CHAPTER ONE

INTRODUCTION

1.0 Background of the Study

Staphylococcus pseudintermedius is an important opportunistic pathogen of companion animals, especially dogs (van Duijkeren *et al*., 2011; Melter *et al*., 2016; Kate *et al*., 2018). It is a well-known cause of dermatologic infections, such as pyoderma, otitis externa, and wound infections (Petersen, 1997; Ruzauskaus *et al*., 2016). It has been isolated from the nares, mouth, anus, groin, forehead, ear, skin, and may also cause infections in other body tissues of dogs (Bannoehr & Guardabassi, 2012; Somayaji *et al*., 2016; Pires dos Santos *et al*., 2017). It has been associated with serious human infections, particularly in people who are in close contact with pets, such as small animal veterinarians and pet owners (Guardabarsi *et al*., 2004; Paul *et al*., 2011; Papadogiannakis *et al*., 2016; Kate *et al*., 2018; Pitchenin *et al*., 2017). However, it has recently emerged as a challenging opportunistic pathogen, comparable to *S. aureus* in humans. Human infections with *S. pseudintermedius* are rare, usually local and associated with bite wounds. However, there are isolated reports of bacteremia, brain abscess, endocarditis, sinusitis, otitis, infected leg ulcers, and pneumonia (Weese and van Duijkeren, 2010; Stegman *et al*., 2010; Ruzauskaus *et al*., 2016; Kate *et al*., 2018; Valentina *et al*., 2017).

Human infection, mainly acquired from dogs, has been reported. The first case of human infection by *S. pseudintermedius* was described in 2006 by Van Hoovels *et al*.; causing endocarditis after the implantation of a cardioverter-defribrillator device (ICD). Since then, human infections have been reported sporadically, including surgical site infections, rhinosinusitis, and catheter-associated bacteremia (Chuang *et al*., 2010; Borjesson *et al*., 2015;

Jurate & Jurate, 2015). Most importantly, methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged as a significant public health problem in veterinary medicine and entails further consequences for humans, as the gene driving the drug resistance is highly mobile and can be transferred between different staphylococcal species, including *S. aureus* (Ruscher, 2009; Borjesson *et al*., 2015; Melter *et al*., 2017).

MRSP has been an emerging concern in veterinary medicine in recent years (Weese and van Duijkeren, 2010; Papadogiannakis *et al*., 2016; Somayaji *et al*., 2016), and similar to *S. aureus*, methicillin resistance is mediated by the *mec*A gene which encodes production of a modified penicillin-binding protein (PBP). The *mec*A gene is located on a mobile element of the bacterial chromosome called the 'staphylococcal chromosomal cassette (SCCmec)' and is readily transferred between different Staphylococcal species. Methicillin resistance conferred by the *mec*A gene results in resistance to the entire β-lactam class of antimicrobials which are the most commonly used drugs in companion animals (Prescott *et al*., 2002; Valentina *et al*., 2017). Moreover, the transmission of *S. pseudintermedius* and *S. aureus* between human and zoonotic hosts is evident and has resulted in severe infections in humans caused by MRSP (Savini *et al*., 2013; Jurate & Jurate, 2015; Verstappen *et al*., 2017). Historically, *S. pseudintermedius* has remarkably remained susceptible to vast classes of antibiotics, but since 2006, there has been a strange worldwide increase in the frequency of methicillin resistance (Barber, 1947; Pitchenin *et al*., 2017).

Consequently, the importance of this bacterium as a pathogen appears to be underestimated (Ruzauskaus *et al*., 2016). Such underestimation has been reflected in genomic data clearly indicating that *S. pseudintermedius* is equipped with genes homologous to those encoding

virulence factors and regulatory systems characteristic of *S. aureus* (Ben Zakour *et al*., 2012; Verstappen *et al*., 2017).

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates are characterized by the presence of the *mecA* gene, which is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) elements and confers resistance to all β-lactam antibiotics. In addition to *mecA*, MRSP may also contain a wide range of antibiotic resistance genes. In addition to β-lactam resistance, resistance to other antibiotic classes has been observed in USA, Denmark, Germany, France, Italy, Sweden, Switzerland, and the Netherlands (Geoghegan *et al*., 2009; Pitchenin *et al*., 2017).

Because the therapeutic options are limited, both for animals and humans, their increasing incidence is an alarming problem. Although the pathogenesis of *S. aureus* has been investigated in human and livestock, the pathogenesis of *S. pseudintermedius* in companion animals is understudied, with only a few virulence factors identified (Fitzgerald, 2009; Pitchenin *et al*., 2017; Somayaji *et al*., 2016). Tissue injury, immunosuppression, and concurrent diseases are purported instigating factors of *S. pseudintermedius* infections in dogs (Melter *et al*., 2017). Hill *et al*. (2016) described a number of putative virulence factors which are involved at multiple stages in the pathogenesis of infection; from adhesion to immune evasion, and then to the spread of infection (Hill & Imai, 2016). These factors are virulence proteins such as fibrinogen binding protein, fibronectin binding protein, and iron-regulated surface determinants which facilitate bacterial adhesion to the natural host (corneocyte) and also in the activation of complement pathway and opsonization of the pathogen. Immunoglobins and cell-mediated immune responses are inactivated by virulence proteins including superantigen and leucocidin. *S. pseudintermedius* also has various virulence factors, some of which are closely related to the virulence factors of *S.*

aureus (Futagawa-Saito *et al*., 2004b; Ruzauskaus *et al*., 2016). It produces enzymes such as coagulase, DNase, protease, thermonuclease and toxins, including haemolysins and exfoliative toxins (Pires dos Santos *et al*., 2017). *S. pseudintermedius* also produces a leucotoxin known as Luk-I, which is very similar to Panton-Valentine leucocidin (PVL) from *S. aureus* (Futagawa-Saito *et al*., 2009, Futagawa-Saito *et al*., 2004a; Borjesson *et al*., 2015; Ruzauskaus *et al*., 2016).

Knowledge of the pathogenesis of *S. pseudintermedius* infections in dogs and dog owners remains yet limited. The resistance of *S. pseudintermedius* depends on geographical distribution as well as on other factors – thus, it is important to obtain data from different countries to better understand the epidemiological spread of *S. pseudintermedius* resistance (Pitchenin *et al*., 2017). This study, was therefore, designed to determine the prevalence, antibiotic resistance profiles, and to molecularly characterize *Staphylococcus pseudintermedius* isolates obtained from dogs and humans in Abakaliki, Ebonyi State, Nigeria.

1.1 Statement of the problem

The increase in antibiotic resistance in staphylococci and transfer of *S. pseudintermedius* from infected pets to humans threaten veterinary medicine and public health worldwide (Paul *et al*., 2011; Kate *et al*., 2018; Pires dos Santos *et al*., 2017). The epidemiological situation of *S. pseudintermedius* infections is further exacerbated as the gene driving the drug resistance is highly mobile and can be transferred between different staphylococcal species colonizing human and zoonotic host (van Duijkeren *et al*., 2011; Jurate & Jurate, 2015; Melter *et al*., 2016; Pitchenin *et al*., 2017). Moreover, the transmission of *S. pseudintermedius* and *S. aureus* between humans and zoonotic hosts is evident and has resulted in severe infections in immunocompromised humans such as catheter-borne bacteremia, sinusitis, infective endocarditis, non-hospital pneumonia and wound infections (Weese & van Duijkeren, 2010;

Verstappen *et al*., 2017; Valentina *et al*., 2017). Further increase in the number of infections is highly possible, due to the fact that *S. pseudintermedius* is well-equipped with various virulence factors such as coagulase, protease, enterotoxins, *siet* exfoliative toxin, *luk*-I leukotoxin, and haemolysins (Bannoehr and Guardabassi, 2012; Ariana *et al*., 2015; Melter *et al*., 2017).

1.2 Justification of the study

Since dogs are in close contact with their owners, the risk of transmission of multidrug-resistant *S. pseudintermedius* between animals and humans as well as the possibility of transfer of resistance and virulence genes from *S. pseudintermedius* to human pathogenic *S. aureus* is a very serious public health problem. However, there is no published information on the prevalence, antibiotic resistance and molecular characterization of *S. pseudintermedius* in dogs and humans in West Africa, including Nigeria. This study will greatly help in understanding the prevalence, antibiotic resistance patterns, and the types of resistance and virulence genes harboured by *S. pseudintermedius* among dogs and humans in Abakaliki, Ebonyi State, Nigeria.

1.3 Aim and objectives of the research

1.3.1 Aim

This study was aimed at determining the prevalence, antibiotic resistance profiles, and to molecularly characterize *Staphylococcus pseudintermedius* isolates obtained from dogs and dog owners (humans) in Abakaliki, Ebonyi State, Nigeria.

1.3.2 Objectives of the research

The specific objectives of this study are:

1. To isolate and identify *S. pseudintermedius* from dogs and humans in Abakaliki, Ebonyi State, Nigeria.

2. To determine the antibiotic susceptibility of the isolates.
3. To determine the antibiotic resistance phenotypes of the *S. pseudintermedius* isolates.
4. To determine the prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) among the isolates obtained.
5. To determine the multiple antibiotic resistance indices (MARI) of the isolates.
6. To classify the antibiotic resistance in the isolates.
7. To characterize tetracycline and chloramphenicol resistance genes from *S. pseudintermedius* isolates using Polymerase Chain Reaction (PCR).
8. To determine and characterize the presence of virulence genes in isolates of *S. pseudintermedius* using PCR.
9. To sequence *tet*M resistance genes of the *S. pseudintermedius* isolates.

1.4 Hypothesis

The hypotheses of this research work were as follows:

1. Null Hypothesis: *S. pseudintermedius* are not harboured by dogs in Abakaliki.

   Alternative Hypothesis: *S. pseudintermedius* are harboured by dogs in Abakaliki.
2. Null Hypothesis: Dog owners are not at risk of harbouring *S. pseudintermedius* than non-dog owners or humans who have no contact with dogs in Abakaliki.

   Alternative Hypothesis: Dog owners are at risk of harbouring *S. pseudintermedius* than non-dog owners or humans who had no contact with dogs in Abakaliki.
3. Null Hypothesis: Dogs and dog owners in Abakaliki do not harbour antibiotic-resistant *S. pseudintermedius*.

   Alternative Hypothesis: Dogs and dog owners in Abakaliki harbour antibiotic-resistant *S. pseudintermedius*.

4. Null Hypothesis: Dogs in Abakaliki do not harbour multi-drug methicillin-resistant *S. pseudintermedius* strains.

   Alternative Hypothesis: Dogs in Abakaliki harbour multi-drug methicillin-resistant *S. pseudintermedius* (MRSP) strains.

5. Null Hypothesis: *S. pseudintermedius* isolates from dogs and dog owners in Abakaliki do not exhibit similar antibiotic resistance patterns which could depict zoonotic transfer.

   Alternative Hypothesis: *S. pseudintermedius* isolates from dogs and dog owners in Abakaliki exhibit similar antibiotic resistance patterns which could depict zoonotic transfer.

6. Null Hypothesis: *S. pseudintermedius* isolates from dogs and dog owners do not harbour various resistance and virulence genes which are major contributors to its pathogenicity.

   Alternative Hypothesis: *S. pseudintermedius* isolates from dogs and dog owners harbour various resistance and virulence genes which are major contributors to its pathogenicity.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Staphylococcus* genus

The word "*Staphylococcus*" comes from the Greek word "Staphule", meaning a bunch of grapes. Taxonomically, the genus *Staphylococcus* is in the bacterial family, Staphylococcaceae, which includes five lesser known genera, *Gemella*, *Jeotgalicoccus*, *Macrococcus*, *Nosocomiicoccus, and Salinicoccus*. There are 47 recognized species of staphylococci and 21 subspecies most of which are found only in lower mammals (Prax *et al.*, 2013; Papadogiannakis *et al*., 2016, Ruzauskas *et al*., 2016). Staphylococci are ubiquitous Gram-positive bacteria about 0.5 – 1.0 µm in diameter and appear as individual coccus, in pairs, tetrads, or in grape-like clusters. They are non-spore formers, non-motile, facultative anaerobes, capsule variable, usually catalase-positive and oxidase-negative. They grow rapidly on blood agar and readily on many other types of media.

They are metabolically active, fermenting carbohydrates and producing pigments that vary from white to deep yellow. They are indole-negative and respire using oxygen as the terminal electron acceptor; although some can reduce nitrate to nitrite (Ghebremedhin *et al*., 2008; Papadogiannakis *et al*., 2016). Staphylococci hydrolyse urea and ferment a number of sugars with the production of acid only. They can survive harsh environmental conditions (high salt media up to 10 % NaCl) and are relatively heat-resistant. They are natural part of skin flora in canines. They have been isolated from the nares, mouth, anus, groin, forehead, ear, skin, and may also cause infections in other body tissues of dogs (Somayaji *et al*., 2016).

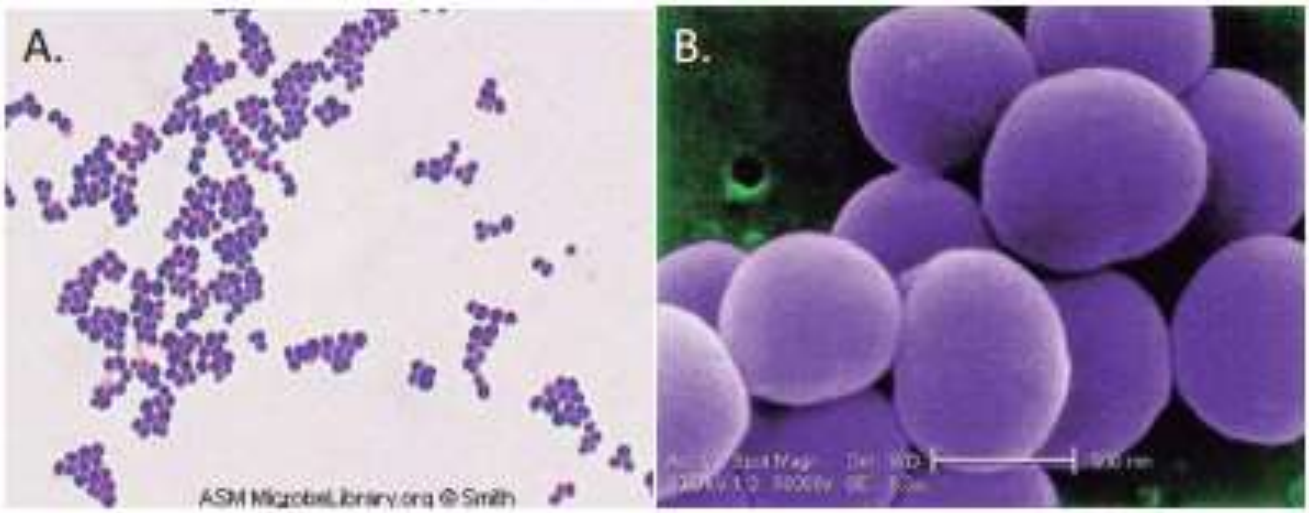

Figure 1a: Morphology and clustering of staphylococci. **i.** Gram tincion that shows the clusters of Staphylococci violet colour due to the retention of crystal violet primary stain. **ii.** Scanning electron micrograph of staphylococci **Source:** phil.cdc.gov

They are also the most common cause of pus-forming infections. Some are members of the normal microbiota of the mucous membranes of humans whereas others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicaemia. Most staphylococci members have preference for a specific host. Eleven members of this species can be isolated from humans as commensals. *S. aureus* (nostrils) and *S. epidermidis* (nostrils, skin) are common commensals and also have the greatest pathogenic potential (Gyles *et al*., 2010; Ruzauskas *et al*., 2016). Many species of *Staphylococcus* have the ability to form biofilms which can then colonize structures such as medical catheters, stents, heart valves, prostheses, shunts, and valves. In recent years, several other *Staphylococcus* species have been implicated in human infections, notably *S. lugdunensis, S. schleiferi, and S. caprae.* In veterinary medicine, the most important species are *S. aureus*, *S. hyicus*, and *S. pseudintermedius*. They are a common and important cause of disease in animals, including abscesses, dermatitis, furunculosis, meningitis, osteomyelitis, food poisoning, and wound infections (Pitchenin *et al*., 2017).

Staphylococcus species which are pathogenic to dogs include *Staphylococcus intermedius* group (SIG), *Staphylococcus pseudintermedius* (Blondeau, 2012; Arianna *et al*., 2015), *Staphylococcus aureus*, *Staphylococcus schleiferi*, *Staphylococcus lugdunensis*, and *Staphylococcus lentus* (Kadlec and Schwarz, 2012; Kate *et al*., 2018; Ruzauskas *et al*., 2016). *S. pseudintermedius* inhabits and sometimes infects the skin of domestic dogs and cats. This organism, too, can carry the genetic material that imparts multiple bacterial resistances. It is rarely implicated in human infections, as zoonosis (Karsten *et al*., 2014). The pathogenic staphylococci often haemolyse blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. The enzymes produced by staphylococcal species include:

- **Catalase**: Catalase converts hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2). *S. pseudintermedius* is catalase-positive (meaning that it can produce the enzyme, catalase) and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci.

- **Coagulase**: Coagulase converts fibrinogen to fibrin and is the primary test used to distinguish staphylococcal species. Six species are currently recognized as being coagulase positive: *S. aureus*, *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, and *S. pseudintermedius.* These species belong to two separate groups – the *S. aureus* (*S. aureus* alone) group and the *S. hyicus-intermedius* group (the remaining five). A seventh species has also been described – *Staphylococcus leei* – from patients with gastritis (Jin *et al*., 2004; Melter *et al*., 2017). *S. pseudintermedius* is coagulase-positive, meaning it produces coagulase. However, while the majority of *Staphylococcus* species are coagulase-positive, some do not produce coagulase. Two major examples of coagulase-negative staphylococci are *S. epidermidis* and *S. saprophyticus*. *S. epidermidis* is a commensal of the skin, but can cause severe infections in immunosuppressed patients and those with central venous catheters. *S. saprophyticus* is part of the normal vaginal flora which is predominantly implicated in genito-urinary tract infections especially in sexually active young women.

- **Hyaluronidases**: Hyaluronidases hydrolyze hyaluronic acids and may contribute to tissue breakdown and spread of staphylococci across tissue barriers.

- **Beta-lactamases**: Beta-lactamases are released by staphylococci and can hydrolyze the beta-lactam ring of penicillins and cephalosporins rendering the antibiotics ineffective. Staphylococci rapidly develop resistance to many antimicrobial agents, which consequently

presents difficult therapeutic problems. The importance of staphylococci as pathogens for humans and animals has been recognized for more than 100 years.

2.2 Evolution of *Staphylococcus pseudintermedius* taxonomy

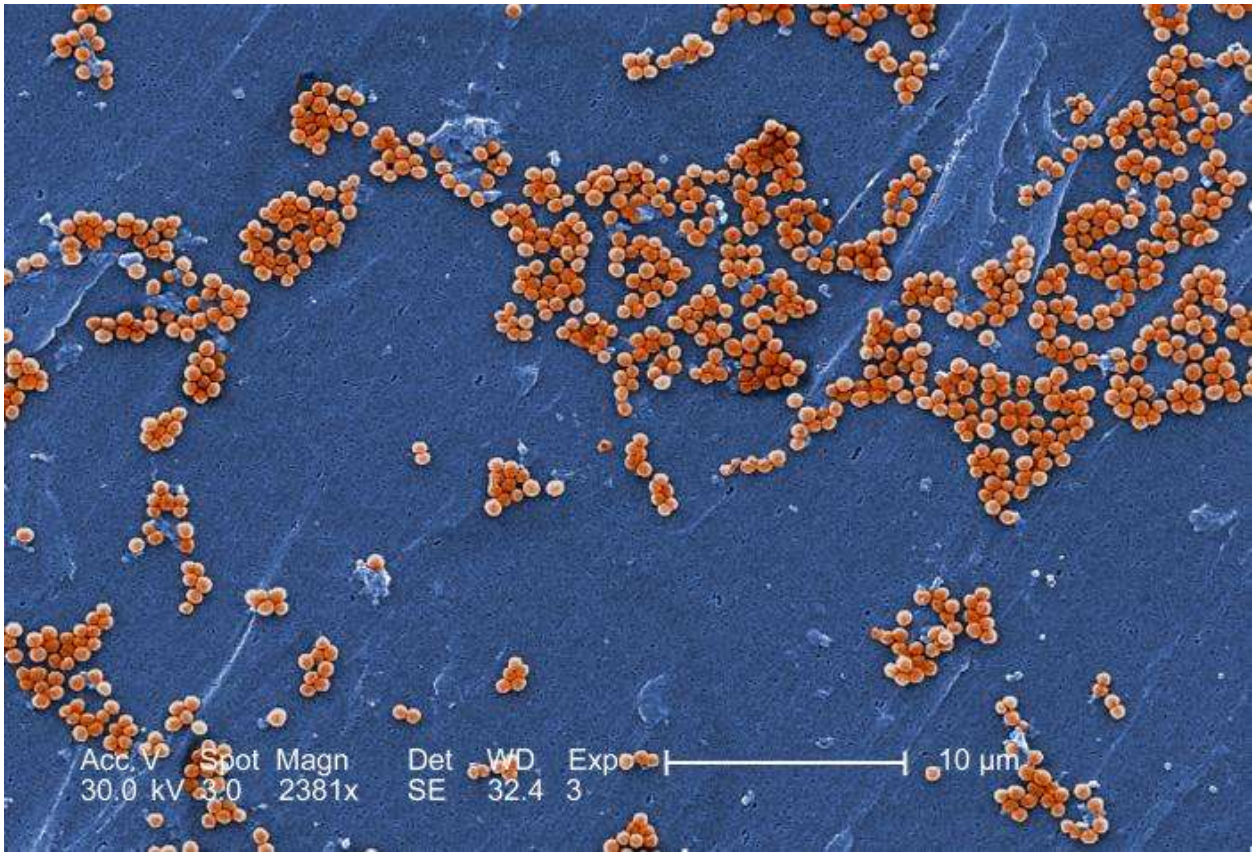

Figure 1b: Electron micrograph of *Staphylococcus pseudintermidius*

Source: CDC, 2013

2.2.1 Scientific classification of *Staphylococcus pseudintermedius*

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species: *S. pseudintermedius* (Devriese *et al*., 2005)

Staphylococcus was first discovered in 1882 by Alex Ogston. Rosenbach subdivided staphylococci based on their colour on culture media in 1884 (Keneeth, 2012; Somayaji *et al*., 2016); where *S. aureus* forms golden colonies, and *S. albus* white ones. Around 1950, Smith observed that in canine samples, not all strains were uniform (Smith, 1947). In 1967, a report proposed a new strain called *S. aureus* var *canis*, which described those differences observed by Smith in the 50's (Meyer, 1967; Pitchenin *et al*., 2017). It wasn't until 1976 that Hajek discovered a new species considered to be the staphylococcal normal flora as well as opportunistic pathogen of dogs, which he named *Staphylococcus intermedius* (Fazakerley, 2009; Jurate & Jurate, 2015; Melter *et al*., 2017). *Staphylococcus intermedius* had been considered the agent causing skin and soft tissue infections in canines for a long time. However, the advance in technology and the development of new molecular techniques with more powerful discriminatory capabilities underpinned the extensive revision of staphylococcal classification and allowed further distinction of 3 different species within *S. intermedius*: *S. intermedius, S. pseudintermedius*, and *S. delphini* (Ghebremedhin *et al*., 2008; Somayaji *et al*., 2016; Arianna *et*

al., 2015). *Staphylococcus pseudintermedius* was first described by Devriese in 2005 following molecular analyses of isolates from a dog, a cat, a horse, and a parrot. It was recognized as the common cause of canine cutaneous infections. Their phenotypic profiles were similar to *S. intermedius* and *Staphylococcus delphini*, a species first isolated in 1988 from skin lesions of dolphins (Bannoehr & Guardabassi, 2012; Papadogiannakis *et al*., 2016).

In 2005, Devriese wrote as follows "the name "*pseudintermedius*" reflects the close genetic relatedness (99 % similar) to *S. intermedius* and the inability of discriminating among the two when phenotypic tests are used". Based on whole genome analysis, the average nucleotide identity (ANI) between these 3 species is 93.61 % (Ben Zakour, 2012; Verstappen *et al*., 20017), very close to the threshold for species delineation (ANI 95 %). Therefore, for differentiation of the species, DNA-DNA hybridization was used, and this determined that most canine isolates phenotypically identified as *S. intermedius*, were, in fact, *S. pseudintermedius* (Sasaki *et al*., 2007; Valentina *et al*., 2017; Verstappen *et al*., 2017). Bannoehr's research work in 2007 affirmed Sasaki's phylogenetical analyses (Bannoehr *et al*., 2007).

Detailed biochemical testing may differentiate *S. intermedius* from *S. pseudintermedius* and *S. delphini*, but the latter two species can only be reliably distinguished by molecular tests, such as sequencing of the thermonuclease (*nuc*) or heat shock protein (*hsp*60) genes (Sasaki *et al*., 2007; Valentina *et al*., 2017). These molecular studies support the introduction of the term "*S. intermedius* group" (SIG), comprising at least three closely related species, *S. intermedius*, *S. delphini*, and *S. pseudintermedius* (Ghebremedhin *et al*., 2008; Borjesson *et al*., 2015). These observations indicate that isolates with traditional phenotypic characteristics of "*S. intermedius*" should be identified as *S. pseudintermedius* when obtained from canines. Isolates from other

species with such characteristics are best identified as bacteria of the "*S. intermedius* group" unless molecular test results are available (Hermans *et al*., 2010; Jurate & Jurate, 2015).

Consequently, since the reclassification of the species, it has been proposed that all canine isolates belonging to the SIG should be considered as *S. pseudintermedius* unless proven otherwise by genetic typing methods (Devriese *et al*., 2009; Borjesson *et al*., 2015). One recent study showed that 100 % (44/44) of the isolates that had been classified as *S. intermedius* based on phenotypic properties and PCR amplification of the *S. intermedius*-specific fragment of the 16S rRNA gene, were reclassified as *S. pseudintermedius* once more discriminatory methods were used (Chrobak *et al*., 2011; Pires dos Santos *et al*., 2016).

2.3 Dogs

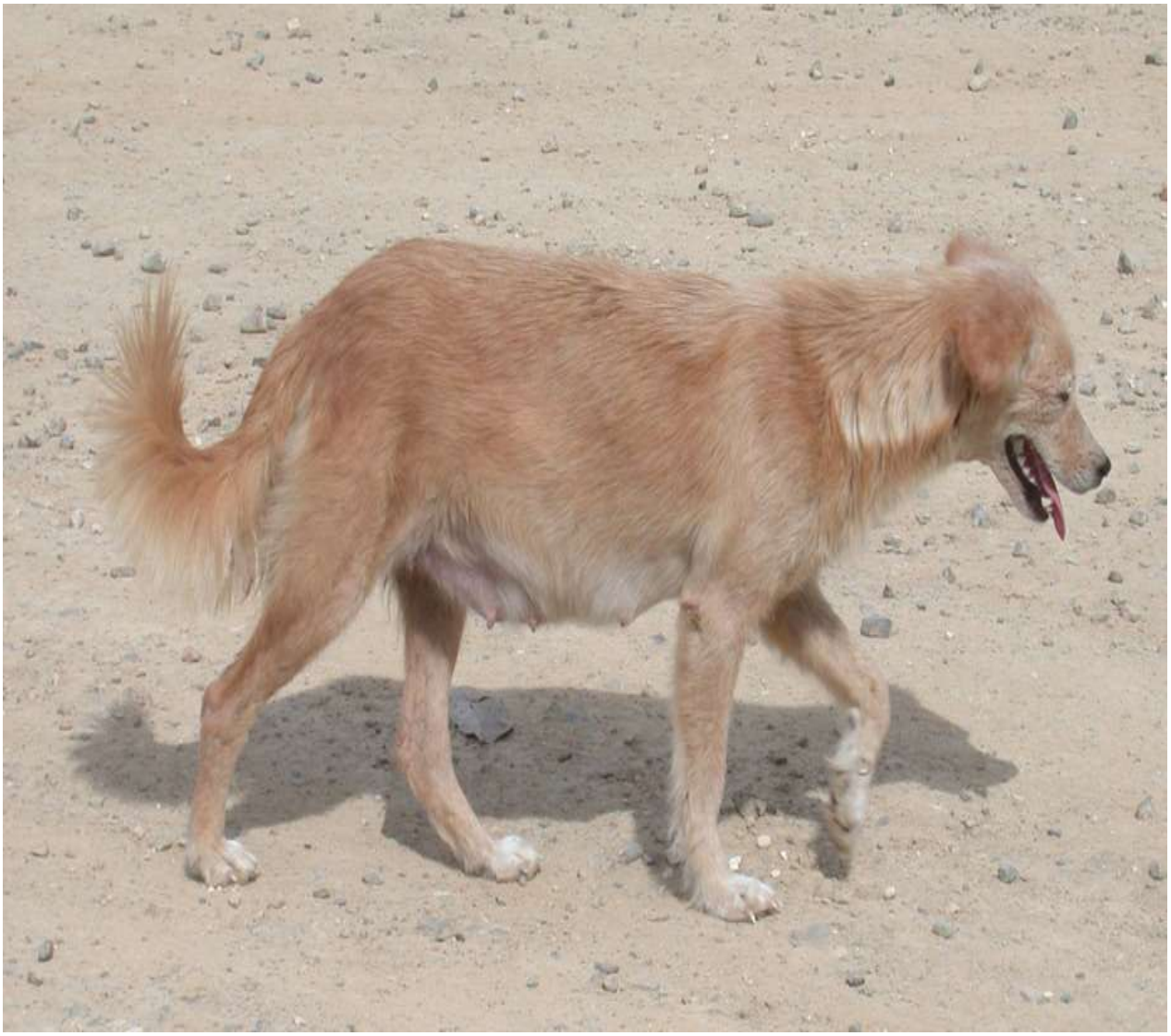

Figure 2a: Picture of a domestic dog (*Canis lupus familiaris*)

Source: (Biokids, 2017)

The domestic dog (*Canis lupus familiaris* or *Canis familiaris*) is a member of genus *Canis* (canines) that forms part of the wolf-like canids, and is the most widely abundant carnivore (Thalmann *et al*., 2013; Biokids, 2017). The dog was the first domesticated species and has been selectively bred over millennia for various behaviours, sensory capabilities and physical attributes. It is of the kingdom Animalia, phylum Chordata, class Mammalia, order Carnivora, suborder Caniformia, family Canidae, genus *Canis*, species *C. lupus* and subspecies *C. l. familiaris*. Their long association with humans has led dogs to be uniquely attuned to human behaviour and they are able to thrive on a starch-rich diet that would be inadequate for other *Canis* species. Dogs vary widely in shape, size, and colours (Axelsson *et al*., 2013; Biokids, 2017).

They perform many roles for people such as hunting, herding, pulling loads, protection, assisting police and military, companionship, and more recently, aiding handicapped individuals. It is widely kept as pet, for work or field sports. This influence on human society has given them the sobriquet, man's best friend (Bridgett *et al*., 2017). The most widespread form of interspecies bonding occurs between humans and dogs and the keeping of dogs as companions, particularly by elites, have a long history. The global dog population is estimated at 900 million and rising. Dogs are scavengers and predators, and like many other predatory mammals, the dog has powerful muscles, fused wrist bones, a cardiovascular system that supports both sprinting and endurance, teeth for catching and tearing (Thalmann *et al*., 2013; Bridgett *et al*., 2017).

2.4 *Staphylococcus pseudintermedius* as a zoonotic pathogen

Staphylococcus pseudintermedius is a coagulase-positive bacterium within the *Staphylococcus intermedius* group (SIG) that was identified as a distinct species in 2005 (Devriese *et al*., 2005; Borjesson *et al*., 2015), where *pseudintermedius* means false (*Staphylococcus*) *intermedius*

because of the high phenotypic similarity to *S. intermedius.* It is Gram-positive cocci predominantly arranged in groups. They are non-pigmented colonies and surrounded by double zone haemolysis on Columbia sheep blood agar. The outer band, which is incompletely haemolytic turns into complete haemolysis after being put at 4 ^{o}C (hot-cold haemolysis), and is typical of staphylococcal β-haemolysin which confirms the pathogenic potential of this bacterium to cause human disease. *S. pseudintermedius* is a normal inhabitant and common opportunistic pathogen of canines and other companion animals including cats and horses (Van Duijkeren *et al*., 2011; Somayaji *et al*., 2016). In dogs, colonization with *S. pseudintermedius* is ubiquitous, occurring in up to 90 % of healthy dogs (Rubin & Chirino-Trejo, 2011; Somayaji *et al*., 2016; Papadogiannakis *et al*., 2016).

Dogs are the natural host of *Staphylococcus pseudintermedius.* This bacterium affects dogs of various breed or any age depending on immunological status of the dogs. Some subjective changes found in infected dogs are fever, pain, loss of appetite (anorexia), skin abscesses, infections of the eyes, skin, ears or respiratory system, itching (pruritus), inflammation marked by pus-filled lesions (pyoderma). The common indicators of dogs infected with the bacterium are wound infections, abscesses on the skin or mouth and pyoderma. Younger dogs are most prone to this infection as their immune systems are still at their nascent stage. Older dogs are also more susceptible as their immune systems become worn out overtime (Weese *et al*., 2012; Pires dos Santos *et al*., 2016).

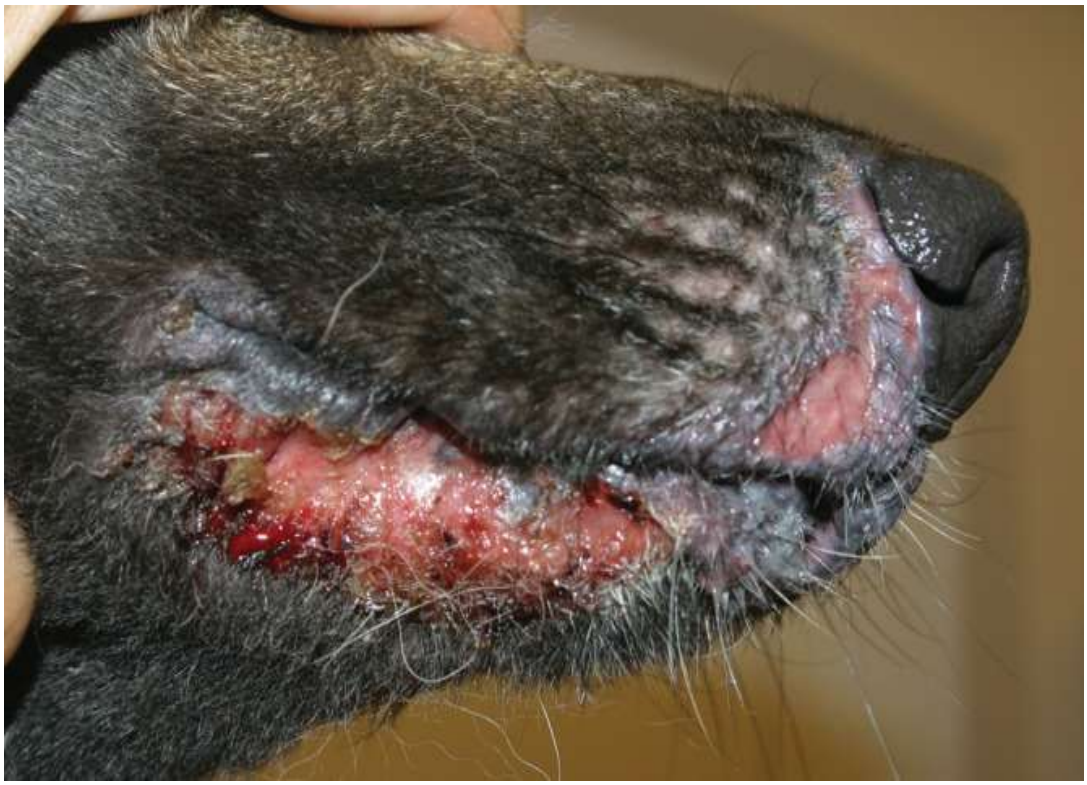

Fig.2b: Mouth infection caused by *Staphylococcus pseudintermedius* in a dog

Source: (Beco, 2013)

Although considerable literature exists on the role and pathogenecity of *S. pseudintermedius* in animal infections, much remains to be learned on its role in human infections. *Staphylococcus pseudintermedius* is a common colonizer of companion animals and particularly dogs and is most frequently associated with skin and soft tissue infections (SSTI) in animals (Rubin & Chirino-Trejo, 2011; Somayaji *et al*., 2016). Similarly, SSTIs occur most commonly with human *S. pseudintermdius* infections likely through zoonotic transmission, though invasive infections can also occur as demonstrated in case reports and more recently in a larger case series (Somayaji *et al*., 2016). As *S. pseudintermedius* has virulence and antimicrobial resistance characteristics in animals and human isolates comparable to *S. aureus*, this pathogen may be a potentially important emerging zoonotic pathogen (Kate *et al*., 2018).

Like *S. aureus* colonization in humans, *S. pseudintermedius* can be recovered from multiple body sites in dogs like the nose, mouth, perineum, groin, otitis etc. (Rubin & Chirino-Trejo, 2011; Van Duijkeren *et al*., 2011; Somayaji *et al*., 2016). Otitis externa and pyoderma; secondary to food allergy, endocrine disease or other immune-suppressive conditions, are very common in dogs, and are most often associated with *S. pseudintermedius* (Bannoehr & Guardabassi, 2012; Melter *et al*., 2017). *Staphylococcus pseudintermedius* is also a common cause of canine urinary tract infections and a frequent cause of opportunistic infections at any site where normal host defenses are disrupted. They are now found in association with humans worldwide and in a wide variety of habitats; temperate, tropical, polar, and terrestrial habitats. Dogs and cats it seems to be the most popular pet animals in Europe, with nearly 24 % of households having a dog (FEDIAF, 2012; Jurate & Jurate, 2015).

The relationship between companion animals and humans has changed throughout the years. While in the past, dogs were maintained outside the households, today, they are often kept inside

the homes. Close physical contact by touching, petting, and licking occurs at high frequency on the basis of the current perception of household pets as actual family members. Companion animals and humans can act as reservoirs of such bacteria. Healthy dogs have *Staphylococcus pseudintermedius* as part of their normal microflora of the skin, coat and mucocutaneous sites like the nose, mouth and anus (Lloyd, 2010; Borjesson *et al*., 2015). Transmission of pathogenic staphylococci may occur between pets and owners. There have been several recent reports describing the carriage of indistinguishable coagulase-positive staphylococci strains in humans and animals (Hanselman, 2009; Papadogiannakis *et al*., 2016).

Although dogs and humans are the natural hosts for *S. pseudintermedius* and *S. aureus* respectively, interspecies transmission of such bacteria can occur between humans and in-contact dogs with a reverse direction. Transmission of *S. pseudintermedius* from the canine to the human host is named (direct) zoonotic interspecies transmission while the transfer of *S. aureus* from the human to the canine host is termed (direct) anthropozoonotic interspecies transmission (or reverse zoonosis) (Figure 2c). In addition, indirect evidence of interspecies transmission occurs when *S. pseudintermedius* is detected in humans and *S. aureus* in dogs. In these cases, the term indirect zoonotic (dog $->$ human) and indirect anthropozoonotic (human $->$ dog) transmission can be used. *Staphylococcus pseudintermedius* does not usually colonize humans, although transmission between pet and owner has been reported (Bannoehr & Guardabassi, 2012; Pires dos Santos *et al*., 2017).

Human beings may become transient carriers if in very close contact with an infected dog (Frank *et al*., 2009; Sasaki *et al*., 2007; Valentina *et al*., 2017; Borjesson *et al*., 2015). The carriage rate of *S. pseudintermedius* was reported to range from 46 % to 92 % (Bannoehr and Guardabassi, 2012). Other transmission of *S. pseudintermedius* can occur in the following ways:

- Vertical or pseudo-vertical transmission: The skin of puppies is normally colonized after birth, probably due to transmission from the female dog species; and *S. pseudintermedius* can be detected as early as 1 day after birth (Saijonmaa-Koulumies *et al*., 2002; Somayaji *et al*., 2016).
- Horizontal transmission between dogs: Not many studies have looked at this type of transmission in dogs. However, in the case of an MRSP infection, healthy pets in contact are at a high risk of habouring the pathogen (van Duijkeren *et al*., 2008; Arianna *et al*., 2015).

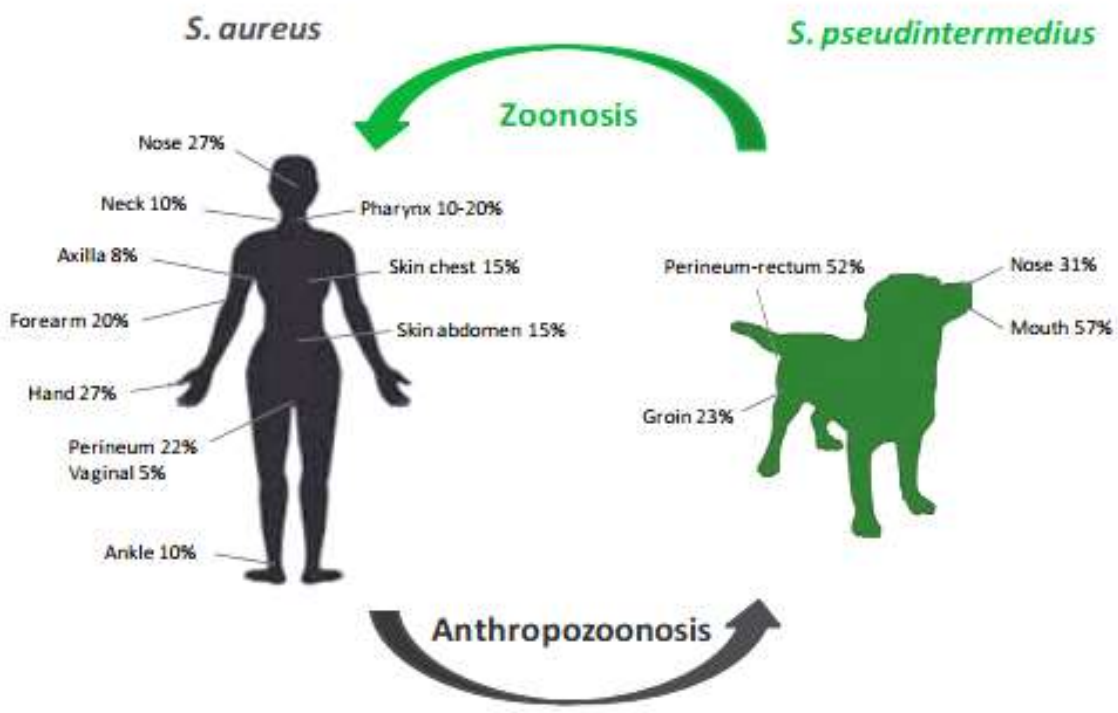

Figure 2c: *S. aureus* and *S. pseudintermedius* carriage rates per body site in humans and dogs, respectively. (Source: Bannoehr & Guardabassi, 2012)

2.5 Clinical significance of *Staphylococcus pseudintermedius*

Staphylococcus pseudintermedius is recognized as the main cause of canine pyoderma, which represents the most common dermatological pathology seen in dogs. It is also associated with infections in other body sites such as ears, urinary tract, surgical sites, wounds infections, arthritis, mammary gland, and endocardium. Other symptoms of *Staphylococcus pseudintermedius* infection may include fever, severe pain at wound site, weakness and lethargy, loss of appetite (anorexia), skin abscesses, itching (pruritis), hypotension, desquamation, osteomyelitis, pneumonia, draining sinus tracts, inflammation marked by pus-filled lesion (pyoderma), infections of the eyes, ears, skin or respiratory systems, diffuse erythroderma, deep-red, "sunburned" appearance and so on (Devriese, 2009; Borjesson *et al*., 2015; Arianna *et al*., 2015). It is a canine commensal and opportunistic pathogen, which is analogous to *S. aureus* in human beings. The bacterium is part of the normal flora of the skin of dogs and typically does not represent a clinical problem. However, if the skin barrier is broken (due to trauma, abrasions, surgery, etc.), or if the animal is immunosuppressed, the organism can become pathogenic (May, 2006; Papadogiannakis *et al*., 2016).

This species are commensal organisms, but they are also a cause of disease such as pyoderma, the urinary tract and otitis externa in dogs (Hanselman *et al*., 2009; Jurate & Jurate, 2015; Valentina *et al*., 2017). Infection usually occurs when skin or mucosal barriers are affected by predisposing factors such as atopic dermatitis, medical and surgical operations. *S. pseudintermedius* can cause clinical infections in humans as well, but these are rare and often misidentified as *S. aureus.* In routine diagnostic bacteriology, *S. aureus* has long been differentiated from other species by means of a single test, most often coagulase or clumping-factor production (Devriese, 2005; Borjesson *et al*., 2015; Somayaji *et al*., 2016). However, *S.*

pseudintermedius and other pathogenic staphylococci are also coagulase-positive (Wang, 2013; Papadogiannakis *et al*., 2016). The incidence of colonization varies significantly among different studies, more than likely due to difference in the number and sites of sample collection. Pets such as dogs and cats are usually colonized with *S. pseudintermedius*. It has been reported that 87 % of atopic dogs are colonized by *S. pseudintermedius*, in contrast to "only" 37 % in healthy dogs (Fazakerley, 2009; Jurate & Jurate, 2015). On the other hand, carriage rates in cats is much lower than in dogs, which may imply that cats are not natural hosts of *S. pseudintermedius*. *S. pseudintermedius* is a nosocomial pathogen in veterinary settings, just like MRSA in human medicine (Chrobak, 2011; Somayaji *et al*., 2016).

Additionally, people working in animal hospitals have been shown to be carriers of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) and therefore could transfer MRSP to animals. Human infections with MRSP have been previously described, however these are uncommon. People can get infected with MRSP after direct contact with pets that are colonized or infected. Also, in one study, similar or non-distinguishable MRSP were isolated from patients, contact animals, and the environment, indicating transmission within the household (Laarhoven, 2011; Pires dos Santos *et al*., 2016; Papadogiannakis *et al*., 2016).

Infection from dog bite wounds has been reported. In certain cases, human infections with MRSP are difficult to treat and have an increased risk of mortality. Another relevant issue of MRSP infection in humans is that MRSP could provide genetic material by the transfer of SCC*mec* and convert methicillin-susceptible *Staphylococcus aureus* (MSSA) into MRSA (Epstein, 2009; Somayaji *et al*., 2016). It is not known if dogs and human beings are either colonized persistently or transiently or if they are just contaminated with MRSP. However, MRSP is rarely isolated from human beings, and very rarely more than once, which suggests either sporadic

contamination or rapid elimination if colonization occurs. On the other hand, MRSP can be repeatedly and intermittently isolated from dogs. MRSP was isolated from one particular dog more than a year after the initial sampling, meaning that MRSP can persist in dogs for a long time (Laarhoven, 2011; Pires dos Santos *et al*., 2016).

2.6 *Staphylococcus pseudintermedius* infections

2.6.1 Pathogenicity

The knowledge of the pathogenesis of *Staphylococcus pseudintermedius* is limited (Somayaji *et al*., 2016; Pitchenin *et al*., 2017). Staphylococci contain peptidoglycan, a polysaccharide polymer, which produces the rigidity to the cell wall and is responsible for the pathogenesis of infection and when this peptidoglycan is linked with teichoic acid, it is antigenic. Cell wall protein A with attached IgG molecule directed against a specific antigen will agglutinate bacteria that have that antigen. In *S. aureus*, enzymes and toxins are involved in the conversion of host tissues into nutrients for bacterial growth in addition to having numerous modulatory effects on the host immune response. *Staphylococus pseudintermedius* has various virulence factors, including some that are closely related to virulence factors of *S. aureus* (Ruzauskas *et al*., 2016).

These virulence factors are involved in almost all processes from colonization of the host to bacterial nutrition and dissemination. *S. pseudintermedius* produces enzymes such as coagulase, DNase, lipases, protease, thermonuclease and toxins, including haemolysins, exfoliative toxins and enterotoxins (Fitzgerald, 2009; Somayaji *et al*., 2016). Exfoliative toxin is a virulence factor implicated in canine pyoderma, and the exfoliative toxin gene is mainly found among *S. pseudintermedius* isolated from skin infections (Iyori *et al*., 2010; Borjesson *et al*., 2015; Melter *et al*., 2017). Dogs injected with purified exfoliative toxin develop clinical signs such as

erythema, exfoliation and crusting, which are signs of canine pyoderma (Terauchi *et al*., 2003; Arianna *et al*., 2015). *S. pseudintermedius* also produces a leucotoxin known as *Luk*-I, which is very similar to Panton–Valentine leucocidin (PVL) from *S. aureus. Luk*-I shows strong leucotoxicity towards various polymorphonuclear cells (Futagawa-Saito *et al.*, 2004; Pitchenin *et al*., 2017, Ruzauskas *et al*., 2016). *S. pseudintermedius* expresses surface proteins that resemble those from *S. aureus*. It has the capacity to bind to fibrinogen, fibronectin, and cytokeratin, which could explain how it adheres to canine corneocytes (Geoghegan *et al*., 2009; Pitchenin *et al*., 2017). *S. pseudintermedius* produces an immunoglobulin-binding protein called staphylococcal protein A (spa), similar to that of *S. aureus* (Moodley *et al*., 2009; Pires dos Santos *et al*., 2016; Kate *et al*., 2018). Like most staphylococci, some *S. pseudintermedius* strains have the capacity to form biofilms (Futagawa-Saito *et al*., 2006; Pitchenin *et al*., 2017; Melter *et al*., 2017).

Accessory gene regulator (agr) homologues were found in *S. pseudintermedius*. The agr quorum-sensing and signal transduction system was first described in *S. aureus* and plays a key role in the regulation of virulence during infection (Dufour *et al*., 2002; Valentina *et al*., 2017). Similarly, to *S. aureus*, *S. pseudintermedius* synthesizes an array of invasion and virulence factors. These include factors enabling adhesion to host's cells or extracellular matrix (clumping factor and biofilm), toxins, and factors modulating host's immune system (haemolysins, leukotoxin, exfoliative toxins and enterotoxins). However, the knowledge on the pathogenesis of *S. pseudintermedius* is very limited; to date, the majority of virulence factors have not been characterized in detail.

2.6.2 Host factors

As a commensal organism, *S. pseudintermedius* does not cause disease unless host defenses are compromised, such as with skin allergies, skin abrasions, or surgical procedures. Thus, host factors play a critical role in the pathogenesis of *S. pseudintermedius* infections. *S. pseudintermedius* has also been isolated from other clinical specimens and has been reported to cause endometritis, cystitis, mastitis, and osteomyelitis in dogs (Bannoehr & Guardabassi, 2012; Jurate & Jurate, 2015; Pires dos Santos *et al*., 2017).

2.6.3 Virulence factors

Information on the virulence factors of *S. pseudintermedius* is limited. *S. pseudintermedius* has numerous virulence factors: haemolysins, leukotoxins, and exfoliative toxins.

2.6.3.1 Haemolysins

The haemolysin toxins produced by *S. pseudintermedius* cause haemolysis of rabbit erythrocytes and hot-cold haemolysis of sheep erythrocytes. They possess a similar enzymatic activity to the haemolysins produced by *S. aureus* but their amino acid compositions are different (Bannoehr & Guardabassi, 2012; Pires dos Santos *et al*., 2017).

2.6.3.2 Leukotoxins

Luk-I, a cytotoxin produced by *S. pseudintermedius*, has similar activity to *S. aureus'* Panton-Valentine leukocidan (PVL), and has been shown to be leukotoxic to polymorphonuclear cells (Bannoehr & Guardabassi, 2012; Jurate & Jurate, 2015; Pires dos Santos *et al*., 2017). One study showed that there was no significant difference in the leukotoxicity of *S. pseudintermedius* isolated from healthy or diseased dogs (Futagawa-Saito *et al*., 2004; Kate *et al*., 2018), while

another showed no significant difference in *Luk*-I presence between methicillin-resistant and methicillin-susceptible strains. The presence of *Luk*-I in both methicillin-resistant and methicillin–susceptible strains isolated from healthy dogs highlights the important role this toxin may have in a commensal *S. pseudintermedius* becoming an opportunistic pathogen (Couto *et al*., 2015).

2.6.3.3 Exfoliative toxins

The *S. pseudintermedius* exfoliative toxin (*siet*) has been identified as a virulence factor in canine skin disease (Bannoehr & Guardabassi, 2012; Arianna *et al*., 2015; Somayaji *et al*., 2016). Injection of purified *siet* in dogs produced symptoms of erythema, exfoliation, and crusting, similar to the symptoms of canine *S. pseudintermedius* pyoderma infections (Fitzgerald, 2009; Ruzauskas *et al*., 2016). One study determined that over half of the *S. pseudintermedius* strains isolated from skin and wound infections were positive for the *siet* encoding genes (Iyori *et al*., 2011; Pitchenin *et al*., 2017; Borjesson *et al*., 2015).

2.6.3.4 Enterotoxins

Staphylococcal enterotoxin (SE) production is most commonly associated with foodborne illness, but has also been associated with cases of human pyoderma and dermatitis (Youn *et al*., 2010; Melter *et al*., 2017). Isolates of *S. pseudintermedius* have demonstrated the ability to produce enterotoxins and to activate T-cell proliferation (Fitzgerald, 2009; Ruzauskas *et al*., 2016). One study reported that 24 % of *S. pseudintermedius* isolates from cases of canine pyoderma were positive for sec_{canine}. Another reported that 12 % of strains tested were sec_{canine}-positive, while in a third study; this enterotoxin was detected in only 0.5 % of isolates tested (Yoon *et al.*, 2010; Melter *et al*., 2017).

2.7 Emergence and spread of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP)

Methicillin-resistant *S. pseudintermedius* (MRSP) have recently emerged as significant pathogens in companion animals (Bannoehr *et al*., 2007; Nienhoff *et al*., 2011; Melter *et al*., 2017; Pires dos Santos *et al*., 2016; Verstappen *et al*., 2017). Having been isolated from cats and dogs, MRSP have also been isolated from humans, highlighting a public health issue for veterinarians and pet owners (Paul *et al*., 2011; Arianna *et al*., 2015; Kate *et al*., 2018). In the past, *S. pseudintermedius* isolates were generally susceptible to penicillinase-stable β-lactam antibiotics, but, since 2006, MRSP has emerged as a significant animal health problem in veterinary medicine (Weese & Van Duijkeren, 2010; Papadogiannakis *et al*., 2016; Kate *et al*., 2018). Since 2006, there has been a significant emergence of MRSP mainly due to clonal spread. As in methicillin-resistant *S. aureus* (MRSA), the methicillin resistance of *S. pseudintermedius* is mediated by the *mecA* gene that encodes production of a modified penicillin binding protein (PBP). Normally, β-lactam antibiotics bind to PBP of *S. pseudintermedius* to prevent cell wall construction by the bacterium. The modified PBP of MRSP has a low affinity for β-lactams and therefore cell wall construction is not prevented by these antimicrobials. The *mecA* gene is located on the chromosome of the bacterium on a mobile element called the 'staphylococcal chromosomal cassette' (SCC*mec*) (Weese & van Duijkeren, 2010; Kate *et al*., 2018). The SCC*mec* element can be transferred between different staphylococcal species (Wielders *et al*., 2001; Arianna *et al*., 2015; Somayaji *et al*., 2016).

Methicillin-resistant *S. pseudintermedius* was first reported in Brazil, and was isolated from the skin of a clinically healthy cat (Lilenbaum *et al*., 1998; Pitchenin *et al*., 2017). Colombini *et al.* in 2000 reported first two isolates of MRSP in the USA obtained from dogs with otitis media.

MRSP emerged in Europe in 2005 in Germany where twelve multi-drug resistant isolates were obtained from 11 dogs and one cat at the veterinary dermatology referral clinic (Loeffler *et al*., 2007; Melter *et al*., 2017; Papadogiannakis *et al*., 2016). The isolates were resistant to oxacillin, enrofloxacin, gentamicin, macrolides, lincosamides, trimethoprim-sulfamethoxazole and most of them to tetracycline while their pulse field gel electrophoresis profiles showed that they were very closely related. Since then, MRSP was reported in other studies in Germany, Italy, Switzerland, Poland, and several other European countries including Sweden, Denmark, Netherlands, Luxemburg, and United Kingdom. Infections with MRSP are more common in dogs than in cats (Morris *et al*., 2006; Borjesson *et al*., 2015; Papadogiannakis *et al*., 2016; Ruzauskas *et al*., 2016).

2.8 Multidrug resistance by *Staphylococcus pseudintermedius*

Multidrug resistance is recognized as resistance to several antimicrobials, usually resistance to at least two different classes of antimicrobials. It is generally caused by the acquisition of different genes that code for resistance to a single drug, in different acquisition events. This accumulation of antibiotic resistance genes generally occurs on resistance plasmids, known as “R plasmids”, that are not only stably maintained, but that are also passed along between bacterial cells at a very high efficiency. A large number of MRSP strains also show multidrug resistance (Chrobak *et al*., 2011; Valentina *et al*., 2017). In one study from South Korea, where 11 different species of *Staphylococcus* were recovered, *S. pseudintermedius* showed the highest rate of multidrug resistance. All multidrug-resistant *S. pseudintermedius* were resistant to antibiotics commonly used in the treatment of pyoderma, otitis, and enterocolitis in dogs (Moon *et al*., 2012; Somayaji *et al*., 2016).

Multidrug resistance is frequent in *S. pseudintermedius* and includes resistance to tetracycline, macrolides, lincosamides, streptogramins, aminoglycosides, aminocyclitols, fluoroquinolones, and methicillin. The genome of a *S. pseudintermedius* methicillin-susceptible strain (ED99) revealed the presence of four transposons containing one or more antibiotic resistance genes, where two of those contained the *bla* operon, which is responsible for beta-lactamase-mediated resistance. The close similarity of transposons found in human-associated staphylococcal species and *S. pseudintermedius* suggests interspecies horizontal transfer of antibiotic resistance. It should be noted that the mentioned strain, ED99, is resistant to penicillin but susceptible to methicillin since it lacks the *mec*A gene. The clinical importance of *S. pseudintermedius* is responsible for a high antibiotic selective pressure, which plays a role in the spread of mobile genetic elements encoding antibiotic resistance (Ben Zakour *et al*., 2012; Verstappen *et al*., 2017).

2.9 Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) infection in dogs

Colonization of the canine skin and mucosa with both methicillin-susceptible and methicillin-resistant strains of *S. pseudintermedius* has been well-documented. Most healthy dogs are colonized with methicillin-susceptible *Staphylococcus pseudintermedius* (MSSP), with rates ranging from 37 % - 92 % (Priyantha *et al*., 2016). Reported prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) colonization of healthy dogs in the community, or at hospital admission, have ranged from 0 - 4.5 %, with one study in Japan reporting MRSP carriage in 30 % of dogs at a veterinary teaching hospital (Garbacz *et al*., 2013; Arianna *et al*., 2015; Melter et al., 2017). A primary limitation discussed by the authors of most colonization studies is the one time sampling approach making it difficult to discriminate between

colonization and contamination (Bannoehr and Guardabassi *et al*., 2012; Somayaji *et al*., 2016; Ruzauskas *et al*., 2016).

The variability in reported colonization rates can be attributed to a number of factors, such as the number of samples collected, the anatomical sites (nasal, perianal, mouth, skin) sampled, differences in study populations (e.g. geographic location, inclusion criteria), and culture methodology (e.g. enrichment broth or selective culture) (Bannoehr & Guardabassi, 2012; Somayaji *et al*., 2016). Two longitudinal studies have reported information on long-term carriage of both MSSP and MRSP (Paul *et al*., 2012; Windahl *et al*., 2012; Ruzauskas *et al*., 2016; Melter *et al*., 2017). Paul *et al*. (2012), investigated *S. pseudintermedius* colonization in Denmark, collected and followed samples from 16 dogs over a six-month period. Six (37.5 %) of the dogs studied were classified as persistent carriers, that is, having *S. pseudintermedius* isolated at all sampling times over the study period. Of the remaining 10 dogs, five were classified as transient carriers (positive in at least three consecutive samplings), four as sporadic carriers (positive in only one or two samplings), and one as a non-carrier (negative in all samplings) respectively (Paul *et al*., 2012; Melter *et al*., 2017).

Windahl *et al*. (2012) investigated MRSP carriage in 31 dogs with a previous history of MRSP infection, over a period of eight months. They reported that dogs remain colonized for several months of post-infection, with 61 % of dogs culturing positive for at least 8 months. Of the dogs that were positive for 8 months, 5 were followed for a total of 14 months and continued to be culture-positive for MRSP. The authors of this study emphasized that re-infection (from other positive dogs, the environment, or humans) rather than prolonged colonization could not be ruled out (Windahl *et al*., 2012; Ruzauskas *et al*., 2016).

2.10 Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) infections in Humans

Until the last decade, reports of *Staphylococcus pseudintermedius* colonization in humans were limited. *Staphylococcus pseudintermedius* is part of the normal microbiota of dogs and cats. Both animals and humans can be contaminated, colonized or infected with methicillin-resistant *S. pseudintermedius*. Colonization is the presence, growth and multiplication of MRSP in one or more body sites without observable clinical signs or immune reaction. The term 'carrier' in animals or humans refers to an individual colonized with MRSP. The most commonly reported site of MRSP colonization in dogs is the nose and the anus, but these are also the most commonly tested sites (Weese & van Duijkeren, 2010; Papadogiannakis *et al*., 2016; Pitchenin *et al*., 2017).

Other sites, such as the pharynx, might also be important, but this has not been thoroughly investigated. Infection is a condition whereby MRSP has invaded a body site, is multiplying in tissue, and is causing clinical manifestations of disease. Contamination of the coat, skin and nose can occur. When an individual is contaminated, the bacteria can be easily washed off, and often only one culture is MRSP-positive, while subsequent cultures are negative. As most studies on MRSP are one-point prevalence studies and only one sample per individual is investigated, it is often unclear whether individuals are colonized or merely contaminated with MRSP. Longitudinal studies involving repeated cultures of the same individuals could help to clarify if animals or humans are colonized or contaminated by MRSP (Rubin *et al*., 2011; Somayaji *et al*., 2016; Papadogiannakis *et al*., 2016).

More recently, there have been a number of studies investigating the zoonotic transmission of *S. pseudintermedius* from dogs to veterinary personnel or their owners. Colonization of humans by

methicillin-susceptible and methicillin-resistant strains of *S. pseudintermedius* is considered rare or uncommon, with prevalence estimates in humans with animal contact ranging from 0.4 % - 8 % (Boost *et al*., 2011; Pires dos Santos *et al*., 2016). The colonization or contamination of veterinarians with MRSP is within the reported estimates of 3.9 % - 32 % (Weese & van Duijkeren, 2010; Pitchenin *et al*., 2017; Valentina *et al*., 2017). Most of the reports were cross-sectional studies that describe a one-time sampling approach; therefore, it is impossible to distinguish between colonization (either persistent or intermittent) and contamination. Resampling approaches for detecting human colonization or contamination with MRSP in households with infected dogs have been reported (Laarhoven *et al*., 2011; Pires dos Santos *et al*., 2016).

One longitudinal study investigated the carriage of MRSP in 16 previously infected dogs, as well as the household environment, contact animals and humans (Laarhoven *et al*., 2011; Pires dos Santos *et al*., 2016). Sample collection started within 7 months of initial diagnosis of a household dog with an MRSP infection. Nasal samples were collected from 25 persons (owners/household members) in 16 households for 6 months. Three humans from different households were positive at the initial sampling, while no humans were positive over the next four samplings. Two humans from the same house were MRSP positive at the last sampling. No human tested positive more than once, including 2 that were positive at the start of the study and remained negative for the duration. In all cases where humans were MRSP positive, MRSP was also isolated from the index dog, in contact animals, and the household environment. Throughout the study, 10 dogs had active clinical disease, suggesting that even when the bacterial load is high, the risk of zoonotic transmission is low. The strains recovered from humans were always identical to strains isolated from the index canine case. Although reports of *S. pseudintermedius* infection in humans

exist, *S. pseudintermedius* is primarily considered a canine pathogen. Infected dog bite wounds are a common cause of *S. pseudintermedius* infections in humans (Bannoehr & Guardabassi, 2012; Somayaji *et al*., 2016; Jurate & Jurate, 2015; Melter *et al*., 2017). Most human clinical laboratories do not routinely take the additional steps to detect *S. pseudintermedius*, so it is possible that these isolates, once being identified as coagulase-positive staphylococci, were mistakenly identified as *S. aureus* (van Hoovels *et al*., 2006; Arianna *et al*., 2015; Somayaji *et al*., 2016). Thus it is conceivable that the true incidence of human *S. pseudintermedius* infections is under-reported (Weese & van Duijkeren, 2010; Ruzauskas *et al*., 2016; Kate *et al*., 2018).

2.11 Epidemiology of *Staphylococcus pseudintermedius*

As mentioned earlier, *S. pseudintermedius* is an opportunistic pathogen that is part of the normal flora of dogs and does not cause disease except when the host is immunosuppressed and/or has alteration of the skin barrier. Therefore, exposure of a healthy dog to an infected one is typically not sufficient to produce clinical disease (Ricardo, 2013; Arinanna *et al*., 2015). MRSP colonization and infection has been described in dogs, cats, horses, birds, and humans (Weese & van Duijkeren, 2010; Kate *et al*., 2018; Somayaji *et al*., 2016). Colonization with MRSP is more prevalent in dogs than in any other mammal. The prevalence of MRSP colonization or contamination has been studied in various dog populations in different countries, with rates of 0 % – 4.5 % in dogs in the community and upon admission to veterinary hospitals; and 0 % – 7 % in dogs with skin disease (Griffeth *et al*., 2008; Somayaji *et al*., 2016; Papadogiannakis *et al*., 2016; Verstappen *et al*., 2017).

A study at Japan reported that 66 % of the *S. pseudintermedius* isolates cultured from dogs with pyoderma visiting two referral hospitals were methicillin-resistant based on the detection of *mec*A (Kawakami *et al.,* 2010; Pires dos Santos *et al*., 2016; Kate *et al*., 2018). In Canada, the prevalence of MRSP colonization in healthy cats was 1.2 % (Hanselman *et al*., 2009; Jurate & Jurate, 2015; Valentina *et al*., 2017). In the United States, the prevalence of MRSP colonization in healthy dogs was 2 % (1/59) (Griffeth *et al*., 2008; Papadogiannakis *et al*., 2016; Verstappen *et al*., 2017). In Germany, the prevalence of MRSP in 16,103 clinical specimens of small animal and equine origin was 0.8 % in dogs (61/7490), 0.1 % in cats (6/3903) and 0.1 % in horses and donkeys (5/4710). MRSP prevalence in dogs was significantly higher than in cats and equines (Ruscher *et al*., 2010; Melter *et al*., 2017).

The epidemiology of MRSP in countries such as Sweden and Norway, have been explored (Kjellman *et al*., 2015). There is evidence that the clonal structure of MRSP is changing in some countries (Duim *et al*., 2016). Since the mid-1980s, an ever increasing number of MRSP isolates have been reported. In the mid-2000s, two predominant MRSP clones, ST71 (sequence type 71) and ST68, spread through Europe and North America, respectively. MRSP isolates are commonly multi-drug resistant and are thus capable of causing infections that do not respond to routinely used antimicrobials. MRSP appeared in the small animal population of Finland in the late 2000s, also causing numerous infections at the Veterinary teaching hospital.

The population of *S. pseudintermedius* is highly diverse and included five major MRSP clonal complexes (CCs). CC71, previously described as the epidemic European clone, is now widespread worldwide. In Europe, CC258, which is more frequently resistant to sulphonamides/trimethroprim than CC71, is increasingly reported in various countries. CC68, previously described as the epidemic North American clone, is frequently reported in Europe,

while CC45 (associated with chloramphenicol resistance) and CC112 are prevalent in Asia (Pires dos Santos *et al*., 2016). MRSP isolation frequency may reach up to 67 % of all clinical *S. pseudintermedius* isolates in certain countries and veterinary hospitals (Kawakami *et al*., 2010; Kate *et al*., 2018). A 272 % increase in MRSP cases was noted in a veterinary laboratory from 2007- 2008 through 2010 - 2011 (Steen, 2011; Valentina *et al*., 2017). Hospitalization, frequent visits to veterinary practices, and prior antimicrobial usage are recognized risk factors for canine MRSP infection and carriage (Frank *et al*., 2009; Pires dos Santos *et al*., 2016; Papadogiannakis *et al*., 2016).

Epidemiologic research of the genetic relations between methicillin-resistant staphylococci is important because it helps to understand the spread of the bacteria as well as the relationship between human and animal infections (Cohn & Middleton, 2010; Arianna *et al*., 2015). Studies on *Staphylococcus pseudintermedius* characterization have been performed in several countries. In China, a large study done in Guangdgong province recovered 144 *Staphylococcus pseudintermedius* isolates from 785 sampled dogs and cats. Almost 50 % of the isolates were classified as MRSP. In this study, 24 different sequence types (STs) were identified, thus demonstrating that MRSP in South China has high genetic diversity (Feng *et al*., 2012; Kjellman *et al*., 2015; Duim *et al*., 2016).

In a study from South Korea, staphylococci were isolated in 55.2 % (111/201) of the samples obtained from staff, hospitalized animals, and medical equipment. The most prevalent species was *S. pseudintermedius* (46.8 %). Of importance, among the MRSP isolates, *SCCmec*V was the most prevalent. The highest detection rate and diversity were found in the staff and not in the animals or equipment, this is a relevant issue since it indicates that people could serve as reservoirs for the dissemination of staphylococci (Gomez-sanz, 2011; Pitchenin *et al*., 2017). The

epidemiology and molecular characteristics of MRSP in countries, such as Sweden and Norway, have already been explored (Borjesson *et al*., 2012; Duim *et al*., 2016). In Finland, a large veterinary hospital outbreak in 2010 was caused by the predominant European MRSP clone, ST71 (Gronthal *et al*., 2014) and ST45.

These two main clones were identified among Finnish guide dogs (Gronthal *et al*., 2015). Transmission of *S. pseudintermedius* from dogs into humans is an understudied and likely under-recognized epidemiological phenomenon; the ubiquity of canine colonization and the frequency of dog ownership provides ample opportunity for transmission. Although inadequately studied, putative within household transmission between people and dogs has been reported; one study found that 5.6 % of dog owners carried *S. pseudintermedius* (Gomez-Sanz *et al*., 2013; Walther *et al*., 2012; Melter *et al*., 2017; Pires dos Santos *et al*., 2016).

The propensity of *S. pseudintermedius* to colonize the pharynx and rectum may facilitate transmission through the fecal-oral routes (Rubin & Chirino-Trejo, 2011; Somayaji *et al*., 2016; Pitchenin *et al*., 2017). Study of other organisms such as *Escherichia coli* have demonstrated that transmission between dogs and humans within households is highly frequent, and thus it is plausible that staphylococci may similarly be transmitted (Johnson *et al*., 2008; Jurate & Jurate, 2015). One study where 146 MRSP isolates from Germany, Netherlands, France, Italy, Austria and Luxembourg were analyzed, showed that ST71 was the main clone detected (145/146), with only one isolate pertaining to a different sequence type (ST5) (Ruscher *et al*., 2010; Melter *et al*., 2017).

Another study conducted in Spain (Gomez-Sanz *et al*., 2011; Borjesson *et al*., 2015; Pitchenin *et al*., 2017) supported the findings that ST71 is the main MRSP lineage in Europe. On the other hand, a more heterogeneous clonal distribution was reported in Norway, where ST106 (8/23)

was the main MRSP clone, followed by ST71 (4/23), ST28 and ST127 (2/23 each), and STs 10, 26, 69, 78, 100, 128 and 129 (1/23 each) (Osland *et al*., 2012; Valentina *et al*., 2017; Pires dos Santos *et al*., 2016). Notably, transmission of *S. pseudintermedius* may also occur from other companion animals to humans and potentially between humans in nosocomial settings (Starlander *et al*., 2014). The populations at risk of infection with *S. pseudintermedius* have not been defined. Working in the veterinary field is another plausible risk factor. In a study of veterinarians, individuals with open wounds or medical devices, who are in close contact with dogs, may also be at increased risk of *S. pseudintermedius* colonization and infection.

2.12 Prevention of *Staphylococcus pseudintermedius* transmission

- **In Veterinary clinics**

The dispersal of *S. pseudintermedius* from the skin of dogs accounts for the frequent occurrence of this bacterium in the environment of veterinary practices (van Duijkeren *et al*., 2008; Kate *et al*., 2018). Rigorous hygienic precautions must be adopted whenever MRSP colonisation or infection is detected or suspected in animal patients to prevent nosocomial infection and further spread of this multi-drug-resistant bacterium (Lloyd, 2010; Kjellman *et al*., 2015; Duim *et al*., 2016). This should include personal hygiene (hand washing, use of masks, gowns and gloves for surgical procedures) and environmental hygiene measures through thorough and regular cleansing and disinfection of all practice areas, as recommended for MRSP (NASPHV, 2008; Somayaji *et al*., 2016, Ruzauskas *et al*., 2016). Guidelines on the management of *S. pseudintermedius* in veterinary practices have been developed by the British Small Animal Veterinary Association (BSAVA, 2007; Ruzauskas *et al*., 2016; Valentina *et al*., 2017). In line with standard infection control principles, animals diagnosed with or suspected of MRSP

infections should be isolated in order to minimize the risk of nosocomial transmission. In veterinary clinics, this includes using barrier nursing precautions and limiting staff contact. This includes wearing protective aprons, overshoes and gloves (Hanselman *et al*., 2009; Borjesson *et al*., 2015; Jurate & Jurate, 2015; Valentina *et al*., 2017). The environment of veterinary hospitals must be cleaned and disinfected regularly. Decolonization of personnel that test MRSP positive repeatedly should be considered. MRSP infected wounds should be covered with clean bandages if possible, in addition to isolation of the patient.

- **In households**

Household transmission from MRSP infected or colonized animals to healthy contact animals has been described (van Duijkeren *et al*., 2011b; Papadogiannakis *et al*., 2016; Somayaji *et al*., 2016). Widespread contamination of the environments of households has also been reported, indicating that direct contact with a patient or colonized animal is not necessary, as indirect transmission through the environment can also occur. It is difficult to clear the organism from the environment as long as the MRSP-infected animal still has clinical signs of MRSP infection and lives in the environment, especially when the infection site is the skin or the ears, because shedding of the organism will continue (van Duijkeren *et al*., 2011b; Somayaji *et al*., 2016).

Proper cleaning and disinfection of the contaminated environment will reduce the number of organisms. Other possible interventions in households with MRSP-positive animals include removing the pet from the household (temporarily) in order to avoid transmission to other pets and even pet owners. Although the risk of zoonotic transmission of MRSP is small and colonization of humans seems to be transient, persons in close contact with infected animals seem to have a higher risk to be MRSP positive. Clearly, for all people having contact with

companion animals, appropriate hygiene is the cornerstone in minimizing the spread of MRSP between animals and humans. One study indicates that routine hand hygiene may be effective in reducing transmission of *S. pseudintermedius* between humans and pets in the household (Hanselman *et al*., 2009; Jurate & Jurate, 2015; Valentina *et al*., 2017).

- **Other good hygiene practices**

Frequent hand washing after contact with the pet is encouraged. Individuals and dog owners should avoid contact with the infected site. Contact with the nose of the infected animal should be reduced, since it may also be carrying the bacterium there. In general, reducing close contact (e.g. snuggling, nuzzling, hugging, and kissing) with dogs during the period of infection is a good preventive measure. Regular washing (in hot air with hot air drying) of pet beds and other items that come into close and frequent contact with the pet will reduce the risk of *S. pseudintermedius* transmission to humans (Worms, 2011; Ariana *et al*., 2015; Verstappen *et al*., 2017).

2.13 Treatment

Staphylococcus pseudintermedius is acknowledged as the main cause of canine pyoderma, which represents the most common dermatological pathology seen in dogs. It is also associated with infections in other body sites such as ears, urinary tract, surgical sites, wounds, mammary gland, and endocardium (Ricardo, 2013; Somayaji *et al*., 2016). Treatment is generally required when infection is caused by MRSP. Treatment for the infection can be topical therapy, combined or not with systemic antibiotics. For the topical treatment, usually lavage and debridement will be done if possible. Conventional treatment relies on antimicrobial ointments such as mupirocin. Unconventional therapy is based on natural products such as oak bark and honey (Cohn & Middleton, 2010; Verstappen *et al*., 2017).

For systemic antibiotic treatment, drugs have to be chosen based on the susceptibility of the isolates. It is also important to know if the antimicrobial will reach therapeutic concentrations at the site of infection. Irrespective of the culture and susceptibility results, MRSP should not be treated with beta-lactams. It is also relevant to know that even if the isolate is susceptible to fluoroquinolones *in vitro*, rapid resistance can develop *in vivo*. Thus, fluoroquinolones are not recommended to treat MRSP (Onuma *et al*., 2012; Valentina *et al*., 2017). Decolonization therapy in dogs is not currently recommended, nasal mupirocin is difficult to administer effectively and does not address enteral colonization. Similarly, the efficacy of systemic decolonization therapies has not been evaluated (Paul *et al*., 2011; Pitchenin *et al*., 2017; Papadogiannakis *et al*., 2016).

2.14 Antibiotics

Antibiotics can be defined as a substance or compound produced by microorganisms, microscopic plants, or chemically synthesized that is capable of killing or inhibiting the growth of other microorganisms. Antibiotics are specifically used to treat infections caused by bacteria, such as *Staphylococcus*, *Streptococcus*, or *E. coli*, and either kill the bacteria (bactericidal) or keep it from reproducing and growing (bacteriostatic). The production of antibiotics has been widespread since the pioneering efforts of Florey and Chain in 1938. Penicillin, the first antibiotic was discovered in 1928 by Alexander Flemming (Tan *et al*., 2015). Antibiotics are used in different forms: as ointment or cream when the infections occurs on the skin surface; if the infection is internal, can be swallowed as tablets and capsules or as injection delivered throughout the body by absorption into the bloodstream (Finberg, 2004; Tan *et al*., 2015). Most antibiotics fall into their individual antibiotic classes that have similar chemical and pharmacological properties. Their chemical structures may look comparable, and drugs within

the same class may kill the same or related bacteria. These antibiotic classes include penicillins, tetracyclines, cephalosporins, fluoroquinolones, lincomycins, macrolides, sulfonamides, glycopeptides, aminoglycosides, etc. (Kingston, 2008; Tan *et al*., 2015).

2.15 Antibiotic resistance

Antibiotic resistance is the ability of bacteria to resist the effects of an antibiotic. Antibiotic resistance occurs when bacteria change in a way that reduces the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply, causing more harm. Antibiotic resistance is one of the biggest threats to global health, food security and development (WHO, 2014). This serious threat is no longer a prediction for the future; it is happening right now in every region of the world and has the potential to affect anyone of any age in any country (WHO, 2014). Antibiotic resistance occurs naturally but misuse of antibiotics in humans and animals is accelerating the process. Antibiotics are extremely important in medicine, but unfortunately, bacteria are capable of developing resistance to them. When bacteria are exposed to the same antibiotics over and over, the bacteria can change and are no longer affected by the drug. Bacteria have a number of ways of becoming antibiotic-resistant. For example, they possess an internal mechanism of changing or mutating their structure so that an antibiotic no longer works. They develop ways to inactivate or neutralize the antibiotic or spontaneously cause resistance because of random mutations (Cassir *et al*., 2014).

The problem of antibiotic resistance is worsened when antibiotics are used to treat disorders in which they have no efficacy (e.g. antibiotics are not effective against infections caused by viruses), and when they are used widely as prophylaxis rather than treatment. Microbes resistant to multiple antimicrobials are called multidrug resistant (MDR) or sometimes superbugs (Leekha

et al., 2011; Cassir *et al*., 2014). The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would have previously been lethal. Antibiotics like penicillin and erythromycin, which used to be one-time miracle cures, are now less effective because bacteria have become more resistant. Timeline of antibiotic deployment and evolution of antibiotic resistance is shown in Figure 2d. Antibiotic use causes evolution of bacteria via the mechanism of natural selection; therefore antibiotic resistance is an outcome of the selection for resistant bacteria. Antibiotic resistance can be either natural or acquired, the latter being a consequence of the selective pressure exerted by antimicrobial drug use or mutation. The mechanisms developed by bacteria in order to acquire resistance as described by Kenneth (2011; Cassir *et al*., 2014), are vertical gene transfer and horizontal gene transfer. A bacterium can acquire resistance by mutations or the acquisition of extra chromosomal DNA (González-Candelas *et al*., 2011; Cassir *et al*., 2014). Thus, mobile genetic elements, which can be transferred horizontally between bacteria, play a major role in acquired antibiotic resistance. For example, bacteria have evolved accessory pieces of DNA such as plasmids that are separate from the chromosome itself. These plasmids are independently duplicating genetic elements, which allow the bacteria to alter its genetic layout in the presence of new conditions. Plasmids are the essential genetic components that allow bacteria to acquire and carry antibiotic resistance. Bacteria can transfer plasmids via a process known as conjugation. Acquiring antibiotic resistance via the transmission of extra-chromosomal DNA including plasmids is known as horizontal transmission. Therefore, horizontal transmission is a common pathway through which *S. pseudintermedius* and *S. aureus* share resistance factors (Youn *et al*., 2010; Melter *et al*., 2017).

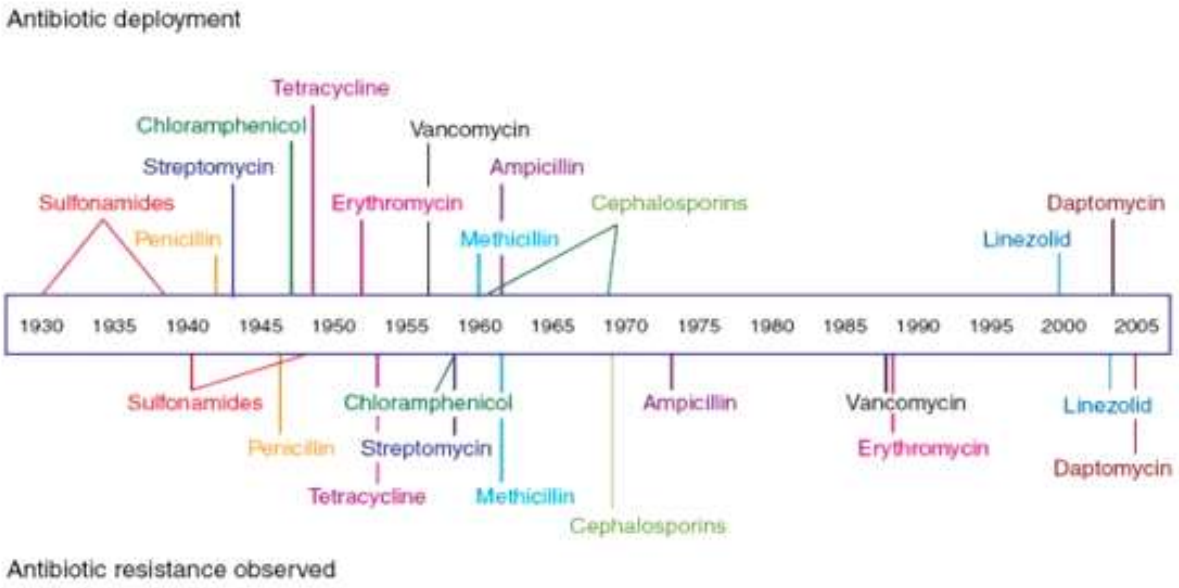

Fig. 2d: Timeline of antibiotic deployment and evolution of antibiotic resistance. The year each antibiotic was deployed is depicted above the timeline and the year resistance to each antibiotic was observed is depicted below the timeline (with the caveat that the appearance of antibiotic resistance does not necessarily imply that a given antibiotic has lost all clinical utility). (Source: Clatworthy *et al*., 2007)

2.15.1 Antibiotic resistance in *Staphylococcus pseudintermedius*

Antibiotic resistance in staphylococci is of great concern due to a continuously increasing incidence of methicillin resistance among *S. pseudintermedius* (Schwarz *et al*., 2006; Somayaji *et al*., 2016). Also, a high rate of multidrug resistance is observed among MRSP strains. Methicillin resistance in *'S. intermedius'* from a canine isolate was first reported in a study published in 1999 (Gortel *et al*., 1999; Papadogiannakis *et al*., 2016). It is important to take into consideration that, since it was not uncommon to misclassify *S. intermedius* as *S. aureus* based on phenotypic tests, MRSP isolates could have been present long before 1999 and erroneously reported as methicillin-resistant *S. aureus* (MRSA). In recent years, an increasing number of MRSP isolates have been identified. A study published in 2006 by Morris *et al*. found that as many as 17 % of the isolates studied were methicillin-resistant (Morris *et al*., 2006; Borjesson *et al*., 2015).

Analogous to that seen in *S. aureus*, the overwhelming majority of resistance to beta-lactamase-resistant penicillin (methicillin being the prototype) in *S. pseudintermedius* isolates is due to the *mec*A gene, which encodes a supernumerary penicillin binding protein (PBP2a) with reduced affinity for beta-lactams (Loeffler *et al*., 2007; Melter *et al*., 2017; Papadogiannakis *et al*., 2016). Resident PBPs play important roles in the formation of the bacterial cell wall peptidoglycans (Berger-Bächi & Rohrer, 2002; Priyantha *et al*., 2016). These PBPs can be inactivated by the presence of beta-lactam antimicrobials, leading to abnormal cell wall synthesis and bacterial death. However, the poor affinity for beta-lactams associated with the carriage of the *mec*A gene, serves as a mechanism of protection for the bacteria, evading disruption of the peptidoglycan layer and preventing bacterial death (Berger-Bächi & Rohrer, 2002; Priyantha *et al*., 2016). A recent study from Youn *et al*. suggests the possibility of horizontal transmission of the *mec*A

gene from *S. pseudintermedius* between different species (Youn *et al.,* 2010; Kjellman *et al*., 2015; Priyantha *et al*., 2016). Methicillin-resistant staphylococci are considered resistant to all beta-lactam antibiotics. Methicillin resistance in *S. pseudintermedius* is based on the expression of the *mec*A gene. Different antimicrobial resistance genes have been identified in *S. pseudintermedius*, most of which have also been detected in other staphylococcal species as well as in a few other Gram-positive bacteria (Kadlec & Schwarz, 2012; Ruzauskas *et al*., 2016; Somayaji *et al*., 2016). The gold standards for determination of methicillin-resistance in *S. pseudintermedius* are *mec*A PCR and PBP2a serology, but other phenotypic methods such as oxacillin and cefoxitin disk diffusion test can also be used (Bemis *et al*., 2012; Papadogiannakis *et al*., 2016). Multidrug resistance is frequent in *S. pseudintermedius* and includes resistance to tetracycline, macrolides, lincosamides, streptogramins, aminoglycosides, aminocyclitols, fluoroquinolones, and methicillin (Ben Zakour *et al*., 2012; Verstappen *et al*., 2017).

2.16 Irrational Use of Antibiotics: A Cause and Risk Factor of Antibiotic Resistance

Development and spread of antimicrobial resistance (AMR) is commonly due to overuse, misuse and indiscriminate use of antimicrobials by doctors, nurses and pharmacists, non-compliance, and self-medication by patients, and use in animal husbandry and agriculture. It is estimated that 70-80 % of prescriptions for antimicrobials are probably advised unnecessarily by the health professionals. In spite of the fact that most common colds and diarrheal episodes are viral in origin, yet, antimicrobials are used indiscriminately (Mishra *et al*., 2012; Arianna *et al*., 2015). Reasons for over prescribing are often lack of confidence, peer pressure, patient pressure, and pharmaceutical company pressure (Kenneth, 2011, Tan *et al*., 2015). Antimicrobial use is a key driver of resistance. Poverty and inadequate access to antibiotics constitute a major factor in the development of resistance. Another common cause of developing resistance is improper

diagnosis. In many instances, dearth of an adequately equipped diagnostic laboratory in the vicinity compels the physician to prescribe antibiotics empirically, thus, increasing the likelihood of the patient receiving a wrong antibiotic. Furthermore, readily availability of antibiotics over-the-counter and sales promotion schemes by the pharmaceutical manufacturers also lead to the promotion of indiscriminate use, thus, increasing the likelihood of development of resistance. Counterfeit drugs are also a problem contributing to the development of resistance. These contain either the wrong ingredient or lesser amount of the active ingredient. In some instances, the medication poisons are capable of causing disability or even death. The impact of the media has also contributed to the development of resistance. Patients often demand antibiotics for their ailment on the basis of advertisements read or seen. Unwitting use of more active drugs at sub-therapeutic doses leads directly to the development of multidrug resistance (Kenneth, 2011; Tan *et al*., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Ebonyi State is in the South East geo-political zone of Nigeria. It is popularly known as "the Salt of the Nation", apparently because of the large deposits of salt water in the state. It derived its name from the Ebonyi River. Abakaliki is the capital city of the present day Ebonyi State in South-eastern Nigeria (Figure 3). The tropical climate is broadly of two seasons: which are rainy season between April and October, and dry season between November and March. Temperature throughout the year ranges between 21 ^{0}C to 34 ^{0}C, and humidity is relatively high. The annual rainfall is about 1,150 mm**.** It lies approximately within latitude 6^0 19' 23.02" N and longitude 8^0 6' 43.24" E. The inhabitants are primarily the Igbos. According to data from the 2006 Population and Housing Census, Ebonyi State has an estimated population of 2.3 million and a land mass of 5,935 km^2. Abakaliki is made up of four clans; namely Ezza Ezekuna, Ngbo, Izzi, and Ikwo. It has an estimated population of 141,438 according to the 2006 census. Nigerians from other communities also reside mainly within the state capital city. Inhabitants are predominantly farmers, civil servants, and traders (The World Gazetteer, 2013).

Figure 3: Map of Nigeria and Ebonyi State, showing Abakaliki, the capital of Ebonyi State (Source: Wikipedia, 2019; https://en.wikipedia.org/wiki/Ebonyi_State).

3.2 Sample Size Calculation

Sample size was determined using the formula;

Sample size = $Z_{1-\alpha/2}^2$ p (1-p) / d^2

Where $Z_{1-\alpha/2}$ = Standard normal variate (at 5 % type 1 error ($p < 0.05$) which is equal to 1.96

p = Expected prevalence in the population based on previous studies. The prevalence of *S. pseudintermedius* among dogs in previous studies ranged from 90 % to 95 %. Rubin *et al*. (2011) reported a 90 % prevalence rate for *S. pseudintermedius* among dogs in Canada (Rubin *et al*., 2011; Somayaji *et al*., 2016). In this study, we calculated the sample size by pegging the expected prevalence at 95 %.

d = Absolute error (0.05)

Therefore, from the sample size formula, **Sample size = $Z_{1-\alpha/2}^2$ p (1-p) / d^2**

Sample size = 1.96^2 x 0.95(1-0.95) / 0.05^2 = **73**

Thus, a minimum of 73 samples were needed for this study but to allow for attrition and a robust sample size, a total of 359 samples were collected from dogs, humans (dog owners), and people who have no contact with dogs.

3.2.1 Study Population

The study population were shelter dogs, and humans [(dog owners), and other people who have no contacts with dogs] living within Abakaliki metropolis, Ebonyi State, South East Nigeria. Three hundred and fifty nine (359) samples were collected in this study from dogs, humans (dog owners), and people who have no contact with dogs.

A total of 112 shelter dogs were sampled (one out of triplicate samples per dog) while 97 dog owners volunteered for this study between January, 2017 and February, 2018. A total of 45, 35, and 32 swab samples were obtained from the perineum, nares, and mouth of dogs respectively. A total of 97 nasal swab samples of dog owners that volunteered for this study were collected. The dogs and dog owners were selected from 69 different households. Also included in the study are 150 people who have no contacts with dogs. All samples were collected in triplicate from each dog and human.

3.3 Equipment and Instruments

The following equipment and instruments were used in the course of this research work; thermocycler (BIO-RAD Finnegan C1000 Touch™, USA), Power pack (BIO-RAD, USA), Molecular Imager® Gel Documentation system (BIO-RAD Gel DOC™XR+, BIO-RAD Laboratories, inc., USA) autoclave, incubator (NL-9052-I, England), McFarland densitometer (Grant Instruments ltd, Shepreth, Cambridgeshire, SG 86 GB, England), Sensititre nephelometer (Grant-bio, England), VWR mixer touch vortex (Henry Troemner, LLC, USA), VWR digital heatblock (Henry Troemner, LLC, USA), Eppendorf AG 22331 centrifuge (USA), refrigerator, water bath, Bunsen burner, petri dishes, conical flasks, test slant, swab sticks, inoculating loop, weighing balance, Bijou bottles etc.

3.4 Chemicals and Reagents

The following reagents and chemicals were used; 10X TBE buffer (Fisher Scientific International inc., USA), gel loading dye, Invitrogen Trackit™ 100 bp DNA ladder (Thermofisher scientific, USA), McFarland reagent (0.5 ml of barium chloride of 0.04 mol/l and 99.5 ml of 0.18 mol/l of sulfuric acid), Gram staining reagent, indole reagent (Oxoid, UK), distilled water, peptone water (Oxoid, UK), and normal saline.

3.5 Culture media

The following culture media were used: Prepared sensititre Mueller-Hinton broth (Oxoid, UK), nutrient agar (Oxoid, UK), DNase agar (Oxoid, UK), CHROMagar *S. aureus* (CHROMagar, Paris, France), Columbia sheep blood agar (Oxoid, UK), trypticase soy broth (Oxoid, UK).

3.5.1 Media preparation

Preparation and sterilization of the various media used in this research was carried out following the manufacturers' instructions. Briefly, this involved weighing the appropriate quantity of each medium, dissolving in the stated solvent using heat and distribution into conical flask for sterilization in the autoclave at 121 ^{o}C for 15 minutes at 15 Psi. Exactly 28 g of nutrient agar powder; 30 g of trypticase soy broth powder; 82.5 g of CHROMagar^{TM} *S. aureus* powder; and 39 g of DNase agar powder were each dissolved in 1 litre of distilled water. All media were dissolved completely by boiling before sterilizing in autoclave at at 121 ^{o}C for 15 minutes at 15 Psi. Also, 39 g of Columbia blood agar base was dissolved in 1 litre of distilled water by boiling. The medium was then sterilized by autoclaving at 121 ^{o}C for 15 minutes at 15 Psi, allowed to cool to 45 ^{o}C before the addition of 5 % v/v sterile defibrinated sheep blood to the sterile cool base. The content of the flasks were aseptically poured into the plates and allowed to set at room temperature.

3.6 Sample Collection

Sterile cotton swab sticks moistened with sterile normal saline (0.85 % NaCl) were used to collect samples from dogs (nares, perineum, and mouth) and humans (nasal swabs). Dog owners and other people (who had no contact with dogs) who volunteered for this study were given instructions for self-collection of nasal swabs. Collected samples (one out of triplicate samples from each dog and human) from dogs and humans were labelled accordingly and immediately

transported within two hours to the Department of Applied Microbiology laboratory, Ebonyi State University, Abakaliki on the same day for bacteriological analysis.

3.7 Ethical Consideration

Ethical clearance for the collection of bacterial isolates from dogs and humans (dog owners and non-dog owners) was obtained from the Ministry of Health, Ebonyi State [Reference number: SMOH/ERC/19/061 **(Appendix XXIV)**]. Informed consent of dog owners and other people (who had no contacts with dogs) were obtained prior to sample collection. This research project was carried out based on a thorough knowledge of the scientific literatures, satisfactory laboratory protocols, and other relevant sources of information guiding this area of research. Every fundamental study was done in line with the World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human and animal subjects, and identifiable human and animal material or data (WMA Declaration of Helsinki, 2004). All bacterial isolates and identifiable human and animal data or material used in undertaking this research project was treated and handled with utmost confidentiality. Appropriate caution was also observed in the conduct of this research work so as to avoid every negative impact of the study on the environment and other human and animal subjects within the vicinity of the research work including the researchers. Results (both positive and negative) of the research work was also taken and recorded accurately based on existing guidelines in relevant scientific literatures, and the results were preserved without any alteration whatsoever.

3.8 Bacteriological Analysis

Each swab sample was streaked onto CHROMagar *S. aureus* (CHROMagar, Paris, France) and incubated at 35 °C for 24 hours. After incubation, small blue coonies (1-3 mm) typical of *Staphylococcus pseudintermedius* were picked and further sub-cultured onto 5 % sheep blood Columbia agar. Suspected *S. pseudintermedius* colonies on Columbia sheep blood agar (small, creamy grey to white, round low convex colonies with a small margin and double zone of hemolysis) were then purified through successive streaking so as to obtain pure cultures. Purified colonies were then subjected to Gram staining and catalase test. Presumptive Staphylococcal colonies (Gram-positive cocci in bunches with positive catalase test) were further evaluated for coagulase production (tube coagulase using rabbit plasma), beta-galactosidase production, acetoin production (MRVP), pyrrolidonyl arylamidase (PYR) test, DNase activity, hyaluronidase test, and some other biochemical tests such as glucose utilization, sucrose utilization, maltose utilization, and mannitol utilization (Cheesebrough, 2006; Rubin *et al*., 2011; Somayaji *et al*., 2016). Identified *S. pseudintermedius* colonies were then preserved at -80 ^{o}C in trypticase soy broth (TSB) with 15 % glycerol for further analysis, including molecular characterization.

3.9 Morphological and Physiological Characterization of the bacterial isolates

Various morphological and physiological tests were carried out on the isolates (Cheesebrough, 2006; Rubin *et al*., 2011; Somayaji *et al*., 2016). They include the following:

3.9.1 Gram staining and microscopy

Smear was prepared from a 24 hour old culture on a grease free slide and this was then heat-fixed. The smear was stained for 2 minutes with crystal violet. The smear was later flooded with

Gram's iodine solution and this was allowed for 1 minute. The smear was decolourized with alcohol until no more violet colour runs from the slide; this lasted for only 10 seconds. The smear was then rinsed with gentle running tap water and counterstained with safranin for 2 minutes. The smear was then washed with gentle running tap water, blotted dry and then observed under oil immersion objective. The organisms that retain the purple coloration were taken as Gram-positive while those that retain the red coloration were taken as Gram-negative.

3.9.2 DNase Test

The isolates were inoculated on DNase agar (a medium containing DNA) and incubated at 37 ^{o}C for 24 hours. The colonies were tested for DNase production by flooding the plates with a weak hydrochloric acid solution. Clearing around colonies within 5 minutes of adding the acid indicates a positive result while a negative test shows no clearing around the colonies.

3.9.3 Catalase Test

An 18-hour old culture of the isolate was emulsified with a loopful of water to a clean slide. A loopful of hydrogen peroxide was then added to the emulsified slide. Active bubbling indicates a positive test as a result of the breakdown of hydrogen peroxide (H_2O_2) to oxygen and water while no bubbling indicates a negative test result. Catalase positive organisms produce gas bubbles on the slide.

3.10 Biochemical Tests

Several biochemical tests which included uninoculated controls were carried out to characterize and identify the isolates.

3.10.1 Beta-haemolysis

A plate of Columbia sheep blood (5 %) agar was streaked with the test organism and incubated at 37 ^{o}C for 24 hours. A clear colourless zone surrounding the colonies indicates total lysis of the red blood cells. This indicates a positive test result while no clear zone indicates a negative test result.

3.10.2 Coagulase Test

In this test, the sample was added to rabbit plasma and held at 37 ^{o}C or a specified period of time, usually about 24 hours. A positive test is the formation of a visible clump, which is the clotted plasma. Samples must be observed for clotting within 24 hours. This is because some strains that produce coagulase also produce an enzyme called fibrinolysin, which can dissolve the clot. The formation of a clot within 12 hours and the subsequent disappearance of the clot within 24 hours could produce a so-called false negative if the test were only observed at the 24 hour time.

3.10.3 Methyl red Voges-Proskauer (MRVP) Test

MRVP medium containing glucose phosphate and peptone dissolved in 100 ml of distilled water was used. Reagents applied in this test include methyl red solution, potassium hydroxide, and creatine. Two tubes labeled MR and VP each containing the MRVP medium were inoculated with a loop of 24 hour old culture and incubated at 37 ^{o}C for 3 days. To the tube labeled MR, 2 drops of 4 % methyl red solution was added. A magenta colour indicated a positive result while a yellow colour indicated a negative result. To the tube labeled VP, 0.5 ml of 6 % α-naphthol solution and 0.5 ml of 16 % KOH were added. The tube was well-shaken and observed for a

colour change. Pink colour indicated a positive result while a negative result was indicated by no colour change.

3.10.4 Oxidase Test

An 18 hour old broth culture of the test organism was streaked onto the dry surface of a nutrient agar plate and incubated at 37 ^{o}C until a reasonable growth was observed. Oxidase medium reagent was then poured over the surface of the agar growth. Oxidase positive colonies developed pink colours which become successively dark-red, purple and black within 15 minutes.

3.10.5 Indole Test

About 5 ml of peptone water was dispensed into test tubes and inoculated with a loopful of broth culture of the bacteria under study and incubated for 6 days at 37 ^{o}C. After incubation, 0.5 ml Kovac's indole reagent was added, shaken gently and then allowed to stand for 5 minutes. The development of a deep red coloration in the presence of indole which separates out in the alcohol layer indicates a positive test, and vice versa indicates negative test result.

3.10.6 Citrate Test

Simmons citrate agar was inoculated lightly on the slant by touching the tip of a needle to a colony that is 24 hours old. It was then incubated at 35 ^{o}C to 37 ^{o}C for 24 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium. The development of blue colour, denoting alkalinisation indicates a positive test result.

3.10.7 Sugar fermentation Test

This detects the ability of an organism to ferment a particular sugar. This test was carried out for glucose, sucrose, lactose, mannitol, and maltose. Each of the sugar media were separately dispensed into test tubes together with a control. Phenol red was used as indicator and this gave each medium its characteristic red coloration. Inverted Durham's tubes were introduced into each test tube. All the test tubes were inoculated with a loopful of the broth culture of the bacteria isolate except the control and incubated at 37 ^{o}C for 7 days. The test tubes were observed for colour change from red to yellow for a positive result, this implied that the organism was able to ferment the particular sugar and produce acid. A negative result showed no colour change.

3.10.8 Beta-galactosidase Test

A loopful of the test bacterial colonies from an 18 -24 hour old pure culture was transferred into a tube containing 0.5 ml of 0.85 % sterile saline. An ONPG disk was then added to the dense bacterial suspension and incubated at 37 ^{o}C for 24 hours. The development of a yellow colour in the tube after 24 hours of incubation indicates a positive test result while no colour change indicates a negative test result.

3.10.9 Pyrrolidonyl arylamidase (PYR) Test

A PYR paper disk was slightly moistened with sterile water and placed in petri dish using sterile forceps. A sterile wooden stick was then used to pick distinct pure culture colonies from 24 hours sheep blood agar plate. A visible heavy inoculum was then gently rubbed onto a small area of the PYR disc. This was allowed to incubate for 2 minutes at room temperature. After the

incubation, 1 drop of PYR reagent (N-N-dimethyl aminocinnamaldehyde) was added and observed for colour change within 1 minute. The development of a deep cherry red or bright pink colour within a minute of addition of the PYR reagent indicates a positive test while a yellow colour, orange colour or no colour change indicates a negative test.

3.10.10 Hyaluronidase Test

The production of hyaluronidase is assessed by the interaction of a test isolate with a pure culture of mucoid *Pasteurella multocida* 29214. *Staphylococcus aureus* 29213 was used as a positive control. A *P. multocida* 29214 streak was made on blood agar and a line of the test organism (*S. pseduintermedius*) was then inoculated adjacent to the *Pasteurella multocida* 29214 streak at 90° without touching it. *S. aureus* 29213 will result in a deviation of the normal *Pasteurella multocida* 29214 colony morphology (flattened, non-mucoid growth) while *S. pseduintermedius* does not. Hyaluronidase production is very useful for differentiating *S. aureus* (positive), from *S. pseudintermedius* (negative) (Rubin & Chirino-Trejo, 2011).

3.11 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, M100-S24 (CLSI, 2014). Minimum Inhibitory Concentration (MIC) was determined by broth microdilution using the sensititre system (ThermoFisher Scientific, Oakwood Village, Ohio, USA). The following antibiotics were tested on all the isolates: penicillin, ampicillin, erythromycin, clindamycin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, moxifloxacin, gentamycin, chloramphenicol, rifampin, nitrofurantoin, vancomycin, linezolid, daptomycin, quinupristin/dalfopristin, and amikacin. *Staphylococcus aureus* ATCC 29213 and *Enterococcus*

faecalis ATCC 29212 were used as quality control strains. An 18 hour old blood agar culture of the *S. pseudintermedius* isolate was standardized to 0.5 McFarland's standard with the aid of McFarland densitometer (Grant Instruments LTD, Cambridgeshire, England) and senstitre nephelometer (Grant Instruments LTD, Cambridgeshire, England). The sensitire machine was turned on and single plating icon was selected. The nephelometer was then calibrated with the standardized inoculum (control was not vortexed) after McFarland standardization with the McFarland densitometer.

The sensititre plate containing the antibiotics was opened and labelled according to the isolate code. Next, 30 µl aliquot of the standardized inoculum was pipetted into Mueller-Hinton broth and vortex. Dosing head was then carefully screwed on the Mueller-Hinton broth tube and place in the sensititre machine to fit the tube holder. Sensitire plate was then placed in the plate holder of the sensititre machine. Next the green single plate screen icon on the machine was selected to aliquot the standardized inoculum into the sensititre plates containing the antibiotics. After inoculum discharge was completed, the Mueller-Hinton broth tube containing the standardized inoculum left over was removed from the machine and discarded. The sensititre plate was then covered with a transparent sticker. The top end of the plate was then labelled with the time of sensititre process completion. After this, the inoculated sensititre plate was then incubated at 35 ^{0}C for 18 hours. After incubation, MICs were determined and interpreted as susceptible or resistant using the CLSI breakpoints for all antibiotics except tigecycline and daptomycin for which the European Committee on Antimicrobial Susceptibility testing (EUCAST) interpretative criteria was used (EUCAST, 2014).

3.11.1 Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) determination

This was done by antimicrobial minimum inhibitory concentration (MIC) determination in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, M100-S24 (CLSI, 2014). Antimicrobial minimum inhibitory concentration (MIC) was determined by broth microdilution using the sensititre system (ThermoFisher Scientific, Oakwood Village, Ohio, USA). Oxacillin antibiotic (with 2 % NaCl) was used in MRSP determination. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains. An 18 hour old blood agar culture of the *S. pseudintermedius* isolate was standardized to 0.5 Mcfarland's standard with the aid of McFarland densitometer (Grant Instruments LTD, Cambridgeshire, England).

The sensitire machine was turned on and single plating icon was selected. Next, the sensititre plate containing the oxacillin antibiotic with 2 % NaCl was opened and labelled according to the isolate code. Next, 30 µl aliquot of the standardized inoculum was pipetted into Mueller-Hinton broth and vortex. Dosing head was then carefully screwed on the Mueller-Hinton broth tube and place in the sensititre machine to fit the tube holder. Sensitire plate was then placed in the plate holder of the sensititre machine. Next, the green single plate screen icon on the machine was selected to aliquot the standardized inoculum into the sensititre plates containing the antibiotics. After inoculum discharge was completed, the Mueller-Hinton broth tube containing the standardized inoculum left over was removed from the machine and discarded. The sensititre plate was then covered with a transparent sticker. The top end of the plate was then labelled with the time of sensititre process completion. After this, the inoculated sensititre plate was then incubated at 35 ^{0}C for 18 hours. After incubation, MICs were determined and interpreted as susceptible or resistant using the CLSI breakpoints for oxacillin antibiotic. Isolates were

considered to be methicillin-resistant *S. pseudintermedius* (MRSP) when they exhibit resistance to oxacillin (MIC ≥ 0.5µg/ml).

3.12 Determination of multiple antibiotic resistance indices (MARI) of the isolates

Multiple Antibiotic Resistance Index (MARI) of isolates was calculated with the technique described by Christopher *et al*. (2013) and Subramani (2012). This was calculated as the number of antibiotics to which the tested isolates were resistant to (a), divided by the total number of antibiotics to which the organisms were tested against (b); that is, MARI = a/b

3.13 Molecular characterization of *Staphylococcus pseudintermedius* isolates

3.13.1 Extraction of Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) was extracted from overnight cultures of *S. pseudintermedius* grown at 37 °C on blood agar plates by boil prep technique using heat block matrix (VWR Digital Heat Block, Henry Troemner, LLC, USA). Sterile Eppendorf tubes were labelled in duplicates according to the isolates' codes; and one set of the tubes was filled with 200 µl of sterile water each. The Eppendorf tubes filled with sterile water were used for isolate inoculation while the second set of Eppendorf tubes without sterile water was used for DNA storage. The first set of the tubes was each inoculated with a loopful of a 24 hour old culture of the isolates. Next, each of the inoculated tube was vortexed for proper mixing. The vortexed tube was tapped occasionally to allow inoculum droplets drain down the tube. After this, the vortexed Eppendorf tube was fixed into the heat block boil prep machine and heated at 100 ^{0}C for 10 minutes and allowed for 3 minutes before centrifuging. The Eppendorf tube with a mixture of the lysed bacterial cells and DNA was removed from the heat block matrix and centrifuged at 10,000 rpm for 2 minutes to isolate the bacterial cell's DNA. After centrifugation, the cell pellets settle at the

bottom of the Eppendorf tube while the cell's DNA remain in the supernatant in the tube. The centrifuged Eppendorf tube was then removed from the centrifuge and the supernatant carefully aliquoted into the second set of pre-labelled empty Eppendorf tube. The second set of the Eppendorf tube which now contains the bacterial DNA was then put in small-sized boxes and kept in the refrigerator at -20 °C for preservation and further use.

3.13.2 Polymerase Chain Reaction assays for antibiotic resistance and virulence genes

All isolates were screened for methicillin resistance (*mec*A and *mec*C) genes by polymerase chain reaction (PCR) amplification using previously published primers (Steggar *et al*., 2012). Tetracycline-resistant isolates were screened for *tet*M, *tet*L, *tet*K, and *tet*O resistance genes while chloramphenicol-resistant isolates were screened for *cfr* resistance gene by PCR using previously described primers (Kehrenberg *et al*., 2005). All isolates were also screened for *siet, sec, exi,* and *luk*D virulence genes by PCR using previously published primers (Lautz *et al*., 2006; Futagawa-saito *et al*., 2004; Melter *et al*., 2017). Annealing temperatures and oligonucleotide primers used for the detection of the various resistance and virulence genes are all shown in Table 1. Validation of all primers was done before mastermix preparation and Polymerase Chain Reaction (PCR).

Table 1: Oligonucleotide primers used

Primer name	Sequence (5'-3')	Size (bp) and T (^{O}C)	Target gene	Source
sec-F	GTCAGACCCAACACCAGACC			
sec-R	CGGCATCAAGTCATACCAGA	598 (66)	*sec*	Gharsa *et al*., 2013
siet-F	TGGCGGTACATATGAAAGTGA			
siet-R	TTTCAACTCTGCACGCAATC	601 (58)	*Siet*	Gharsa *et al*., 2013
lukD-F	GGCCAAATGCAAGAGACTTT			
lukD-R	CCAACCAGCATTCATGATTTT	513 (55)	*luk*D	Couto *et al*., 2016a
mecA-F	TCCAGATTACAACTTCACCAGG			
mecA-R	CCACTTCATATCTTGTAACG	167 (52)	*mec*A	Stegger *et al*., 2012
mecC-F	GCTCCTAATGCTAATGCA			
mecC-R	TAAGCAATAATGACTACC	167 (59)	*mec*C	Stegger *et al*., 2012
exi F	TGCAGTTGGGACTGTTTTTG			
exi R	AACGTCCCCCTTTACCTACG	512 (61)	*exi*	Iyori *et al*., 2011
tetM-F	ACACGCCAGGACATATGGAT			
tetM-R	GCAAAGTTCAGACKGACCTC	530 (54)	*tet* (M)	Rubin J. E. designs
tetK-F	ATCTGCTGCATTCCCTTCAC			
tetK-R	GCAAACTCATTCCAGAAGCA	818 (53)	*tet* (K)	Rubin J. E. designs
tetL-F	CTGCATTTCCAGCACTCGTA			
tetL-R	ATTCCCCCACAAAGAACTCC	448 (64)	*tet* (L)	Rubin J. E. designs
tetO-F	GATGTRTGTTCCGACAAACG			
tetO-R	CCATAAAGAACCCCCTCCAT	573 (59)	*tet* (O)	Rubin J. E. designs
cfrK-F	TGAAGTATAAAGCAGGTTGGGAGTCA			
cfrK-R	ACCATATAATTGACCACAAGCAGC	746 (48)	*cfr*	Kehrenberg *et al*., 2005

3.13.3 Polymerase Chain Reaction conditions for the molecular detection of methicillin resistance genes

Amplification reaction was carried out in a 25 µl PCR mixture containing 17.55 µl of PCR water, 2.5 µl of 10x buffer, 1.25 µl of 50 mM $MgCl_2$, 0.5 µl of 2 mM dNTP, 1 µl primer 1 (Forward), 1 µl primer 2 (Reverse), 0.2 µl of *Taq* polymerase, and 1 µl of the genomic DNA. The thermocycler (BIO-RAD Finnegan C1000 Touch^{TM}, USA) was programmed for optimum conditions. The PCR mixture was poured into microcentrifuge tubes and vortexed for proper mixing before loading them into the thermocycler. The PCR reaction for *mec*A gene was performed as follows: an initial denaturation at 95 °C for 15 minutes, 30 cycles of denaturation at 94 °C for 60 seconds, annealing temperature of 52 °C for 30 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C. The PCR reaction for *mec*C gene was performed as follows: an initial denaturation at 94 °C for 15 minutes, 30 cycles of denaturation at 94 °C for 60 seconds, annealing temperature of 59 °C for 60 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.

3.13.4 Polymerase Chain Reaction conditions for the molecular detection of tetracycline resistance genes

Amplification reaction was carried out in a 25 µl PCR mixture containing 17.55 µl of PCR water, 2.5 µl of 10x buffer, 1.25 µl of 50 mM $MgCl_2$, 0.5 µl of 2 mM dNTP, 1 µl primer 1 (Forward), 1 µl primer 2 (Reverse), 0.2 µl of *Taq* polymerase, and 1 µl of the genomic DNA. The thermocycler (BIO-RAD Finnegan C1000 Touch^{TM}, USA) was programmed for optimum conditions. The PCR mixture was poured into microcentrifuge tubes and vortexed for proper mixing before loading them into the thermocycler. The PCR reaction conditions for *tet*M, *tet*L, *tet*K, and *tet*O resistance genes are shown in Table 2.

Table 2: Polymerase Chain Reaction conditions for tetracycline resistance genes

Genes	PCR reaction conditions
*Tet*M	Initial denaturation at 95 °C for 5 minutes, 40 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 54 °C for 45 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.
*Tet*L	Initial denaturation at 95 °C for 5 minutes, 30 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 64 °C for 60 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.
*Tet*K	Initial denaturation at 95 °C for 5 minutes, 35 cycles of denaturation at 94 °C for 60 seconds, annealing temperature of 53 °C for 60 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.
*Tet*O	Initial denaturation at 95 °C for 5 minutes, 35 cycles of denaturation at 94 °C for 60 seconds, annealing temperature of 59 °C for 60 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.

3.13.5 Polymerase Chain Reaction conditions for the molecular detection of chloramphenicol resistance (*cfr*) genes

Amplification reaction were carried out in a 25 µl PCR mixture containing 17.05 µl of PCR water, 2.5 µl of 10x buffer, 1.25 µl of 50 mM $MgCl_2$, 1 µl of 2 mM dNTP, 1 µl primer 1 (Forward), 1 µl primer 2 (Reverse), 0.2 µl of *Taq* polymerase, and 1 µl of the genomic DNA. The thermocycler (BIO-RAD Finnegan C1000 Touch^{TM}, USA) was programmed for optimum conditions. The PCR mixture was poured into microcentrifuge tubes and vortexed for proper mixing before loading them into the thermocycler. The PCR reaction was performed as follows: an initial denaturation at 94 °C for 3 minutes, 40 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 48 °C for 60 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.

3.13.6 Polymerase Chain Reaction conditions for the molecular detection of virulence (*siet, sec, exi,* and *luk*D) genes

Amplification reaction were carried out in a 25 µl PCR mixture containing 17.55 µl of PCR water, 2.5 µl of 10x buffer, 1.25 µl of 50 mM $MgCl_2$, 0.5 µl of 2 mM dNTP, 1 µl primer 1 (Forward), 1 µl primer 2 (Reverse), 0.2 µl of *Taq* polymerase, and 1 µl of the genomic DNA. The thermocycler (BIO-RAD Finnegan C1000 Touch^{TM}, USA) was programmed for optimum conditions. The PCR mixture was poured into microcentrifuge tubes and vortexed for proper mixing before loading them into the thermocycler. The PCR reaction conditions for *siet, sec, exi,* and *luk*D virulence genes are shown in Table 3.

Table 3: Polymerase Chain Reaction conditions for virulence genes

Genes	PCR reaction conditions
Siet	Initial denaturation at 95 °C for 5 minutes, 40 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 58 °C for 30 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.
Sec	Initial denaturation at 95 °C for 5 minutes, 40 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 66 °C for 30 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.
Exi	Initial denaturation at 95 °C for 5 minutes, 40 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 61 °C for 30 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.
*luk*D	Initial denaturation at 95 °C for 5 minutes, 40 cycles of denaturation at 94 °C for 30 seconds, annealing temperature of 55 °C for 30 seconds, elongation at 72 °C for 60 seconds and final 10 minutes extension period at 72 °C.

3.14 Agarose gel electrophoresis

Gel electrophoresis was used to detect amplified DNA products. DNA fragments were analyzed by electrophoresis in 1x TBE buffer (Fisher Scientific International Inc., USA) on a 1 % UltraPure agarose gel (Invitrogen, UK) stained with ethidium bromide. A 1 % (w/v) agarose gel was used to resolve the amplified Polymerase Chain Reaction (PCR) products of the *S. pseudintermedius* isolates. The 1 % (w/v) agarose gel was prepared by combining 1 g agarose with 100 ml of 1X TBE ((Tris-borate-EDTA) buffer in a 250 ml conical flask and heated in a microwave for about 2 minutes until the agarose was completely dissolved. The agarose solution was allowed to cool to about 60 °C. After cooling, 1µl of ethidium bromide (10 mg/ml) was added to the dissolved agarose solution with swirling to mix. The melted agarose was then poured into a gel electrophoresis mould and the casting combs were inserted. It was allowed to gel for 30 minutes. The casting comb was then carefully removed after the gel had completely solidified. The solidified agarose gel was then carefully placed in the gel electrophoresis tank. One times concentration (1X) TBE electrophoresis buffer was then added to the Gel electrophoresis tank until the buffer just covered the agarose gel. Exactly 3 µl of amplified PCR products was mixed with 2 µl of gel tracking dye (bromophenol blue) and loaded in the sample wells of the prepared agarose gel (the marker was loaded on lane 1, followed by the controls, and later followed by the samples)**.**

The electrophoresis tank was then covered and the electrodes were connected to the power pack (BIO-RAD, BIO-RAD Laboratories, USA) in such a way that the negative terminal is at the end where the samples have been loaded. The agarose gel was then subjected to electrophoresis at 90 volts for 30 minutes. TrackIt^{TM} 100 bp DNA ladder (Invitrogen, ThermoFisher Scienctific, USA) was used as the molecular weight marker. At the completion of electrophoresis, electrodes were

then disconnected and the power pack was turned-off. After this, the gel was removed from the buffer and the band pattern images of the DNA fragments in the gel were viewed using the Molecular Imager® (Gel Doc™ XR, BIO-RAD Laboratories, inc., USA) documentation system.

3.15 Polymerase Chain Reaction product purification/clean-up and deoxyribonucleic acid quantification by NanoDrop Spectrophotometer

Two enzymes; alkaline phosphatase, and exonuclease I was removed from the freezer and kept on ice while preparing for the clean-up reaction. Exactly 5 µl aliquot of the completed amplified PCR product was pipetted into a 1.5 ml Eppendorf tube and 1 µl each of the enzymes; alkaline phosphatase, and exonuclease I were added to the Eppendorf tube respectively. The tube was then incubated at 37 °C for 15 minutes. Next, the tube was again incubated at 80 °C for 15 minutes to inactivate the enzymes. After this incubation, the cleaned amplified PCR product was then prepped for sequencing. DNA content was checked by a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

3.16 Gene Sequencing

The identity of the purified amplified PCR product was confirmed by direct DNA sequencing at Macrogen (Korea). Sequencing results were identified using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) against highly similar sequences (megablast) and protein-protein BLAST (blastp) using the corresponding databases. The DNA sequences were compared with the GenBank database using a BLAST search on the NCBI server (http: www.ncbi.nlm.nih.gov/BLAST). Isolates with 99 – 100 % nucleotide identity with the reference nucleotide on the database were deemed to be positive for that gene. Nucleotide sequences of the isolates were deposited in the National Center for Biotechnology Information (NCBI) GenBank database with their respective accession numbers.

3.17 Statistical Analysis

Statistical analysis was performed using SPSS 17.0 version statistical software package. Comparison between categorical variables was calculated using the T-test, ANOVA, and Tukey post-hoc multiple comparison test. Results were considered statistically significant if the p value is less than 0.05 ($p < 0.05$).

CHAPTER FOUR

RESULTS

4.1 Cultural, Morphological, and Biochemical characteristics of bacterial isolates

The cultural characteristics of the isolates showed that they were small blue colonies approximately 1-3 mm in diameter when grown on CHROMagar *S. aureus* while they were small, creamy grey to white, round low convex colonies with a small margin and beta-haemolysis on 5 % sheep blood Columbia agar (Table 4, Appendix XIII). The morphological characteristics of the isolates showed that they are Gram-positive cocci in bunch / groups (Table 4). They were positive for catalase, tube coagulase, DNase, and pyrrolidonylarylamidase (PYR) tests but negative for hyaluronidase production, acetoin production, indole test, and oxidase test (Table 4). They utilized citrate as sole carbon source. They also utilized glucose, maltose, mannitol, and sucrose with the production of acid (Table 4).

Table 4: Cultural, Morphological, and Biochemical characteristics of bacterial isolates

Growth on CHROMagar *S. aureus*	Growth on 5 % sheep blood Columbia agar	Gram's reaction	Catalase	Tube coagulase	Voges-proskauer	Beta-haemolysis	DNase	Hyaluronidase	Oxidase	Citrate	Indole	Pyrrolidonylarylamidase	Beta-galactoosidase	Glucose	Maltose	Mannitol	Sucrose
Small blue colonies approximately 1-3 mm in diameter	Small, creamy grey to white, round low convex colonies with a small margin and double zone of haemolysis	Gram-positive cocci in bunches / groups	+	+	-	+	+	-	-	+	-	+	+	+	+	+	+

Key: + = positive; - = negative

4.2 Prevalence of *Staphylococcus pseudintermedius* isolates in dogs and dog owners (Humans)

Out of the 45 perineum swab samples, 42 (93.3 %) were positive for *S. pseudintermedius* while 25 (71.4 %) out of the 35 nares swab samples were positive for *S. pseudintermedius*. A total of 19 (59.4 %) swab samples were positive for *S. pseudintermedius* out of the 32 mouth swab samples obtained from dogs. In total, 86 (76.8 %) dogs were positive for *S. pseudintermedius* (Table 5). Out of the 97 volunteers, 13 (13.4 %) harboured for *S. pseudintermedius* (Table 6). The result of the one sample t-test conducted showed that there was no statistically significant difference in the prevalence of *S. pseudintermedius* in the perineum, nares, and mouth [mean = 2.000, SD = 1.000, t = 3.44, df = 2, p = 0.074 (at $p < 0.05$) (Appendix V)]. The varying differences in prevalence frequency of *S. pseudintermedius* in the three sample sites; perineum, nares, and mouth was also graphically confirmed by the plot of means using one-way anova (Appendix V).

Table 5: Prevalence of *Staphylococcus pseudintermedius* isolates in dogs in relation to sample site

Sample site	Total number of dog sampled	Total number of *S. pseudintermedius* isolates obtained n (%)
Perineum	45	42 (93.3 %)
Nares	35	25 (71.4 %)
Mouth	32	19 (59.4 %)
Total	112	86 (76.8 %)

Table 6: Prevalence of *Staphylococcus pseudintermedius* isolates obtained from the nasal swabs of humans (dog owners)

Total number of dog owners sampled	Total number of *Staphylococcus pseudintermedius* isolates obtained n (%)
97	13 (13.4 %)

4.3 Minimum Inhibitory Concentration (MIC) distributions of antibiotics tested and percentage resistance occurrence of *Staphylococcus pseudintermedius* isolates from dogs

The Minimum inhibitory concentration (MIC) distribution of twenty antibiotics tested on the *S. pseudintermedius* isolates from dogs is shown in Appendix II & Table 8 while the summary of their percentage resistance occurrence is shown in Tables 7 & 8.

Antibiotic susceptibility tests results from the MIC values of antibiotics tested on the *S. pseudintermedius* isolates from dogs revealed that they were highly resistant to penicillin and ampicillin with resistance frequencies of 95.3 % and 94.2 % respectively (Tables 7 & 8). Results also showed that 46 (53.5 %) of the dog isolates were methicillin-resistant strains as they were resistant to oxacillin with 2 % NaCl. Equal resistance frequency of 51.2 % was each observed to erythromycin, clindamycin, trimethoprim/sulfamethoxazole, levofloxacin, and moxifloxacin. Resistance was also observed to gentamycin, chloramphenicol, tetracycline, and tigecycline with resistance frequencies of 46.5 %, 23.1 %, 19.8 %, and 8.1 % respectively. No resistance to nitrofurantoin, rifampin, daptomycin, vancomycin, quinupristin/dalfopristin, linezolid, and amikacin was observed (Tables 7 & 8). Two isolates from dogs were completely susceptible to all the antibiotics tested (Appendix II). The result of the one sample t-test conducted showed that there was a statistically significant difference in the mean percentage resistances of the *S. pseudintermedius* isolates from dogs [mean = 49.731, SD = 24.880), t = 7.207, df = 12, p = 0.000 ($p < 0.05$) (Appendix VI)].

Table 7: Percentage occurrence of resistance in the isolates

Drug (µg/ml)	Number (%) of isolates		Total number (%) of isolates (n = 99)
	Dog (n = 86)	Human (n = 13)	
PEN	95.3	100	97.7
AMP	94.2	92.3	93.3
OXA	53.5	46.2	49.9
ERY	51.2	46.2	48.7
CLI	51.2	46.2	48.7
TET	19.8	0	19.8
TGC	8.1	0	8.1
SXT	51.2	46.2	48.7
CIP	50	46.2	48.1
LEV	51.2	46.2	48.7
MXF	51.2	46.2	48.7
GEN	46.5	46.2	46.4
CHL	23.1	23.1	23.1
NIT	0	0	0
RIF	0	0	0
VAN	0	0	0
LZD	0	0	0
DAP*	0	0	0
SYN	0	0	0
AMK	0	0	0

Key: PEN = Penicillin, **AMP** = Ampicillin, **OXA** = Oxacillin + 2 % NaCl, **ERY** = Erythromycin, **CLI** = Clindamycin, **TET** = Tetracyline, **TGC** = Tigecycline, **SXT** = Trimethoprim/Sulfamethoxazole, **CIP** = Ciprofloxacin, **LEV** = Levofloxacin, **MXF** = Moxifloxacin, **GEN** = Gentamycin, **CHL** = Chloramphenicol, **NIT** = Nitrofurantoin, **RIF** = Rifampin, **VAN** = Vancomycin, **LZD** = Linezolid, **DAP** = Daptomycin, **SYN** = Quinupristin/dalfopristin, **AMK** = Amikacin, * = non-susceptible.

Table 8: Minimum Inhibitory Concentration (MIC) breakpoints of antibiotics tested and percentage resistance occurrence of *Staphylococcus pseudintermedius* isolates from dogs (n =86)

Drug (µg/ml)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	% Resistant Isolates (n=86)
PEN		3	1	2		1	2		3	74			95.3
AMP			4	1	4	2	4	4	10	57			94.2
OXA				40	1		2	1	42				53.5
ERY				2	31	8	1		44				51.2
CLI					41	1		44					51.2
TET							68		1	1	16		19.8
TGC	1	1	41	36	6	1							8.1
SXT					32	9	1	3	41				51.2
CIP						41	2	43					50
LEV				39	1	1	1		44				51.2
MXF				40	2			39	5				51.2
GEN							43		3	6	34		46.5
CHL									26	46	14		23.1
NIT											86		0
RIF					86								0
VAN					3	81	2						0
LZD						3	54	29					0
DAP*					85	1							0
SYN					85	1							0
AMK				2		34	28	22					0

Antimicrobial minimum inhibitory concentration (MIC_{90}) distribution of the Staphylococcus pseudintermedius isolates (n=86). Shaded cells indicate the MIC breakpoints for the antibiotics tested against the Staphylococcus pseudintermedius isolates. The numbers of isolates inhibited were noted in each cell.

Key: PEN = Penicillin, **AMP** = Ampicillin, **OXA** = Oxacillin + 2 % NaCl, **ERY** = Erythromycin, **CLI** = Clindamycin, **TET** = Tetracyline, **TGC** = Tigecycline, **SXT** = Trimethoprim/Sulfamethoxazole, **CIP** = Ciprofloxacin, **LEV** = Levofloxacin, **MXF** = Moxifloxacin, **GEN** = Gentamycin, **CHL** = Chloramphenicol, **NIT** = Nitrofurantoin, **RIF** = Rifampin, **VAN** = Vancomycin, **LZD** = Linezolid, **DAP** = Daptomycin, **SYN** = Quinupristin/dalfopristin, **AMK** = Amikacin, * = non-susceptible.

4.4 Minimum Inhibitory Concentration (MIC) distributions of antibiotics and percentage resistance occurrence of *Staphylococcus pseudintermedius* isolates from humans

The Minimum inhibitory concentration (MIC) distribution of twenty antibiotics tested on the *S. pseudintermedius* isolates from humans is shown in Table 10 & Appendix III while the summary of their percentage resistance occurrence is shown in Tables 7, 9, & 10.

Antibiotic susceptibility tests results from the MIC values of antibiotics tested on the *S. pseudintermedius* isolates from humans revealed that all the human *S. pseudintermedius* isolates were completely resistant (100 %) to penicillin while 92.3 % were resistant to ampicillin (Tables 7 & 10). Exactly 6(46.2 %) of the human *S. pseudintermedius* were methicillin-resistant as they were also resistant to oxacillin with 2 % NaCl. Resistance frequency of 46.2 % was each observed to erythromycin, clindamycin, trimethoprim/sulfamethoxazole, levofloxacin, moxifloxacin, and gentamycin. Resistance to chloramphenicol was also observed with a resistance frequency of 23.1 %. None of the human isolates was resistant to tetracycline, tigecycline, nitrofurantoin, rifampin, daptomycin, vancomycin, quinupristin/dalfopristin, linezolid, and amikacin (Tables 7, 9 & 10). One sample t-test conducted showed that there was a statistically significant difference in the mean percentage resistances of the *S. pseudintermedius* isolates from humans [mean = 53.1818, SD = 22.399), t = 7.875, df = 10, p = 0.000 ($p < 0.05$) (Appendix VII)]. Results of the independent samples t-test conducted to compare the percentage resistances frequencies of *S. pseudintermedius* from dogs to those from humans (dog owners) showed that there was no statistically significant difference in the mean percentage resistance (mean =49.731, SD = 24.880) of the *S. pseudintermedius* from dogs and those from humans (mean = 53.182, SD = 22.399); t = -0.354, df = 22, p = 0.727 (at $p < 0.05$) (Appendix VIII). One-way anova result also showed that there was no statistically significant difference between the

mean resistance of *S. pseudintermedius* from dogs when compared to those from humans (F (1, 22) = 0.125, P = 0.727. The difference in the mean resistance of *S. pseudintermedius* between dogs and humans was also graphically confirmed by the plot of means using one-way anova (Appendix IX).

4.5 Multiple Antibiotic Resistance Indices (MARI) values of *S. pseduintermedius* isolates

The multiple antibiotic resistance index (MARI) values of the *S. pseudintermedius* isolates ranged from 0.1 – 0.6 while the average MARI value of the *S. pseudintermedius* isolates was 0.3, thus depicting multidrug resistance (Appendix IV, Table 11).

Table 9: Resistance Phenotypes of the isolates

S/N	Resistance antibiotypes	Number (%) of isolates		
		Dog isolate n (%)	Human isolate n (%)	Total n (%)
1	CHL	1 (1.2 %)	-	1 (1.2 %)
2	PEN	2 (2.3 %)	1 (7.7 %)	2 (2.3 %)
3	TGC	1 (1.2 %)	-	1 (1.2 %)
4	AMP PEN	19 (22.1 %)	4 (30.8 %)	19 (22.1 %)
5	CHL AMP PEN	3 (3.5 %)	2 (15.4 %)	3 (3.5 %)
6	SXT AMP PEN	1 (1.2 %)	-	1 (1.2 %)
7	**MRSP**	2 (2.3 %)	-	2 (2.3 %)
8	TET AMP PEN	8 (9.3 %)	-	8 (9.3 %)
9	CHL AMP PEN TGC	1 (1.2 %)	-	1 (1.2 %)
10	CHL TET AMP PEN TGC	1 (1.2 %)	-	1 (1.2 %)
11	CHL TET ERY **MRSP** CLI	1 (1.2 %)	-	1 (1.2 %)
12	SXT ERY **MRSP** LEV CIP MXF	1 (1.2 %)	-	1 (1.2 %)
13	CHL SXT TET AMP PEN LEV CIP MXF TGC	1 (1.2 %)	-	1 (1.2 %)
14	GEN ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)	-	1 (1.2 %)
15	TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)	-	1 (1.2 %)
16	GEN SXT ERY **MRSP** LEV CIP MXF CLI	31 (36 %)	5 (38.5 %)	31 (36 %)
17	SXT TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)	-	1 (1.2 %)
18	GEN SXT TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)	-	1 (1.2 %)
19	CHL GEN SXT ERY **MRSP** LEV CIP MXF CLI	4 (4.7 %)	1 (7.7 %)	4 (4.7 %)
20	GEN SXT ERY **MRSP** LEV CIP MXF CLI TGC	1 (1.2 %)	-	1 (1.2 %)
21	CHL GEN SXT TET **MRSP** LEV CIP MXF CLI TGC	1 (1.2 %)	-	1 (1.2 %)
22	CHL GEN SXT TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)	-	1 (1.2 %)

Table 10: Minimum Inhibitory Concentration (MIC) breakpoints of antibiotics tested and percentage resistance occurrence of *S. pseudintermedius* isolates from humans (n =13)

Drug (µg/ml)	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	% Resistant Isolates (n=13)
PEN				1						12			100
AMP				1					2	10			92.3
OXA				7					6				46.2
ERY					4	3			6				46.2
CLI					7			6					46.2
TET							13						0
TGC		2	6	5									0
SXT					4	2	1		6				46.2
CIP						6	1	6					46.2
LEV				6	1				6				46.2
MXF				7				6					46.2
GEN							7				6		46.2
CHL									3	7	3		23.1
NIT											13		0
RIF					13								0
VAN				1		12							0
LZD							9	4					0
DAP*					12	1							0
SYN					13								0
AMK						2	1	10					0

Antimicrobial minimum inhibitory concentration (MIC_{90}) distribution of the Staphylococcus pseudintermedius isolates (n=13). Shaded cells indicate the MIC breakpoints for the antibiotics tested against the Staphylococcus pseudintermedius isolates. The numbers of isolates inhibited were noted in each cell.

Key: PEN = Penicillin, **AMP** = Ampicillin, **OXA** = Oxacillin + 2 % NaCl, **ERY** = Erythromycin, **CLI** = Clindamycin, **TET** = Tetracyline, **TGC** = Tigecycline, **SXT** = Trimethoprim/Sulfamethoxazole, **CIP** = Ciprofloxacin, **LEV** = Levofloxacin, **MXF** = Moxifloxacin, **GEN** = Gentamycin, **CHL** = Chloramphenicol, **NIT** = Nitrofurantoin, **RIF** = Rifampin, **VAN** = Vancomycin, **LZD** = Linezolid, **DAP** = Daptomycin, **SYN** = Quinupristin/dalfopristin, **AMK** = Amikacin, * = non-susceptible.

Table 11: Multiple Antibiotic Resistance Index (MARI) values of *Staphylococcus pseudintermedius* isolates

	Number of isolates (%)	
MARI	Dog isolates	Human isolates
0.6	8 (9.3 %)	1 (7.7 %)
0.5	35 (40.7 %)	5 (38.5 %)
0.4	2 (2.3 %)	0 (0.0 %)
0.3	1 (1.2 %)	0 (0.0 %)
0.2	20 (23.3 %)	4 (30.8 %)
0.1	18 (20.9 %)	3 (23. 1 %)

4.6 Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates among dog and humans (dog owners) isolates

A total of 52 (52.5 %) out of all the 99 isolates from both shelter dogs and humans were methicillin-resistant *S. pseudintermedius* (MRSP) as they were resistant to oxacillin with 2 % NaCl (Table 12).

Out of the 52 MRSP isolates, 46 were from dogs while six (6) were from humans (dog owners) as shown in Table 12. The result of the one sample t-test conducted showed that there was no statistically significant difference in the prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) isolates between dogs and humans [mean = 26.000, SD = 28.284, t = 1.300, df = 1, p = 0.417 (at $p < 0.05$) (Appendix X)]. The difference in the prevalence frequency of MRSP isolates between dogs and humans was also graphically confirmed by the plot of means using one-way anova (Appendix XI).

Table 12: Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates among dog and humans (dog owners) isolates

Sample source	Total number (%) of MRSP isolates
Dogs	46 (53.5 %)
Humans (Dog owners)	6 (46.2 %)
Total	52 (52.5 %)

4.7 Antibiotic resistance phenotypes of *Staphylococcus pseudintermedius* isolates

A total of 84 *S. pseudintermedius* isolates out of the 86 isolates obtained from dogs exhibited 22 different resistance antibiotypes (Table 13). Ampicillin + penicillin resistance antibiotype (AMP^{R} PEN^{R}) was the most prevalent antibiotype as it was present in 19 out of the 22 resistance antibiotypes observed (Table 13).

In contrast, all the 13 *S. pseudintermedius* isolates obtained from humans (dog owners) exhibited 5 different resistance antibiotypes (Table 14). Ampicillin + penicillin resistance antibiotype (AMP^{R} PEN^{R}) was also the most prevalent antibiotype as it was present in 4 out of the 5 resistance antibiotypes observed (Table 14).

Table 13: Resistance antibiotypes of *Staphylococcus pseudintermedius* isolates from dogs

S/N	Resistance antibiotypes	Number of isolates n (%)
1	CHL	1 (1.2 %)
2	PEN	2 (2.3 %)
3	TGC	1 (1.2 %)
4	AMP PEN	19 (22.1 %)
5	CHL AMP PEN	3 (3.5 %)
6	SXT AMP PEN	1 (1.2 %)
7	**MRSP**	2 (2.3 %)
8	TET AMP PEN	8 (9.3 %)
9	CHL AMP PEN TGC	1 (1.2 %)
10	CHL TET AMP PEN TGC	1 (1.2 %)
11	CHL TET ERY **MRSP** CLI	1 (1.2 %)
12	SXT ERY **MRSP** LEV CIP MXF	1 (1.2 %)
13	CHL SXT TET AMP PEN LEV CIP MXF TGC	1 (1.2 %)
14	GEN ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)
15	TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)
16	GEN SXT ERY **MRSP** LEV CIP MXF CLI	31 (36 %)
17	SXT TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)
18	GEN SXT TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)
19	CHL GEN SXT ERY **MRSP** LEV CIP MXF CLI	4 (4.7 %)
20	GEN SXT ERY **MRSP** LEV CIP MXF CLI TGC	1 (1.2 %)
21	CHL GEN SXT TET **MRSP** LEV CIP MXF CLI TGC	1 (1.2 %)
22	CHL GEN SXT TET ERY **MRSP** LEV CIP MXF CLI	1 (1.2 %)

Key: CHL = Chloramphenicol**, GEN** = Gentamycin**, TET** = Tetracyline**, SXT** = Trimethoprim/Sulfamethoxazole, **ERY** = Erythromycin, **MRSP =** OXA AMP PEN, **LEV** = Levofloxacin, **CIP** = Ciprofloxacin**, MXF** = Moxifloxacin, **CLI** = Clindamycin, **TGC** = Tigecycline**.**

Table 14: Resistance antibiotypes of *S. pseudintermedius* isolates from humans

S/N	Resistance antibiotypes	Number of isolates n (%)
1	PEN	1 (7.7 %)
2	AMP PEN	4 (30.8 %)
3	CHL AMP PEN	2 (15.4 %)
4	GEN SXT ERY **MRSP** LEV CIP MXF CLI	5 (38.5 %)
5	CHL GEN SXT ERY **MRSP** LEV CIP MXF CLI	1 (7.7 %)

Key: CHL = Chloramphenicol**, GEN** = Gentamycin**, SXT** = Trimethoprim/Sulfamethoxazole, **ERY** = Erythromycin, **MRSP =** OXA AMP PEN, **LEV** = Levofloxacin, **CIP** = Ciprofloxacin**, MXF** = Moxifloxacin, **CLI** = Clindamycin.

4.8 Frequency of tetracycline and chloramphenicol resistance genes among *S. pseudintermedius* isolates

Seventeen isolates (17.2 %) out of the 99 isolates obtained from both dogs and humans were resistant to tetracycline (Table 15). These isolates were screened for different tetracycline resistance genes such as *tet*M, *tet*L, *tet*K, and *tet*O. Twelve (70.6 %) out of the 17 tetracycline resistant isolates harboured *tet*M gene while none harboured *tet*L, *tet*K, and *tet*O genes (Table 15; Figures 4, 5, 6, and 7). The DNA sequences of the *tet*M gene for the 12 tetracycline-resistant *S. pseudintermedius* isolates in our study exhibited 99-100 % nucleotide similarity to the *tet*M reference nucleotide sequences of *Staphylococcus aureus* subsp. *aureus* in the GenBank database using a BLAST search on the NCBI server **(Appendix XXIII)**.

Seventeen isolates that were also resistant to chloramphenicol were screened for *cfr* resistance gene but none of the isolates harboured this particular type of chloramphenicol resistance gene (Table 16, Figure 8).

Table 15: Frequency of tetracycline resistance genes among *S. pseudintermedius* isolates

Tetracycline resistant	**Tetracycline resistance genes screened**			
isolates [n (%)]	*tet*M [n (%)]	*tet*L [n (%)]	*tet*K [n (%)]	*tet*O [n (%)]
17 (17.2 %)	12 (70.6 %)	0 (0 %)	0 (0 %)	0 (0 %)

Table 16: Frequency of chloramphenicol resistance genes among *S. pseudintermedius* isolates

Chloramphenicol resistant isolates [n (%)]	**Chloramphenicol resistance gene screened**
	cfr [n (%)]
17 (17.2 %)	0 (0.0 %)

***tet*M resistance gene (530 bp)**

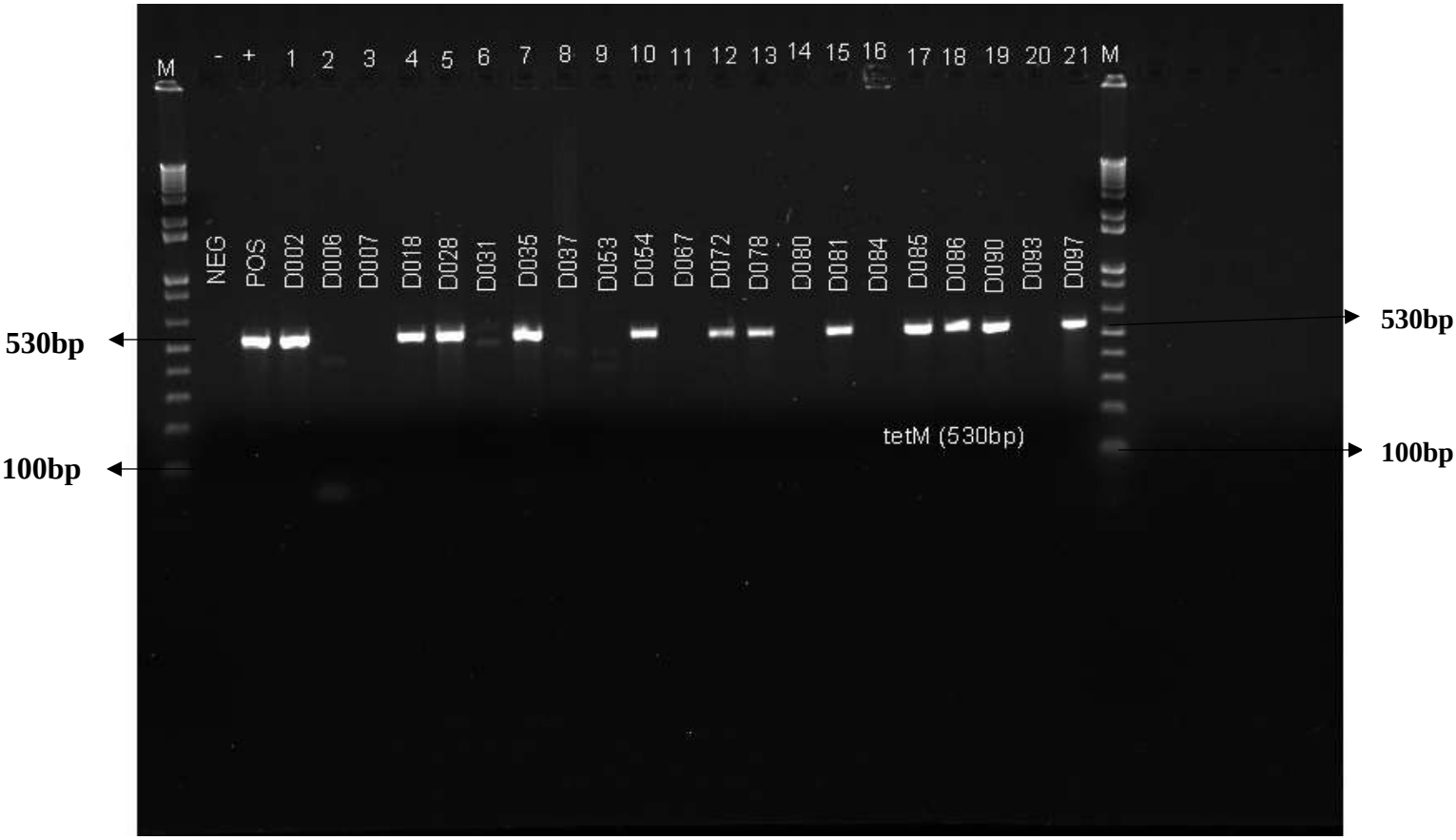

Figure 4: Polymerase Chain Reaction amplification gel of *tet*M resistance gene (530 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-21)

Lane M=100 bp marker; - = negative control without DNA; + = *S. pseudintermedius* SP06 (positive control).

Lanes 1, 4, 5, 7, 10, 12, 13, 15, 17, 18, 19, and 21 were positive for *tet*M resistance gene.

***tet*O resistance gene (573 bp)**

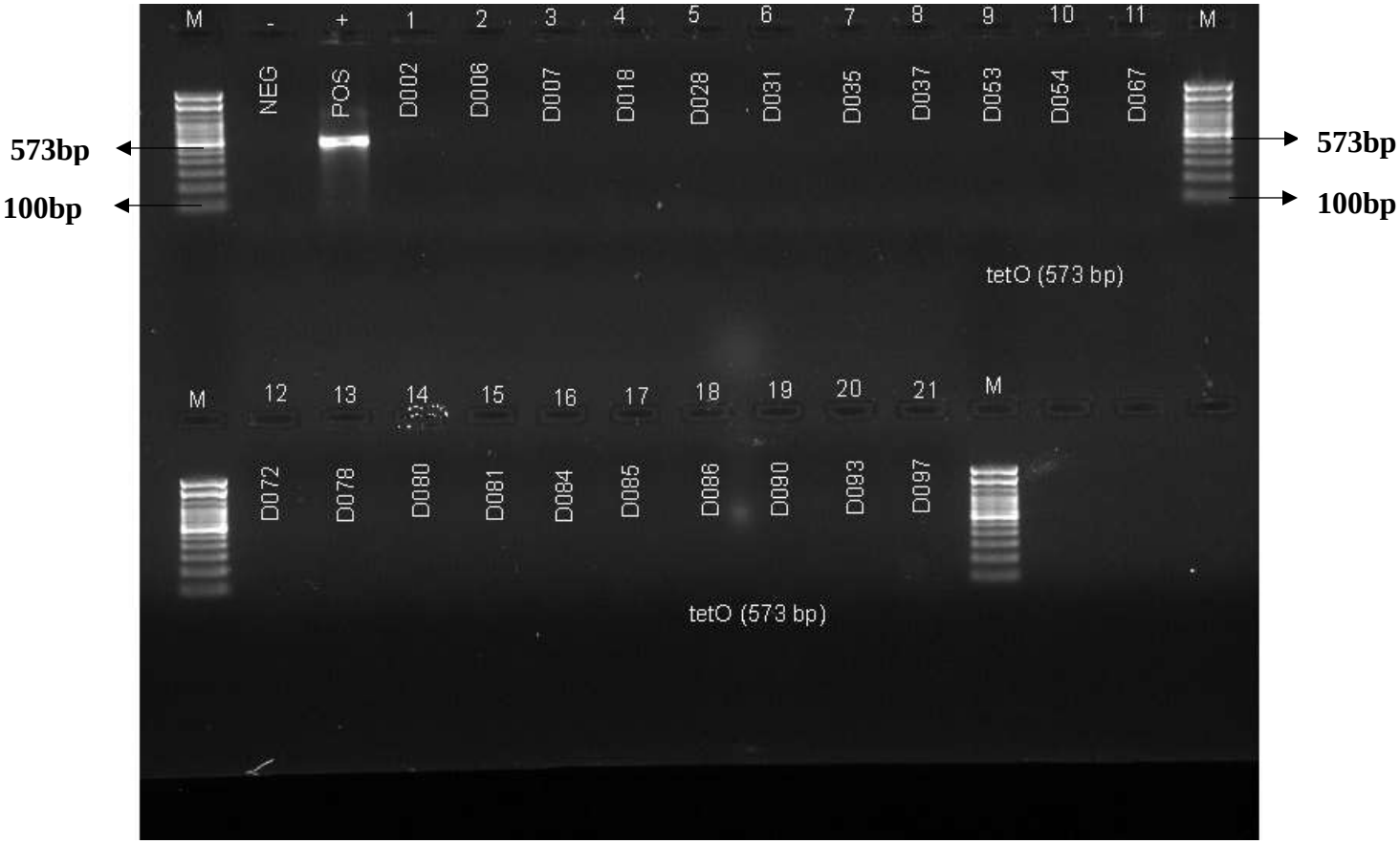

Figure 5: Polymerase Chain Reaction amplification gel of *tet*O resistance gene (573 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-21)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SP42 (positive control).

All tetracycline-resistant *S. pseudintermedius* isolates were negative for *tet*O resistance gene.

***tet*K resistance gene (818 bp)**

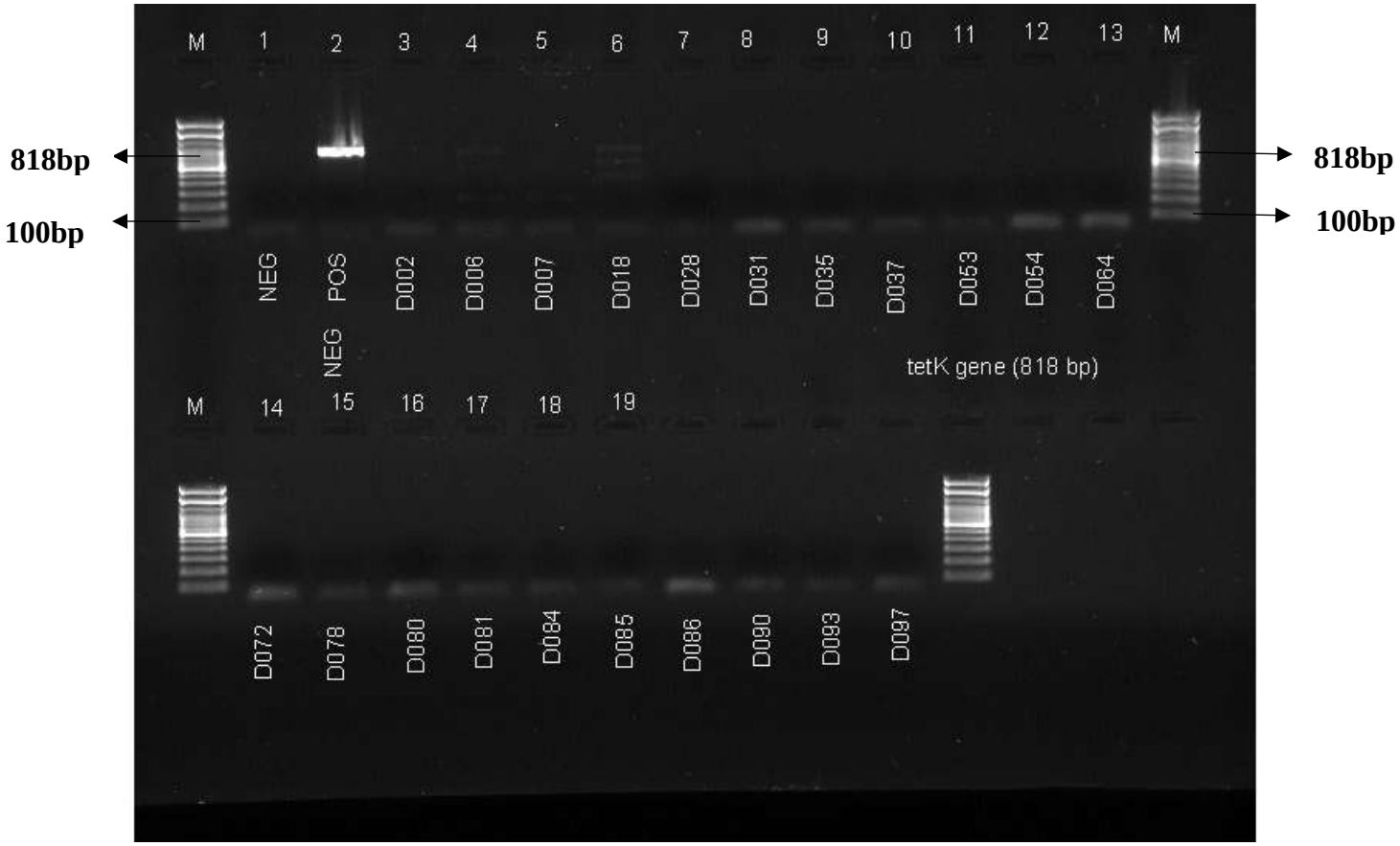

Figure 6: Polymerase Chain Reaction amplification gel of *tet*K resistance gene (818 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-21)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MRSP 11 (positive control).

All tetracycline-resistant *S. pseudintermedius* isolates were negative for *tet*K resistance gene.

***tet*L resistance gene (448 bp)**

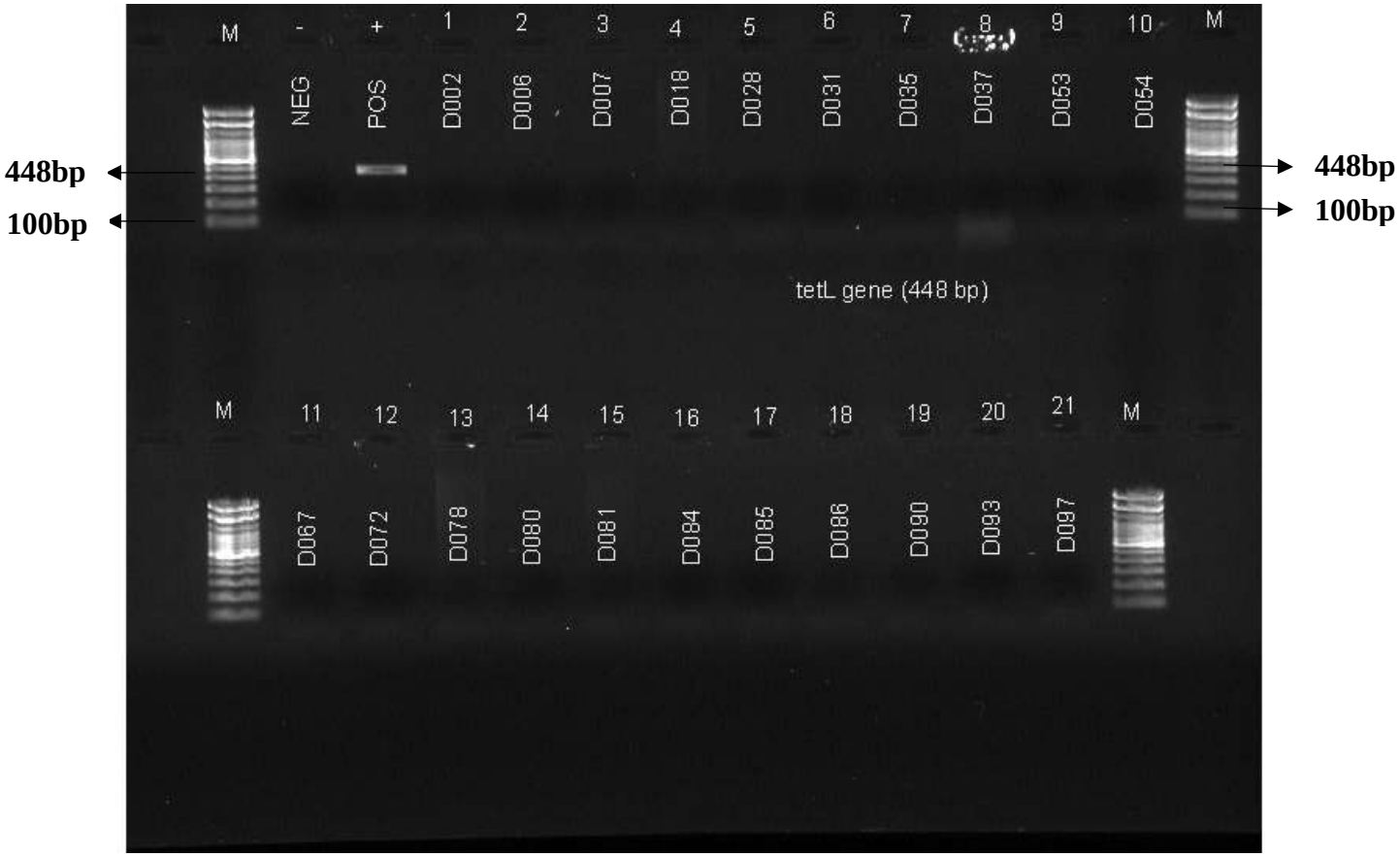

Figure 7: Polymerase Chain Reaction amplification gel of *tet*L resistance gene (448 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-21)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* RSP63A1c (positive control).

All the tetracycline-resistant *S. pseudintermedius* isolates were negative for *tet*L resistance gene.

***cfr* resistance gene (746 bp)**

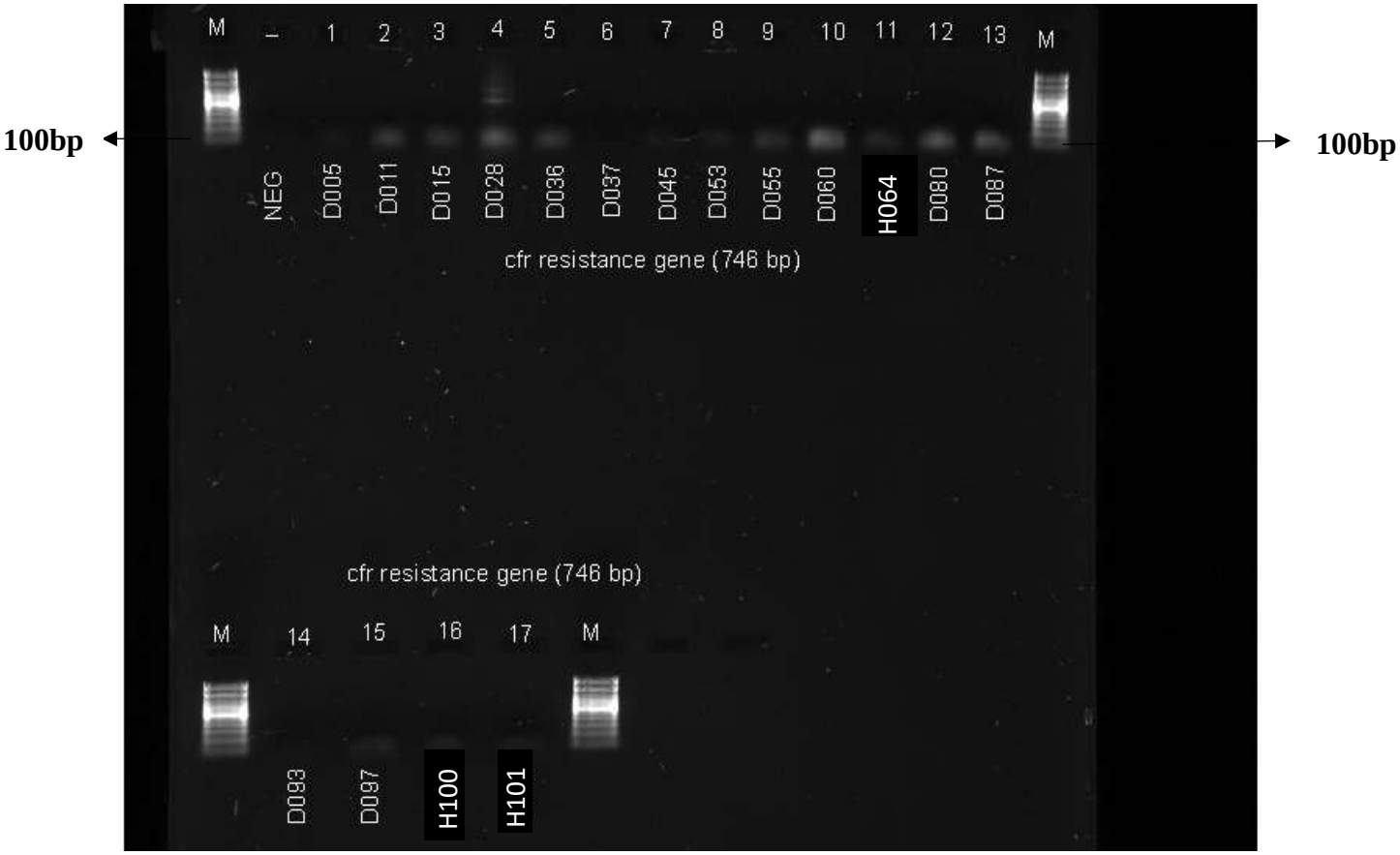

Figure 8: Polymerase Chain Reaction amplification gel of *cfr* resistance gene (746 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-21)

Lane M=100 bp marker; **-** = negative control without DNA

All the chloramphenicol-resistant *S. pseudintermedius* isolates were negative for *cfr* resistance gene.

4.9 Frequency of *mec* genes among the methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates

Out of the 52 MRSP isolates identified among all the dog and human isolates, *mec*A gene was found in 41 (78.9 %) of them (Table 17, Figures 9a & 9b). A total of 36 out of the 46 MRSP isolates obtained from dogs harboured *mec*A gene while five (5) out of the 6 MRSP isolates obtained from dogs harboured *mec*A gene. No *mec*C gene was found in any of the isolates obtained from dogs and humans (Table 16, Figure 10).

Table 17: Frequency of *mec* genes among the methicillin-resistant *S. pseudintermedius* (MRSP) isolates from dogs and humans (Dog owners)

		Number (%) of *mec* genes	
Number (%) of MRSP isolates	Isolate source	*mec*A	*mec*C
	Dogs	36 (87.8 %)	0 (0.0 %)
	Humans	5 (12.2 %)	0 (0.0 %)
52 (52.5 %)		41 (78.9 %)	0 (0.0 %)

***mec*A gene (167 bp)**

`

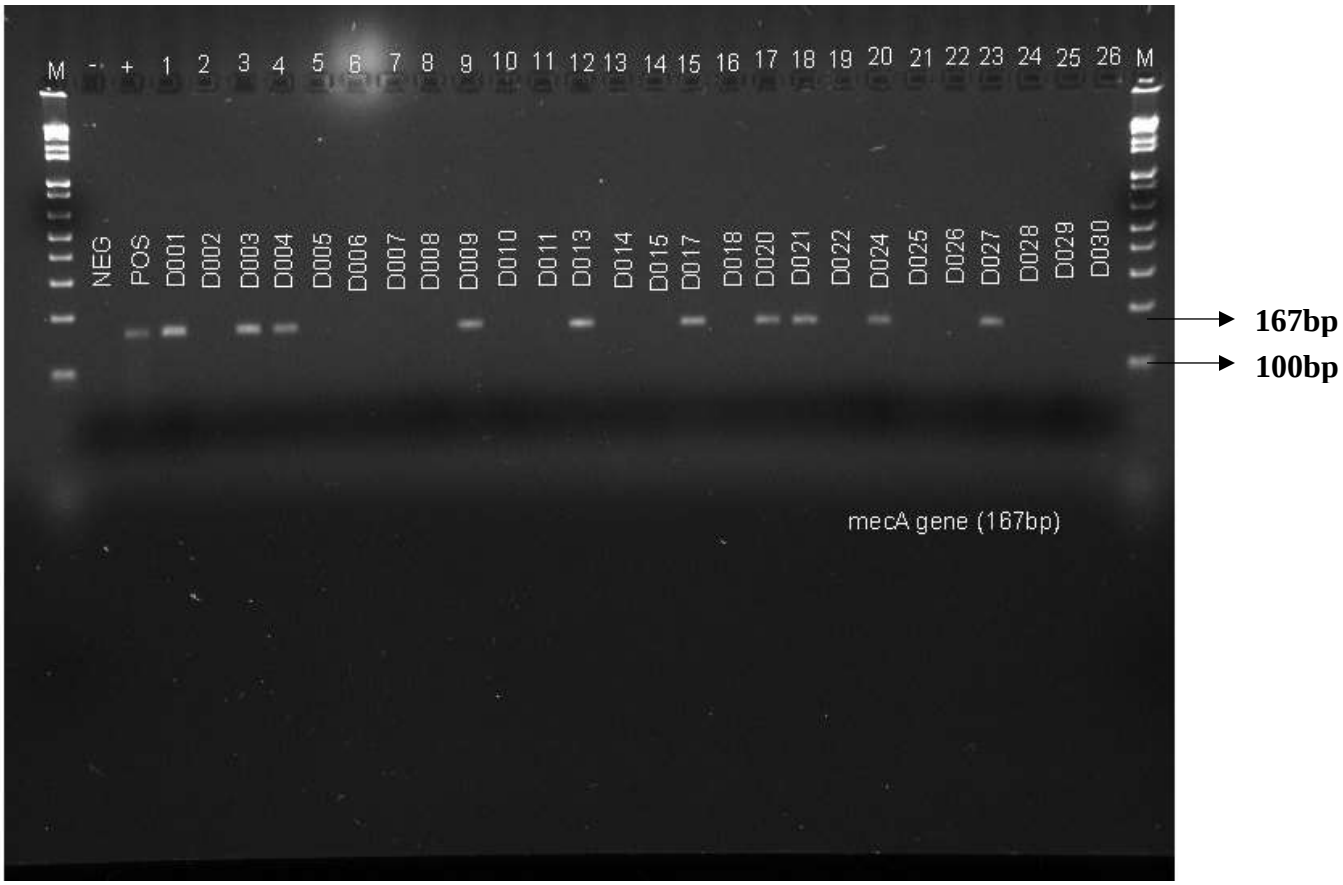

Figure 9a: Polymerase Chain Reaction amplification gel of *mec*A gene (167 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-26)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MRSP 24 (positive control).

Lanes 1, 3, 4, 9, 12, 15, 17, 18, 20, and 23 were positive for ***mec*A gene.**

***mec*A (167 bp)**

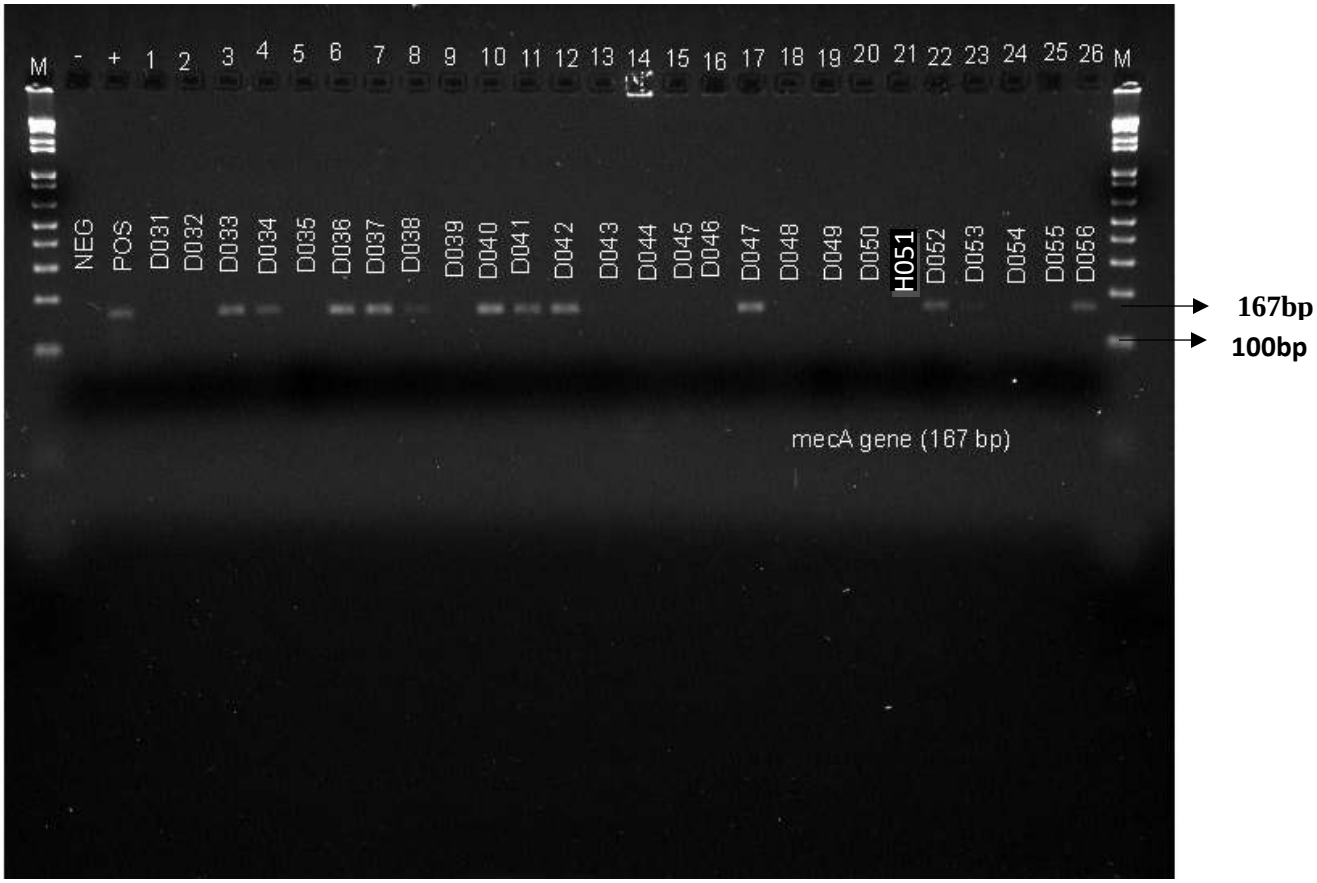

Figure 9b: Polymerase Chain Reaction amplification gel of *mec*A (167 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-26)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MRSP 24 (positive control).

Lanes 3, 4, 6, 7, 8, 10, 11, 12, 17, 22, 23, and 26 were positive for ***mec*A gene.**

***mec*C gene (167 bp)**

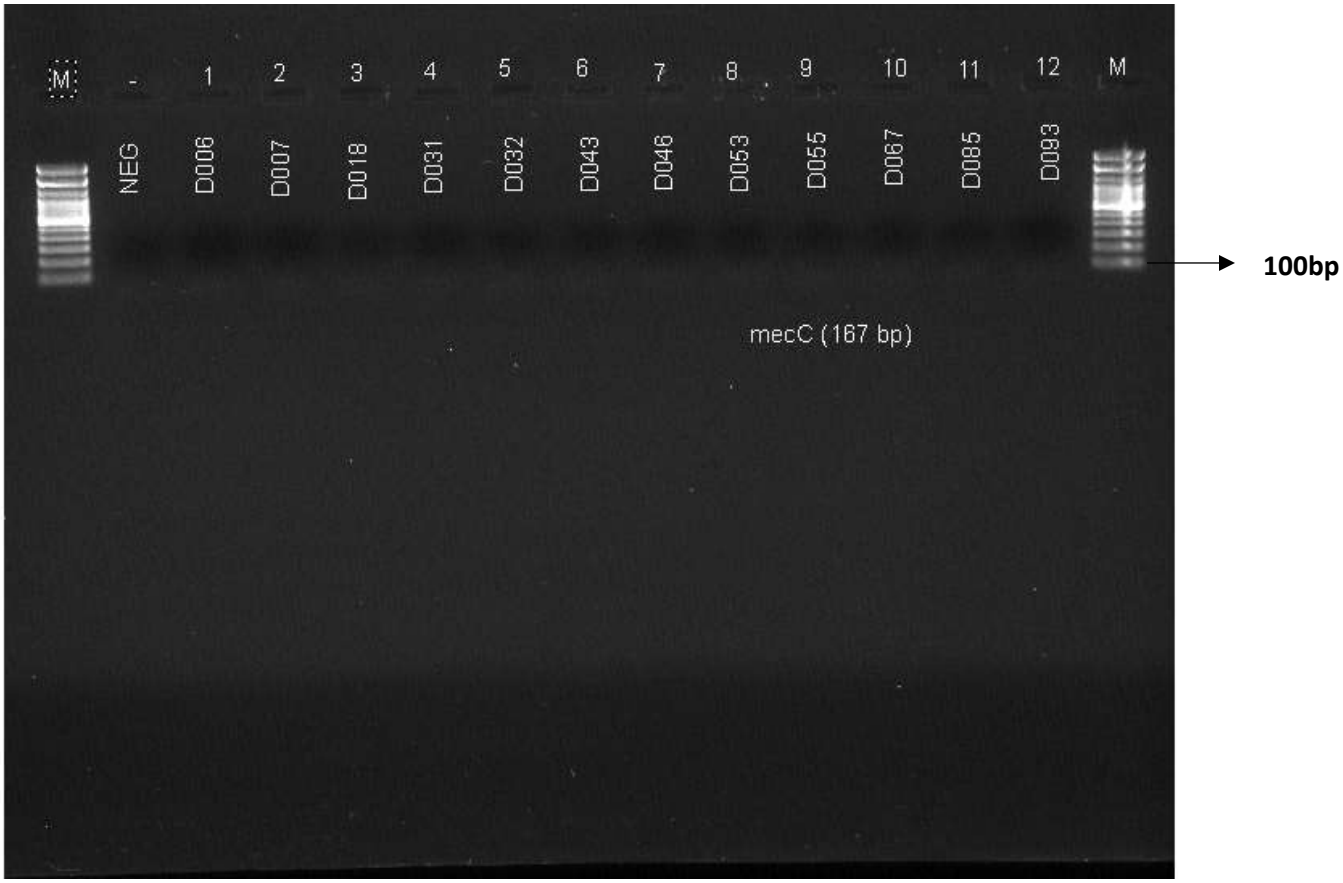

Figure 10: Polymerase Chain Reaction amplification gel of *mec*C gene (167 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-12)

Lane M=100 bp marker; **-** = negative control without DNA

All the *S. pseudintermedius* isolates were negative for ***mec*C gene.**

4.10 Prevalence of virulence genes among the *Staphylococcus pseudintermedius* isolates from dogs and humans (Dog owners)

A total of 73 (73.7 %), 2 (2 %), 62 (62.6 %), and 55 (55.6 %) isolates haboured *sec*, *exi*, *siet*, and *luk*D virulence genes respectively (Table 18, Figures 11 - 14). Out of the 73 *sec* virulence genes, 62 (84.9 %) were found in the dog isolates while 11 (15.1 %) were found in the human (dog owners) isolates. One *exi* virulence gene was present in both a dog (D014) and human (D103) isolates. *Siet* virulence gene was present in 53 (85.5 %) isolates while 9 (14.5 %) were present in the human isolates. Out of the 55 *luk*D virulence genes, 48 (87.3 %) were found in the dog isolates while 7 (12.7 %) were present in the human isolates (Table 18). *Sec* gene, mostly implicated in dog pyoderma cases was the most predominant virulence gene detected among the 99 isolates as 73(73.7 %) of the isolates were positive (Table 18, Figures 11a and 11b). This was closely followed by *siet* gene [62 (62.6 %] and *luk*D gene [55 (55.6 %] while *exi* gene [2(2 %)] was the least predominant (Table 18, Figures 11 - 14). The results of the one-way anova conducted showed that there was no statistically significant difference in the prevalence frequencies among the 4 virulence genes; *sec*, *exi*, *siet*, and *luk*D [$F(3, 4) = 0.640$, $P = 0.628$ (at $p < 0.05$) **(Appendix XII)**]. Tukey post-hoc multiple comparison test conducted to determine the statistically significant difference in the prevalence of the virulence genes from one another also showed that the prevalence of *sec* gene does not significantly differ from *exi* gene ($p = 0.621$), *siet* gene ($p = 0.997$), and *luk*D gene ($p = 0.987$). Tukey post-hoc test also showed that the prevalence of *exi* gene does not significantly differ from *siet* gene ($p = 0.721$), and *luk*D gene ($p = 0.783$). Tukey post-hoc test also showed that the prevalence of *siet* gene does not significantly differ from *luk*D gene ($p = 0.999$) **(Appendix XII)**. The difference in the virulence gene prevalence frequencies among the *S. pseudintermedius* isolates was also confirmed by the plot of means **(Appendix XII)**.

Table 18: Prevalence of virulence genes among *Staphylococcus pseudintermedius* isolates from dogs and humans (Dog owners)

Virulence gene	Isolate source	Number of positive isolates (%)	Total number of positive isolates (%)
Sec	Dogs	62 (84.9 %)	73 (73.7 %)
	Humans	11(15.1 %)	
Exi	Dogs	1 (50 %)	2 (2 %)
	Humans	1 (50 %)	
Siet	Dogs	53 (85.5 %)	62 (62.6 %))
	Humans	9 (14.5 %)	
LukD	Dogs	48 (87.3 %)	55 (55.6 %)
	Humans	7 (12.7 %)	

***sec* virulence gene (598 bp)**

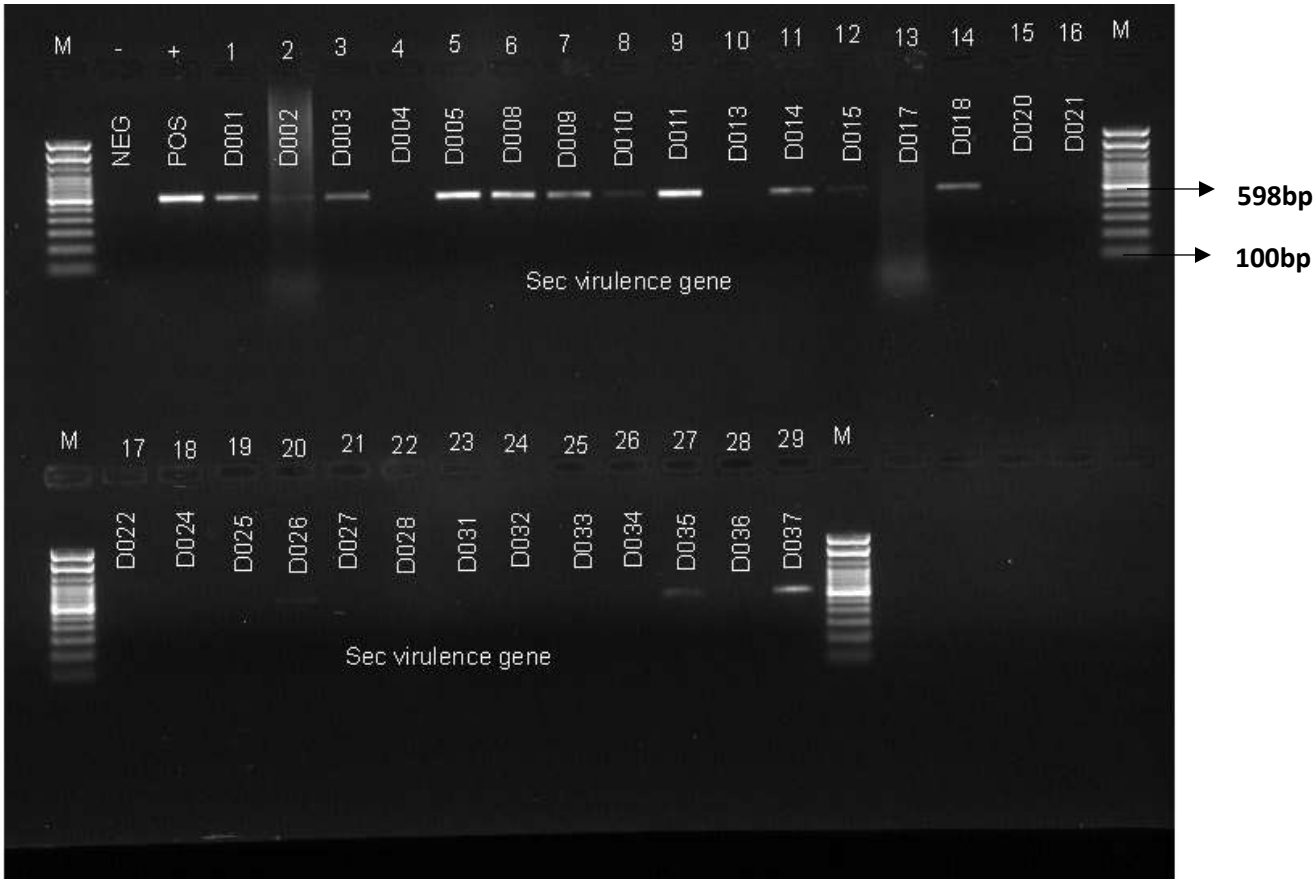

Figure 11a: Polymerase Chain Reaction amplification gel of *sec* virulence gene (598 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-37)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SP31 (positive control).

Lanes 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 14, 20, 27, and 29 were positive for ***sec* virulence gene.**

***sec* virulence gene (598 bp)**

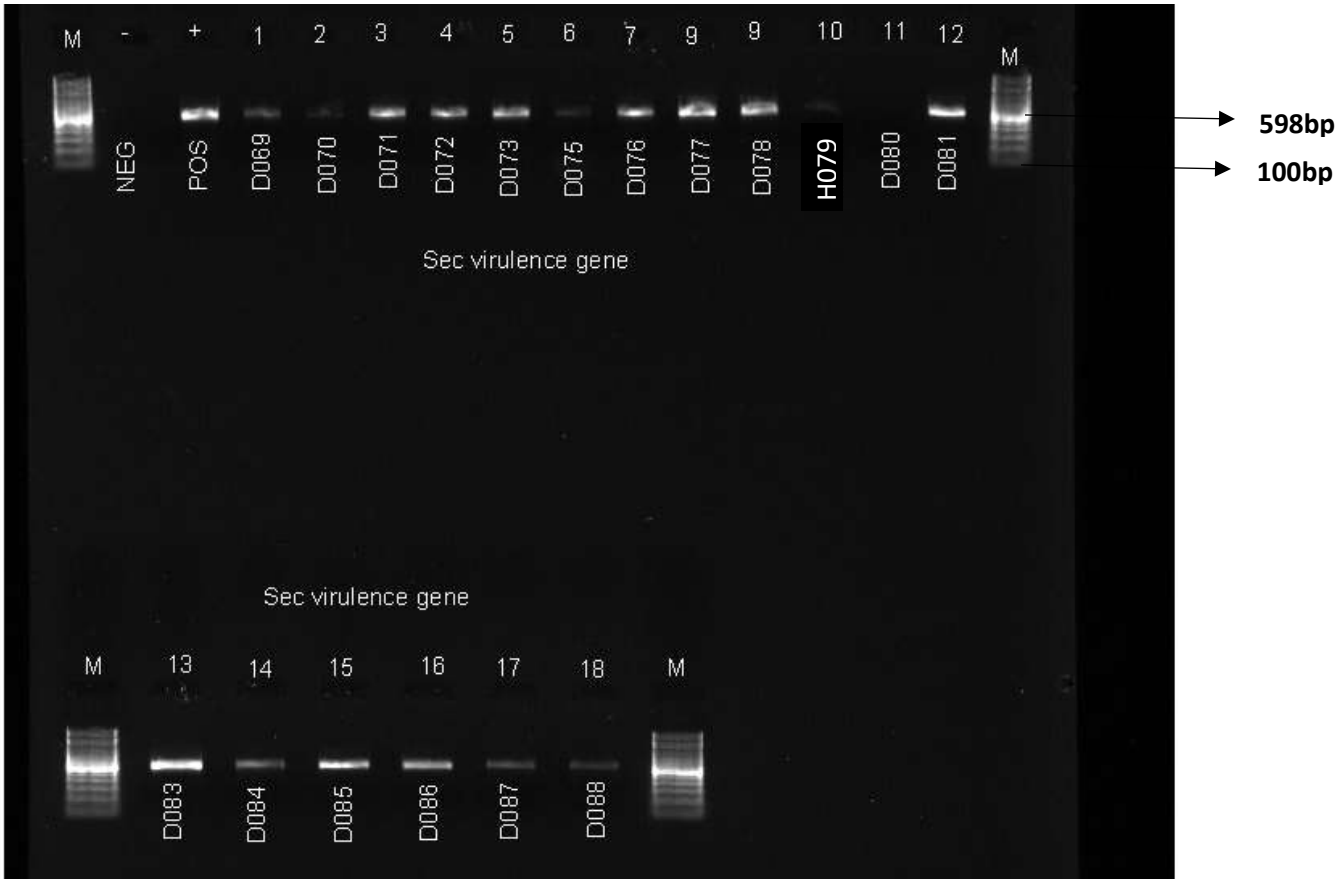

Figure 11b: Polymerase Chain Reaction amplification gel of *sec* virulence gene (598 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SP31 (positive control).

Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, and 18 were positive for ***sec* virulence gene.**

***exi* virulence gene (512 bp)**

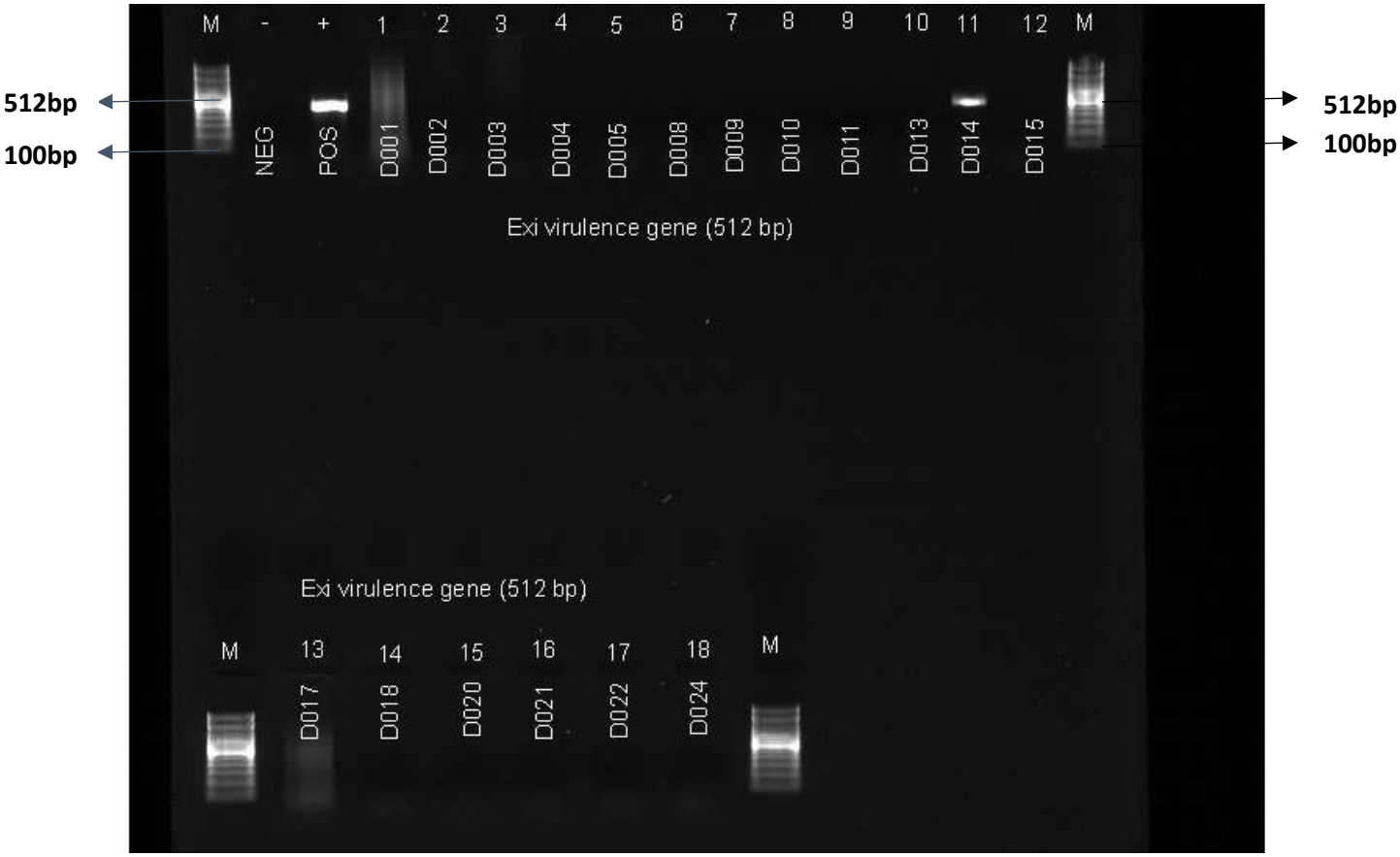

Figure 12a: Polymerase Chain Reaction amplification gel of *exi* virulence gene (512 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MSSP19 (positive control).

Lane 11 was positive for ***exi* virulence gene.**

***exi* virulence gene (512 bp)**

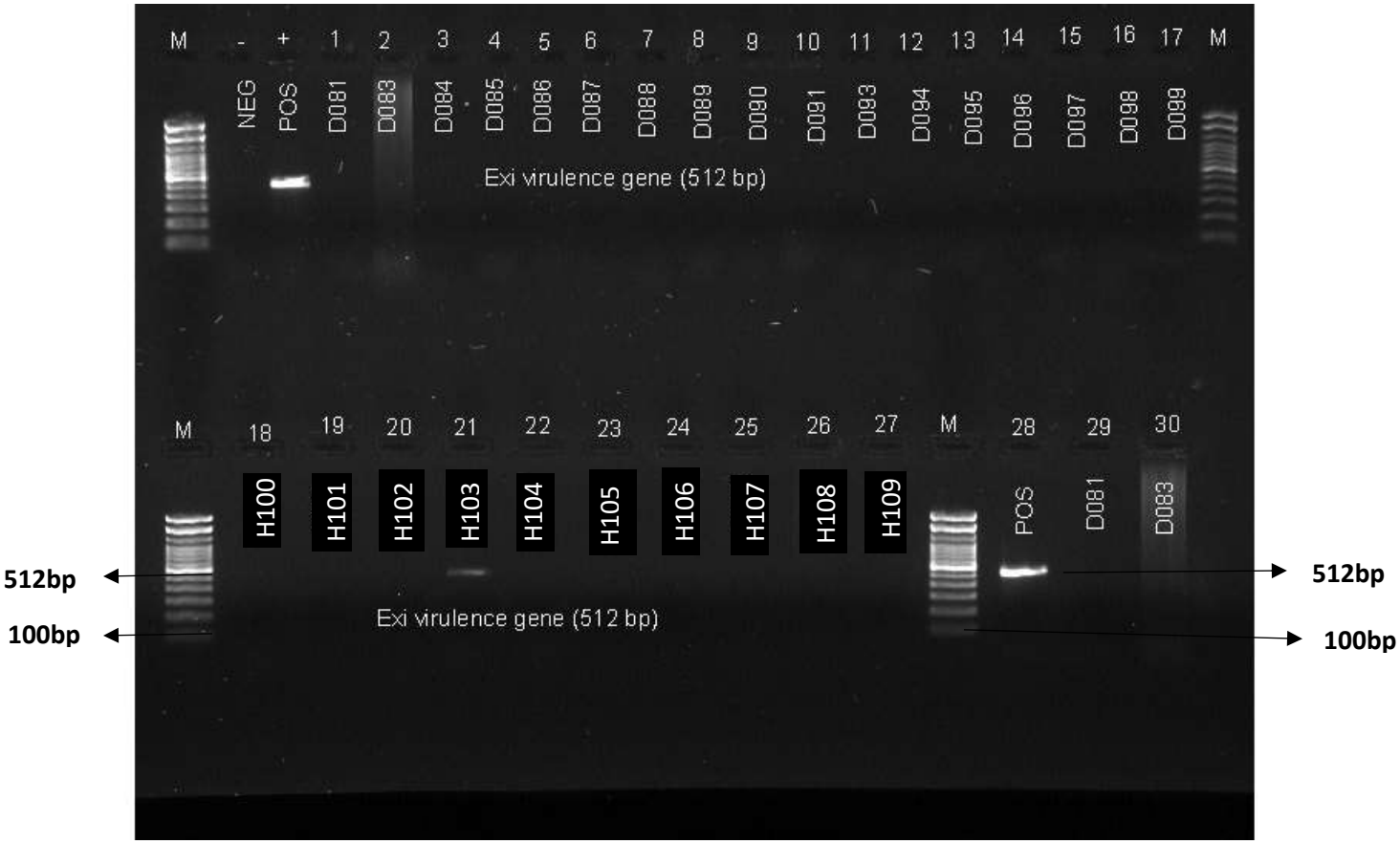

Figure 12b: Polymerase Chain Reaction amplification gel of *exi* virulence gene (512 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-30)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MSSP19 (positive control).

Lane 21 was positive for ***exi* virulence gene.**

***siet* virulence gene (601 bp)**

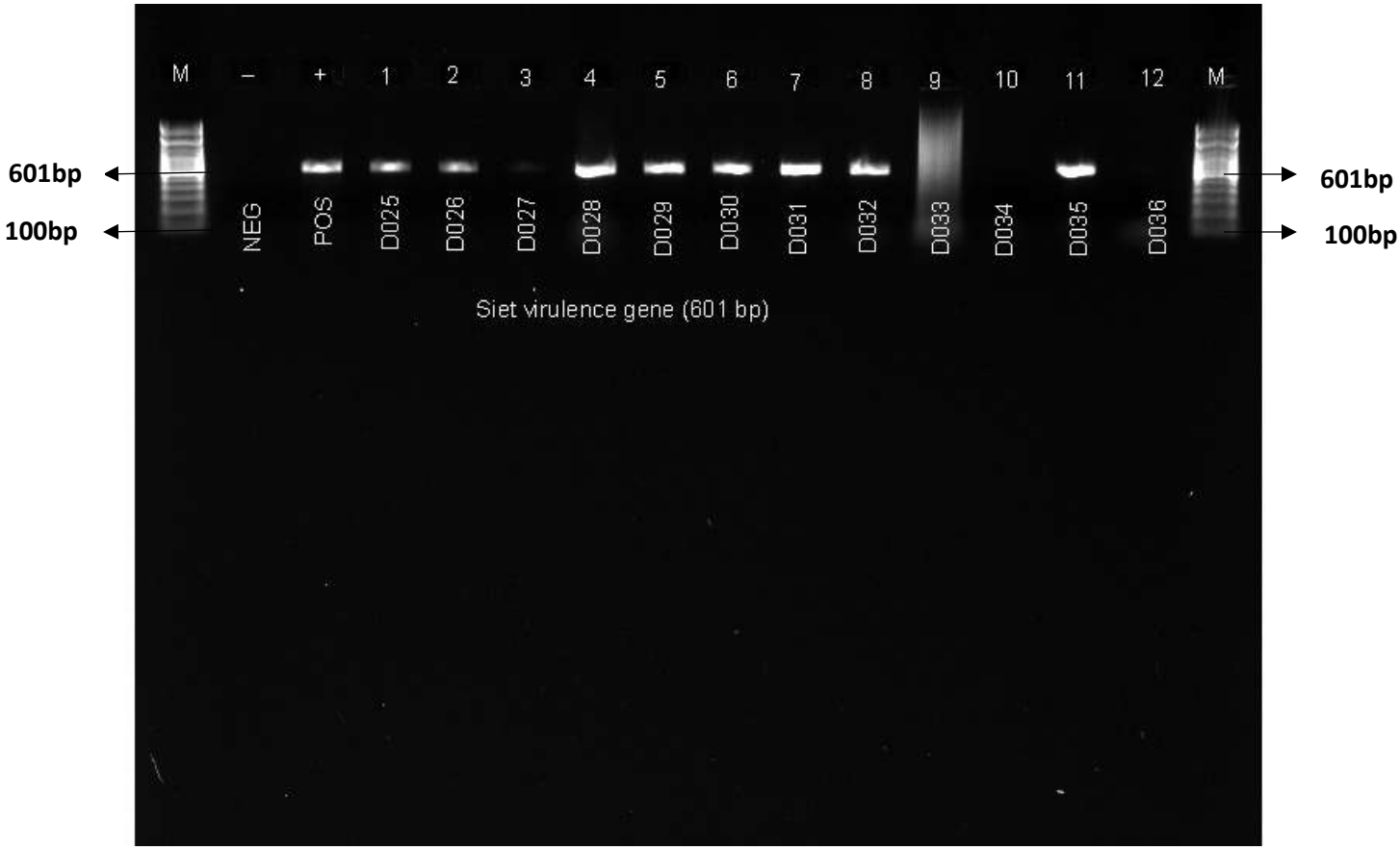

Figure 13a: Polymerase Chain Reaction amplification gel of *siet* virulence gene (601 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-12)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC20 (positive control).

Lanes 1, 2, 3, 4, 5, 6, 7, 8, and 11 were positive for ***siet* virulence gene.**

***siet* virulence gene (601 bp)**

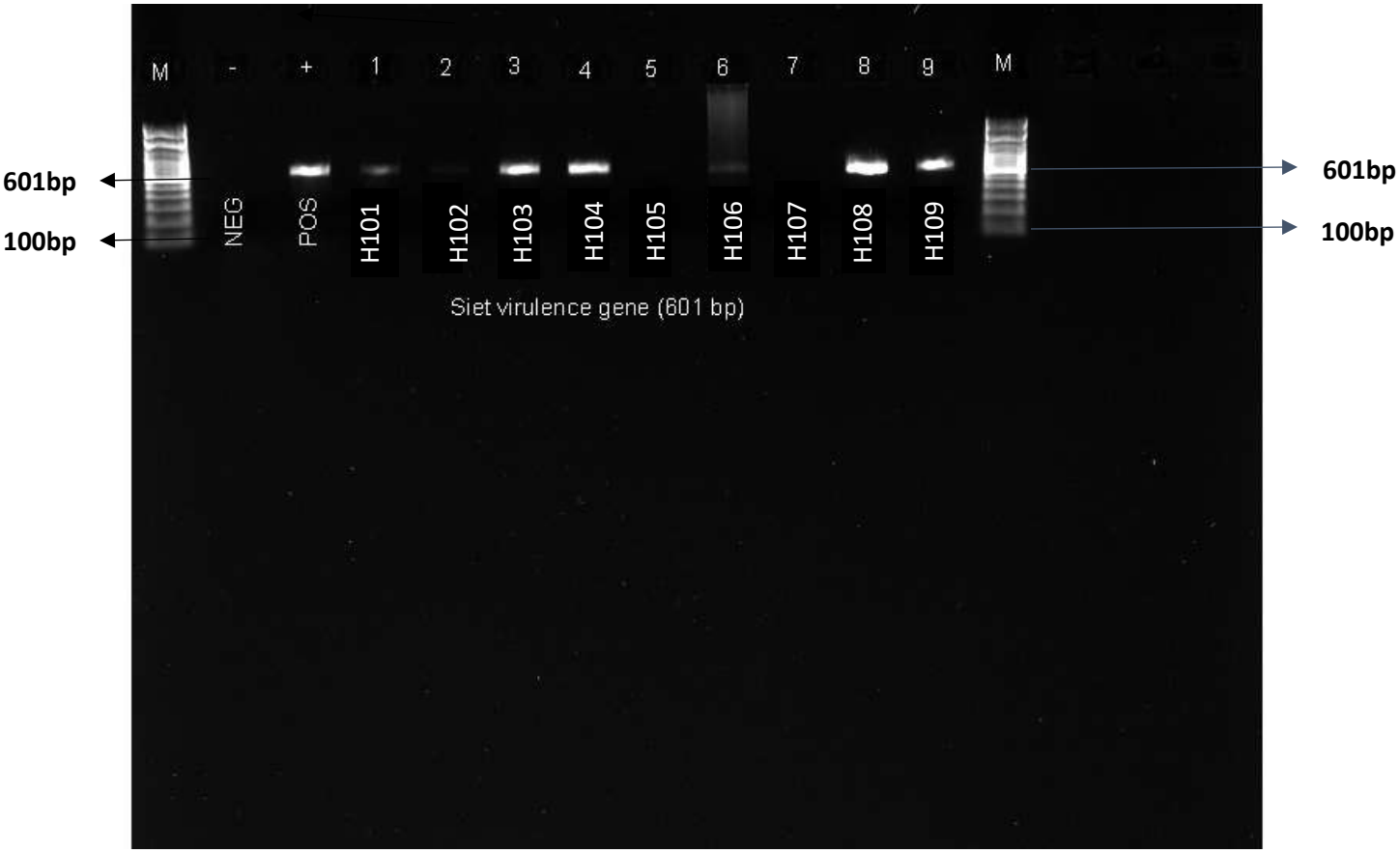

Figure 13b: Polymerase Chain Reaction amplification gel of *siet* virulence gene (601 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-9)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC20 (positive control).

Lanes 1, 2, 3, 4, 6, 8, and 9 were positive for ***siet* virulence gene.**

***luk*D virulence gene (513 bp)**

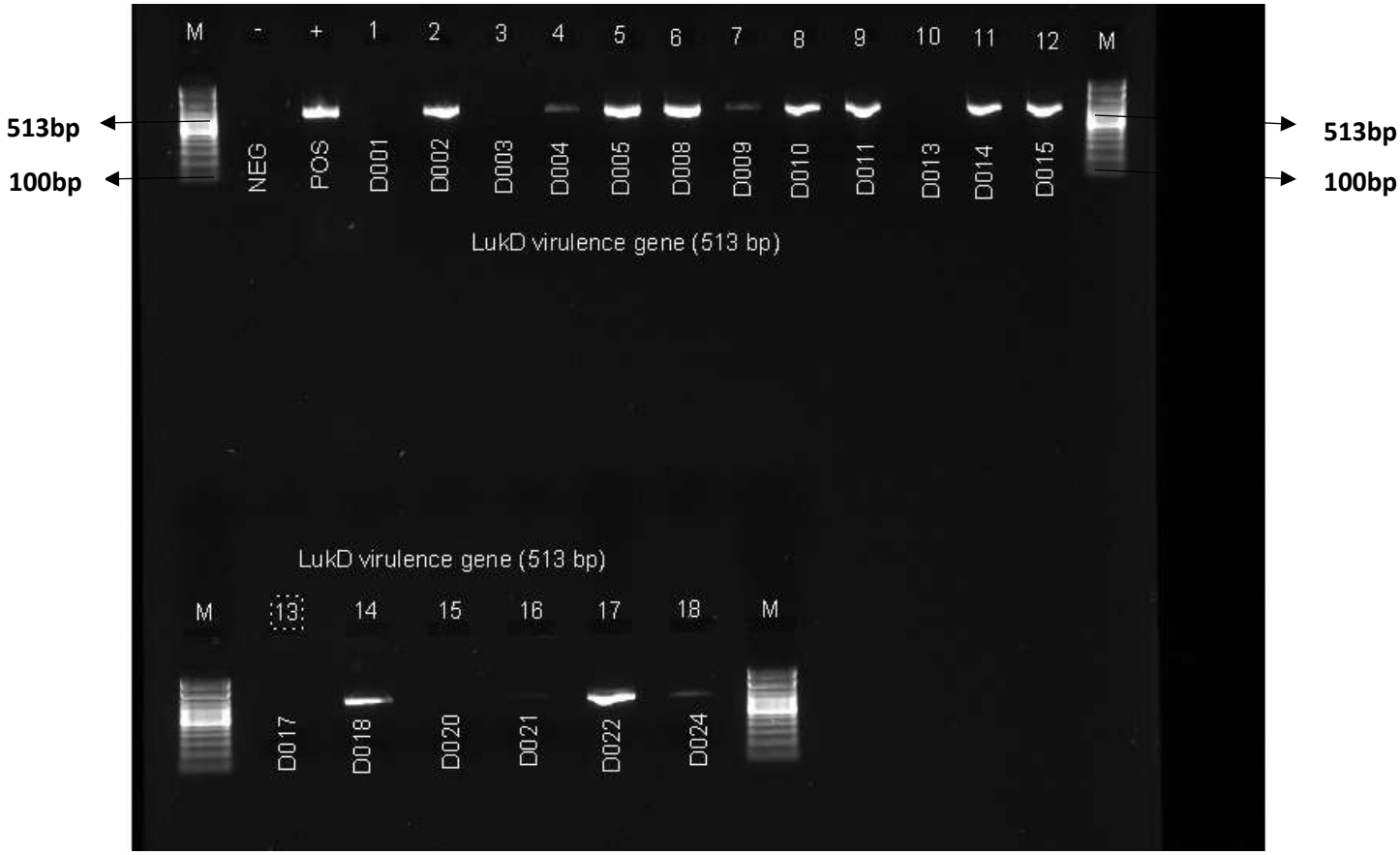

Figure 14a: Polymerase Chain Reaction amplification gel of *luk*D virulence gene (513 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC06 (positive control).

Lanes 2, 4, 5, 6, 7, 8, 9, 11, 12, 14, 16, 17, and 18 were positive for ***luk*D virulence gene.**

***luk*D virulence gene (513 bp)**

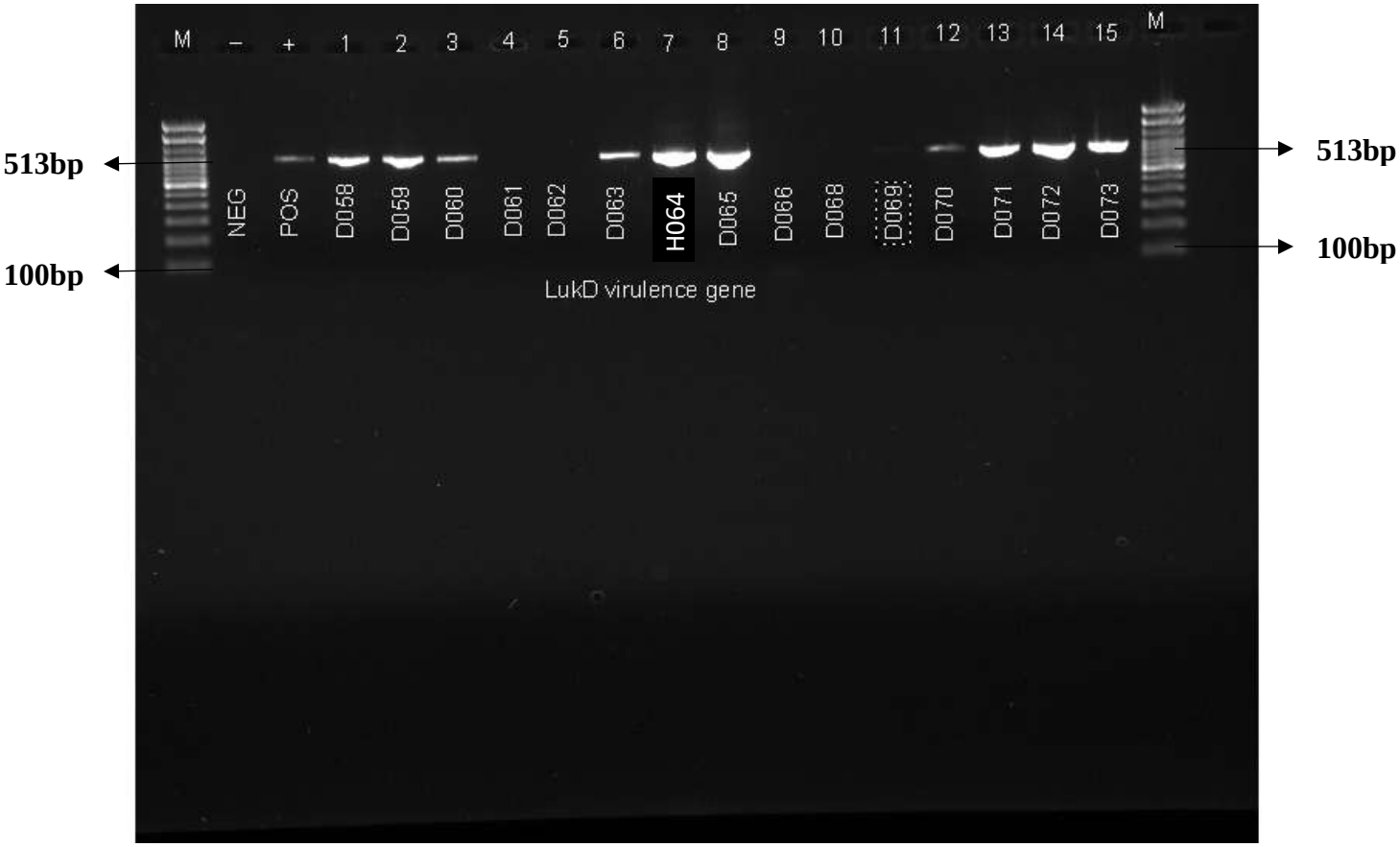

Figure 14b: Polymerase Chain Reaction amplification gel of *luk*D virulence gene (513 bp) among the *Staphylococcus pseudintermedius* isolates (lanes 1-15)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC06 (positive control).

Lanes 1, 2, 3, 6, 7, 8, 11, 12, 13, 14, and 15 were positive for ***luk*D virulence gene.**

4.11 Antibiotypes and identified genes in *Staphylococcus pseudintermedius* isolates from dogs and humans based on houesholds

There was phenotypic homogeneity in the antibiotic resistance profiles of isolates obtained in each of the 69 households that were sampled (Table 19). Similarity in antibiotic resistance profiles was also observed between human and dog isolates in each household (Table 19).

Table 19: Antibiotypes and identified genes in *Staphylococcus pseudintermedius* isolates from dogs and humans based on houesholds

Household	Isolate source	Isolate code	Resistance phenotypes	Genes
#1	Dog 1	D001	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Dog 2	D003	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#2	Dog	D002	TET AMP PEN	*tet*M, *sec*, *siet*, *luk*D
#3	Dog	D004	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *siet*, *luk*D
	Human	D100	CHL AMP PEN	*sec*, *siet*, *luk*D
#4	Dog	D005	CHL AMP PEN	*sec*, *siet*, *luk*D
	Human	D064	CHL AMP PEN	*sec*, *siet*, *luk*D
#6	Dog	D008	PEN	*sec*, *siet*, *luk*D
#7	Dog 1	D009	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*, *luk*D
	Dog 2	D070	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*, *luk*D
#8	Dog	D010	AMP PEN	*sec*, *siet*, *luk*D
	Human	D103	AMP PEN	*sec*, *siet*, *exi*, *luk*D
#9	Dog	D011	CHL AMP PEN	*sec*, *siet*, *luk*D
#10	Dog 1	D013	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *siet*
	Dog 2	D017	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
#11	Dog	D014	AMP PEN	*sec*, *exi*, *siet*, *luk*D
	Human	D104	AMP PEN	*sec*, *siet*, *luk*D
#12	Dog	D015	CHL AMP PEN TGC	*sec*, *luk*D
#13	Dog	D018	TET ERY **MRSP** LEV CIP MXF CLI	*tet*M, *sec*, *siet*, *luk*D

Key: CHL = Chloramphenicol**, GEN** = Gentamycin**, TET** = Tetracyline**, SXT** = Trimethoprim/Sulfamethoxazole, **ERY** = Erythromycin, **MRSP =** OXA AMP PEN, **LEV** = Levofloxacin, **CIP** = Ciprofloxacin**, MXF** = Moxifloxacin, **CLI** = Clindamycin, **TGC** = Tigecycline**.**

Table 19 (contd..): Antibiotypes and identified genes in *Staphylococcus pseudintermedius* isolates from dogs and humans based on houesholds

Household	Isolate source	Isolate code	Resistance phenotypes	Genes
#14	Dog 1	D020	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *siet*
	Dog 2	D024	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *luk*D
	Human	D107	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#15	Dog	D025	SXT AMP PEN	*siet*, *luk*D
#16	Dog	D026	AMP PEN	*sec*, *siet*, *luk*D
#17	Dog	D027	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *siet*
#18	Dog	D028	CHL SXT TET AMP PEN LEV CIP MXF TGC	*tet*M, *siet*, *luk*D
#19	Dog	D029	TGC	*siet*, *luk*D
#20	Dog	D030	AMP PEN	*siet*, *luk*D
#21	Dog	D031	GEN SXT TET ERY **MRSP** LEV CIP MXF CLI	*siet*, *luk*D
#22	Dog	D032	SXT ERY **MRSP** LEV CIP MXF	*siet*, *luk*D
#23	Dog 1	D033	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
	Dog 2	D085	SXT TET ERY **MRSP** LEV CIP MXF CLI	*tet*M, *sec*, *siet*, *luk*D
	Dog 3	D094	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
#24	Dog 1	D021	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
	Dog 2	D034	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
#25	Dog	D035	TET AMP PEN	*tet*M, *sec*, *siet*, *luk*D
#26	Dog	D036	CHL GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
	Human	D101	CHL GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*, *siet*
#27	Dog	D037	CHL GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*

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Table 19 (contd..): Antibiotypes and identified genes in *Staphylococcus pseudintermedius* isolates from dogs and humans based on houesholds

Household	Isolate source	Isolate code	Resistance phenotypes	Genes
#28	Dog	D038	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
	Human	D079	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#29	Dog	D039	AMP PEN	*sec*, *siet*, *luk*D
#30	Dog 1	D040	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Dog 2	D046	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*sec*, *siet*, *luk*D
	Dog 3	D047	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Human	D102	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#31	Dog	D041	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Human	D106	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, siet
#32	Dog 1	D042	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Dog 2	D056	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#33	Dog	D043	**MRSP**	*sec*, *siet*
#34	Dog	D044	Completely susceptible to all the antibiotics tested	-
#35	Dog	D045	CHL	-
#36	Dog	D048	**MRSP**	*sec*, *luk*D
#37	Dog	D049	Completely susceptible to all the antibiotics tested	-
#38	Dog	D050	AMP PEN	*sec*, *siet*, *luk*D
#39	Dog 1	D052	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A
	Dog 2	D093	CHL GEN SXT TET ERY **MRSP** LEV CIP MXF CLI	-
#40	Dog	D053	CHL GEN SXT TET **MRSP** LEV CIP MXF CLI TGC	*sec*, *siet*, *luk*D
#41	Dog	D054	TET AMP PEN	*tet*M, *sec*, *siet*, *luk*D

Key: CHL = Chloramphenicol**, GEN** = Gentamycin**, TET** = Tetracyline**, SXT** = Trimethoprim/Sulfamethoxazole, **ERY** = Erythromycin, **MRSP =** OXA AMP PEN, **LEV** = Levofloxacin, **CIP** = Ciprofloxacin**, MXF** = Moxifloxacin, **CLI** = Clindamycin, **TGC** = Tigecycline**.**

Table 19 (contd..): Antibiotypes and identified genes in *Staphylococcus pseudintermedius* isolates from dogs and humans based on houesholds

Household	Isolate source	Isolate code	Resistance phenotypes	Genes
#42	Dog	D055	CHL GEN SXT ERY **MRSP** LEV CIP MXF CLI	*sec*, *siet*, *luk*D
#43	Dog 1	D057	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*, *siet*
	Dog 2	D061	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#44	Dog 1	D058	AMP PEN	*sec*, *siet*, *lukD*
	Dog 2	D059	AMP PEN	*sec*, *siet*, *luk*D
	Human	D051	AMP PEN	*sec*, *siet*, *luk*D
#45	Dog	D060	CHL AMP PEN	*sec*, *siet*, *luk*D
#46	Dog 1	D062	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Dog 2	D068	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#47	Dog	D063	AMP PEN	*sec*, *siet*, *luk*D
#48	Dog	D065	AMP PEN	*sec*, *siet*, *luk*D
#49	Dog 1	D066	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Dog 2	D069	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*, *luk*D
	Dog 3	D098	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *siet*
#50	Dog	D071	AMP PEN	*sec*, *siet*, *luk*D
#51	Dog	D072	TET AMP PEN	*tet*M, *sec*, *siet*, *luk*D
#52	Dog	D073	AMP PEN	*sec*, *siet*, *luk*D
#53	Dog	D075	GEN ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
#54	Dog	D076	AMP PEN	*sec*, *siet*, *luk*D

Key: CHL = Chloramphenicol**, GEN** = Gentamycin**, TET** = Tetracyline**, SXT** = Trimethoprim/Sulfamethoxazole, **ERY** = Erythromycin, **MRSP =** OXA AMP PEN, **LEV** = Levofloxacin, **CIP** = Ciprofloxacin**, MXF** = Moxifloxacin, **CLI** = Clindamycin, **TGC** = Tigecycline**.**

Table 19 (contd..): Antibiotypes and identified genes in *Staphylococcus pseudintermedius* isolates from dogs and humans based on houesholds

Household	Isolate source	Isolate code	Resistance phenotypes	Genes
#55	Dog	D077	AMP PEN	*sec*, *siet*, *luk*D
#56	Dog	D078	TET AMP PEN	*tet*M, *sec*, *siet*, *luk*D
#57	Dog	D080	CHL TET ERY **MRSP** CLI	*mec*A
#58	Dog	D081	TET^R AMP PEN	*tet*M, *sec*, *siet*
#59	Dog	D083	AMP PEN	*sec*, *siet*, *luk*D
	Human	D109	AMP PEN	*sec*, *siet*, *luk*D
#60	Dog	D084	GEN SXT ERY **MRSP** LEV CIP MXF CLI TGC	*mec*A, *sec*, *siet*
#61	Dog	D086	TET AMP PEN	*tet*M, *sec*, *siet*, *luk*D
#62	Dog	D087	CHL GEN SXT ERY **MRSP** LEV MXF CLI CIP	*mec*A, *sec*
#63	Dog 1	D088	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*, *siet*
	Dog 2	D096	GEN SXT ERY **MRSP** LEV CIP MXF CLI	*mec*A, *sec*
	Human	D105	GEN SXT ERY **MRSP** LEV CIP MXF CLI	-
#64	Dog	D089	AMP PEN	*sec*, *siet*, *luk*D
#65	Dog	D090	TET AMP PEN	*tet*M, *sec*, *siet*, *luk*D
#66	Dog	D091	AMP PEN	*sec*, *siet*, *luk*D
#67	Dog	D095	PEN	*sec*, *siet*, *luk*D
#68	Dog	D097	CHL TET AMP PEN TGC	*tet*M, *sec*, *siet*
#69	Dog	D099	AMP PEN	-
	Human	D108	PEN	*sec*, *siet*, *luk*D
#70	Dog	D022	AMP PEN	*siet*, *luk*D

Key: CHL = Chloramphenicol**, GEN** = Gentamycin**, TET** = Tetracyline**, SXT** = Trimethoprim/Sulfamethoxazole, **ERY** = Erythromycin, **MRSP =** OXA AMP PEN, **LEV** = Levofloxacin, **CIP** = Ciprofloxacin**, MXF** = Moxifloxacin, **CLI** = Clindamycin, **TGC**=Tigecycline**.**

4.12 *Tet*M DNA sequences of the *Staphylococcus pseudintermedius* isolates

The DNA sequences of the *tet*M genes for the 12 tetracycline-resistant *S. pseudintermedius* isolates (D002, D018, D028, D035, D054, D072, D078, D081, D085, D086, D090, D097) in our study exhibited 99-100 % nucleotide similarity to the *tet*M reference nucleotide sequences of *Staphylococcus aureus* subsp. *aureus* in the GenBank database using a BLAST search on the National Centre for Biotechnology Information (NCBI) server (**Appendix XXIII**).

CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 Discussion

Increasing antimicrobial resistance by *S. pseudintermedius* especially MRSP strains in dogs represents a major challenge for veterinarians in terms of antibiotic therapy (Paul *et al*., 2011; Kate *et al*., 2018; Papadogiannakis *et al*., 2016) and is a concern for both animal and public health. The epidemiological situation of *S. pseudintermedius* infections is further exacerbated as the gene driving the drug resistance is highly mobile and can be transferred between different staphylococcal species colonizing human and zoonotic host (van Duijkeren *et al*., 2011; Arianna *et al*., 2015; Jurate & Jurate , 2015). This is the first study in West Africa, including Nigeria which reports *S. pseudintermedius* colonization in both dogs and dog owners. The *S. pseudintermedius* isolates from this study, including the MRSP strains were multi-drug resistant and more prevalent in dogs than among dog owners. Isolates were notably more resistant than those reported in literature and haboured genes encoding various pathogenic factors. Interestingly, there was phenotypic homogeneity in the antibiotic resistance profiles of isolates from both dogs and dog owners in each household that was sampled, thus depicting a possible zoonotic transmission event from dogs to their owners.

The carriage frequency of *S. pseudintermedius* in our study among the 112 shelter dogs sampled was 76.8 % as 86 of the dogs were positive for *S. pseudintermedius*. Feng *et al*. (2012) reported a prevalence of 18.3 %, while Garbacz *et al*. (2013) described it to be 51.8 % of tested animals (Arianna *et al*., 2015; Melter *et al*., 2017). The carriage frequency of *S. pseudintermedius* among

dogs in our study was higher than the 55 % carriage frequency reported by Gharsa *et al*. (2013) in Tunisia, North Africa where 55 out of the 100 nasal swabs of dogs were positive for *S. pseudintermedius*. However, our finding is less than the 90 % carriage frequency reported by Rubin and Chirino-Trejo (2011) in Western Canada (Borjesson *et al*., 2015; Somayaji *et al*., 2016).

S. pseudintermedius is not a part of the human resident microbiota; it is therefore suggested that people acquire this organism through canine contact such as licking or handling of pets, although the transmission of this organism has not been adequately studied (Gharsa *et al*., 2013; Borjesson *et al*., 2015). People who suffer from concurrent immunosuppressive conditions are more susceptible to the secondary infections and may be over-represented among human patients with *S. pseudintermedius* infections (Kuan *et al*., 2016). Among patients with *S. pseudintermedius* infections, close association with dogs is commonly reported, suggesting zoonotic transmission (Gomez-Sanz *et al*., 2013b; Borjesson *et al*., 2015; Pires dos Santos *et al*., 2017; Melter *et al*., 2017)). Because dogs are frequently pharyngeally colonized, contaminated saliva provides ample opportunity for *S. pseudintermedius* to infect damaged tissues following dog bite wounds (Borjesson *et al*., 2015). Although human infections with *S. pseudintermedius* are a recognized public health risk associated with canine contact, the magnitude of this risk has not been quantified. The carriage of *S. pseudintermedius* in humans has been reported to be sporadic, and colonization or infection with this bacterium is likely zoonotic (Paul *et al*., 2011; Paul, 2015). The first reported case of human infection with *S. pseudintermedius* was a cardiac device pocket infection initially misidentified as *S. aureus* (Van Hoovels *et al*., 2006; Jurate & Jurate, 2015; Arianna *et al*., 2015; Somayaji *et al*., 2016). The frequency of human *S. pseudintermedius*

infections may be under-reported and under-appreciated due to its misidentification in diagnostic laboratories because of its morphological and biochemical similarity to *S. aureus***.**

The carriage frequency of *S. pseudintermedius* among the 97 dog owners that volunteered for this study was 13 (13.4 %), while 6 (46.2 %) out of the 13 *S. pseudintermedius* isolates obtained from humans (dog owners) were MRSP. Although few human cases have been described (Chuang *et al*., 2010; Savini *et al*., 2013; Stegmann *et al*., 2010; Borjesson *et al*., 2015; Verstappen *et al*., 2017), the presumptive transmission of *S. pseudintermedius* from dogs to dog owners has also been reported as 3.9 % to 13 % of those humans have been found to harbour *S. pseudintermedius* (Chanchaithong *et al*., 2014; Paul *et al*., 2015).

This report is similar to our study where *S. pseudintermedius* carriage frequency of 13 (13.4 %) was observed among the 97 dog owners that were sampled. Another study reported *S. pseudintermedius* carriage frequency of 5.6 % among dog owners (Gomez-Sanz *et al*., 2013; Pires dos Santos *et al*., 2016) which was lower than what we observed in our study. *S. pseudintermedius* has primarily been associated with infections in companion animals, and acquisition of this bacterium in humans suggests a possible zoonotic transmission event from dogs. Among the three major dog sites that were sampled (perineum, nares, and mouth) for *S. pseudintermedius*, the perineum was the most colonized site with a recovery frequency of 93.3 %. This was closely followed by the nares and the mouth with recovery frequencies of 71.4 % and 59.4 % respectively. There was no statistically significant difference in the prevalence of *S. pseudintermedius* in the perineum, nares, and mouth [$p = 0.074$ (at $p < 0.05$)].

The ability to recover *S. pseudintermedius* from a dog depends on multiple factors including whether single or multiple anatomical sites are sampled, and which specific sites are included (Rubin & Chirino-Trejo, 2011; Somayaji *et al*., 2016). The recovery rate of *S. pseudintermedius*

was reported to be high from the pharynx (81.1 % of colonized dogs) compared to the recovery rate from the nares (47.7 %) (Rubin & Chirino-Trejo, 2011; Somayaji *et al*., 2016). In one study which included only nasal swabs, staphylococci were recovered from 37 % of dogs (Han *et al*., 2016). The reported recovery rate (37 %) of *S. pseudintermedius* from the nares of dogs by Han *et al*. (2016) was lower than the 71.4 % recovery rate from nares in our study. As such, including multiple sampling sites improves recovery of *S. pseudintermedius* in dogs (Rubin & Chirino-Trejo, 2011; Somayaji *et al*., 2016). However, methodological inconsistencies between studies, including site and number of samples collected and media used to isolate *S. pseudintermedius* make the identification of global resistance trends difficult. Differences in the prevalence of *S. pseudintermedius* might also depend on study design, identification methods, sampling, animal health status, and other factors (Ruzauskas *et al*., 2016). The high prevalence of *S. pseudintermedius* in our study might be due to the large sample size (359 swab samples) and the site of dogs (perineum, nares, and mouth) sampled.

Antimicrobial resistance appears to be emerging among *S. pseudintermedius* colonizing shelter dogs and dog owners in Abakaliki, Nigeria. The emergence of antimicrobial resistance, including methicillin resistance in *S. pseudintermedius*, is increasingly challenging the treatment of infections. Since 2006, there has been a dramatic worldwide increase in the frequency of MRSP among clinical canine infections (Moodley *et al*., 2014; van Duijkeren *et al*., 2011a; Melter *et al*., 2017). More than 50 % of the *S. pseudintermedius* isolates in our study exhibited methicillin resistance traits in addition to being multi-drug resistant to other antibiotic classes such as aminoglycosides, sulfonamides, macrolides, beta-lactams, fluoroquinolones, and lincosamides. Rising methicillin resistance in *S. pseudintermedius* and the ability for resistance traits transfer

between Staphylococcal species may have significant implications for rising antimicrobial resistance and infection management.

The resistance of *S. pseudintermedius* depends on geographical distribution as well as on other factors – thus, it is important to obtain data from different countries to better understand the epidemiological spread of resistance (Geoghegan *et al*., 2009; Pitchenin *et al*., 2017). The recurring emergence of antibiotic resistance exhibited by *S. pseudintermedius* is a serious public health problem as it poses a great challenge to antimicrobial therapy for animals and even humans. The ability of staphylococci to adapt to selection pressure in antibiotic use has been recognized since the first ever description of penicillin-resistant *S. aureus* in 1940 by Ball *et al*. (2008).

The antibiotic susceptibility testing results of the *S. pseudintermedius* isolates from dogs in this study revealed that the highest observed resistance was to penicillin (95.3 %) and ampicillin (94.2 %) respectively which are both beta-lactam antibiotics. These dog isolates exhibited multi-drug resistant traits as they were resistant to at least three different classes of antibiotics except for two isolates that were completely susceptible to all the antibiotics tested. Multi-drug resistant *S. pseudintermedius* is on the increase and has resulted in very limited treatment options (Vigo *et al*., 2015; Zur *et al*., 2016). Other studies have also reported multi-drug resistant *S. pseudintermedius* (Detwiler *et al*., 2013; Garbacz *et al*., 2013; Arianna *et al*., 2015; Somayaji *et al*., 2016). There was a statistically significant difference in the mean percentage resistances of the *S. pseudintermedius* isolates from dogs ($p < 0.05$).

Amoxicillin, penicillin, tetracycline, clindamycin, and sulphamethoxazole-trimethoprim are the first antibiotic choices for treating Gram-positive bacterial infections in companion animals (Summers *et al*., 2014; Prescott *et al*., 2002; Papadogiannakis *et al*., 2016). The *S.*

pseudintermedius isolates in our study exhibited high resistance traits to these first line antibiotics for treating bacterial infections in companion animals. Among *S. pseudintermedius,* resistance is emerging to the β-lactams, with resistance to penicillin reported in over 70 % of *S. pseudintermedius* isolates colonizing healthy dogs and 95 % of clinical isolates (Kang *et al*., 2014; Yoon *et al*., 2010; Melter *et al*., 2017). A recent systemic review found that between 1980 and 2013 there was a trend of increasing resistance to penicillin and ampicillin among methicillin susceptible *S. pseudintermedius* (MSSP) (Moodley *et al*., 2014). Resistance to other antibiotic classes such as the fluoroquinolones, aminoglycosides, and chloramphenicol also increased during that period (Moodley *et al*., 2014). Antimicrobial resistance limits the ability of clinicians to select appropriate antimicrobials for the treatment of bacterial infections.

Even though the resistance frequencies of the *S. pseudintermedius* isolates from dogs observed in our study is higher than those reported in other studies, our study is still in agreement with report of Gharsa *et al*. (2013) who also reported resistance to penicillin (56.4 %), tetracycline (40 %), trimethoprim-sulfamethoxazole (23.7 %), chloramphenicol (1.8 %), erythromycin (1.8 %), clindamycin (1.8 %), and ciprofloxacin (1.8 %). This study is also in agreement with the study of Matthew *et al*. (2017) who reported resistance frequencies of 74.5 %, 68.4 %, 55.1 %, 34.1 %, and 23.5 % to trimethoprim-sulfamethoxazole, erythromycin, clindamycin, gentamycin, and chloramphenicol respectively.

Methicillin-resistant *S. pseudintermedius* first emerged in the late 1990s in Europe and North America and is increasingly reported globally (Damborg *et al*., 2016; Hensel *et al*., 2016; Kasai *et al*., 2016). From 2004 through 2013, the incidence of canine MRSP infections increased sevenfold at a veterinary diagnostic lab in Utrecht, the Netherlands (Duim *et al*., 2016). Moreover, *S. pseudintermedius* is often reported as methicillin-resistant with co-resistance to

different classes of antimicrobials other than beta-lactams (Perreten *et al*., 2010, Melter *et al*., 2017; Verstappen *et al*., 2017; Valentina *et al*., 2017). This was also observed in our study as MRSP isolates exhibited resistance to different classes of antibiotics such as aminoglycosides, sulfonamides, macrolides, beta-lactams, fluoroquinolones, and lincosamides which are not beta-lactams. Exactly 46(53.5 %) of the dog isolates were resistant to oxacillin with 2 % NaCl, depicting methicillin-resistance. Similar MRSP resistance to beta-lactams, fluoroquinolones, aminoglycosides, macrolides, trimethoprim/sulfamethoxazole in a community-associated urinary tract infection was reported in an otherwise healthy, neutered male pub dog in 2009 (Rubin *et al*., 2011; Somayaji *et al*., 2016).

Tetracycline resistance by *S. pseudintermedius* in dogs has also been reported in both Australia and United Kingdom with resistance frequencies of 50 % and 35 % respectively (Siak *et al*., 2014; Maluping *et al*., 2014). However, these reported frequencies were a bit higher than the tetracycline resistance frequency of 19.8 % in our study. Elsewhere, increasing antimicrobial resistance to other antimicrobials other than beta-lactams has also been reported in MRSP isolates, thus leaving few therapeutic options available in many instances (Perrenten *et al*., 2010; Priyantha *et al*., 2016; Verstappen *et al*., 2017; Valentina *et al*., 2017). Resistance to drugs such as vancomycin, daptomycin, linezolid, and quinupristin/dalfopriston which are active against methicillin-resistant isolates has not yet been reported in animal or human isolates (van Duijkeren *et al*., 2011; Priyantha *et al*., 2016). This is in agreement with our study as isolates from both dogs and humans were completely susceptible to nitrofurantoin, rifampin, vancomycin, quinupristin/dalfopristin, daptomycin, linezolid, and amikacin which are considered drugs of last resort in the treatment of *S. pseudintermedius* infections. Interestingly, a recent study at a Texas Veterinary Medical Teaching Hospital in the United States reported an amikacin

resistance frequency of 36 % among MRSP isolates from dogs (Gold *et al*., 2014), whereas amikacin resistance was not observed among the dog and human isolates in our study. In contrast to the dog isolates, the isolates from humans (dog owners) were completely resistant (100 %) to penicillin while the resistance frequency to ampicillin was 92.3 % which is very similar to the resistance frequency observed among the dog isolates. All the *S. pseudintermedius* obtained from humans were also multi-drug resistant. They exhibited equal resistance frequency of 46.2 % each to aminoglycosides, macrolides, lincosamides, sulfonamides, and fluoroquinolones. Resistance frequency of the human isolates to chloramphenicol was 23.1 %. Interestingly, this was the same resistance frequency observed among the dog isolates, and this depicts phenotypic homogeneity among the dog and human isolates, thus suggesting a zoonotic transfer.

The indiscriminate use of antimicrobials might select for antibiotic-resistant bacteria (van Duijkeren *et al*., 2011; Arianna *et al*., 2015). Ruzauskas (2016) reported that 10 MRSP strains were isolated in two large breeding kennels from dogs with reproductive disorders in which the owners irregularly used antimicrobials for ‘‘better reproductive performance”. Such inappropriate use of antimicrobials possibly led to high resistance rates of MRSP in these kennels especially during mating (Ruzauskas *et al*., 2016). Attention should be paid to this problem since methicillin-resistant staphylococci pose a risk not only to animals but also to humans (Catry *et al*., 2010; Stegmann *et al*., 2010, Ruzauskas et al., 2016; Kate *et al*., 2018). Interestingly, none of the *S. pseudintermedius* isolates recovered from dog owners exhibited resistance to tetracycline unlike the dog isolates in which resistance frequency of 19.8 % was observed.

The high frequency of resistance to antibiotics in our study area could due to indiscriminate use of antibiotics, especially broad-spectrum antibiotics in treating dog infections without sending clinical material to a laboratory for diagnosis and antibiogram.

The MRSP isolates in a study in Luthiania were susceptible only to those antimicrobials (linezolid, vancomycin, and daptomycin) reserved for treating human infections but banned from veterinary use (Ruzauskas *et al*., 2016). Our study is in agreement with this Lithuanian study as no resistance was observed to tigecycline, nitrofurantoin, rifampin, vancomycin, Quinupristin/dalfopristin, linezolid, daptomycin, and amikacin among the *S. pseudintermedius* isolates from dog owners (humans). There was a statistically significant difference in the mean percentage resistances of the *S. pseudintermedius* isolates from humans ($p < 0.05$). The multiple antibiotic resistance index (MARI) values of the *S. pseudintermedius* isolates in our study ranged from 0.1 – 0.6 while the average MARI value of the *S. pseudintermedius* isolates was 0.3, thus further depicting multidrug resistance traits. Our study showed that 84 *S. pseudintermedius* isolates out of the 86 isolates obtained from dogs exhibited 22 different resistance antibiotypes. Ampicillin + penicillin resistance antibiotype ($AMP^{\mathbf{R}}$ $PEN^{\mathbf{R}}$) was the most prevalent antibiotype as it was present in 19 out of the 22 resistance antibiotypes observed.

In contrast to the isolates from dogs, all the 13 *S. pseudintermedius* isolates obtained from humans (dog owners) exhibited 5 different resistance antibiotypes. Ampicillin + penicillin resistance antibiotype ($AMP^{\mathbf{R}}$ $PEN^{\mathbf{R}}$) was also the most prevalent antibiotype as it was present in 4 out of the 5 resistance antibiotypes observed. The highest resistance antibiotype observed among the *S. pseudintermedius* isolates from dogs and humans was a combined resistance to 10 different antibiotics (GEN^R SXT^R ERY^R OXA^R AMP^R PEN^R LEV^R CIP^R MXF^R CLI^R) which belong to 6 different antibiotic classes (aminoglycosides, sulfonamides, macrolides, beta-lactams,

fluoroquinolones, and lincosamides) with a resistance frequency of 36 % and 38.5 % for dog and human isolates respectively. This was closely followed by a combined resistance to beta-lactams (AMP^{R} PEN^{R}) with a resistance frequency of 22.1 % and 30.8 % for dog and human isolates respectively. No combined resistance to these 6 different classes of antibiotics by *S. pseudintermedius* from dogs and humans has ever been reported anywhere in the world according to literature. There was no statistically significant difference between the mean resistance of *S. pseudintermedius* from dogs when compared to those from humans ($p = 0.727$ (at $p < 0.05$). Over the last decade, MRSP has emerged as a major opportunistic pathogen in dogs and as a leading cause of skin diseases, otitis, surgical site infections, and wound infections (Perrenten *et al*., 2010; Weese & van Duijkeren, 2010; Arianna *et al*., 2015; Somayaji *et al*., 2016). Although methicillin resistance is not the product of beta-lactamase production even as the addition of beta-lactamase inhibitors such as clavulanic acid does not restore susceptibility; methicillin resistance, which is rapidly emerging among *S. pseudintermedius* in dogs and recently in humans is a serious threat to the efficacy of the most frequently used antibiotics in human and veterinary medicine, especially the beta-lactams (Prescott *et al*., 2002; Perrenten *et al*., 2010; Ruzauskas *et al*., 2016; Kate *et al*., 2018). *S. pseudintermedius* carried especially by healthy dogs in Saskatoon, Canada have been remarkably susceptible even as a 2008 study didn't identify canines carrying MRSP (Rubin *et al*., 2011; Ball *et al*., 2008; Somayaji *et al*., 2016). However, a recently published study reported an MRSP carriage frequency of 7 % in Saskatoon (Priyantha *et al*., 2016) which was higher than the 4.5 % prevalence reported in some studies in North America and Europe (Kawakami *et al*., 2010; Gingrich *et al*., 2011; Papadogiannakis *et al*., 2016; Melter *et al*., 2017). Our study revealed that 46 (53.5 %) of the dog isolates were

methicillin-resistant strains while 6 (46.2 %) of the human *S. pseudintermedius* were methicillin-resistant.

The prevalence frequency of MRSP among dogs in our study is far higher than the 4.5 % prevalence frequency reported in some studies in North America and Europe and the 7 % prevalence frequency reported by Priyantha *et al*. (2016) in Saskatoon, Western Canada. Similarly, the MRSP prevalence frequency we observed among dogs in our study is far higher than the 0.8 % (61/7490) and 2 % (1/59) MRSP prevalence frequency reported by Ruscher *et al*. (2010) and Griffeth *et al*. (2008) in Germany and the United States respectively (Melter *et al*., 2017; Papadogiannakis *et al*., 2016). MRSP in North America are reportedly often susceptible to chloramphenicol while isolates from Europe are frequently resistant (van Duijkeren *et al*., 2011b; Kjellman *et al*., 2015; Duim *et al*., 2016). Interestingly, the *S. pseudintermedius* isolates in our study exhibited similar resistance patterns to those isolates reported in both North America and Europe. There was no statistically significant difference in the prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) isolates between dogs and humans [$p = 0.417$ (at $p < 0.05$)]. Our study is in total concord with other studies from Asia where 32 % - 45 % MRSP carriage frequency was reported in Thailand, Japan, and Hong Kong (Sasaki *et al*., 2007; Epstein *et al*., 2009; Chanchaithong *et al*., 2014; Jurate & Jurate, 2015; Verstappen *et al*., 2017). Rising methicillin reistance in *S. pseudintermedius* and the ability of resistance and virulence factor to be exchanged between staphylococcal species could have serious implications for the rising antibiotic resistance and infection management. These high antibiotic resistances could be attributed to grievous abuse and misuse of antimicrobials.

A total of 47 (47.5 %) out of all the 99 isolates from both dogs and humans were methicillin-susceptible *S. pseudintermedius* (MSSP) as they were susceptible to oxacillin with 2 % NaCl.

Our study doesn't agree with the work of Gharsa *et al*. (2013) in Tunisia who reported that all the 55 *S. pseudintermedius* isolates they recovered from the 100 nasal swab samples of dogs were MSSP and none was MRSP. All the 99 isolates from dogs and humans were screened for *mec*A genes by PCR. Exactly 41 (78.9 %) out of the 52 MRSP isolates haboured *mec*A gene. All the MRSP isolates that were negative for *mec*A gene were also screened for *mec*C gene. Results showed that all the *mec*A-negative MRSP isolates do not possess *mec*C gene. As expected, all the 47 MSSP isolates were also negative for both *mec*A and *mec*C genes.

*Tet*M is a ribosomal protection protein which acts on the amino acyl-tRNA ribosome complex and confers resistance by displacing tetracycline (Chopra & Roberts, 2001; Priyantha *et al*., 2016; de Vries *et al*., 2016). The *tet*M gene has also been found in both the Tn916 and Tn5801 transposons which are integrative and conjugative elements (ICE) (de Vries *et al*., 2016). These conjugative transposons have a broad host range and have also been shown to play a role in the dissemination of chloramphenicol, kanamycin, and erythromycin resistance genes (Chopra & Roberts, 2001; de Vries *et al*., 2016). Interestingly, 17 isolates that exhibited resistance to tetracycline also exhibited resistance to chloramphenicol in our study. According to literature, *tet*M gene is the most prevalent tetracycline resistance genes among *S. pseudintermedius*. Our study is in agreement with other studies from literature as our study revealed that 12 (70.6 %) out of 17 tetracycline-resistant isolates harboured *tet*M gene. The respective accession numbers of the *tet*M DNA sequences for the 12 *S. pseudintermedius* isolates were deposited on the NCBI database. Our study is also in agreement with the work of Gharsa *et al*. (2013) who also reported *tet*M gene among the 40 tetracycline-resistant *S. pseudintermedius* isolates from dogs in their study. Our study also showed that none of the 17 chloramphenicol-resistant isolates from both

dogs and humans harboured *cfr* resistance gene. These chloramphenicol-resistant isolates might be habouring other types of chloramphenicol resistance genes such as $cat_{pC221.}$

Putative virulence factors found in *S. aureus* have been assumed to be present in *S. pseudintermedius*, although their role in disease has not been demonstrated (Fitzgerald, 2009; Ruzauskas *et al*., 2016; Somayaji *et al*., 2016; Pitchenin *et al*., 2017).

The leucocidin toxin (*luk*) secreted by *S. pseudintermedius* inactivates mononuclear cells and neutrophils (Hill & Imai, 2016). Exfoliative toxin (*exi*) digest the Dsg 1 protein in the canine skin causing acantholysis in canine skin (Iyori *et al*., 2011; Borjesson *et al*., 2015; Melter *et al*., 2017). *Sec*, *siet*, and *exi* virulence genes have been reported to be mostly implicated in toxic shock syndrome and dog pyoderma cases (Tanabe *et al*., 2013; Hill & Imai, 2016). Other Studies have also shown that these virulence genes are involved in the breaking down of the skin epidermis where they cause soft tissue and ear infections, as well as infections of other body tissues or cavities in canines (Iyori *et al*., 2011; Abraham *et al*., 2007; Somayaji *et al*., 2016; Melter *et al*., 2017).

Luk gene in *S. pseudintermedius* is similar in function to the Panton and Valentine Leucocidin (PVL) gene found in certain strains of *S. aureus* as these *luk* genes code for pore-forming leukotoxin that causes leukocyte destruction and tissue necrosis. *Luk* genes in *S. pseudintermedius* are also cytotoxic to various polymorphonuclear cells, monocytes, and macrophages, thus suppressing the host's cellular immunity (Futagawo-Saito *et al*., 2004a; Hill & Imai, 2016; Ruzauskas *et al*., 2016).

Ruzauskas *et al*. (2016) also reported *luk* virulence gene encoding leukotoxin in 29 % of *S. pseudintermedius* isolates in their study. However, this reported *luk* virulence gene prevalence

frequency by Ruzauskas *et al*. is lower than the 55.6 % prevalence frequency we observed in our study. Pitchenin *et al*. (2017), in Brazil, reported a higher prevalence frequency of 95 % for *luk* gene among the *S. pseudintermedius* isolates in their study. This frequency is higher than the 55.6 % frequency we observed in our study. Other studies involving *S. pseudintermedius* from dogs were also positive for *luk* genes (Garbacz *et al*., 2013; Gharsa *et al*., 2013; Matanovic *et al*., 2012; Pires dos Santos *et al*., 2016; Pitchenin *et al*., 2017). Interestingly, the *siet* gene, responsible for the production of an exfoliative toxin, mostly associated with skin infections in dogs were detected in our study with a high prevalence frequency of 62.6 %. Our study is in total concord with the 69 % prevalence frequency for *siet* gene reported in Lithuania by Ruzauskas in 2016. Pitchenin *et al*. also reported a frequency of 91 % for *siet* gene among the *S. pseudintermedius* isolates in their study in 2017. This reported prevalence frequency for *siet* gene is a bit higher than the 62.6 % frequency we observed for *siet* virulence gene in our study. The *siet* virulence gene prevalence frequency we observed in our study corroborates with the studies of canine *S. pseudintermedius* isolates in Korea (Terauchi *et al*., 2003), Poland (Garbacz *et al*., 2013), and Tunisia (Gharsa *et al*., 2013). Interestingly, most of the isolates that harboured *luk*D gene also harboured *siet* gene. This was also reported in Lithuania by Ruzauskas (2016). Borjesson *et al*. also reported in 2015 that all the 12 *S. pseudintermedius* isolates (100 %) in their study harboured *luk* and *siet* gene. This report is in agreement with our study except that we observed lesser frequency values of 55.6 % and 62.6 % for *luk*D and *siet* virulence genes respectively. In contrast, Borjesson *et al*. (2015) reported that only one isolate (8 %) out of the 12 isolates they molecularly characterized harboured *sec* gene. This frequency value of 1(8 %) observed by Borjesson *et al*. in 2015 does not completely agree with our study as the *S. pseudintermedius* isolates in our study possessed more of the *sec* virulence gene with the highest

prevalence frequency of 73(73.7 %) among all the 4 virulence genes that we molecularly characterized. There was no statistically significant difference in the prevalence frequencies among the 4 virulence genes; *sec*, *exi*, *siet*, and *luk*D [P = 0.628 (at $p < 0.05$).

The occurrence of virulence genes encoding toxins among the *S. pseduintermedius* isolates in our study is ubiquitous with the exception of *exi* gene which was present in just 2 % of the isolates. The high prevalence of *sec*, *siet*, and *luk*D virulence genes in *S. pseduintermedius* isolates obtained from dogs and humans in our study indicates that these toxin genes may play an important role in the pathogenesis of infection and could be very important in active infection. High prevalence of isolates positive for a significant number of toxin genes suggests that these toxins may play an important role in infection caused by *S. pseudintermedius*. Knowledge about the toxigenic profile of *S. pseudintermedius* strains will greatly help in understanding the pathogenesis of infection caused by this microbe. Interestingly, there was phenotypic homogeneity in the antibiotic resistance profiles of isolates obtained in each of the 69 households that were sampled. Similarity in antibiotic resistance profiles were also observed between human and dog isolates in each household, thus depicting a possible zoonotic transfer between the dogs and their owners.

5.2 Conclusion

This study is the first study that will be reporting *S. pseudintermedius* colonization in both dogs and humans (dog owners) in West Africa, including Nigeria. The *S. pseudintermedius* isolates from this study were more prevalent in dogs than among dog owners. Our study showed that humans who had no contact with dogs did not harbour *S. pseudintermedius*. MRSP strains were also prevalent among the *S. pseudintermedius* isolates from dogs and dog owners. Isolates from this study were multi-drug resistant and notably more resistant than those reported in literature.

The highest resistance antibiotype observed among the *S. pseudintermedius* isolates from dogs and humans in this study was resistance to 10 different antibiotics (Gentamycin, trimethoprim/sulfamethoxazole, erythromycin, oxacillin, ampicillin, penicillin, levofloxacin, ciprofloxacin, moxifloxacin, and clindamycin) which belong to 6 different antibiotic classes (aminoglycosides, sulfonamides, macrolides, beta-lactams, fluoroquinolones, and lincosamides).

Interestingly, isolates from both dogs and humans were completely susceptible to nitrofurantoin, rifampin, vancomycin, quinupristin/dalfopristin, daptomycin, linezolid, and amikacin which are considered drugs of last resort in the treatment of *S. pseudintermedius* infections. The average multiple antibiotic resistance index (MARI) value of the *S. pseudintermedius* isolates in our study was 0.3; thus further depicting multi-drug resistance traits. Our study also showed that *mec*A, *tet*M, *sec*, *siet*, *exi*, and *luk*D genes encoding various pathogenic factors were the genes harboured by the *S. pseudintermedius* isolates. The DNA sequences of the *tet*M gene for the 12 tetracycline-resistant *S. pseudintermedius* isolates in our study exhibited 99-100 % nucleotide similarity to the *tet*M reference nucleotide sequences of *Staphylococcus aureus* subsp. *aureus* in the GenBank database using a BLAST search on the NCBI server. There was phenotypic homogeneity in the antibiotic resistance profiles of isolates from both humans and dogs in each of the 69 households that were sampled, thus depicting a possible zoonotic transfer from dogs to their owners. Hence, appropriate hygienic measures such as hand washing after attending to dogs should be adopted. Monitoring of antimicrobial resistance in dogs should be performed routinely and should include control options to avoid the spread of resistance.

5.3 Recommendations

1. Proper hygienic practices are advised to reduce the risk of infection. Hygienic practices include proper hand washing after contact with pets, regular washing (in hot air with hot

air drying) of dogs' bodies and items that come in close contact with dogs, adequate cleaning and disinfection of the environment.

2. Monitoring of antimicrobial resistance in dogs should be performed routinely and must include control options such as judicious use of antibiotics in the treatment of MRSP infections to avoid the spread of resistance.
3. It is recommended that dogs diagnosed with or suspected with *S. pseudintermedius* infections should be isolated in order to minimize the risk of transmission.
4. It is therefore of utmost interest that proper sensitization, veterinary education on the recent taxonomical and resistance evolutions with regards to MRSP be done so as to avert health challenges associated with it.
5. To ensure prudent antimicrobial therapy, the importance of culture and antimicrobial susceptibility testing should be stressed to both veterinarians and physicians. This will greatly reduce the incidence of multi-drug resistant bacterial strains.
6. Further longitudinal studies in shelter dogs and among dog owners are required to assess the population diversity, antimicrobial resistance profiles and persistence of *S. pseudintermedius* especially, MRSP strains in dogs and dog owners or people who are in close contact with dogs.
7. High prevalence of isolates positive for a significant number of toxin genes suggests that these toxins may play an important role in infection caused by *S. pseudintermedius*. Knowledge about the toxigenic profile of *S. pseudintermedius* strains will greatly help in understanding the pathogenesis of infection caused by this microbe.
8. Relatively little is known about the epidemiology, frequency of zoonotic transmission, and antimicrobial resistance of *S. pseudintermedius* colonizing dogs. Continued

surveillance of antimicrobial resistance among staphylococci, including *S. pseudintermedius,* is required to actively monitor the emergence and dissemination of resistance among human and veterinary staphylococci.

9. Collaboration between veterinarians and human health professionals is essential to further our understanding of the ecology of this potential pathogen. This present study will provide a template for future research in West Africa.

5.4 Contribution to Knowledge

1. This is the first study in West Africa in which *mec*A, *tet*M, *sec*, *siet, exi,* and *luk*D genes encoding for various pathogenic factors will be isolated from *S. pseudintermedius* isolates obtained from dogs and dog owners.
2. The high prevalence of *sec, siet*, and *luk*D virulence genes in *S. pseduintermedius* isolates obtained from dogs and humans in our study indicates that these toxins may play an important role in the pathogenesis of infection and could be very important in active infection.
3. The interesting homogeneity in the antibiotic resistance phenotypes of *S. pseudintermedius* observed between dogs and dog owners in each household that was sampled suggest a possible zoonotic transfer between dogs and their owners.

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APPENDICES

APPENDIX I: Antibiotic classes and Minimum Inhibitory Concentration (MIC) break points for *Staphylococcus pseudintermedius*

S/N	Antibiotics	Antibiotic classes	MIC breakpoints
1	Penicillin (PEN)	Beta-lactams	≥ 0.25
2	Ampicillin (AMP)	Beta-lactams	≥ 0.5
3	Oxacillin (OXA)	Beta-lactams	≥ 0.5
4	Erythromycin (ERY)	Macrolides	≥ 8
5	Clindamycin (CLI)	Lincosamides	≥ 4
6	Tetracycline (TET)	Tetracyclines	≥ 16
7	Tigecycline (TGC)	Glycycline	≥ 0.5
8	Sulfamethoxazole/Trimethoprim (SXT)	Sulfonamides	≥ 4/76
9	Ciprofloxacin (CIP)	Fluoroquinolones	≥ 4
10	Levofloxacin (LEV)	Fluoroquinolones	≥ 4
11	Moxifloxacin (MFX)	Fluoroquinolones	≥ 2
12	Gentamycin (GEN)	Aminoglycosides	≥ 16
13	Chloramphenicol (CHL)	Amphenicols	≥ 32
14	Nitrofurantoin (NIT)	Nitrofurans	≥ 128
15	Rifampin (RIF)	Rifamycins	≥ 4
16	Vancomycin (VAN)	Glycopeptides	≥ 32
17	Linezolid (LZD)	Oxazolidinones	≥ 8
18	Daptomycin (DAP)	Cyclic peptides	≥ 1
19	Quinupristin/dalfopristin (SYN)	Streptogramins	≥ 4
20	Amikacin (AMK)	Aminoglycosides	≥ 64

APPENDIX II: Minimum Inhibitory Concentrations (MICs) of antibiotics tested on the *S. pseudintermedius* isolates from dogs

S/N	Isolate Code	Antibiotics (MICs)																		
		CHL	DAP	GEN	LZD	RIF	SXT	SYN	TET	ERY	OXA+	AMP	PEN	VAN	LEV	TGC	MXF	CLI	CIP	NIT	AMK
1	D001	16	≤ 0.5	16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.06	4	4	4	≤ 128	≤ 64
2	D002	16	≤ 0.5	≤ 2	4	≤ 0.5	1/19	≤ 0.5	>16	2	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
3	D003	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
4	D004	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
5	D005	32	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
6	D008	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	≤0.12	0.25	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
7	D009	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
8	D010	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
9	D011	32	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
10	D013	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
11	D014	8	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	≤ 2	0.5	≤0.25	0.5	1	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
12	D015	32	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	≤0.25	0.5	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
13	D017	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
14	D018	16	≤ 0.5	≤ 2	2	≤ 0.5	2/38	≤ 0.5	>16	8	0.5	> 8	> 8	1	>4	≤0.03	4	4	4	≤ 128	≤ 64
15	D020	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
16	D021	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
17	D022	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	8	> 8	0.5	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
18	D024	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
19	D025	16	≤ 0.5	≤ 2	4	≤ 0.5	4/76	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
20	D026	16	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
21	D027	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	8	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
22	D028	32	≤ 0.5	≤ 2	4	≤ 0.5	>4/76	≤ 0.5	>16	0.5	≤0.25	2	> 8	1	>4	0.5	>4	≤ 0.5	4	≤ 128	≤ 64
23	D029	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	≤0.12	≤0.06	1	≤0.25	0.5	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
24	D030	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	0.5	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
25	D031	8	≤ 0.5	16	2	≤ 0.5	>4/76	≤ 0.5	16	8	4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64

Key: CHL = Chloramphenicol, **DAP** = Daptomycin, **GEN** = Gentamycin, **LZD** = Linezolid, **RIF** = Rifampin, **SXT** = Trimethoprim/Sulfamethoxazole, **SYN** = Quinupristin/dalfopristin, **TET** = Tetracyline, **ERY** = Erythromycin, **OXA+** = Oxacillin + 2 % NaCl; **AMP** = Ampicillin, **PEN** = Penicillin, **VAN** = Vancomycin, **LEV** = Levofloxacin, **TGC** = Tigecycline; **MXF** = Moxifloxacin, **CLI** = Clindamycin, **CIP** = Ciprofloxacin, **NIT** = Nitrofurantoin, **AMK** = Amikacin

APPENDIX II (Contd..): Minimum Inhibitory Concentrations (MICs) of antibiotics tested on the *S. pseudintermedius* isolates from dogs

S/N	Isolate Code	Antibiotics (MICs)																		
		CHL	DAP	GEN	LZD	RIF	SXT	SYN	TET	ERY	OXA+	AMP	PEN	VAN	LEV	TGC	MXF	CLI	CIP	NIT	AMK
26	D032	16	≤ 0.5	8	2	≤ 0.5	4/76	≤ 0.5	≤ 2	8	>4	8	> 8	1	>4	0.12	>4	4	2	≤ 128	≤ 64
27	D033	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
28	D034	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
29	D035	8	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	>16	0.5	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
30	D036	32	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
31	D037	32	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	>16	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
32	D038	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
33	D039	16	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
34	D040	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
35	D041	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
36	D042	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
37	D043	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	≤0.25	2	0.5	0.25	1	1	0.12	0.5	1	≤ 1	≤ 128	≤ 64
38	D044	16	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	≤0.12	≤0.06	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
39	D045	32	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	≤0.12	≤0.06	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
40	D046	16	≤ 0.5	16	≤ 1	≤ 0.5	4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
41	D047	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
42	D048	16	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	1	2	1	2	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
43	D049	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	0.25	0.12	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
44	D050	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	0.5	2	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
45	D052	16	≤ 0.5	> 16	≤ 1	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
46	D053	32	≤ 0.5	16	4	≤ 0.5	>4/76	≤ 0.5	>16	8	>4	8	8	1	>4	>0.5	>4	4	4	≤ 128	≤ 64
47	D054	8	≤ 0.5	≤ 2	≤ 1	≤ 0.5	≤0.5/9.5	≤ 0.5	>16	0.5	≤0.25	8	8	2	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
48	D055	32	≤ 0.5	16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.5	>4	4	4	≤ 128	≤ 64
49	D056	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
50	D057	16	≤ 0.5	16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64

Key: CHL = Chloramphenicol, **DAP** = Daptomycin, **GEN** = Gentamycin, **LZD** = Linezolid, **RIF** = Rifampin, **SXT** = Trimethoprim/Sulfamethoxazole, **SYN** = Quinupristin/dalfopristin, **TET** = Tetracyline, **ERY** = Erythromycin, **OXA+** = Oxacillin + 2 % NaCl; **AMP** = Ampicillin, **PEN** = Penicillin, **VAN** = Vancomycin, **LEV** = Levofloxacin, **TGC** = Tigecycline; **MXF** = Moxifloxacin, **CLI** = Clindamycin, **CIP** = Ciprofloxacin, **NIT** = Nitrofurantoin, **AMK** = Amikacin

APPENDIX II (Contd..): Minimum Inhibitory Concentrations (MICs) of antibiotics tested on the *S. pseudintermedius* isolates from dogs

S/N	Isolate Code	Antibiotics (MICs)																	
		CHL	DAP	GEN	LZD	RIF	SXT	SYN	TET	ERY	OXA+	AMP	PEN	VAN	LEV	TGC	MXF	CLI	CIP	NIT	AMK
51	D058	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
52	D059	16	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
53	D060	32	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
54	D061	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
55	D062	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
56	D063	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	4	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
57	D065	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	2	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
58	D066	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
59	D068	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
60	D069	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
61	D070	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
62	D071	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
63	D072	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	>16	0.5	≤0.25	8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
64	D073	16	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
65	D075	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
66	D076	8	≤ 0.5	≤ 2	4	≤ 0.5	1/19	≤ 0.5	≤ 2	0.5	≤0.25	1	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
67	D077	8	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
68	D078	8	≤ 0.5	≤ 2	4	≤ 0.5	1/19	≤ 0.5	>16	0.5	≤0.25	> 8	> 8	1	0.5	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
69	D080	32	≤ 0.5	8	2	≤ 0.5	≤0.5/9.5	≤ 0.5	>16	8	>4	8	8	0.5	2	0.12	0.5	4	2	≤ 128	≤ 64
70	D081	8	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	>16	0.5	≤0.25	8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
71	D083	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	≤0.25	≤0.25	4	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
72	D084	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
73	D085	16	≤ 0.5	8	4	≤ 0.5	>4/76	≤ 0.5	>16	8	>4	> 8	> 8	1	>4	0.25	>4	4	4	≤ 128	≤ 64
74	D086	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	>16	0.5	≤0.25	8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
75	D087	32	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64

Key: CHL = Chloramphenicol, **DAP** = Daptomycin, **GEN** = Gentamycin, **LZD** = Linezolid, **RIF** = Rifampin, **SXT** = Trimethoprim/Sulfamethoxazole, **SYN** = Quinupristin/dalfopristin, **TET** = Tetracyline, **ERY** = Erythromycin, **OXA+** = Oxacillin + 2 % NaCl; **AMP** = Ampicillin, **PEN** = Penicillin, **VAN** = Vancomycin, **LEV** = Levofloxacin, **TGC** = Tigecycline; **MXF** = Moxifloxacin, **CLI** = Clindamycin, **CIP** = Ciprofloxacin, **NIT** = Nitrofurantoin, **AMK** = Amikacin

APPENDIX II (Contd..): Minimum Inhibitory Concentrations (MICs) of antibiotics tested on the *S. pseudintermedius* isolates from dogs

S/N	Isolate Code	Antibiotics (MICs)																		
		CHL	DAP	GEN	LZD	RIF	SXT	SYN	TET	ERY	OXA+	AMP	PEN	VAN	LEV	TGC	MXF	CLI	CIP	NIT	AMK
76	D088	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
77	D089	8	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	2	≤0.25	0.5	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
78	D090	16	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	>16	0.5	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
79	D091	8	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	4	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
80	D093	32	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	>16	8	>4	> 8	> 8	0.5	>4	0.25	4	4	4	≤ 128	≤ 64
81	D094	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
82	D095	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	4	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
83	D096	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
84	D097	32	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	>16	0.5	≤0.25	> 8	> 8	1	≤0.25	0.5	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
85	D098	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
86	D099	16	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64

Key: CHL = Chloramphenicol, **DAP** = Daptomycin, **GEN** = Gentamycin, **LZD** = Linezolid, **RIF** = Rifampin, **SXT** = Trimethoprim/Sulfamethoxazole, **SYN** = Quinupristin/dalfopristin, **TET** = Tetracyline, **ERY** = Erythromycin, **OXA+** = Oxacillin + 2 % NaCl; **AMP** = Ampicillin, **PEN** = Penicillin, **VAN** = Vancomycin, **LEV** = Levofloxacin, **TGC** = Tigecycline; **MXF** = Moxifloxacin, **CLI** = Clindamycin, **CIP** = Ciprofloxacin, **NIT** = Nitrofurantoin, **AMK** = Amikacin

APPENDIX III: Minimum Inhibitory Concentrations (MICs) of antibiotics tested on *S. pseudintermedius* isolates from humans

S/N	Isolate Code	Antibiotics (MICs)																		
		CHL	DAP	GEN	LZD	RIF	SXT	SYN	TET	ERY	OXA+	AMP	PEN	VAN	LEV	TGC	MXF	CLI	CIP	NIT	AMK
1	H051	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	0.5	0.12	≤0.25	≤ 0.5	2	≤ 128	≤ 64
2	H064	32	≤ 0.5	≤ 2	4	≤ 0.5	2/38	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
3	H079	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
4	H100	32	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	≤0.25	0.06	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
5	H101	32	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
6	H102	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.25	4	4	4	≤ 128	≤ 64
7	H103	16	≤ 0.5	≤ 2	2	≤ 0.5	1/19	≤ 0.5	≤ 2	1	≤0.25	> 8	> 8	1	≤0.25	0.12	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
8	H104	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	8	>8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
9	H105	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
10	H106	16	≤ 0.5	> 16	4	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	≤0.25	>4	0.25	4	4	4	≤ 128	≤ 64
11	H107	16	≤ 0.5	> 16	2	≤ 0.5	>4/76	≤ 0.5	≤ 2	8	>4	> 8	> 8	1	>4	0.12	4	4	4	≤ 128	≤ 64
12	H108	16	≤ 0.5	≤ 2	4	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	1	≤0.25	0.25	0.25	1	≤0.25	0.06	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64
13	H109	8	≤ 0.5	≤ 2	2	≤ 0.5	≤0.5/9.5	≤ 0.5	≤ 2	0.5	≤0.25	> 8	> 8	1	≤0.25	0.25	≤0.25	≤ 0.5	≤ 1	≤ 128	≤ 64

Key: CHL = Chloramphenicol, **DAP** = Daptomycin, **GEN** = Gentamycin, **LZD** = Linezolid, **RIF** = Rifampin, **SXT** = Trimethoprim/Sulfamethoxazole, **SYN** = Quinupristin/dalfopristin, **TET** = Tetracyline, **ERY** = Erythromycin, **OXA+** = Oxacillin + 2 % NaCl; **AMP** = Ampicillin, **PEN** = Penicillin, **VAN** = Vancomycin, **LEV** = Levofloxacin, **TGC** = Tigecycline; **MXF** = Moxifloxacin, **CLI** = Clindamycin, **CIP** = Ciprofloxacin, **NIT** = Nitrofurantoin, **AMK** = Amikacin

APPENDIX IV: Multiple Antibiotic Resistance Index (MARI) values of *Staphylococcus pseudintermedius* isolates

S/N	Isolate code	MARI value	S/N	Isolate Code	MARI value	S/N	Isolate code	MARI value	S/N	Isolate code	MARI value
1	D001	0.5	26	D032	0.4	51	D058	0.2	76	D088	0.5
2	D002	0.2	27	D033	0.5	52	D059	0.2	77	D089	0.2
3	D003	0.5	28	D034	0.5	53	D060	0.2	78	D090	0.2
4	D004	0.5	29	D035	0.1	54	D061	0.5	79	D091	0.1
5	D005	0.2	30	D036	0.6	55	D062	0.5	80	D093	0.6
6	D008	0.1	31	D037	0.6	56	D063	0.2	81	D094	0.5
7	D009	0.5	32	D038	0.5	57	D065	0.2	82	D095	0.1
8	D010	0.1	33	D039	0.1	58	D066	0.5	83	D096	0.5
9	D011	0.2	34	D040	0.5	59	D068	0.5	84	D097	0.3
10	D013	0.5	35	D041	0.5	60	D069	0.5	85	D098	0.5
11	D014	0.1	36	D042	0.5	61	D070	0.5	86	D099	0.1
12	D015	0.2	37	D043	0.2	62	D071	0.1	**Average MARI(Dogs) = 0.3**		
13	D017	0.5	38	D044	0	63	D072	0.2	**HUMANS (Dog owners)**		
14	D018	0.5	39	D045	0.1	64	D073	0.1	1	H051	0.2
15	D020	0.5	40	D046	0.5	65	D075	0.5	2	H064	0.2
16	D021	0.5	41	D047	0.5	66	D076	0.1	3	H079	0.5
17	D022	0.1	42	D048	0.2	67	D077	0.1	4	H100	0.2
18	D024	0.5	43	D049	0	68	D078	0.2	5	H101	0.6
19	D025	0.2	44	D050	0.2	69	D080	0.4	6	H102	0.5
20	D026	0.1	45	D052	0.5	70	D081	0.2	7	H103	0.1
21	D027	0.5	46	D053	0.6	71	D083	0.1	8	H104	0.2
22	D028	0.5	47	D054	0.2	72	D084	0.6	9	H105	0.5
23	D029	0.1	48	D055	0.6	73	D085	0.5	10	H106	0.5
24	D030	0.1	49	D056	0.5	74	D086	0.2	11	H107	0.5
25	D031	0.6	50	D057	0.5	75	D087	0.6	12	H108	0.1
									13	H109	0.1
									Average MARI (Humans) = 0.3		

Average MARI value for all the *S. pseudintermedius* isolates = 0.3

APPENDIX V (STATISTICAL ANALYSIS RESULTS)

```
T-TEST
  /TESTVAL=0
  /MISSING=ANALYSIS
  /VARIABLES=S_pseud
  /CRITERIA=CI(.95).
```

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
S_pseud	3	2.0000	1.00000	.57735

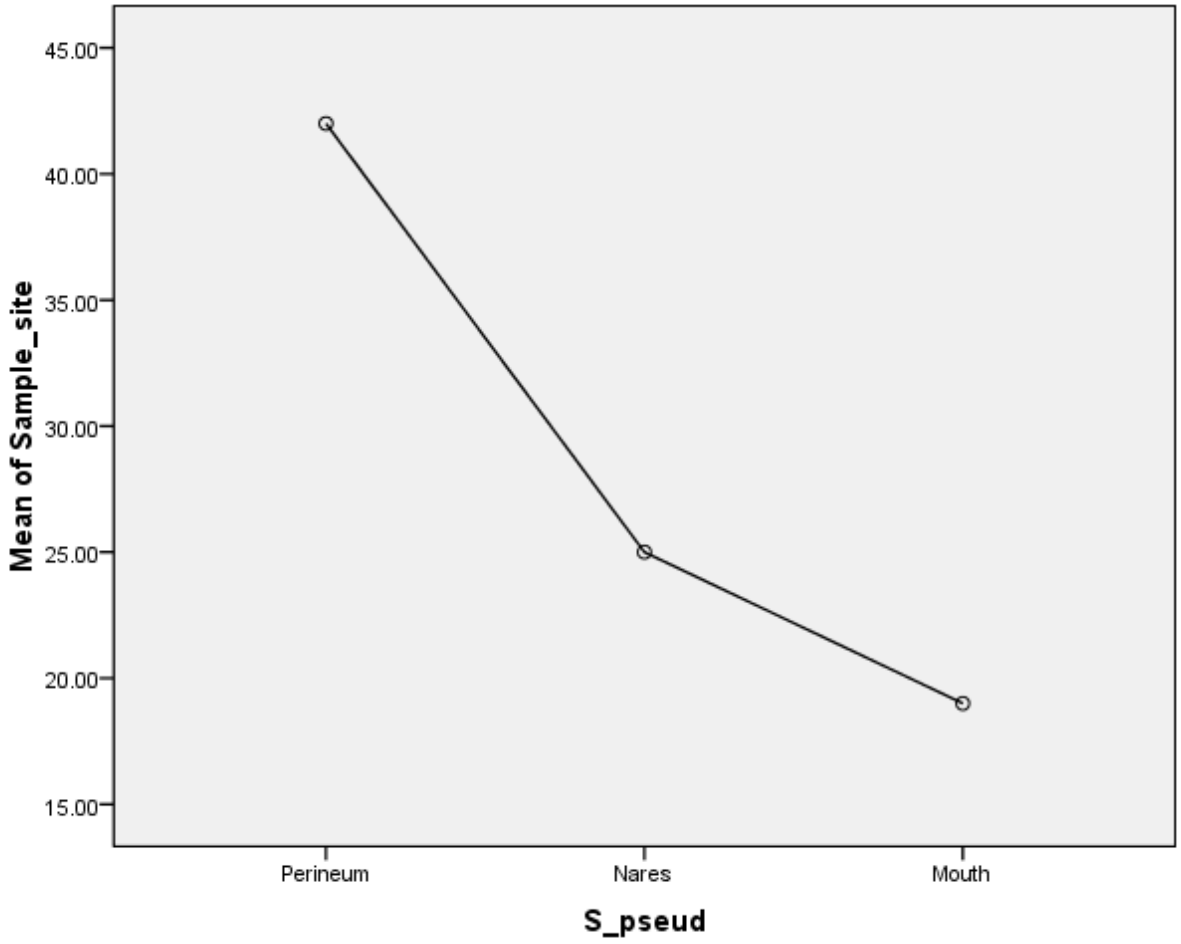
One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
S_pseud	3.464	2	.074	2.00000	-.4841	4.4841

mean = 2.000, SD = 1.000, t = 3.44, df = 2, p = 0.074

One-way ANOVA

Means Plots



APPENDIX VI

```
T-TEST
/TESTVAL=0
/MISSING=ANALYSIS
/VARIABLES=Resistance
/CRITERIA=CI(.95).
```

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Resistance	13	49.7308	24.87929	6.90027

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Resistance	7.207	12	.000	49.73077	34.6964	64.7652

mean = 49.731, SD = 24.880), t = 7.207, df = 12, p = 0.000

APPENDIX VII

```
T-TEST
/TESTVAL=0
/MISSING=ANALYSIS
/VARIABLES=Resistance
/CRITERIA=CI(.95).
```

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Resistance	11	53.1818	22.39879	6.75349

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Resistance	7.875	10	.000	53.18182	38.1341	68.2295

mean = 53.1818, SD = 22.399), t = 7.875, df = 10, p = 0.000

APPENDIX VIII

```
T-TEST
GROUPS=S_pseud (1 2)
/MISSING=ANALYSIS
/VARIABLES=Resistance
/CRITERIA=CI(.95).
```

T-Test

Group Statistics

	S_pseud	N	Mean	Std. Deviation	Std. Error Mean
Resistance	Resistance_dogs	13	49.7308	24.87929	6.90027
	Resistance_Humans	11	53.1818	22.39879	6.75349

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Resistance	Equal variances assumed	.000	.998	-.354	22	.727	-3.45105	9.74362	-23.65808	16.75598
	Equal variances not assumed			-.357	21.894	.724	-3.45105	9.65523	-23.48040	16.57830

S. pseudintermedius Dogs: mean = 49.731, SD = 24.880)

S. pseudintermedius from Humans: mean = 53.182, SD = 22.399

t = -0.354, df = 22, p = 0.727

APPENDIX IX

```
ONEWAY Resistance BY S_pseud
/STATISTICS DESCRIPTIVES
/PLOT MEANS
/MISSING ANALYSIS.
```

Oneway

Descriptives

Resistance

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Resistance_dogs	13	49.7308	24.87929	6.90027	34.6964	64.7652	8.10	95.30
Resistance_Humans	11	53.1818	22.39879	6.75349	38.1341	68.2295	23.10	100.00
Total	24	51.3125	23.32732	4.76167	41.4622	61.1628	8.10	100.00

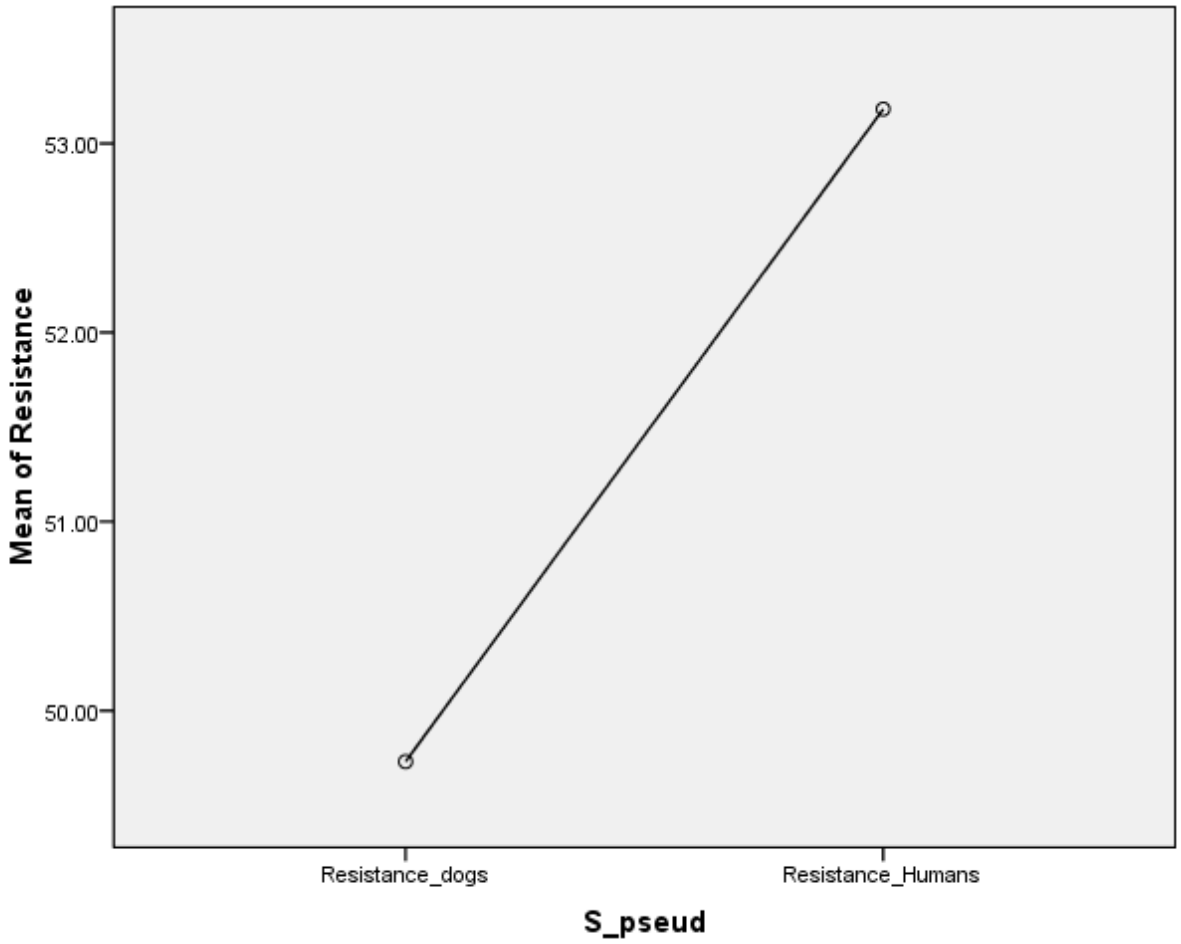
ANOVA

Resistance

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	70.962	1	70.962	.125	.727
Within Groups	12444.804	22	565.673		
Total	12515.766	23			

F (1, 22) = 0.125, P = 0.727

Means Plots


Mean of Resistance
53.00
52.00
51.00
50.00
Resistance_dogs
Resistance_Humans
S_pseud

APPENDIX X

```
T-TEST
/TESTVAL=0
/MISSING=ANALYSIS
/VARIABLES=Prevalence
/CRITERIA=CI(.95).
```

T-Test

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Prevalence	2	26.0000	28.28427	20.00000

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Prevalence	1.300	1	.417	26.00000	-228.1241	280.1241

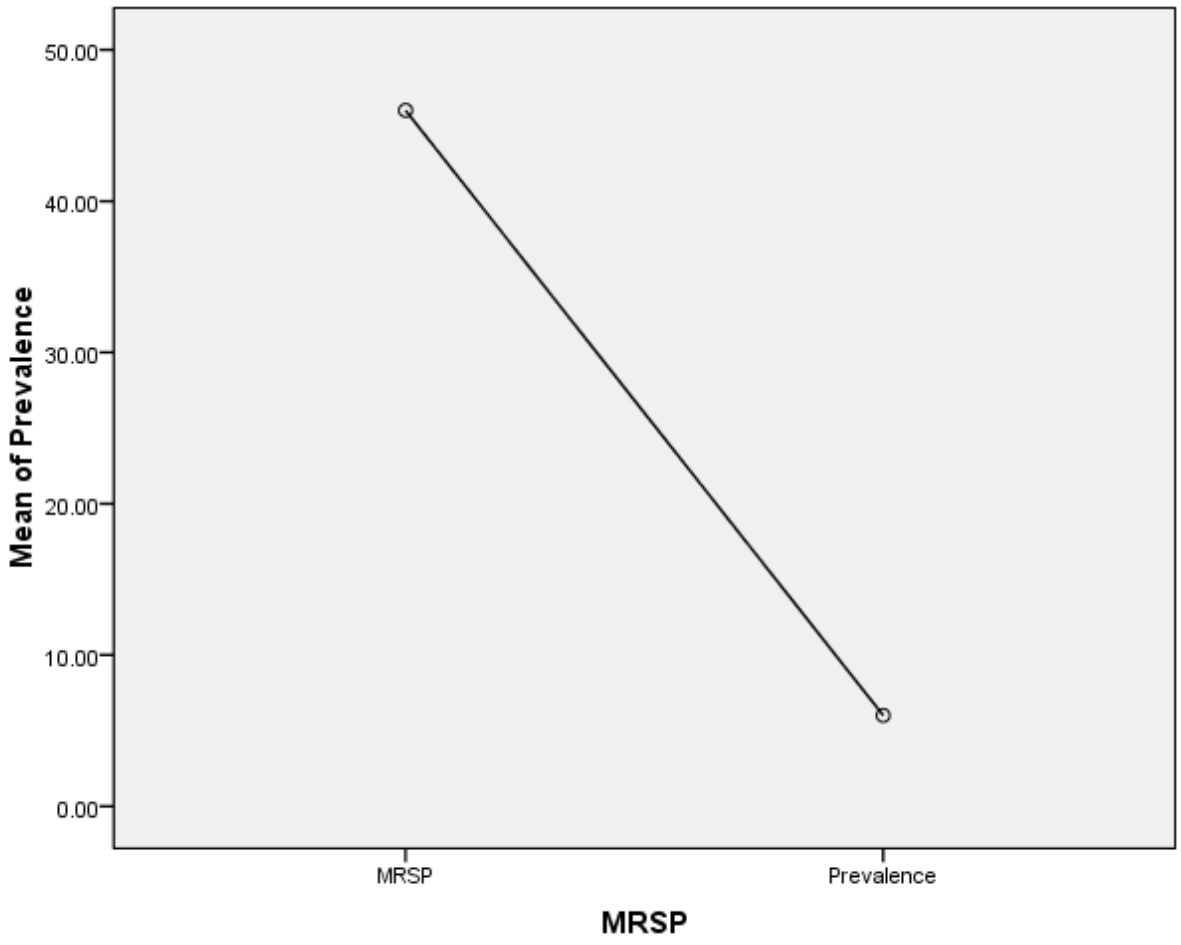
mean = 26.000, SD = 28.284, t = 1.300, df = 1, p = 0.417

APPENDIX XI

```
ONEWAY Prevalence BY MRSP
  /STATISTICS DESCRIPTIVES
  /PLOT MEANS
  /MISSING ANALYSIS.
```

Oneway

Means Plots



APPENDIX XII

```
ONEWAY Virulence_gene BY S_pseud
  /STATISTICS DESCRIPTIVES
  /PLOT MEANS
  /MISSING ANALYSIS
  /POSTHOC=TUKEY ALPHA(0.05).
```

Oneway

Descriptives

Virulence_gene

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Sec	2	36.5000	36.06245	25.50000	-287.5082	360.5082	11.00	62.00
Exi	2	1.0000	.00000	.00000	1.0000	1.0000	1.00	1.00
Siet	2	31.0000	31.11270	22.00000	-248.5365	310.5365	9.00	53.00
LukD	2	27.5000	28.99138	20.50000	-232.9772	287.9772	7.00	48.00
Total	8	24.0000	25.64037	9.06524	2.5641	45.4359	1.00	62.00

ANOVA

Virulence_gene

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1493.000	3	497.667	.640	.628
Within Groups	3109.000	4	777.250		
Total	4602.000	7			

F (3, 4) = 0.640, P = 0.628

Post Hoc Tests

Multiple Comparisons

Virulence_gene

Tukey HSD

(I) S_pseud	(J) S_pseud	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Sec	Exi	35.50000	27.87920	.621	-77.9922	148.9922
	Siet	5.50000	27.87920	.997	-107.9922	118.9922
	LukD	9.00000	27.87920	.987	-104.4922	122.4922
Exi	Sec	-35.50000	27.87920	.621	-148.9922	77.9922
	Siet	-30.00000	27.87920	.721	-143.4922	83.4922
	LukD	-26.50000	27.87920	.783	-139.9922	86.9922
Siet	Sec	-5.50000	27.87920	.997	-118.9922	107.9922
	Exi	30.00000	27.87920	.721	-83.4922	143.4922
	LukD	3.50000	27.87920	.999	-109.9922	116.9922
LukD	Sec	-9.00000	27.87920	.987	-122.4922	104.4922
	Exi	26.50000	27.87920	.783	-86.9922	139.9922
	Siet	-3.50000	27.87920	.999	-116.9922	109.9922

Homogeneous Subsets

Virulence_genes

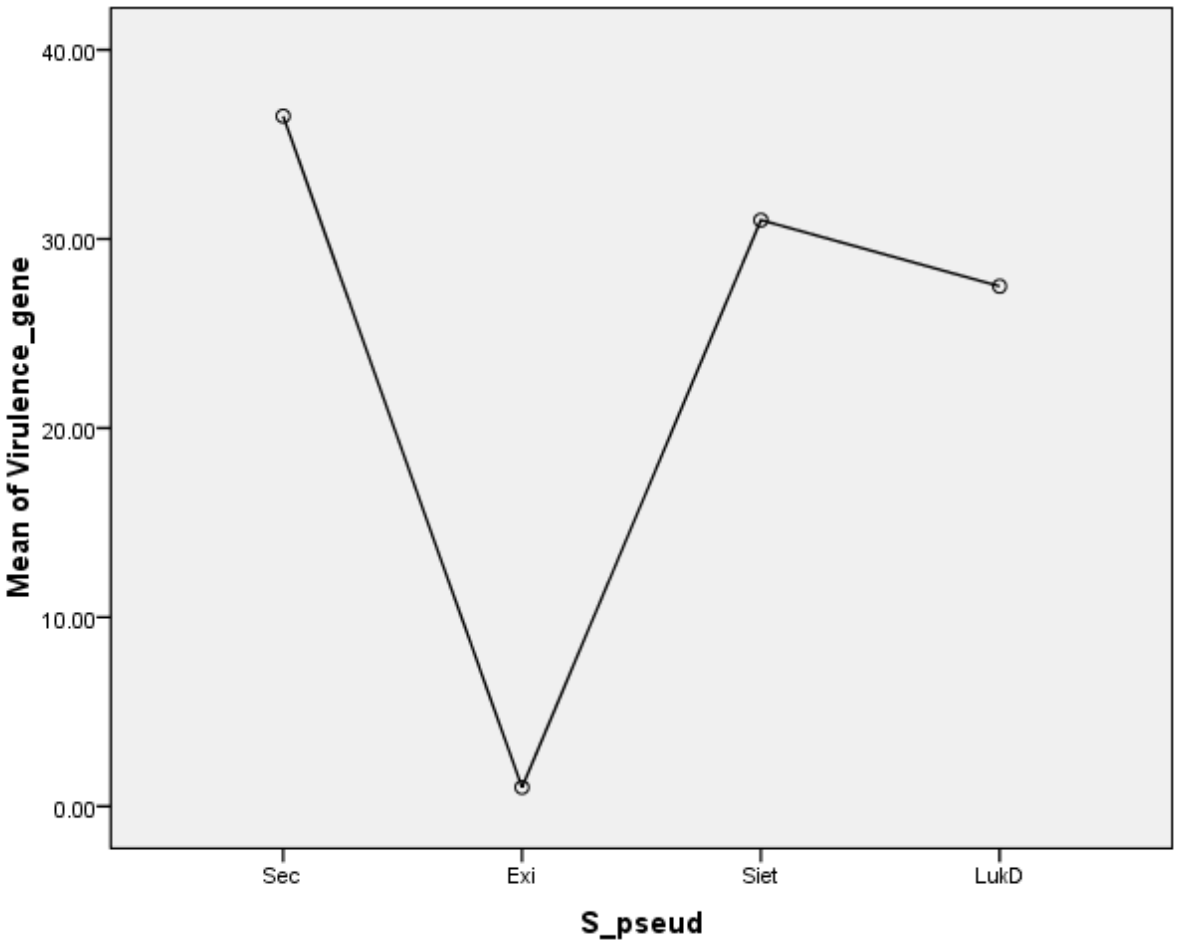
Tukey HSD[a]

S_pseud	N	Subset for alpha = 0.05
		1
Exi	2	1.0000
LukD	2	27.5000
Siet	2	31.0000
Sec	2	36.5000
Sig.		.621

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Means Plots


40.00
30.00
20.00
10.00
0.00
Mean of Virulence_gene
Sec
Exi
Siet
LukD
S_pseud

APPENDIX XIII: Cultural morphology of *S. pseudintermedius* on Columbia sheep blood agar

Appendix XIV: **Prevalence of *S. pseudintermedius* isolates in dogs based on sample site**

Sample site	Total number of dog sampled	Total number of *S. pseudintermedius* isolates obtained n (%)	Isolate codes
Perineum	45	42 (93.3 %)	D001, D002, D003, D004, D005, D008, D009, D010, D011, D014, D015, D018, D020, D022, D024, D025, D026, D028, D029, D030, D033, D034, D035, D039, D041, D042, D043, D044, D045, D046, D048, D052, D053, D055, D057, D059, D061, D070, D075, D080, D085, D087
Nares	35	25 (71.4 %)	D017, D021, D027, D031, D032, D049, D050, D054, D056, D058, D062, D066, D068, D069, D071, D072, D073, D076, D077, D078, D081, D088, D093, D096, D097
Mouth	32	19 (59.4 %)	D013, D036, D037, D038, D040, D047, D060, D063, D065, D083, D084, D086, D089, D090, D091, D094, D095, D098, D099
Total	112	86 (76.8 %)	

Key: D = Dog isolate

Appendix XV: Prevalence of *S. pseudintermedius* isolates obtained from the nasal swabs of humans (dog owners)

Total number of dog owners sampled	Total number of *S. pseudintermedius* isolates obtained n (%)	Isolate codes
97	13 (13.4 %)	H051, H064, H079, H100, H101, H102, H103, H104, H105, H106, H107, H108, H109

Key: H = Human isolate

Appendix XVI: Prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) isolates among dog and humans (dog owners) isolates

Sample source	Total number of MRSP isolates	Isolate codes
Dogs	46	D001, D003, D004, D009, D013, D017, D018, D020, D021, D024, D027, D031, D032, D033, D034, D036, D037, D038, D040, D041, D042, D043, D046, D047, D048, D052, D053, D055, D056, D057, D061, D062, D066, D068, D069, D070, D075, D080, D084, D085, D087, D088, D093, D094, D096, D098
Humans (Dog owners)	6	H079, H101, H102, H105, H106, H107
Total	52 (52.5 %)	

Key: D = Dog isolate; H = Human isolate

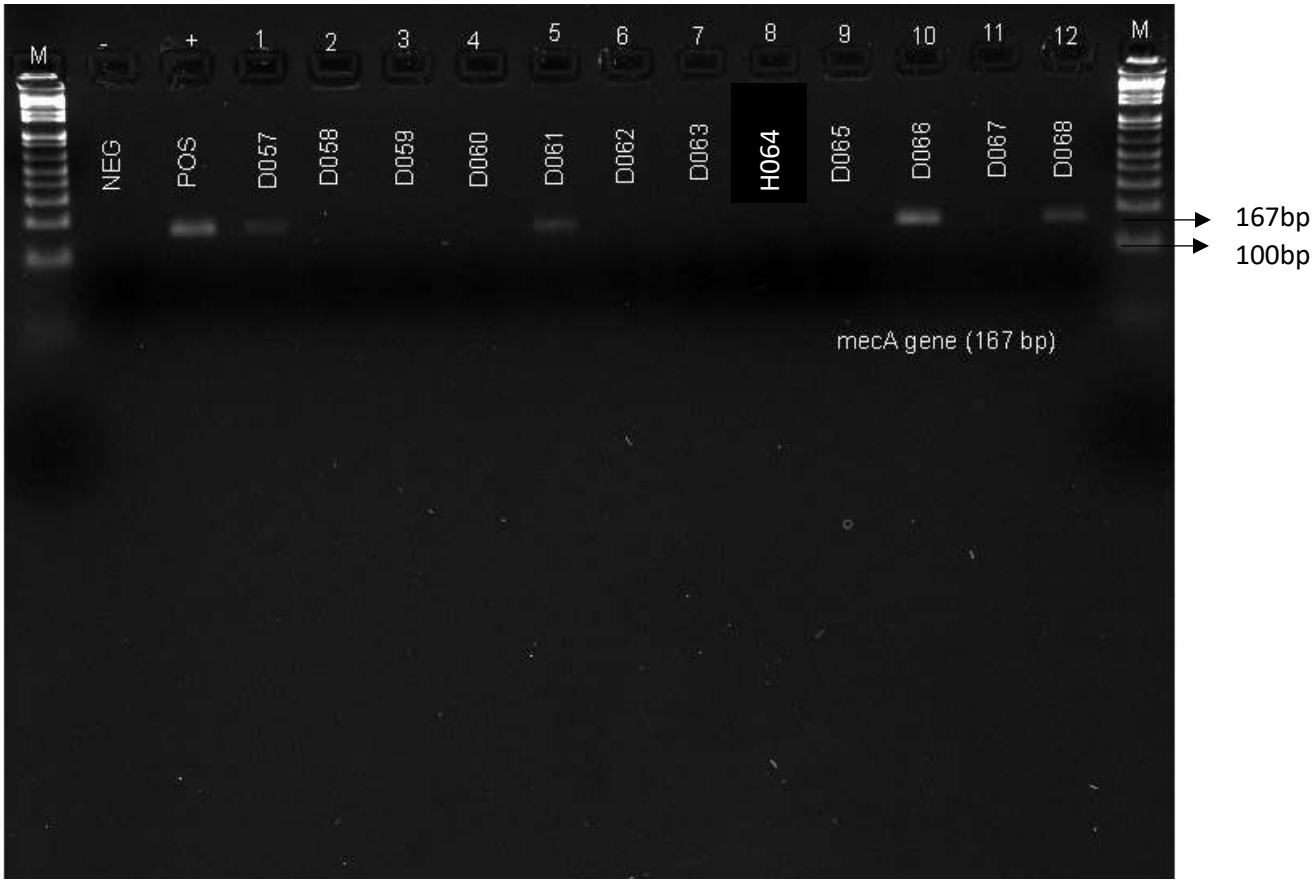
Appendix XVII: Prevalence of virulence genes among *S. pseudintermedius* isolates from dogs and humans (Dog owners)

Virulence gene	Isolate source	Total number of positive isolates n (%)	Isolate codes
Sec	Dogs	62 (84.9 %)	D001, D002, D003, D005, D008, D009, D010, D011, D014,D015, D018, D026, D035, D037, D033, D039, D040, D041, D042, D043, D044, D046, D047, D048, D049, D050, D053, D054, D055, D056, D057, D058, D059, D060, D061, D062, D063, D065, D066, D068, D069, D070, D071, D072, D073, D075, D076, D077, D078, D081, D083, D084, D085, D086, D087, D088, D089, D090, D091, D095, D096, D097
	Humans	11(15.1 %)	H051, H064, H079, H100, H101, H102, H103, H104, H107, H108, H109
Exi	Dogs	1 (50 %)	D014
	Humans	1 (50 %)	H103
Siet	Dogs	53 (85.5 %)	D002, D004, D005, D008, D010, D011, D013, D014, D018, D020, D022, D025, D026, D027, D028, D029, D030, D031, D032, D035, D039, D043, D044, D046, D049, D050, D053, D054, D055, D057, D058, D059, D060, D063, D065, D071, D072, D073, D076, D077, D078, D081, D083, D084, D085, D086, D088, D090, D091, D095, D097, D098, D098
	Humans	9 (14.5 %)	H051, H064, H100, H101, H103, H104, H106, H108, H109
LukD	Dogs	48 (87.3 %)	D002, D004, D005, D008, D009, D010, D011, D014, D015, D018, D022, D024, D025, D026, D028, D029, D030, D031, D032, D035, D039, D044, D046, D048, D049, D050, D053, D054, D055, D058, D059, D060, D063, D065, D069, D070, D071, D072, D073, D076, D077, D083, D085, D086, D089, D090, D091, D095
	Humans	7 (12.7 %)	H051, H064, H100, H103, H104, H108, H109

Key: D = Dog isolate; H = Human isolate

Appendix XVIII: PCR amplification gel of *mec*A gene (167 bp) among the *S. pseudintermedius* isolates

***mec*A (167 bp)**

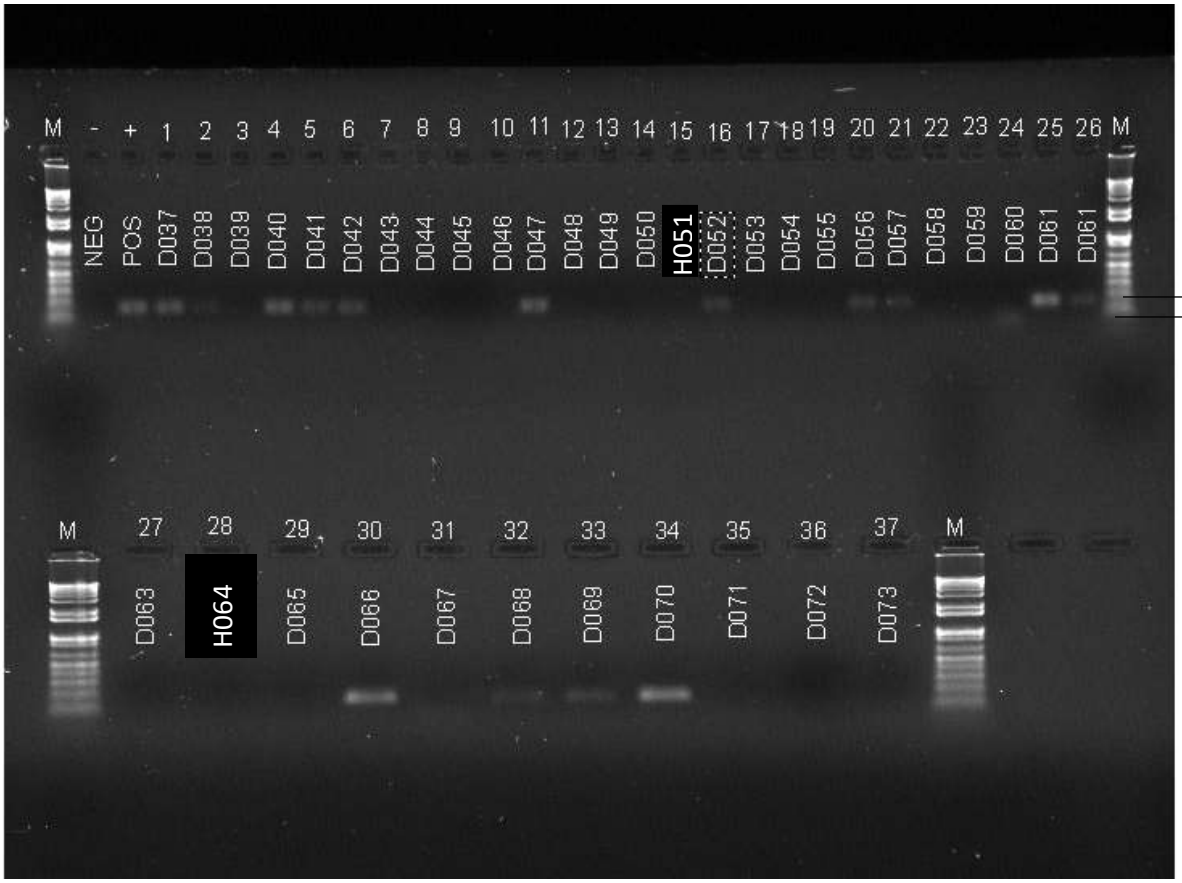


PCR amplification gel of *mec*A (167 bp) among the *S. pseudintermedius* isolates (lanes 1-12)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MRSP 24 (positive control).

Lanes 1, 5, 10, and 12 were positive for ***mec*A gene.**

***mec*A (167 bp)**

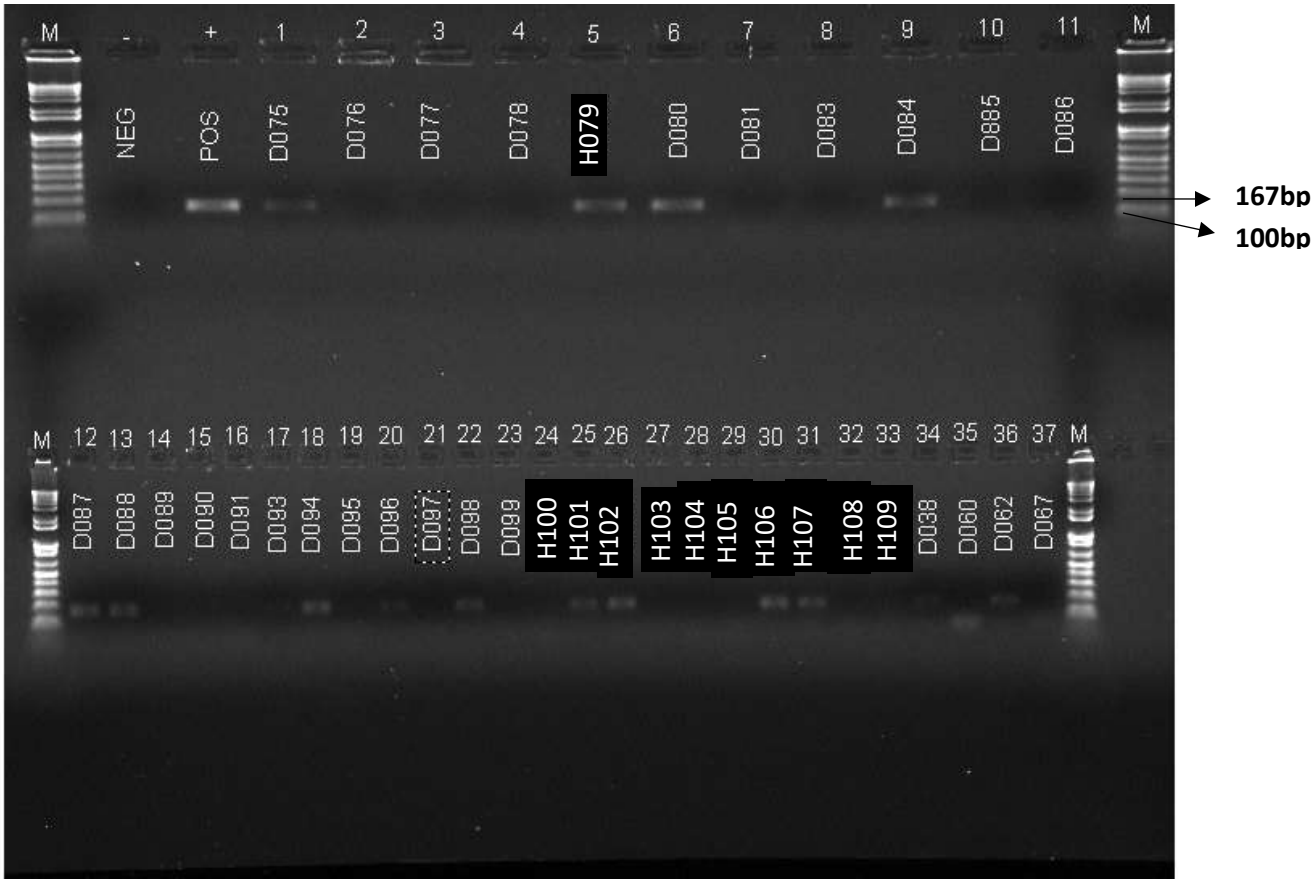


PCR amplification gel of *mec*A (167 bp) among the *S. pseudintermedius* isolates (lanes 1-37)

Lane M=100 bp marker; - = negative control without DNA; + = *S. pseudintermedius* MRSP 24 (positive control).

Lanes 1, 2, 4, 5, 6, 11, 16, 20, 21, 25, 26, 30, 32, 33, and 34 were positive for ***mec*A gene.**

***mec*A (167 bp)**



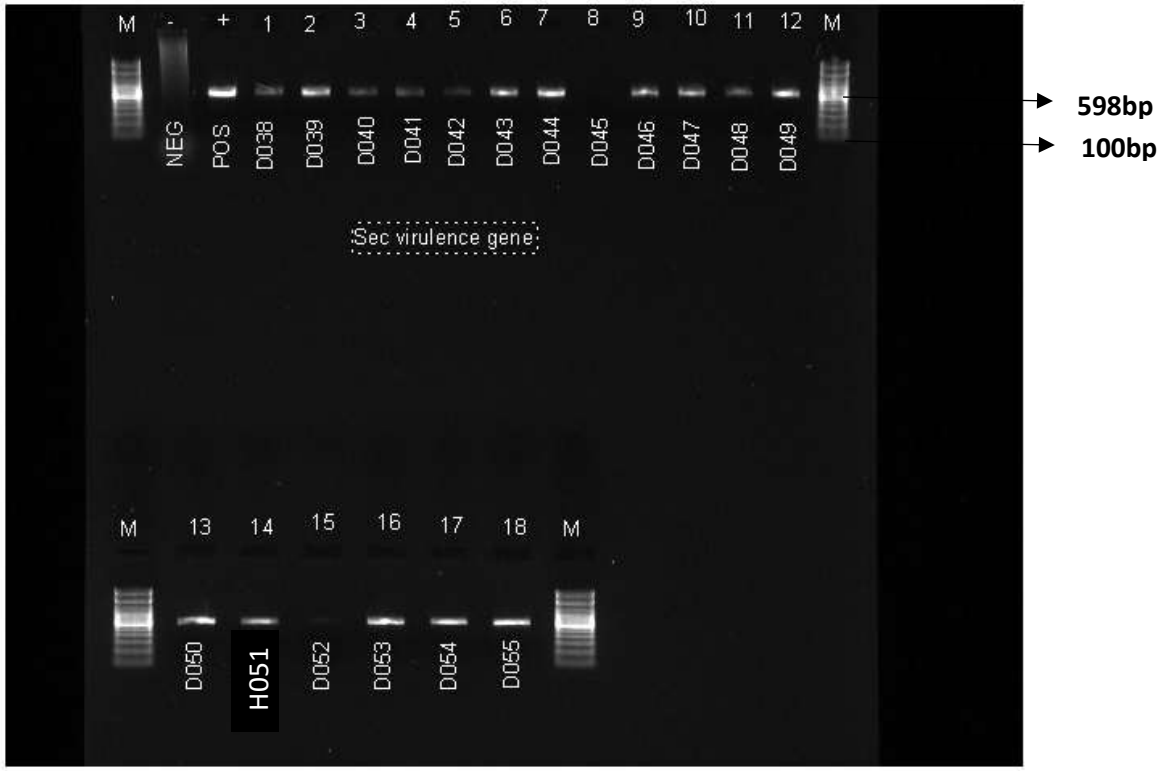
PCR amplification gel of *mec*A (167 bp) among the *S. pseudintermedius* isolates (lanes 1-37)

Lane M=100 bp marker; - = negative control without DNA; + = *S. pseudintermedius* MRSP 24 (positive control).

Lanes 1, 5, 6, 9, 12, 13, 17, 18, 20, 22, 25, 26, 30, 31, 34, and 36 were positive for ***mec*A gene.**

Appendix XIX: PCR amplification gel of *sec* virulence gene (598 bp) among the *S. pseudintermedius* isolates

***sec* virulence gene (598 bp)**

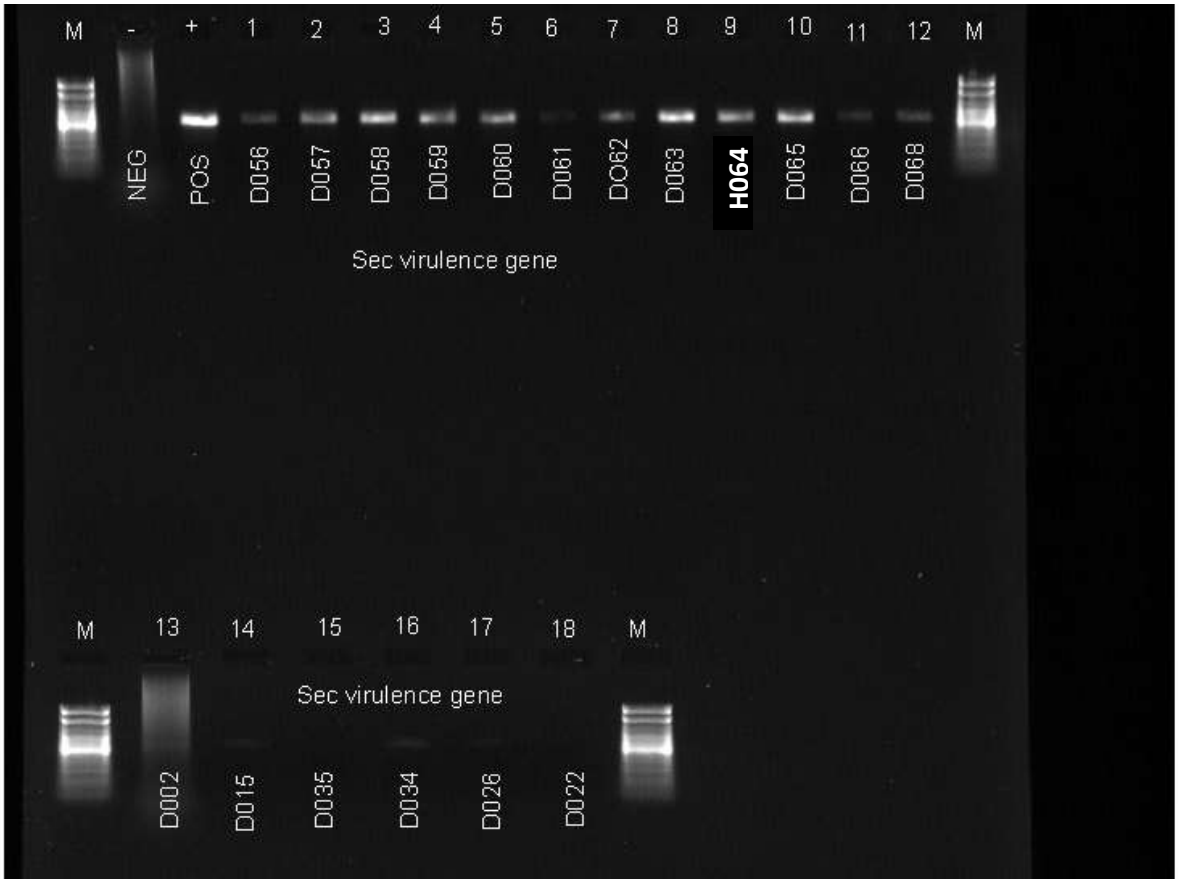


PCR amplification gel of *sec* virulence gene (598 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SP31 (positive control).

Lanes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, and 18 were positive for ***sec* virulence gene.**

***sec* virulence gene (598 bp)**

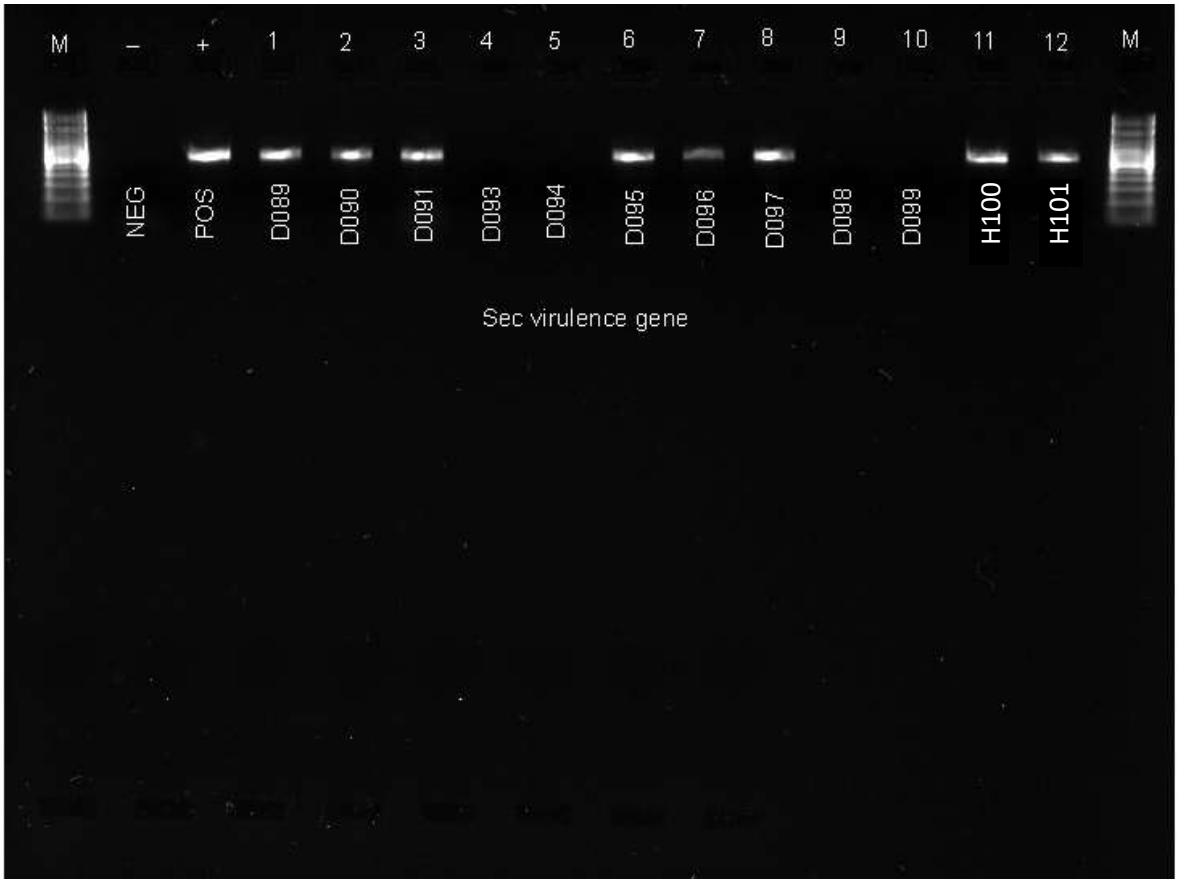


PCR amplification gel of *sec* virulence gene (598 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SP31 (positive control).

Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, and 17 were positive for ***sec* virulence gene.**

***sec* virulence gene (598 bp)**

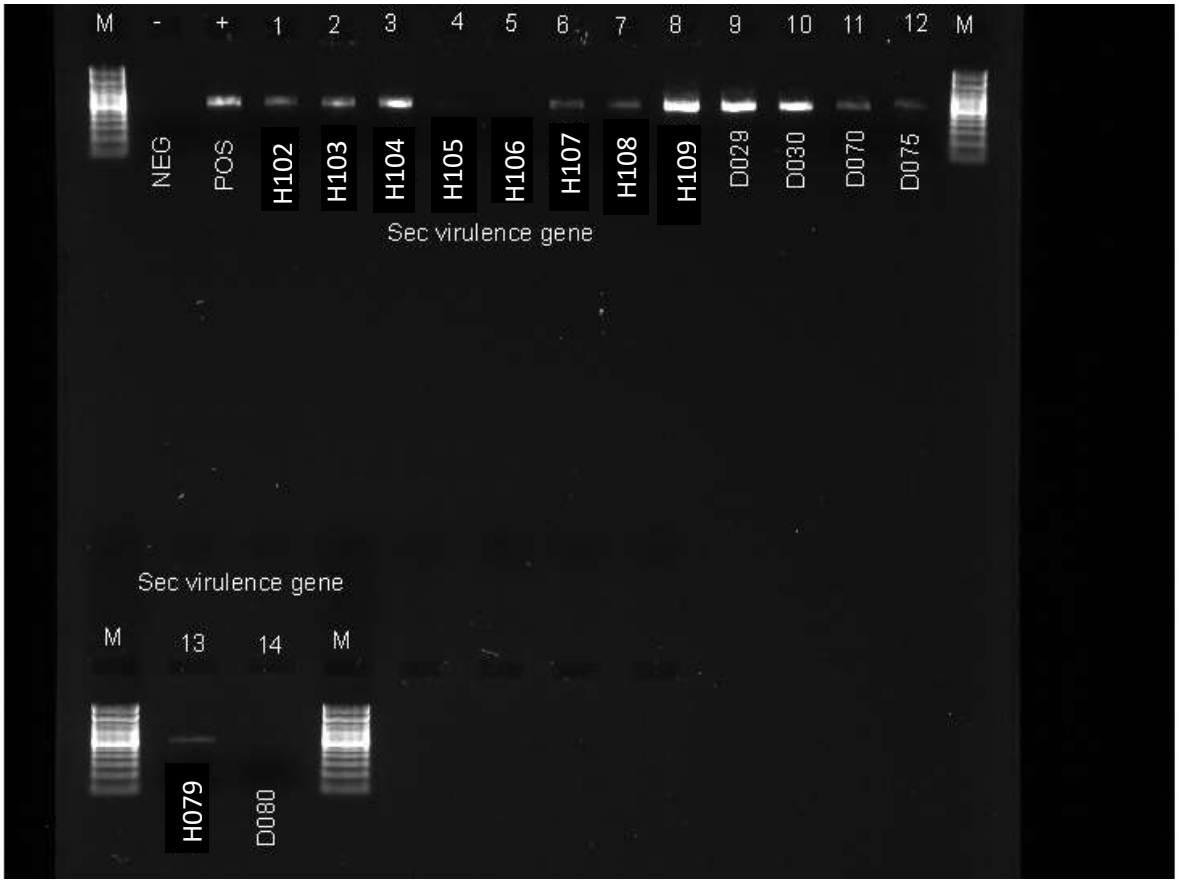


PCR amplification gel of *sec* virulence gene (598 bp) among the *S. pseudintermedius* isolates (lanes 1-12)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SP31 (positive control).

Lanes 1, 2, 3, 6, 7, 8, 11, and 12 were positive for ***sec* virulence gene.**

***sec* virulence gene (598 bp)**



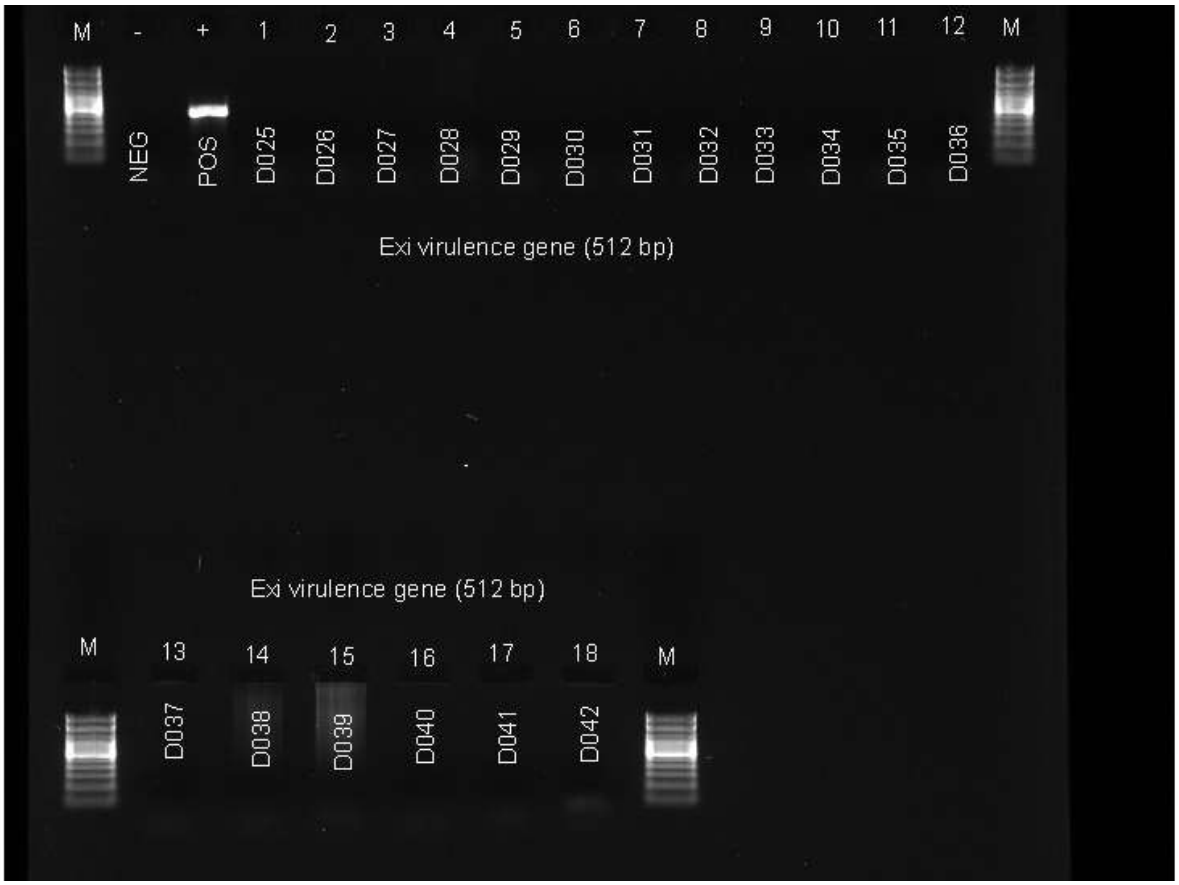
PCR amplification gel of *sec* virulence gene (598 bp) among the *S. pseudintermedius* isolates (lanes 1-14)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SP31 (positive control).

Lanes 1, 2, 3, 6, 7, 8, 9, 10, 11, 12, and 13 were positive for ***sec* virulence gene.**

Appendix XX: PCR amplification gel of *exi* virulence gene (512 bp) among the *S. pseudintermedius* isolates

***exi* virulence gene (512 bp)**

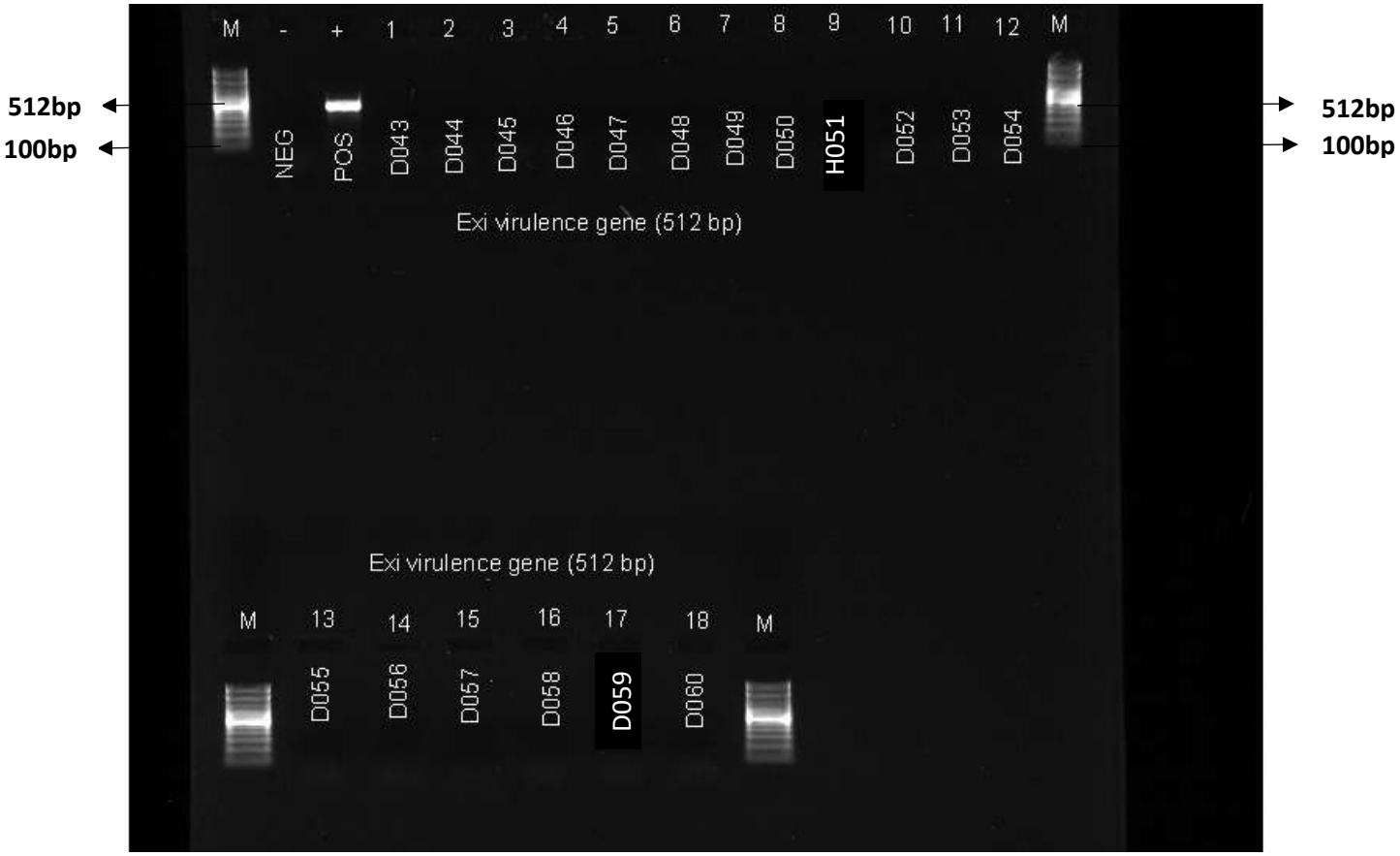


PCR amplification gel of *exi* virulence gene (512 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MSSP19 (positive control).

All the *S. pseudintermedius* isolates were negative for ***exi* virulence gene.**

***exi* virulence gene (512 bp)**

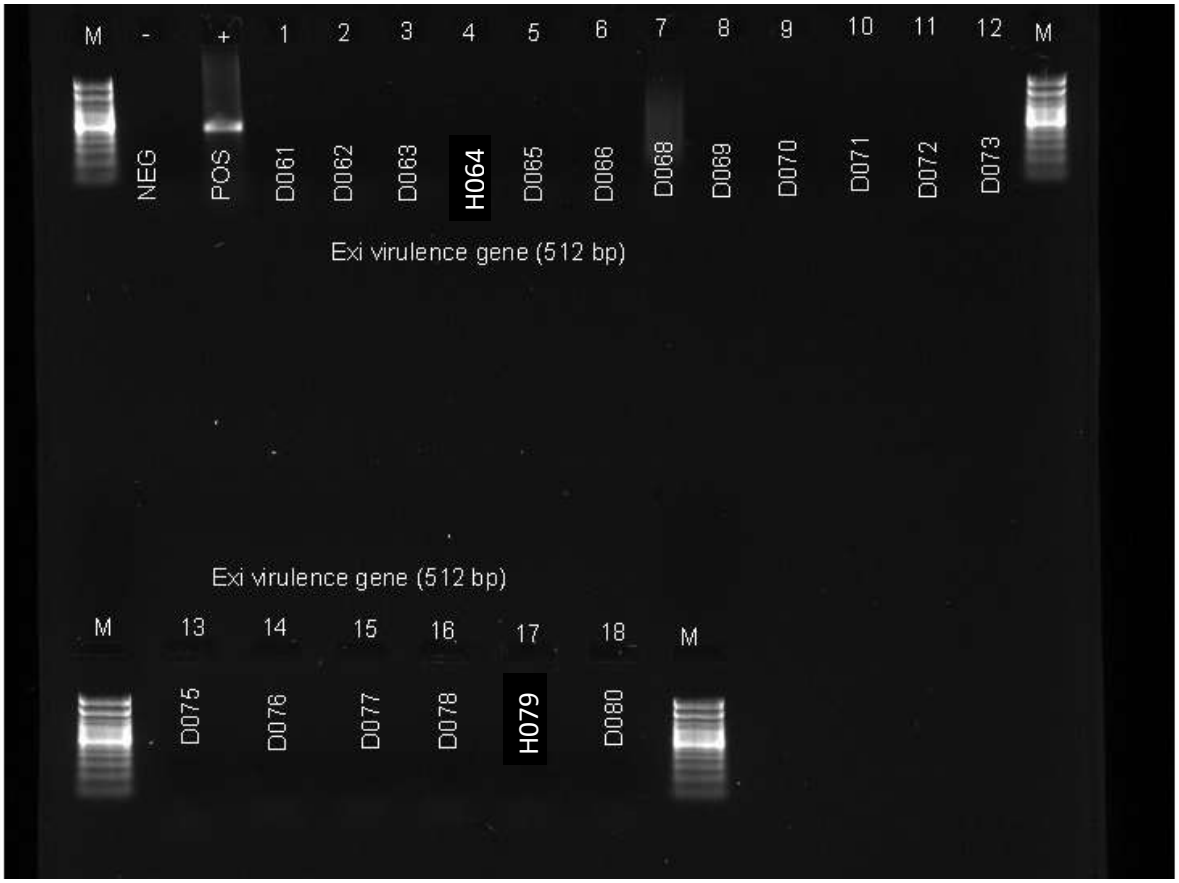


PCR amplification gel of *exi* virulence gene (512 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MSSP19 (positive control).

All the *S. pseudintermedius*isolates were negative for ***exi* virulence gene.**

***exi* virulence gene (512 bp)**



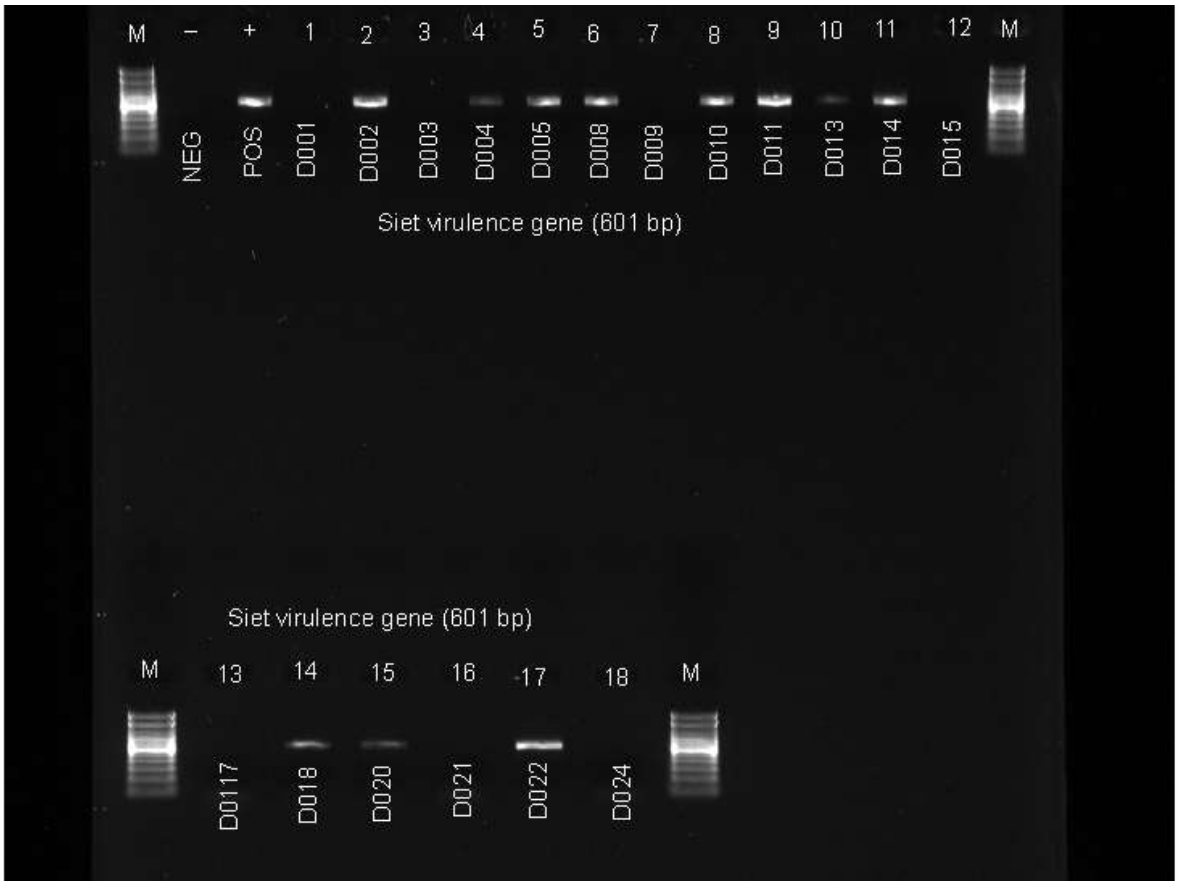
PCR amplification gel of *exi* virulence gene (512 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* MSSP19 (positive control).

All the *S. pseudintermedius*isolates were negative for ***exi* virulence gene.**

Appendix XXI: PCR amplification gel of *siet* virulence gene (601 bp) among the *S. pseudintermedius* isolates

***siet* virulence gene (601 bp)**

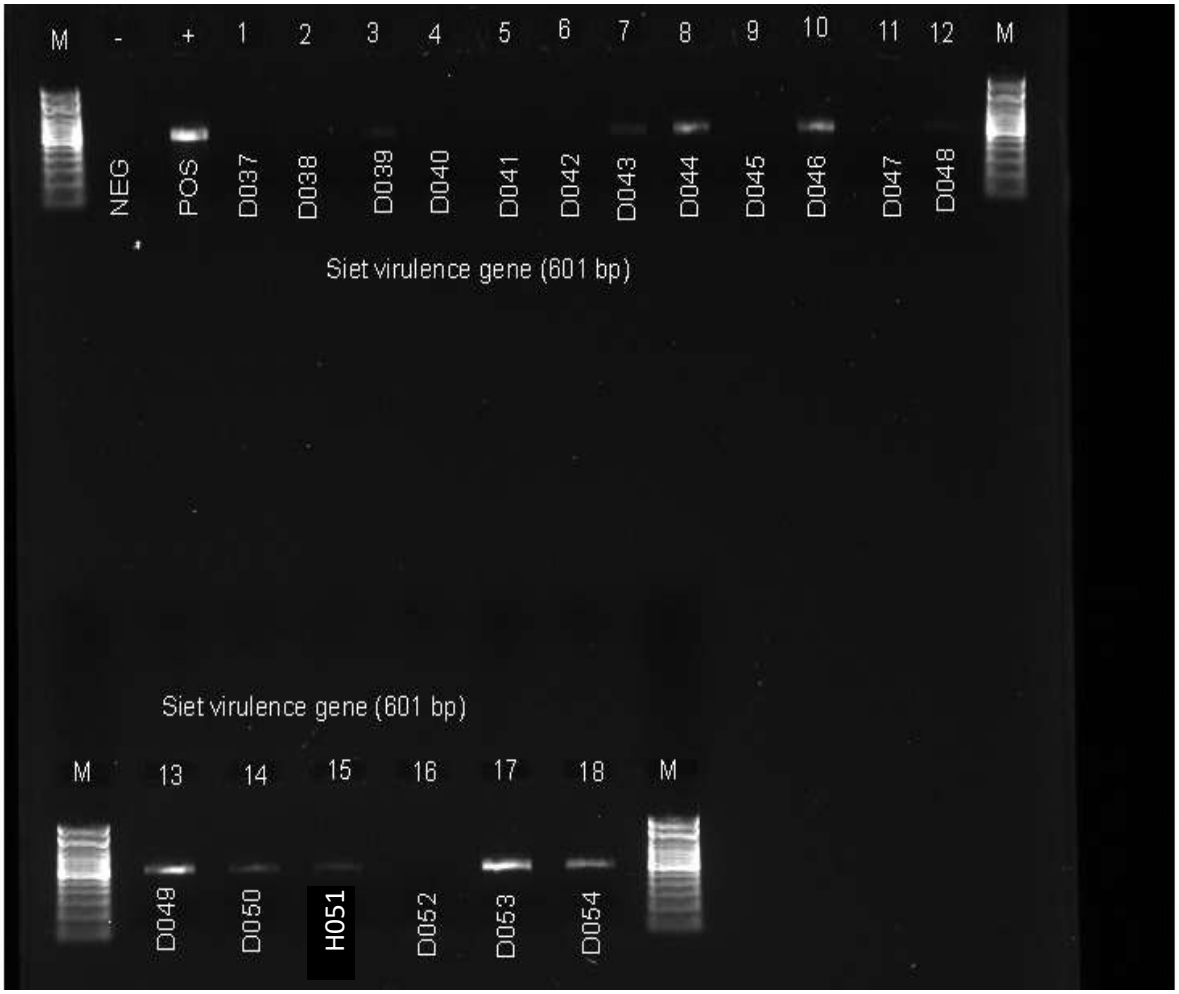


PCR amplification gel of *siet* virulence gene (601 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC20 (positive control).

Lanes 2, 4, 5, 6, 8, 9, 10, 11, 14, 15, and 17 were positive for ***siet* virulence gene.**

***siet* virulence gene (601 bp)**

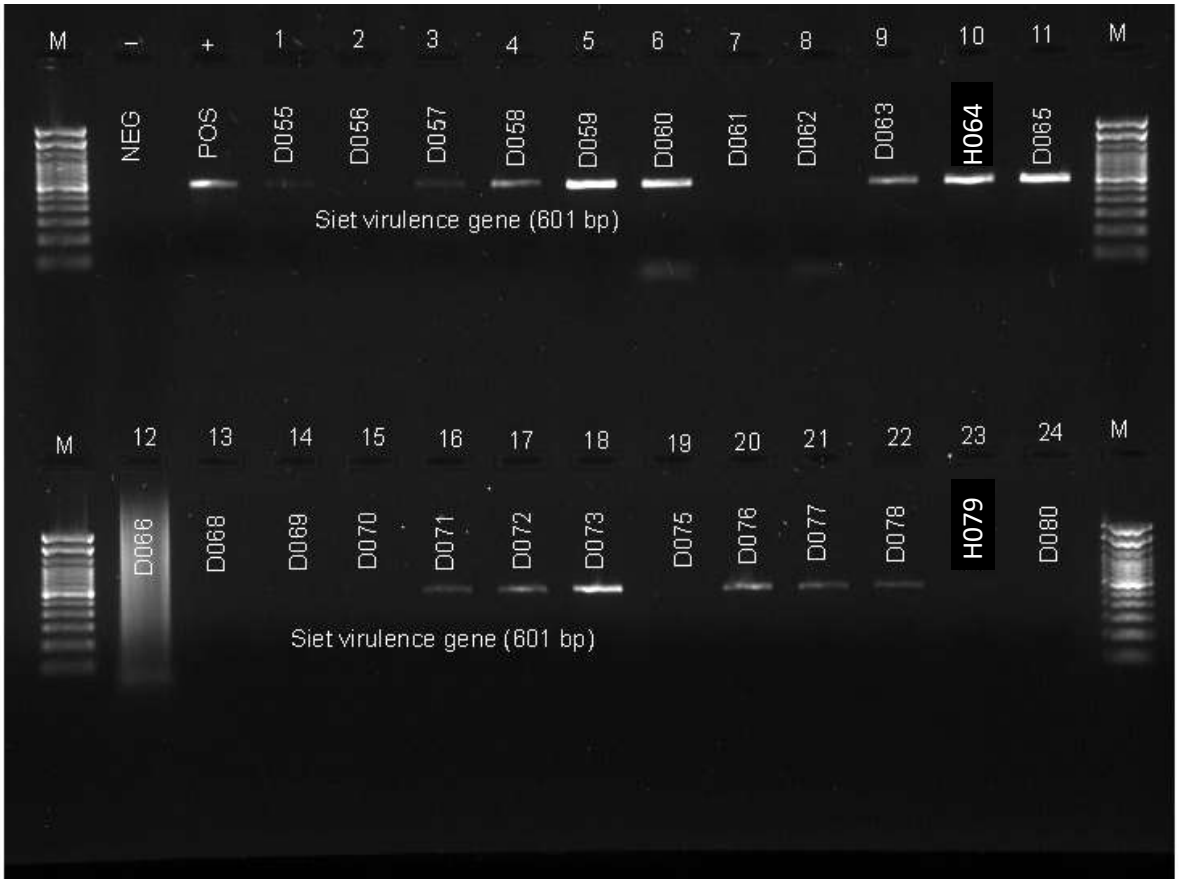


PCR amplification gel of *siet* virulence gene (601 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC20 (positive control).

Lanes 3, 7, 8, 10, 12, 13, 14, 15, 17, and 18 were positive for ***siet* virulence gene.**

***siet* virulence gene (601 bp)**

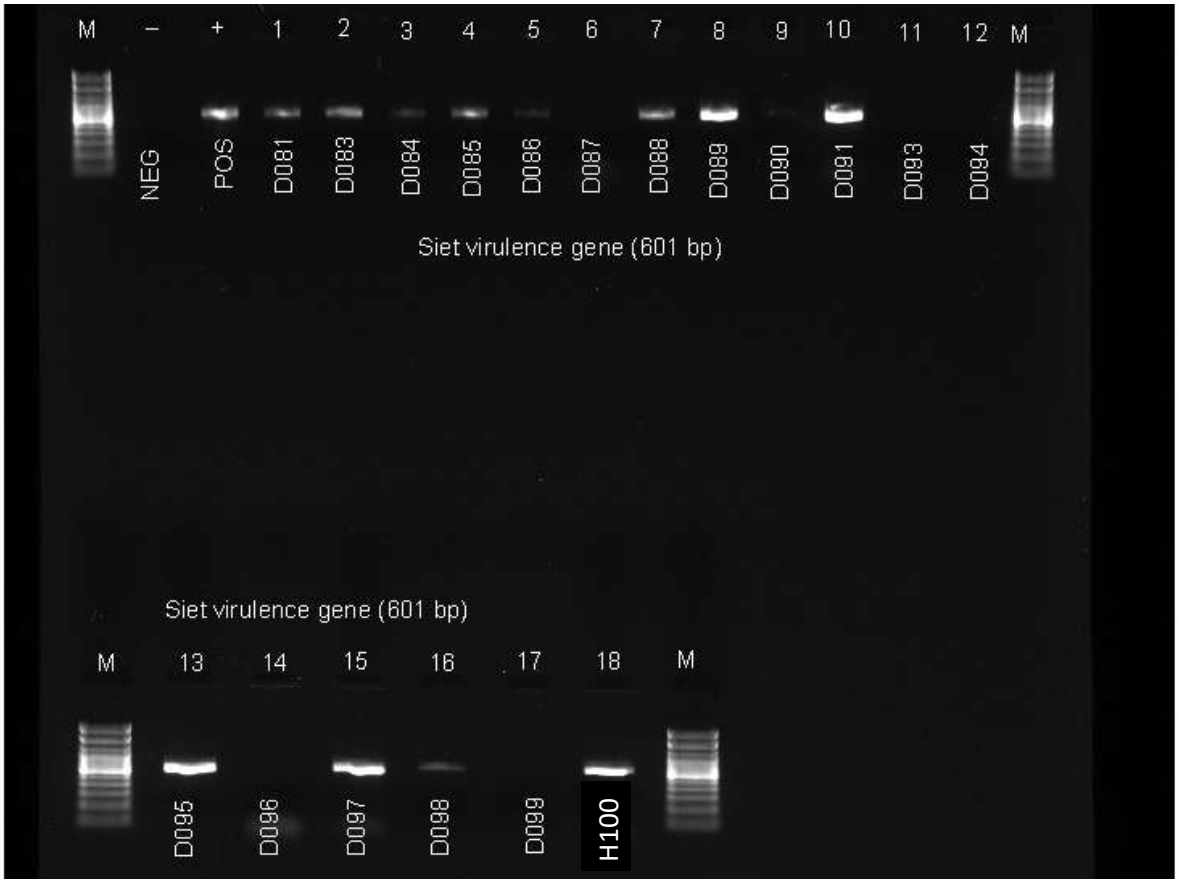


PCR amplification gel of *siet* virulence gene (601 bp) among the *S. pseudintermedius* isolates (lanes 1-24)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC20 (positive control).

Lanes 1, 3, 4, 5, 6, 9, 10, 11, 16, 17, 18, 20, 21, 22 and 23 were positive for ***siet* virulence gene.**

***siet* virulence gene (601 bp)**



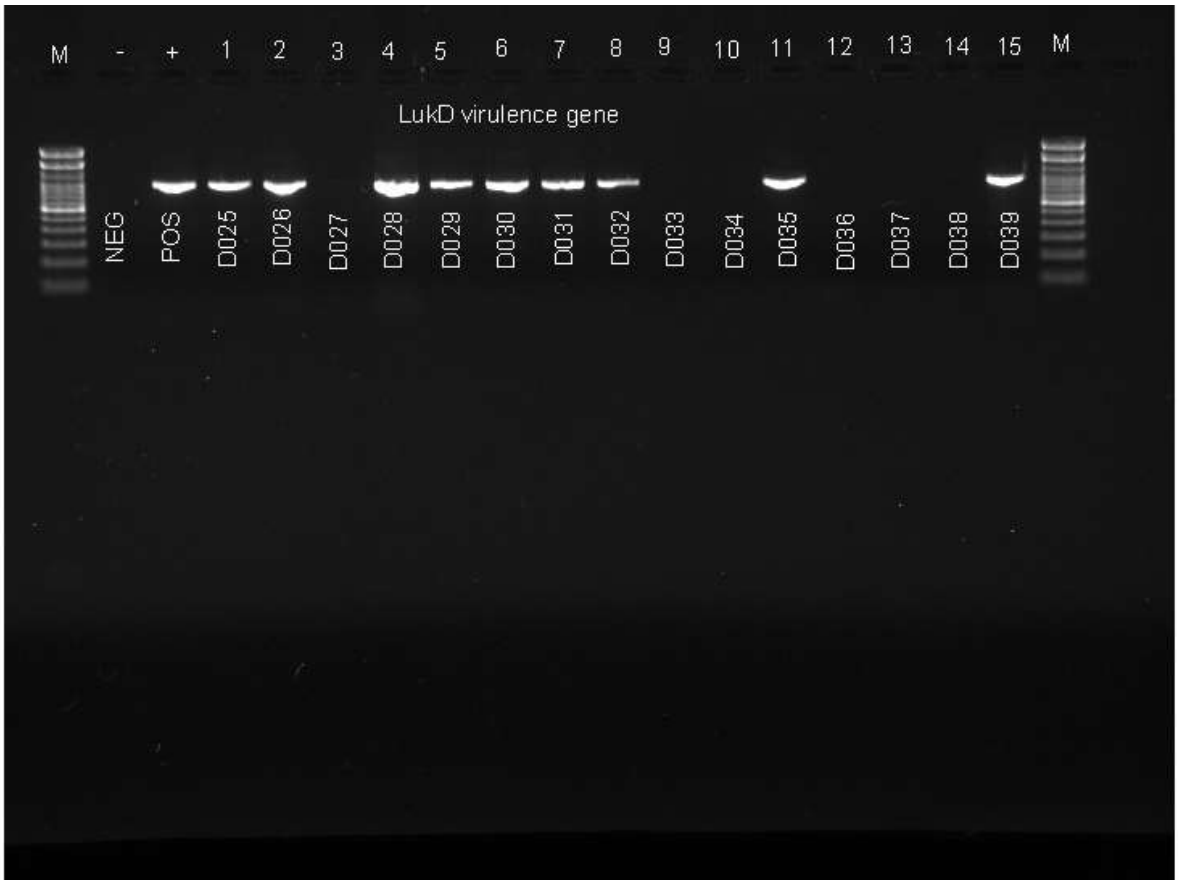
PCR amplification gel of *siet* virulence gene (601 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC20 (positive control).

Lanes 1, 2, 3, 4, 5, 7, 8, 9, 10, 13, 15, 16, and 18 were positive for ***siet* virulence gene.**

Appendix XXII: PCR amplification gel of *luk*D virulence gene (513 bp) among the *S. pseudintermedius* isolates

***luk*D virulence gene (513 bp)**

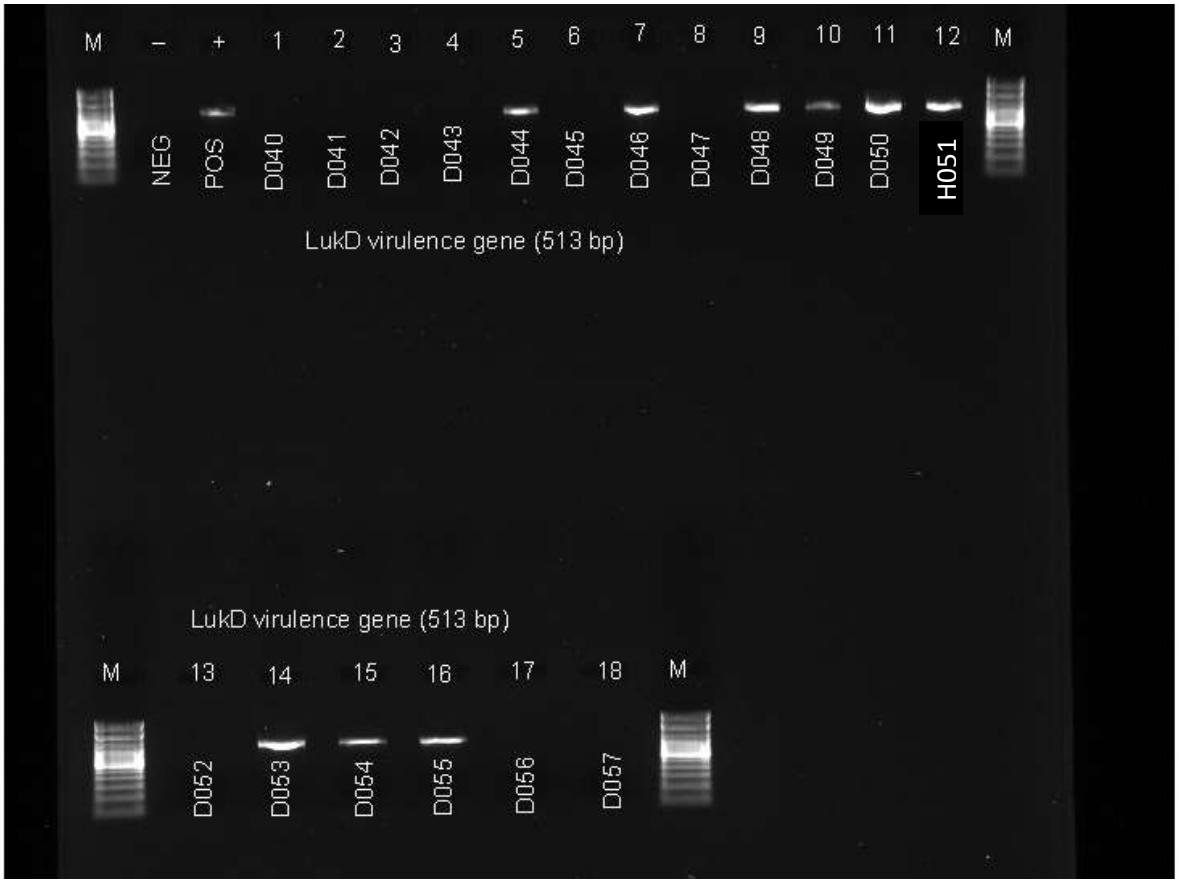


PCR amplification gel of *luk*D virulence gene (513 bp) among the *S. pseudintermedius* isolates (lanes 1-15)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC06 (positive control).

Lanes 1, 2, 4, 5, 6, 7, 8, 11, and 15 were positive for ***luk*D virulence gene.**

***luk*D virulence gene (513 bp)**

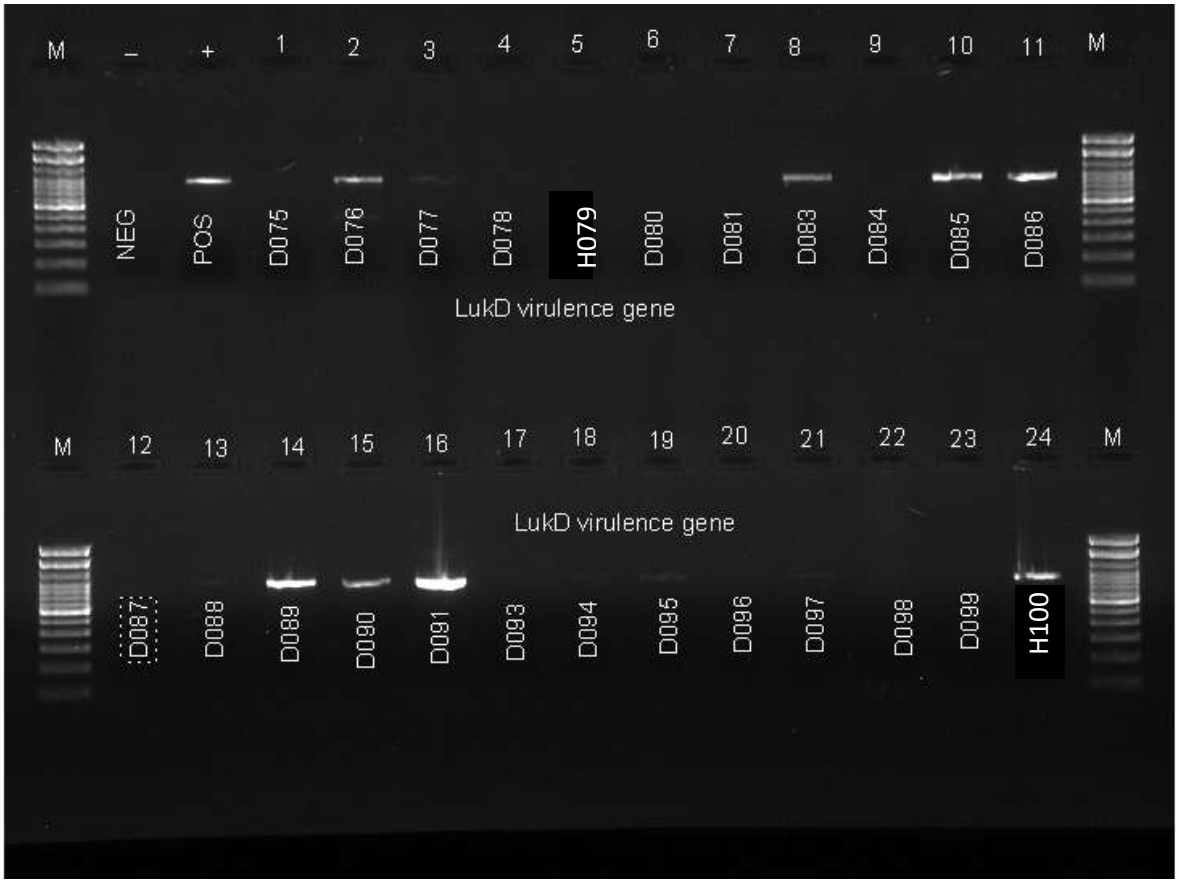


PCR amplification gel of *luk*D virulence gene (513 bp) among the *S. pseudintermedius* isolates (lanes 1-18)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC06 (positive control).

Lanes 5, 7, 9, 10, 11, 12, 14, 15, and 16 were positive for ***luk*D virulence gene.**

***luk*D virulence gene (513 bp)**

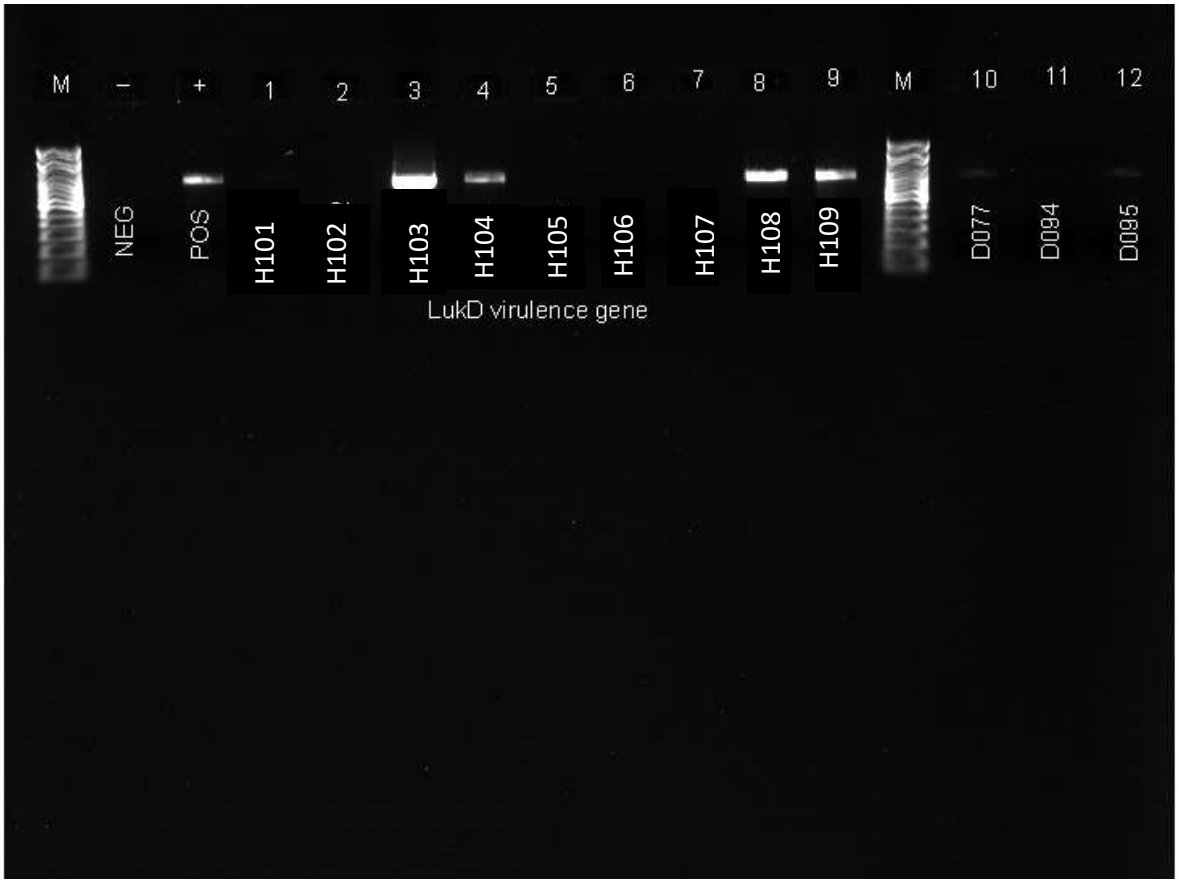


PCR amplification gel of *luk*D virulence gene (513 bp) among the *S. pseudintermedius* isolates (lanes 1-24)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC06 (positive control).

Lanes 2, 3, 8, 10, 11, 13, 14, 15, 16, 19, 21, and 24 were positive for ***luk*D virulence gene.**

***luk*D virulence gene (513 bp)**



PCR amplification gel of *luk*D virulence gene (513 bp) among the *S. pseudintermedius* isolates (lanes 1-12)

Lane M=100 bp marker; **-** = negative control without DNA; **+** = *S. pseudintermedius* SPC06 (positive control).

Lanes 3, 4, 8, 9, 10, and 12 were positive for ***luk*D virulence gene.**

APPENDIX XXIII: *Tet*M DNA sequences of the *S. pseudintermedius* isolates

The DNA sequences of the *tet*M genes for the 12 tetracycline-resistant *S. pseudintermedius* isolates in our study exhibited 99-100 % nucleotide similarity to the *tet*M reference nucleotide sequences of *Staphylococcus aureus* subsp. *aureus* in the GenBank database using a BLAST search on the NCBI server.

D002 DNA Sequence

gcagaagtatatcgttcattatcagttttagatggggcaattctactgatttctgcaaaa

gatggcgtacaagcacaaactcgtatattatttcatgcacttaggaaaatggggattccc

acaatctttttatcaataagattgaccaaaatggaattgatttatcaacggtttatcag

gatattaaagagaaactttctgccgaaattgtaatcaaacagaaggtagaactgtatcct

aatatgtgtgtgacgaactttaccgaatctgaacaatgggatacggtaatagagggaaac

gatgacctttagagaaatatatgtccggtaaatcattagaagcattggaactcgaacaa

gaggaaagcataagatttcagaattgttctctgttccctctttatcatggaagtgcaaaa

agtaatatagggattgataaccttatagaagttattactaataaattttattcatcaaca

catcgaggtccgtcg

BLAST Search result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Query: 99%

Ident: 100%

Sequence ID: NG_048252.1

D018 DNA Sequence

atggatttcttagcagaagtatatcgttcattatcagttttagatggggcaattctactg

atttctgcaaaagatggcgtacaagcacaaactcgtatattatttcatgcacttaggaaa

atggggattcccacaatctttttttatcaataagattgaccaaaatggaattgatttatca

acggtttatcaggatattaaagagaaactttctgccgaaattgtaatcaaacagaaggta

gaactgtatcctaatatgtgtgtgacgaactttaccgaatctgaacaatgggatacggta

atagagggaaacgatgacctttttagagaaatatatgtccggtaaatcattagaagcattg

gaactcgaacaagaggaaagcataagatttcagaattgttctctgttccctctttatcat

ggaagtgcaaaaagtaatatagggattgataaccttatagaagttattactaataaattt

tattcatcaacacatcgaggtccgtctgaactttg

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

Query: 100 %

Ident: 100 %

D028 DNA Sequence

atttcttagcagaagtatatcgttcattatcagttttagatggggcaattctactgattt

ctgcaaaagatggcgtacaagcacaaactcgtatattatttcatgcacttaggaaaatgg

ggattcccacaatctttttttatcaataagattgaccaaaatggaattgatttatcaacgg

tttatcaggatattaaagagaaactttctgccgaaattgtaatcaaacagaaggtagaac

tgtatcctaatgtgtgtgtgacgaactttaccgaatctgaacaatgggatacggtaatag

agggaaacgatgacctttagagaaatatatgtccggtaaatcattagaagcattggaac

tcgaacaagaggaaagcataagatttcagaattgttctctgttccctctttatcatggaa

gtgcaaaaagtaatatagggattgataaccttatagaagttattactaataaattttatt

catcaacacatcgaggt

BLAST Search Result on NCBI database: *Staphylococcus aureus* strain UW1055 tetracycline resistance protein *Tet*M (*tet*M) gene, partial cds

Sequence ID: AY057893.1

QUERY: 100%

IDENT: 100%

D035 DNA Sequence

acgccaggacatatggatttcttagcagaagtatatcgttcattatcagttttagatggg

gcaattctactgatttctgcaaaagatggcgtacaagcacaaactcgtatattatttcat

gcacttaggaaaatggggattcccacaatctttttttatcaataagattgaccaaaatgga

attgatttatcaacggtttatcaggatattaaagagaaactttctgccgaaattgtaatc

aaacagaaggtagaactgtatcctaatatgtgtgtgacgaactttaccgaatctgaacaa

tgggatacggtaatagagggaaacgatgacctttagagaaatatatgtccggtaaatca

ttagaagcattggaactcgaacaagaggaaagcataagatttcagaattgttctctgttc

cctctttatcatggaagtgcaaaaagtaatatagggattgataaccttatagaagttatt

actaataaattttattcatcaacacatcgaggtccgtctgaactt

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %

D054 DNA Sequence

atatggatttcttagcagaagtatatcgttcattatcagttttagatggggcaattctac

tgatttctgcaaaagatggcgtacaagcacaaactcgtatattatttcatgcacttagga

aaatggggattcccacaatctttttttatcaataagattgaccaaaatggaattgatttat

caacggtttatcaggatattaaagagaaactttctgccgaaattgtaatcaaacagaagg

tagaactgtatcctaatatgtgtgtgacgaactttaccgaatctgaacaatgggatacgg

taatagagggaaacgatgacctttttagagaaatatatgtccggtaaatcattagaagcat

tggaactcgaacaagaggaaagcataagatttcagaattgttctctgttccctctttatc

atggaagtgcaaaaagtaatatagggattgataaccttatagaagttattactaataaat

tttattcatcaacacatcgaggtccgtctg

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100%

IDENT: 100%

D072 DNA Sequence

agacggacctcgatgtgttgatgaataaaatttattagtaataacttctataaggttatc

aatccctatattacttttgcacttccatgataaagagggaacagagaacaattctgaaa

tcttatgctttcctcttgttcgagttccaatgcttctaatgatttaccggacatatattt

ctctaaaaggtcatcgtttccctctattaccgtatcccattgttcagattcggtaaagtt

cgtcacacacatattaggatacagttctaccttctgtttgattacaatttcggcagaaag

tttctctttaatatcctgataaaccgttgataaatcaattccattttggtcaatcttatt

gataaaaaagattgtgggaatccccattttcctaagtgcatgaaataatatacgagtttg

tgcttgtacgccatcttttgcagaaatcagtagaattgccccatctaaaactgataatga

acgatatacttctgctaagaaat

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %

D078 DNA Sequence

acggacctcgatgtgttgatgaataaaatttattagtaataacttctataaggttatcaa

tccctatattacttttgcacttccatgataaagagggaacagagaacaattctgaaatc

ttatgctttcctcttgttcgagttccaatgcttctaatgatttaccggacatatatttct

ctaaaaggtcatcgtttccctctattaccgtatcccattgttcagattcggtaaagttcg

tcacacacatattaggatacagttctaccttctgtttgattacaatttcggcagaaagtt

tctctttaatatcctgataaaccgttgataaatcaattccattttggtcaatcttattga

taaaaaagattgtgggaatccccattttcctaagtgcatgaaataatatacgagtttgtg

cttgtacgccatcttttgcagaaatcagtagaattgccccatctaaaactgataatgaac

gatatacttctgctaagaaatccatatg

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %

D081 DNA Sequence

acggacctcgatgtgttgatgaataaaatttattagtaataacttctataaggttatcaa

tccctatattacttttgcacttccatgataaagagggaacagagaacaattctgaaatc

ttatgctttcctcttgttcgagttccaatgcttctaatgatttaccggacatatatttct

ctaaaaggtcatcgtttccctctattaccgtatcccattgttcagattcggtaaagttcg

tcacacacatattaggatacagttctaccttctgtttgattacaatttcggcagaaagtt

tctctttaatatcctgataaaccgttgataaatcaattccattttggtcaatcttattga

taaaaaagattgtgggaatccccattttcctaagtgcatgaaataatatacgagtttgtg

cttgtacgccatcttttgcagaaatcagtagaattgccccatctaaaactgataatgaac

gatatacttctgctaagaaatccata

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %

D085 DNA Sequence

tataaggttatcaatccctatattacttttgcacttccatgataaagagggaacagaga

acaattctgaaatcttatgctttcctcttgttcgagttccaatgcttctaatgatttacc

ggacatatatttctctaaaaggtcatcgtttccctctattaccgtatcccattgttcaga

ttcggtaaagttcgtcacacacatattaggatacagttctaccttctgtttgattacaat

ttcggcagaaagtttctctttaatatcctgataaaccgttgataaatcaattccattttg

gtcaatcttattgataaaaaagattgtgggaatccccattttcctaagtgcatgaaataa

tatacgagtttgtgcttgtacgccatcttttgcagaaatcagtagaattgccccatctaa

aactgataatgaacgatatacttctgctaagaaatccatatgtcct

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %

D086 DNA Sequence

CACCTTACCCGGTGACTTCGTGTCCTATGTTTCTTCTGGGACGTTTCTAC

TGCCTTCTTACTTTTGGCCTTCCATGATAACTAGGGAATTATTTCATGCA

CTTAAATAAAATGGCTTTCCTCACGTTCTTTTTCCTCAGTATCAATGAAC

TTACTGGAAATGATTTTCTCTAAGTTGTCCTCGATTCCCTCTATAAACTT

TCTGCCTAAATTGTATTCAACAAAATTCATCACTGTATCTAATATGTGTG

TCCGAACTTTACCTAATCTGATTATGGGATACGGTTCTCTTTGAAACGAT

GACCTTTTATGAAAATCTGTCCGGTTTTTCACTATATTCATTGTAACTCA

AACAGTAGGAATCCACATTATTTCAAAATTGTTCTCTGTTCCCTCATTAT

CATGGAAGTGCAAAATCTTATACAGAGATCGATAAACTTATCCAATTTAT

TACTAATAATGTATATTCATCTTCACCTAAAGGTCCCTCATGCCTTTGCG

TGTAAGA

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %

D090 DNA Sequence

catatggatttcttagcagaagtatatcgttcattatcagttttagatggggcaattcta

ctgatttctgcaaaagatggcgtacaagcacaaactcgtatattatttcatgcacttagg

aaaatggggattcccacaatctttttttatcaataagattgaccaaaatggaattgattta

tcaacggtttatcaggatattaaagagaaactttctgccgaaattgtaatcaaacagaag

gtagaactgtatcctaatatgtgtgtgacgaactttaccgaatctgaacaatgggatacg

gtaatagagggaaacgatgacctttagagaaatatatgtccggtaaatcattagaagca

ttggaactcgaacaagaggaaagcataagatttcagaattgttctctgttccctctttat

catggaagtgcaaaaagtaatatagggattgataaccttatagaagttattactaataaa

ttttattcatcaacacatcgagg

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %

D097 DNA Sequence

tcgatgtgttgatgaataaaatttattagtaataacttctataaggttatcaatccctat

attacttttgcacttccatgataaagagggaacagagaacaattctgaaatcttatgct

ttcctcttgttcgagttccaatgcttctaatgatttaccggacatatatttctctaaaag

gtcatcgtttccctctattaccgtatcccattgttcagattcggtaaagttcgtcacaca

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aatatcctgataaaccgttgataaatcaattccattttggtcaatcttattgataaaaaa

gattgtgggaatccccattttcctaagtgcatgaaataatatacgagtttgtgcttgtac

gccatcttttgcagaaatcagtagaattgccccatctaaaactgataatgaacgatatac

ttctgc

BLAST Search Result on NCBI database: *Staphylococcus aureus* subsp. *aureus* TW20 *tet* (M) gene for tetracycline resistance ribosomal protection protein *Tet* (M), complete CDS

Sequence ID: NG_048252.1

QUERY: 100 %

IDENT: 100 %