Emergence of methicillin-resistant *Staphylococcus pseudintermedius* in domestic animals

by

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Abstract

Staphylococcus pseudintermedius is a pathogenic bacterium of concern within the veterinary sector, and it is involved in numerous types of infections, including localized cutaneous infections, such as canine pyoderma, as well as systemic infections in the urinary, respiratory and reproductive systems. The emergence and high prevalence of methicillin resistant *Staphylococcus pseudintermedius* (MRSP) infections is becoming a growing concern in canine patients. Therefore, it is crucial to understand the involvement of *S. pseudintermedius* in canine disease pathology in order to gain a better understanding of its impact and provide insights into novel treatment strategies. A case study is reviewed in this report, which focuses on a MRSP-infected canine patient with pyoderma, and it discusses some of the common challenges in implementing effective treatments for this infection. Current and future treatment options against *S. pseudintermedius* are also discussed. Since this bacterium has caused a significant impact in the veterinary field, further research is needed to reduce the incidence and recurrence of MRSP infections in canine patients. This will be essential in order to understand how to improve control measures of this infection when there are a limited number of effective treatments.

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Chapter 1 Introduction

This chapter will discuss on the background information regarding antimicrobial resistant bacteria, primarily *Staphylococcus* species. It will introduce *Staphylococcus pseudintermedius* reviewing features about this organism, including the characterization of the organism, and known mechanisms of antimicrobial resistance. The epidemiology of disease caused by *S. pseudintermedius* and its relevance in veterinary medicine will also be highlighted.

Antimicrobial Resistant Bacteria

The emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance (AMR), continues to threaten our ability to treat common infections (WHO, 2020). The rapid global spread of multi- and pan-resistant bacteria (also known as "superbugs") that cause infections that are not treatable with existing antimicrobial medicines has become especially alarming (WHO, 2020).

Antimicrobial resistance occurs when bacteria, viruses, fungi and parasites evolve over time, and they can no longer respond to medicines making infections harder to treat. Increasing the risk of disease spread, severe illness, and death. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become increasingly difficult or impossible to treat (WHO, 2020).

Antimicrobial resistance poses a serious global threat of growing concern to human, animal, and environment health (Hashmi et al., 2020). The environment plays a significant part in spreading antibiotic resistance. During the last 20 years, antibiotic concentrations in the soil and water ecosystems have been gradually increasing as a result of the advancements in the field of environmental sciences (Carvalho et al., 2016). The impact of these antibiotics on

environment is perilous as these drugs are not fully metabolized in the human and animal bodies and they get excreted in large concentration via urine and feces (Hashmi et al., 2020).

These unaltered active compounds get to wastewater treatment plants. Research studies have indicated the emergence of resistant microbes in the wastewater treatment plants. Most often, treatment plants are not conventionally built to eliminate the incoming antibiotics, and as a result, they may be released directly into the receiving niche causing serious concerns for the surrounding habitats (Halling-Sørensen et al., 1998).

Similarly, the unmonitored disposal of drugs and the application of reclaimed water and animal manure in the agricultural sector may also contribute to the spread of antibiotic resistant and bacteria and their genes in the environment (Hashmi et al., 2020). The impact of the antibiotic-resistant strains on natural resources and environment is getting precarious and immediate action plans need to be devised to manage the concerning situation (Hashmi et al., 2020). The presence of antibiotic resistant bacteria in the environment can prove to be a major factor in transferring and strengthening the resistant strains in human hosts (Bengtsson-Palme et al., 2015).

Since the inception of antimicrobial drug use in the practice of medicine, different species of staphylococcus bacteria have evolved in response to the presence of antimicrobial drugs in biological systems. High rates of AMR have been seen in several other bacterium including, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, and *Neisseria gonorrhoeae* (WHO, 2020). Furthermore, drug resistance in viruses is an increasing concern, as well as resistance in fungi, mycobacterium tuberculosis, and malaria parasites (WHO, 2020).

If left untreated any infection can lead to sepsis, and without timely treatment with antimicrobials, sepsis can rapidly lead to tissue damage, organ failure and death (CDC, 2020). Therefore, the cost of AMR to national economies and their health systems is significant as it affects productivity of patients or their caretakers through prolonged hospital stays and the need for more expensive and intensive care (CDC, 2020).

Epidemiological studies have demonstrated a direct relationship between antibiotic consumption and the emergence and dissemination of resistant bacteria strains (Nature, 2013). Resistance of these bacteria can occur based on the organism's genetic makeup and previous or current exposure to antibiotics (Weese, 2012). In bacteria, genes are inherited from relatives or can be acquired from non-relatives on mobile genetic elements such as plasmids (Read et al., 2014). Plasmid transmission describes the transfer genes of antibiotic resistance to the host cell (Lushniak, 2014).

Additionally, resistance development and dissemination can be consequences of horizontal gene transfer (HGT), which describes the lateral movement of genetic information between organisms (Haug et al., 2011). Horizontal gene transfer enables new, antibiotic-resistant variants to arise without the need for genetic mutation (Summers et al., 2006). Furthermore, horizontal gene transfer can allow antibiotic resistance to be transferred among different species of bacteria (Read et al., 2014). Resistance can also occur spontaneously through mutation (Read et al., 2014). Resistance within bacteria is spread by natural selection when antibiotics fail to halt their reproduction while removing their drug-sensitive competitors (Read et al., 2014).

The development of antibiotic resistance to methicillin is a good example how this could occur. Methicillin is a semi-synthetic, penicillinase-resistant penicillin that was developed to overcome penicillin resistance mediated by staphylococcal penicillinases (Morris et al., 2017).

Penicillinases are bacterial enzymes that deactivate both natural penicillin's and aminopenicillins by breaking the core structure of these antibiotics (Morris et al., 2017). Shortly after the introduction of methicillin in human medicine, *S. aureus* developed resistance by acquisition of *mec*A, a gene encoding a specific penicillin-binding protein with low affinity to all beta-lactams antibiotics (Morris et al., 2017). Even though methicillin is no longer used in clinical practice, the term "methicillin-resistant" has persisted and has been used to indicate strains that are resistant to all beta-lactams except the newest generation of cephalosporins, which were specifically developed for treatment of methicillin resistant *Staphylococcus aureus* (MRSA) infections (Berger-Bachi et al., 2002). Methicillin resistance expresses co-resistance to other antimicrobials including aminoglycosides, fluoroquinolones, lincosamides, macrolides, tetracyclines, potentiated sulfonamides, chloramphenicol and rifampicin (Kadlec et al., 2012). When a methicillin resistant strain expresses co-resistance to at least two additional antimicrobial classes, it may be referred to as multidrug resistant (MDR) (Kadlec et al., 2012). Multidrug resistant strains of bacteria have emerged worldwide in veterinary clinics among methicillin resistant isolates from dog and cat cases involving infections. These strains include *S. intermedius*, *S. pseudintermedius*, *S. aureus*, *S. schleiferi* v. *coagulans*, and *S. schleiferi* v. *schleiferi* (Detwiler A et al., 2006-2011).

Staphylococcus **Bacteria**

Staphylococcus spp. naturally colonize a large proportion of the healthy human and mammal populations (Ansari et al., 2019). *Staphylococcus* bacteria are Gram-positive, facultatively anaerobic cocci that are involved in a variety of infections due to their virulence factors, surface proteins, polysaccharides, enzymes, and toxins (Cadieux et al., 2014).

The genus S*taphylococcus* includes many species. The *Staphylococcus* genus comprises 48 species and 21 subspecies (Talon et al., 2015). It is divided into two distinct groups: the coagulase-positive staphylococci (CoPS), such as *Staphylococcus aureus* and six other species, and the coagulase-negative staphylococci (CoNS) (Bonar et al., 2018). Staphylococcal coagulases are secretory proteins that cause blood clotting through the activation of prothrombin (Savini, 2018). The coagulate test is used to categorize the species of staphylococci. The CoNS include *S. epidermidis* and *S. saprophyticus* where *S. epidermidis* is the most prominent (Savini, 2018).

Coagulase positive staphylococci are well recognized as important human and animal pathogens, while the role of CoNS as primary pathogens or opportunistic bacteria is still under discussion, since they have been historically thought to be "non-pathogenic" and yet it is recommended that they should be considered and treated as pathogens (Bertelloni et al., 2021).

Staphylococcus Bacteria in Human Medicine

Staphylococcus aureus is an eminent human pathogen that can colonize the human host and cause severe life-threatening illnesses (Balasubramanian et al., 2017). *S. aureus* is a leading cause of endocarditis, bacteremia, osteomyelitis and skin and soft tissue infections in humans (Harkins et al., 2017). With the rise of hospital-based medicine, *S. aureus* quickly became a leading cause of health-care-associated infections as well. Penicillin offered short-lived relief; however, antibiotic resistance arose in the 1940s that was mediated by the beta-lactamase gene *bla*Z (Harkins et al., 2017).

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first observed in 1960, less than one year after the introduction of the second-generation beta-lactam antibiotic, methicillin (Balasubramanian et al., 2017). Epidemiological evidence suggests that resistance arose during

this time period, when the *mec*A gene encoding methicillin resistance was horizontally transferred to an intrinsically sensitive strain of *S. aureus* (Harkins et al., 2017).

Furthermore, MRSA infections have spread worldwide, appearing at a high incidence in several countries including Europe, the Americas, and the Asia-Pacific region (Ventola, 2015). These multidrug infections can be very serious and are the most frequent occurring among of all antibiotic-resistant threats (Ventola, 2015). Methicillin resistant *S. aureus* is resistant to penicillin-like beta-lactam antibiotics (Sengupta et al., 2013). Several drugs still retain activity against MRSA, including glycopeptides (e.g., vancomycin and teicoplanin), linezolid, tigecycline, daptomycin, and even some new beta-lactams, such as ceftaroline and ceftobiprole (Ventola, 2015). However, MRSA has shown outstanding versatility at emerging and spreading in different epidemiological settings over time including hospitals, the community, and, more recently, in animals (Harkins et al., 2017).

Several studies showed that MRSA clones circulating in cats and dogs are similar to the ones identified in humans and belong mostly to hospital-acquired clones (Loeffler et al., 2010). This is clinically important since hospital acquired MRSA isolates usually carry more virulence genes, specifically genes that code for enterotoxins or the toxic shock syndrome toxin (Mutters et al., 2016). This human-related epidemiology suggests that humans may be the source of MRSA isolated in cats and dogs, however these animals may also act as a secondary reservoir capable of human re-infections in specific contexts (Harrison et al., 2014). At this time, available data on MRSA transmission between humans and companion animals is limited and the public health impact of such transmission needs to be the subject of more detailed epidemiological studies (Loeffler et al., 2010).

Staphylococcus intermedis group (SIG) in Domestic Animals

First described in 2005 as a novel species, *Staphylococcus pseudintermedius* along with two other CoPS species, *S. intermedius* and *S. delphini*, forms the *S. intermedius* group (SIG). Members of the SIG have been identified in a variety of animal species, either as colonizers or as causative agents of diseases, most commonly cutaneous skin infections (Morris et al., 2017). Other CoPS and CoNS can be isolated and cause diseases in dogs including *S. aureus*, *S. schleiferi*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. sciuri*, and *S. warneri* (Bertelloni et al., 2021). Of this group, the primary canine and feline pathogen is now known to be *S. pseudintermedius* (Fitzgerald, 2009).

Studies have confirmed that *S. pseudintermedius* is the most frequently isolate detected in dogs (Beck et al., 2012). It is known that puppies are colonized by maternal staphylococcal flora during the neonatal period and often maintain the strain transferred from the dam for many months after they are separated (Baddour et al., 2010). As adult dogs, it is not common for them to harbor two or more genetically unrelated strains of *S. pseudintermedius* simultaneously, but on different body locations (Iverson et al., 2015). The mouth and nose appear to be the most consistent site for staphylococcal carriage in canines and felines, followed by the perineum (Iverson et al., 2015). Several studies have isolated *S. pseudintermedius* from 46-92% of healthy dogs' nasal, oral, and perineum mucosa (Lynch, 2021).

In canines, *S. pseudintermedius* is also an important pathogen of skin and ear infections (Beck et al., 2012). Several *Staphylococcus* species serve different roles as commensal bacteria and opportunistic pathogens capable of causing serious infections of the skin and many other tissues (Faires et al., 2010). When a cutaneous or systemic disease disrupts the skin’s surface defense mechanisms, the result is either a skin infection (bacterial pyoderma) or otitis externa.

Otitis externa is defined as an inflammatory disease of the external ear canal, including the ear pinna (Bajwa, 2019).

In the case of canine superficial bacterial pyoderma, infection is typically caused by the same strain of *Staphylococcus* that is present at the site of infection (Pinchbech et al., 2007). Additionally, the potential for pathogenicity is determined by the virulence factors expressed by any given *Staphylococcus* strain. Virulence factors include expression of adhesins that the bacterium bind to cells and extracellular matrix, formation of the biofilm that protects the bacterium from the immune response, production of toxins (cytolytic, exfoliative, enterotoxigenic and super- antigenic toxins) and expression of factors that assist in evasion of the host's immune response (Laabei et al., 2014). It should be noted that genetic expression of antimicrobial resistance is not a true virulence factor; therefore, a resistant strain is not necessarily more invasive or pro-inflammatory than a susceptible strain. In fact, acquisition of certain antimicrobial resistance genes may come at a cost to the bacterium. For example, in some strains of MRSA, the methicillin resistant gene is associated with reduced production of biofilm and cytolytic toxins, which would be harmful to the bacterium (Becerio et al., 2013).

Staphylococcus pseudintermedius **in Veterinary Medicine**

Staphylococcus pseudintermedius is an opportunistic pathogen that has emerged as a significant health problem in canine and feline patients because of the development of multidrug resistance. Antibiotic resistance of these bacteria can occur based on the organism's genetic makeup, specifically the presence of the *mec*A gene and previous or current exposure to antibiotics (Weese, 2012). Antibiotic resistance strains of *S. pseudintermedius* is highly associated with the overuse and misuse of antibiotics in human and veterinary medicine, agriculture and industry (Silva et al., 2020).

In 1976, *S. pseudintermedius* was first isolated from rabbits, minks, pigeons, dogs and horses in Palacky University, Olomouc, Czechoslovakia (Hajek, 1976). It was originally identified as *S. intermedius* due to the morphological similarities (Hajek, 1976). In 2005, using a DNA–DNA hybridization technique on *S. intermedius*, *S. pseudintermedius* was identified as a novel species (Ulrika et al., 2016).

Mechanism of Resistance of Methicillin-resistant *Staphylococcus pseudintermedius*

As previously mentioned, MRSA is a critically important pathogen in human medicine. Like MRSA, methicillin- resistant Staphylococcus *pseudintermedius* (MRSP) strains carry the *mec*A gene, which encodes for a modified penicillin-binding protein (PBP) (Moodley et al., 2013). The *mec*A gene is known to alter the affinity of PBP of all beta-lactam antimicrobials, thus creating beta-lactam resistance. In general, beta-lactam antibiotics include penicillin's, cephalosporins, and carbapenems (Silva et al., 2020). Methicillin-resistant *S. pseudintermedius* was first reported in Europe in 2006, and the whole genome sequence of MRSP was reported in 2013 (Moodley et al., 2013).

Methicillin-resistant *Staphylococcus pseudintermedius* in Veterinary Medicine

Methicillin resistant *S. pseudintermedius* is a growing concern in veterinary medicine due to the discovery that isolates from canine patients had a high occurrence of resistance to a wide range of antimicrobials in addition to beta- lactam antibiotics (Lynch, 2021). Along with *S. pseudintermedius*, *S. schleiferi* and *S. aureus* as the primary pathogens encountered in small animal practice, clinical isolates of all three species commonly express methicillin resistance and multidrug resistance (Detwiler et al., 2011).

A recent study reported that 63% of *S. pseudintermedius* strains isolated from sick dogs were methicillin-resistant, with 78% of these isolates were also described as multidrug resistant,

in that isolates were resistant to three or more antibiotic classes (Lynch, 2021). Additionally, it has been reported that up to 97.8% of MRSP isolates show multidrug resistance to three or more antibiotics routinely used in veterinary medicine (Hartantyo et al., 2018). Similarity, MRSP has been increasingly reported as a cause of infections, hence a major concern in clinical practice worldwide.

Epidemiology of Methicillin-resistant *Staphylococcus pseudintermedius*

Regarding transmission, MRSP can be transmitted from sick to healthy canines via direct contact or indirect environmental transmission (Bryan et al., 2012). *S. pseudintermedius* transmission and subsequent colonization may be associated with infections located in different organ systems, skin infections being the most common (Bryan et al., 2012). In general, clones of methicillin resistant *staphylococcus* bacteria are spreading worldwide, and affecting the health of both humans and animals. This makes it an important focus in the development of effective treatment and prevention strategies, in order to decrease the circulation of methicillin resistant staphylococcal infections. It has been hypothesized that MRSP strains are highly clonal, comparable to the evolution of MRSA. Recent studies of the population genetic structure of *S. pseudintermedius* infection isolates obtained from animals in North America, Europe and Japan have proven this hypothesis (Morris et al., 2017). Two major clonal lineages have disseminated throughout Europe [Sequence Type (ST) 71], North America (ST 68) and Japan (ST 71) and other less common clonal lineages may be emerging (Morris et al., 2017).

Compared to MRSA, the emergence of MRSP is of greater concern for veterinary patients as *S. pseudintermedius* is the primary staphylococcal species that colonize healthy dogs and cats (Bryan et al., 2012).

In summary, methicillin resistance has been identified in another species of staphylococci bacteria that was first reported nearly 50 years ago and found in worldwide locations. Infections caused by MRSP pose a major clinical challenge in veterinary medicine, especially for the effective treatment of canine cases of pyoderma. In the following chapters, the focus of this report will be to review the clinic relevance of MRSP infections in canine patients and discuss the current challenges associated with its treatment.

Chapter 2 Methicillin-Resistant *Staphylococcus pseudintermedius* in Canines

This chapter will focus on the emergence, prevalence, transmission and clinical impact of methicillin- resistant *Staphylococcus pseudintermedius* (MRSP). It will also highlight common diagnostic tools, treatment protocols, and prognosis of MRSP infections in canines as well as explain the importance of prevention of this multidrug resistant bacterium.

Emergence

Staphylococcus pseudintermedius is associated with numerous infections in animals, and it is a common pathogen in multiple other canine disease pathologies, including urinary tract, respiratory, and reproductive tract infections. It is also a major issue in canine cutaneous infections, and this will be elaborated further in this chapter.

Urinary Tract Infections

Urinary tract infections (UTI) are a common diagnosis within veterinary practices (Lynch, 2021). Numerous bacteria species have been isolated previously from canine UTI cases, including *Enterococcus* spp., *Proteus* spp., *Staphylococcus* spp. and *Streptococcus* spp., with *Escherichia coli* identified as the most common pathogens (Lynch, 2021). These pathogens are isolated in up to 51% of cases of canine UTIs (Roberts et al., 2019). *S. pseudintermedius* has been shown as the most common *Staphylococcal* spp. isolated in canine UTIs, with studies reporting a variable frequency of *S. pseudintermedius* isolation in 6.3–94.7% of canine UTIs (Windahl et al., 2014).

Recent studies report high rates of MRSP and multidrug resistant (MDR) *S. pseudintermedius* from UTIs in canines, with significant increases in methicillin and gentamicin resistance in *S. pseudintermedius*, and significant increases in fluoroquinolone antibiotic

resistance over a 7-year period (Roberts et al., 2019). Therefore, antimicrobial resistance in *S. pseudintermedius* is impacting the resolution of UTIs that often, result in major therapeutic limitations.

Respiratory Tract Infections

Respiratory tract infections (RTI) in canines are relatively common and encompass various diseases etiologies including bacterial pneumonia, canine infectious respiratory disease complex (CIRDC) and viral infections. Additionally, these microorganisms are readily passed between dogs in social settings such as dog parks and boarding kennels (Moyaert et al., 2019). Several bacterial species are associated with RTI, particularly *Staphylococcus* spp., including *S. pseudintermedius*, which is isolated in 9.3–60% of RTI in canines (Hoekstra et al., 2002). The heightened prevalence of *S. pseudintermedius* is likely due to the presence of *S. pseudintermedius* in the mouths of healthy canines that enter the lungs and cause infection (Moyaert et al., 2019). Trends of antibiotic resistance in respiratory isolates are being reported; therefore, studies suggest reducing the use of broad-spectrum antibiotics and avoiding the use of previously prescribed antibiotics that, may limit the treatment availability for bacterial RTI in canines in the future (Moyaert et al., 2019).

Reproductive Tract Infections

Staphylococcus pseudintermedius infections have been associated with the reproductive tract system, specifically the uterus and the mammary glands where this bacterium has been linked with canines pyometra and mastitis, respectively (Rota et al., 2011). Canine pyometra is an infection within the uterus of breeding female dogs with *E. coli* and *Staphylococcus* spp. predominantly isolated from these cases (Hagman, 2018). Clinical mastitis in canines has been

associated with *S. pseudintermedius*; however, the prevalence of *S. pseudintermedius* in mastitis cases has not been well researched (Rota et al., 2011).

Prevalence

The prevalence of MRSP infections in dogs has increased in recent years (Hartantyo et al., 2018). Recent reports of the prevalence of canine MRSP-associated infections vary worldwide, and depending on the country, it ranges from 7.4 to 68.1% (Hartantyo et al., 2018).

Transmission

Although, *S. pseudintermedius* has been identified as part of the normal flora of canines, it is unclear whether this commensal species initiates the infection or if it is associated with secondary infections. Thus, more studies are needed to understand the role of *S. pseudintermedius* infections and whether transmission of the bacteria has contributed to the increasing prevalence in canine cases of MRSP infections. This information would be important to understand in order to develop effective strategies to prevent the spread of this infection.

Like most staphylococcus bacteria, MRSP can be transmitted through direct or indirect environmental contamination (Laarhoven et al., 2011). Humans, other companion animals, and certain home environments, such as pet bedding have been associated with staphylococcal carriage or infection in dogs and cats (Davis, 2015). Transmission of MRSP by infected pets to other individuals in the home or community is known to occur, but the results from studies are inconclusive (Davis, 2015). Therefore, limiting dog-to-dog contact as individuals or as defined groups, avoiding contact with animals that are at increased risk and avoiding contact during periods of heightened risk are recommended (Morris et al., 2017). Heightened risks would include atopic flares, where animals who are pruritic, and/or develop ulcerated skin lesions. Transmission of MRSP from both healthy and sick pets to owners probably occurs regularly,

although *S. pseudintermedius* is not a normal bacterial inhabitant of people, and it is not well adapted to cause disease in humans. However, a recent study found that of 24 human clinical cases of *S. pseudintermedius* infection, 91.7% had confirmed contact with dogs temporally with *S. pseudintermedius* infections, which reflect the possibility of zoonotic transmission of this species (Somayaji et al., 2016). Therefore, *S. pseudintermedius* is becoming increasingly recognized as a potential zoonotic organism of canine origin (Somayaji et al., 2016). This is a serious concern, since emerging antimicrobial resistant infections are becoming more prevalent and difficult to treat with available antimicrobials in both in animals and humans (Ventola, 2015).

Clinical Presentation of Canine Pyoderma

Staphylococcus pseudintermedius is known for its pathogenic properties in canine infections, but it is also a primary commensal bacteria species of canines. As discussed previously, this bacterium is the primary infectious agent in a wide range of infections, including external ear otitis, abscess formation, urinary tract infections, mastitis, endocarditis, with pyoderma being of particular importance.

Canine pyoderma is a bacterial skin infection commonly diagnosed in companion animal veterinary clinics. It is often associated with moderate to severe ulcerated skin lesions, redness, pain and inflammation (Morris et al., 2017). Pyoderma can be the result of underlying factors such as sensitivities to environmental/food related allergens, endocrinopathies, and ectoparasites (Lynch, 2021). These sensitivities initiate the colonization of pathogenic *S. pseudintermedius* and lead to infection by disrupting the epidermal barrier and/or alter the immune system (Noli et al., 2014). Other bacterial pathogens that may colonize the skin including streptococcus, *Proteus mirabilis* and *Escherichia coli*, and *Pseudomonas aeruginosa*, and fungal agents like *Malassezia*

pachydermatis (Noli et al., 2014). These are occasionally isolated from skin infections but are thought to occur as opportunistic infections, secondary to *S. pseudintermedius* primary infections (Noli et al., 2014). Colonization implies that a bacterial population is self-sustaining for an extended period of time in the absence of disease (Lynch, 2021). Identifying and controlling the underlying cause is *critical* for effective treatment and prevention of recurrence.

In Chapter 3, a clinical case of canine pyoderma caused by MRSP will be discussed highlighting some common and important features of this infection.

Diagnosis

Once pyoderma is suspected and in order to identify the causative agent of infection, general diagnostic tools include phenotypic tests such as bacterial isolation, antimicrobial susceptibility testing, and species confirmation by polymerase chain reaction (PCR) or matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF). Polymerase chain reaction is a very sensitive technique that allows rapid amplification of a specific segment of DNA, which allows detection and identification of gene sequences using visual techniques based on size and charge of the amplified DNA fragment (Garibyan et al., 2013). While, MALDI-TOF mass spectrometry (MS) can measure the mass of molecules from a sample that has been embedded in a matrix by using a laser to ablate and desorb the molecules with minimal fragmentation (Singhal et al., 2015). This diagnostic tool allows for the measurement of these charged molecules (Singhal et al., 2015). Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth *in vitro* (Khan et al., 2019). It specifies effective antibiotic dosage and formulates a profile of empirical therapy for the proper management of an individual (Khan et al., 2019). Furthermore, selective culture media would allow for presumptive identification of MRSP as early as 24 hours after receipt of a

specimen, compared to at least 48 or 72 hours using traditional culture methods (Silva, 2015). Molecular assays have been designed for rapid detection of the gene, *mec*A, which is considered the gold standard for methicillin-resistance testing. The *mec*A gene is responsible for beta-lactam resistance in staphylococcus bacteria (Loeffler et al., 2010). However, these assays cannot readily differentiate between staphylococcal species without bacterial culture and are not practical in smaller diagnostic laboratories. Thus, aerobic culture and antimicrobial susceptibility testing are the most practical and cost-effective diagnostic tools, typically sent to and performed in commercial bacteriology and mycology laboratories.

Treatment Strategies

Therapeutic recommendations for MRSP infections include topical and systemic antimicrobials. Topical treatments can easily access and penetrate the skin. Common topical therapy consists of chlorhexidine shampoo, solution and spray, ketoconazole shampoo, wipes and flush, mupirocin ointment, and benzoyl peroxide shampoo (Noli et al., 2014). A systematic review of topical therapy for canine skin infections treatments concluded that, based on randomized controlled trials, topical therapies were sparse. However, there was evidence supporting the use of shampoo containing 2–3% chlorhexidine for bacterial skin infections (Kadlec et al., 2012).

Although systemic antimicrobial therapy is recommended for superficial pyoderma with or without added topical medication, the recommendation can be risky given the issue of increased antimicrobial resistance in human and animal medicine. Recent studies have provided evidence that topical therapy as the sole antibacterial treatment can be effective in superficial pyoderma, reducing the need for systemic therapy in some cases. (Kadlec et al., 2012). Additionally, alternative topical therapies, such as hypochlorite (bleach), and manuka honey

have been introduced as successful alternate topical therapies. Manuka honey originates from the manuka flower. A recent study provided evidence that low concentrations of manuka honey can inhibit the growth of clinical isolates of *S. pseudintermedius* with a bactericidal mode of action, and when combined with selected antibiotics, it can increase the efficacy of treatment (Brown et al., 2020). Household bleach (sodium hypochlorite) has also been found to decrease bacterial load, reduce clinical lesion severity, and improve owner assessment scores from dogs with superficial pyoderma associated with *S. pseudintermedius* (Fadok et al., 2019). Topical therapy should be used as the primary treatment for superficial infections when the canine patient and owner are willing to comply with this recommendation.

Systemic treatment is indicated for those animals with deep, widespread, and severe pyoderma, as well as in animals that are not amenable to topical therapies (Frank et al., 2012). The efficacy of systemic antibacterial therapy for MRSP infections depends predominantly on susceptibility of the organism, but it will also be determined by appropriate drug administration including accurate dosing, owner compliance and clinical variables such as the severity of disease, the causative agent, and concurrent diseases (Kadlec et al., 2012).

Due to the extensive MDR associated with all MRSP, treatment choices for systemic therapy are substantially limited. Currently, it is common to isolate MRSP that is susceptible to very few antimicrobials. Susceptibility is usually limited to amikacin, rifampicin, vancomycin and linezolid (Jones, 2007).

Amikacin is an aminoglycoside that is not typically used for treating dogs with skin infections because of its parenteral administration; however, it is being used more frequently now, with the emergence of MRSP (Frank et al., 2012). The major potential adverse effect of amikacin is nephrotoxicity, and its use is contraindicated with renal disease, and prolonged use in

linked to deafness (Frank et al., 2012). Use of this antibiotic should be based on culture and susceptibility results when no other antibiotic is effective (Frank et al., 2012).

Rifampicin is a bactericidal antibiotic with broad spectrum of activity against many Gram-negative and most Gram-positive microorganisms and is the most active antibiotic known against staphylococci (Frank et al., 2012). Rifampicin is potentially hepatotoxic, and this adverse effect appears to occur more commonly in dogs than in people (Frank et al., 1990). Mild increases in alkaline phosphatase activity occur frequently and appear to be benign; however, treatment should be discontinued if there are concurrent increases in other hepatic enzyme activities, because acute hepatic toxicity and death have been described (Frank et al., 2012). Other rare signs associated with rifampicin administration in dogs include thrombocytopenia, hemolytic anemia, anorexia, vomiting and diarrhea (Frank et al., 2012).

Vancomycin and linezolid are two antimicrobials developed to treat methicillin resistant *Staphylococcus aureus* infections in humans when no other antimicrobials are effective (Frank et al., 2012). Vancomycin is a glycopeptide antimicrobial that is administered parenterally via slow intravenous infusion, and linezolid is a synthetic antibiotic that can be administered orally (Frank et al., 2012). While linezolid has been used effectively to treat methicillin-resistant staphylococcal infections in dogs and cats, there are no published reports of the effective use of vancomycin in animals in a veterinary clinical setting (Murphy, 2008). Use of these antimicrobials in animals, however, must be strongly discouraged in light of their place in the treatment of serious, potentially life-threatening MRSA infections in people (Weese, 2012).

Evidently, there is a therapeutic dilemma with the antimicrobials listed above. There is a potential for drug toxicities and ethical use considerations (Frank et al., 2012).

Currently, topical treatments are most likely used in combination with systemic antimicrobials, since topical treatments rapidly resolve lesions, show low rates of resistance and reduce the frequency and duration of antibiotics that may decrease the evolution of antibiotic resistance (Hillier et al., 2014). Current published advice on the duration of treatment includes 3 weeks for cases of superficial pyoderma with an additional 2 weeks of treatment beyond clinical resolution and 4–6 weeks for deep pyoderma with an additional 2 weeks of treatment beyond clinical resolution (Beco et al., 2013). If treatment regimens are prescribed for 3 weeks or longer duration, the patient should be reevaluated prior to discontinuation of therapy.

There is little evidence for a difference in outcome between MRSP and MSSP in animals, and the prognosis for MRSP skin infections in pets is good, depending on the underlying cause and co-morbidities (Morris et al., 2017).

In conclusion, the emergence of MRSP has become an important focus in veterinary medicine as well as a human concern due to its zoonotic potential. Treatment of canine pyoderma cause by MRSP is extremely challenging given the limited number of effective antibiotics. Treatment of MRSP cutaneous infections in canines should target the underlying disease that leads to pyoderma. A case study of canine pyoderma cause by MRSP will be discussed in Chapter 3, highlighting strategies for the diagnosis, treatment, and prevention.

Chapter 3 Case Study

A neutered male, 5-year-old cocker spaniel was referred to a specialty veterinary dermatology clinic for chronic, recurrent skin problems. The primary concern was the non-healing cutaneous lesions associated with pruritus. The patient had a history of recurring atopic dermatitis over the past 2 years that had been treated with antimicrobials including cefpodoxime, a third-generation cephalosporin antibiotic. A summary of each visit to the clinic is outlined below. It should be noted that all cytologic samples were obtained using a tape and glass slide impression smear technique (Layne et al., 2017), and interpreted by a board-certified dermatologist.

Initial Examination

On September 15, 2020, the patient was presented for chronic dermatitis. Clinical findings included multi focal collarettes over the dorsum and some on the chest/abdomen. Epidermal collarettes are secondary lesions from a ruptured pustule (Englar, 2019). Epidermal collarettes have a common appearance of circular rims that outline the location of the ruptured pustule, and it consists of peeling skin with flakes (Figure 3.1). The patient had developed collarettes and pustules along the dorsum (Figures 3.2 and 3.3). In addition, dry, crusty scabs were also present that appeared to be composed of a mixture of purulent discharge, serum, and dried exudate. These scabs can be the result of ruptured pustules and often adhere to the hair coat (Englar, 2019). At this time, the clinician began treatment with a 21-day course of cefpodoxime (5–10 mg/kg every 24 hours orally). With client compliance, the patient returned 3 weeks later for a follow up examination.

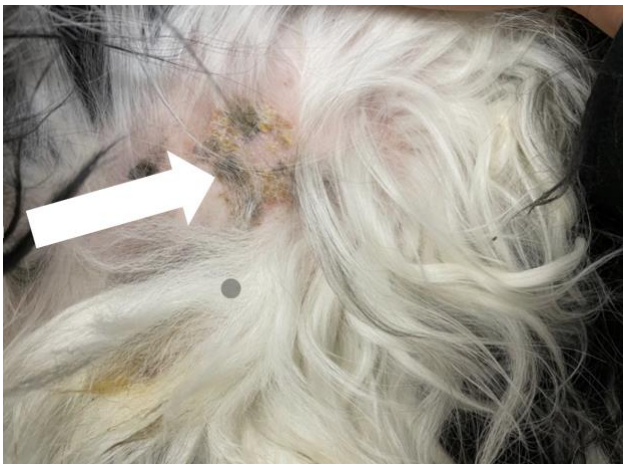

Figure 3.1 Epidermal collarette located on the patient's abdomen on 9/15/20. The white arrow indicates the location of an epidermal collarette.

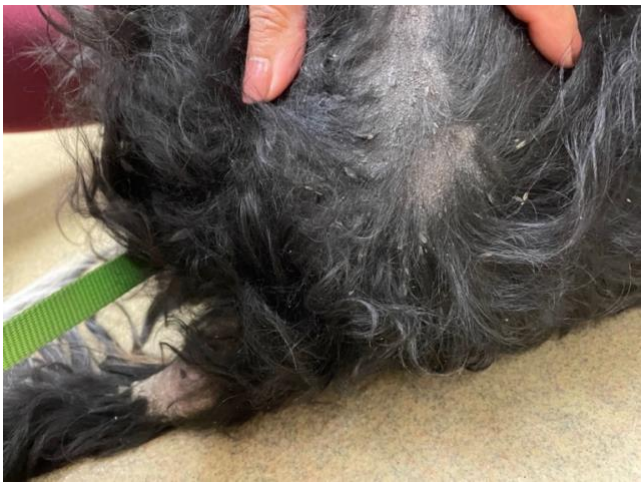

Figure 3.2 Pictured are secondary lesions, such as epidermal collarettes, and crusty scabs located on the patient's caudal dorsum on 9/15/20. Hair loss as a result of epidermal collarettes developed on caudal dorsum.

Figure 3.3 Epidermal collarette on the caudal dorsum of patient on 9/15/2020. Note: Green structure in image is the leash.

Follow up Examination: 3 Weeks After Initial Presentation

On October 6, 2020, the patient presented for a re-examination of the pyoderma. Clinical findings included multi focal scaling collarettes over the dorsum, flanks, occasionally on chest and groin. Several collarettes were still active, erythematous, scaley, and overall, there had been very little improvement following treatment. Diagnostic testing, specifically skin cytology, revealed numerous red blood cells, neutrophils, and bacterial cocci. The lesions showed no signs of improvement since initial presentation. Therefore, a sample of the skin lesions was collected, for culture and isolation as well as antimicrobial susceptibility testing.

Laboratory Findings

One week after the recheck examination (October 13, 2020), the results from the aerobic bacterial culture and antimicrobial sensitivity assays yielded *Staphylococcus pseudintermedius* as the primary organism.

Table 3.1 lists the antimicrobials and the minimum inhibitory concentrations (MICs) and of each of the bacteria isolated from this case. The MIC is the lowest concentration (μg/mL) of an antibiotic that inhibits the growth of a bacterial isolate (Kowalska-Krochmal, 2021). As outlined in Table 3.1, the MIC of antimicrobials can vary. The range of dilutions differs by drug and bacterial species; therefore, comparing MICs of different antibiotics is not based solely on the numerical value, but also, it is based on how far the MIC is from the site of the infection, and other considerations, such as the age, species, and health of the animal (Noli et al., 2014).

Table 3.1 Minimum inhibitory concentrations (MICs) of *S. pseudintermedius*

Antibiotic	Interpretation	MIC (μg/mL)
Amoxicillin (Clavamox)	Resistant	16
Amikacin	**Susceptible**	<4.0
Baytril (Enrofloxacin)	Intermediate	1.0
Cefoxitin (2nd gen.)	Resistant	16.0
Cefazolin (1st gen.)	Resistant	16.0
Ceftazidime (3rd gen.)	Resistant	16.0
Cefpodoxime (3rd gen.)	Resistant	8.0
Cefovecin (3rd gen.)	Resistant	8.0
Ceftiofur (3rd gen.)	Resistant	>4.0
Chloramphenicol	**Susceptible**	<4.0
Ciprofloxacin	**Susceptible**	<4.0
Clindamycin	Resistant	4.0
Doxycyline	Resistant	>0.5
Erythromycin	Resistant	>4.0
Gentamicin	**Susceptible**	2.0
Imipenem	Resistant	8.0
Marbofloxacin	**Susceptible**	0.5
Mupirocin	**Susceptible**	—
Oxacillin	Resistant	>4.0
Rifampin	**Susceptible**	—
Trimethoprim Sulfa	**Susceptible**	0.5/9.5
Ticarcillin	Resistant	16.0
Ticarcillin/Clavamox	Resistant	16.0/2.0
Tobramycin	**Susceptible**	—

*Dashed lines indicate MIC lower than <4.0.

Based on these results, it was recommended to discontinue cefpodoxime as the organism *S. pseudintermedius* in this case was determined to be resistant to this antimicrobial. Based on culture and antimicrobial susceptibility results, *S. pseudintermedius* was also resistant to the following antimicrobials: amoxicillin, cefoxitin, cefazolin, ceftazidime, cefpodoxime, cefovecin, ceftiofur, clindamycin, doxycycline, erythromycin, imipenem, oxacillin, ticarcillin, and ticarcillin/clavamox. In contrast, *S. pseudintermedius* was susceptible to the following antibiotics: amikacin, chloramphenicol, ciprofloxacin, gentamicin, marbofloxacin, mupirocin, rifampin, trimethoprim sulfamethoxazole, and tobramycin. Therefore, it was advised to initiate the antibiotic, trimethoprim sulfamethoxazole at 18mg/kg twice daily, and apply mupirocin ointment to the affected lesions twice daily for 21 days.

Follow up Examination: Six Weeks After Initial Presentation (11/3/2020)

Three weeks after the initiation of trimethoprim sulfamethoxazole and mupirocin treatments (November 3, 2020), the patient presented for a follow up examination after the completion of the 21-day course of oral trimethoprim sulfamethoxazole (18mg/kg twice daily) and topical mupirocin (applied twice daily to the affected areas). Pruritus had improved as reported by the owner. Clinical examination revealed a few remaining epidermal collarettes that were not fully healed, primarily on the inguinal area, caudal thighs and mid abdomen. Skin cytology was performed, and as before, it revealed occasional bacterial cocci (Figure 3.4). It was recommended to extend the duration of treatment with trimethoprim sulfamethoxazole for an additional 14 days as well as continue topical application of mupirocin ointment application to the remaining lesions. After the 14-day course treatment, the owner noted the resolution of the patient's skin lesion.

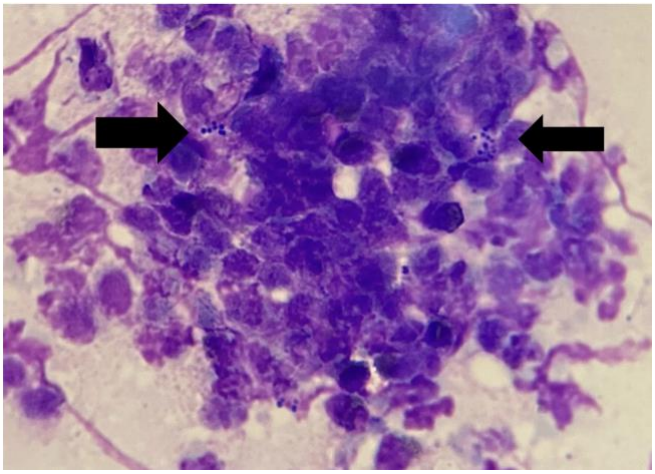

Figure 3.4 Microscopic view of cocci bacteria collected from a pustule lesion on 11/3/20 (100x magnification). Clusters of cocci bacteria are indicated with the black arrows.

Follow up Examination: 10 Months After Initial Presentation

On August 18, 2021, the patient presented for recurrence of a bacterial skin infection. Clinical findings included multi focal scaling collarettes over the dorsum. Skin cytology of epidermal collarettes revealed numerous neutrophils and cocci. These lesions appeared similar to as the lesions during the first examination on September 15th, 2020. Due the effectiveness of these treatments in 2020, it was advised to re-start another 21-day course of trimethoprim sulfamethoxazole (18 mg/kg twice daily), as well as topical bleach rinses and the topical application of mupirocin ointment. Bleach rinses consist of the use of equal parts hypochlorite (bleach) and water as a topical remedy. This topical treatment is commonly mixed in a 32 oz spray bottle and applied to the skin lesions daily.

Follow up Examination: 11 Months After Initial Presentation

The patient was reexamined 4 weeks after the completion of treatment (September 15, 2021). There was no change in pruritus and physical exam revealed several active epidermal

collarettes, specifically on the rump, abdomen, and cranial dorsum, skin cytology of caudal dorsum collarettes revealed numerous neutrophils and cocci. Due to the patient's poor response to treatment, the antimicrobial treatment recommendation was adjusted based on the previous antimicrobial susceptibility results. Therefore, it was advised to administer oral chloramphenicol (50 mg/kg every eight hours) and continue with daily bleach rinses and the topical application of mupirocin to affected areas twice daily.

Follow up Examination: 12 Months After Initial Presentation

On October 13, 2021, after 4 weeks of treatment, there was marked improvement in the clinical presentation after the administration of chloramphenicol with near resolution of the pyoderma and cessation of pruritus. There were no recommended treatments following this examination, and as of October 18th, 2021, there have been no more recurrences of pyoderma in this patient. It was recommended to the owner that the next steps would be to investigate any underlying causes of the patient's recurrent infection.

For this case, recurrent pyoderma primarily occurred in the summer and fall seasons. This suggested that seasonal allergic sensitivities may have led to the recurrence of pyoderma in this patient. Since allergy testing is considered the gold standard in the selection of allergens, intradermal allergy testing was performed on the patient to reveal specific environmental inhalant allergies. Results indicated that the patient has several pollen allergies, including fall weeds and summer grasses. These allergens were selected based on the correlation of the test results and clinical history (Noli et al., 2014). The results of the allergy test in this patient correlated well with the history of summer and fall allergies.

To reduce the underlying cause of pyoderma in this patient, allergen-specific immunotherapy (ASIT) was initiated to desensitize the immune system to the pollen allergens.

Canine ASIT is administered subcutaneously and requires patience and excellent client communication for an optimal outcome. Canine ASIT has been associated with an increase of allergen-specific serum IgG within the first 6 months of treatment (Noli et al., 2014). The allergen specific IgG antibodies are "blocking antibodies", and thought to decrease availability of antigen for mast cell-bound allergen specific IgE antibodies in canines (Noli et al., 2014). Several studies in canines have described a decrease in allergen specific IgE after a year of successful immunotherapy treatment (Noli et al., 2014). The importance of IgE in canine atopic dermatitis will be further discussed in Chapter 4.

Discussion

Methicillin-resistant staphylococci (MRS) can be resistant to multiple classes of antimicrobials other than methicillin itself; therefore, this makes cases of pyoderma very difficult to treat due to the limited selection of antimicrobials susceptible to this bacterium. A major concern of MRS is that methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) continues to spread globally at a worrying rate, and this has implications for the pool of antimicrobial resistance genes amongst bacterial pathogens and potentially for human health (WHO, 2020).

Prescribed antibiotics are becoming increasingly ineffective as drug-resistance spreads globally leading to more difficult to treat infections and resultant death. New antibacterial treatments are urgently needed; however, if society does not change the way antibiotics are currently used, then the effectiveness of any new antibiotics will suffer the same fate as current ones (WHO, 2020).

In this study, the patient was effectively treated with the antimicrobial chloramphenicol as well as with the topical treatments of bleach rinses and an antimicrobial ointment. This regimen was chosen based on the culture and susceptibility results, which identified the primary

organism as *S. pseudintermedius*. However, the patient's lack of response to this antimicrobial required additional diagnostics to inform a more effective treatment regimen.

The antimicrobial resistance characteristics of the patient's isolates are shown in Table 3.1. As shown, the findings revealed resistance to over 10 common antibiotics in veterinary medicine. Overall, the accurate and rapid culture and antimicrobial susceptibility testing of MRSP is essential for delivering effective antimicrobial therapy and implementation of appropriate infection control measures in a timely manner. Additionally, these results revealed strong resistance to the antimicrobials cefpodoxime. Although treatment of the patient's MRSP infection initially appeared to be responsive to trimethoprim sulfamethoxazole (as determined in October 2020), it took less than a year for the bacterium to develop resistance. As mentioned in Chapter 1, antimicrobial resistance occurs naturally over time, usually through genetic changes (WHO, 2020). Additionally, antimicrobial resistant organisms are found in people, animals, food, plants and the environment such as, water, soil and air (WHO, 2020). They can spread from person to person or between people and animals. While the exact mechanism behind *S. pseudintermedius* resistance was unknown in this case, perhaps competition of strains with different antimicrobial sensitivities should be considered as a possible explanation.

Ideally, a second culture and susceptibility panel should have been performed after the lack of improvement after the first course of trimethoprim sulfamethoxazole, but the assay was not financially feasible by the owner.

Based on the original culture and susceptibility results, it was elected to initiate systemic treatment with chloramphenicol. In the literature, chloramphenicol has been associated with not only gastrointestinal adverse effects, but it has also been shown to cause bone marrow suppression and hepatotoxicity (Bryan et al., 2012). The patient tolerated the treatment regimen

of chloramphenicol well with no side effects; however, this is not the case in most canine patients. Thus, proper monitoring should be considered upon choosing this potentially toxic antimicrobial for treatment. Monitoring of clinical signs such as limping, difficulty walking, difficulty standing, and lethargy are all signs that owners and veterinarians should monitor during treatment. Cats are more susceptible than dogs to adverse effects with this antibiotic, and so, a lower dose should be recommended in felines (Frank et al., 2012).

In contrast to the other susceptible antibiotics identified in the culture and susceptibility assay, chloramphenicol was elected because the side effects were less cumbersome. Other susceptible antibiotics included rifampin, amikacin and marboquin. Like chloramphenicol, these have been linked to toxicities such as nephrotoxicity, and hepatoxicity (Frank et al., 2012).

Overall, this case study describes a typical presentation of canine pyoderma, in which the selection of antibiotic treatment depends on the results of the diagnostics assays. Given the issues related to the transmission of the antibiotic resistance genes and the limitations of available antibiotics, the most effective strategy in successfully managing MRSP-infected pyoderma cases should focus on the underlying cause (or causes) of the infections.

Chapter 4 Discussion and Future Directions

This chapter will discuss the management and treatment strategies of methicillin resistant *Staphylococcus pseudintermedius* (MRSP) infections in canines. It will also review potentially effective treatment options for use in canine cases in the future and these are based on current treatment strategies used for methicillin resistant *Staphylococcus aureus* (MRSA) in humans.

Treatment Strategies

With the recognition of the complexity of the pathogenesis of canine pyoderma and MRSP, it is now recognized that treatment approaches must be personalized, flexible, and use several different modes of therapy. A new multifaceted and proactive approach to treatment includes correcting the underlying pathogenesis of the disease where possible, preventing acute flares, and forestalling the development of chronic inflammatory changes in the skin (Noli et al., 2014). Important elements within this approach include identification and elimination of relevant allergens, modification of immune response through allergen -specific immunotherapy (ASIT), controlling secondary infections, that contribute to discomfort and augment the allergic and inflammatory responses, and restoring the epidermal barrier to reduce entry of allergens and the colonization by microorganisms including *S. pseudintermedius*. (Noli et al., 2014). It should be noted that this new approach includes proactive therapies that yield gradual results rather than immediate ones; therefore, client education is essential in order to have the best opportunity in controlling this life-long disease. Bacterial infections of the skin are invariably a secondary event that occurs in response to a reduction in the protective properties of the skin, and canine atopic dermatitis (CAD) is a common cause of this. Therefore, it is crucial to further investigate the pathogenesis of CAD to better manage atopic dermatitis.

Management of Atopic Dermatitis

Allergen-specific immunotherapy (ASIT) remains one of the single most valuable and proven long-term treatments for CAD (Noli et al., 2014). Canine atopic dermatitis can be defined as a predisposed inflammatory and pruritic allergic skin disease associated with IgE antibodies directed against environmental allergens (Noli et al., 2014).

Pathogenesis of Canine Atopic Dermatitis

Research studies that focus on CAD have progressed over the years, where it is now known that atopic dogs have a genetically inherited defect of their skin barrier (Noli et al., 2014). This defect leads to allergic sensitization, commonly to environmental allergens. This creates a cascade of lymphocytic response and a release of cytokines that result in the overproduction of the allergen specific IgE and fulminant eosinophilic response (Frank et al., 2012). As a result, mast cell degranulation and chemokines stimulate the inflammatory response and yield the release of pruritogenic cytokines, such as interleukin (IL)-31, leading to self-trauma by the canine and deterioration of the skin barrier (Noli et al., 2014). *Staphylococcus pseudintermedius,* being an opportunistic pathogen, uses this trauma to colonize and produce infection. These changes in the epidermis favor the establishment of secondary infections and further initiate the vicious cycle of allergic sensitization (Messman et al., 2016).

Pruritus and erythema are the presenting features of CAD, therefore the treatment goal of allergen- specific immunotherapy treatment is to reverse the underlying pathogenesis of the disease, and it has an excellent safety profile (Noli et al., 2014). Other treatments to control pruritus in CAD include oral and topical corticosteroids, antihistamines, and Oclacitinib (Apoquel®). Oclacitinib inhibits the inflammation and pruritus that is mediated by cytokines including IL-31 (Frank et al., 2012). These medications have clear evidence of effectiveness for

CAD, which could eliminate the subsequent occurrence of pyoderma and lessen the need for systemic antimicrobials for treatment.

Topical Treatments

Conservative use of systemic antimicrobials has become crucial due to the concern for the development antimicrobial resistance, and so, topical therapy is often sought as an alternative or supplemental treatment for canine pyoderma (Messman et al., 2016). Current guidelines created by the American and European Colleges of Veterinary Dermatology state that canine pyoderma caused by *S. pseudintermedius* should be treated at the minimum with topical and/or systemic antimicrobial therapies (Lynch, 2021). Topical treatments used in combination with systemic antimicrobials are preferred, since topical treatments rapidly resolve lesions, show low rates of resistance and reduce the frequency and duration of systemic antibiotics (Hillier et al., 2014).

In the case study described in Chapter 3, client compliance played a large role in the patient's successful resolution of disease. The owner was diligent with the recommended topical treatments of daily bleach rinses and the application of mupirocin ointment to the affected areas twice per day. In particular, bleach rinses have shown to be an effective regimen. With the dilution of equal parts water and household bleach, this is an inexpensive treatment and bleach is highly effective at killing even resistant bacteria. It is typically well tolerated and non-irritating to skin lesions.

Mupirocin ointment is a topical bacteriostatic antimicrobial and is the most widely prescribed topical treatment for MRSA colonization in humans (Park et al., 2018). In animals, mupirocin ointment remains an effective treatment option for canine pyoderma. However, in a recent study it was determined that MRSP previously exposed to mupirocin exhibited a high-

level of resistance in phenotype and genotype (Park et al., 2018). In particular, resistance genes and increasing minimum inhibitory concentrations (MICs) in staphylococcal isolates from humans and animal cases have been described (Morris et al., 2017).

Although the argument in favor of topical antibacterial therapy for canine pyoderma is convincing, the choice of drug, particularly in creams, gels and ointments, is more complicated as some topical treatments, like mupirocin, are not licensed for use in dogs in other countries, given its important role and use in human medicine (Park et al., 2018). Therefore, continuous monitoring for mupirocin resistance is important in small animal practice in order to reduce the potential transmission of these resistant genes to bacteria that impact human health.

The management of canine pyoderma with topical treatments is further complicated by the hair coat and hair follicles as target treatment sites of canine pyoderma (Bajwa, 2019). The use of topical therapy is more difficult in areas of haired skin as the hair can obstruct the skin lesions from treatment (Löflath et al., 2007). Since the mouth, nose and anus are important sources of *S. pseudintermedius,* this suggests that the organism is seeded to different locations on the body that are covered in hair (Lynch, 2021).

Regarding the hair follicles, the distal hair shaft may also act as a trap for bacteria in the environment and it has been shown that more bacteria can be isolated from the distal hair shaft than from the skin at multiple locations on the body (Kerk et al., 2018). It is possible that the presence of residual topical antimicrobial agents on the hair shaft may inhibit infection and bacterial reproduction. Thus, topical antimicrobial products may serve two functions: to treat the pyoderma and to help prevent reinfection from bacteria present on the hair shafts (Messman et al., 2016).

Alternative Therapeutics

There is a significant need for alternative therapeutics. Two progressive areas of research for the treatment of canine pyoderma are vaccines and phage therapy. Both have been successful in different animal diseases and offer promising alternative therapies for canine pyoderma and other *S. pseudintermedius* related diseases (Lynch, 2021). There has also been some work involving nature-derived therapeutics. Several alternative therapeutics for MRSP infections are discussed below.

Vaccines

Vaccines for use as therapeutics may include bacterins and subunit vaccines. Bacterins are inactivated vaccines consisting of whole cell or lysed suspensions of bacterial strains and with additional of adjuvants that, aim to elicit an immune response within the host (Lynch, 2021). The commercialization of a bacterin vaccine, known as Staphage Lysate (SPL®) has been an addition to treatment options for canine pyoderma. Staphage lysate is prepared by lysing cultures of *S. aureus* using staphylococcal bacteriophages, followed by bacterial sterilization (Borku et al., 2007). One study found that SPL injections resulted in significant decreases in pruritus levels at 12-23 weeks following treatment (Borku et al., 2007). The likelihood of pyoderma is reduced by decreasing the pruritus in canines. It appears bacterin therapy may be used in combination with alternative antimicrobial strategies for the management of staphylococcal canine pyoderma. However, further development of this vaccine will be required to produce a product that will completely protect against pyoderma caused by *S. pseudintermedius* (Lynch, 2021).

Subunit vaccines generally consist of specific parts of the target pathogen that are highly antigenic. The goal is to use the target pathogens to illicit an immune response within the host (Jorge, 2017). Although, vaccines against staphylococcal infections have proven to be difficult to

develop and generally do not elicit a protective immune response against staphylococcal infections (Thammavongsa et al., 2015).

Phage Therapy

Phage therapy is a therapeutic application of interest due to its success in both human and animal clinical trials. Phage therapy involves the use of bacterial viruses, called bacteriophages, to kill specific strains of bacteria and has potential as an alternative treatment option for canine pyoderma (El-Shibiny et al., 2017). Phages are highly abundant, viruses that are host specific and infect and replicate within their bacterial host (El-Shibiny et al., 2017). It is less likely to disrupt the host's normal flora, thus reducing the likelihood of symbiosis or secondary infections. This is important as *S. pseudintermedius* is a both commensal bacterium and an opportunistic pathogen. In addition, phage therapy has been shown to be versatile in administration forms such as liquids, ointments, tablets and injectables (Malik et al., 2017). This is helpful as *S. pseudintermedius* can occur in various regions of the body in animals. Therefore, if similar phages could be used in altering formulations, this would significantly reduce barriers to the clinical use of these new therapeutics for *S. pseudintermedius* canine infections.

Natural Therapeutics

Recent studies reported topical treatment with mangosteen. Mangosteen is a tropical evergreen fruit tree from Southeast Asia, India and Sri Lanka with a long history of use as a traditional medicine for the treatment of chronic diarrhea, infected wounds, skin infections, and dysentery (Larsuprom et al., 2019). A study demonstrated that mangosteen crude extract had antibacterial activity against clinical isolates of *S. pseudintermedius* from dogs, which suggests that mangosteen crude extract might be a good alternative treatment for canine pyoderma and warrants further investigation (Larsuprom et al., 2019).

There are several potential therapies for effectively managing CAD, pyoderma and MRSP infections in canines. In particular, implementing a new multifaceted approach in managing and treating of multidrug resistant pyoderma in canines will be essential in effectively controlling this life-long disease.

Conclusion

This report has highlighted the impact of *S. pseudintermedius* in veterinary medicine. This bacterium can be isolated from healthy canines as part of the normal flora; however, *S. pseudintermedius* is also associated with a multitude of moderate to severe infections in dogs, particularly canine pyoderma. The indiscriminative use of different antibiotics over the years has led to the emergence of multi-resistant staphylococcal strains due to mutations in genes that encode target proteins and through the acquisition and accumulation of genes that confer resistance to antibiotics (Silva et al., 2020). While the presented case study focused on a successful treatment of MRSP with systemic chloramphenicol in a canine, effective systemic antibiotics for MRSP are limited for use in veterinary medicine. It is now well known that *S. pseudintermedius* infections in canines are frequent and are commonly treated with antibiotics; however, the misuse and overuse of antibiotics in human and veterinary medicine has contributed to the global rise in antibiotic resistant bacteria, particularly MRSP. Thus, further research in alternative treatments and management strategies of canine atopic dermatitis is warranted. Additionally, *S. pseudintermedius* can be transmitted between dogs, humans, and the environment, thus showing that the presence of MRSP is an ever-growing problem that will impact the availability of future treatment options for both veterinary and human patients. Recent research endeavors have introduced the field to some promising areas for treatment, such as

vaccines and phage therapy, that could have an impact on MRSP infections in canines and other mammalian species in the future.

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