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Investigation on the Epidemiology and Pathogenesis Characteristics of *Staphylococcus pseudintermedius*-Associated Veterinary Hospital Infections

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Abstract

A retrospective analysis of the diagnostic laboratory submissions from 2016 to 2020 was performed to assess the antibiotic resistance of *Staphylococcus pseudintermedius* and determine locations in the hospital which might be acting as environmental source(s). Previous studies have identified a significant increase in infections with *S. pseudintermedius*. Samples were taken from the hospital environment by swabbing areas in the intensive care unit and anesthesia preparation room and bacterial species identified. Isolates obtained from patients were then examined regarding the ability to form biofilm, an important phenotype on hospital-related infection. In addition, veterinary hospital associated strains of *S. pseudintermedius* were used to determine the bactericidal effect of the used disinfectant, applying the hospital current protocol, by comparing the efficacy against *S. pseudintermediius* and a strain of *Staphylococcus aureus* from a dog. The isolates identified were resistant to commonly used antibiotics such as enrofloxacin and cephalosporins, and 45% percent of those were methicillin resistant. The environmental survey in the hospital identified *S. pseudintermedius* in the pre-anesthesia area, although the isolate was killed by the current used disinfection protocol. A few disease associated bacteria were evaluated for biofilm formation in comparison to a dog isolate of *Staphylococcus aureus*, demonstrating strong ability to form biofilms.

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Keywords

Staphylococcus pseudintermedius, Veterinary Hospital Infection, Epidemiology, Biofilm, Antibiotic Susceptibility, Environment

1. Introduction

Staphylococcus pseudintermedius is a coagulase-positive cocci belonging to the *Staphylococcus intermedius* group. *S. pseudintermedius* is a commensal bacterium encountered on the skin and mucous membranes of small animal patients, frequently seen on dogs and occasionally on cats [1] [2]. The bacterium is a component of the normal microbiota of dogs, being isolated colonizing the nose of 30%, the mouth of approximately 55% and the perineum-rectum in 52% of samples cultured from dogs [1] [3] [4]. Some additional epidemiologic works place the prevalence of colonization in dogs to 46.2% [5]. In fact, the number of infections caused by *S. pseudintermedius* in dogs and in cats has been increasing since the year 2000, likely connected to the ability of the organism to develop biofilms. Associated with the increase in number of infections is the observation that many of those *S. pseudintermedius* isolates are methicillin resistant [6] [7] [8]. In a large study in Europe by Menandro and colleagues, *S. pseudintermedius* was linked to 76% of the *Staphylococcus* infections diagnosed in dogs and cats, and 36% of those isolates were methicillin resistant [6] [8].

Recently, the Veterinary Teaching Hospital at Oregon State University has seen an increase in the number of infections involving *S. pseudintermedius,* including methicillin-resistant *S. pseudintermedius* (MRSP) strains [9]. This observation agrees with other epidemiologic studies in the northern hemisphere [6] [8]. For example, a study by Nienhoff and colleagues in Germany found that 7.1% of the dogs admitted in the hospital were colonized with methicillin-resistant *S. pseudintermedius* [7].

Antibiotic resistance is a major obstacle to the treatment of bacterial infections in veterinary medicine. It has been previously demonstrated that hospital environment often acts as a reservoir for bacteria, especially those that are adept at forming biofilms, like *S. pseudintermedius* [10] [11].

The patients most at risk for developing an infection are those that have compromised immune systems, such as those that have recently undergone surgery, or are experiencing increased stress due to prolonged periods in the hospital. Prolonged stays in the hospital also enhance the chances that a staff member could transfer bacteria from a patient, or from a reservoir within the hospital.

This study was conducted in order to better understand the epidemiology of *S. pseudintermedius* in the hospital environment. The pathogen is known to form biofilm in the environment, as well as to have the tendency to establish biofilms in host tissues, as an common example, in bones [8], which creates a very difficult challenge for the treatment with antibiotics [12] [13] [14]. For instance,

work by Pompilio and colleagues demonstrated that *S. pseudintermedius* can establish biofilms in conditions with different pH, and develop resistance to treatment with several different antibiotics [14]. Although the knowledge about the pathogenesis of *S. pseudintermedius* is not extensive, some information is available about toxins, such as, beta-hemolysin, coagulase, DNAse, leukotoxin, exfoliative toxin, enterotoxin [1] [15]. In addition, not many studies have addressed the similarities and differences of biofilm formation between *S. aureus* and *S. pseudintermedius*.

Some of the questions we had, pertained to the hospital environment and the source of infection for the patients. Recent published study has characterized the different strains of *S. pseudintermedius* according to the function of a quorum sensing gene regulator, *agr*, which is also important for regulation of gene expression in *S. aureus* [16]. The investigators showed that among the 4 *agr* identified groups of *S. pseudintermedius*, the examination of biofilm formation, toxin gene carriage and antimicrobial resistance, did not show significant difference among the phenotypes.

Epidemiologic study in our hospital for the past several years has identified a significant increase of *S. pseudintermedius* infection, many of them associated with surgery. In a previous study conducted between 2012 and 2015 at the same facility, it was noticeable that the number of *staphylococcus* cases were clearly becoming more common, as well as the percent of methicillin-resistant *S. pseudintermedius* [9] [16]. Since the pathogen is a common colonizer of dogs, and may, like *S. aureus*, have the ability to survive in the environment of the hospital, this study was intended to identify the potential sources of infection, and to confirm some of the characteristics of the pathogen, which allow it to survive in different environments and infect hosts. The study showed that between 2016 and 2020 a large number of cases were diagnosed, that the pathogen can be isolated from surfaces in the hospital, and form biofilms.

2. Materials and Methods

Retrospective Study

All records involving bacterial culture submissions from the years 2016 to 2020 (5 years) were obtained, and the cases specific to *S. pseudintermedius* were examined. The categories analyzed included, bacterial species, antibiotic resistance/susceptibility, the site of the infection, locations visited in the hospital, sex, age, and treatment and outcome of the patient.

Bacteria

Three different isolates of *S. psedintermedius* (D1, DS1, DW), obtained from infections in dogs submitted to surgery or isolated from urine (they were part of the isolates tested for antibiotic susceptibility during the period reported in the study) were culture in Mueller-Hinton agar plates (VWR). Also, a strain of *Staphylococcus aureus* isolated from a dog,, was also cultured on Muller-Hinton agar plates. Prior to the assays, 48 hrs old colonies were suspended in Hanks Ba-

lanced Salt Solution (HBSS) and the solution was adjusted to approximately 3 x 10^8 CFU/mL using a McFarland standard as reported before [9] [17]. The final concentration of bacteria depended on the assay performed.

Environmental Bacteria Isolates

Bacterial swabs were obtained from the Oregon State University Veterinary Hospital (VTH). The samples were collected from ICU, from a computer keyboards, faucet handles, kennel handles, whiteboard markers, door handles and clipboards. The swabs from the anesthesia prep room were from faucet handles, disinfectant bottles, anesthesia machines, clippers, cabinet handles, microwave, anesthesia monitors, clipboards, calculator, light handles, kennel handles, Suntech vet 25 machine, and door handles. Sterile swabs were used to swab the desired surface, then the swab was placed in a sterile tube containing Luria-Bertani (LB) nutrient broth. A 0.1 mL direct sample from the broth was then plated onto LB agar and incubated for up to 72 hours at 37˚C. Cultures that showed growth were then streaked to isolate bacterial colonies. Isolated colonies were collected and added to microcentrifuge tubes filled with 200 µL of disruption beads and 1 mL sterile DI water. The tubes were then vortex agitated and placed in a homogenizer for 35 seconds. Then, the suspension were added to a microcentrifuge tubes on ice and spun down for 60 seconds at 18˚C on maximum speed. Without disturbing the beads or bacterial pellet, 450 µL of supernatant were transferred to a graduated microcentrifuge tube and refrigerated at 4˚C.

Sequencing

The DNA obtained from individual colonies were cleaned and concentrated using a DNA Clean and Concentrator −5 kit (Qiagen) prior to be submitted for sequencing. One hundred fifty µL of bacterial isolate of DNA were combined with 300 µL of DNA binding buffer in a column, then centrifuged at 11,000× g for 30 seconds. The filtrate was then discarded and 200 µL of DNA Wash Buffer was added to the column and then the tube was submitted to centrifugation. This last step was then repeated. The DNA was then eluted by adding 30 µL of sterile molecular water to the column. The column was allowed to stand for 1 minute, and then centrifuged. A Tecan instrument (280/260 wavelengths) was then used to calculate the concentration of the DNA that was been eluted. The purified DNA (concentration was then amplified using a Master Mix for 16 s PCR as reported [9]. This was carried out by combining 5 µL of Mastermix (Bio-Rad), 0.1 µL each of Forward and Reverse Primers (16S V4 region forward primer: GTG YCA GCM GCC GCG GTA A; Reverse: GGA CTA CNV GGG TWT CTA AT obtained from Invitrogen) and 3.8 µL of sterile molecular water in each tube, along with 1 µL of the selected isolated DNA for each sample. The tubes were briefly vortex-agitated before being placed in the BioRad T100 thermocycler for amplification following the reactions conditions: 59˚C annealing temperature, 60 s extension time, and 39 total cycles. Amplified DNA was run on agarose gel (1% in EDTA) for electrophoresis to ensure the correct size of DNA was obtained. The 16s DNA was cut from the gel and put through a gel extraction kit to concentrate and clean the amplified DNA, then submitted to be sequenced at the

Center for Quantitative Life Sciences [9].

Resistance to Disinfectant

We chose six bacteria (representing each one of the morphologically different colonies) from those isolated from the environmental swabs (*R. mucosa*, *B. megaterium*, *A. gandavensis*, *N. terrae*, *S. pseudintermedius* and *S. aureus*) along with a strain of *S. pseudintermedius* 21.153.8 cultured from a bone screw from the VTH (isolated in 2018) and used them to create inoculant solutions. Approximately 3×10^8 bacteria in HBSS were added to 48-well plates, then allowed to be in a lab drawer for five days. After the period, the supernatant was removed and 1.0 mL Accel Ready to Use disinfectant solution or Accel Diluted disinfectant solution was added to two wells of each bacteria. For the Ready to Use disinfectant, at time points of 1, 2, and 5 minutes the disinfectant was removed and replaced with 1.0 mL HBSS. For the Dilute disinfectant, at time points of 5, 7, and 10 minutes the disinfectant was removed and replaced with 1.0mL HBSS. The wells were then agitated to dislodge the bacteria and 0.1 mL was plated directly on LB agar, then left to incubate overnight.

Minimal Inhibitory Concentration

Specimens for culture were collected from canine patients at the Lois Bates Acheson Veterinary Teaching Hospital (Oregon State University, Corvallis, Oregon) and submitted to the associated Oregon Veterinary Diagnostic Laboratory (OVDL).

The protocol used followed the CLSI guidelines. Routine culture set up included inoculation onto Tryptic soy agar (TSA) plates containing 5% sheep blood (Remel), MacConkey agar plates (Hardy Diagnostics), and Thioglycolate broth (Remel). Inoculated media was incubated for a minimum of 18 hours at 35˚C and 6% CO_2.

Agar plates were observed for growth over a total of 48 hours of incubation. The Thioglycolate broth was sub-cultured onto TSA w/5% sheep blood and incubated if no growth was observed in the primary agar plates. Colonies were isolated and identified using established phenotypic methods and criteria using traditional biochemical tests as described previously [18]. Beginning in December of 2019, a Vitek MS MALDI-TOF (Biomerieux) was used for identification in conjunction with phenotypic methods.

When requested, antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Individual isolates standardized to 0.5 McFarland by the Prompt™ inoculation system (BD BBL-Thermo Fisher) were plated onto Mueller Hinton agar (Remel) to achieve a bacterial lawn, and antimicrobial disks (BD-Remel or Hardy Diagnostics) were placed by hand or using a stamper (BD-Remel). The plates were then incubated for 16 to 24 hours, depending on the disks, at 35C ambient air. Disk diffusion zones were measured by the BIOMIC V3 Reader (Giles Scientific). Susceptibility categories were determined using the most current Clinical and Laboratory Standards Institute (CLSI) guidelines, and minimum inhibitory concentration (ug/mL) were reported when available.

Bacterial isolates identified as S*taphylococcus pseudintermedius* or *Staphyloccocus aureus* were screened for methicillin resistance via either a latex agglutination assay for penicillin-binding protein PBP2' encoded by the mecA gene (Oxoid-Thermo Fisher), or via Kirby-Bauer disk diffusion of oxacillin for *S. pseudintermedius* isolates and cefoxitin for *S. aureus* isolates. Per CLSI Vet08 4th edition: *S. pseuintermedius* isolates yielding ≤ 17 mm against oxacillin, and *S. aureus* isolates yielding ≤ 21 mm against cefoxitin disks were considered to be methicillin resistant.

The following antimicrobials were routinely tested: amikacin, amoxicillin-clavulanic acid, clindamycin, chloramphenicol, cefovecin, enrofloxacin, gentamicin, marbofloxacin, penicillin G, trimethoprim-sulfamethoxazole, tetracycline, and erythromycin. amoxicillin-clavulanic acid, cefovecin, and penicillin were reported as resistant for isolates determined to be methicillin resistant regardless of actual MIC. Methicillin resistant isolates were also susceptibility tested for: azithromycin, ceftaroline, nitrofurantoin, rifampin, streptomycin, vancomycin, and doxycycline. Vancomycin was not included on client reports. Chloramphenicol, clindamycin, and erythromycin was not reported for isolates recovered from urine specimens.

Biofilm

In order to test how resilient the bacteria were in different hospital environments, they were stimulated to grow biofilms by simulating five different conditions encountered in hospitals: LB broth, HBSS, sterile water, serum, and dry surface. Each bacteria, three strains of *S. pseudintermedius* and one strain of *S. aureus* for comparison, were submitted to all five conditions. This was accomplished by using two 48-well plates with two strains per plate. Three wells were filled with a 1.0 mL aliquot of each condition for every strain, with 0.1 mL of 3×10^5 CFU/mL or 1×10^8 CFU/mL suspension of bacteria in HBSS added to each well, dependent on the assay performed. In the case of the dry condition, 0.1 mL of 3×10^5 bacteria in HBSS was added to an empty well. The well plates were incubated in a drawer in the lab at room temperature, with CFU collected on days 1, 2, 7 and 9. To measure CFU, 0.1 mL of supernatant from each well except the dry surface was diluted 1:10 with HBSS and then plated onto an LB agar and incubated overnight. The dry surface wells were only measured on day 9, which was accomplished by adding 1.0 mL HBSS to the well, drawing up 0.1 mL of supernatant and diluting 1:10 with HBSS and then plating on LB agar. On day 9 the mass of biofilm formed in each well was then measured. The supernatant was removed from each well and the biofilm was washed with 0.2 mL sterile DI water. We then added 0.2 mL of 0.1% crystal violet to every well. After waiting 10 minutes the wells were washed twice with 0.2 mL sterile distilled water. Finally, 0.2 mL of 30% acetic acid was added to each well and left for 10 minutes at 24˚C. The supernatant was then transferred to a new 48-well plate and the absorbance was read at 550 nm with an Epoch spectrophotometer.

In order to establish a biofilm on a dry surface, as many times seen in hospital environments, we established a 1×10^8 suspension of *S. aureus* and similar sus-

pensions of 2 different strains of *S. pseudintermedius*. We evaluated several variations of the inoculum and the most consistent was used. Then, 0.1 ml of each suspension was pipetted and inoculated into different wells of a 48-well tissue culture plates. Plates were then incubated in the dark for two weeks at room temperature. Then, biofilm mass and CFU were determined as described [9] [19].

Scanning Electron Microscopy (SEM) preparations

Samples were prepared for scanning electron microscopy observation. Sterile coverslips were placed on 6-well tissue culture plates. We added 1 ml of a suspension of 3×10^5 bacteria (*S. pseudintermedius* or *S. aureus*) and 1 ml of LB broth, then incubated the plates in the dark at room temperature for 2 and 7 days. For the biofilms grown on a dry surface, 1 ml of the same suspension was added to each well and allowed to dry out for 7 days. By the timepoints, the supernatant was removed and the coverslips were fixed with 4% paraformaldehyde and 1% formaldehyde solution as described [20].

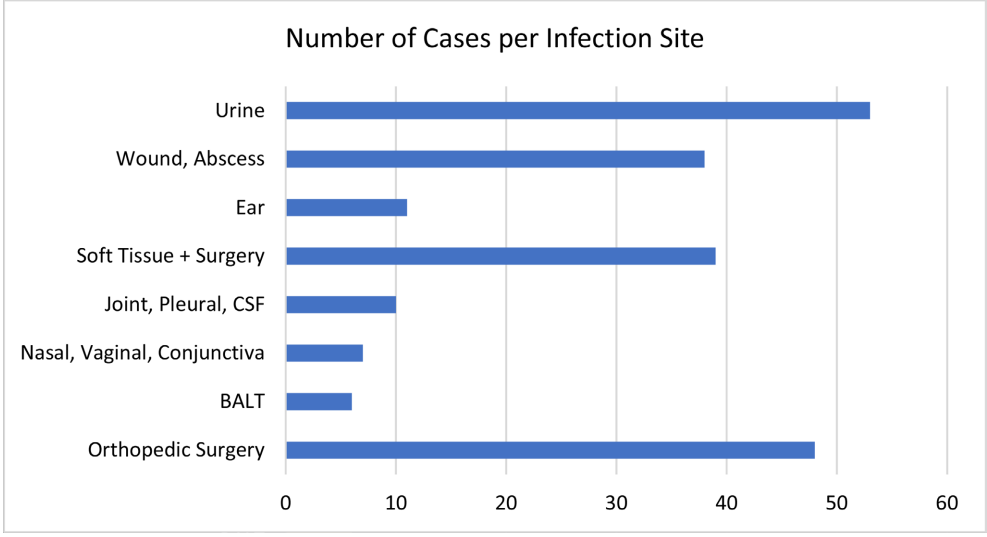
Statistical Analysis

The experiments were performed independently 3 times and the GraphPad-PRISM version 8.0 was used for analysis. The results are presented as mean +/− standard deviation from three biological experiments, unless otherwise indicated. The significance level was determined by using Student's t-test or ANOVA. A p-value of <0.05 was considered statistically significant.

3. Results

Antibiotic Susceptibility

A total of 213 isolates of *S. pseudintermedius* were submitted from 2016-2020 from 159 animals. Two species were represented, with 153 dogs sampled and 6 cats sampled. An almost equal distribution between males and females (48% and



CSF: Cerebrospinal fluid; BALT: Bronchoalveolar lavage.

Figure 1. Number of cases per infection site, from January 2016 to December 2020.

52%) was observed, with 71% of males castrated and 66% of females spayed. Among all of the cases examined, 40.9% were associated with a surgical site incision or hardware (screws, TPLO plates, stents), 25% were associated with a urine culture, 17.9% were associated with a non-surgical wound site, and 9% of isolates had an unidentified etiology. Of all the patients examined in this study, 75.2% had previously undergone a surgical procedure within the year. Out of 213 isolates, 45% were methicillin-resistant strains of *S. pseudintermedius.*

The susceptibility to a number of antibiotics was determined. As seen in Table 1, the percentage of resistance to commonly used antibiotics is shown, a total of 213 isolates from 159 animals. Among, 159 were dogs, 6 were cats. The distribution

Table 1. Percentage of Antimicrobial resistance of *S. pseudintermedius* isolates from animal sources.

Site	Orthopedic Surgery	BALT	Nasal/Vaginal Conjunctiva	Joint/Pleura	Soft tissue Surgery	Ear	Wound Abscess	Urine
Number of cases	48	6	7	10	39	11	38	53
Amp	62	83	57	60	69	54	68	64
Pen	81	83	100	80	84	91	82	77
Amox.	46	33	14	30	38	54	29	38
TMP/S.	52	50	71	40	41	82	26	48
Cefov	35	36	14	30	33	54	37	40
Cefpo	37	33	0	10	33	45	29	34
Ceph	31	33	0	10	31	27	24	28
Chlora	19	17	0	30	21	27	16	19
Clinda	40	17	14	50	31	64	21	ND
Enro	38	17	14	30	33	45	24	34
Marbo	35	17	14	30	26	45	16	30
Oxa	29	34	0	10	31	45	24	28
Genta.	33	17	14	40	28	27	32	28
Amik	6	0	0	0	5	9	5	9
Tobra	27	17	0	10	26	27	26	23
Tetra	51	50	29	30	33	45	26	34
Azyth	0	100	0	100	90	100	75	91
Ceftar	8	0	ND	0	0	0	0	0
Erythr	ND	100	ND	ND	ND	ND	67	ND
Rif	7	0	ND	0	0	0	0	0

Abbreviations: Amp: ampicillin; Pen: Penicillin; Amox/cla: amoxicillin/clavulanic acid; TPM/sulfa: trimethoprim/sulfamethoxazole, Cefov: cefovecin; Cefpo: cefpodoxime; Ceph: cephalotin; Chlora: chloramphenicol; Clinda: clindamycin; Enro: enrofloxacin; Marbo: marbofloxacin; Oxa: oxacillin; Genta: gentamycin; Amik: amikacin, Trobra: tobramycin; Tetra: tetracycline; Azith: azithromycin; Ceftar: ceftaroline; Erytho: erythromycin; Rif: Rifampin. ND: Not Done; BALT: Bronchial alveolar lavage.

by sex was 48% males and 52% females. Out of the patients, 75.2% has undergone anesthesia within a year. Remarkably, 45% of the isolates were methicillin resistant (MRSP).

Environmental Samples

Of the six locations swabbed in the ICU, 5 bacterial isolates were sequenced (4 *S. aureus* and 1 *Curtobacterium*). Of the fifteen locations swabbed in the anesthesia prep room, 20 bacterial isolates were sequenced (5 *S. aureus,* 3 *Micrococcus luteus*, 2 each *of A. gandavensis, Bacillus spp., Bacillus megaterium, and Bacillus pumilus* and 1 each *of Bacillus subtilis, Nocardiopsis terrae, Roseomonas mucosa,* and a *S. pseudintermedius*). The isolated *S. pseudintermedius* was then submitted to the Microbiology diagnostic laboratory for antibiotic resistance and susceptibility testing.

Resistance to Disinfectant

According to the product description, Accel Ready to Use (RTU) solution (1:40 dilution) should be killing off all of the bacteria within 1 minute, and the Dilute solution within 5 minutes. Of the seven bacteria tested, both the Dilute and RTU disinfectants killed at least 99% of both *S. pseudintermedius* strains and one strain of *S. aureus*. The other strain of *S. aureus* proved resilient with only 45.7% killed at the 5 minute time point for RTU, and 24% killed at the 10

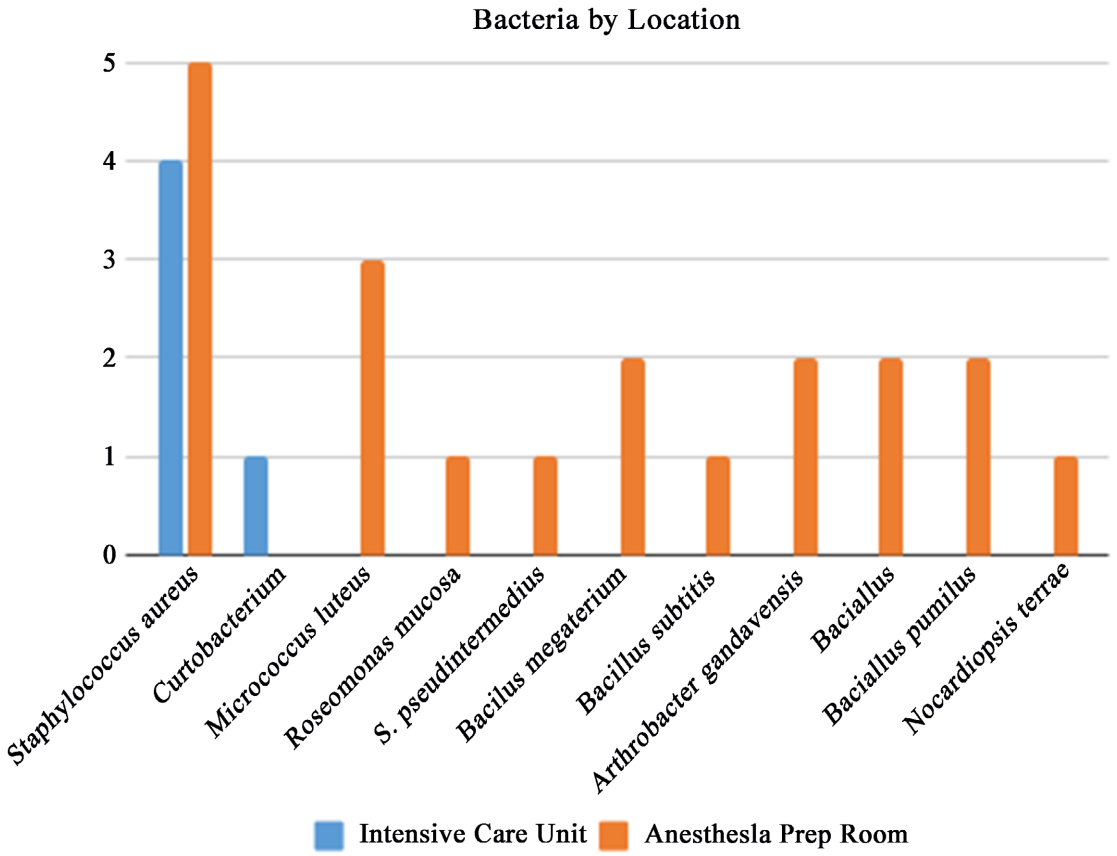


Figure 2. Number and species of bacteria isolated by two hospital locations (critical care and pre-anesthesia areas).

minute time point for Diluted disinfectant. Similarly, *N. terrae* showed no appreciable decrease in numbers until the 5 minute RTU (79.8% killed) and 10 minute Dilute (33.6% killed) time points. Only 25.9% of *A. gandavensis* was killed off in RTU 1 minute wells, and 53.4% in the RTU 5 minute wells. In the Dilute wells, 41.9% and 67.5% were killed off in the 5 minute and 10 minute wells respectively.

The determination if a strain of *S. pseudintermidius*, obtained from the hospital environment, was resistant to antibiotics, we evaluated the antibiotic susceptibility. Table 2 shows that the strain was methicillin-resistant, but was susceptible to many other compounds.

Biofilm:

S. pseudintermedius is known to form biofilm in the environment, much like *S. aureus*. To determine whether *S. pseudintermedius* can establish biofilm, and if those biofilms in the condition used were more or less robust than biofilms established by *S. aureus*, we compared the bacteria abilities. As shown in Table 3, *S. pseudintermidius* strains were more efficient in biofilm formation then *S. aureus*, under the conditions used. Electron micrographs of the biofilms, appear

Table 2. Susceptibility of Environmental obtained *S. pseudintermedius* to antibiotics.

Antibiotic	MIC (mg/mL)	Comment
Azithomycin	1	Susceptible
Doxycycline	3	Susceptible
Amoxicillin/Clavulinate	3	Resistant
Amikacin	6	Susceptible
Cefovecin	_	Resistant
Enrofloxacin	> 24	Resistant
Gentamicin	> 48	Resistant
Penicillin	> 12	Resistant
Trimethoprim/Sulfa.	> 12	Resistant
Tetracycline	32	Resistant
Oxacillin	_	Resistant

Table 3. Biofilm formation by two different strains of *S. pseudintermedius* and *S. aureus* on plastic surface. Biofilm was allowed to form for 4 days in broth, and then the biofilm mass was stained and quantified as described in methods. The results were generated in at least 3 different experiments. In other assays, bacteria were added to the plastic surface and were allowed to dry.

	Blank Control	*S. aureus*	*S. pseud* D1	*S. pseud* DS1
Broth OD	0.169 ± 0.027	2.501 ± 0.493	3.902 ± 0.038*	3.945 ± 0.025*
Dry OD	0.156 ± 0.031	0.731 ± 0.186	0.904 ± 0.211*	0.978 ± 0.194*

*$p < 0.05$ compared to the biomass of *S. aureus* biofilm.

to indicate that biofilm formed by *S. pseudintermedius* is not exactly similar to biofilm formed by *S. aureus*. In addition, biofilm were allowed to for in a dry surface, in order to verify the ability to for biofilms in hospital surfaces (Figure 3). As shown in Table 3, biofilm formed in presence of broth has a significant greater biomass than biofilm formed in a dry surface.

4. Discussion

S. pseudintermedius is a commensal bacterium routinely found in dogs, and can

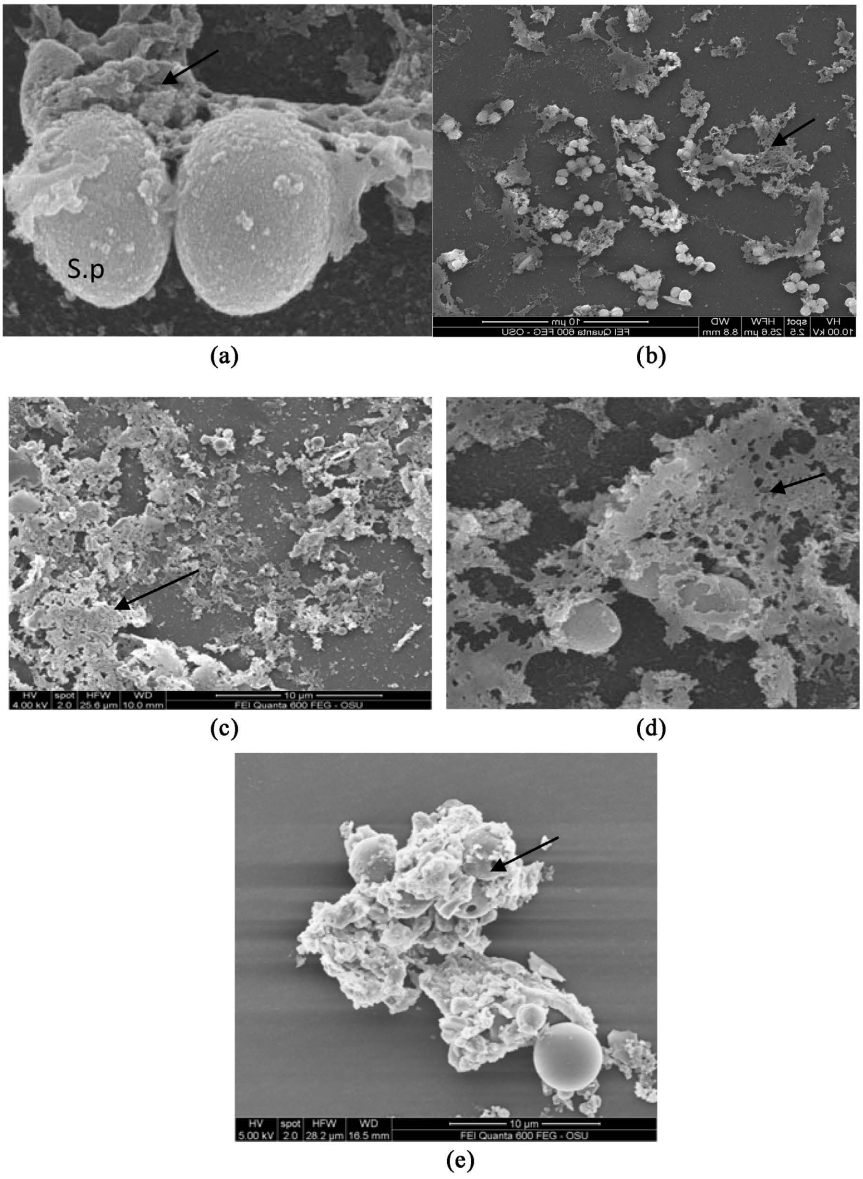


Figure 3. Scanning electron micrographs of *S. pseudintemedius* ((a) and (b)) and *S. aureus* ((c) and (d)) biofilms at 24 hours and 72 hours respectively. In (a), a high magnification of *S. speudintermedius* (S.p) attached to biofilm. In (b), one can observe many S.p and biofilm on a surface. In (c) and (d) *show S. aureus* biofilm and bacteria, which are amplified in the image (d). In (e), the image shows a biofilm of *S. pseudintermedius* in dry surface. The black arrows point to biofilm matrix

become an opportunistic pathogen, causing infections within a hospital setting [1] [4] [21]. In dogs, *S. pseudintermedius* is a common agent associated with canine pyoderma [1] [7] [21] [22]. In addition, the bacterium can be frequently isolated from wound and ear infections [7] [8] [9]. It is important to consider that *S. pseudintermedius* very likely evolved with the canine host, and therefore is well adapted to the conditions existing in the host [1] [2].

Due to increased stress in patients and the high amount of hands-on work performed on the patients in the hospital, besides being exposed to a strange environment, hospitalized animals are increasingly susceptible to pathogens which colonize their own skin or survive in the hospital environment. For example, it has been reported that up to 74% of infections isolated from surgical sites can be attributed to *Staphylococcus* species [9] [14] [23], from which the great majority of cases were associated with *S. pseudintermidius* [7] [11].

The current study, looking at infections in a veterinarian hospital in the last 5 years, demonstrated that 34% of the reported *S. pseudintermedius* infections at the OSU VTH were associated with surgery incisions or metals introduced in the animal (TPLO plates, screws, stents etc.). In addition, 75.2% of patients that had cultured positive for *S. pseudintermedius* had undergone a previous anesthetic procedure (within one year), suggesting a possible correlation between invasive procedure, prior exposure to hospital environment and infection with the bacterium. Also, of interest, was the large number of urinary tract infections identified in this population of patients. *S. pseudintermedius* is known to colonize the perineum region, and that association might correlate with the infection, once the animal has developed some degree of immunosuppression [24]. One important characteristic of the pathogen is the resistance to many of the available antibiotics, inducing veterinarians to use recent and more potent medication to treat the infections. Past studies have reported an increased resistance of *S. pseudintermedius* to antibiotics [14] [24], and as shown in **Table 1**, in this epidemiological surveillance, only amikacin, rifampin and ceftaroline were effective in vitro against 90% or more of the isolated strains. It is also of note that cephalotin, a first generation cephalosporin, commonly used for surgery prophylaxis, showed to be inactive against approximately 30% of the isolates from surgical infections. Finally, is paramount to realize that an antibiotic used in humans to treat MRSA, such as TMP/SMX, had very low percent of activity against strains of *S. pseudintermidius* [23] [25].

Veterinary hospital related infections usually are a combination of host susceptibility and well adapted pathogens. We do not know much about the pathogenic aspects of *S. pseudintermedius*. Recent reports showed that the bacterium expresses two surface proteins, SpsD and SpsO which have being shown to participate in the adherence to dog's keratinocytes by binding to host fibronectin [26] [27]. In addition, the ability to form biofilm has been reported to be greater than the ability of *S. intermedius* to establish biofilm [13] [23]. In fact, in our investigation it was clear that *S pseudintermedius* was significantly more effi-

cient at forming biofilm the clinical isolate (from a dog) of *S. aureus*. The results indicated that the biofilms of *S. pseudintermedius* and *S. aureus* are also different, as the images of the scanning microscopy suggest. The findings also demonstrate that *S. pseudintermedius* can be very effective in forming biofilms in dry surfaces, which may explain how some of the infection can be associated with the physical environment. In addition, the observation also supports the isolation of *S. pseudintermedius* from the hospital sites. In fact, the bacterium was isolated from the pre-anesthesia room, although when tested regarding the susceptibility to the disinfectant used in the hospital, the isolates were shown to be killed. This observation raise a couple possibilities, lie the protocol is not been followed as expected or the disinfectant is not been applied to the surfaces as frequent as needed. Those aspects will require additional study.

Human cases of *S. pseudintermedius* infection have been reported [7] [28], although colonization of dogs that share the home with humans, as far is now known, does not commonly leads to owner colonization or infection by *S. pseudintermedius* [13] [29]. Human cases are usually associated with skin infection or ear infection, and the fact that the organism is capable of colonizing the skin of humans, a potential source of hospital infection can be associated with human transmission. The observation that dogs may be colonized by different strains of *S. pseudintermedius* in different body sites, and that carriers of the bacterium can be an important source of infection, establishes another layer of complexity to the understanding of the epidemiology of the infection [30]. Certainly, the development of a rapid test, to aid the decision of which animals should receive preventive measures prior to the procedure, may be a necessary development. The observation that prophylactic use of measures in humans have led to a significant decrease in hospital infections by *S. aureus* [31], may have similar application in dogs. The percent of *S. pseudintermedius* causing infections in the veterinary hospital has increased over the years, as the number of isolates that showed resistance to a large number of antibiotics [9].

A limitation of this study is that only a few veterinary hospital-associated strains were investigated, although the pattern of the results was similar among the strains.

In summary, this study shows that clinical strains of *S. pseudintermedius* are well-suited to survive in the hospital environment, and be capable of infect hospital veterinary patients. In addition, the pattern of isolates' susceptibility is clearly becoming a concern, with increased resistant to many antibiotics. Future studies addressing approaches to control the infection are warranted.

5. Conclusion

The epidemiologic study of infections caused by *S. pseudintermedius* in a veterinary hospital setting confirmed that the pathogen, a common colonizer of dog skin and mucosal areas, is also capable living on surfaces in the hospital. Over the years, this characteristic has been associated with increase of antibiotic resis-

tance, and the ability to cause surgical infections in which host tissue biofilm occurs, that are very difficult to treat.

6. Simple Summary

The incidence of hospital infections caused by *Staphylococcus pseuditermedius* has increased significantly. The study investigated the infections caused by the bacterium for the last 5 years, and identified surgeries as the most common site of infection. Because *S. pseudintermedius* is a common bacterium encountered in skin and mucosal surfaces of dogs, we examined if the bacteria would be able to form robust biofilms in the environment of the hospital, as well as the most common hospital locations where it could be found. It was determined that in the last 5 years, there was an increased resistance of *S. pseudintermedius* isolates to antibiotics and that the bacterium can be cultured from surfaces in the hospital environment. The data suggests that the development of more efficacious approaches to prevent the infections is needed.

Participation

SM: performed assays, analyzed data, wrote the paper: JB: performed assays, analyzed data and wrote the paper, JJ: performed assays, wrote the paper, interpreted the data; SS: performed assays, interpreted the data; ALP: performed assays, wrote the paper, LEB: designed the study, performed assays, analyzed the data, wrote the paper.

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Institutional Review Board

Not required, since the study only uses clinical laboratory data.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Bannoehr, J. and Guardabassi, L. (2012) *Staphylococci pseudintermedius* in the Dog: Taxonomy, Diagnostics, Ecology, Epidemiology and Pathogenicity. *Veterinary Dermatology*, **23**, 253-266. https://doi.org/10.1111/j.1365-3164.2012.01046.x

[2] Rota, A., Milani, C., Drigo, I., Drigo, M. and Corrò, M. (2011) Isolation of Methicillin-Resistant *Staphylococcus pseudintermedius* from Breeding Dogs. *Theriontology*, **75**, 115-121. https://doi.org/10.1016/j.theriogenology.2010.07.016

[3] Carroll, K.C., Burnham, C.A.D. and Westblade, L.F. (2021) From Canines to Humans: Clinical Importance of *Staphylococcus pseudintermedius*. *PLOS Pathogens*, **17**, e1009961. https://doi.org/10.1371/journal.ppat.1009961

[4] Lynch, S.A. and Helbig, K.J. (2021) The Complex Diseases of *Staphylococcus pseudintermidius* in canines: Where to Next? *Veterinary Sciences*, **8**, Article 11. https://doi.org/10.3390/vetsci8010011

[5] Hanselman, B.A., Kruth, S.A., Rousseau, J. and Weese, J.S. (2009) Coagulase Positive Staphylococcus Colonization of Humans and Their Household Pets. *Canadian Veterinary Journal*, **50**, 954-958.

[6] Menandro, M.L., Dotto, G., Mondin, A., Martini, M., Ceglie, L. and Pasotto, D. (2019) Prevalence and Characterization of Methicillin-Resistant *Staphylococcus pseudintermedius* from Symptomatic Companion Animals in Northern Italy: clonal Diversity and Novel Sequence Types. *Comparative Immunology, Microbiology and Infectious Diseases*, **66**, Article ID: 101331. https://doi.org/10.1016/j.cimid.2019.101331

[7] Nienhoff, U., Kadlec, K., Chaberny, I.F., Verspohl, J., Gerlach, G.F., Kreienbrock, L., Schwarz, S., Simon, D. and Nolte, I. (2011) Methicillin-Resistant *Staphylococcus pseudintermedius* among Dogs Admitted to a Small Animal Hospital. *Veterinary Microbiology*, **150**, 191-197. https://doi.org/10.1016/j.vetmic.2010.12.018

[8] Pires Dos Santos, T., Damborg, P., Moodley, A. and Guardabassi, L. (2016) Systematic Review on Global Epidemiology of Methicillin-Resistant *Staphylococcus pseudintermedius*: Inference of Population Structure from Multilocus Sequence Data. *Frontiers in Microbiology*, **7**, Article 1599. https://doi.org/10.3389/fmicb.2016.01599

[9] Shoen, H.R.C., Rose, S.J., Ramsey, S.A., de Morais, H. and Bermudez, L.E. (2019) Analysis of *Staphylococcus* Infections in a Veterinary Teaching Hospital from 2012 to 2015. *Comparative Immunology, Microbiology and Infectious Diseases*, **66**, Article ID: 101332. https://doi.org/10.1016/j.cimid.2019.101332

[10] Han, J.I., Yang, C.-H. and Park, H.-M. (2015) Emergence of Biofilm-Producing *Staphylococcus pseudintermedius* Isolated from Health Dogs Is South Korea. *Veterinary Quarterly*, **35**, 207-210. https://doi.org/10.1080/01652176.2015.1096429

[11] Singh, A., Walker, M., Rouseau, J. and Weese, J.S. (2013) Characterization of the Biofilm Forming Ability of *Staphylococcus pseudintermedius* from Dogs. *BMC Veterinary Research*, **9**, Article No. 93. https://doi.org/10.1186/1746-6148-9-93

[12] Kumar, A., Alam, A., Rani, M., Ehtesham, N.Z. and Hasnain, S.E. (2017) Biofilms: Survival and Defense Strategy for Pathogens. *International Journal of Medical Microbiology*, **307**, 481-489. https://doi.org/10.1016/j.ijmm.2017.09.016

[13] Velázquez-Guadarrama, N., Olivares-Cervantes, A.L., Salinas, E., Martínez, L., Escorcia, M., Oropeza, R. and Rosas, I. (2017) Prevalence of Environmental Coagulase-Positive Staphylococci, Their Clonal Relationship, Resistance Factors, and the Ability to Form Biofilm. *Revista Argentina de Microbiología*, **49**, 15-23. https://doi.org/10.1016/j.ram.2016.08.006

[14] Pompilio, A., De Nicola, S., Crocetta, V., Guarnieri, S., Savini, V., Carretto, E. and Di Bonaventura, G. (2015) New Insights in *Staphylococcus pseudintermedius* Pathogenicity: Antibiotic-Resistant Biofilm Formation by a Human Wound-Associated Strain. *BMC Microbiology*, **15**, Article No. 109. https://doi.org/10.1186/s12866-015-0449-x

[15] Bannoehr, J., Ben Zakour, N.L., Reglinski, I.N.F., *et al.* (2011) Genomic and Surface Proteomic Analysis of a Canine Pathogen *Staphylococcus pseudintermedius* Reveals Proteins that Mediate Adherence to the Extracellular Matrix. *Infection and Immunity*, **79**, 3074-3086. https://doi.org/10.1128/IAI.00137-11

[16] Little, S.V., Bryan, L.K., Hillhouse, A.E., Cohen, N.D. and Lawhon, S.D. (2019)

Characterization of *agr* Groups of *Staphylococcus pseudintermedius* Isolates from Dogs in Texas. *mSphere*, **4**, e00033-19. https://doi.org/10.1128/mSphere.00033-19

[17] Danelishvili, L., Amrstrong, E., Miyasako, E., Jeffrey, B. and Bermudez, L.E. (2020) Exposure of *Mycobacterium avium subsp homonissuis* to Metal Concentrations of the Phagosome Environment Enhances the Selection of Persistent Subpopulation of Antibiotic Treatment. *Antibiotics*, **9**, Article 927. https://doi.org/10.3390/antibiotics9120927

[18] Murray, P.R., Phaler, A., Tenover, F.C. and Yolken, R.H., Eds. (2007) Manual of Clinical Microbiology. 9th Edition, ASM, Washington DC.

[19] Rose, S. and Bermudez, L.E. (2016) Identification of Bicarbonate as the Trigger and Genes Involved in the Export of eDNA by Pathogenic *Mycobacterium avium*. *mBio*, **7**, e01597. https://doi.org/10.1128/mBio.01597-16

[20] Sangari, F., Goodman, J. and Bermudez, L.E. (2000) Ultrastructural Study of *Mycobacterium avium*_Infection of HT-29 Human Intestinal Epithelial Cells. *Journal of Medical Microbiology*, **49**, Article ID: 139. https://doi.org/10.1099/0022-1317-49-2-139

[21] Gonzalez-Martin, M., Corbera, J.A., Suarez-Bonnet, A. and Teljedor-Junco, M.T. (2020) Virulence Factors in Coagulase Negative Staphylococci of Veterinary Interest other than *Staphylococcus aureus*. *Virulence*, **40**, 118-131. https://doi.org/10.1080/01652176.2020.1748253

[22] Tabatabaei, S., Najafifar, A., Askari, M., Zahraei, T., Ashrafi, I., Khaksar, E., Abbassi, M.S. and Ghazisaeedi, F. (2019) Genetic Characterization of Methicillin-Resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in Pets and Veterinary Personnel in Iran: New Insights into Emerging Methicillin-Resistant *S. pseudintermedius*. *Journal of Global Antimicrobial Resistance*, **16**, 6-10. https://doi.org/10.1016/j.jgar.2018.08.022

[23] Garbacz, K., Zarnowska, S., Piechowicz, L. and Haras, K. (2013) Pathogenicity Potential of *Staphylococcus pseudintermedius* Strains Isolated from Canine Carriers and Dogs with Infected Signs. *Virulence*, **4**, 255-259.

[24] Rubin, J.E. and Gaunt, M.C. (2011) Urinary Tract Infection Caused by Methicillin-Resistant *Staphylococcus pseudintermidius* in a Dog. *Canadian Veterinary Journal*, **52**, 162-164.

[25] Windahl, U., Gren, J., Holst, B.S. and Borjesson, S. (2016) Colonization with Methicillin-Resistant *Staphylococcus pseudintermedius* in Multi-Dog Households: A Longitudinal Study Using Whole Genome Sequencing. *Veterinary Microbiology*, **189**, 8-14. https://doi.org/10.1016/j.vetmic.2016.04.010

[26] Pietrocola, G., Gionotti, V., Richards, A., Nobile, G., Geoghengan, J.A., Rindi, S., Monk, I.R., *et al.* (2015) Fibronectin Binding Proteins SpsD and SpsL both Support Invasion of Canine Epithelial Cells by *Staphylococcus pseudintermedius*. *Infection and Immunity*, **83**, 4093-4102. https://doi.org/10.1128/IAI.00542-15

[27] Diribe, O., Thomas, S., AbuOun, M., Fitzpatrik, N. and La Ragione, R. (2015) Genotypic Relatedness and Characterization of *Staphylococcus pseudintermedius* Associated with Post-Operative Surgical Infections in Dogs. *Journal of Medical Microbiology*, **64**, 1074-1081. https://doi.org/10.1099/jmm.0.000110

[28] Starlander, G., Borjesson, S., Gronlund-Andersson, U., Tellgren-Roth, C. and Melhus, A. (2014) Cluster of Infectious Caused by Methicillin-Resistant *Staphylococcus pseudintermedius* in Humans in a Tertiary Hospital. *Journal of Clinical Microbiology*, **52**, 3118-3120. https://doi.org/10.1128/JCM.00703-14

[29] Somayaji, R., Rubin, J.E., Priyantha, M.A.R. and Church, D. (2016) Exploring *Sta-*

phylococcus pseudintermedius: An Emerging Zoonotic Pathogen? Editorial. *Future Microbiology*, **11**, 1371-1374. https://doi.org/10.2217/fmb-2016-0137

[30] Kalra, S., Amin, A., Albert, N., Cadwell, C., Edmonson, C., Gaynes, R., Hand, M., Marinella, M., Morely, C., Sieck, S. and Vasudevan, R.S. (2021) Stethoscope Hygiene: A Call to Action. Recommendations to Update the CDC Guidelines. *Infection Control & Hospital Epidemiology*, **42**, 740-742. https://doi.org/10.1017/ice.2021.115

[31] Kluytmans, J.A.J.W. and Wertheim, H.F.L. (2005) Nasal Carriage of *Staphylococcus aureus* and Prevention of Nosocomial Infections. *Infection*, **33**, 3-8. https://doi.org/10.1007/s15010-005-4012-9