation of T cells (i.e., ETA and ETB), and leukocidin. 17–19 TSST-1 and the enterotoxins are known as *pyrogenic toxin superantigens*. Superantigens bind directly to the class II major histocompatibility complex of antigen-presenting cells outside the normal antigen-presenting groove, resulting in nonspecific stimulation of T cells. As a result, as many as 20% of T cells may be activated by these superantigens, whereas only 0.01% of T cells are stimulated during normal antigen presentation (Figure 1).

**CLINICAL DISEASE AND ANTIMICROBIAL RESISTANCE**

Superantigens are responsible for most of the severe clinical syndromes caused by *S. aureus* infection. Staphylococci...
**Clinical Cases**

The following case summaries involve two horses that presented to The University of Georgia Veterinary Medical Teaching Hospital with *S. aureus* pneumonia, which was believed to be secondary to catheter-related thrombophlebitis. The *S. aureus* in these cases was not methicillin resistant, but one manifestation of staphylococcal disease and the challenges of management in horses are demonstrated.

**Case 1**

An 18-year-old Arabian gelding presented with high fever (104.5˚F [40.3˚C]; normal: 99˚F to 101.5˚F [37.2˚C to 38.6˚C]) and left jugular thrombophlebitis. The gelding had been discharged from the hospital 4 days earlier with mild thrombophlebitis secondary to intravenous catheterization (using a 14-gauge, 3.5-inch, polyurethane, over-the-needle catheter; MILA International) and fluid therapy for medical treatment of colic. During examination, the gelding was alert and responsive. The rectal temperature was 103.3˚F (39.6˚C); the pulse and respiratory rates were within normal limits. The left jugular vein was swollen and painful in the proximal one-third of the neck.

Auscultation of the thorax with a rebreathing bag revealed increased bronchovesicular sounds; however, no cough was elicited. Rectal examination findings and microscopic examination of fluid obtained from the abdomen via abdominocentesis were within normal limits. Laboratory examination of peripheral blood revealed leukocytosis (leukocyte count: 18,300/µl) characterized by mature neutrophilia (neutrophil count: 17,200/µl). The plasma fibrinogen concentration was increased (i.e., 800 [normal: ≤400] mg/dl). Analysis of a serum biochemical profile revealed no significant abnormalities. Submission of transtracheal aspirate for cytology revealed moderate to marked mucopurulent inflammation with no evidence of sepsis. The fluid was also submitted for culture and subsequently grew *S. aureus*. Further diagnostic testing was declined because of financial constraints of the owner. The gelding was discharged with owner instructions to administer trimethoprim–sulfamethoxazole (20 mg/kg PO q12h) and procaine penicillin G (22,000 U/kg IM q12h) and return for reevaluation in 3 days.

During reexamination, the rectal temperature was increased (102.3˚F [39.1˚C]). The owner reported persistently high fevers of 105˚F to 106˚F (40.6˚C to 41.1˚C), which were managed with flunixin meglumine (1.1 mg/kg IV q12h). The gelding continued to have a good attitude and appetite and to defecate normally. Mild tachycardia was present (heart rate: 56 bpm; normal: 28 to 48 bpm), and pronounced nostril flaring was noted. The left jugular vein remained moderately swollen, and thrombophlebitis had extended proximally to the bifurcation of the vein. Thoracic radiography revealed a diffuse multifocal nodular pattern (A). Based on the persistent fever, clinical signs, and radiographic findings, the owner elected euthanasia and necropsy examination.

Postmortem examination revealed multifocal, subacute, severe pulmonary abscesses (>50). Histopathologic examination of the abscesses and jugular thrombosis revealed numerous large colonies of bacterial cocci. The jugular thrombophlebitis was characterized as chronic, suppurative, and severe, with numerous intravascular colonies of bacterial cocci and adjacent cellulitis. The vein was not cultured. A light growth of *S. aureus* was obtained from the transtracheal aspirate collected 3 days earlier; however, a heavy growth was isolated directly from the pulmonary abscesses. The antimicrobial sensitivity pattern of the lung isolate revealed this strain of *S. aureus* to be susceptible in vitro to amikacin, ceftiofur, cephalothin, chloramphenicol, amoxicillin–clavulanate, clindamycin, enrofloxacin, erythromycin, gentamicin, oxacillin, tetracycline, and trimethoprim–sulfamethoxazole. The organism was resistant to ampicillin and penicillin. This *S. aureus* isolate was coagulase positive, β-hemolytic, and negative for superantigens A, B, C, D, E, G, H, J, and I as well as ETA, ETB, and TSST-1.

**Case 2**

A 2½-month-old Oldenburg filly presented with high fever, thrombophlebitis, and pneumonia. The filly had been an outpatient 2½ weeks previously and had been diagnosed with *Streptococcus zooepidemicus* pneumonia by physical examination, thoracic radiography, and culture of transtracheal aspirate.
The filly was treated with ceftiofur (4.4 mg/kg q12h) administered through an intravenous catheter (14-gauge, 20-cm, polyurethane guidewire–style catheter; MILA International). The owner noted fever and marked left jugular thrombophlebitis at the catheter site 2 days before re-presentation, at which time the same type of catheter was replaced in the right jugular vein by a University of Georgia staff veterinarian on the farm. Local therapy for thrombophlebitis was begun and ceftiofur administration continued.

During examination at the second presentation, the filly was quiet and responsive. The rectal temperature was 101.7˚F (38.7˚C; normal: 99˚F to 102˚F [37.2˚C to 38.9˚C]), and the respiratory rate was increased (i.e., 42 breaths/min; normal: 20 to 40 breaths/min). Mild soft wheezes were auscultated bilaterally with a rebreathing bag. Moderately severe left jugular vein thrombophlebitis was present in the rostral two-thirds of the neck. A complete blood cell count revealed mature neutrophilia (8,690 neutrophils/µl) with 220 band cells/µl. The plasma fibrinogen concentration was 800 mg/dl. Thoracic radiographic findings were similar to those obtained 2 weeks earlier, with evidence of cranioventral pneumonia.

The filly was treated with chloramphenicol (50 mg/kg PO q6h) for thrombophlebitis and maintained on intravenous ceftiofur. Flunixin meglumine was administered as needed for pyrexia, with the dose not exceeding 1.1 mg/kg IV q12h. Supportive care included hot packing the skin over the left jugular vein and intravenous administration of polyionic fluids at 50 ml/kg/day.

Crackles and wheezes became more severe during serial rebreathing examinations over the next 2 days, and pyrexia persisted, with the rectal temperature reaching 105˚F (40.6˚C) approximately every 12 hours. Transtracheal aspirate collected 48 hours after hospitalization had cytologic evidence of septic suppurative inflammation with bacterial cocci in clusters. Antimicrobial therapy was changed to potassium penicillin (22,000 U/kg IV q6h) and gentamicin (6.6 mg/kg IV q24h). Culture of the transtracheal aspirate yielded heavy growth of *S. aureus* sensitive to amikacin, ampicillin, ceftiofur, cephalothin, chloramphenicol, amoxicillin + clavulanate potassium, clindamycin, enrofloxacin, gentamicin, oxacillin, penicillin, tetracycline, and trimethoprim + sulfadiazine, with intermediate susceptibility to erythromycin. Five days after hospitalization, the filly developed marked effusion of the right hind metatarsophalangeal joint. The respiratory rate and effort were increased, and pyrexia was persistent. The owner elected euthanasia.

Postmortem examination revealed multifocal pulmonary abscesses (B) containing degenerate neutrophils and large colonies of bacterial coci. The left jugular vein (C) contained a thrombus composed of fibrin, neutrophils, and erythrocytes; *S. aureus* was cultured from this site. This isolate was compared with the one from case 1 using the Box A technique (a polymerase chain reaction genotyping technique) and was determined to be different. It was coagulase positive, β-hemolytic, and had negative results for superantigens A, B, C, D, E, G, H, J, and I as well as ETA, ETB, and TSST-1. Diffuse distribution of the pulmonary abscesses and culture of the same organism from the thrombus and transtracheal aspirate suggested a hematogenous route for the pneumonia. Culture of the lungs at necropsy yielded no growth.
Pathogenesis of *Staphylococcus aureus* Pneumonia

Bacteriococcal food poisoning in humans results from ingestion of one or more preformed enterotoxins on food contaminated with *S. aureus*. This food poisoning typically results in emesis, which is self-limiting in 24 to 48 hours. TSST-1 induces fever, erythroderma, cutaneous desquamation, hypotension, and multisystemic involvement. A TSST-1–secreting *Staphylococcus* sp has recently been isolated from a horse with pneumonia. The affected horse developed vasculitis, skin sloughing, and a fever that was unresponsive to NSAID therapy; the horse survived after a prolonged course of antimicrobial therapy.

The primary defense mechanism protecting the host against staphylococcal infection is phagocytosis of the organisms. Additional protective responses of the host include production of antibodies to neutralize the toxins and promote opsonization of the organism. From the bacterium’s perspective, infection of the host is facilitated by minimizing the ability of antimicrobials to reach the organism and development of antimicrobial resistance. There are four mechanisms of bacterial resistance against antimicrobials:

- Enzymatic inactivation of the drug
- Alterations within the organism that prevent the drug from binding to its target site
- Accelerated drug efflux to prevent accumulation of effective concentrations of the antimicrobial in the bacterium
- A bypass mechanism whereby an alternative drug-resistant version of the target within the bacterium is expressed

Antibiotic resistance is mediated by acquisition of extrachromosomal plasmids, transposons, or other types of DNA insertion and by mutations in chromosomal genes.

Staphylococcal organisms have a remarkable ability to become resistant to antimicrobials, as evidenced by the acquisition of drug resistance genes soon after the organisms were exposed to new antimicrobials. The structural gene for methicillin (oxacillin) resistance, *mecA*, encodes an altered penicillin-binding protein that has reduced affinity for β-lactam antimicrobials. The original source of *mecA* in staphylococci is unknown because the gene has not been identified outside this genus. Oxacillin rather than methicillin is used to detect methicillin resistance because oxacillin is more resistant to degradation and is more likely to detect heteroresistant strains. Therefore, oxacillin-resistant *S. aureus* is also methicillin resistant. Most MRSA isolates are resistant to many other antimicrobial classes besides β-lactam antimicrobials. Resistance to antiseptics and disinfectants, such as quaternary ammonium compounds, may aid the survival of *S. aureus* in hospitals. Successful treatment of *S. aureus* infection can be hindered by antimicrobial resistance as well as abscess and scar-tissue formation, which makes it difficult to achieve effective drug concentrations at the site of infection.

*S. aureus* can cause several diseases (e.g., mastitis, arthritis, suppurative disease, folliculitis, septicemia, cellulitis) in animals. Manifestations of *S. aureus* infection in horses include, but are not limited to, thrombophlebitis, pneumonia, and cellulitis. *S. aureus* pneumonia is rare in horses, and secondary staphylococcal infection can be terminal in horses and humans. Most life-threatening diseases caused by *S. aureus* infection in humans are hospital acquired and often associated with indwelling vascular devices or catheters. In animal hospitals, infections typically occur secondary to exposure of open skin or wounds to staphylococci, which normally colonize human nasal passages and are commensal organisms on human skin.

**DISCUSSION**

To our knowledge, *S. aureus* pneumonia presumed to be secondary to septic thrombophlebitis has been reported in only one equine case other than those presented in the box on page 214. The patient was treated with intravenous antibiotics through an indwelling catheter following hospital discharge and subsequently developed thrombophlebitis, a complication associated with venous catheterization and fluid therapy. Clinical signs of thrombophlebitis may include heat, swelling,
pain, presence of exudate at the catheter site, and palpation of a thrombus within the affected vessel.

The results of a retrospective study\textsuperscript{26} of horses determined that venous thrombosis was correlated with the use of locally produced fluids, fever, diarrhea, and duration of treatment. Catheter type has not been associated with phlebitis in previous studies,\textsuperscript{26} and similar types of catheters were used in the two cases reported in this article (see box on page 214). Our teaching hospital uses locally produced intravenous fluids to treat hospitalized patients. Although the Arabian gelding was administered locally produced polyionic fluids before the development of thrombophlebitis, the Oldenburg filly was not initially administered fluids. To decrease the risk for thrombophlebitis, all catheters in our hospital are placed while sterile gloves are worn after surgical preparation of the skin using chlorhexidine and alcohol. Extension sets are used to minimize handling of the catheter at the entrance on the skin, and catheter caps are changed daily. Catheter sites are evaluated by a clinician at least once daily, and the catheter is removed if there is evidence of local cellulitis. However, once cellulitis and phlebitis are clinically evident, secondary hematogenous spread of bacteria may be difficult to prevent. In one study\textsuperscript{27} summarizing culture results obtained from the tips of intravenous catheters, 50% of horses with normal-appearing veins had bacterial colonization of the catheter tip, whereas horses with evidence of phlebitis had a 73% frequency of bacterial colonization. Of the microorganisms isolated from catheters in that study, 19.5% were staphylococcal species—the most represented group.\textsuperscript{27}

Although strict catheter maintenance and aseptic technique may reduce the risk for infection, they do not eliminate the possibility altogether. The cases presented in this article also illustrate the severity and persistence of secondary staphylococcal infection, even in strains that demonstrate broad antimicrobial susceptibility in vitro. Although long-term antimicrobial therapy was not attempted because of financial considerations of each owner, pyrexia and malaise persisted during short-term administration of appropriate drugs based on culture and sensitivity results. In vitro sensitivities did not reveal highly resistant organisms or MRSA. The cultures revealed different strains of \textit{S. aureus} in each case, which made the lack of a short-term response to antimicrobials disappointing. Long-duration antimicrobial treatment of staphylococcal infections is frequently required, and antibiotic choice is still best guided by the results of culture and sensitivity testing. In one report\textsuperscript{1} of horses with \textit{S. aureus} infection, only one of five \textit{S. aureus} isolates from horses with pneumonia or pleuropneumonia was susceptible to penicillin or ampicillin. However, 80% or more of isolates in that study were susceptible to β-lactamase-resistant antimicrobials, including cephalothin, aminoglycosides, and trimethoprim-sulfamethoxazole.\textsuperscript{1} Although primary pneumonia is not commonly caused by \textit{S. aureus}, secondary infection from a jugular-vein catheter may be a more significant health threat to the patient. The zoonotic threat of MRSA in the veterinary population must be a concern of veterinary professionals.\textsuperscript{9,28,29}

The primary defense mechanism protecting the host against staphylococcal infection is phagocytosis of the organisms.\textsuperscript{28,29} The evaluation of MRSA transmission between humans and animals initially revolved around the belief that MRSA was a humanotic disease—one that is transferred from humans to animals.\textsuperscript{6} More recent evidence indicates that animals can transmit MRSA to veterinary personnel, resulting in human colonization or infection.\textsuperscript{9} The current role of the environment in transmission of MRSA infection to horses or humans is unclear.\textsuperscript{11} However, relatively widespread contamination of the hospital environment occurring when infected horses are hospitalized suggests that the environment may be an important source of MRSA infection. Weese et al\textsuperscript{11} isolated MRSA from 62% of surfaces, including walls, doors, water bowls, feed bowls, and hay nets, in stalls housing MRSA-infected horses. Infection-control protocols in veterinary hospitals should be strict and well defined and must include isolation of infected patients as well as strict barrier control.

CONCLUSION

\textit{S. aureus} infection can cause a variety of clinical syndromes in humans and animals. The pathogenicity is
multifactorial and is complicated by production of pyrogenic superantigens. Strains that are not multidrug resistant in vitro may still produce pyrexia and pneumonia that are difficult to control with short-term NSAID and antimicrobial therapy. MRSA is emerging with an increased frequency in veterinary populations and warrants further research. 9, 31 Although staphylococcal organisms are uncommon primary pathogens in the equine respiratory tract, their presence should not be overlooked because severe clinical disease may result.

REFERENCES


1. S. aureus is a gram-positive spherical coccus and
   a. a member of the Micrococcaceae family of bacteria.
   b. a facultative anaerobe.
   c. always catalase positive.
   d. all of the above

2. Which surface protein(s) promotes attachment of staphylococci to host proteins and forms a part of the extracellular matrix?
   a. laminin and fibronectin
   b. fibrinectin and capsular polysaccharide
   c. laminin and protein A
   d. fibrinogen/fibrin-binding protein

3. Most severe clinical syndromes caused by S. aureus are a result of
   a. hemolysins.
   b. superantigens.
   c. capsular polysaccharide.
   d. laminin.

4. Which is the primary defense mechanism protecting the host against staphylococcal infection?
   a. antibody production
   b. opsonization
   c. phagocytosis
   d. neutralization

5. mecA is significant because it
   a. is a protein that codes for penicillin resistance.
   b. is the gene for methicillin resistance.
   c. promotes abscess and scar-tissue formation.
   d. is a superantigen.

6. Manifestations of S. aureus infection in horses include
   a. pneumonia.
   b. cellulitis.
   c. wound infection.
   d. all of the above

7. Which statement regarding thrombophlebitis is correct?
   a. Aseptic technique and the use of sterile gloves for catheter placement eliminate the risk for thrombophlebitis.
   b. Catheter type is associated with the development of thrombophlebitis.
   c. It is a potential complication associated with venous catheterization and intravenous fluid therapy.
   d. none of the above

8. The most common site of colonization with MRSA appears to be the
   a. skin in horses and nasal passages in humans.
   b. nasal passages in horses and the skin in humans.
   c. distal limbs in horses and mucous membranes in humans.
   d. mucous membranes in both horses and humans.

9. Which statement regarding S. aureus is incorrect?
   a. All S. aureus are coagulase positive.
   b. S. aureus is a facultative anaerobe that grows by aerobic respiration or fermentation that yields lactic acid.
   c. S. aureus is a common primary cause of equine pneumonia.
   d. S. aureus produces a variety of exoproteins.

10. TSST-1 usually results in the following combination of clinical signs:
   a. fever, erythroderma, and cutaneous desquamation
   b. emesis, lethargy, and heart failure
   c. arthritis, liver disease, and fever
   d. seizures, emesis, and cutaneous desquamation