ESTONIAN UNIVERSITY OF LIFE SCIENCES
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THE OCCURRENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS* SPP. AND *ACINETOBACTER* SPP. IN THE ENVIRONMENT OF SMALL ANIMAL CLINICS IN ESTONIA

METITSILLIINIRESISTENTSETE STAFÜLOKOKKIDE JA ATSINETOBAKTERITE ESINEMINE VÄIKELOOMAKLIINIKUTE KESKKONNAS EESTIS

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Abstract of Final Thesis

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Healthcare-associated infections (HAIs) are frequently associated with methicillin-resistant staphylococci (MRS) and *Acinetobacter*. The veterinary clinical environment is considered to have a key role in the spread of nosocomial pathogens among small animal patients. In 2017-2018, 358 surface samples from 23 Estonian small animal clinics were collected to describe the occurrence of MRS and *Acinetobacter* in the clinic environment. In addition, biosafety methods implemented by each clinic were described through a questionnaire.

22.9% of the samples collected were positive for either MRS or Acinetobacter. MRS was isolated from samples of 17 clinics and *Acinetobacter* from samples of 15 clinics. Out of 22 MRS samples tested for antimicrobial susceptibility, resistance was most commonly observed against fusidic acid (72.2%), ciprofloxacin (68.8%), and tetracycline (68.8%). In total, 15 samples were multi-drug resistant. Proportionally, the procedure room and reception had the most positive bacterial samples. The implementation of biosafety methods varied between clinics and only 9 clinics out of 23 had biosafety protocol in place. Biosafety protocols are needed to be implemented in veterinary facilities to ensure appropriate infection control.

Keywords: methicillin-resistant staphylococci, acinetobacteria, small animal clinics

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Haigla ehk nosokomiaalsed infektsioonid on sagedasti põhjustatud metitsilliinresistentsete stafülokokkide (MRS) või atsinetobakterite poolt. Väikeloomakliinikute keskkonda on samuti peetud üheks nosokomiaalinfektsioonide tekkekohaks, mille kaudu võivad mikroobid levida väikeloomadest patsientidele. Käesoleva uurimistöö eesmärgiks oli hinnata MRS ja atsinetobakterite esinemust eesti väikeloomakliinikute keskkonnas ning kirjeldada kliinikutes kasutuses olevaid bioturvalisuse meetmeid.

Kokku uuriti 2017.-2018. aastal 23st väikeloomakliiniku keskkonnast 358 pinnaproovi, kus 22,9% proovidest olid positiivsed kas MRS või atsinetobakterite suhtes. MRS isoleeriti kokku 17st ja atsinetobaktereid 15st väikeloomakliinikust. Antibiootikumitundlikkus määrati 22le MRS isolaadile. Isoleeritud MRS olid resistentsed fusidiinhappe (72.2%), tsiprofloksatsiini (68.8%) ja tetratsükliini (68.8%) suhtes. Multiresistentseid isolaate leiti 15. Kõige enam positiivseid proove leiti protseduuritubadest ning retseptisoonist. Bioturvalisuse meetmed erinesid kliinikute lõikes suurel määral. Üheksal kliinikus 23st olid koostatud bioturvalisuse protokollid väikeloomakliiniku jaoks.

Märksõnad: metitsilliinresistentsed stafülokokid, atsinetobakterid, väikeloomakliinikud

TABLE OF CONTENTS

LIST OF ABBREVIATIONS **6**

INTRODUCTION **7**

1. LITERATURE REVIEW **9**

1.1 Methicillin-resistant opportunistic pathogens **10**

1.1.1. *Staphylococcus* spp. 10

1.1.2. *Acinetobacter* spp. 10

1.2 The development of resistance and description of resistance mechanisms **11**

1.3 The effect of drug resistance on human health **13**

1.4 Hospital-associated infections in the small animal clinic **13**

1.5 Risk factors associated with nosocomial infections in small animal clinics **15**

1.5.1 Environmental factors 16

1.5.2 Veterinary personnel 16

1.5.3 Use of antimicrobial agents 16

1.6 Biosafety methods in small animal medicine **17**

1.7 Conclusions of literature review **18**

2. AIMS OF THE STUDY **19**

3. MATERIALS AND METHODS **20**

3.1 Data management and agreement of study **20**

3.2 Collection of study materials **20**

3.2.1 Procedure of sample collection from the clinic environment 20

3.2.2 Questionnaire 21

3.2 Laboratory analysis of study materials **21**

3.2.1 Isolation and identification of methicillin-resistant staphylococci 21

3.2.2 Isolation and identification of *Acinetobacter* spp. 22

3.2.3 Antimicrobial susceptibility testing of staphylococci 22

3.3 Statistical analysis 23

4. RESULTS 24

4.1 Distribution of methicillin-resistant staphylococci and *Acinetobacter* spp. in the environment of the small animal clinic 24

4.2 Description of positive samples collected from animal and human surfaces26

4.3 Antimicrobial susceptibility of methicillin-resistant staphylococci 27

4.4 Implementation of biosafety measures 28

..... 29

5. DISCUSSION 30

CONCLUSIONS 33

ACKNOWLEDGMENTS 34

REFERENCES 35

APPENDICES 48

Appendix 1. Questionnaire form 49

Appendix 2. Non-exclusive licence for depositing the final thesis and opening it for the public and the supervisor's (supervisors') confirmation for allowing the thesis for the defence 50

Appendix 3. Non-exclusive licence for depositing the final thesis and opening it for the public (restricted access) and the supervisor's (supervisors') confirmation for allowing the thesis for the defence 51

Appendix 4. Non-exclusive licence for depositing the final thesis (indefinite restriction) and the supervisor's (supervisors') confirmation for allowing the thesis for the defence 52

LIST OF ABBREVIATIONS

A. - *Acinetobacter*

ACB – *Acinetobacter calcoaceticus-A. baumannii*

ESBL – extended-spectrum beta-lactamase

HAI – healthcare associated infection

MDR – multi-drug resistance

MRS – methicillin-resistant staphylococci

MRSA – methicillin-resistant *Staphylococcus aureus*

MRSP – methicillin-resistant *Staphylococcus pseudintermedius*

PBP – penicillin-binding protein

S. – *Staphylococcus*

SCC*mec* – staphylococcal cassette chromosome *mec*

SSI – surgical site infection

VFL – Veterinary and Food Laboratory

INTRODUCTION

Healthcare-associated infections (HAIs) or nosocomial infections are frequently associated with colonisation with drug resistant pathogens (Francey *et al.*, 2000; Faires *et al.*, 2010; Stegmann *et al.*, 2010; Nazarali *et al.*, 2015; Hazard *et al.*, 2021). Infection with drug resistant bacteria has been linked with a high mortality among human patients (Kanyo *et al.*, 2021; Kharaba *et al.*, 2021; Matsushima *et al.*, 2021). Although similar findings have not been confirmed in animals, a retrospective study by Kuzi *et al.* (2016) noted a mortality rate of 70% in a population of 23 animals during a severe outbreak of drug-resistant *Acinetobacter calcoaceticus-A. baumannii* (ACB) complex bacteria. In addition, HAIs in small animal patients have frequently been associated with drug resistant pathogens (Francey *et al.*, 2000; Nazarali *et al.*, 2015; Raske *et al.*, 2015). According to Ruple-Czerniak *et al.* (2013) 16.3% of dogs and 12% of cats in critical care units in the small animal referral hospitals in the US develop nosocomial infections during their admission.

Colonisation with drug resistant pathogens among companion animals may pose a risk to human health (Stegmann *et al.*, 2010; Somayaji *et al.*, 2016). In a study by Somayaji *et al.* (2016), 92.1% of patients with confirmed *S. pseudintermedius* infection had had contact with dogs near the time of infection.

Environmental surfaces act as reservoirs for micro-organisms and have been identified as a major source of contamination in the veterinary clinic (Churak *et al.*, 2021). Murphy *et al.* (2010) attribute this to animal exploratory behaviour, and methods of management and housing. Isolates collected from surfaces in high traffic areas in contact with animals, such as floors, scales, kennels, and examination tables have yielded common nosocomial pathogens (Murphy *et al.*, 2010; Hamilton *et al.*, 2012; Feßler *et al.*, 2018). In addition, veterinary personnel have been recognised with a higher risk of drug resistant bacteria colonisation, more notably methicillin-resistant *S. aureus* (MRSA) (O'Mahony *et al.*, 2005; Loeffler *et al.*, 2010; Feßler *et al.*, 2018). Although direct transmission between small animal patients and veterinary staff has not been confirmed, Churak *et al.* (2021) emphasised the role of veterinary staff in the spread of pathogens within the clinic environment.

Among the most important nosocomial pathogens in veterinary medicine are considered to be methicillin-resistant staphylococci (MRS) and *Acinetobacter* spp. (Ishihara *et al.*, 2010; Kuzi *et al.*, 2016; Grönthal *et al.*, 2017; Unnerstad *et al.*, 2018). Most common MRS associated with HAIs are MRSA and *S. pseudintermedius* (MRSP), although other

drug resistant staphylococci, including *S. haemolyticus, S. epidermidis,* and *S. schleiferi* have been isolated from infection sites in small animal patients (Abraham *et al.*, 2007; Moon *et al.*, 2012; Detwiler *et al.*, 2013; Ruzauskas *et al.*, 2015). *Acinetobacter baumannii* has frequently been associated with nosocomial outbreaks and infections (Bou *et al.*, 2000; Francey *et al.*, 2000; Brachelente *et al.*, 2007; Nocera *et al.*, 2020). In addition, other species of *Acinetobacter* have been linked to HAIs in human hospitals (Lee *et al.*, 2013). Similar findings have been collected in Japan, where extended-spectrum beta-lactamase (ESBL) - producing *A. lwoffii* and *A. radioresistens* have been isolated from infections in small animals (Shimada *et al.*, 2012; Kimura *et al.*, 2017).

The aim of the study was to investigate the occurrence of methicillin-resistant staphylococci and *Acinetobacter spp.* in the environment of small animal clinics, to determine the phenotypic antimicrobial resistance of isolated strains, and to describe implementation of biosafety methods in small animal clinics in Estonia.

1. LITERATURE REVIEW

The main aim of this literature review is to describe the bacterial species and resistance mechanism causing healthcare-associated infections in small animal veterinary medicine and to analyse the impact on animal and human health.

Drug-resistant pathogens are a major challenge for human and veterinary medicine (Francey *et al.*, 2000; Stegmann *et al.*, 2010; Chuang *et al.*, 2011; Fitzpatrick *et al.*, 2015; Menezes *et al.*, 2022). Due to development of resistance mechanisms, the spectrum of action of antibiotics is limited. Pathogens may produce antibiotic inactivating enzymes (Lee *et al.*, 2005), efflux system decrease the concentration of antibiotics within the cell (Costa *et al.*, 2019), amino acid codon mutations may affect the attachment of antibiotics and mutations on the outer membrane proteins inhibit drug influx (Catel-Ferreira *et al.*, 2011). These resistance mechanisms are encoded for by genes, which may be transferred between pathogens (Oppegaard *et al.*, 2001; Ribera *et al.*, 2003).

Healthcare-associated infections (HAIs) or nosocomial infections are associated with medical procedures, such as surgical-site infections (SSIs), urinary catheter site infections, or central venous catheter site infections (Francey *et al.*, 2000; Faires *et al.*, 2010). There are various risk factors affecting development of HAIs, such as hygiene, length of stay in hospital, but most notably colonisation with drug resistant pathogens provide a challenge to healthcare as infections which previously have been successfully treated may not respond to antimicrobial therapy (Bergström *et al.*, 2012; Grönthal *et al.*, 2017; Churak *et al.*, 2021). Methicillin-resistance is one of the most notable contributors to serious illness leading to high mortality as resistance to methicillin implies resistance to other beta-lactam drugs (Cosgrove *et al.*, 2003; Velasco *et al.*, 2005). In contrast to HAIs, communal infections with pathogens are considered to occur due to pressure outside of the healthcare environment (Klevens *et al.*, 2006).

Drug resistant pathogens are of special concern in veterinary medicine, as the human-animal contact has been associated with a risk of transmission of pathogens and associated resistance coding genes between strains and species (Oppegaard *et al.*, 2001; Guardabassi *et al.*, 2004; Stegmann *et al.*, 2010; Paul *et al.*, 2011; Price *et al.*, 2012; Somayaji *et al.*, 2016). More so, animals colonised with drug resistant pathogens may prove to be a health risk for immunosuppressed owners (Stegmann *et al.*, 2010; Somayaji *et al.*, 2016). In small animal medicine, colonisation with drug resistant pathogens has been associated with previous

treatment with antimicrobial drugs, previous hospitalisation, and contact with veterinary personnel (Paul *et al.*, 2011; van Duijkeren *et al.*, 2011; Lehner *et al.*, 2014).

Most common pathogens associated with HAIs in veterinary medicine are *Staphylococcus* spp., *Acinetobacter* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecium*, which all have presented with drug resistance to varying degrees (Seguin *et al.*, 1999; Brachelente *et al.*, 2007; Andrade *et al.*, 2016; Salavati *et al.*, 2018; Churak *et al.*, 2021). In this current study, we will focus on methicillin-resistant bacteria *Staphylococcus* spp. and *Acinetobacter* spp.

1.1 Methicillin-resistant opportunistic pathogens

Methicillin-resistance is considered to be indicative of resistance to other beta-lactam antibiotics (Velasco *et al.*, 2005) and is associated with increased mortality (Cosgrove *et al.*, 2003). Both *Staphylococcus* spp. and *Acinetobacter* spp. are associated with methicillin-resistance (Diancourt *et al.*, 2010; Moon *et al.*, 2012; Price *et al.*, 2012; Maboni *et al.*, 2020).

1.1.1. Staphylococcus spp.

Staphylococci are clustering Gram-positive, nonmotile, non-spore forming facultatively anaerobic cocci that classified in two main groups, coagulase-positive and coagulase-negative (Thorberg and Brändström, 2000; Sasaki *et al.*, 2010). Most commonly *Staphylococcus (S.) aureus* and *S. pseudintermedius* are associated with methicillin resistance and nosocomial infections, however various other staphylococcal species have been identified with resistance to beta-lactam antibiotics, such as *S. epidermidis* and *S. schleiferi* (Griffeth *et al.*, 2008; Moon *et al.*, 2012; Couto *et al.*, 2016). *S. aureus* is a known human pathogen and plays a role in human HAIs and food contamination issues (Cosgrove *et al.*, 2003; Unnerstad *et al.*, 2018). *S. pseudintermedius* is considered to be part of the common flora of the small animal mucous membranes and skin and is often identified as a source of skin and wound infections (Loeffler *et al.*, 2007; Beck *et al.*, 2012). Staphylococci are important nosocomial and zoonotic pathogens (Stegmann *et al.*, 2010; van Duijkeren *et al.*, 2011; Somayaji *et al.*, 2016).

1.1.2. Acinetobacter spp.

Acinetobacter is a genus of Gram-negative bacilli (Nemec *et al.*, 2011). *Acinetobacter (A.) baumannii* is considered an emerging nosocomial pathogen and has been identified with

multidrug resistance (Fitzpatrick *et al.*, 2015; Elbehiry *et al.*, 2021). In addition to *A. baumannii*, two closely related genomic species *A. pittii* (3) and *A. nosocomialis* (13TU) have been associated with HAIs and outbreaks (Fitzpatrick *et al.*, 2015; Lee *et al.*, 2013). Together with *A. calcoaceticus* these *Acinetobacter* species are called "*A. calcoaceticus-A. baumannii* complex" (ACB complex) due to the phenotypic similarities (Nemec *et al.*, 2011). However, only *A. baumannii* strains have been associated with multi-drug resistance and worse clinical outcome (Fitzpatrick *et al.*, 2015; Lee *et al.*, 2013). *Acinetobacter* are identified as biofilm-forming environmental pathogens, which is thought to contribute to the virulence (Antunes *et al.*, 2011). *A. baumannii* is considered to have flagella-independent motility (Skiebe *et al.*, 2012). According to Skiebe *et al.* (2012), *A. baumannii* isolates are motile on wet surfaces due to manipulation and polyamine synthesis.

1.2 The development of resistance and description of resistance mechanisms

The methods of antibiotic resistance in both *Staphylococcus* spp. and *Acinetobacter* spp. are numerous, including enzymes involved with inactivating antibiotics, efflux pumps, amino acid codon mutations and changes in outer-membrane protein production (Villegas-Estrada *et al.*, 2008; Catel-Ferreira *et al.*, 2011; Costa *et al.*, 2019). Efflux systems decrease drug concentrations within the bacterial cell by expulsion (Costa *et al.*, 2019). Mutations in the outer-membrane proteins alter the membrane permeability which leads to inhibition of drug influx (Catel-Ferreira *et al.*, 2011).

Development of resistance is considered to be the result of mutations, selective pressure, and transfer of genes (Oppegaard *et al.*, 2001; Ribera *et al.*, 2003; Price *et al.*, 2012). In addition, research by Price *et al.* (2012) suggests that transfer of bacteria between species may potentiate development of resistance due to adaptation. Genes can be transferred across bacteria species, which is a major concern with the development of multi-drug resistant bacteria (Ribera *et al.*, 2003). A ribosomal protection gene, *tet*M, isolated from *A. baumannii* has been identified to be homologous to the *S. aureus* gene and may present transfer of resistance mechanisms between the two pathogens (Ribera *et al.*, 2003).

The presence of the *mec*A gene located on staphylococcal cassette chromosome *mec* (SCC*mec*) elements has been associated with resistance to beta-lactam antibiotics (Couto *et al.*, 2016). The penicillin-binding protein (PBP) 2a is encoded by *mec*A and despite beta-lactam antibiotics inactivating native PBPs, the *mec*A gene expression allows the cell wall

synthesis to continue (Villegas-Estrada *et al.*, 2008). The *mec*A gene can be transferred between different species of staphylococci. The *bla*Z gene has been identified to encode a beta-lactamase and is found as a part of the *bla* operon (Couto *et al.*, 2016). The *bla* operon is widely spread among Gram-positive bacteria (Couto *et al.*, 2016). In addition to afore mentioned, *S. aureus* contains the *nor*A efflux system, which is associated with fluoroquinolone and disinfectant resistance (Costa *et al.*, 2019). Perreten *et al.* (2010) identified two major clonal MRSP lineages isolated, ST71-J-t02-II-III in Europe and ST68-C-t06-V in North America. SCC*mec* typing has revealed five types (II – III, III, IV, V and VII-241) and two non-typeable cassettes (Perreten *et al.*, 2010).

Acinetobacter baumannii has been associated with epidemic spread and outbreak strains are often multidrug resistant, including resistance to penicillins and cephalosporins, often aminoglycosides and fluoroquinolones (Sheikhalizadeh *et al.*, 2017). Most recently, carbapenem and colistin resistance has been identified (Zhang *et al.*, 2017). *A. baumannii* strain typing has recognised clones responsible for hospital outbreaks (Diancourt *et al.*, 2010). Pathogenic strains have been distinguished by comparing cell envelope protein profiling, ribotyping and AFLP genomic fingerprinting (Diancourt *et al.*, 2010). These strains are divided into three major groups international clones I, II and III (Diancourt *et al.*, 2010). Diancourt *et al.* (2010) found that these three major clones comprised almost exclusively multi-drug resistant strains. Beta-lactam resistance has been identified to be due to beta-lactamase production, which is seen in all *A. baumannii* strains due to chromosomal genes encoding beta-lactamase (Smiline *et al*., 2018). In addition, extended-spectrum beta-lactamases *bla*TEM, *bla*SHV and *bla*CTX-M have been isolated from *A. baumannii* strains (Smiline *et al*., 2018). More recently, carbapenemases have been isolated from *A. baumannii*, including metallo-beta-lactamases (MBLs) which are broadly potent and inactivate all beta-lactam class antibiotics (Lee *et al.*, 2005). In addition, *A. baumannii* present nonenzymatic mechanisms of beta-lactam resistance, including efflux pumps, changes in membrane porin channels and alteration in the PBPs (Bou *et al.*, 2000).

Tetracycline class resistance is mediated via efflux pumps and ribosomal protection mechanisms. Efflux pumps in *A. baumannii* are divided into two categories, the resistance-nodulation-division, which is a non-specific pump and has affinity to aminoglycosides, chloramphenicol, erythromycin, fluoroquinolones, tetracyclines, trimethoprim and some beta-lactams – and *tet*A and *tet*B gene encoded, which are associated with plasmids, transposons and integrons (Fernando *et al*., 2013). *Tet*A is associated with resistance to tetracyclines and *tet*B with resistance to minocycline (Fernando *et al*., 2013).

Fluoroquinolone resistance in *A. baumannii* is considered to be primarily due to mutations in the fluoroquinolone-resistance determining regions of the target enzymes DNA gyrase and DNA topoisomerase IV (Park *et al.*, 2011). In addition, efflux systems and decreased production of outer-membrane proteins have been identified to affect the susceptibility to fluoroquinolones (Park *et al.*, 2011).

1.3 The effect of drug resistance on human health

According to a recent surveillance report by WHO Regional Office for Europe/European Centre for Disease Prevention and Control (2022), antimicrobial resistance among nosocomial pathogens remains a serious threat to human health. Nosocomial infections with MRSA have been linked with high mortality (Kanyo *et al.*, 2021; Matsushima *et al.*, 2021). *A. baumannii* infection has been frequently associated with outbreaks in the intensive care unit globally and especially mechanical ventilation posed a risk for infection (Ayobami *et al.*, 2019; Kharaba *et al.*, 2021). Although information is limited, it is suggested that HAI with drug resistant pathogens occurs to a degree in paediatric neonatal patients (Lee *et al.*, 2021; Weiner-Lastinger *et al.*, 2020). In a study by Brown *et al.* (2019), a nurse in contact with neonatal patients in the intensive care unit was a source of methicillin-resistant *S. aureus* (MRSA) infection. Spread of drug resistant *Acinetobacter* and staphylococci occurs both through HAI and communal infections (Moore *et al.*, 2009; Kim *et al.*, 2018). In MRSA, nosocomial infections have been associated with higher mortality and severe illness than community acquired (Klevens *et al.*, 2006; Moore *et al.*, 2009). In comparison, communal *A. baumannii* was associated with wider drug susceptibility in comparison to healthcare acquired strains (Chen *et al.*, 2018; Kim *et al.*, 2018). However, Chen *et al.* (2018) noted increased mortality in community acquired infections.

1.4 Hospital-associated infections in the small animal clinic

Healthcare-associated infections, sometimes called nosocomial infections, are associated with medical procedures. HAIs are present only after treatment at hospital or clinic and should not be present before arrival. Healthcare-associated infections are a rising concern, not only in human medicine but also veterinary medicine. HAIs have been associated with the spread of drug resistant opportunistic pathogens and often occur during outbreaks. In veterinary medicine, the most common HAI are surgical site infections, urinary catheter site infections and central venous catheter site infections (Francey *et al.*, 2000; Faires *et al.*,

2010). Methicillin-resistant staphylococci (MRS) as well as extended spectrum beta-lactamase (ESBL) -producing *Enterobacteriaceae* and *Acinetobacter baumanii* are most recently associated with healthcare-associated infections in veterinary medicine (Holm *et al.*, 2002; Faires *et al.*, 2010; Tjon-A-Tsien *et al.*, 2010; Endimiani *et al.*, 2011; van Duijkeren *et al.*, 2011; Salavati *et al.*, 2018; Nocera *et al.*, 2020).

MRS has been identified as a cause of HAI in veterinary hospitals internationally and most commonly MRS infections have been associated with *S. aureus* and *S. pseudintermedius* (Holm *et al.*, 2002; Loeffler *et al.*, 2007; Bergström *et al.*, 2012), although other strains have been identified with methicillin resistance, such as *S. epidermidis* (Moon *et al.*, 2012). According to a study in South Korea, *S. pseudintermedius* was recognised with the highest multidrug resistance rate, followed by *S. epidermidis* and S. *warneri* (Moon *et al.*, 2012). Multidrug-resistance was mostly associated with methicillin-resistance (Moon *et al.*, 2012). There appears to be differences in risks affecting infections with MRSA and methicillin-resistant *S. pseudintermedius* (MRSP) in small animals (Soares Magalhães *et al.*, 2010; Lehner *et al.*, 2014). In a study by Bergström *et al.* (2012), MRSP colonisation has been associated with hospital admission. The findings are similar to other studies where the effect of environmental pressure on MRSP colonisation risk has been identified (van Duijkeren *et al.*, 2011; Lehner *et al.*, 2014). According to Lehner *et al.* (2014), MRSP may be well adapted to the canine skin and colonise with little selective pressure, though more research is needed. In contrast, MRSA infections in small animals have been linked to previous treatments with antimicrobial drugs (Soares Magalhães *et al.*, 2010). In addition, animals with MRSA infections may have had previous contact with humans who have had previous admission in hospitals (Soares Magalhães *et al.*, 2010).

Acinetobacter baumannii has been frequently isolated from HAI sites during outbreaks in small animal hospitals, such as urogenital tracts, infected wounds, and respiratory tracts of intensive care unit patients (Francey *et al.*, 2000). Infections with *A. baumannii* have been associated with both systemic and focal illness in small animals (Francey *et al.*, 2000; Brachelente *et al.*, 2007). Infections with A. baumannii are commonly associated with environmental contact with the pathogen and the pathogen has been cultured from the environment during outbreaks. However recent findings may indicate a potential transfer between humans and animals (Nocera *et al.*, 2020). Treatment of *A. baumannii* infections have posed a challenge due to the multidrug resistance presented by the strain (Francey *et al.*, 2000).

Although MRSP is most commonly associated with HAI in small animals, studies have found MRSA to be isolated from infections in canine, feline, and equine patients (Vincze *et al.*, 2014; Raske *et al*., 2015). *S. aureus* is not considered to be naturally occurring bacteria of canines and felines. Thus, contraction of *S. aureus* is considered to be due to a "spill-over" effect from human medicine. Spill-over is of significant concern due to the possibility of transfer of resistance coding genes between staphylococci in human and animal contact (Oppegaard *et al.*, 2001; Price *et al.*, 2012). In multiple studies, veterinary staff have been identified as potential reservoirs for methicillin-resistant staphylococci and suspected to be source of infection of hospitalised animals (Seguin *et al.*, 1999; O'Mahony *et al.*, 2005; Worthing *et al.*, 2018). More so, Paul *et al.* (2011) identified MRSP carriage in veterinary dermatologists, suggesting a potential occupational hazard to veterinarians. In addition, a major concern is the transfer of resistance mechanisms between pathogens, which could result in reduced spectrum of use of antibiotics (Ribera *et al*., 2003). The safety risks relating to antimicrobial resistance in the veterinary environment is considered to be presents not only to the hospitalised animal patients but veterinary personnel and animal owners as well (Paul *et al.*, 2011; Han *et al*., 2016). There are cases of reported transfer of antimicrobial resistant bacteria between animals and their owners (Guardabassi *et al*., 2004; Weese *et al.*, 2006; Stegmann *et al.*, 2010; Somayaji *et al.*, 2016). Especially dermatological patients and their owners have been linked with transmission of drug resistant pathogens (Guardabassi, *et al*., 2004). Of special concern, human patients with MRSP isolated infections have been linked with previous contact with small animals (Stegmann *et al.*, 2010; Somayaji *et al.*, 2016).

1.5 Risk factors associated with nosocomial infections in small animal clinics

Although there is little data about drug-resistant pathogens and prevention in veterinary medicine, recently multiple studies have been performed to track MRSA, MRSP and ESBL in major veterinary hospitals around the world (Seguin *et al.*, 1999; Moon *et al.*, 2012; Stull *et al*., 2012; Grönthal *et al.*, 2017; Worthing *et al.*, 2018). Risk factors affecting the drug resistant pathogen colonisation within a veterinary clinic setting are considered to be primarily related to the hospital environment and veterinary personnel (Tjon-A-Tsien *et al.*, 2010; Churak *et al.*, 2021), although other factors, such as previous treatment with antibiotics play a role in contracting MDR bacteria (Faires *et al.*, 2010; Grönthal *et al.*,

2017). There are recorded instances of potential outbreaks of HAI (Boerlin *et al.*, 2001; Brachelente *et al.*, 2007; Endimiani *et al.*, 2011), whereas formalised training to control outbreaks may be limited among veterinary staff (Benedict *et al.*, 2008).

1.5.1 Environmental factors

Environmental surfaces act as reservoirs for several micro-organisms and has been identified as a source of contamination within the veterinary environment and to a limited extent in human medicine (Churak *et al.*, 2021). Murphy *et al.* (2010) postulate animal behaviour (*i.e.*, exploration), management and housing have an effect on the relevance of environmental contamination. In a study by Murphy *et al*. (2010), isolates collected from kennels, floors, tables, and isolation units yielded opportunistic pathogens, including *E. coli*, *C. difficile*, MRSA and MRSP. Hamilton *et al.* (2012) identified similar sites of high traffic likely to isolate opportunistic pathogens, specifically scales and treatment floors. The effectiveness of daily cleaning and sanitations plays a key role in removal of pathogens from the environment (Bastos *et al.*, 2021; Churak *et al.*, 2021). Boerlin *et al.* (2001) suggest that methods of control may need to vary based on suspected pathogen. According to the study, infections with *A. baumannii* were controlled with thorough cleaning, whereas infections with *S. pseudintermedius* persisted despite cleaning (Boerlin *et al.*, 2001).

1.5.2 Veterinary personnel

Churak *et al.* (2021) identified veterinarians and other veterinary staff to play an important role in the spread of pathogens within the clinic environment. Multiple studies indicate that veterinary personnel are at a higher risk of drug resistant bacteria colonisation (O'Mahony *et al.*, 2005; Loeffler *et al.*, 2010; Morris *et al.*, 2010; Boost *et al*., 2011; Feßler *et al.*, 2018). More so, companion animals of veterinary personnel have been associated with a high colonisation risk (Morris *et al.*, 2010; Worthing *et al.*, 2018). Studies have provided varying results on the pathogen species and genetic structure isolated from veterinary personnel and their companion animals and the relationship between the colonisations is unclear (Worthing *et al.*, 2018), however possibility of a cycle of colonisation and recolonisation cannot be ruled out.

1.5.3 Use of antimicrobial agents

Use of antimicrobial drugs, specifically beta-lactams and fluoroquinolones has been linked to MRS carriage in canine patients (Faires *et al.*, 2010; Soares Magalhães *et al.*, 2010;

Grönthal *et al.*, 2017). In addition, dermatological referral patients have been associated with higher rates of infection with MRS pathogens (Loeffler *et al.*, 2007; Bryan *et al.*, 2012). Bryan *et al.* (2012) suggest this may be due to primary treatment of antibiotics without testing for antibiotic sensitivity. Holm *et al.* (2002) identified repeated courses of antibiotic treatment to be related with increased antimicrobial resistance in *S. pseudintermedius* isolated from deep pyoderma of canine patients. However, Lehner *et al.* (2014) suggest that MRSP may need little selective pressure for colonisation of canine skin.

There are several both national and international guidelines on prudent antimicrobial use, although implementation of these guidelines are under individual discretion (Hopman *et al.*, 2018; Jessen *et al.*, 2018). According to Hopman *et al.* (2018), the reasons behind antimicrobial use by veterinarians is multifactorial and often influenced by other factors beyond theory.

1.6 Biosafety methods in small animal medicine

Although there are no specific requirements for the biosafety methods implemented in small animal clinics, infection control methods have been described by institutions such as American Animal Hospital Association and Ontario Animal Health Network (Stull *et al.*, 2018; Anderson *et al.*, 2020). Stull *et al.* (2018) emphasise the importance of implementing infection control protocol regardless of clinic size or type. Furthermore, a staff member should oversee the implementation of the protocol and identify areas of improvement (Anderson *et al.*, 2020). Proper hand hygiene, use of personal protective equipment and checklists of key tasks are focal in infection prevention (Stull *et al.*, 2018; Anderson *et al.*, 2020).

Despite aforementioned recommendations, in a survey by (Murphy *et al.*, 2010) none of the participating 101 small animal practices in Southern Ontario had an infection control protocol. Survey participants described aspects of biosafety protocols implemented in the clinic suggesting a basic understanding of infection control (Murphy *et al.*, 2010). However, Weese *et al.* (2012) postulate that compliance with infection control may be reliant on clearly stated protocol. Furthermore, in a study by Benedict *et al.* (2008), formalised training on infection control was limited among personnel in veterinary teaching hospitals, which may affect the ability of veterinary personnel to manage and adapt to HAI outbreak conditions (Murphy *et al.*, 2010).

Surveillance is recognised as a valuable method to evaluate the effectiveness of infection control (Stull *et al.*, 2018). Fluorescent tagging has been commonly used to assess cleaning and disinfection of surfaces in veterinary hospitals (Weese *et al.*, 2012; Langdon *et al*., 2020; Hunter *et al.*, 2021). In their study, Weese *et al.* (2012) identified human contact surfaces as areas commonly neglected by routine cleaning. The finding is further corroborated by Langdon *et al.* (2020). Surveillance of microbial contamination may be conducted by bacterial culture, although the method does not identify fungal and viral contamination (Weese *et al.*, 2012; Hunter *et al.*, 2021). In a study by Hunter *et al.* (2021), bacterial cultures were used to evaluate the environmental *Staphylococcus* spp. contamination over a period of 6 months in a new veterinary hospital. The study identified highest portion of staphylococcal contamination to coincide with a period of reduced cleaning compliance, although a direct correlation could not be established (Hunter *et al.*, 2021).

1.7 Conclusions of literature review

Antimicrobial resistance is a threat to the current methods of bacterial infection management within healthcare and recent discoveries underline the role of veterinary medicine in prevention and control of MDR pathogens. Pathogens carry genes which potentiate the transfer of resistance mechanisms between different strains, species, and genus. More so, the zoonotic potential of MDR pathogens is of special concern. There are notable instances of infection in humans with drug resistant pathogens of animal origin.

The veterinary clinic as an environment may act as a vector for notable pathogens in veterinary medicine, including MRSA, MRSP, MDR *A. baumannii* and ESBL *E. coli*. More so, veterinarians and veterinary personnel are at a risk of not only carrying but transmitting pathogens to animal patients. There is still limited data on the occurrence of drug-resistant bacteria within the veterinary clinic environment. However, there are studies suggesting that environmental control has an effect on spread of drug resistant pathogens. Many studies focus on veterinary teaching hospitals, where referral patients previously treated with antimicrobials have been identified at a risk of carrying drug-resistant bacteria. In order to understand the risks in small animal veterinary medicine, it is necessary to perform studies with private small animal clinics, where animal patients often receive first-hand care.

2. AIMS OF THE STUDY

The aim of this study is to analyse the occurrence of methicillin-resistant *Staphylococcus* spp. and *Acinetobacter* spp. in the environment of small animal clinics in Estonia and determine the phenotypic antimicrobial resistance of isolated strains. In addition, to describe the implementation of biosafety measures in small animal clinics in Estonia.

3. MATERIALS AND METHODS

3.1 Data management and agreement of study

The study was conducted in Estonia during 2017-2018 by the PhD student Aleksandr Dedjulja. Initially, 36 small animal clinics were approached with written proposal to participate in the study. The information of clinics was collected by using the personal communication of Estonian Small Animal Association. In total, 23 small animal clinics (63.8%) agreed to participate, representing approximately 25% of the total number of clinics in Estonia at the time. Before the study was conducted, a confidentiality agreement between the representative of research group and the representative of the clinic was signed.

3.2 Collection of study materials

3.2.1 Procedure of sample collection from the clinic environment

Environmental surface samples were collected after routine sanitation of the rooms and surfaces in accordance with the normal sanitation procedure of each clinic. Samples were collected in different rooms and equipment based on the characteristics and design of the clinic. Surfaces and equipment were categorised based on whether human or animal contact was primarily intended in use as presented in Table 1.

Sterile samples of surfaces were collected using commercial sponge wipes (*3M™ Hydrated-Sponges,* US*)* by using the maximal surface area of the wipe over the determined surface. After sample collection, the wipe was placed in a sterile package. Boot swab methods (*MDR Boot Swab,* UK) used in salmonella testing of poultry farms was used to collect environmental samples from the clinic floors. The entire clinic floor was walked through starting from the most sterile rooms, *i.e.*, surgery, towards the most contaminated areas. In addition, separate samples were taken from reception and surgery hall floor. Sampling boots were packed into sterile caps after use.

Each sample package contained the following information: clinic number, sampling date, and surface description. All collected samples were stored at 4-8°C and transported within 4-24 hours to the Estonian Veterinary and Food Laboratory (VFL) for bacteriological analysis.

Table 1. Classification of samples based on area and type of contact associated with each surface.

Room description	Sampling areas	Human/animal contact
Consultation room	Examination table	Animal
	Cabinets	Human
	Computer	Human
	Door	Human
	Faucet	Human
Imaging room	Computer	Human
	Ultrasound machine	Animal
	X-ray table	Animal
Isolation unit	Isolation cage	Animal
Operating room	Inhalation tubes	Animal
	Instrument desk	Human
	Operating table	Animal
	Walls	Neither
Procedure room	Computer	Human
	Door	Human
	Equipment	Human
	Examination table	Animal
	Faucet	Human
	Infusion stand	Human
Reception	Animal weight	Animal
	Computer	Human
	Desk	Human
	Phone	Human
	Seats	Animal/human
Ward	Cages	Animal
	Cage door	Animal
	Examination table	Animal
	Faucet	Human

3.2.2 Questionnaire

During the same visit as the sample collection, the clinic veterinarian or chief veterinary technician filled a questionnaire. The questionnaire included 25 questions about biosafety measures and hygiene procedures used on daily bases at the clinic. In addition, information on the main cleaning entity used by each clinic was collected. The questionnaire form is presented in supplementary files (Appendix 1).

3.2 Laboratory analysis of study materials

Isolation, identification of bacterial strains and antimicrobial susceptibility testing were performed in Estonian VFL by the veterinary microbiologist.

3.2.1 Isolation and identification of methicillin-resistant staphylococci

For isolation of methicillin-resistant staphylococci, *CHROMagar™ MRSA* (CHROMagar™, France) was used in accordance with the EURL-AR laboratory protocol (EURL-AR, 2018). Inoculated *CHROMagar™ MRSA* plates were incubated for 18-24 hours at 37°C. Presumptive MRS colonies were sub-cultured on blood agar and incubated for 24 hours at 35-37°C. The staphylococcal species was confirmed using the MALDI-TOF method.

3.2.2 Isolation and identification of Acinetobacter spp.

The isolation of *Acinetobacter spp.* was performed using the standard microbiological method (*MacConkey agar No 3 (CM0115B)*, Oxoid, UK). Plates were incubated for 22-24 hours at 35-37°C. Different species of *Acinetobacter spp.* were confirmed using MALDI-TOF methods.

3.2.3 Antimicrobial susceptibility testing of staphylococci

Broth microdilution method was used for the phenotypic antimicrobial susceptibility testing. was done by broth microdilution and the commercial microtiter plate panels *TREK EUST* (Sensi Titre, Thermofisher Scientific) were used. For interpretation of results for susceptibility testing of *S. aureus* and CNS, epidemiological cut-off values (ECOFF) issued by the EUCAST were used (Table 2).

Table 2. Antimicrobial agents and interpretation criteria of *S. aureus* and CNS collected for the small animal clinic environment

Antimicrobial agent	Breakpoint for resistance (µg/ml)	
	S. aureus	CNS
Clindamycin	≤0,25	≤0,25
Erythromycin	≤1	≤2
Tetracycline	≤1	≤1
Ciprofloxacin	≤1	≤1
Rifampin	≤0,032	≤0,5
Cefoxitin	≤4	NA
Streptomycin	≤16	≤16
Tiamulin	≤2	≤2
Linezolid	≤4	≤2
Fucidate	≤0,5	≤0,5
Quinupristin/ dalfopristin	≤1	≤2
Mupirocin	≤1	≤1
Penicillin	NA	NA
Vancomycin	≤2	≤2
Sulfamethoxazole	≤128	≤128
Chloramphenicol	≤16	≤8
Gentamycin	≤2	≤1
Kanamycin	≤8	≤8
Trimethoprim	≤2	≤4

The possible presence of methicillin resistance coding gene *mec*A was analysed by PCR in accordance with EURL-AR protocol (EURL-AR, 2012) at VFL molecular analysis department.

3.3 Statistical analysis

Different databases were created in MS Excel for further analysis. Descriptive statistics were done in MS Excel. The proportions of different species of staphylococci and *Acinetobacter* spp. with 95% CI were calculated. The association of methicillin-resistance (0 = no; 1 = yes) and human or animal surface was analysed by using chi-square test. All analysed isolates were categorized S (sensitive) and R (resistant) according to resistance cut-off value. The proportion of antibiotic resistance of MRS isolates with 95% CI were calculated.

4. RESULTS

4.1 Distribution of methicillin-resistant staphylococci and *Acinetobacter* spp. in the environment of the small animal clinic

In total, 358 surface samples were collected from 23 small animal clinics during 2017-2018. The median number of samples per clinic was 15.5, ranging between 10 samples at minimum and at most 17 samples collected per clinic. On average, 24.0% of the samples collected per clinic were positive, ranging between 0.0% at minimum and 69.2% positive samples per clinic.

Out of 358 samples, methicillin-resistant staphylococci were isolated in 54 (15.1%; 95% CI 11.5-19.2) and *Acinetobacter spp*. in 28 (7.8%; 95% CI 5.3-11.1) surface samples. MRS was isolated from samples of 17 clinics (73.9%) and *Acinetobacter* spp. from samples of 15 clinics (65.2%). In 2 clinics, no bacteria were isolated from collected samples. Eight staphylococcal species were identified, and among the most prevalent species were *S. haemolyticus* (46.2%), *S. epidermidis* (24.1%), *S. warneri* (9.3%), *S. sciuri* (5.6%) and *S. hominis* (Figure 1). Phenotypic MRSA was found in only four (7.4%) of the samples. The most common species of *Acinetobacter* was *A. lwoffii* (50%), followed by *A. radioresistens* (17.9%) and equal proportions (14.3%) of *A. schinderi* and *A. calcoaceticus*, respectively (Figure 2).

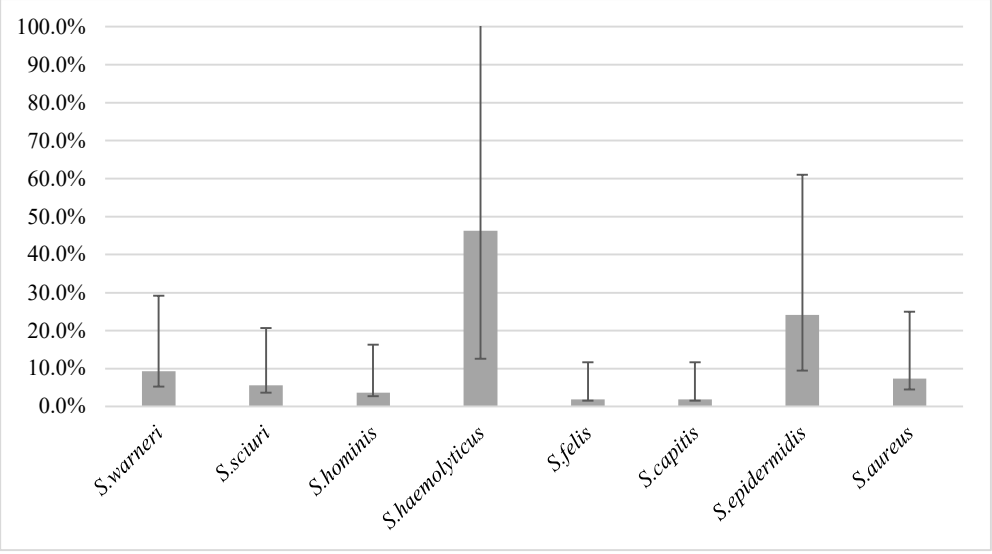


Figure 1. Proportion and 95% confidence interval of isolated phenotypic staphylococcal species. Proportion was calculated from the total of *Staphylococcus* spp. (n = 54) samples.

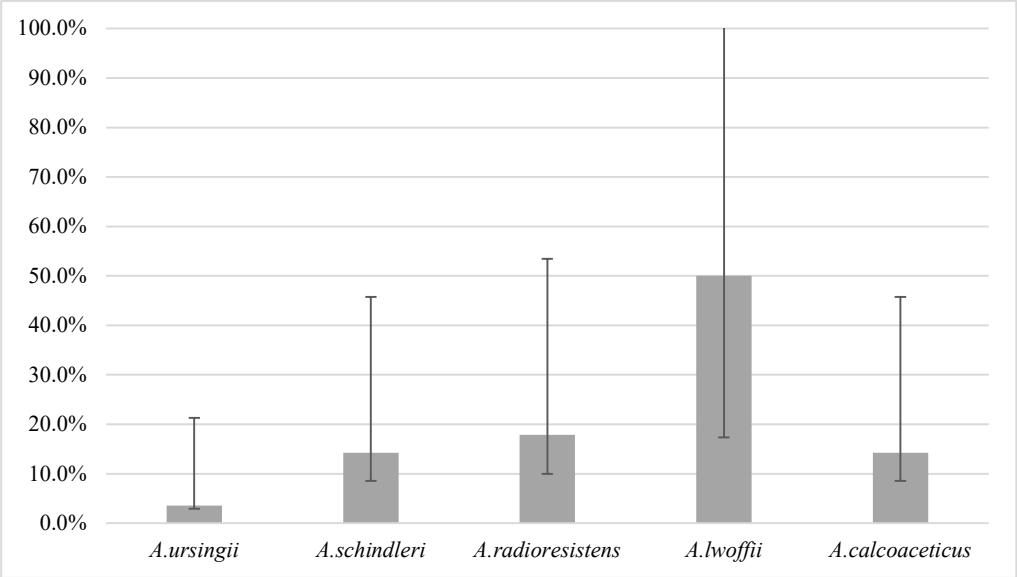
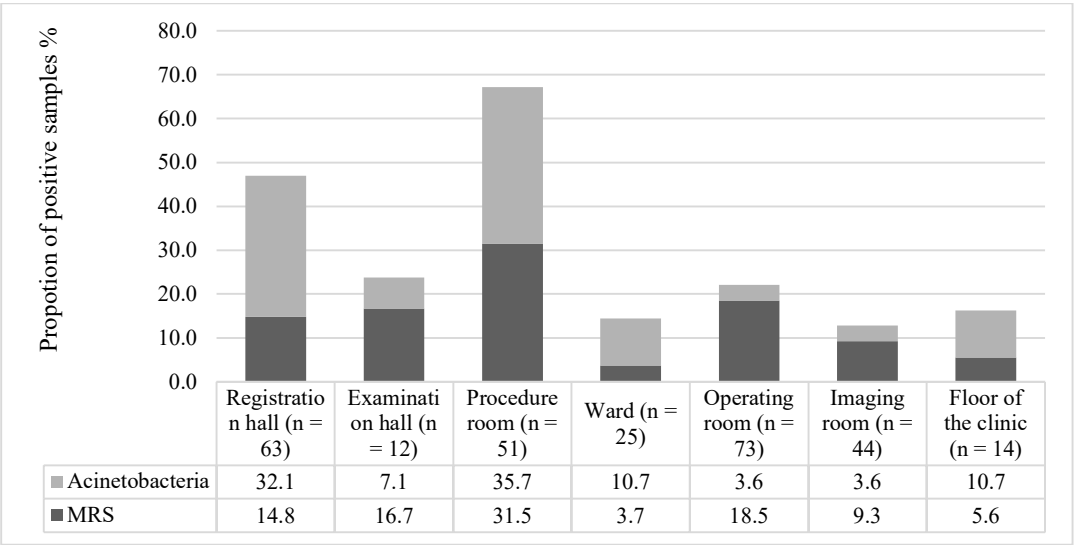


Figure 2. Proportion and 95% confidence interval of isolated phenotypic *Acinetobacter* species. Proportion was calculated from the total of *Acinetobacter* spp. (n = 28) samples.

The largest portion of MRS and *Acinetobacter* spp. positive samples were found among the samples collected from the procedure room (67.2%) and registration hall (46.9%), followed by examination room (23.8%) (Figure 3).



	Registration hall (n = 63)	Examination hall (n = 12)	Procedure room (n = 51)	Ward (n = 25)	Operating room (n = 73)	Imaging room (n = 44)	Floor of the clinic (n = 14)
Acinetobacteria	32.1	7.1	35.7	10.7	3.6	3.6	10.7
MRS	14.8	16.7	31.5	3.7	18.5	9.3	5.6

Figure 3. Distribution of positive samples according to sampling room. Isolation units did not yield any positive samples and are excluded.

4.2 Description of positive samples collected from animal and human surfaces

The distribution of MRS and *Acinetobacter* spp. positive samples according to surface contact type varied between sampling rooms (Figure 4). From the samples collected in the procedure room, 58.8% of the human contact surfaces and 41.2% of the animal contact surfaces were positive for MRS or *Acinetobacter* spp. In the operating room, human contact surfaces were negative for bacteria and 27.7% of the animal contact surfaces were positive for MRS or *Acinetobacter* spp.

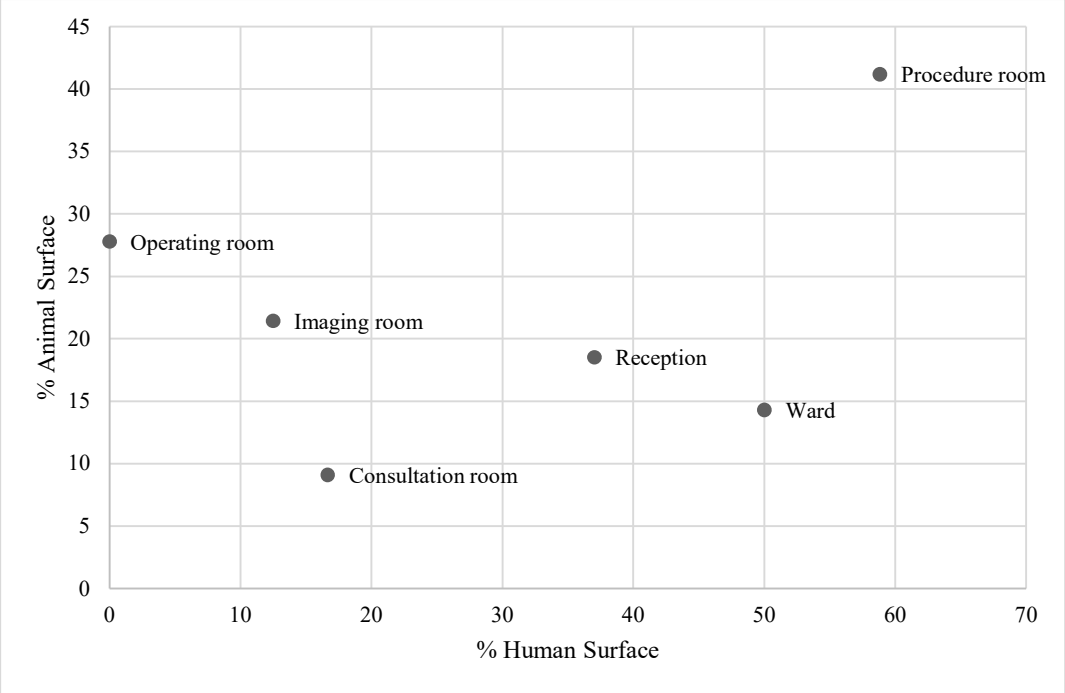


Figure 4. Distribution of MRS and *Acinetobacter* spp. positive samples according to surface type in each sampling room. Floor samples were excluded, as they represented both contact types.

The distribution of MRS was compared between human and animal surfaces with a chi-square test of independence on Excel. In total, 141 samples were defined as human surface areas and 169 animal surface areas. Floor samples (n= 48) were excluded, as they represented both contact types. MRS was isolated from 27 samples collected from human contact surfaces, and from 23 samples from animal contact surfaces. There was no statistically significant association ($\chi^2 = 1.74$, $p = 0.2$) between surface type and presence of MRS (Figure 5).

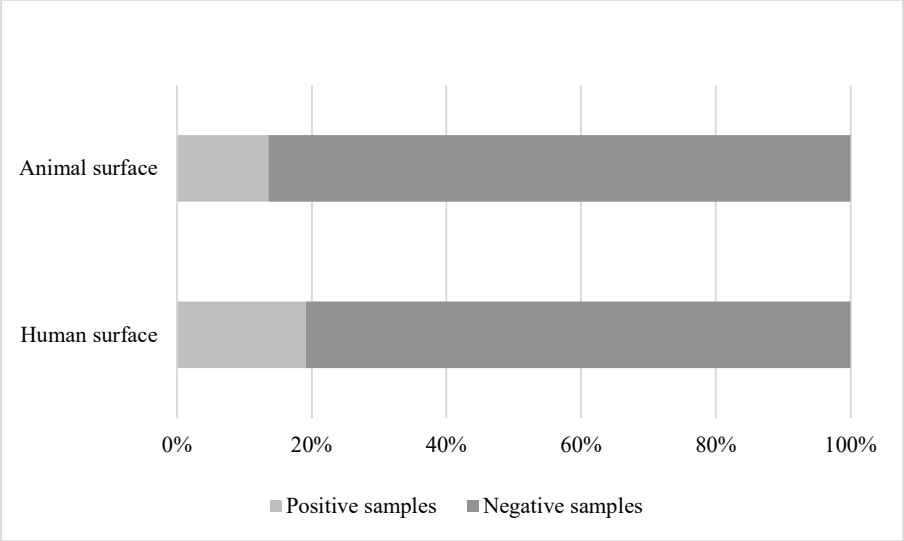


Figure 5. Distribution of MRS according to surface type. In total, 19.1% of the human surfaces were contaminated with MRS and 13.6% of the animal surfaces.

4.3 Antimicrobial susceptibility of methicillin-resistant staphylococci

Out of 22 methicillin-resistant staphylococci, the resistance to at least one antimicrobial was detected in 20 isolates (Table 3). Most commonly resistance was observed against fusidic acid (n=16; 72.2%), ciprofloxacin (n=15; 68.8%), and tetracycline (n=15; 68.8%). All isolates were susceptible to vancomycin, quinupristin/dalfopristin, chloramphenicol and mupirocin. In total, 15 isolates (68.2%) were multidrug-resistant.

Table 3. Antimicrobial resistance of methicillin-resistant staphylococci (n=22).

Antimicrobial	Cut-off values for resistance (mg/l)	% (95% CI) of resistant isolates
Erythromycin	>1	54.5 (0.322; 0.756)
Clindamycin	>0.25	50.0 (0.282; 0.718)
Gentamycin	>2	27.3 (0.107; 0.502)
Kanamycin	>8	22.7 (0.078; 0.454)
Streptomycin	> 16	31.8 (0.139; 0.549)
Fusidic acid	> 0.5	72.7 (0.498;0.839)
Chloramphenicol	>16	0
Tetracycline	>1	68.2 (0.451;0.861)
Ciprofloxacin	>1	68.2 (0.451;0.861)
Sulfamethoxazole *S. aureus* Non-aureus staphylococci	 > 128 > 256	45.5 (0.244; 0.678)
Rifampin *S. aureus* Non-aureus staphylococci	 > 0.03 > 0.064	0
Vancomycin *S. aureus* Non-aureus staphylococci	 > 2 > 4	0
Quinupristin/dalfopristin	> 1	0
Mupirocin	> 1	0

4.4 Implementation of biosafety measures

Out of the 23 clinics, 17 clinics (73.9%) used specialised companies, whereas 4 clinics (17.4%) used their own personnel for routine cleaning and two clinics (8.7%) used both. Only 9 clinics (39.1%) reported having a biosafety protocol. In addition, in 17 per cent of the clinics (n = 4), cleaning and disinfection of the consultation room does not occur after every patient according to their reporting. Questionnaire results are further described in Table 4.

Table 4. Description of questionnaire results (n = 23).

		At least once	More than once
1.	**How many times per day do you clean clinic rooms?**	2 (8.7%)	21 (91.3%)
		Yes	**No**
2.	**Do you clean and disinfect the consultation room after every patient?**	19 (82.6%)	4 (17.4%)
3.	**Do you use always gloves during clinical examination?**	15 (65.2%)	8 (34.8%)
4.	**Do you wash and disinfect hands after handling every patient?**	20 (87.0%)	3 (13.0%)
5.	**Do your personal staff use extra protective clothing during special procedures (wound infection, dental procedures, etc)?**	12 (52.2%)	11 (47.8%)
6.	**Do you clip hair before cannula insertion?**	16 (69.6%)	7 (30.4%)
7.	**Do you follow aseptic rules during cannula insertion?**	22 (95.7%)	1 (4.3%)
8.	**Do you disinfect mouthpiece after use?**	13 (56.5%)	10 (43.5%)
9.	**Do you have a special UV-light in the procedure room?**	11 (47.80%)	12 (52.2%)
10.	**Do you have a special room for preparing patients before surgery?**	18 (78.30%)	5 (21.7%)
11.	**Do you have UV-light or quartz light in the operating room?**	21 (91.30%)	2 (8.7%)
12.	**Do you replace UV-lights according to requirements?**	10 (43.50%)	13 (56.5%)
13.	**Do you use sterile equipment for dental procedures?**	17 (73.90%)	6 (26.1%)
14.	**Do you sterilize surgery instruments and additional materials?**	22 (95.70%)	1 (4.3%)
15.	**Do you use special boots in the operating room?**	5 (21.70%)	18 (78.3%)
16.	**Do you use different clothing during surgical operations?**	11 (47.80%)	12 (52.2%)
17.	**Do you use separate cleaning equipment for operating rooms?**	12 (52.20%)	11 (47.8%)
18.	**Are table surfaces in the examination, procedure, and operating rooms easy to clean and disinfect?**	18 (78.30%)	5 (21.7%)
19.	**Are animal cages easy to clean and disinfect?**	15 (65.20%)	8 (34.8%)
20.	**Do you have separate recovery room for post-operative patients?**	8 (34.8%)	15 (65.2%)
21.	**Do you have an isolation unit in the clinic?**	2 (8.7%)	21 (91.3%)
22.	**Do you have separate ward for cats?**	2 (8.7%)	21 (91.3%)
23.	**Do you reuse endotracheal tubes?**	14 (60.9%)	9 (39.1%)
24.	**Do you have a separate room for staff?**	19 (82.6%)	4 (17.4%)
25.	**Do you have protocol or biosafety regulation in your clinic?**	9 (39.1%)	14 (60.9%)

5. DISCUSSION

In this study, the occurrence of MRS was 15.1% and *Acinetobacter* spp. was 7.8% in the small animal clinic environment. These findings are similar in other studies (Hamilton *et al.*, 2012; Nakbubpa *et al.*, 2021). According to Nakbubpa *et al.* (2021), occurrence of *Acinetobacter* spp. was 6.0%. In a study by Hamilton *et al.* (2012), occurrence of MRS was 15.8%. In addition, this study identified various species of MRS and *Acinetobacter* in the clinical environment, including eight staphylococcal species and four *Acinetobacter* species. Most commonly isolated staphylococci include *S. haemolyticus*, *S. epidermidis* and *S. warneri*. These species have been noted as potential causes of infection in small animals and in human patients (Abraham *et al.*, 2007; Moon *et al.*, 2012; Detwiler *et al.*, 2013; Ruzauskas *et al.*, 2015; Lourtet-Hascoët *et al.*, 2018). Phenotypic MRSA was found in only four samples, representing 1.1% of all samples, and MRSP was not isolated at all. The result is unexpected, as similar studies have found MRSA in 8.6-9.0% of the surface samples and MRSP in 2-7% (Colleen P Murphy *et al.*, 2010; van Balen *et al.*, 2014; Bastos *et al.*, 2021). The recorded difference may be due to non-outbreak conditions at the time of sampling (Hunter *et al.*, 2021).

The results describe multidrug resistance to be commonly found in staphylococci isolated from the clinic environment. Most commonly resistance was observed against fusidic acid (72.2%), ciprofloxacin (68.8%) and tetracycline (68.8%). However, in other studies, the incidence of resistance to these antibiotics in MRS has been recorded to be lower. According to Hamilton *et al.* (2012), staphylococci samples collected from the environment yielded 10% resistance to ciprofloxacin and 15% resistance to tetracycline. Similarly, Couto *et al.* (2016) noted from clinical staphylococcal samples isolated from small animals, resistance to ciprofloxacin, tetracycline and fusidic acid were recorded in 12.3%, 34.8% and 4.1% of the samples, respectively. However, Grönthal *et al.* (2017) found a similar rate of resistance to tetracycline (74%) in *S. pseudintermedius* samples isolated from small animals in Finland.

In this study, the highest proportion of positive samples was collected from the procedure room (67.2%), followed by the reception (46.9%), whereas the isolation unit did not provide any positive samples. These results are similar to other studies, where areas of high traffic were associated with higher bacterial contamination, and areas where patients were most likely to contract HAIs were least contaminated (Feßler *et al.*, 2018; Hunter *et al.*, 2021). In studies evaluating effectiveness of cleaning, ICU appeared to be one of the

most thoroughly cleaned area, whereas reception and treatment areas were most neglected during cleaning (Weese *et al.*, 2012; Langdon *et al*., 2020). Although direct conclusions cannot be made with the available data, it is possible that cleaning methods and high traffic may have affected the bacterial contamination of surfaces. In addition, due to the versatile nature of the procedure room, it is likely to be frequently contaminated with various bodily fluids, such as blood, faecal matter, and saliva.

In our study, most clinics (73.9%) used external cleaning companies for routine cleaning, whereas only 9 clinics (39.1%) reported having biosafety protocols. In a study by Weese *et al.* (2012), the employment of cleaning companies and unclear guidelines on cleaning responsibilities was suspected to be the cause of improper cleaning of equipment and surfaces. Weese *et al.* (2012) postulate that without clear guidelines on cleaning responsibility, personnel may neglect cleaning and disinfection of some surfaces. The phenomenon was further observed by Hunter *et al.* (2021). The items and surfaces not listed in a routine cleaning list were most likely to be neglected and were most likely to be contaminated by staphylococci during an outbreak (Hunter *et al.*, 2021). In addition, in our study, 4 clinics (17%) did not clean and disinfect the consultation room between patients. However, according to the AAHA guidelines on infection control, animal-contact surfaces should be routinely cleaned and disinfected between patients (Stull *et al.*, 2018). Our study did not record further details on reasons behind the questionnaire results. A misinterpretation of the question is possible, or a clinic representative may have given an answer based on current personnel compliance rather than based on the cleaning protocol in use at given clinic. The findings may be due to the lack of knowledge on the routine protocol. In a study by Murphy *et al.* (2010), many veterinarians and veterinary technicians in charge of decision making within the clinic were not aware of cleaning or disinfection methods used at the practice. In addition, the time of routine cleaning within each clinic may have affected the sampling results. More so, as our study does not analyse the cleaning and disinfections methods instituted within each clinic, it is not possible to rule out insufficient cleaning and disinfection methods affecting the sampling results (Weese *et al.*, 2012; Langdon *et al.*, 2020).

The study provides evidence on the presence of MRS and *Acinetobacter* spp. in the veterinary clinic environment. In isolated strains, multidrug resistance appears to be common. In addition, the study describes preliminary information on biosafety methods instituted in small animal clinics in Estonia. However, due to the large amount of variables, conclusive data on correlation between individual variables was limited. The differences in

bacterial isolation between clinics and rooms may be affected by cleaning and disinfection time and methods, and the time of sampling. In addition, the questionnaire results are dependent on framing of the questions and the biosafety knowledge of the representative.

Future research is needed to describe variables affecting the occurrence of methicillin-resistant bacteria in the clinic environment. Comparison of environmental samples and samples collected from patients and personnel may provide more information on the relevance of antimicrobial resistance seen in the environmental staphylococci. In addition, studies involving fluorescent tagging of surfaces to evaluate effectiveness of cleaning and disinfection to further provide information on biosafety implementations in Estonian small animal practices are needed.

CONCLUSIONS

This study provides information on the occurrence of MRS and *Acinetobacter* in the small animal clinic environment. In total, 15 of the 22 isolated *Staphylococcus* spp. strains were multidrug resistant. Further research is needed to determine the implications of the antimicrobial resistance seen among environmental staphylococci. Areas most commonly associated with positive samples were the procedure room and reception hall. This study provides preliminary information on biosafety methods instituted in small animal clinics in Estonia. In this study, implementation of biosafety methods varied between clinics and only 9 clinics out of 23 had biosafety protocol in place. Biosafety protocols are needed to be implemented in veterinary facilities to ensure appropriate infection control.

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REFERENCES

Abraham, J.L., Morris, D.O., Griffeth, G.C., Shofer, F.S. and Rankin, S.C. (2007) "Surveillance of healthy cats and cats with inflammatory skin disease for colonization of the skin by methicillin-resistant coagulase-positive staphylococci and Staphylococcus schleiferi ssp. schleiferi," *Veterinary Dermatology*, 18(4), pp. 252–259. doi:10.1111/J.1365-3164.2007.00604.X.

Anderson, M.E.C., Wimmers, M. and Weese, J.S. (2020) *Infection Prevention and Control Best Practices For Small Animal Veterinary Clinics, 2nd ed.* Guelph: Ontario Animal Health Network. Available at: https://uoguelph.eu.qualtrics.com/j.

Andrade, N., Schmiedt, C.W., Cornell, K., Radlinsky, M.G., Heidingsfelder, L., Clarke, K., Hurley, D.J. and Hinson, W.D. (2016) "Survey of Intraoperative Bacterial Contamination in Dogs Undergoing Elective Orthopedic Surgery," *Veterinary Surgery*, 45(2), pp. 214–222. doi:10.1111/VSU.12438.

Antunes, L.C.S., Imperi, F., Carattoli, A. and Visca, P. (2011) "Deciphering the multifactorial nature of acinetobacter baumannii pathogenicity," *PLoS ONE*, 6(8). doi:10.1371/JOURNAL.PONE.0022674.

Ayobami, O., Willrich, N., Harder, T., Okeke, I.N., Eckmanns, T. and Markwart, R. (2019) "The incidence and prevalence of hospital-acquired (carbapenem-resistant) Acinetobacter baumannii in Europe, Eastern Mediterranean and Africa: a systematic review and meta-analysis," *Emerging Microbes and Infections*, 8(1), pp. 1747–1759. doi:10.1080/22221751.2019.1698273.

van Balen, J., Mowery, J., Piraino-Sandoval, M., Nava-Hoet, R.C., Kohn, C. and Hoet, A.E. (2014) "Molecular epidemiology of environmental MRSA at an equine teaching hospital: Introduction, circulation and maintenance," *Veterinary Research*, 45(1). doi:10.1186/1297-9716-45-31.

Bastos, A., Surendran Nair, M., Horsman, S., Rynhoud, H., Zhou, X., Soares Magalhães, R.J., Gibson, J.S. and Meler, E. (2021) "Environmental Recovery of Nosocomial Bacteria in a Companion Animal Shelter Before and After Infection Control Procedures," *Frontiers in Veterinary Science*, 7(608901). doi:10.3389/fvets.2020.608901.

Beck, K.M., Waisglass, S.E., Dick, H.L.N. and Weese, J.S. (2012) "Prevalence of meticillin-resistant Staphylococcus pseudintermedius (MRSP) from skin and carriage sites of dogs after treatment of their meticillin-resistant or meticillin-sensitive staphylococcal pyoderma," *Veterinary Dermatology*, 23(4). doi:10.1111/J.1365-3164.2012.01035.X.

Benedict, K.M., Morley, P.S. and van Metre, D.C. (2008) "Characteristics of biosecurity and infection control programs at veterinary teaching hospitals," *Journal of American Veterinary Medical Association*, 233(5), pp. 767–773. doi:10.2460/javma.233.5.767.

Bergström, A., Gustafsson, C., Leander, M., Fredriksson, M., Grönlund, U. and Trowald-Wigh, G. (2012) "Occurrence of methicillin-resistant Staphylococci in surgically treated dogs and the environment in a Swedish animal hospital," *Journal of Small Animal Practice*, 53(7), pp. 404–410. doi:10.1111/J.1748-5827.2012.01238.X.

Boerlin, P., Eugster, S., Gaschen, F., Straub, R. and Schawalder, P. (2001) "Transmission of opportunistic pathogens in a veterinary teaching hospital," *Veterinary Microbiology*, 82(4), pp. 347–359. doi:10.1016/S0378-1135(01)00396-0.

Boost, M. v., So, S.Y.C. and Perreten, V. (2011) "Low rate of methicillin-resistant coagulase-positive staphylococcal colonization of veterinary personnel in Hong Kong," *Zoonoses and Public Health*, 58(1), pp. 36–40. doi:10.1111/J.1863-2378.2009.01286.X.

Bou, G., Gonzalo, C., Domínguez, M.A., Quereda, C. and Martínez-Beltrán, J. (2000) "Characterization of a Nosocomial Outbreak Caused by a Multiresistant Acinetobacter baumannii Strain with a Carbapenem-Hydrolyzing Enzyme: High-Level Carbapenem Resistance in A. baumannii Is Not Due Solely to the Presence of-Lactamases," *Journal of Clinical Microbiology*, 38(9), pp. 3299–3305. Available at: https://journals.asm.org/journal/jcm.

Brachelente, C., Wiener, D., Malik, Y. and Huessy, D. (2007) "A case of necrotizing fasciitis with septic shock in a cat caused by Acinetobacter baumannii," *Veterinary Dermatology*, 18(6), pp. 432–438. doi:10.1111/j.1365-3164.2007.00624.x.

Brown, N.M., Reacher, M., Rice, W., Roddick, I., Reeve, L., Verlander, N.Q., Broster, S., Ogilvy-Stuart, A.L., D'Amore, A., Ahluwalia, J., Robinson, S., Thaxter, R., Moody, C., Kearns, A., Greatorex, J., Martin, H., Török, M.E. and Enoch, D.A. (2019) "An outbreak of meticillin-resistant Staphylococcus aureus colonization in a neonatal intensive care unit: use of a case–control study to investigate and control it and lessons learnt," *Journal of Hospital Infection*, 103(1), pp. 35–43. doi:10.1016/J.JHIN.2019.05.009.

Bryan, J., Frank, L.A., Rohrbach, B.W., Burgette, L.J., Cain, C.L. and Bemis, D.A. (2012) "Treatment outcome of dogs with meticillin-resistant and meticillin-susceptible Staphylococcus pseudintermedius pyoderma," *Veterinary Dermatology*, 23(4). doi:10.1111/J.1365-3164.2012.01034.X.

Catel-Ferreira, M., Coadou, G., Molle, V., Mugnier, P., Nordmann, P., Siroy, A., Jouenne, T. and Dé, E. (2011) "Structure-function relationships of CarO, the carbapenem resistance-

associated outer membrane protein of Acinetobacter baumannii," *Journal of Antimicrobial Chemotherapy*, 66(9), pp. 2053–2056. doi:10.1093/jac/dkr267.

Chen, C.T., Wang, Y.C., Kuo, S.C., Shih, F.H., Chen, T.L., How, C.K., Yang, Y.S. and Lee, Y.T. (2018) "Community-acquired bloodstream infections caused by Acinetobacter baumannii: A matched case–control study," *Journal of Microbiology, Immunology and Infection*, 51(5), pp. 629–635. doi:10.1016/J.JMII.2017.02.004.

Chuang, Y.C., Sheng, W.H., Li, S.Y., Lin, Y.C., Wang, J.T., Chen, Y.C. and Chang, S.C. (2011) "Influence of genospecies of Acinetobacter baumannii complex on clinical outcomes of patients with acinetobacter bacteremia," *Clinical Infectious Diseases*, 52(3), pp. 352–360. doi:10.1093/CID/CIQ154.

Churak, A., Poolkhet, C., Tamura, Y., Sato, T., Fukuda, A. and Thongratsakul, S. (2021) "Evaluation of nosocomial infections through contact patterns in a small animal hospital using social network analysis and genotyping techniques," *Scientific Reports*, 11(1647). doi:10.1038/s41598-021-81301-9.

Cosgrove, S.E., Sakoulas, G., Perencevich, E.N., Schwaber, M.J., Karchmer, A.W. and Carmeli, Y. (2003) *Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus Bacteremia: A Meta-analysis*, *Clinical Infectious Diseases*. Available at: https://academic.oup.com/cid/article/36/1/53/283567.

Costa, S.S., Clark, T.G., Couto, I., Santos Costa, S., Sobkowiak, B., Parreira, R., Edgeworth, J.D. and Viveiros, M. (2019) "Genetic Diversity of norA, Coding for a Main Efflux Pump of Staphylococcus aureus," *Frontiers in Genetics*, 9(710). doi:10.3389/fgene.2018.00710.

Couto, N., Monchique, C., Belas, A., Marques, C., Gama, L.T. and Pomba, C. (2016) "Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period," *Journal of Antimicrobial Chemotherapy*, 71(6), pp. 1479–1487. doi:10.1093/JAC/DKW029.

Detwiler, A., Bloom, P., Petersen, A. and Rosser, E.J. (2013) "Multi-drug and methicillin resistance of staphylococci from canine patients at a veterinary teaching hospital (2006–2011)," *Veterinary Quarterly*, 33(2), pp. 60–67. doi:10.1080/01652176.2013.799792.

Diancourt, L., Passet, V., Nemec, A., Dijkshoorn, L. and Brisse, S. (2010) "The Population Structure of Acinetobacter baumannii: Expanding Multiresistant Clones from an Ancestral Susceptible Genetic Pool," *PLoS ONE*, 5(4), p. 10034. doi:10.1371/journal.pone.0010034.

van Duijkeren, E., Kamphuis, M., van der Mije, I.C., Laarhoven, L.M., Duim, B., Wagenaar, J.A. and Houwers, D.J. (2011) "Transmission of methicillin-resistant Staphylococcus pseudintermedius between infected dogs and cats and contact pets, humans and the

environment in households and veterinary clinics," *Veterinary Microbiology*, 150(3–4), pp. 338–343. doi:10.1016/J.VETMIC.2011.02.012.

Elbehiry, A., Marzouk, E., Moussa, I.M., Dawoud, T.M., Mubarak, A.S., Al-Sarar, D., Alsubki, R.A., Alhaji, J.H., Hamada, M., Abalkhail, A., A. Hemeg, H. and Zahran, R.N. (2021) "Acinetobacter baumannii as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic of biofilm formation and phenotypic pattern of antimicrobial resistance," *Saudi Journal of Biological Sciences*, 28(1), pp. 1158–1166. doi:10.1016/J.SJBS.2020.11.052.

Endimiani, A., Hujer, K.M., Hujer, A.M., Bertschy, I., Rossano, A., Koch, C., Gerber, V., Francey, T., Bonomo, R.A. and Perreten, V. (2011) "Acinetobacter baumannii isolates from pets and horses in Switzerland: molecular characterization and clinical data," *Journal of Antimicrobial Chemotherapy*, 66(10), pp. 2248–2254. doi:10.1093/jac/dkr289.

EURL-AR (2012) *Protocol For PCR Amplification of mecA, mecC (mecALGA251), SPA and PVL*. Available at: https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/279_pcr-spa-pvl-meca-mecc-sept12.pdf (Accessed: April 19, 2022).

EURL-AR (2018) *Isolation of methicillin-resistant Staphylococcus aureus (MRSA) from food-producing animals and farm environment*, *National Food Institute*. European Centre for Disease Prevention and Control (ECDC). Available at: https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/430_mrsa-protocol-final-19-06-2018.pdf (Accessed: April 19, 2022).

Faires, M.C., Traverse, M., Tater, K.C., Pearl, D.L. and Weese, J.S. (2010) "Methicillin-resistant and -susceptible Staphylococcus aureus infections in dogs," *Emerging Infectious Diseases*, 16(1), pp. 69–75. doi:10.3201/eid1601.081758.

Fernando, D., Zhanel, G. and Kumar, A. (2013) "Antibiotic resistance and expression of resistance-nodulation-division pump- And outer membrane porin-encoding genes in acinetobacter species isolated from canadian hospitals," *Canadian Journal of Infectious Diseases and Medical Microbiology*, 24(1), pp. 17–21. doi:10.1155/2013/696043.

Feßler, A.T., Schuenemann, R., Kadlec, K., Hensel, V., Brombach, J., Murugaiyan, J., Oechtering, G., Burgener, I.A. and Schwarz, S. (2018) "Methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus pseudintermedius (MRSP) among employees and in the environment of a small animal hospital," *Veterinary Microbiology*, 221, pp. 153–158. doi:10.1016/J.VETMIC.2018.06.001.

Fitzpatrick, M.A., Ozer, E., Bolon, M.K. and Hauser, A.R. (2015) "Influence of ACB complex genospecies on clinical outcomes in a U.S. hospital with high rates of multidrug resistance," *Journal of Infection*, 70(2), pp. 144–152. doi:10.1016/J.JINF.2014.09.004.

Francey, T., Gaschen, F., Nicolet, J. and Burnens, A.P. (2000) *The Role of Acinetobacter baumannii as a Nosocomial Pathogen for Dogs and Cats in an Intensive Care Unit*, *J Vet Intern Med*.

Griffeth, G.C., Morris, D.O., Abraham, J.L., Shofer, F.S. and Rankin, S.C. (2008) "Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and Staphylococcus schleiferi in dogs with healthy and inflamed skin," *Veterinary Dermatology*, 19(3), pp. 142–149. doi:10.1111/J.1365-3164.2008.00663.X.

Grönthal, T., Eklund, M., Thomson, K., Piiparinen, H., Sironen, T. and Rantala, M. (2017) "Antimicrobial resistance in Staphylococcus pseudintermedius and the molecular epidemiology of methicillin-resistant S. pseudintermedius in small animals in Finland," *Journal of Antimicrobial Chemotherapy*, 72(4), pp. 1021–1030. doi:10.1093/jac/dkw559.

Guardabassi, L., Loeber, M.E. and Jacobson, A. (2004) "Transmission of multiple antimicrobial-resistant Staphylococcus intermedius between dogs affected by deep pyoderma and their owners," *Veterinary Microbiology*, 98(1), pp. 23–27. doi:10.1016/j.vetmic.2003.09.021.

Hamilton, E., Kaneene, J.B., May, K.J., Kruger, J.M., Schall, W., Beal, M.W., Hauptman, J.G. and DeCamp, C.E. (2012) "Prevalence and antimicrobial resistance of Enterococcus spp and Staphylococcus spp isolated from surfaces in a veterinary teaching hospital," *Journal of the American Veterinary Medical Association*, 240(12), pp. 1463–1473. doi:10.2460/JAVMA.240.12.1463.

Han, J.I., Yang, C.H. and Park, H.M. (2016) "Prevalence and risk factors of Staphylococcus spp. carriage among dogs and their owners: A cross-sectional study," *The Veterinary Journal*, 212, pp. 15–21. doi:10.1016/J.TVJL.2015.10.059.

Hazard, D., von Cube, M., Kaier, K. and Wolkewitz, M. (2021) "Predicting Potential Prevention Effects on Hospital Burden of Nosocomial Infections: A Multistate Modeling Approach," *Value in Health*, 24(6), pp. 830–838. doi:10.1016/J.JVAL.2021.02.002.

Holm, B.R., Petersson, U., Mörner, A., Bergström, K., Franklin, A. and Greko, C. (2002) "Antimicrobial resistance in staphylococci from canine pyoderma: A prospective study of first-time and recurrent cases in Sweden," *Veterinary Record*, 151(20), pp. 600–605. doi:10.1136/VR.151.20.600.

Hopman, N.E.M., Hulscher, M.E.J.L., Graveland, H., Speksnijder, D.C., Wagenaar, J.A. and Broens, E.M. (2018) "Factors influencing antimicrobial prescribing by Dutch companion animal veterinarians: A qualitative study," *Preventive Veterinary Medicine*, 158, pp. 106–113. doi:10.1016/J.PREVETMED.2018.07.013.

Hunter, N.D., Hoet, A.E., van Balen, J. and Stull, J.W. (2021) "Longitudinal environmental Staphylococcus contamination in a new small animal veterinary hospital and utility of cleaning checklists," *Zoonoses Public Health*, 68(8), pp. 947–954. doi:10.1111/zph.12887.

Ishihara, K., Shimokubo, N., Sakagami, A., Ueno, H., Muramatsu, Y., Kadosawa, T., Yanagisawa, C., Hanaki, H., Nakajima, C., Suzuki, Y. and Tamura, Y. (2010) "Occurrence and Molecular Characteristics of Methicillin-Resistant Staphylococcus aureus and Methicillin-Resistant Staphylococcus pseudintermedius in an Academic Veterinary Hospital," *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, 76(15), pp. 5165–5174. doi:10.1128/AEM.02780-09.

Jessen, L.R., Damborg, P., Spohr, A., Goericke-Pesch, S., Langhorn, R., Houser, G., Willesen, J., Schærff, M., Eriksen, T., Sørensen, T.M., Jensen, V.F., Obling, F. and Guardabassi, L. (2018) *Antibiotic Use Guidelines for Companion Animal Practice*. 2nd edn. Edited by P. Damborg. Frederiksberg: Companion Animal Group, Danish Veterinary Association.

Kanyo, E.C., Nowacki, A.S., Gordon, S.M. and Shrestha, N.K. (2021) "Comparison of mortality, stroke, and relapse for methicillin-resistant versus methicillin-susceptible Staphylococcus aureus infective endocarditis: a retrospective cohort study," *Diagnostic Microbiology and Infectious Disease*, 100(4), p. 115395. doi:10.1016/J.DIAGMICROBIO.2021.115395.

Kharaba, A., Algethamy, H., Hussein, M., Al-Hameed, F.M., Alghamdi, A., Hamdan, A., Fatani, J., Elhazmi, A., Alkhalaf, H., Barghash, B., Elsawy, A. and Arabi, Y.M. (2021) "Incidence, outcomes, and predictors of Acinetobacter infection in Saudi Arabian critical care units," *Journal of Critical Care*, 66, pp. 109–116. doi:10.1016/J.JCRC.2021.08.010.

Kim, Y.A., Kim, J.J., Won, D.J. and Lee, K. (2018) "Seasonal and temperature-associated increase in community-onset Acinetobacter baumannii complex colonization or infection," *Annals of Laboratory Medicine*, 38(3), pp. 266–270. doi:10.3343/ALM.2018.38.3.266.

Kimura, Y., Miyamoto, T., Aoki, K., Ishii, Y., Harada, K., Watarai, M. and Hatoya, S. (2017) "Analysis of IMP-1 type metallo-β-lactamase-producing Acinetobacter radioresistens isolated from companion animals," *Journal of Infection and Chemotherapy*, 23(9), pp. 655–657. doi:10.1016/J.JIAC.2017.03.011.

Klevens, R.M., Morrison, M.A., Fridkin, S.K., Reingold, A., Petit, S., Gershman, K., Ray, S., Harrison, L.H., Lynfield, R., Dumyati, G., Townes, J.M., Craig, A.S., Fosheim, G., McDougal, L.K. and Tenover, F.C. (2006) "Community-associated methicillin-resistant Staphylococcus aureus and healthcare risk factors," *Emerging Infectious Diseases*, 12(12), pp. 1991–1993. doi:10.3201/EID1212.060505.

Kuzi, S., Blum, S.E., Kahane, N., Adler, A., Hussein, O., Segev, G. and Aroch, I. (2016) "Multi-drug-resistant Acinetobacter calcoaceticus-Acinetobacter baumannii complex infection outbreak in dogs and cats in a veterinary hospital," *Journal of Small Animal Practice*, 57(11), pp. 617–625. doi:10.1111/JSAP.12555.

Langdon, G., Hoet, A.E. and Stull, J.W. (2020) "Fluorescent tagging for environmental surface cleaning surveillance in a veterinary hospital," *Journal of Small Animal Practice*, 61(2), pp. 121–126. doi:10.1111/jsap.13090.

Lee, K., Yum, J.H., Yong, D., Lee, H.M., Kim, H.D., Docquier, J.D., Rossolini, G.M. and Chong, Y. (2005) "Novel acquired metallo-β-lactamase gene, blaSIM-1, in a class 1 integron from Acinetobacter baumannii clinical isolates from Korea," *Antimicrobial Agents and Chemotherapy*, 49(11), pp. 4485–4491. doi:10.1128/AAC.49.11.4485-4491.2005.

Lee, Y.H., Chen, C.J., Lien, R.I. and Huang, Y.C. (2021) "A longitudinal molecular surveillance of clinical methicillin-resistant Staphylococcus aureus isolates in neonatal units in a teaching hospital, 2003–2018," *Journal of Microbiology, Immunology and Infection* [Preprint]. doi:10.1016/J.JMII.2021.10.007.

Lee, Y.-T., Kuo, S.-C., Yang, S.-P., Lin, Y.-T., Chiang, D.-H., Tseng, F.-C., Chen, T.-L. and Fung, C.-P. (2013) "Bacteremic nosocomial pneumonia caused by Acinetobacter baumannii and Acinetobacter nosocomialis: A single or two distinct clinical entities?," *Clinical Microbiology and Infection*, 19(7), pp. 640–645. doi:10.1111/j.1469-0691.2012.03988.x.

Lehner, G., Linek, M., Bond, R., Lloyd, D.H., Prenger-Berninghoff, E., Thom, N., Straube, I., Verheyen, K. and Loeffler, A. (2014) "Case–control risk factor study of methicillin-resistant Staphylococcus pseudintermedius (MRSP) infection in dogs and cats in Germany," *Veterinary Microbiology*, 168(1), pp. 154–160. doi:10.1016/J.VETMIC.2013.10.023.

Loeffler, A., Linek, M., Moodley, A., Guardabassi, L., Sung, J.M.L., Winkler, M., Weiss, R. and Lloyd, D.H. (2007) "First report of multiresistant, mecA-positive Staphylococcus intermedius in Europe: 12 Cases from a veterinary dermatology referral clinic in Germany," *Veterinary Dermatology*, 18(6), pp. 412–421. doi:10.1111/J.1365-3164.2007.00635.X.

Loeffler, A., Pfeiffer, D.U., Lloyd, D.H., Smith, H., Soares-Magalhaes, R. and Lindsay, J.A. (2010) "Meticillin-resistant Staphylococcus aureus carriage in UK veterinary staff and

owners of infected pets: new risk groups," *Journal of Hospital Infection*, 74(3), pp. 282–288. doi:10.1016/J.JHIN.2009.09.020.

Lourtet-Hascoët, J., Félicé, M.P., Bicart-See, A., Bouige, A., Giordano, G. and Bonnet, E. (2018) "Epidemiology and Infection Species and antimicrobial susceptibility testing of coagulase-negative staphylococci in periprosthetic joint infections," *Epidemiology and Infection*, 146, pp. 1771–1776. doi:10.1017/S0950268818001437.

Maboni, G., Seguel, M., Lorton, A. and Sanchez, S. (2020) "Antimicrobial resistance patterns of Acinetobacter spp. of animal origin reveal high rate of multidrug resistance," *Veterinary Microbiology*, 245, p. 108702. doi:10.1016/J.VETMIC.2020.108702.

Matsushima, A., Kuroki, Y., Nakajima, S., Kojima, H., Seki, T. and Ueyama, M. (2021) "The incidence of toxic shock syndrome due to nosocomial methicillin-resistant Staphylococcus aureus infection in burn patients: A four-year retrospective cohort study," *Burns*, 47(2), pp. 402–407. doi:10.1016/J.BURNS.2020.07.005.

Menezes, M.P., Borzi, M.M., Ruaro, M.A., Cardozo, M. v., Rabelo, R.C., Verbisck, N. v. and Moraes, P.C. (2022) "Multidrug-resistant bacteria isolated from surgical site of dogs, surgeon's hands and operating room in a veterinary teaching hospital in Brazil," *Topics in Companion Animal Medicine*, p. 100638. doi:10.1016/J.TCAM.2022.100638.

Moon, B.Y., Youn, J.H., Shin, S., Hwang, S.Y. and Park, Y.H. (2012) "Genetic and phenotypic characterization of methicillin-resistant staphylococci isolated from veterinary hospitals in South Korea," *Journal of Veterinary Diagnostic Investigation*, 24(3), pp. 489–498. doi:10.1177/1040638712440985.

Moore, C.L., Hingwe, A., Donabedian, S.M., Perri, M.B., Davis, S.L., Haque, N.Z., Reyes, K., Vager, D. and Zervos, M.J. (2009) "Comparative evaluation of epidemiology and outcomes of methicillin-resistant Staphylococcus aureus (MRSA) USA300 infections causing community- and healthcare-associated infections," *International Journal of Antimicrobial Agents*, 34(2), pp. 148–155. doi:10.1016/J.IJANTIMICAG.2009.03.004.

Morris, D.O., Boston, R.C., O'Shea, K. and Rankin, S.C. (2010) "The prevalence of carriage of meticillin-resistant staphylococci by veterinary dermatology practice staff and their respective pets," *Veterinary Dermatology*, 21(4), pp. 400–407. doi:10.1111/j.1365-3164.2010.00866.x.

Murphy, Colleen P, Reid-Smith, R.J., Boerlin, P., Weese, J.S., Prescott, J.F., Janecko, N., Hassard, L. and Mcewen, S.A. (2010) "Escherichia coli and selected veterinary and zoonotic pathogens isolated from environmental sites in companion animal veterinary hospitals in southern Ontario," *Canadian Veterinary Journal*, 51(9), pp. 963–972.

Murphy, C.P., Reid-Smith, R.J., Weese, J.S. and Mcewen, S.A. (2010) "Evaluation of Specific Infection Control Practices Used by Companion Animal Veterinarians in Community Veterinary Practices in Southern Ontario," *Zoonoses and Public Health*, 57(6), pp. 429–438. doi:10.1111/j.1863-2378.2009.01244.x.

Nahrgang, S., Gelormini, M. and Merk, H. (2022) *WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022 - 2020 data*. Copenhagen. doi:10.2900/112339.

Nakbubpa, K., Janchawna, R., Thumchop, W., Panboonthong, A. and Pornsukarom, S. (2021) "Prevalence, species, and antimicrobial resistance of Acinetobacter in surgical practice and laboratory dog husbandry room environments," *Korean Journal of Veterinary Research*, 61(3). doi:10.14405/KJVR.2021.61.E26.

Nazarali, A., Singh, A., Moens, N.M.M., Gatineau, M., Sereda, C., Fowler, D., Kim, S.E., Kisiel, A., Reynolds, D., Ringwood, B.R., Bruce, C.W., Gibson, T.W.G., Rousseau, J. and Weese, J.S. (2015) "Association between methicillin-resistant Staphylococcus pseudintermedius carriage and the development of surgical site infections following tibial plateau leveling osteotomy in dogs," *Journal of American Veterinary Medical Association*, 247(8), pp. 909–916.

Nemec, A., Krizova, L., Maixnerova, M., van der Reijden, T.J.K., Deschaght, P., Passet, V., Vaneechoutte, M., Brisse, S. and Dijkshoorn, L. (2011) "Genotypic and phenotypic characterization of the Acinetobacter calcoaceticus–Acinetobacter baumannii complex with the proposal of Acinetobacter pittii sp. nov. (formerly Acinetobacter genomic species 3) and Acinetobacter nosocomialis sp. nov. (formerly Acinetobacter genomic species 13TU)," *Research in Microbiology*, 162(4), pp. 393–404. doi:10.1016/J.RESMIC.2011.02.006.

Nocera, F.P., Addante, L., Capozzi, L., Bianco, A., Fiorito, F., de Martino, L. and Parisi, A. (2020) "Detection of a novel clone of Acinetobacter baumannii isolated from a dog with otitis externa," *Comparative Immunology, Microbiology and Infectious Diseases*, 70, p. 101471. doi:10.1016/J.CIMID.2020.101471.

O'Mahony, R., Abbott, Y., Leonard, F.C., Markey, B.K., Quinn, P.J., Pollock, P.J., Fanning, S. and Rossney, A.S. (2005) "Methicillin-resistant Staphylococcus aureus (MRSA) isolated from animals and veterinary personnel in Ireland," *Veterinary Microbiology*, 109(3–4), pp. 285–296. doi:10.1016/J.VETMIC.2005.06.003.

Oppegaard, H., Steinum, T.M. and Wasteson, Y. (2001) "Horizontal Transfer of a Multi-Drug Resistance Plasmid between Coliform Bacteria of Human and Bovine Origin in a Farm

Environment," *Applied and Environmental Microbiology*, 67(8), pp. 3732–3734. doi:10.1128/AEM.67.8.3732-3734.2001.

Park, S., Lee, K.M., Yoo, Y.S., Yoo, J.S., Yoo, J. il, Kim, H.S., Lee, Y.S. and Chung, G.T. (2011) "Alterations of gyrA, gyrB, and parC and Activity of Efflux Pump in Fluoroquinolone-resistant Acinetobacter baumannii," *Osong Public Health and Research Perspectives*, 2(3), pp. 164–170. doi:10.1016/J.PHRP.2011.11.040.

Paul, N.C., Moodley, A., Ghibaudo, G. and Guardabassi, L. (2011) "Carriage of methicillin-resistant Staphylococcus pseudintermedius in small animal veterinarians: Indirect evidence of zoonotic transmission," *Zoonoses and Public Health*, 58(8), pp. 533–539. doi:10.1111/J.1863-2378.2011.01398.X.

Perreten, V., Kadlec, K., Schwarz, S., Andersson, U.G., Finn, M., Greko, C., Moodley, A., Kania, S.A., Frank, L.A., Bemis, D.A., Franco, A., Iurescia, M., Battisti, A., Duim, B., Wagenaar, J.A., van Duijkeren, E., Weese, J.S., Fitzgerald, J.R., Rossano, A. and Guardabassi, L. (2010) "Clonal spread of methicillin-resistant Staphylococcus pseudintermedius in Europe and North America: An international multicentre study," *Journal of Antimicrobial Chemotherapy*, 65(6), pp. 1145–1154. doi:10.1093/JAC/DKQ078.

Price, L.B., Stegger, M., Hasman, H., Aziz, M., Larsen, J., Skytt Andersen, P., Pearson, T., Waters, A.E., Foster, J.T., Schupp, J., Gillece, J., Driebe, E., Liu, C.M., Springer, B., Zdovc, I., Battisti, A., Franco, A., Schwarz, S., Butaye, P., Jouy, E., Pomba, C., Concepción Porrero, M., Ruimy, R., Smith, T.C., Ashley Robinson, D., Scott Weese, J., Sofia Arriola, C., Yu, F., Laurent, F., Keim, P., Skov, R., Aarestrup, F.M., Zooprofilattico Sperimentale delle Regioni Lazio Toscana, I. and Fernando Baquero, E. (2012) "Staphylococcus aureus CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock," *mBio*, 3(1). doi:10.1128/mBio.00305.

Raske, M., Mcclaran, J.K. and Mariano, A. (2015) "Short-term wound complications and predictive variables for complication after limb amputation in dogs and cats," *Journal of Small Animal Practice*, 56(4), pp. 247–252. doi:10.1111/JSAP.12330.

Ribera, A., Ruiz, J. and Vila, J. (2003) "Presence of the Tet M Determinant in a Clinical Isolate of Acinetobacter baumannii," *ANTIMICROBIAL AGENTS AND CHEMOTHERAPY*, 47(7), pp. 2310–2312. doi:10.1128/AAC.47.7.2310-2312.2003.

Ruple-Czerniak, A., Aceto, H.W., Bender, J.B., Paradis, M.R., Shaw, S.P., van Metre, D.C., Weese, J.S., Wilson, D.A., Wilson, J.H. and Morley, P.S. (2013) "Using Syndromic Surveillance to Estimate Baseline Rates for Healthcare-Associated Infections in Critical

Care Units of Small Animal Referral Hospitals," *Journal of Veterinary Internal MEdicine*, 27, pp. 1392–1399. doi:10.1111/jvim.12190.

Ruzauskas, M., Couto, N., Siugzdiniene, R., Klimiene, I., Virgailis, M., Vaskeviciute, L., Mockeliunas, R. and Pomba, C. (2015) "Methicillin-resistant coagulase-negative Staphylococcus spp. prevalence in Lithuanian dogs: a cross-sectional study: Methicillin-resistant coagulase-negative Staphylococcus spp. prevalence in Lithuanian dogs: a cross-sectional study," *Veterinarski Arhiv*, 85(2), pp. 175–187.

Salavati, S., Taylor, C.S., Harris, J.D. and Paterson, G.K. (2018) "A canine urinary tract infection representing the first clinical veterinary isolation of Acinetobacter ursingii," *New Microbes and New Infections*, 22, pp. 4–5. doi:10.1016/J.NMNI.2017.11.007.

Sasaki, T., Tsubakishita, S., Tanaka, Y., Sakusabe, A., Ohtsuka, M., Hirotaki, S., Kawakami, T., Fukata, T. and Hiramatsu, K. (2010) "Multiplex-PCR method for species identification of coagulase-positive staphylococci," *Journal of Clinical Microbiology*, 48(3), pp. 765–769. doi:10.1128/JCM.01232-09.

Seguin, J.C., Walker, R.D., Caron, J.P., Kloos, W.E., George, C.G., Hollis, R.J., Jones, R.N. and Pfaller, M.A. (1999) "Methicillin-resistant Staphylococcus aureus outbreak in a veterinary teaching hospital: Potential human-to-animal transmission," *Journal of Clinical Microbiology*, 37(5), pp. 1459–1463. doi:10.1128/JCM.37.5.1459-1463.1999.

Sheikhalizadeh, V., Hasani, Alka, Ahangarzadeh Rezaee, M., Rahmati-yamchi, M., Hasani, Akbar, Ghotaslou, R. and Goli, H.R. (2017) "Comprehensive study to investigate the role of various aminoglycoside resistance mechanisms in clinical isolates of Acinetobacter baumannii," *Journal of Infection and Chemotherapy*, 23(2), pp. 74–79. doi:10.1016/J.JIAC.2016.09.012.

Shimada, E., Miyamoto, T. and Hatoya, S. (2012) "Isolation of Metallo-β-Lactamase-Producing Acinetobacter lwoffii from Three Dogs and a Cat," *Journal of the Japan Veterinary Medical Association*, 65(5), pp. 365–369. doi:10.12935/JVMA.65.365.

Skiebe, E., de Berardinis, V., Morczinek, P., Kerrinnes, T., Faber, F., Lepka, D., Hammer, B., Zimmermann, O., Ziesing, S., Wichelhaus, T.A., Hunfeld, K.P., Borgmann, S., Gröbner, S., Higgins, P.G., Seifert, H., Busse, H.J., Witte, W., Pfeifer, Y. and Wilharm, G. (2012) "Surface-associated motility, a common trait of clinical isolates of Acinetobacter baumannii, depends on 1,3-diaminopropane," *International Journal of Medical Microbiology*, 302(3), pp. 117–128. doi:10.1016/J.IJMM.2012.03.003.

Smiline, A.S.G., Vijayashree, J.P. and Paramasivam, A. (2018) "Molecular characterization of plasmid-encoded blaTEM, blaSHV and blaCTX-M among extended spectrum β-

lactamases [ESBLs] producing Acinetobacter baumannii," *British Journal of Biomedical Science*, 75(4), pp. 200–202. doi:10.1080/09674845.2018.1492207.

Soares Magalhães, R.J., Loeffler, A., Lindsay, J., Rich, M., Roberts, L., Smith, H., Lloyd, D.H. and Pfeiffer, D.U. (2010) "Risk factors for methicillin-resistant Staphylococcus aureus (MRSA) infection in dogs and cats: A case-control study," *Veterinary Research*, 41(5). doi:10.1051/VETRES/2010028.

Somayaji, R., Priyantha, M.A.R., Rubin, J.E. and Church, D. (2016) "Human infections due to Staphylococcus pseudintermedius, an emerging zoonosis of canine origin: report of 24 cases," *Diagnostic Microbiology and Infectious Disease*, 85(4), pp. 471–476. doi:10.1016/J.DIAGMICROBIO.2016.05.008.

Stegmann, R., Burnens, A., Maranta, C.A. and Perreten, V. (2010) "Human infection associated with methicillin-resistant Staphylococcus pseudintermedius ST71," *Journal of Antimicrobial Chemotherapy*, 65(9), pp. 2047–2048. doi:10.1093/JAC/DKQ241.

Stull, J.W., Bjorvik, E., Bub, J., Dvorak, G., Petersen, C. and Troyer, H.L. (2018) "2018 AAHA Infection Control, Prevention, and Biosecurity Guidelines," *Journal of the American Animal Hospital Association*, 54(6), pp. 297–326. doi:10.5326/JAAHA-MS-6903.

Stull, J.W., Slavić, D. and Weese, J.S. (2012) "Methicillin-Resistant Staphylococcus aureus (MRSA) Surveillance in Horses at a Veterinary Teaching Hospital," *Journal of Equine Veterinary Science*, 32(10), pp. S3–S4. doi:10.1016/J.JEVS.2012.08.015.

Thorberg, B.-M. and Brändström, B. (2000) "Evaluation of Two Commercial Systems and a New Identification Scheme Based on Solid Substrates for Identifying Coagulase-Negative Staphylococci from Bovine Mastitis," *Journal of Veterinary Medicine Series B*, 47, pp. 683–691.

Tjon-A-Tsien, A.M., Vos, M.C., Duijkeren, E.V., Wannet, W.J., Gotz, H.M., Schop, W. and Richardus, J.H. (2010) "Cluster of MRSA in cats and staff of a veterinary clinic: Follow-up and possible implications for control," *International Journal of Infectious Diseases*, 14, p. e166. doi:10.1016/J.IJID.2010.02.1849.

Unnerstad, H.E., Mieziewska, K., Börjesson, S., Hedbäck, H., Strand, K., Hallgren, T., Landin, H., Skarin, J. and Bengtsson, B. (2018) "Suspected transmission and subsequent spread of MRSA from farmer to dairy cows," *Veterinary Microbiology*, 225, pp. 114–119. doi:10.1016/J.VETMIC.2018.09.017.

Velasco, D., del Mar Tomas, M., Cartelle, M., Beceiro, A., Perez, A., Molina, F., Moure, R., Villanueva, R. and Bou, G. (2005) "Evaluation of different methods for detecting methicillin

(oxacillin) resistance in Staphylococcus aureus," *Journal of Antimicrobial Chemotherapy*, 55(3), pp. 379–382. doi:10.1093/jac/dki017.

Villegas-Estrada, A., Lee, M., Hesek, D., Vakulenko, S.B. and Mobashery, S. (2008) "Co-opting the Cell Wall in Fighting Methicillin-Resistant Staphylococcus aureus: Potent Inhibition of PBP 2a by Two Anti-MRSA-Lactam Antibiotics," *Journal of American Chemical Society*, 130, pp. 9212–9213. doi:10.1021/ja8029448.

Vincze, S., Brandenburg, A.G., Espelage, W., Stamm, I., Wieler, L.H., Kopp, P.A., Lübke-Becker, A. and Walther, B. (2014) "Risk factors for MRSA infection in companion animals: Results from a case–control study within Germany," *International Journal of Medical Microbiology*, 304(7), pp. 787–793. doi:10.1016/J.IJMM.2014.07.007.

Weese, J.S., Dick, H., Willey, B.M., McGeer, A., Kreiswirth, B.N., Innis, B. and Low, D.E. (2006) "Suspected transmission of methicillin-resistant Staphylococcus aureus between domestic pets and humans in veterinary clinics and in the household," *Veterinary Microbiology*, 115(1–3), pp. 148–155. doi:10.1016/J.VETMIC.2006.01.004.

Weese, S., Papers, J., Lowe, T. and Walker, M. (2012) "Use of fluorescent tagging for assessment of environmental cleaning and disinfection in a veterinary hospital; Use of fluorescent tagging for assessment of environmental cleaning and disinfection in a veterinary hospital," *Veterinary Record*, 171(9), pp. 217–217. doi:10.1136/vr.100796.

Weiner-Lastinger, L.M., Abner, S., Benin, A.L., Edwards, J.R., Kallen, A.J., Karlsson, M., Magill, S.S., Pollock, D., See, I., Soe, M.M., Walters, M.S. and Dudeck, M.A. (2020) "Antimicrobial-resistant pathogens associated with pediatric healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network, 2015-2017," *Infection Control and Hospital Epidemiology*, 41(1), pp. 19–30. doi:10.1017/ICE.2019.297.

Worthing, K.A., Brown, J., Gerber, L., Trott, D.J., Abraham, S. and Norris, J.M. (2018) "Methicillin-resistant staphylococci amongst veterinary personnel, personnel-owned pets, patients and the hospital environment of two small animal veterinary hospitals," *Veterinary Microbiology*, 223, pp. 79–85. doi:10.1016/J.VETMIC.2018.07.021.

Zhang, W., Aurosree, B., Gopalakrishnan, B., Balada-Llasat, J.-M., Pancholi, V. and Pancholi, P. (2017) "The role of LpxA/C/D and pmrA/B g ene systems in colistin-resistant clinical strains of Acinetobacter baumannii," *Frontiers in Laboratory Medicine*, 1(2), pp. 86–91. doi:10.1016/j.flm.2017.07.001.

APPENDICES

Appendix 1. Questionnaire form

1. Who is responsible for cleaning of the clinic?	0 – Special company 1 – Internal team
2. How many times per day do you clean clinic rooms?	0 – Once 1 – More than once
3. Do you clean and disinfect the consultation room after every patient?	0 – Yes 1 - No
4. Do you use always gloves during clinical examination?	0 – Yes 1 - No
5. Do you wash and disinfect hands after handling every patient?	0 – Yes 1 - No
6. Do your personal staff use extra protective clothing during special procedures (wound infection, dental procedures, etc)?	0 – Yes 1 - No
7. Do you clip hair before cannula insertion?	0 – Yes 1 - No
8. Do you follow aseptic rules during cannula insertion?	0 – Yes 1 - No
9. Do you disinfect mouthpiece after use?	0 – Yes 1 - No
10. Do you have a special UV-light in the procedure room?	0 – Yes 1 - No
11. Do you have a special room for preparing patients before surgery?	0 – Yes 1 - No
12. Do you have UV-light or quartz light in the operating room?	0 – Yes 1 - No
13. Do you replace UV-lights according to requirements?	0 – Yes 1 - No
14. Do you use sterile equipment for dental procedures?	0 – Yes 1 - No
15. Do you sterilize surgery instruments and additional materials?	0 – Yes 1 - No
16. Do you use special boots in the operating room?	0 – Yes 1 - No
17. Do you use different clothing during surgical operations?	0 – Yes 1 - No
18. Do you use separate cleaning equipment for operating rooms?	0 – Yes 1 - No
19. Are table surfaces in the examination, procedure, and operating rooms easy to clean and disinfect?	0 – Yes 1 - No
20. Are animal cages easy to clean and disinfect?	0 – Yes 1 - No
21. Do you have separate recovery room for post-operative patients?	0 – Yes 1 - No
22. Do you have an isolation unit in the clinic?	0 – Yes 1 - No
23. Do you have separate ward for cats?	0 – Yes 1 - No
24. Do you reuse endotracheal tubes?	0 – Yes 1 - No
25. Do you have a separate room for staff?	0 – Yes 1 - No
26. Do you have protocol or biosafety regulation in your clinic?	0 – Yes 1 - No

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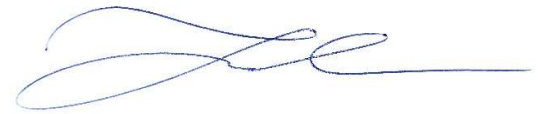
Author of the final thesis 

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