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How clean is clean enough?

Infection prevention and control in animal healthcare

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Abstract

Microorganisms in the environment and on equipment in animal healthcare facilities may constitute a risk of healthcare-associated infections (HAIs). Knowledge of the bacterial load as well as factors influencing the bacterial load is helpful in establishing infection prevention and control (IPC) routines to reduce the risk of HAIs. Cleaning and disinfection of environmental surfaces and equipment is an important IPC measure to reduce the risk of environmental spread of pathogens and incidence of HAIs.

The overall aim of the thesis was to improve IPC in animal healthcare by studying the bacterial load, before and after cleaning and disinfection, in the environment and on equipment, by passive air and surface sampling.

The bacterial load was generally low in air and on high-touch and near-patient surfaces in the operating room (OR) and the ultrasound room (UR). On near-patient surfaces in dog cages the bacterial load was generally low after decontamination, except after microfibre cleaning of the floor. The bacterial load was high on near-patient surfaces and dental handpieces after equine dental care, and after decontamination the bacterial load on near-patient surfaces was generally low, but on dental handpieces the bacterial load was still high. Genes conveying resistance to chlorhexidine and quaternary ammonium compounds were identified in environmental staphylococci from the OR and the UR.

In conclusion, this thesis present a generally low bacterial load, except after microfibre-cleaning of the floor in dog cages and after decontamination of dental handpieces. This indicates a need for evidence-based cleaning and disinfection routines for environmental surfaces and equipment in animal healthcare, to reduce the risk of HAIs.

Keywords: animal hospital, antibacterial resistance, antimicrobial resistance, bacterial reduction, biosecurity, cleaning, disinfection, healthcare-associated infection, hygiene, veterinary clinic

Hur rent är rent nog? Vårdhygien inom djursjukvården

Abstract

Mikroorganismer i miljön och på utrustning i djursjukvården kan utgöra en risk för vårdrelaterade infektioner (VRI). Kunskap om bakteriebördan och faktorer som påverkar bakteriebördan är nödvändiga för att ta fram vårdhygienrutiner för att minska risker för VRI. Rengöring och desinfektion av ytor och utrustning är en viktig vårdhygienåtgärd för att minska risken för miljöspridning av patogener och incidens av VRI.

Det övergripande syftet med avhandlingen är att förbättra vårdhygienen i djursjukvården genom att studera bakteriebördan, före och efter rengöring och desinfektion, i miljön och på utrustning, genom passiv luft- och ytprovtagning.

Bakteriebördan var generellt låg i luft och på tagytor, så väl som patientnära ytor i operationssalen och ultraljudsrummet. På patientnära ytor i hundburar var bakteriebördan generellt låg efter rengöring och desinfektion, förutom efter golvet rengjorts med en fuktad mikrofibermopp. Bakteriebördan var hög på patientnära ytor och på dentala handstycken efter hästtandvård. Efter dekontaminering var bakteriebördan på patientnära ytor mestadels låg, men på de dentala handstyckena var bakteriebördan fortfarande hög. Gener som bär på resistensegenskaper mot klorhexidin och kvartära ammoniumföreningar hittades hos stafylokocker i miljöprover från operationssalen och ultraljudsrummet.

Sammanfattningsvis var bakteriebördan generellt låg, förutom efter mikrofiberrengöring av golvet i hundburar och efter dekontaminering av dentala handstycken. Det indikerar ett behov av evidensbaserade rengörings- och desinfektionsrutiner för ytor och utrustning i djursjukvården, för att minska risken för VRI.

Nyckelord: antibakteriell resistens, antimikrobiell resistens, bakteriereduktion, biosäkerhet, desinfektion, djursjukhus, hygien, rengöring, vårdrelaterad infektion, veterinärklinik

To my family

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Alsing-Johansson, T., Bergström, K., Sternberg-Lewerin, S., Bergh, A., Östlund, E. & Penell, J. (2024). Environmental bacterial load during surgical and ultrasound procedures in a Swedish small animal hospital. Acta Vet Scand, 66 (43), 2024. https://doi.org/10.1186/s13028-024-00768-4

II. Alsing-Johansson, T., Pedersen, A., Bergström, K., Sternberg-Lewerin, S., Penell, J. & Bergh, A (2021). Bacterial Contamination of Equine Dentistry Equipment—Effect of Cleaning and Disinfection. Animals,11 (8), 2021. https://doi.org/10.3390/ani11082320

III. Alsing-Johansson, T., Nilsson-Torstensson, E., Bergström, K., Sternberg-Lewerin, S., Bergh, A & Penell, J. A comparison of two cleaning methods applied in a small animal hospital. Manuscript.

All published papers are published open access.

The contribution of Todd Alsing Johansson to the papers included in this thesis was as follows:

I. Participated in planning the study. Executed bacteriological sampling. Processed and analysed the samples except for antibiotic susceptibility testing and whole genome sequencing. Analysed and interpreted data with support from one co-author. Drafted the manuscript, with support from one co-author, and finalised it with input from the co-authors. Corresponded with the journal.

II. Took major responsibility for planning the study. Executed bacteriological sampling together with one co-author. Performed laboratory analysis, analysed and interpreted data. Drafted the manuscript and finalised it with input from the co-authors. Corresponded with the journal.

III. Took major responsibility for planning the study. Executed bacteriological sampling together with one co-author. Performed laboratory analysis. Analysed and interpreted data with support from one co-author. Drafted the manuscript and finalised it with input from the co-authors.

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Abbreviations

CFU   colony-forming unit
DSB   dry surface biofilm
EPS   extracellular polymeric substance
HAI   healthcare-associated infection
ICU   intensive care unit
IMA   the standard index of microbial air contamination
IPC   infection prevention and control
MRSA   methicillin-resistant Staphylococcus aureus
MRSP   methicillin-resistant Staphylococcus pseudintermedius
OR   operating room
QAC   quaternary ammonium compound
UR   ultrasound room

1. Introduction

1.1 Infection prevention and control in animal healthcare

Infection prevention and control (IPC) is a cornerstone of patient safety in animal healthcare, with the purpose of preventing healthcare-associated infections (HAIs) (Weese 2011; Sebola *et al.* 2022). Infection prevention and control is considered an important part of Swedish animal healthcare (The Swedish Veterinary Association and the committee for Veterinary Medicine (SVF & SVS) 2012; SVF & SVS 2017; SJVFS 2021:5). Gathering many sick animals together in a small area, as in an animal hospital, increases the risk of spread of pathogens between patients. Patients with increased susceptibility to infections are especially vulnerable (Weese 2011). Progression in animal healthcare has led to advanced treatments being given to the patients. With some advanced treatments, e.g. implant surgery, comes increased risks of HAIs (Weese 2011). Studies from animal healthcare have shown that HAIs can prolong hospital stays and lead to increased healthcare costs, morbidity and mortality (Dallap Schaer *et al.* 2010; Bergström *et al.* 2012; Willemsen *et al.* 2019). The need for structured IPC work in animal healthcare has been acknowledged due to problems with HAIs, including HAIs caused by resistant bacteria (Weese 2011; Sebola *et al.* 2022; Singaravelu *et al.* 2023).

Since 2014 it has been required by law for animal healthcare businesses in Sweden to have an IPC plan (SJVFS 2021:5). This legislation was introduced after increased incidence of HAIs with methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) in animal healthcare in Sweden. The IPC plan should include hand hygiene routines, prevention and control of outbreaks

of HAIs, routines for environmental cleaning and disinfection, and cleaning, disinfection and sterilization of instruments and equipment.

Previous research in IPC in animal healthcare has mainly focused on control of outbreaks of HAIs, hand hygiene and occurrence of pathogens or potential pathogens in the animal healthcare environment. Environmental microorganism on surfaces in human healthcare facilities constitute a risk of HAI (Dancer *et al.*, 2009; Weber *et al.*, 2010; Suleyman *et al.*, 2018) and presumably also in animal healthcare facilities (Sebola et al., 2022; Singaravelu et al., 2023). This thesis focuses on bacteria occurring in the animal healthcare environment and the effect of cleaning and disinfection of environmental surfaces and equipment. With this knowledge cleaning and disinfection routines can be improved, leading to a reduced risk of HAIs.

1.2 Bacteria in the environment and on equipment

1.2.1 Measurement of bacterial load

Air

The bacterial load in air is measured with passive or active air sampling. For passive air sampling, settle plates are kept open for a specific time so that bacteria, carried on inert particles, fall onto the surface used (Pasquarella *et al.* 2000; Swedish Standards Institute (SIS) 2015). For passive air sampling, results are commonly reported in colony-forming units (CFU)/area/h (e.g. $CFU/dm^2/h$), CFU/plate/h (generally plates with a diameter of 90 or 140 mm are used) or IMA (the standard index of microbial air contamination), which is the same as CFU/plate/h when using a settle plate with 90 mm diameter (Pasquarella *et al.* 2000).

In active air samplers a known volume of air is blown through a gelatine filter or onto an agar plate (Pasquarella *et al.* 2000; SIS 2015). For active air sampling, results are commonly reported in CFU/m^3 (Pasquarella *et al.* 2000).

Studies in human healthcare show that the bacterial load from passive and active air sampling correlates and that the European Union's Guidelines on Good Manufacturing Practice ratio of 1:8 between passive and active air sampling is valid in operating rooms (ORs) (Napoli *et al.* 2012; Pasquarella *et al.* 2023). The use of settle plates, i.e. passive air sampling, in ORs, during

surgery, has been reported to be a more relevant indicator of surgical site contamination (Friberg *et al.* 1999a, 1999b).

Surfaces

For surface sampling of bacterial load, various collection methods can be used such as dip slides, gauze pads, contact plates, electrostatic cloths, sponges and swabs (Lemmen *et al.* 2001; Otter *et al.* 2009; Thom *et al.* 2012; Ibfelt *et al.* 2014; Ruple-Czerniak *et al.* 2014; Goeman *et al.* 2018; Li *et al.* 2023). It is important to choose a sampling method that suits the surfaces to be sampled, e.g. smoothness and evenness of the surface (Buttner *et al.* 2007).

Comparing surface sampling methods shows that their performance sometimes differs between studies, the performance of, for example, contact plates, gauze pads and swabs differs between studies (Lemmen *et al.* 2001; Brauge *et al.* 2020; Sultan *et al.* 2021; Li *et al.* 2023). A study from human healthcare, did on the other hand show no difference in total bacterial load between one contact plate and four dip slides (Ibfelt *et al.* 2014). One explanation for differences between studies could be that neutralizer (which inhibits the effect of disinfectants) in broths used for extraction of samples can impact the bacterial recovery rate (Downey *et al.* 2012). Neutralizers in contact plates can also be assumed to impact the bacterial recovery rate. Another explanation could be that the type of material in sampling swabs can impact the bacterial load detected, which was reported in a laboratory study sampling biofilms (Watson *et al.* 2024). Sampling protocol can also affect the detected bacterial load (Griffith 2005).

1.2.2 Threshold values

Threshold values for bacterial load on surfaces are used in the food industry, and threshold values for both air and surfaces are used in human healthcare (Griffith *et al.* 2000; Pasquarella *et al.* 2000; Dancer 2004; Lewis *et al.* 2008; Cunningham *et al.* 2011; Mulvey *et al.* 2011; Pasquarella *et al.* 2012; SIS 2015; Ching *et al.* 2021; Moazzami *et al.* 2023). The focus, in human healthcare, is on surfaces with an increased risk of contamination, e.g. high-touch and near-patient surfaces, and air in rooms where the risk of a patient to acquiring an HAI is increased, for example in the OR (Dancer 2004; Lewis *et al.* 2008; Mulvey *et al.* 2011; SIS 2015). The purpose of using threshold values in human healthcare for bacterial load is to reduce the risk of

spreading pathogens to patients and staff (Dancer 2004; Mulvey *et al.* 2011; SIS 2015). Unfortunately there is a lack of evidence for the effect on incidence of HAI in human healthcare for the suggested threshold values as well as for the criteria for microbial cleanliness of equipment.

In human healthcare studies ventilation systems with a laminar airflow generally provides a lower bacterial load in air than turbulent airflow ventilation systems (Whyte *et al.* 1982; Lidwell *et al.* 1983; Erichsen Andersson *et al.* 2014; Birgand et al. 2015; Alsved *et al.* 2018; Knudsen *et al.* 2021). Some older studies have shown that a lower bacterial load in air (provided by laminar airflow ventilation) reduced the wound contamination (Whyte *et al.* 1982; Lidwell *et al.* 1983), while a more recent study did not find a relationship between the bacterial load in air and wound contamination (Birgand et al. 2015). The bacterial load in air from ORs with turbulent airflow ventilation in the study by Birgand *et al.* (2015) was though clearly lower than in the studies by Whyte *et al.* (1982) and Lidwell *et al.* (1983) which may explain at least part of the different results. In line with these inconclusive results, the study by Lidwell *et al.* (1983) showed a reduced risk of HAI in ORs with laminar airflow ventilation, while other studies did not (Zheng *et al.* 2014; Bischoff *et al.* 2017). This indicate a complex situation, with several factors influencing the risk of HAI after surgeries.

Applied threshold values

There are no suggested threshold values for bacterial load in air or on surfaces in animal healthcare, and suggested threshold values from guidelines or recommendations in human healthcare were therefore applied in Studies I-III. An overview of applied threshold values is presented in Table 1.

Table 1. Applied threshold values in Studies I-III (modified from Alsing-Johansson et al. 2024)

Study	Type of sample	Sampling site	Threshold value	Type of threshold value[a]	Type of publication	Reference
I	Passive air during surgery	OR	≤19 CFU/dm²/h	Suggested mean value per surgery	Swedish guidelines, technical specification	SIS 2015
I	Passive air during surgery	OR	39 CFU/dm²/h	Suggested highest value during surgery	"	SIS 2015
I	Passive air in an empty OR	OR	2 CFU/dm²/h	Suggested target value	Observational study suggesting and comparing threshold values	Pasquarella et al. 2012
I	Passive air in an empty OR	OR	5 CFU/dm²/h	Suggested alert value	"	Pasquarella et al. 2012
I	Passive air in medium-risk environments	UR	≤79 CFU/dm²/h	Suggested threshold value	Review presenting the index of microbial air contamination	Pasquarella et al. 2000
I, II, III	High-touch and near-patient surfaces	UR, dental practice and dog cages	< 2.5 CFU/cm²	Suggested threshold value	Observational studies suggesting threshold values	Mulvey et al. 2011; Lewis et al. 2008; Griffith et al. 2000
III	Near-patient surfaces	Medical and surgical wards	< 5 CFU/cm²	Suggested threshold value	Review proposing microbiological standards for surface hygiene	Dancer 2004
I	High-touch and sterile field surfaces	OR	≤0.21 CFU/cm²	Suggested expected level	Italian guidelines	ISPESL[b] 2009
I	High-touch and sterile field surfaces	OR	≤0.63 CFU/cm²	Suggested acceptable level	"	ISPESL 2009

[a]. According to the reference. [b]. Department of Occupational Hygiene

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1.2.3   Environmental bacteria in healthcare

Environmental bacteria in human healthcare, in relation to human healthcare threshold values

In human healthcare there is wide variation in bacterial load from the OR: samples from ORs with laminar airflow ventilation were generally below the threshold value used for clean surgery ($\leq$ 10 CFU/m^3), while more than half of the samples from ORs with turbulent airflow ventilation were above it (Erichsen Andersson *et al.* 2014; Alsved *et al.* 2018; Knudsen *et al.* 2021). In a systematic review, the mean bacterial load in air in restricted areas, such as the OR and the intensive care unit (ICU), varied between 36 and 388 CFU/m^3 depending on ventilation system, the lowest value being from using enhanced heating, ventilation and air conditioning and the highest for rooms without heating, ventilation and air conditioning (Dai *et al.* 2021). Results in line with this from an ICU showed that the majority of active air samples were > 2-40 CFU/m^3, while the majority of passive air samples were 0.5-4 CFU/dm^2/h (Adams & Dancer 2020). While another study reported a mean bacterial load of ~2,500 CFU/m^3 in the gynaecology and paediatrics wards, factors influencing the bacterial load were found to be low cleaning frequency and room temperature and ventilation influenced (Atalay *et al.* 2023). In an ICU, 47% of high-touch surfaces showed a bacterial load above the threshold value (< 2.5 CFU/cm^2) (Adams & Dancer 2020), while in another study the bacterial load on high-touch surfaces in the ICU and OR varied between the hospitals included, from all ten surfaces showing a mean bacterial load below the threshold value (5 CFU/cm^2) to only one surface below it (Khanduker *et al.* 2021).

Environmental bacteria in animal healthcare, in relation to human healthcare threshold values

In animal healthcare there have been only a few small studies on bacterial load in air, most of them from outside Europe. When sampling in the morning prior to the first patient of the day, the bacterial load in ORs often exceeded the suggested threshold values for human healthcare, independently of active or passive air sampling (Harper *et al.* 2013; Jeong *et al.* 2017; Bagcigil *et al.* 2019). For details about threshold values selected for the comparison, see Table 2. Harper *et al.* (2013) reported mean values below the suggested threshold value for human healthcare during surgery.

After surgery, before cleaning and disinfection, Bagcigil *et al.* (2019) reported that all samples exceeded suggested threshold value for human healthcare.

Two studies, however, reported mean bacterial load in active air samples below the suggested threshold value for human healthcare in the rooms used for cats, examination, treatment and radiology during activity in the room (Harper *et al.* 2013; Viegas *et al.* 2018). In another study, from a military working dog clinic, the mean bacterial load in active air samples exceeded the suggested threshold value for human healthcare when there were more than two dogs in a clinic room (Kim *et al.* 2020).

In a study from a small and large animal hospital 22% of high-touch samples showed a bacterial load above or equal to 2.5 CFU/cm^2, while 50% of samples from the dog kennel and 33% of the floor samples showed a bacterial load above or equal to 2.5 CFU/cm^2 (Singaravelu *et al.* 2023). Surface samples from the floor around the small animal clinic showed wide variation, the range being 2-388 CFU/cm^2 (Viegas *et al.* 2018).

Table 2. Threshold values selected for comparison between the results in Study I and results in other studies in animal healthcare

Study	Type of sample	Sampling site	Threshold value	Type of threshold value[a]	Type of publication	Reference
Kim _et al._, 2020	Active air in medium-risk environment	Clinic room	632 CFU/m^3	Suggested threshold value	Review presenting the index of microbial air contamination	Pasquarella _et al._ 2000
Harper _et al._, 2013; Bagcigil _et al._, 2019	Active air in an empty OR	OR	12 CFU/m^3	Suggested target value	Observation study suggesting and comparing threshold values	Pasquarella _et al._ 2012
Harper _et al._, 2013; Bagcigil _et al._, 2019	Active air in an empty OR	OR	32 CFU/m^3	Suggested alert value	"	Pasquarella _et al._ 2012
Harper _et al._, 2013	Active air during surgery	OR	≤ 100 CFU/m^3	Suggested mean value per surgical procedure	Swedish guidelines, technical specification	SIS 2015
Harper _et al._, 2013	Active air during surgery	OR	200 CFU/m^3	Suggested highest value during surgery	"	SIS 2015

[a.] According to the reference

Some studies in animal healthcare have not measured the total bacterial load in air or on environmental surfaces but rather the quantity of one or more pathogens. Even though these studies contribute to increased knowledge of the prevalence of pathogens in air and on environmental surfaces in animal healthcare, the studies do not provide information about the total bacterial load and only limited information about cleanliness. It is important to have knowledge of the total bacterial load to estimate the effect of, for example, cleaning and disinfection. To determine what cleaning and/or disinfection methods to use, knowledge of the total bacterial load is valuable, a high

bacterial load requiring different methods than a low bacterial load. When only focusing on one or more pathogens, it is impossible to estimate whether these pathogens constitute a high or low proportion of the bacterial load. Pathogens have been shown to often, but not always, be found on surfaces with a high total bacterial load in both animal and human healthcare (Adams & Dancer 2020; Singaravelu *et al.* 2023). This shows that it is beneficial to combine sampling of total bacterial load with sampling of pathogens, at least initially, even if the goal is longitudinal sampling of prevalence of pathogens.

Environmental bacterial flora in human healthcare

Some bacteria genera have been reported as common (> 10% of bacterial load) on environmental surfaces and equipment and in air in human healthcare. The following genera are the most commonly reported, in decreasing order: *Staphylococcus* spp., *Acinetobacter* spp., *Escherichia* spp., *Bacillus* spp., *Pseudomonas* spp., *Flavobacterium* spp., *Klebsiella* spp., and *Micrococcus* spp. (Karigoudar *et al.* 2020; Sebre *et al.* 2020; Shi *et al.* 2020; Khanduker *et al.* 2021; Teklehaimanot *et al.* 2021; Atalay *et al.* 2023).

Environmental bacterial flora in animal healthcare

Staphylococcus spp. and *Micrococcus* spp. have been reported as common bacteria (> 10% of bacterial load) in environmental air samples in animal healthcare (Kim *et al.*, 2020; Jeong *et al.*, 2017; Harper *et al.*, 2013). In studies of contamination of equipment in animal healthcare *Staphylococcus* spp. was generally reported as common (> 10%), while *Bacillus* spp. was common in two of the referenced studies (Mount *et al.* 2016; Gustafsson *et al.* 2024).

Biofilm

Some bacteria have an increased capacity to attach to surfaces, for example through the presence of fimbriae and flagella, and production of extracellular polymeric substances (EPSs) (Donlan 2002). Attached bacteria produce EPSs for protection, they grow and divide, and planktonic (free-floating) microorganisms are entrapped in the EPS, together forming a biofilm (Lindsay & von Holy 2006). Microorganisms in biofilms use quorum sensing (intercellular signalling) for communication to adapt to the environment by varied gene expression and exchange genes (Donlan 2002). Bacteria in biofilms are difficult to remove from the biofilm; a recent laboratory study showed that only between 3 and 30% of the dry surface biofilm (DSB)

bacteria were detected in swab samples (Watson *et al.* 2024). Bacteria in biofilms are also difficult to culture, making it difficult to identify them (Watson *et al.* 2023; Rayner *et al.* 1998). Furthermore, microorganisms in biofilms show increased resistance or tolerance to antimicrobials and heavy metals (Teitzel & Parsek 2003; Souza *et al.* 2020; Brunke *et al.* 2022). Bacteria in multi-species DSBs have been reported to survive for ≥12 months without nutrition (Hu *et al.* 2015). Microorganisms in biofilm can detach from the biofilm and spread to the environment (Donlan 2002). This happens both by quorum sensing and, for example, by cleaning when the biofilm is not removed but disrupted (Fernando *et al.* 2019; Donlan 2002).

Biofilms, generally multi-species, are found on many surfaces in human healthcare, including, for example, on high-touch surfaces such as computer keyboards, drains, equipment and furnishings (Vickery *et al.* 2012; McLean *et al.* 2013; Hu *et al.* 2015; Johani *et al.* 2018; Ledwoch *et al.* 2018; Costa *et al.* 2019; Watson *et al.* 2023; Hayward *et al.* 2024). Wet surfaces such as sink drains and washbasins have been shown to act as reservoirs for multidrug-resistant organisms including carbapenemase-producing *Enterobacterales* clones carrying carbapenemase genes as well as *Pseudomonas aeruginosa*, which may cause occasional HAIs and outbreaks of HAIs (Tofteland *et al.* 2013; Fernando *et al.* 2019; Perkins *et al.* 2019; Aracil-Gisbert *et al.* 2024).

Common (> 10% of total bacterial load) bacteria frequently found in DSBs include: *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp. and *Bacillus* spp (Hu *et al.* 2015; Johani *et al.* 2018; Ledwoch *et al.* 2018; Costa *et al.* 2019). Resistant bacteria frequently reported in DSB include: MRSA, vancomycin-resistant *Enterococcus* (VRE), and bacteria producing extended-spectrum beta-lactamase (Vickery *et al.* 2012; Hu *et al.* 2015; Johani *et al.* 2018; Ledwoch *et al.* 2018; Costa *et al.* 2019; Ledwoch *et al.* 2021a).

Unfortunately, there are no studies of the prevalence of biofilm on surfaces in animal healthcare. There are, however, laboratory studies showing that bacterial isolates, mainly *Staphylococcus aureus* from veterinarians, animals and animal healthcare environments produce biofilms (Olson *et al.* 2002; Chen *et al.* 2020; Silva *et al.* 2022; Šmitran *et al.* 2023).

1.2.4 Transmission of pathogens

Most studies on transmission of pathogens have been conducted in human healthcare or in a laboratory setting. An association between bacterial load and frequency of hand contact by staff and visitors was seen in the ICU in human healthcare (Adams *et al.* 2017). In the study by Adams *et al.* (2017), most of the S*taphylococcus aureus* detected were present on highly contaminated surfaces. Results in line with that study were presented in a study from animal healthcare, where the total bacterial load was high on near-patient surfaces in dog kennels (Singaravelu *et al.* 2023).- Enterococci were detected on 50% of those surfaces and *Escherichia coli* was detected on 20% of the surfaces. To sample relevant surfaces efficiently in order to save time and resources, it is beneficial to know where to sample. By identifying highly contaminated surfaces among 113 surfaces sampled, nine surfaces were identified to be sampled in a longitudinal study (Singaravelu *et al.* 2023). Faecal organisms were, in fact, detected more than once on seven out of nine surfaces in the longitudinal study, indicating that the selection process for sampling sites was successful.

In the study by Singaravelu *et al.* (2023), staff movements between the large- and small animal hospitals and low compliance with hygiene routines were likely explanations for cross-contamination in the animal hospital. In a hospital with endemic MRSA, it was as common for staff to contaminate their hands from near-patient surfaces as from direct patient contact (Creamer *et al.* 2010). In human healthcare there is an increased risk of acquiring a specific pathogen infection if the previous occupant was infected or colonized with the same pathogen (Mitchell *et al.* 2023).

Wiping DSBs with 1000 ppm sodium hypochlorite significantly reduced, but did not eliminate, the transfer of bacteria compared to control DSBs (which were not wiped) in a laboratory study (Ledwoch *et al.* 2019). The transfer test imitated the touch of a finger on a surface, biofilm discs (discs where biofilms were grown before the experiment started) being pressed onto the agar plate (Ledwoch *et al.* 2019). In DSBs formed in the presence of organic matter, bacterial transfer, after wiping with 1000 ppm sodium hypochlorite, was significantly higher than in clean DSBs (Ledwoch *et al.* 2019). Bacteria, presumably from DSBs, were found to be transferable at a high level from hospital keyboards, in human healthcare, despite surface disinfection with 1000 ppm sodium hypochlorite (Ledwoch *et al.* 2021a). It has also been reported, in laboratory studies, that both bare and gloved hands

can transfer a large number of bacteria from DSBs to multiple surfaces and may serve as an environmental reservoir of pathogens and spread HAIs (Chowdhury *et al.* 2018; Tahir *et al.* 2019; Ledwoch *et al.* 2021b).

A study from human healthcare revealed multiresistance region plasmids in environmental bacteria of different genera (Betteridge *et al.* 2012). This indicates that residential bacteria may cause HAIs by spread of clones carrying multiresistance region plasmids (Betteridge *et al.* 2012).

1.2.5   Resistance to disinfectants

Bacteria that have an increased tolerance of, or are resistant to, disinfectants such as quaternary ammonium compounds (QAC) and chlorhexidine have been reported in laboratory studies (Boyce 2023; Fernandes Â *et al.* 2024). QAC genes found for example in *Staphylococcus* spp. and *Enterococcus* spp. encode multidrug efflux pumps (Lyon & Skurray 1987; Bjorland *et al.* 2003). QAC genes may spread with plasmids encoding resistance to QACs (Bjorland *et al.* 2003). However, in human clinical settings resistance to QACs seems uncommon (Boyce 2023). In animal healthcare an outbreak of HAI was caused by a chlorhexidine-resistant *Serratia marcescens* from a container in which gauze, used for pre-operative skin disinfection, was moisturised (Keck *et al.* 2020). A systematic review and meta-analysis found no evidence of reduced susceptibility to chlorhexidine in staphylococci or streptococci isolates of human origin (Aftab *et al.* 2023).

1.3   Cleanliness

1.3.1   Classification of medical equipment based on infection risk

In human healthcare, medical equipment has been classified based on its use and the infection risk it poses for patients since the 1950s, when this system was introduced (Spaulding 1957). The classification included the categories of non-critical, semi-critical and critical (Spaulding 1957). Today, the main focus internationally is on describing reprocessing processes (i.e. cleaning, disinfection and sterilization) for each category of medical equipment, while in Sweden the focus has been on both microbial cleanliness and reprocessing process (The Public Health Agency of Sweden (Fohm) 2006; SIS 2006; U.S. Centers for Disease Control and Prevention (CDC) 2008; SVF & SVS 2012; SVF & SVS 2017; WHO 2018). In Sweden there are two categories of

criteria for microbial cleanliness of semi-critical equipment, based on use, a stricter one for e.g. endoscopes and a less strict one for e.g. compresses used for wound care (Fohm 2006). The Spaulding classification, guidelines and standards are presented in Table 3.

Table 3. Classification of medical equipment based on infection risk to patients. A combination of Spaulding's classification and Swedish guidelines

Category[a]	Use[abc]	Example of equipment and products[bc]	Microbial cleanliness[b]	Reprocessing process[abc]
Non-critical	In contact with intact skin	Stethoscopes	Visibly clean	Cleaning or low- or intermediate level[d] disinfection
Semi-critical	In contact with damaged skin or intact mucous membranes	Endoscopes	Free from pathogenic microorganisms. Less than one microorganism on 1000 items[e]	High-level disinfection[dg]
		Compresses	Free from pathogenic microorganisms and occurrence of occasional vital microorganisms[f]	
Critical	In contact with sterile tissue	Surgical instruments	Free from viable microorganisms and less than one microorganism on 1,000,000 items	Sterilization[h]

[a] From Spaulding (1957). [b] From Swedish guidelines and standards (Fohm 2006; SIS 2006; SVF & SVS 2012; SVF & SVS 2017) [c] From international guidelines (CDC 2008; WHO 2018) [d] Disinfectants are grouped in the levels of low, intermediate, and high based on what microorganisms they can inactivate, from enveloped viruses, which are easiest to inactivate to mycobacteria and bacterial spores, which are the most difficult to inactivate (Spaulding 1957). [e] The strictest criteria for microbial cleanliness was defined as 0 CFU/equipment or product in the thesis. [f] The less strict criteria for microbial cleanliness was defined as ≤ 1 CFU/equipment or product in the thesis [g] Before disinfection the equipment needs to be cleaned, [h] Before sterilization the equipment needs to be cleaned and disinfected

1.3.2 Cleaning and disinfection methods

The Sinner's circle (Sinner 1960), describes the interaction between four components needed for a satisfactory result from cleaning: chemical action, mechanical action, temperature, and time, see Figure 1. If one factor is reduced another needs to increase to reach a satisfactory cleaning result.
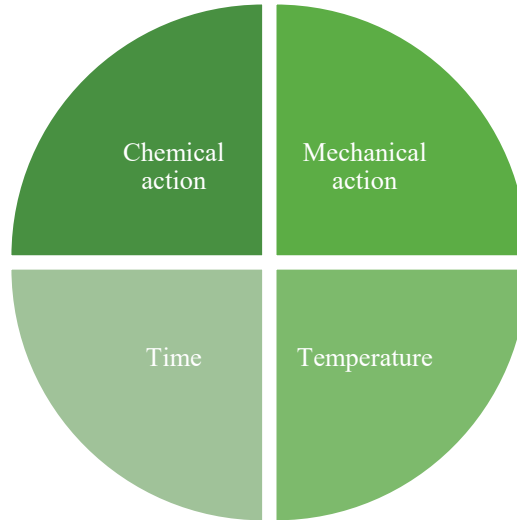
Figure 1. The Sinner's circle showing the four interacting factors in cleaning

Environmental surfaces

In human healthcare, surface cleaning of floors and walls is usually carried out in the form of moist cleaning with detergent on mops or cloths made of cotton, viscose or microfibre (The Swedish Association for Infection Control (SFVH) 2020). Wet cleaning with mops or cloths with detergent and water are only used on heavily soiled areas (SFVH 2020). Similarly, in animal healthcare, cloths or scrubbing brushes with detergent are recommended for cleaning heavily soiled surfaces and can be followed by rinsing with water (SVF & SVS 2012). There is, however, a risk of splashes and aerosol production which can spread pathogens, as well as a risk of slipping on wet floors during wet cleaning and cleaning with a scrubbing brush (SVF & SVS 2012; SFVH 2020). If wet cleaning including rinsing is used, rinsing with a soft water jet, to minimize the aerosol production, and to wipe up excess water or squeegeeing is recommended (SVF & SVS 2012). In all surface

cleaning, it is important to work from clean to dirty surfaces, i.e. on walls starting high and scrubbing or wiping downwards (SVF & SVS 2017).

A common cleaning method in small animal healthcare in Sweden, even though not recommended in guidelines, is cleaning with moist microfibre mops and moist microfibre cloths. Little is known about the effect on bacterial load of cleaning with moist microfibre mops and cloths in animal healthcare, but in a laboratory study only one out of six different damp microfibre cloths was shown to perform better than a damp paper towel (Moore & Griffith 2006). Furthermore, microfibre cloths performed better with detergent/disinfectant in another laboratory study (Robertson *et al.* 2019).

Surfaces are generally disinfected with surface disinfectants (a liquid disinfectant applied on surfaces) but whole room disinfectants (aerosolized or gaseous hydrogen peroxide or UV light) can also be used (SVF & SVS 2012; SFVH 2020). In both surface cleaning and disinfection it is important to physically wipe or scrub the surface (SVF & SVS 2017).

Equipment

Medical equipment is preferably cleaned and disinfected in a washer-disinfector (SVF & SVS 2012; SVF & SVS 2017). If that is not an option, due to lack of a washer-disinfector or, for example, heat-sensitive material, the equipment should be cleaned in an ultrasound cleaner or manually cleaned, preferably completely dispersed in water to lower the risk of splashes, and then soaked in a disinfectant (SVF & SVS 2012; SVF & SVS 2017).

1.3.3   Effect of cleaning and disinfection

Effect of environmental cleaning and disinfection in human healthcare

All included studies on effect of environmental cleaning and disinfection in human healthcare cover the effect of cleaning and disinfection of environmental surfaces. Detergent cleaning, using one disposable detergent wipe per site, of near-patient surfaces resulted in 0.29 $\log_{10}$ reduction of bacterial load after 4 h (Bogusz *et al.* 2013). For *Staphylococcus aureus* load, the detergent cleaning resulted in 0.78 $\log_{10}$ reduction after 4 h (Bogusz *et al.* 2013). All surfaces, except the overbed tables, had a bacterial load < 5 CFU/cm^2 in all samplings ≤ 24 h after cleaning (Bogusz *et al.* 2013). Another study showed that, although the bacterial load on bed rails in the

ICU was generally below 2.5 CFU/cm^2 after disinfection (with a QAC or isopropanol), the bacterial load was above 2.5 CFU/cm^2 within 2 h after disinfection (Attaway *et al.* 2012). Enhanced detergent cleaning of high-touch surfaces in a surgical ward with endemic MRSA resulted in a 0.17 log$_{10}$ reduction of the bacterial load (Dancer *et al.* 2009). It also resulted in a 0.14 log$_{10}$ reduction in new MRSA infections and thereby saved the hospital an estimated < 80,000 euro (Dancer *et al.* 2009).

Effect of environmental cleaning and disinfection in animal healthcare

All studies of effect of environmental cleaning and disinfection in animal healthcare includes effect of cleaning and disinfection of equipment. Studies showed that 51-81% of clipper blades from hair clippers used in clinical practice remained contaminated after decontamination (Mount *et al.* 2016; Gustafsson *et al.* 2024). Fewer of the clipper blades remained contaminated after disinfection with an alcohol-based clipper disinfectant than those decontaminated with lubricant-based clipper cleaners (Mount *et al.* 2016). In a laboratory study, disinfectants with a higher alcohol content or chlorhexidine gluconate resulted in all clipper blades being free from contamination, while disinfectants with a lower alcohol content and saline resulted in contaminated clipper blades (Ley *et al.* 2016). In a laboratory study on disinfection of endoscopes, a disinfectant containing ethanolamine, isopropanol and QACs, as well as a disinfectant containing ortho-phthalaldehyde, resulted in no culture-positive endoscopes (Svonni *et al.*, 2020). Disinfection with ethanol resulted in 17% contaminated endoscopes (Svonni *et al.* 2020). When endoscopes were used for examination of patients, of which 37% were identified as carriers of *Streptococcus equi,* disinfection with ortho-phthalaldehyde also resulted in no culture positive endoscopes (Svonni *et al.* 2020).

Effect of biofilms on environmental cleaning and disinfection

In a laboratory study *Staphylococcus aureus* DSB was significantly harder to remove using a wipe moistened with sterile water than dried planktonic bacteria (Parvin *et al.* 2019). One wiping action reduced the *S. aureus* load of planktonic bacteria by > 3 log$_{10}$ while 50 wiping actions reduced the *S. aureus* in DSB by 1.4 log$_{10}$ (Parvin *et al.* 2019).

Terminal cleaning including cleaning with neutral detergent followed by disinfection with 500 ppm sodium hypochlorite resulted in biofilm detection in 83-93% of the surface samples (Vickery *et al.* 2012; Hu *et al.* 2015;

Ledwoch *et al.* 2018). There was no evaluation of the prevalence before terminal cleaning in the studies by Vickery *et al.* (2012) and Hu *et al.* (2015). The high prevalence of DSBs after disinfection with sodium hypochlorite corresponds to the results in a laboratory study in which 1000-20,000 ppm sodium hypochlorite reduced DSB *S. aureus* by > 7 $\log_{10}$ and > 95% of the biomass in the biofilm; however, the remaining viable bacteria regrew and formed biofilms (Almatroudi *et al.* 2016). In another laboratory study, most chemicals (9 out of 13) reduced bacterial viability by $\geq 4\log_{10}$, but only 5 out of 13 prevented bacteria transfer from treated surfaces, and only one (a peracetic acid) delayed biofilm recovery (Ledwoch *et al.* 2021b).

In a laboratory study, biofilm of an outbreak strain of *Klebsiella pneumoniae* was tolerant to peracetic acid (PAA) disinfection (Brunke *et al.* 2022). The duodenoscopes which were spreading *K. pneumoniae* were disinfected with PAA, which can be assumed to explain the tolerance (Brunke *et al.* 2022). In a burns unit, the detection of carbapenemase-producing *Enterobacteriacae* from wet surfaces, floor drains and sluices increased after vigorous scrubbing with a sodium hypochlorite disinfectant indicating possible disruption of wet surface biofilms (Fernando *et al.* 2019).

2. Main aims of the thesis

The overall aim of the thesis was to improve infection prevention and control in animal healthcare by studying the bacterial load, before and after cleaning and disinfection, in the environment and on equipment.

Specific objectives were to:
- Assess the bacterial load in air, on environmental surfaces and on equipment in animal healthcare.
- Compare the bacterial load in air and on surfaces with threshold values for bacterial load suggested for use in human healthcare and the bacterial load on equipment with criteria for microbial cleanliness.
- Evaluate the effect of cleaning and disinfection of near-patient surfaces and equipment.
- Characterise the genetic relationship between selected bacterial species, from operating and ultrasound rooms, to assess clonal dissemination.
- Investigate factors associated with bacterial load during surgery and on near-patient surfaces.

3. Comments on material and methods

This chapter provides a summary and comments on the material and methods section in Papers I-III. The selection of sampling methods and their user-friendliness for in-house sampling in animal healthcare are discussed. Detailed information on the material and methods section can be found in the individual papers.

3.1 Study design

Studies I and II were prospective observational studies, while Study III was a prospective, randomized study with a parallel group design. Study I was carried out in an OR and an ultrasound room (UR) in a private small animal hospital. Study II was carried out in a dental practice in a private equine hospital, while Study III was carried out in a mixed medical and surgical ward at a university small animal hospital. Before the start of each study a pilot study was carried out to identify relevant sampling techniques and sampling locations.

3.2 Cleaning and disinfection

In Study I the abdominal positioner cushion was either cleaned or disinfected. In Study II different cleaning and/or disinfection methods were used for decontamination of dental handpieces and the head support. In Study III two cleaning methods for cleaning dog cages were compared, and all dog cages were disinfected after cleaning. An overview of applied cleaning and disinfection methods in Studies I-III is presented in Table 4. For pictures of the surfaces cleaned and disinfected in Studies I-III, see Figure 2.

Table 4. Cleaning and disinfection methods used in studies I-III

Study	Equipment/surface	Cleaning methods	Disinfection methods
I	Abdominal positioner cushion	Microfibre cloth[a] moistened with water	Wiping paper moistened with isopropanol with surfactant[b]
II	Dental handpieces	Cleaning wipes with surfactants[c]	Disinfection wipes with nitrogen based disinfectant[d]
		Dish-washing brush with standard washing liquid	
II	Head support	Cleaning wipes with surfactants	Disinfection wipes with nitrogen based disinfectant
			Wiping paper moistened with alcohol and surfactant[e]
III	Floor and wall	Scrubbing brush[f] with surfactants[g]	Mop[i] moistened with peroximonosulfate[j]
		Microfiber mop[h] moistened with water	

[a] Duotex® Microfibre Cloths, Micro System Duotex AB, Solna, Sweden. [b] Liv Des +45, Liv By Clemondo, Helsingborg, Sweden. [c] ICA Städservett, ICA, Solna, Sweden. [d] Wet Wipe Triamin Disinfection, Wet Wipes A/S, Vallensbæk, Denmark. [e] Dax 75+, KiiltoClean AB, Täby, Sweden or LiV72+, Clemondo, Helsingborg, Sweden. [f] Deck Scrub, waterfed, 270 mm, Very hard, White, Vikan A/S, Skive, Denmark. [g] Allotol Natur, Nordexia AB, Bromma, Sweden. [h] Duotex® Shine Plus Mop 30 cm, ID MSD420TRW, Micro System Duotex AB, Solna, Sweden [i] Vileda UltraMax Refill, Vileda, Freudenberg Home and Cleaning Solutions GmbH, Weinheim, Germany. [j] DesiDos, SeptiChem ApS, Holte, Denmark

Figure 2. Photographs of environmental surfaces and equipment which were cleaned and disinfected during Studies I-III. a. abdominal positioner cushion (Study I), b. low-speed handpiece, surgical low-speed handpiece and high-speed handpiece (Study II), c. Dog cage (Study III) (photographs by author)

3.2.1 Selection of cleaning and disinfection methods

The purpose of Studies I-III was to evaluate hygiene routines, e.g. cleaning and disinfection routines, which are used in animal healthcare to gain an understanding of how well they reduce the bacterial load. The applied hygiene routines in Studies I-III include some hygiene routines that, based on knowledge from laboratory and human healthcare studies, could be assumed to not result in sufficiently clean surfaces or equipment.

In Study I (OR and UR), the animal hospital's in-house cleaning and disinfection routines were applied, both methods being common in small animal healthcare. In Study II (dental practice), the hospital's in-house disinfection routines, which are common in animal healthcare, as well as manual cleaning routines, were tested in different combinations for both dental handpieces and the head support. In Study III (dog cages), the bacterial reduction of two common cleaning methods, one of them being the university small animal hospital's in-house cleaning routine, for cleaning dog cages were compared. Dog cages were disinfected, following the hospital's in-house disinfection routine, after cleaning by both cleaning methods.

3.3 Data collection

3.3.1 Bacterial sampling

The bacterial load in air was analysed by passive air sampling in Study I (OR and UR). In Studies I-III, the bacterial load on surfaces was analysed by three surface sampling methods. Sampling equipment is shown in Figure 3. An overview of sampling methods used in Studies I-III is presented in Table 5 and details of sampling are given in Table 6. Petrifilms were used for cultivation of the broth from sampling sponges and swabs in Studies II (dental practice) and III (dog cages).
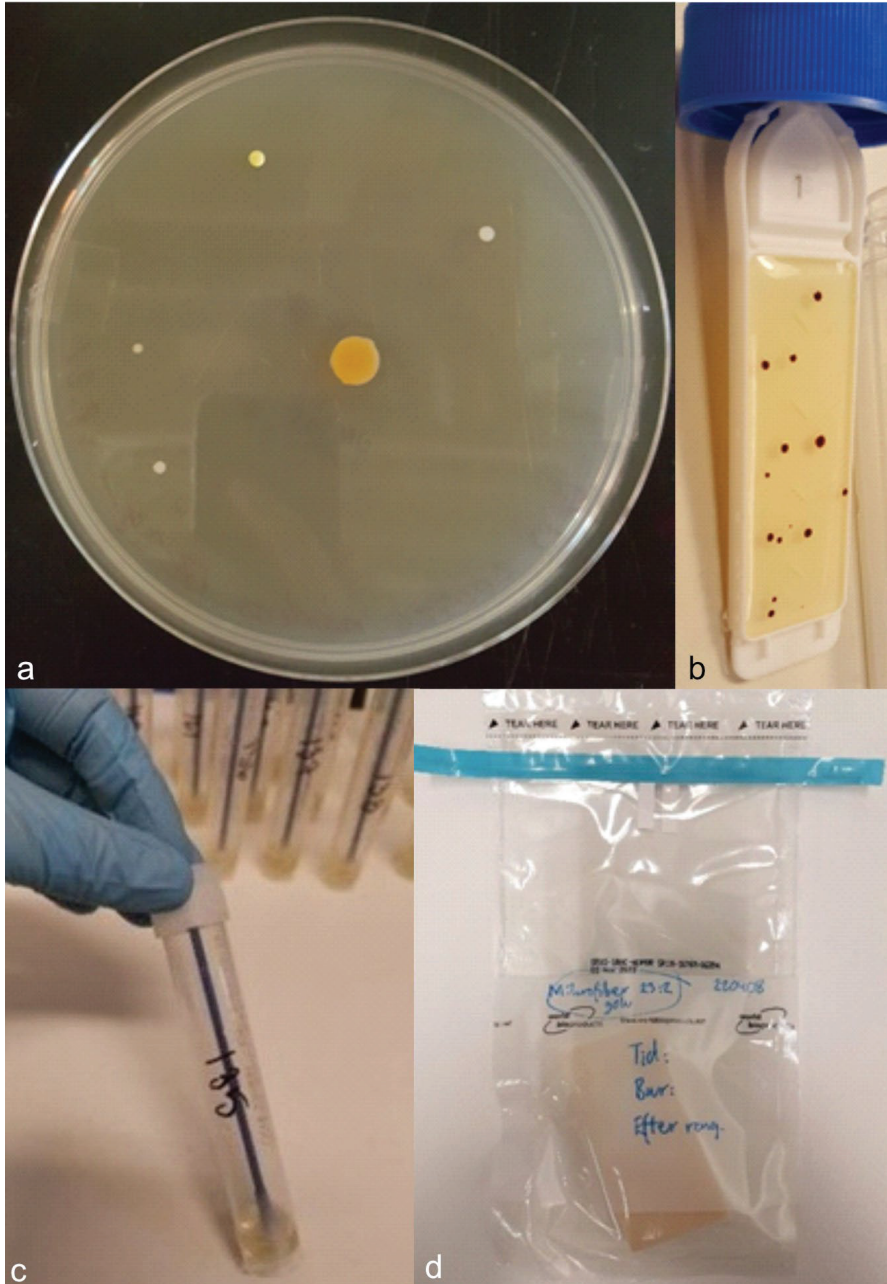
Figure 3. Sampling methods used: a. settle plate (photograph by Ingrid Hansson), b. dip slide (photograph by author), c: swab sampler (photograph by Johanna Persson), and d. sampling sponge (photograph by Elin Torstensson)

Table 5. Overview of sampling methods used in Studies I-III

Study	Type of sample	Sampling method	Medium
I	Passive air	Settle plates[a]	TSA[b]
I, II	Surface	Dip slides[c]	TSA/TSA+neutralizers
II	Surface	Swab[d]	Letheen broth
III	Surface	Sponge[e]	HiCap neutralizing broth

[a.] 14 cm in diameter; 80 mL TSA in-house production. [b.] Tryptic Soy Agar. [c.] Envirocheck® Dip Slide DC Disinfection Control, 9.4 cm^2 per side, Merck KGaA, Darmstadt, Germany. [d.] 3M^{TM} Swab Sampler, 3M, Saint Paul, Minnesota, USA. [e.] SampleRight™ sponge sampler, World Bioproducts, Libertyville, Illinois, USA

Table 6. Details about samplings in Studies I-III

Study	Sampling site	Extent of study	Sampling method	Number of samples
I	Operating room	27 surgeries	Settle plates	114
I			Dip slides	188
I	Ultrasound room	25 ultrasound examinations	Settle plates	42
I			Dip slides	99
II	Dental practice	Dental care of 11 patients	Swab	146
II			Dip slides	14
III	Care unit	46 dog cages	Sponge	276

Sampling before, during and after activities

Sampling prior to the first patient of the day was performed (either in empty rooms or during staff preparations) in air (Study I), on near-patient (Study I and II) and high-touch surfaces (Study I), and on equipment (Study II). During activities (surgery and ultrasound examination) air was sampled, changing settle plates every hour (Study I). After the activities, equipment (Study II), near-patient surfaces (Studies I and II) and surfaces in the sterile field (Study I) were sampled. It was only possible to sample surfaces in the sterile field after surgery due to the risk of contamination from sampling. The sampling took place immediately after the surgery ended to obtain as representative samples as possible for contamination during surgery (Study I).

Evaluation of the effect of surface cleaning and disinfection

The effect of surface cleaning and disinfection of near-patient surfaces was evaluated in the UR (Study I, OR and UR), the dental practice (Study II), and

the mixed medical and surgical ward (Study III, dog cages). In Study II the effect of surface cleaning and disinfection of equipment, i.e. dental handpieces, was also evaluated.

3.3.2 Discussion of selected methods for measuring bacterial load

Passive air sampling

Passive air sampling was chosen in Study I (OR and UR) due to settle plates being easy to use, cheap, standardized, with no need for extra sampling equipment, and considered relevant to use in hospital environments (Pasquarella *et al.*, 2023; Napoli *et al.*, 2012; Pasquarella *et al.*, 2000). Settle plates are recommended for microbiological measurements of the air in peripheral parts of ORs with a turbulent airflow system and 17-20 air changes/h (SIS, 2015). Pasquarella *et al.* (2000) suggest using the 1/1/1 schedule: settle plate open for 1 h, 1 m above the floor, and 1 m from the wall. In Study I, the settle plates were kept open for up to 1 h, replaced every hour, during surgery as well as in the UR. For practical reasons, i.e. not to disturb the ongoing work in the OR and UR, the settle plates were placed ~0.9-1.1 m above the floor and less than 1 m from the walls. The air change in the OR was ~21 air changes/h, measured during a mandatory ventilation control during Study I.

Surface sampling

Several methods for surface sampling were used in Studies I-III. Dip slides, sampling swabs and sponges are all commonly used for surface sampling in human healthcare and the food industry (Moazzami *et al.*, 2023; Maes *et al.*, 2017; Lewis *et al.*, 2008; White *et al.*, 2008; Griffith *et al.*, 2000). The reason for using different sampling methods was to apply a suitable sampling method for environmental surfaces or equipment sampled. A positive effect of using different sampling methods was the possibility of assessing how user-friendly different sampling methods could be for animal healthcare staff for in-house evaluation for example of the effect of cleaning.

Dip slides

Dip slides were convenient to use for sampling of smooth even surfaces, such as the sampled high-touch surfaces in Study I. Those surfaces were usually touched, i.e. contaminated, by the dorsal part of the fingers or the palmar wrist but not by the fingertips. However, due to the selected method some

high-touch surfaces, usually touched by fingertips and the palmar part of the hand (such as door handles, computer keyboard and machine buttons) were not sampled. It is likely that the results would have differed if a sampling method suitable for sampling of high-touch surface usually touched by fingertips had been used, and those surfaces had consequently been sampled.

Sampling swabs

Swabs were easy to use for sampling of smooth, even surfaces such as high-touch and near-patient surfaces (tested in a pilot during Study I and in Study II, dental practice) and on smooth and mostly even surfaces such as external parts of dental handpieces. However, sampling was more difficult to perform on abrasive surfaces (pilot during Study I) due to difficulty in using the correct sampling technique when the swab almost became stuck in the surface.

Swab sampling of the shaft of the surgical low-speed handpiece (Study II) was easy to perform, as it was a smooth, even surface, while sampling of couplings of dental low-speed handpieces and dental high-speed handpieces was difficult to perform. The couplings were narrow and uneven and had small parts which make it difficult to sample with a swab. The couplings and the shaft were, however, concluded to be non-relevant to sample in order to assess whether internal surfaces are contaminated or not as there are components which have a higher risk of being exposed to contamination (since their surfaces may be directly contaminated during dental care) and that are more difficult to decontaminate. For future studies relevant components such as turbines, spray channels and inner gears should be sampled with a suitable sampling method, as was done by Smith & Smith (2014).

Sampling sponges

Sponges worked well for sampling of non-smooth non-even anti-slip surfaces such as the floor, as well as of smooth even surfaces on the wall (Study III, dog cages). A sampling sponge made of polyurethane was chosen as it should be resistant to crumbling and tearing (World Bioproducts no date), which was important as the floor in the dog cages have an anti-slip surface layer to prevent dogs from slipping on the floor.

Neutralizers

Neutralizers are added to agar and broths to neutralize the effect of remnants of disinfectants used on the surface sampled (Russell *et al.* 1979). By inhibiting the effect of disinfectants, viable, but inhibited, bacteria can be cultured (Russell *et al.* 1979). Different agar and broths were used for culturing of the surface samples in Studies I-III, all with some neutralizers for disinfectants. In Studies I (OR and UR) and II (dental practice), a two-sided dip slide was used, with neutralizers on one of the sides.

When the disinfection effect is evaluated, it is important to choose a neutralizer that inhibits the disinfectant used. This can, unfortunately, be difficult as there are new surface disinfectants on the animal healthcare market and information about neutralization of them, using commercial neutralizers, is sparse. For this reason, information from producers of agar mediums and broths have been included when no information has been possible to find. The used neutralizers and their neutralising effect are presented in Table 7.

Table 7. Neutralizers in Studies I-III and their neutralizing effect on disinfectants that are on the animal healthcare market

Disinfectant	Neutralizing agents		
	TSA + neutralizers[a] (Studies I (OR and UR) and II (dental practice)	Letheen broth (Study II)	HiCap neutralizing broth (Study III, dog cages)
Peroximonosulphate	Peroxyacetic acid[b] (Liofilchem 2021)[c]	At very low concentration (Ward, 2013)	Peroxyacetic acid (Ward, 2013)
Sodium hypochlorite dioxide	Sodium hypochlorite and sodium chlorite[d] (Sutton *et al.*, 2002)	Sodium hypochlorite and sodium chlorite (Ward, 2013; Sutton *et al.*, 2002)	Sodium hypochlorite and sodium hypochlorite compounds (Ward, 2013)
Hydrogen peroxide	Peroxyacetic acid (Liofilchem)	At very low concentration (Ward, 2013)	(Ward, 2013)
Hypochlorous acid	Sodium hypochlorite and sodium chlorite (Sutton *et al.*, 2002)	Sodium hypochlorite and sodium chlorite (Ward, 2013; Sutton *et al.*, 2002)	Sodium hypochlorite and sodium hypochlorite compounds (Ward, 2013)
Chloramine	Sodium hypochlorite and sodium chlorite (Sutton *et al.*, 2002)	Sodium hypochlorite and sodium chlorite (Ward, 2013; Sutton *et al.*, 2002)	Sodium hypochlorite and sodium hypochlorite compounds (Ward, 2013)
Ethanol	(Johnston *et al.*, 2002; Sutton *et al.*, 2002)	(Sutton *et al.*, 2002)	(Ward, 2013)
Isopropanol	(Russell *et al.*, 1979)[e]	(Johnston *et al.*, 2002; Russell *et al.*, 1979; Rosenkranz *et al.*, 1965; Treick & Konetzka, 1964)[e]	(Ward, 2013)
Nitrogen			

Green indicates that the neutralizer can neutralize the disinfectant. Yellow indicates that the neutralizer may neutralize the disinfectant. Red indicates that it is unknown whether the neutralizer neutralizes the disinfectant. [a.] Neutralizers include polysorbat 80, sodium thiosulfate, lecithin and histidine (Merck, 2019). [b.] Peroximonosulphate and peroxyacetic acid are both persulphates, and it can be assumed that the neutralizing effect is similar. [c.] The reference is a producer of culture media and broths. [d.] It depends on the substance left on the surface, if any, and if the neutralizer has effect on that substance. [e.] According to Russell et al. (1979), polysorbates have some neutralizing effect on isopropanol, and dilution would probably neutralize isopropanol as it does with ethanol (Johnston et al., 2002; Rosenkranz et al., 1965; Treick & Konetzka, 1964). Dilution is not, however, possible using dip slides (Studies I and II).

In Studies I (OR and UR) and II (dental practice), the bacterial load overall was numerically higher on the side of the dip slide with neutralizers added to the agar. The result indicates that neutralizers inhibited the isopropanol used for disinfection in Study I, in line with what was suggested by (Russell et al. 1979). The result in Study II indicates that the added neutralizers inhibited at least some of the disinfectants (ethanol, isopropanol and nitrogen) used on the head support. Based on the result in Study I and in the studies by Johnston et al. (2002) and Sutton et al. (2002), it can be assumed that ethanol and isopropanol were inhibited. It is, however, impossible to know whether the nitrogen disinfectant was inhibited or not. The nitrogen-based disinfectant was also used for disinfection of dental handpieces (Study II), and since Letheen broth was used for all samples it is impossible to know if the disinfectant was inhibited. Nitrogen-based disinfectants are fairly new on the market, and it has not been possible to find any studies on the inhibitory effect of neutralizers on them. In Study III (dog cages) HiCap neutralizing broth was used for all samples, and it is therefore impossible to evaluate its inhibitory effect in the study, but it can be assumed to have inhibited peroximonosulphate, as it has been reported to do (Ward 2013).

Petrifilm

Initially petrifilms were tested in a pilot during Study I (OR and UR), for cultivation of broth from swabs. The swabs were used for sampling of different high-touch surfaces including both high-touch surfaces sampled with dip slides in Study I and, for example, the light switch and the bottle with ultrasound gel. Hydrated petrifilm plates were also tested in the pilot, for direct surface sampling of near-patient and high-touch surfaces, both surfaces being sampled in Study I and, for example, the door handle and the focus tracking ball on the ultrasound machine. Hydrated petrifilm plates were finally also tested for passive air sampling, kept open for 15 min, in the pilot (Study I). Petrifilms were considered an easy and fast method using only a minimum of laboratory equipment, for analyses of bacterial load. Beside the quite high price of petrifilms, the main drawback was that it was hard to re-cultivate bacteria from the petrifilm agar, which was tested in a pilot study for Study II (dental practice). Hydrated petrifilm plates were easy to use for direct surface sampling on even and fairly smooth surfaces, as well as for passive air sampling. A drawback when used for passive air sampling was, however, that it was recommended to stay open for only 15 min, which makes it difficult to use for long-term sampling due to the work load, the

potential interruption of activities for example in the OR, and the number (i.e. expense) of used petrifilms.

User-friendliness for in-house monitoring

Settle plates were found to be user-friendly due to being easy to use for sampling, and only an incubator and decent light (for counting CFUs) is needed (Study I, OR and UR). The settle plates do, however, take up a lot of space in the refrigerator (before sampling) and in the incubator, which can make them difficult to use for in-house monitoring. In Studies I and II (dental practice), dip slides were found to be user-friendly due to being fast and easy to use for sampling. Only an incubator and decent light are also needed for dip slides. The experience from Studies II and III (dog cages) is that swabs are easy to use for sampling of smooth and even/fairly even surfaces, while sponges are easy to use also on unsmooth and uneven surfaces. The experience from Study I is that hydrated petrifilm plates can also be a good option for direct surface sampling. Particularly if petrifilms are already used for cultivation of swab or sponge samples, they can be a relevant choice as no extra material is needed for the sampling. In Studies I-III, petrifilms were found to be quick and easy to use, as only a 1000 µl pipette, an incubator and decent light were needed as long as the samples did not need to be homogenized or diluted.

All the methods used in Studies I-III can be considered to provide reliable results, but unfortunately there are many things that can affect the detected bacterial load, among other things the bacterial species, the material in sampling equipment, nutrients and neutralizer in broths and agar, sampling protocols and surface characteristics (Lemmen *et al.* 2001; Griffith 2005; Buttner *et al.* 2007; Downey *et al.* 2012; Li *et al.* 2023; Watson *et al.* 2024) For sampling of environmental bacterial load it is important to use a standardized sampling protocol, which was shown by Napoli *et al.* (2012), who was able to demonstrate that passive and active air sampling were correlated by using a standardized sampling protocol. The protocol should include a plan for surfaces to sample, locations for air sampling, sampling time and information to collect (e.g. number of staff in the OR) (Napoli *et al.* 2012). Using a standardized sampling protocol will ensure repeatability of the sampling and result in higher reliability of the results. For longitudinal monitoring, the same sampling method(s), including material in sampling equipment and type of broth or agar etc., should be used for the results to be reliable and possible to use, for example, for trend analyses.

3.4 Bacteriological and molecular analyses

In Study III (dog cages) samples were diluted to reduce the bacterial concentration, thus enabling CFU counting. Settle plates (Study I, OR and UR), dip slides (Studies I and II, dental practice), and petrifilms (Study III) were incubated in 37 ±1 °C for 48 ±2 h while petrifilms (Study II) were incubated in 30 ±1 °C for 48 ±2 h. After incubation, CFU was counted manually from settle plates (Study I), dip slides (Studies I and II) and petrifilms (Studies II and III), see Figure 4.
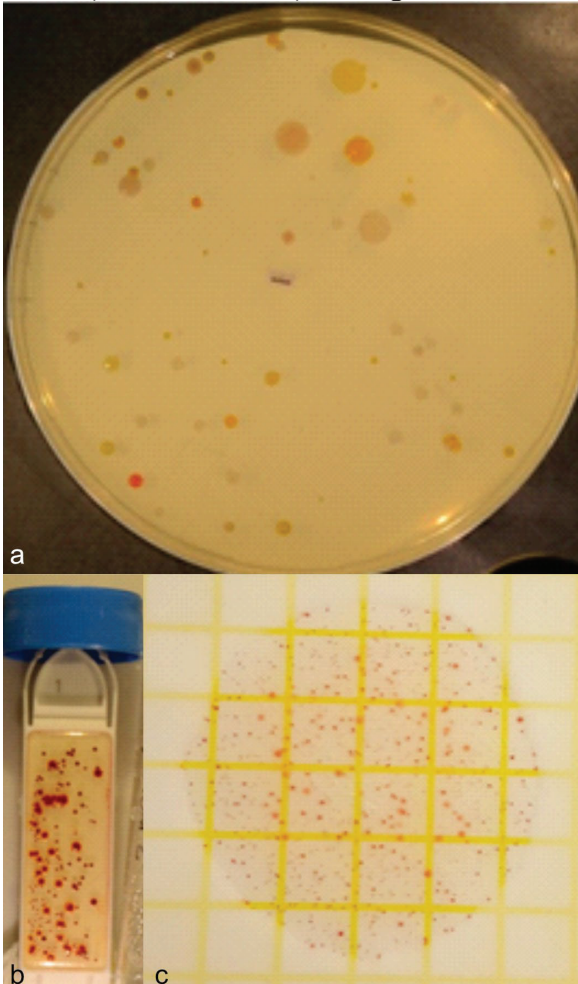

Figure 4. Photographs of a. settle plate (photograph by Ingrid Hansson), b. dip slide (photograph by author) and c. petrifilms (photograph by author) from which CFUs were counted

Matrix Assisted Laser Desorption – Time Of Flight (MALDI-TOF) was used for detection of bacterial species identification (Study I). Antimicrobial susceptibility testing (microdilution) was performed to identify staphylococci isolates with a similar antibiotic susceptibility pattern (Study I). Selected staphylococci isolates (with similar antibiotic susceptibility pattern) were sequenced, typed by multilocus sequence typing and screened for resistance genes (Study I). Single nucleotide polymorphism (SNP) analysis was used to analyse relationships between isolates (Study I).

3.5   Data analysis

For data management and descriptive statistics, Microsoft $^®$ Excel 2016 (16.0.5134.1000) (Microsoft Corporation, Redmond, Washington, USA) was used (Studies I-III). For statistical analysis Stata SE 16.1 (StataCorp LLC, 4905 Lakeway Drive, College Station, Texas 77845 USA) (Study I, OR and UR) and RStudio version 2021.9.0.351 (RStudio Team (2021). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA) with packages ggplot2 (v3.4.3; (Hadley, 2016)), mgcv (v1.8-36; (Wood, 2011)) emmeans (v. 1.8.9 (Lenth, 2023)) and predictmeans (v. 1.0.9 (Luo, 2023)) (Study III, dog cages) were used.

In Study I, linear regression was performed for univariate analyses of the association between each bacterial load outcome in the OR (on settle plates, at three sampling locations) and the potential factors: acute/elective surgery, number of staff in the OR, degree of staff movement during surgery, surgery length, door openings and persons in and out of the OR.

In Study III, bacterial counts and reductions were log_{10}-transformed for the data to, if possible, be normally distributed. A generalized additive model was performed for analysis of the association between bacterial load on the floor before cleaning and time the patient spent in the cage, time the cage was empty before cleaning and the bacterial load on the wall. Spearman's rank correlation was performed for analysis of the correlation between the bacterial load on the floor and the wall before cleaning. For comparisons of bacterial load and effect of cleaning and disinfection between surfaces and methods, the Welch two-sample t-test (comparison between two groups) or one-way Anova (comparison between three groups) were used for normally distributed data, and the Wilcoxon rank sum test (comparison between two groups) was used for non-normally distributed data.

4. Results and discussion

4.1 Bacterial load in air, and on surfaces and equipment

4.1.1 Bacterial load in air

The bacterial load in the air was generally below suggested threshold values for human healthcare (Table 1, page 21) in both the OR and the UR (study I) (Department of Occupational Hygiene (ISPESL) 2009; Pasquarella *et al.* 2000; Pasquarella *et al.* 2012; SIS 2015). Bacterial loads in the OR and UR are presented in Table 8 together with results from other environmental air sample studies in animal healthcare.

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Table 8. Bacterial load in air samples from operating rooms and ultrasound or radiology rooms in animal healthcare

	Prior to the first patient of the day in the OR[a], empty room	Prior to the first patient of the day in the OR, staff in the room	During surgery	After surgery, before cleaning	Prior to the first patient of the day UR[b], empty room	Prior to the first patient of the day UR, staff in the room	During ultrasound examination	During lunch, empty room
Study I, OR and UR (Alsing-Johansson et al. 2024)	Median: 0 CFU/dm²/h, 100% < target value	Median: 1.9 CFU/dm²/h, 44% ≤ target value, 67% < alert value	Median: 7.7 CFU/dm²/h, 100% < threshold value	NA[c]	Median: 0.32 CFU/dm²/h	Median: 4.0 CFU/dm²/h	Median: 27.9 CFU/dm²/h, 95% < threshold value	Median: 0.9 CFU/dm²/h,
Bagcigil et al., 2019	Median: 230 CFU/m³, 7% < target value, 18% < alert value	NA	NA	Median: 680 CFU/m³, 100% > alert value	NA	NA	NA	NA
Harper et al., 2013	NA	Geometrical mean: ~60 CFU/m³, > alert value	Geometrical mean ~15 CFU/m³, < threshold value	NA	NA	Geometrical mean: ~58 CFU/m³	NA	NA
Jeong et al., 2017	Mean 6.81 CFU/dm²/h, > alert value	NA	NA	NA	Mean 19.4 CFU/dm²/h	NA	NA	NA
Viegas et al., 2018	NA	NA	NA	NA	NA	NA	~108 CFU/m³, > threshold value	NA

[a.]Operating room. [b.] Ultrasound room. [c.] Not applicable.

The low bacterial load in the OR and UR suggests that the risk of environmental spread of pathogens between patients is reduced. This is the case at least if relevant horizontal surfaces are decontaminated between patients, otherwise there is a risk that particles carrying bacteria may whirl up in the air when the room is in use again.

For practical reasons, in the sampling before the first patient of the day the bacterial load was most commonly measured during staff preparation (Study I, OR and UR). It can be assumed that the bacterial load would have been lower if the UR and OR had been empty during sampling.

The bacterial load in the OR both during preparations and in the empty OR in Study I met the suggested threshold values to a higher degree than the bacterial load reported by Harper *et al.* (2013) and Bagcigil *et al.* (2019). Both Harper *et al.* (2013) and Bagcigil *et al.* (2019) used active air sampling, and based on that studies have shown a correlation between passive and active air sampling; the difference can be assumed to be accurate (Napoli *et al.* 2012; Pasquarella *et al.* 2023). Due to lack of information among other things on the ventilation system in the studies by Harper *et al.* (2013) and Bagcigil *et al.* (2019), it is difficult to speculate about the reasons for noted differences in bacterial load.

The bacterial load during surgery (Study I) was below the suggested threshold values, a result that resemble that reported by Harper *et al.* (2013). It is not yet known if meeting these threshold values substantially reduces the risk of HAI in animal healthcare. Due to this, it is important to be cautious in not just assuming that the lowest possible bacterial load is what we need to strive for even though it can be assumed that a low bacterial load contributes to a reduced risk of HAI. For the future we need to gain more knowledge of the correlation between environmental bacterial load and incidence of HAI so that a cost-benefit analysis can be performed and threshold values correlated with low incidence of HAI can be identified. Otherwise the risk is that lower threshold values than needed to keep HAI incidence low will be applied at a high cost. One example of a simple cost-benefit analysis from human healthcare showed that enhanced detergent cleaning of high-touch surfaces saved the hospital <80,000 euro (Dancer *et al.* 2009).

The bacterial load in the UR (Study I) was generally below the threshold values suggested for human healthcare, compared to the bacterial load reported by Viegas *et al.* (2018), which was above the suggested threshold

value. However, that study included only one passive air sample from the radiology room, so it is difficult to tell if the bacterial load measured was representative or not. There are no suggested threshold value for empty URs or radiology rooms. Comparing the results from Study I with studies by Harper *et al.* (2013) and Jeong *et al.* (2017), the bacterial load was lower in Study I.

4.1.2 Bacterial load on surfaces and equipment before decontamination

The bacterial load on surfaces before decontamination was generally below the suggested threshold values (Table 1, page 21) in Study I (OR and UR), to a quite small degree in Study III (dog cages), but not at all in Study II (dental practice). In Study II most of the dental handpieces not used (but placed close by) during dental care had a bacterial load above the less strict Swedish criteria for microbial cleanliness of semi-critical equipment, while all dental handpieces used during dental care had a bacterial load high above the criteria (Fohm 2006), see Table 9 (ISPESL 2009; Griffith *et al.* 2000; Dancer 2004; Lewis *et al.* 2008; Mulvey *et al.* 2011).

Table 9. Bacterial load on environmental surfaces and equipment before decontamination in Studies I-III

Surface	Study I	Study II	Study III
High-touch UR[a][b]	Median: 0.53 CFU/cm^2, 88% < threshold value	NA[c]	NA
Near-patient[b]	Abdominal position cushion median: 0.43 CFU/cm^2, 84% < threshold value	Head support median: TNTC[d], 0% < threshold value[e]	Floor median: 9.1 CFU/cm^2, 15%< threshold value Wall median: 2.6 CFU/cm^2, 48% < threshold value
Equipment[f]	NA	Dummy[g] median: 87 CFU/cm^2, 9% < criteria for microbial cleanliness of semi-critical equipment.	NA
Sterile field[h]	Median: 0 CFU/cm^2, 93% ≤ expected value	NA	NA

[a.] Ultrasound room. [b.] Threshold value < 2.5 CFU/cm^2. [c.] Not applicable. [d.] Too numerous to count. [e.] Based on bacterial loads on petrifilms. [f.] The less strict criteria for microbial cleanliness was defined as ≤1 CFU/handpiece in the thesis. [g.] An extra dental low-speed handpiece used for assessment of environmental contamination. The dummy was not used during dental care but was placed close by, where dental handpieces that may be needed during dental care were placed. [h.] Expected value ≤ 0.21 CFU/cm^2

All near-patient and high-touch surfaces in the OR and UR during mornings and after lunch were below suggested threshold values (Study I), compared to the head support (Study II) where just one of two morning samples was below the suggested threshold values. The head support sample above the suggested threshold value showed an uncountable quantity of bacteria. The high bacterial load on the head support compared to the abdominal position cushion in the UR (Study I) can probably be explained by a higher contamination rate on the head support. Even though the bacterial load may be higher in equine clinics than in small animal clinics, the higher bacterial load on the head support is probably explained by contamination from dental care equipment (Singaravelu *et al.* 2023). When in operation, motorised dental care equipment such as dental handpieces causes aerosols that can spread several metres, producing contamination of many surfaces in dental

care rooms (Ionescu *et al.* 2020; Innes *et al.* 2021). The difference in bacterial load, on the head support, may be explained by the use of the dental care room before sampling. On the sampling day with low bacterial load, the dental care room had not been used for several days, compared to the other sampling day when the room had been used the previous day. Although bacteria can survive for a long time on environmental surfaces, it is likely that the load may decrease over time, since survival depends for example on the amount of protein, serum, and sputum on the surface (Kramer *et al.* 2006; Hu *et al.* 2015). Results in line with this were noted in Study III, a tendency towards bacterial load reduction on surfaces being found with increasing vacancy of dog cages, before cleaning. Based on this a quarantine period can be an IPC tool to consider in case of increased risk of spread of pathogens, for example during an outbreak of HAI.

The higher bacterial load on near-patient surfaces and dental handpieces, before cleaning, in Studies II and III compared to Study I is probably explained by high contamination of the head support and dental handpieces (Study II) and constant patient contact with the cage floor, for 12 h - 7 days, on which the patient for example eats, drinks and sometimes defaecates, urinates and vomits (Study III). The bacterial load, and presumably the risk of spread of pathogens, increased with longer stay in the dog cage. The time of stay should be considered together with the visible degree of cleanliness when choosing cleaning and disinfection methods.

In Study II we suggested to use the less strict criteria for microbial cleanliness of semi-critical dental low-speed handpieces and dental surgical low-speed handpieces, as it may be achievable and measurable (Alsing-Johansson *et al.* 2021) which was why it was applied in the thesis. The less strict criteria for microbial cleanliness was defined as $\leq$ 1 CFU/handpiece in the thesis.

For surfaces in the sterile field in the OR (Study I), it can be assumed most of the contamination happened during surgery, but it is not possible to know for certain. People and, to some extent, patients in the OR will provide particles and bacteria from their skin to the air, which will fall on surfaces, including sterile surfaces. Skin bacteria were dominant in Study I, which indicates that the bacteria found in the sterile field were probably provided by staff or patients. There are no studies of bacterial load on surfaces in the sterile field to compare with, but it can be assumed that the result of Study I is fairly good since the expected value is very low.

4.2 Environmental bacterial flora

4.2.1 Dominant bacterial flora

In Study I (OR and UR) the environmental bacterial flora was dominated by skin flora, i.e. *Staphylococcus* spp. and *Micrococcus* spp., as also reported previously in studies of environmental surfaces in animal healthcare (Harper *et al.* 2013; Jeong *et al.* 2017; Gentile *et al.* 2020; Kim *et al.* 2020). Equipment, in animal healthcare, have been reported to be dominated by *Staphylococcus* spp. (Mount *et al.* 2016; Gustafsson *et al.* 2024). *Bacillus* spp. frequently occurred in the studies by Mount *et al.* (2016) and Gustafsson *et al.* (2024), in line with the results in the UR in Study I. For details about the environmental bacterial flora in different studies, see Table 10.

Table 10. Frequently occurring environmental bacterial flora in animal healthcare

	Study I	Kim *et al.* 2020	Jeong *et al.* 2017	Harper *et al.* 2013	Gentile *et al.* 2020	Gustafsson *et al.* 2024	Mount *et al.* 2016
Type of sample	Air, surface	Air	Air	Air	Surface	Clipper blades	Clipper blades
Sampling method	Settle plate, dip slide	Impac-tor	Settle plate	Impact-or	Swab	Swab	Swab
Staphylococcus **spp.**	x[ab]	x	x	x	x[abc]	x[ab]	x[ab]
Micrococcus **spp.**	x	x	x	x			
Bacillus **spp.**	x			x		x	
Coliform bacteria							
Corynebacterium **spp.**				x			
Kocuria **spp.**		x					
Macrococcus **spp.**		x					
Glutamicibacter **spp.**		x					
Granulicatella **spp.**		x					

[a.] *Staphylococcus* spp. include, but are not limited to, *S. aureus.* [b.] *Staphylococcus* spp. include, but are not limited to, *S. pseudintermedius*. [c.] *S. pseudintermedius* includes occasional MRSP.

4.2.2 Residential bacteria

To look for residential bacteria in the environment, frequently occurring *Staphylococcus* spp. and methicillin-resistant *Staphylococcus* spp. were

further analysed in Study I (OR and UR), but no relationship was found among the analysed staphylococci. This indicates introduction of the staphylococci by staff, animal owners and/or patients. Moreover, due to the narrow selection of isolates for analysis and the lack of analysis of biofilm prevalence on surfaces in Study I, it is possible that residential bacteria were undetected. In human healthcare, DSBs have been found on 83-95% of terminal cleaned surfaces (Vickery *et al.* 2012; Hu *et al.* 2015; Ledwoch *et al.* 2018). *Staphylococcus* spp. have been reported as the most frequently occurring bacteria in DSBs in human healthcare (Hu *et al.* 2015; Johani *et al.* 2018; Ledwoch *et al.* 2018; Costa *et al.* 2019), for details of commonly occurring bacteria see Table 11.

Table 11. Frequently occurring bacterial flora in dry surface biofilm in human healthcare

Study	Costa *et al.* 2019	Johani *et al.* 2018	Ledwoch *et al.* 2018	Hu *et al.* 2015
Staphylococcus spp.	x	x	x	x
Bacillus spp.		x	x	
Propionibacterium spp.		x		x
Pseudomonas spp.	x	x		x
Enterococcus spp.		x		
Streptococcus spp.		x		
Acinetobacter spp.	x	x		
Faecalibacterium spp.				x
Massilia spp.				x

The environmental bacterial flora in Study I resembles the bacterial flora in DSBs in human healthcare, with *Staphylococcus* spp. and sometimes *Bacillus* spp. frequently occurring, except for the lack of *Micrococcus* spp. in DSB in human healthcare. *Micrococcus* spp. was, however, only reported as frequently occurring (> 10% of bacterial load) in one of the studies from human healthcare (Karigoudar *et al.* 2020; Sebre *et al.* 2020; Shi *et al.* 2020; Khanduker *et al.* 2021; Teklehaimanot *et al.* 2021; Atalay *et al.* 2023).

4.3   Effect of cleaning and disinfection

4.3.1   High-touch and near-patient surfaces

After decontamination of high-touch and near-patient surfaces, the majority of the samples in Studies I-III except for samples from the floor after microfibre cleaning (Study III, dog cages) were below suggested threshold values (Table 1, page 21) (ISPESL 2009; Griffith *et al.* 2000; Dancer 2004; Lewis *et al.* 2008; Mulvey *et al.* 2011). The median bacterial load on environmental surfaces after decontamination in study I-III is presented in Table 12 together with results from one other environmental surface sampling study in animal healthcare.

Table 12. Bacterial load on environmental and surfaces after decontamination

Surface	Study I (OR[a] and UR[b])	Study II (dental practice)	Study III (dog cages)	Singaravelu *et al.* 2023
High-touch OR[c]	Median: 0 CFU/cm², 89% < expected level	NA[d]	NA	NA
High-touch UR[e]	Median: 0.53 CFU/cm², 88% < threshold value	NA	Na	Different rooms[f] median: 0.5 CFU/cm², 78% < threshold value
Near-patient[e]	Median: 0.11 CFU/cm², 100% < threshold value	Head support median: 0.07 CFU/cm², 82%[g] < threshold value,	Floor: after scrub cleaning median: 1.3 CFU/cm², 70% ≤ threshold value, after microfibre cleaning median: 3.7 CFU/cm², 35% < threshold value, after disinfection (both cleaning methods) median: 0.7 CFU/cm², 78% < threshold value Wall: after scrub cleaning median: 0.2 CFU/cm², 100% ≤ threshold value, after microfibre cleaning median: 0.7 CFU/cm², 70% < threshold value, after disinfection (both cleaning methods) median: 0.1, 98% < threshold value.	Dog kennel median: 3.1 CFU/cm², 50% < threshold value

[a.] Operating room. [b.] Ultrasound room. [c.] Expected level ≤ 0.21 CFU/cm². [d.] Not applicable. [e.] Threshold value < 2.5 CFU/cm². [f.] sampling in different rooms including for example UR, radiology room and OR. [g.] Based on bacterial load on petrifilms.

The median bacterial load, including the interquartile range, on high-touch surfaces after decontamination was almost identical in Study I (OR and UR) and the study by Singaravelu *et al.* (2023). However, 10% more of the samples in that study were above the suggested threshold value. This might indicate higher contamination of surfaces in the study by Singaravelu *et al.* (2023), which might be due to different surfaces being sampled in the two studies. The results in the two studies emphasise that investigation of the percentage of samples meeting the threshold values can provide valuable information in IPC work. The percentage meeting threshold values can be a good tool for trend analysis in an animal healthcare facility. By identifying highly contaminated surfaces, relevant surfaces for continuous sampling for both bacterial load and pathogens can be determined, as was shown by Singaravelu *et al.* (2023). On high-touch surfaces 22% of the samples showed a total bacterial load above or equal to 2.5 CFU/cm^2 while pathogens were found on 44% of the samples, indicating compliance with hand hygiene routines needs to be reviewed (Singaravelu *et al.* 2023).

Information such as the percentage meeting threshold values could be excellent help in IPC work. To some extent, relevant surfaces to sample can probably be general between animal healthcare clinics. Previously identified relevant surfaces can be used as a starting point together with a risk analysis of risk surfaces or areas of spread of pathogens in the current clinic to identify potentially relevant surfaces for sampling. By sampling a larger number of surfaces initially, the most relevant surfaces in the specific clinic can be determined.

In the UR patients were often placed on a patient-bound blanket on the abdominal position cushion which may have decreased the contamination of the abdominal position cushion as long as the blanket was clean (Study I). Since there were seldom signs of fur or dirt after the ultrasound examination and most surface samples were below suggested threshold values, it can be assumed the blankets were at least fairly clean. A low contamination grade, as was seen on the abdominal position cushion in the UR, increases the probability of the bacterial load after cleaning and/or disinfection staying low. On the contrary, the head support (Study II) was generally heavily contaminated after each patient, which can probably explain the sometimes high bacterial load after decontamination, especially since aerosols for example from dental handpieces can spread far and fall onto surfaces for at

least up to 30 min after dental care (Ionescu *et al.* 2020; Ahmed & Jouhar 2021; Innes *et al.* 2021).

Comparing scrub and microfibre cleaning of dog cages (Study III), scrub cleaning generally reduced the bacterial load on the floor and wall more than microfibre cleaning. It is not possible to distinguish exactly what detail(s) in the scrub cleaning or a combination of them made it more efficient. Rinsing before cleaning rinses away loose particles and makes the surface wet, which may help in the cleaning process. Microfibre cleaning with a detergent/disinfectant has been shown by Robertson *et al.* (2019) to be more effective than microfibre cleaning with only water. This in line with the Sinner's circle (Sinner 1960), that chemical action is needed and interacts with mechanical action, temperature and time to achieve a satisfactory cleaning result. It is thereby realistic to assume that the detergent increased the effect of the scrub cleaning. In human healthcare, the mechanical cleaning effect from wet scrubbing using a scrubbing machine was more effective in reducing coagulase-positive staphylococci than vacuum cleaning followed by damp mopping with detergent (White *et al.* 2007). There was, however, no significant difference in bacterial reduction between cleaning methods, on the total bacterial load (White *et al.* 2007). A scrubbing brush has the mechanical ability to reach pockets in the surface, which is beneficial on an anti-slip surface such as on the floor cleaned in Study III. Based on the referenced studies, mechanical abilities of scrubbing brushes and the result in Study III it can be assumed the scrubbing brush accounted for at least part of the greater effect from scrub cleaning. Rinsing after cleaning will rinse off dirt and microorganisms that have detached from the surface during scrub cleaning. A study in human healthcare showed that cleaning PVC and porcelain grès floors with a damp mop with detergent followed by dry mopping significantly reduced the bacterial load (De Lorenzi *et al.* 2006). It is possible that the effect of microfibre cleaning would have increased if it had been followed by rinsing or dry mopping. It can, however, be assumed that rinsing anti-slip floors is more effective than dry mopping them.

The results indicate that using a detergent, rinsing and scrubbing, especially on rough surfaces, can increase the cleaning effect. Based on this, detergent cleaning and rinsing or dry mopping after cleaning should be considered for environmental cleaning independent of cleaning equipment used. Scrubbing with a scrubbing brush should be considered for rough surfaces, and possibly also heavily soiled surfaces. Risk surfaces for spread

of pathogens, such as the cage floor, can be managed by targeted cleaning and/or disinfection.

4.3.2 Dental equipment

The effect of cleaning or disinfection of dental handpieces (Study II, dental practice) was insufficient as only a few of the used dental handpieces met the Swedish criteria for microbial cleanliness of semi-critical equipment. While a majority, but not all, of the unused handpieces met the criteria. The effect of decontamination of dental handpieces in Study II is presented in Table 13 together with results from other studies evaluating the effect of decontamination of equipment in animal healthcare.

Table 13. Effect of decontamination of equipment used in animal healthcare

Study	Type of study	Equipment
Study II, dental practice (Alsing-Johansson *et al.* 2021)	Prospective observational study	External surface of low-speed dental handpieces and surgical low-speed dental handpieces: median: 94 CFU/cm^2, 21% ≤ criteria for microbial cleanliness of semi-critical equipment[a].
		External surface of high-speed dental handpieces: median: 1420 CFU/cm^2, 0% ≤ criteria for microbial cleanliness of semi-critical equipment[b].
		External surface dummy[c], median: 0 CFU/cm^2, 73% ≤ criteria for microbial cleanliness of semi-critical equipment[a]
Gustafsson *et al.*, 2024	Multicentre, prospective observational study	81% contaminated clipper blades after decontamination, 14% ≤ criteria for microbial cleanliness of semi-critical equipment[ad]
Mount *et al.*, 2016	Multicentre, prospective observational study	51% contaminated clipper blades after decontamination[de]
Ley *et al.*, 2016	Laboratory study	No recovered bacteria on clipper blades after soaking in 70% iso[f] or 2% chg[g] or spraying with 63.2% eth[h]. S. aureus was recovered on clipper blades after soaking in saline. *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were recovered on clipper blades after spraying with 45.6% isopr[i] or 44.25% eth.
Svonni *et al.*, 2020	Laboratory and prospective observational study	Laboratory study, endoscope: 17% of *Streptococcus equi* was recovered after soaking in ethanol. No *S. equi* was recovered after soaking in isopropanol and QAC[j], ortho-phthalaldehyde or an endoscope washer-disinfector[k].
		Prospective observational study, endoscopes: No bacterial contamination after soaking in ortho-phthalaldehyde.

[a.] The less strict criteria for microbial cleanliness was defined as ≤1 CFU/handpiece in the thesis. [b.] The strictest criteria for microbial cleanliness was defined as 0 CFU/handpiece in the thesis. [c.] An extra low-speed handpiece used for assessment of environmental contamination. The dummy was not used during dental care but was placed close by, where dental handpieces that may be needed during dental care were placed. [d.] After decontamination according to routines of each individual practice. [e] Fewer clipper blades were contaminated after decontamination with clipper disinfectant (alcohol + o-phenylphenol + surfactant (in one of them)) compared to clipper cleaners (lubricants without disinfectant). [f.] Isopropyl alcohol. [g.] Chlorhexidine gluconate. [h.] Ethanol. [i] Isopropanol. [j.] Quaternary ammonium compound [k.] Poka Yoke AER with Aperlan Poka-Yoke Agent A and Agent B, Getinge Group, Gothenburg, Sweden

All the studies referred to in Table 11 showed problems with remaining bacteria after decontamination to some extent, and did so to a higher degree on dental handpieces and clipper blades (Ley *et al.* 2016; Mount *et al.* 2016; Svonni *et al.* 2020; Alsing-Johansson *et al.* 2021; Gustafsson *et al.* 2024). Dental handpieces, clipper blades and endoscopes are considered semi-critical equipment, providing they are used for non-sterile procedures, which means that they should meet the Swedish criteria for microbial cleanliness of semi-critical equipment (CDC 2003; SVF & SVS 2017; SFVH 2019). Decontaminating dental handpieces and clipper blades in a washer-disinfector would probably reduce the contamination, although dental handpieces can also be hard to decontaminate in machines intended for decontamination of dental handpieces (Offner *et al.* 2019). There are still animal healthcare clinics that do not have a washer-disinfector, as it is a somewhat large investment, and endoscope washer-disinfectors are uncommon in animal healthcare. If dental handpieces and clipper blades are cleaned and disinfected in a washer-disinfectors, more equipment is needed since the washer-disinfector programme will take more time than chemical disinfection. Investment in a washer-disinfector and equipment will increase the costs. For small clinics, especially ambulatory ones, that do not have a washer-disinfector, it is important to develop more effective manual decontamination protocols for dental handpieces and clipper blades.

Generally dental handpieces cannot be soaked in water, which would have been the first choice for manual cleaning. Cleaning dental handpieces under cold running water is a recommendation from a manufacturer (Nakanishi Inc. no date) that could be tested, at least if a detergent is added when cleaning. Although there is a risk of aerosol production and potential spread of pathogens from cleaning under running water, that risk is already present during dental care and appropriate protective gear should be used. Dental handpieces generally cannot be soaked in a disinfectant, which would have been preferable. Instead the external surface of the dental handpieces can be disinfected, the first choice being a disinfectant with a high effect on microorganisms. The suggested cleaning and disinfection methods will only have an effect on the external surface of the dental handpieces, so the internal surfaces need to be decontaminated as well. There are cleaning and disinfection sprays for internal surfaces of dental handpieces on the market which should be tested.

Using manual cleaning and disinfection methods, especially those that can be used with dental handpieces, cannot be expected to be as efficient as cleaning and disinfection in a washer-disinfector. However, all measures that increase the effect of cleaning and disinfection will reduce the risk of outbreaks of, for example, resistant bacteria or strangles, and thereby have a positive impact on animal welfare and save costs.

In a human healthcare study, sampling high-touch surfaces with a moistened gauze pad showed higher sensitivity than sampling with a moistened swab (Sultan *et al.* 2021). A laboratory study, on the other hand, showed no difference in sensitivity between the methods when sampling mono-species biofilms, with *Listeria monocytogenes* and *Pseudomonas flourescens*, on stainless steel or polyurethane (Brauge *et al.* 2020). Sampling external parts of dental handpieces with a moistened gauze pad after human dental care resulted in lower bacterial load than in our Study II, although the validation of the sampling method showed quite promising results (Pinto *et al.* 2017). This could be due, for example, to a lower bacterial load, a lower sensitivity of the sampling method or lack of neutralizer. Based on the results of Study II and the study by Pinto *et al.* (2017), it would be interesting to contaminate dental handpieces with relevant bacteria, e.g. *Streptococcus equi* or *Staphylococcus aureus,* and compare the sensitivity of the sampling methods. It would be beneficial to include an evaluation of the number of times the surface of the handpiece should be swiped or rubbed to extract a maximum proportion of the bacteria on dental handpieces.

4.3.3 Biofilm

In one case in Study III (dog cages), the floor had a substantially higher bacterial load after microfibre cleaning, which may have been caused by disruption of a biofilm that had not been removed. This can be compared to a study from human healthcare where disruption of wet biofilm was the suspected cause of increased detection of carbapenemase-producing *Enterobacterales* after cleaning (Fernando *et al.* 2019). After disinfection of the dog cage floor mentioned above, the bacterial load was reduced to below 2.5 CFU/cm^2, indicating that the disinfectant had the expected effect on at least culturable aerobic bacteria. This is in line with the effect of peracetic acid in the laboratory study by Ledwoch *et al.* (2021b), which is reasonable since the potassium monosulphate used in Study III also belongs to the persulphate group and could be expected to have similar properties. There is

a clear need for more knowledge about the effect cleaning and disinfection have on biofilm in animal healthcare and how that affect risk of spread of pathogens. It is possible detergent cleaning may be favourable over disinfection since enhanced detergent cleaning may reduce the risk of HAI (Dancer *et al.* 2009). While disinfection seem to reduce but not eliminate biofilm (Ledwoch *et al.* 2021b) and there is a risk of resistance to disinfectants (Souza *et al.* 2020; Brunke *et al.* 2022). Another option may be cleaning with probiotics which showed a significant reduction of the amount of antibiotic resistant genes in sinks when compared to disinfection (Klassert *et al.* 2022).

4.3.4 Risk of spread of pathogens

The bacterial load in the OR and UR in Study I was generally below threshold values suggested for human healthcare. Based on that, the risk of environmental spread of pathogens should be considered to be low. On a more general level, the risk of environmental spread of pathogens should be considered quite high in the OR, depending, for example, on the type of surgeries performed, ventilation and number of staff in the OR, and quite low in the UR. Monitoring the bacterial load in the air and on surfaces in the OR in animal healthcare can provide valuable information to reduce the risk of spread of pathogens. This information may be especially valuable in animal hospitals performing advanced surgeries, sometimes on immunocompromised patients, with increased risk of HAIs. Monitoring of the bacterial load can also be a useful tool in HAI outbreak investigation.

At the beginning of Study I, patients who were isolated due to an infection or potential infection were examined in the same UR as other patients (including out-patients), which may increase the risk of spread of pathogens between patients. Later in the study, a separate UR was instead used for isolated patients. Isolation of infectious or potentially infectious patients decreases the risk of spreading pathogens between patients. If there are no separate room for ultrasound examination, surgery, etc. for such patients, examinations and surgeries could preferably be performed at the end of the day. Before another patient enters, the room needs to be thoroughly cleaned and disinfected. To protect immunocompromised patients, if the UR/OR is shared with infectious and potentially infectious patients, they could be planed as the first patient of the day.

The bacterial load on dental handpieces were generally above the threshold value for semi-critical equipment. That, together with the high bacterial load on the head support in one morning, indicates a risk of spread of pathogens, such as strangles and resistant bacteria, between patients. To reduce the risk of spread of pathogens, effective cleaning and disinfection methods are needed, especially such methods as can be applied in ambulatory practices. Unused dental handpieces need to be cleaned and disinfected between patients or, if possible, kept protected from contamination during dental care. In dental practices horizontal surfaces should be cleaned and/or disinfected in the morning prior to the first patient since aerosol generated when inter alia dental handpieces are used may fall down on surfaces for at least up to 30 min after dental care (Ahmed & Jouhar 2021).

The majority of near-patient surfaces in dog cages (Study III) sampled after decontamination showed a bacterial load below suggested threshold values, except for samples from the floor after microfibre cleaning. The bacterial load overall was higher on the floor than on the wall. The difference can probably be explained by a floor being difficult to clean due to an anti-slip surface and the patient having constant contact with the floor, on which body fluids and food particles may be present. This indicates that the floor may be a vector for transmission of pathogens between patients. In line with human healthcare (Mitchell *et al.* 2023), there may be an increased risk of acquiring a pathogen if the previous cage occupant was infected or colonized with that pathogen. Aerosol from scrubbing or rinsing with a soft water jet may spread pathogens in the environment (Thoroddsen *et al.* 2012; Fernando *et al.* 2019) which can increase the risk of environmental spread of pathogens e.g. during cleaning. Risk surfaces for spread of pathogens can be managed by targeted cleaning and/or disinfection and should be considered relevant surfaces for longitudinal monitoring of surface bacterial load.

4.4 Resistance to disinfectants

Genes conveying resistance to disinfectants, e.g. chlorhexidine and QACs, were revealed in 12 of 35 staphylococci isolates (Study I, UR and OR). Identified genes were: *qacA, qacB and qacJ*. Most of the isolates with these identified genes were collected in the OR. For detailed information, see Table 14.

Table 14. Staphylococcus spp. isolated from a small animal hospital carrying qacA, qacB or qacJ genes

Staphylococcus isolate	Sampling site	Type of sample	Sampling time point	Sampling week and year	qacA	qacB	qacJ
S. epidermidis	Surgical light handle	Dip slide	After surgery 25	Week 25 2020	x		
S. hominis	Surgical drape, near the anaesthesia machine	Dip slide	After surgery 17	Week 23 2020	x		
S. hominis	Instrument table	Dip slide	After surgery 17	Week 23 2020	x		
S. hominis	Below the focus tracking ball on the ultrasound machine	Dip slide	Before the first patient, sampling day 6	Week 24 2020	x		
S. hominis	Below the focus tracking ball on the ultrasound machine	Dip slide	After the first patient, sampling day 6	Week 24 2020	x		
S. capitis	Behind the cupboard handle	Dip slide	Before surgery 12	Week 22 2020		x	
S. capitis	Computer table	Settle plate	During surgery 23	Week 25 2020		x	
S. capitis	Shelf	Settle plate	During surgery 25	Week 25 2020		x	
S. hominis	Anaesthesia machine	Settle plate	During surgery 19	Week 24 2020		x	
S. hominis	Computer table	Settle plate	During surgery 16	Week 22 2020		x	
S. hominis	Anaesthesia machine	Settle plate	During surgery 13	Week 22 2020		x	
S. epidermidis	Instrument table	Dip slide	After surgery 7	Week 45 2019			x

Since no relationship was found between the staphylococci with genes conveying resistance to disinfectants and most of the species are known as being human-associated, there is an obvious risk that staff working in the OR have spread such bacteria from their skin. Repeated contact with chlorhexidine and possibly, for example, cleaning products with QACs may explain the prevalence of genes conveying resistance to disinfectants. Based on the results of Study I, it would be interesting to analyse the prevalence of genes conveying resistance to disinfectants in skin bacteria from staff, patients, and environmental samples from air and surfaces including surface biofilms in the OR. By doing so the dynamics of how these genes, if found, spread can be analysed. It would also be interesting to compare the minimum bactericidal concentration of chlorhexidine in these isolates and the human isolates included in the meta-analysis by Aftab *et al.* (2023). Knowledge about prevalence and spread of genes conveying resistance to disinfectants, and minimum bactericidal concentration of chlorhexidine can provide important information for IPC work in the OR. Chlorhexidine with alcohol is recommended by, for example, WHO (2018) for skin disinfection prior to surgery, and is widely used. If there is an increased risk of resistance to chlorhexidine in isolates from OR staff, patients and/or the environment, it is important to review IPC routines. Improving IPC routines can reduce the risk of spread of resistant clones and plasmids, as demonstrated by Keck *et al.* (2020) who found that updating inadequate IPC routines reduced the spread of chlorhexidine-resistant *Serratia marcescens*.

4.5 Clinical implications

These points are based on results from Studies I-III as well as previously published studies by other authors.

- Dip slides, sampling swabs, and sponges were considered user-friendly and reliable, and only a limited amount of laboratory equipment was needed for analysis of bacterial load on surfaces. These sampling methods can therefore easily be used both in clinical studies and for in-house monitoring of bacterial load on various environmental surfaces and equipment. In particular, this would be useful for example when evaluating change of cleaning and disinfection routines, during outbreaks of HAI/increased incidence of HAI.

- Swab sampling of the shaft of surgical low-speed dental handpieces, and sampling of couplings of low- and high-speed dental handpieces were concluded to be non-relevant for evaluation of bacterial load on internal surfaces in dental handpieces. Instead, internal parts with a higher risk of contamination during dental care such as turbines, spray channels and inner gears should be sampled.
- Longitudinal monitoring of the bacterial load in air in the OR and on relevant surfaces in the animal healthcare facilities, can provide valuable information about the effect of IPC routines. This can be used to identify routines that need to be reviewed and actions that need to be taken to reduce the environmental bacterial load. Trend analysis can help identify changes over time, which can be helpful for early detection of an increased risk of spread of pathogens.
- Generally, the risk of environmental spread of pathogens is quite high in the OR, while it is quite low, but not negligible, in the UR. Adequate air change and IPC routines including isolation of infectious and potentially infectious patients can lower the risk of pathogen spread. If isolation is not possible for all examinations and/or surgeries, adequate measures to reduce the risk of pathogen spread should be taken.
- Dental handpieces did not meet the criteria for microbial cleanliness of semi-critical equipment, which means that they can be a vector for pathogen spread between patients, and in ambulatory practice also between stables. Unused dental handpieces kept nearby during dental procedures should be decontaminated between patients. There is an urgent need for more efficient methods for cleaning and disinfection of dental handpieces, especially in ambulatory practice.
- In the dog cages, the bacterial load was higher overall on the floors than on the walls. The anti-slip surface on the floor, in combination with constant patient contact and spillage of body fluids and food particles, can probably explain the difference. This indicates that the floor may be a vector for transmission of pathogens between patients. Based on this, the cage floor, can be expected to be a relevant surface for longitudinal monitoring of surface bacterial load. Risk surfaces of pathogen spread, such as the cage floor, can be managed by targeted cleaning and/or disinfection.
- Scrub cleaning of the wall and floor in the dog cages was generally more effective in reducing bacterial load than microfibre cleaning. The results

demonstrate that using a detergent, rinsing and scrubbing (especially on rough surfaces) can increase the cleaning effect. Based on this, detergent cleaning and rinsing after cleaning should be considered for environmental cleaning, regardless of the cleaning equipment used. Scrubbing with a scrub brush should be considered for rough surfaces, and possibly for heavily soiled surfaces.

- The bacterial load, and presumably the risk of spread of pathogens, in dog cages increased with length of stay. In clinical practice, this is a factor to take into account together with the visible degree of cleanliness when cleaning and disinfecting patient cages.
- There was a tendency for the bacterial load to decrease with longer vacancy of the cages. This indicates that a quarantine period can be an IPC tool to consider during an outbreak of HAI or after the cage has been used by a highly infectious patient.
- The presence of genes conveying resistance to disinfectants in environmental bacteria indicate that resistant bacteria may persist in the animal healthcare environment, thereby increasing the risk of HAIs. In IPC work, knowledge about presence of such genes can be helpful both to enable early detection of a potential spread of pathogens with resistance to disinfectants and when making decisions for example about cleaning and disinfection products.

5. Conclusions

- Although the bacterial load was below the suggested threshold values for human healthcare in most sites, some surfaces need more dedicated cleaning and disinfection. Microfibre cleaning was not sufficient and scrub cleaning should be applied on anti-slip surfaces, such as cage floors, and heavily contaminated surfaces. Dental handpieces did not meet the criteria for microbial cleanliness of semi-critical equipment after decontamination, efficient decontamination routines are needed to reduce the risk of pathogen spread.
- A relationship was found between contact time with the cage floor and the bacterial load on the cage floor. Contact time should be considered when choosing surface cleaning and disinfection methods.
- Defining threshold values for bacterial load to prevent HAIs as well as evidence-based cleaning and disinfection routines for environmental surfaces and equipment is crucial for monitoring and IPC measures.

6. Future perspectives

This thesis has contributed to increase the knowledge about bacterial load in the environment and on equipment, and the effect of cleaning and disinfection of environmental surfaces and equipment in animal healthcare. It has also prompted ideas for future research studies:

- Investigation of the correlation between environmental bacterial load on risk surfaces/areas and incidence of HAIs in animal healthcare, with the goal of performing a cost-benefit analysis to identify threshold values for bacterial load correlated with low incidence of HAIs
- Improving manual cleaning and disinfection of dental handpieces, with the focus on ambulatory practice
- Investigation of the presence of biofilms in animal healthcare facilities, on both dry and wet surfaces. To gain knowledge about how biofilms affect bacterial load on surfaces and contributes to pathogen spread and finally how we should clean and disinfect to reduce the risk of spread of pathogens in presence of biofilm
- Development of reliable quick tests for monitoring the level of cleanliness on surfaces and equipment
- Investigation of climate-friendly cleaning methods

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Popular science summary

Microorganisms in air as well as on surfaces and equipment, for example surfaces often touched and equipment used for examination of patients, in animal healthcare facilities may constitute a risk of patients acquiring infections during or soon after they have received healthcare, for example surgery. These infections are known as healthcare-associated infections (HAIs). Knowledge about the number of bacteria, bacterial species and factors influencing the number of bacteria is helpful in establishing infection prevention and control (IPC) routines, the purpose of which is to reduce the risk of HAIs. Cleaning and disinfection of surfaces and equipment is an important IPC measure to reduce the risk of environmental spread of microorganisms which can cause disease (so called pathogens) and incidence of HAIs.

The overall aim of the thesis is to improve IPC in animal healthcare by studying the number of bacteria, before and after cleaning and disinfection, in air as well as on surfaces and equipment.

The number of bacteria was generally low in air and on surfaces often touched, as well as surfaces near patients in the operating room (OR) and the ultrasound room (UR). The number of bacteria was generally low on the walls and the floors in the dog cages after cleaning and disinfection, except for after cleaning the floor with a microfibre mop moistened with water. The number of bacteria was high on surfaces near patients and dental equipment after dental care of horses. After cleaning and/or disinfection the number of bacteria on surfaces near patients was generally low, but the number of bacteria on dental equipment was generally high. Bacteria carrying resistance trait against disinfectants commonly used in animal healthcare were identified from air and surfaces in the OR and the UR.

In conclusion, this thesis present a generally low bacterial load, except after microfibre-cleaning of the floor in dog cages and after cleaning or disinfection of dental equipment. This indicates a need for research to identify satisfying cleaning and disinfection routines for surfaces and equipment in animal healthcare, to reduce the risk of HAIs.

Populärvetenskaplig sammanfattning

Mikroorganismer i luften så väl som på ytor och utrustning, till exempel ytor som ofta tas på och utrustning som används för undersökning av patienter, i djursjukvårdsmiljön kan utgöra en risk för att patienter ska drabbas av en infektion i samband med vård, till exempel kirurgi. Dessa infektioner kallas vårdrelaterade infektioner (VRI). Kunskap om antalet bakterier, bakteriearter som förekommer och faktorer som påverkar antalet bakterier för att ta fram vårdhygienrutiner, vilkas syfte är att minska risker för VRI. Rengöring och desinfektion av ytor och utrustning är en viktig vårdhygienåtgärd för att minska risken för spridning av sjukdomsalstrande mikroorganismer (så kallade patogener) i djursjukvårdsmiljön, vilka kan orsaka vårdrelaterade infektioner.

Det övergripande syftet med avhandlingen är att förbättra vårdhygienen i djursjukvården genom att studera antalet bakterier, före och efter rengöring och desinfektion, i luft och på ytor och utrustning.

Antalet bakterier var generellt lågt i luft och på ytor som ofta tas på, så väl som ytor nära patienter in operationssalen och ultraljudsrummet. Antalet bakterier var generellt lågt på väggar och golv i hundburarna efter rengöring och desinfektion, förutom efter att golvet rengjorts med en fuktad mikrofibermopp. Antalet bakterier var högt på ytor nära patienterna och på tandutrustning efter hästtandvård. Efter rengöring och/eller desinfektion var antalet bakterier på ytor nära patienterna mestadels lågt, men antalet bakterier på tandutrustningen var generellt högt. Bakterier som bär på resistensegenskaper mot desinfektionsmedel som ofta används i djursjukvården hittades i prover från luft och ytor i operationssalen och ultraljudsrummet.

Sammanfattningsvis var antalet bakterier generellt lågt, förutom efter mikrofiberrengöring av golvet i hundburar och efter dekontaminering av

tandutrustning. Det indikerar ett behov av rengörings- och desinfektionsrutiner för ytor och utrustning, baserade på evidens från forskningsstudier i djursjukvård, för att minska risken för VRI.

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Environmental bacterial load during surgical and ultrasound procedures in a Swedish small animal hospital

Todd Alsing-Johansson[1*] , Karin Bergström[2], Susanna Sternberg-Lewerin[3], Anna Bergh[1], Emma Östlund[4] and Johanna Penell[1]

Abstract

Background Environmental bacteria in animal healthcare facilities may constitute a risk for healthcare-associated infections (HAI). Knowledge of the bacterial microflora composition and factors influencing the environmental bacterial load can support tailored interventions to lower the risk for HAI. The aims of this study were to: (1) quantify and identify environmental bacteria in one operating room (OR) and one ultrasound room (UR) in a small animal hospital, (2) compare the bacterial load to threshold values suggested for use in human healthcare facilities, (3) characterise the genetic relationship between selected bacterial species to assess clonal dissemination, and (4) investigate factors associated with bacterial load during surgery.

Settle plates were used for passive air sampling and dip slides for surface sampling. Bacteria were identified by Matrix Assisted Laser Desorption—Time Of Flight. Antimicrobial susceptibility was determined by broth microdilution. Single nucleotide polymorphism-analysis was performed to identify genetically related isolates. Linear regression was performed to analyse associations between observed explanatory factors and bacterial load.

Results The bacterial load on settle plates and dip slides were low both in the OR and the UR, most of the samples were below threshold values suggested for use in human healthcare facilities. All settle plates sampled during surgery were below the threshold values suggested for use in human clean surgical procedures.

Staphylococcus spp. and *Micrococcus* spp. were the dominating species. There was no indication of clonal relationship among the sequenced isolates. Bacteria carrying genes conveying resistance to disinfectants were revealed.

Air change and compliance with hygiene routines were sufficient in the OR. No other factors possibly associated with the bacterial load were identified.

Conclusions This study presents a generally low bacterial load in the studied OR and UR, indicating a low risk of transmission of infectious agents from the clinical environment. The results show that it is possible to achieve bacterial loads below threshold values suggested for use in human healthcare facilities in ORs in small animal hospitals and thus posing a reduced risk of HAI. Bacteria carrying genes conveying resistance to disinfectants indicates that resistant bacteria can persist in the clinical environment, with increased risk for HAI.

Keywords Antibacterial resistance, Antimicrobial resistance, Biosecurity, Contamination, Healthcare-associated infection, Hygiene, Infection prevention and control, Veterinary clinic

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Background

Recent studies suggest that environmental contamination, including pathogenic microorganisms on surfaces in direct contact with or near the patient, present a risk for healthcare-associated infections (HAI) in human healthcare facilities [1, 2] and presumably so also in animal healthcare facilities. Reported consequences of HAI in animal healthcare include prolonged hospital stays, as well as increased healthcare costs, morbidity and mortality [3]. Outbreaks of resistant bacteria such as carbapenemase-producing *Escherichia coli* [4], clonal spread of a chlorhexidine-resistant *Serratia marcescens* [5], and dissemination of carbapenemase-producing Enterobacterales [6] have been reported in animal healthcare facilities, all related to poor infection prevention and control. Even so, there is a lack of evidence-based threshold values for acceptable environmental bacterial load to minimize the risk of HAI in both animal and human healthcare facilities.

Animals, animal owners and staff bring more or less pathogenic microorganisms into animal healthcare facilities. Bacteria can then be transmitted from e.g. surfaces, the air, humans, or directly between the animals. Therefore, knowledge about the presence and amount of viable and potentially pathogenic bacteria in the environment and how the bacterial load changes during various daily activities is helpful in establishing optimal hygiene routines to prevent infection transmission and HAI. People and animals together with e.g. air-conditioning, heating and ventilation systems as well as outdoor factors, including air quality are important sources of airborne microorganisms in the indoor environment [7]. Thus, it may be assumed that the indoor environment in animal healthcare facilities may vary with geographic location, and local studies are therefore needed, ideally taking also seasonal changes into account. Only a few studies, mainly outside of Europe, have reported data on bacterial loads in animal healthcare facilities [8–12]. Also, most studies have only reported bacterial load at one time point. Only one study investigated the bacterial load before and during clinical procedures, e.g. surgery [9] despite the usefulness of knowing how the bacterial load changes depending on activity and cleaning procedures.

In addition to quantification and bacterial species identification, genetic mapping can be used to trace sources of infectious disease outbreaks and for comparison between outbreaks. Knowledge of the environmental microflora composition, possible genetic relationships between bacteria (outbreak and/or house flora) and factors influencing these parameters can support tailored interventions to improve hygiene routines to lower the risk for HAI.

The aims of this study were to:

1) Quantify and identify the environmental bacterial load in air and on surfaces in one operating room and one ultrasound room in a small animal hospital in Sweden,
2) Compare the bacterial load with threshold values suggested for use in human healthcare facilities,
3) Characterise the genetic relationship between selected bacterial species to assess clonal dissemination, and
4) Investigate factors associated with bacterial load during surgery.

Methods

Study design

This prospective observational study was carried out in a small animal hospital in Sweden, with approximately 30,000 patient appointments per year. In February 2019, a pilot study was carried out to identify relevant sampling locations for the main study taking practical considerations into account, including that the data collector would not interfere with the workflow. The data collection for the main study took place in May 2019 to June 2020.

Data collection

The first author was responsible for collecting data, except in November 2019 when a trained hospital staff member collected data twice in the operating room (OR). Settle plates (14 cm in diameter; 80 mL Tryptic Soy Agar (TSA); produced in-house under aseptic conditions) were used for passive air sampling and dip slides (Envirocheck® Dip Slide DC Disinfection Control, 9.4 cm^2 per side, Merck KGaA, Darmstadt, Germany) for surface sampling. The dip slides have a CASO (tryptic soy) agar (called CASO agar further on) on one side and on the other side a CASO agar containing neutralizers that neutralizes the disinfectants hexachlorophene, mercurial compounds, halogen compounds, chlorhexidine, aldehydes and phenolic compounds (called CASO + agar further on). Neutralizers help viable bacteria that were held in bacteriostasis after disinfection to grow on the dip slide [13]. Sampling locations are shown in Fig. 1 and described in Table 1. During sampling in the OR and ultrasound room (UR), the data collector sat by the computer table, in the upper right corner in Fig. 1a, moving every hour only to exchange the settle plates. Furthermore, before the first patient of the day was admitted the data collector sat outside the OR noting staff movements in the OR. The same procedure was repeated for the UR with the addition of ensuring no staff movements in the UR during lunchtime.
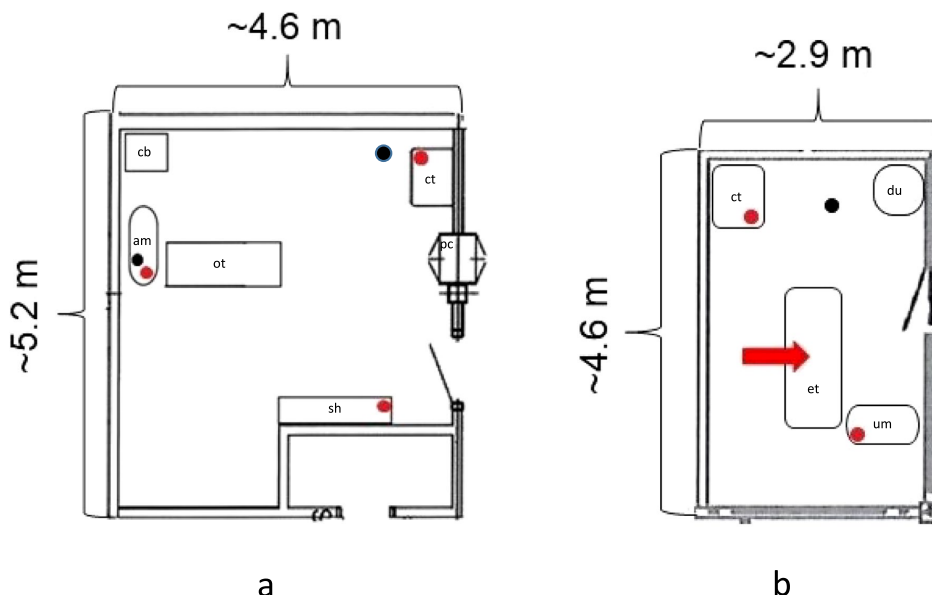
Fig. 1 Layout of **a** the operating room (OR) and **b** the ultrasound room (UR). Red circles show location of settle plates, black circles location of negative control settle plate and dip slide, and red arrow approximate location for near-patient surface (dip slides) sampled. Distances from the midpoint of the surgical table (OR) to the location of settle plates were: ~ 1.6 m (anaesthesia machine), ~ 2 m (computer table), and ~ 2 m (shelf). Distances from the location for near-patient surface sampling (UR) to location of settle plates were: ~ 2.5 m (computer table) and ~ 1 m (ultrasound machine). Settle plate sampling locations were at a height of ~ 0.9-1.1 m. Equipment (size not according to scale): *am* anaesthesia machine, *cb* cupboard, *ct* computer table, *du* drawer unit, *et* examination table, *ot* operating table, *pc* pass-through cabinet, *sh* shelf, *um* ultrasound machine

Table 1 Dip slide sampled surfaces in the operating and ultrasound room

Room	Category of surface	Sampling surface
OR[a]	High-touch[b]	Behind the cupboard handle
OR	High-touch	Behind the handle of the pass-through cabinet
OR	Sterile field[c]	Instrument table
OR	Sterile field	Surgical drape near surgeon
OR	Sterile field	Surgical drape near anaesthesia machine
OR	Sterile field	Handle of surgical light near anaesthesia machine
OR	Sterile field	Handle of surgical light near the door
UR[d]	High-touch	Near the roller mouse, where the wrists have contact with the surface, on the anaesthesia machine
UR	High-touch	Near the keyboard, where the wrists have contact with the surface, at the computer table
UR	Near-patient[e]	In the middle of the patient positioning on the abdominal position cushion

[a] Operating room. [b] Surfaces that are frequently touched by staff and patients. [c] The area close to the incision covered by surgical drapes, the instrument table, and the sterile surgical light handles. [d] Ultrasound room [e] Surface in direct contact with or near the patient

Bacterial sampling, OR

In the study, one OR and one procedure was selected to standardize the bacteriological sampling. There were five ORs in the hospital, and the selected OR was the most frequently used OR for the chosen procedure,

ovariohysterectomy (OHE). Passive air sampling was carried out during both emergency and elective OHE procedures in dogs, between 8am and 10 pm. Three settle plates were placed (Fig. 1a) before each surgery, opened during the initial 2–3 min routine team review of the

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procedures before surgery started and left open until the incision was sutured, then immediately closed. For surgeries exceeding one hour, plates were exchanged for new ones every hour (± 2 min).

In addition, seven selected surfaces in the OR were sampled for each surgery. A dip slide was applied with a contact time of 15 s as previously described [14] on two high-touch surfaces (surfaces that are frequently touched by healthcare workers and patients as defined by Centers for Disease Control and Prevention [15]) before the patient came into the OR, and on five sterile field surfaces sampled after completed surgery (Table 1).

As negative controls, one closed settle plate was placed on a decided spot on the anaesthesia machine and one sealed dip slide on a decided spot on the floor (Fig. 1a). The negative controls were placed just before sampling of high-touch surfaces and they were removed after sampling of the sterile field.

Bacterial sampling, UR

The selected UR was mainly used for abdominal ultrasound examinations of dogs and cats. Sampling was carried out during midmornings when both dogs and cats were examined. Settle plates were placed in two sampling locations and were exchanged for new ones approximately every hour (± 10 min) (Fig. 1b). At lunch break (35–60 min), when the room was empty, plates were also placed for sampling. A negative control, a closed settle plate, was placed on the floor at the start of the study in the morning and collected after the lunch break.

For surface sampling, three surfaces were sampled with dip slides directly after each ultrasound examination; two high-touch surfaces and one near-patient surface (Fig. 1 and Table 1). The near-patient surface, an abdominal positioner cushion, was also sampled after routine disinfection (replaced by routine cleaning during 2020) of the cushion (Table 1). A negative control, a sealed dip slide, was placed on the floor at study start in the morning and collected after the lunch break.

Applied threshold values for bacterial loads

There are no suggested threshold values for bacterial loads for passive air sampling or surface sampling for animal healthcare facilities, so all applied threshold values are from guidelines or recommendations for human healthcare facilities. Reference threshold values from the literature for settle plates were, when needed, transformed from colony forming units (CFU)/plate/h to a more standardized measure CFU/dm^2/h. The plate diameter was given in all references and after calculating the plate area in dm^2 the CFU/dm^2/h was calculated.

Reference threshold values varied with location and use of the room. For settle plates in the OR, suggested threshold values for clean surgical procedures without increased susceptibility for infections were used [16]. For settle plates in the empty OR, threshold values expressed as suggested target and alert values were used [17]. The UR was considered a medium risk environment (such as hospital wards and outpatient clinics) and since there are no suggested threshold values specifically for URs in human healthcare facilities, suggested threshold values for medium risk environment were used [18]. For surface sampling in the OR, including high-touch surfaces, the only available suggested threshold values in human medicine are from Italy. The Italian guidelines for surgical units are expressed as expected level and acceptable level in a closed OR left empty for at least 30–60 min following cleaning and disinfection after surgery [19]. The suggested threshold value for high-touch surfaces, including near-patient surfaces in human healthcare facilities used in this study, was < 2.5 CFU/cm^2 [20–22]. Details about the different threshold values are presented in Table 2.

Bacterial culture, count, and identification

Settle plates and dip slides were incubated in 37 ± 1 ℃ for 48 ± 2 h in the hospital laboratory immediately after sampling, except for samples collected during Thursdays and Fridays. The latter were refrigerated until the end of the sampling day and then transported for ~ 1–1.5 h in room temperature, before incubation at 37 ± 1 ℃ for 48 ± 2 h in the laboratory of the Swedish University of Agricultural Sciences. Colonies were counted manually, and numbers transformed to CFU/dm^2/h for settle plates and CFU/cm^2 for dip slides. For settle plates total CFU/dm^2/h per sampling location, per surgery/midmorning with patients in the UR, was calculated using Eq. 1. Morphology was noted and colonies with different morphology originating from plates from the same surgery were subcultured on bovine blood agar, 5% (B341960; National Veterinary Institute (SVA), Uppsala, Sweden) at 37 ± 1 ℃ for ~ 24 h. Isolates with poor growth were incubated for another ~ 24 h. Due to excessive growth on many UR plates and therefore too many isolates to handle in the study, colonies for subculture were selected from a period of sampling, one or two midmornings, instead of every sampling day. For frequently occurring colony types (with similar morphology), multiple colonies were subcultured, while from rarely occurring colony types, one colony was selected. Bacterial species identification was performed by analysing each isolate in duplicate (technical replicates) using Matrix Assisted Laser Desorption

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Table 2 Applied threshold values, from human healthcare, for bacterial loads

Type of sample	Room	Threshold value	Type of threshold value according to the reference	Type of publication	Reference
Passive air sample during surgery	OR[a]	≤ 19 CFU/dm^2/h	Suggested mean value per surgery	Swedish guidelines	16
Passive air sample during surgery	OR	≤ 39 CFU/dm^2/h	Suggested highest value during surgery	"	16
Passive air sample in an empty OR	OR	2 CFU/dm^2/h	Suggested target value	Prospective observational study	17
Passive air sample in an empty OR	OR	5 CFU/dm^2/h	Suggested alert value	"	17
Passive air sample in medium risk environments (e.g. hospital wards and outpatient clinics)	UR[b]	≤ 79 CFU/dm^2/h	Suggested threshold value	Review	18
Surface sample; high-touch and sterile field	OR	≤ 0.21 CFU/cm^2	Suggested expected level	Italian guidelines	19
Surface sample; high-touch and sterile field	OR	≤ 0.63 CFU/cm^2	Suggested acceptable level	"	19
Surface sample; high-touch and near-patient	UR	< 2.5 CFU/cm^2	Suggested threshold value	Prospective observational studies	20–22

[a] Operating room. [b] Ultrasound room

– Time Of Flight (MALDI-TOF) (Bruker Daltonics, Billerica, MA, USA). If identification failed, formic acid (70%) was added to increase the chance of genus/species identification [23]. Colonies that did not grow after 48 h or that were unidentified by MALDI-TOF were classified as genus/species unknown.

Equation 1. Calculation of total CFU/dm^2/h per sampling location, per surgery/midmorning in the UR

$$(CFU_1 + CFU_2 + \cdots + CFU_n) \div \left(0,7^2 \times \pi\right) \div \left(\frac{T_1 + T_2 + \cdots + T_n}{60}\right) \tag{1}$$

CFU$_n$ is the number of CFU on settle plate n and T$_n$ the time in min settle plate n was kept open. The number of plates varying from 1 to 5.

Antibiotic susceptibility testing

As an initial phenotyping method, antimicrobial susceptibility testing was performed on frequently detected staphylococci and staphylococci known to carry resistance of particular interest, such as methicillin resistance. Single colonies were inoculated on 5% bovine blood agar (B341960; SVA), incubated for ~ 24 h at 37 ± 1 °C and tested by broth microdilution (Mueller Hinton broth 321,300, SVA) according to the Clinical and Laboratory Standards Institute guidelines, using microdilutions panels (ThermoFisher, Waltham, MA, USA, Sensititre STAF-STR). As a quality control *S. aureus* CCUG 15915, (ATCC 29213) was used. The panel included the following substances; penicillin, cephalothin, cefoxitin, enrofloxacin, fusidic acid, erythromycin, clindamycin, gentamicin, nitrofurantoin, tetracycline, and trimethoprim/

sulphmethoxazole. Penicillinase-production in staphylococci was tested by the cloverleaf test [24].

Whole genome sequencing

Isolates were subcultured from single colonies to ensure pure cultures, inoculated on 5% bovine blood agar (B341960; SVA) and incubated for ~ 24 h at 37 ± 1 °C. DNA was prepared by mixing bacterial colonies with 190 mL G2 buffer (EZ1 DNA Tissue Kit; Qiagen, Hilden, Germany), adding 10 μl lysostaphin (5 mg/mL) and centrifuged at 350 rpm for 1 h 30 min at 37 °C, as slightly modified from the manufacturer's instructions for pretreatment of gram-positive bacteria. DNA was extracted using the IndiMag Pathogen kit (Indical) on a Maelstrom-9600 automated system. Library preparation was performed using Nextera chemistry, and sequencing performed as 2 × 150 bp paired-end reads using an Illumina NovaSeq instrument at SciLifeLab Clinical Genomics, Solna, Sweden. Samples were assembled, typed with multilocus sequence typing (MLST) and screened for resistance genes. Samples with the same multilocus sequence type or allele combination were compared with single nucleotide polymorphism (SNP)-analysis to identify possibly related isolates. All *S. capitis* samples were compared with each other as no MLST scheme for the species was available. Details including program versions and parameters are provided in Additional file 1.

Factors associated with bacterial load during surgery

Staff movements, patient data and other information connected to the surgery were registered: temperature and humidity; staff movement from incision to closed incision; number of staff; opening of the door; staff walking in or out of the OR; air change/h. Staff movements were registered as spaghetti diagrams. To translate movement into numeric values for statistical analyses movements were categorised as short, medium, or long. Short movements without actual walking were defined as 0 movement, medium long movements ($\sim$ < 2 m) as 1 movement and long movements ($\sim$ > 2 m) were defined as 2 movements. The OR had a turbulent airflow ventilation system. Air change/h was based on a mandatory ventilation control 27 May 2019 as part of the routine quality assurance procedures. The bacterial load in the OR was based on three outcomes: bacterial load on the computer table, the anaesthesia machine, and the shelf. Factors assessed for potential association with these outcomes were: acute/elective surgery, number of staff in the OR, degree of staff movement during surgery, surgery length, door openings and persons in and out of the OR.

Hygiene routines

The hospital's OR hygiene routines included aseptic preparation of patient and staff, compliance with correct protective wear (surgical cap and mask for everyone, sterile surgical gown, and sterile gloves for surgeon/-s). Before surgery, the sterilisation wrapping used for the instrument set, was confirmed to be intact. Special scrubs with tight-fitting cuffs were introduced by the animal hospital as an update in the IPC routine during the study period, but not worn by everyone during all surgeries.

Data analysis

Microsoft® Excel 2016 (16.0.5134.1000) (Microsoft Corporation, Redmond, Washington, USA) was used for data management and descriptive statistics. Linear regression was performed for the association between each bacterial load outcome in the OR (measured at three sampling locations with settle plates) and potential explanatory variables in univariate analyses. Skewness and kurtosis tests for normality were used to evaluate the normality assumption using the residuals. Regression analysis was done using Stata SE 16.1 (StataCorp LLC, 4905 Lakeway Drive, College Station, Texas 77,845 USA). The Bonferroni correction was applied adjusting the P-value for statistical significance by dividing 0.05 with the number of analyses to compensate for multiple analyses and avoid overestimation of statistically significant results. The
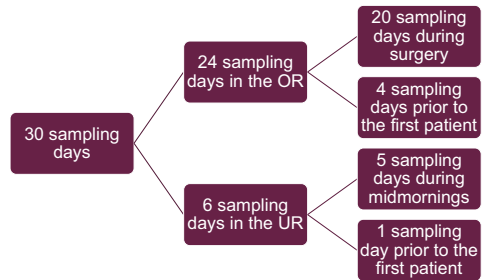

Fig. 2 Sampling days in the study. OR = operating room, UR = ultrasound room, prior to the first patient = before the first patient of the day, during midmornings = sampling during the part of the midmorning when patients were examined

Fig. 3 Settle plates used in the study. OR = operating room, UR = ultrasound room, prior to the first patient = before the first patient of the day, during midmornings = sampling during the part of the midmorning when patients were examined, during lunch = when the UR was empty during lunch time
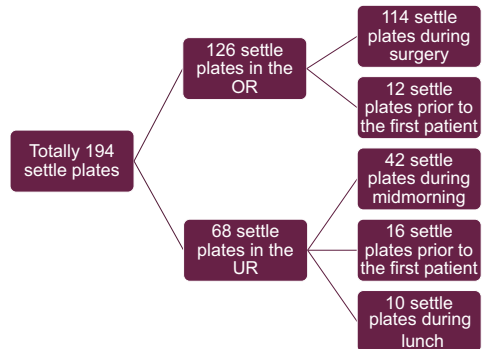
number of analyses were three outcomes * five explanatory factors = 15, thus the P-value for statistical significance was set to 0.05/15 = 0.0033.

Results

Number and type of procedures

Sampling took place during 30 days, for details see Fig. 2. In the OR, data was collected on four occasions prior to the first patient of the day and during 27 OHE procedures. Twenty-one OHEs were emergency procedures, i.e. pyometra (n = 19, 1 ruptured), hydrometra (n = 1), and metritis (n = 1), the remaining were elective OHE.

UR data was collected during five midmornings, from the hour prior to the first patient of the day until
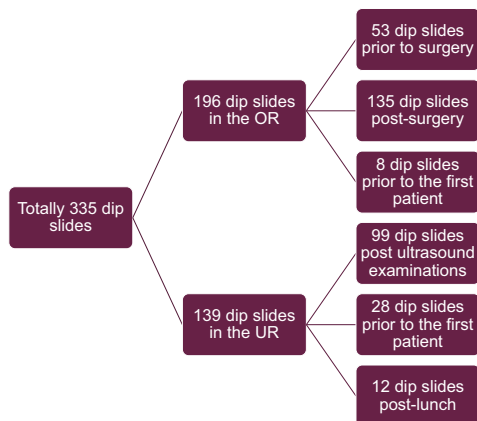
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Fig. 4 Dip slides used in the study. OR=operating room, UR=ultrasound room, prior to surgery=during preparations for the surgery, post-surgery=immediately after the surgery finished, prior to the first patient=before the first patient of the day, post ultrasound examinations=after the ultrasound examination of each patient including before and after decontamination of the abdominal position cushion, post-lunch=when the UR had been empty during lunch time and before the next patient arrived

the end of lunch break, and one morning only prior to the first patient of the day. The ultrasound patients included 12 dogs and 13 cats, most of the patients underwent an abdominal ultrasound examination while the eye was examined in one case and the neck/chest in another. Invasive sampling was performed on spleen

(n=1), liver (n=1) and prostate (n=1). In the study 194 settle plates and 336 dip slides were used for sampling, for details see Figs. 3 and 4.

Bacterial load
S*taphylococcus* spp. and *Micrococcus* spp. were the dominating bacterial genera in both the OR and the UR (Tables 3 and 4, and Additional file 2).

Passive air sampling, settle plates
For the passive air sampling in the OR, the bacterial load varied depending on the use of the room, samples were taken before surgery, during preparation of the room, and during the surgeries. In the empty OR (1 sampling occasion) the bacterial load on all settle plates was below the suggested target value [17]; varying between 0 and 1 CFU/dm^2/h, with a median of 0 CFU/dm^2/h. The bacterial load when staff prepared the OR prior to the first surgery of the day (3 sampling occasions) was higher and varied between 0 and 13 CFU/dm^2/h with a median of 2 CFU/dm^2/h. Forty-four percent of the samples were below the suggested target value and 67% below the suggested alert value [17]. During surgery, the bacterial loads on settle plates were all below both the suggested mean value per surgery and the suggested highest value [16] (Tables 2 and 3). Additional bacterial load data is provided in Additional file 3.

In the empty UR, prior to the first patient, the bacterial load was between 0 and 1 CFU/dm^2/h (1 sampling occasion). The bacterial load while staff were preparing for the day, was between 2 and 15 CFU/dm^2/h (6

Table 3 Bacterial air sampling with settle plates during surgery and ultrasound examination

Room	Sampling location	Median[a] (25th-75th percentile) CFU/dm^2/h	Bacteria[b]
OR[c]	Anaesthesia machine (38[d]/29[e])	3 (2–6)	**48.3% *Staphylococcus* spp.** (21.4% *S. epidermidis*, 14.3% S. spp., 14.3% *S. hominis*, 14.3% *S. pseudintermedius*, 14.3% *S. saprophyticus*, 7.1% *S. capitis*, 7.1% *S. caprae*, 7.1% *S. warneri*), **17.2% *Micrococcus* spp.** (100% *M. luteus*)
OR	Computer Table (38/61)	13 (8–18)	**42.6% *Staphylococcus* spp.** (26.9% S. spp., 26.9% *S. epidermidis*, 23.1% *S. capitis*, 11.5% *S. hominis*, 3.8% *S. cohnii*, 3.8% *S. equorum*, 3.8% *S. saprophyticus*), **26.2% *Micrococcus* spp.** (81.3% *M. luteus*, 12.5% *M. flavus*, 6.3% *M. cohnii*)
OR	Shelf (38/47)	9 (5–11)	**38.3% *Staphylococcus* spp.** (38.9% S. spp., 22.2% *S. capitis*, 16.7% *S. epidermidis*, 11.1% *S. aureus*, 5.6% *S. hominis*, 5.6% *S. lugdunensis*), **34.0% *Micrococcus* spp.** (68.8% *M. luteus*, 12.5% *M. flavus*, 6.3% M. spp., 6.3% *M. lylae*, 6.3% *M. terreus*)
UR[f]	Computer Table (21/53)	31 (30–38)	**35.8% *Staphylococcus* spp.** (36.8% *S. epidermidis*, 21.5% *S. capitis*, 15.8% *S. hominis*, 5.3% *S. equorum*, 5.3% *S. saprophyticus*), **26.4% *Micrococcus* spp.** (100% *M. luteus*)
UR	Ultrasound machine (21/55)	32 (31–38)	**36.4% *Staphylococcus* spp.** (30% *S. hominis*, 25% S. spp., 20% *S. epidermidis*, 10% *S. lugdunensis*, 5% *S. aureus*, 5% *S. capitis*, 5% *S. petrasii*), **18.2% *Micrococcus* spp.** (60% M. luteus, 20% M. spp., 20% M. flavus), **14.5% *Bacillus* spp.** (50% B. spp., 12.5% *B. licheniformis*, 12.5% *B. megaterium*, 12.5% *B. pumilus*, 12.5% *B. weihenstaphanensis*)

[a] Median bacterial load per sampling occasion (surgery or midmorning in the UR). [b] Frequently (>10%) occurring bacteria, includes samples taken before, during and after procedures. [c] Operating room. [d] Number of plates for bacterial count. [e] Number of isolates for bacterial identification. [f] Ultrasound room

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Table 4 Bacterial surface sampling with dip slides during surgery and ultrasound examination

Room	Surface	Medium	Median[a] (25th-75th percentile) CFU/cm^2	Bacteria[b]
OR[c]	High-touch[d] (53[e]/23[f])	CASO[g]	0 (0–0)	**60.9% *Staphylococcus* spp.** (35.7% *S. epidermidis*, 21.4% *S. hominis*, 14.3% *S. capitis*, 7.1% S. spp., 7.1% *S. haemolyticus*, 7.1% *S. pseudintermedius*, 7.1% *S. warneri*)
		CASO+[h]	0 (0–0.11)	
OR	Sterile field[h] (135/30)	CASO	0 (0–0)	**56.7% *Staphylococcus* spp.** (35.3% *S. hominis*, 29.4% *S.* spp., 11.8% *S. epidermidis*, 11.8% *S. pseudintermedius*, 5.9% *S. haemolyticus*, 5.9% *S. saprophyticus*), **20% *Micrococcus* spp.** (100% *M. luteus*)
		CASO+	0 (0–0)	
UR	High-touch (50/34)	CASO	0.21 (0.11–0.53)	**58.8% *Staphylococcus* spp.** (45% *S. hominis*, 25% *S.* spp., 15% *S. epidermidis*, 10% *S. haemolyticus*, 5% *S. xylosus*)
		CASO+	0.53 (0.24–1.33)	
UR[i]	Near-patient[j] after examination (25/22)	CASO	0.21 (0–0.85)	**45.5% *Staphylococcus* spp.** (30% *S. saprophyticus*, 20% *S.* spp., 10% *S. aureus*, 10% *S. capitis*, 10% *S. equorum*, 10% *S. felis*, 10% *S. warneri*), **18.2% *Macrococcus* spp.** (50% *M. canis*, 25% *M. brunensis*, 25% *M.* spp.), **13.6% *Bacillus* spp.** (66.7% *B.* spp., 33.3% *B. licheniformis*)
		CASO+	0.43 (0.11–1.91)	
UR	Near-patient after decontamination (24/12)	CASO	0 (0–0.03)	**41.7% *Bacillus* spp.** (60% *B. cereus*, *B.* spp. 20% *B. licheniformis*), **25% *Staphylococcus* spp.** (33.3% *S. capitis*, 33.3% *S. felis*, 33.3% *S. haemolyticus*), **16.7% *Kocuria* spp.** (100% *K.* spp.)
		CASO+	0.11 (0–0.27)	

[a] Median bacterial load per dip slide. [b] Frequently (> 10%) occurring bacteria, includes samples taken before, during and after procedures. [c] Operation room. [d] Surfaces that are frequently touched by staff and patients. [e] Number of dip slides for bacterial count. [f] Number of isolates for bacterial identification. [g] CASO (tryptic soy) agar. [h] CASO agar containing neutralizers that neutralize hexachlorophene, mercurial compounds, halogen compounds, chlorhexidines, aldehydes and phenolic compounds. [i] The area close to the incision covered by surgical drapes, instrument table and sterile surgical light handles. [j] Ultrasound room. [k] Surface in direct contact with or near the patient

sampling occasions) with a median of 5 CFU/dm^2/h. During midmornings when patients were examined, 95% of the settle plate samples were below the suggested threshold value for medium risk environments (e.g. human hospital wards and outpatient clinics) [18] (Tables 2 and 3). The two samples with bacterial load exceeding the threshold value were taken during the same data collection hour, one plate in each sampling location. During lunch (empty UR) the bacterial load was between 0 and 4 CFU/dm^2/h (5 sampling occasions) with a median of 1 CFU/dm^2/h. Additional bacterial load data is provided in Additional file 3.

The change in bacterial load in the UR is presented in Fig. 5. In the hour before arrival of the first patient in the UR when staff were preparing for the day, the bacterial load on settle plates was low. A numerical increase in bacterial load was seen during the first hours of ultrasound examinations followed by a decrease later during the midmorning. During lunch, when the UR was empty, the bacterial load was lower than during the hour before the arrival of the first patient.

For the negative controls, some were suspected to have been contaminated during production or handling. In the OR, nine of 31 negative controls (six from samplings during surgery and three from sampling in the empty OR) were contaminated with a few colonies. In the UR, one of six negative controls was contaminated with one colony. *Sphingomonas paucimobilis*

was identified as one of the contaminating bacterial species and colonies with similar morphology were therefore excluded from the bacterial counts on the same batch of plates.

Surface sampling, dip slides

Most of the surface samples from the OR and the UR were below the suggested threshold values. In the OR, using disinfectant neutralizers (CASO+agar), 89% of high-touch samples and 93% of sterile field met the expected level (Tables 2 and 4). The acceptance level was met to 98% on high-touch surfaces and to 99% in the sterile field. In total, 14 of 16 of the high-touch surface samples taken prior to the first patient of the day were negative and all samples met the expected level. Additional bacterial load data is provided in Additional file 4.

In the UR, using disinfectant neutralizers, 88% of high-touch surface samples and 100% of the near-patient surfaces after decontamination met the threshold value (Tables 2 and 4). All surface samples taken prior to the first patient of the day or at the end of the lunch break met the threshold value. Additional bacterial load data is provided in Additional file 4.

Antibacterial resistance and sequencing

A total of 78 isolates of frequently occurring species were frozen (−70/−80 °C) for further analyses. Of them, 51 frequently detected staphylococci (*S. aureus, S. capitis, S. epidermidis, S. hominis,* and *S. pseudintermedius*)

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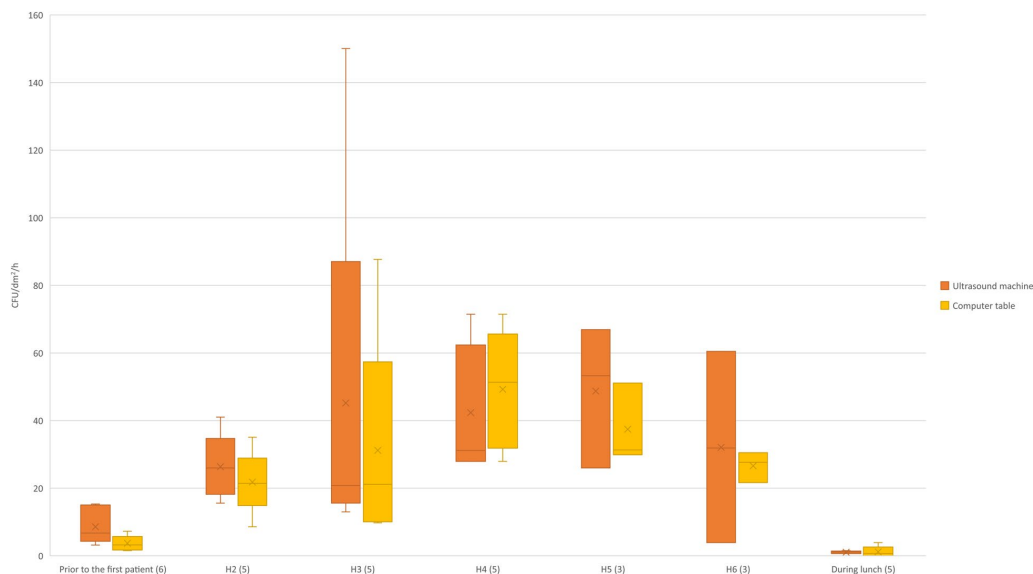
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Fig. 5 Bacterial load, in passive air samples, during mid mornings in the ultrasound room. Bacterial load reported as CFU/dm²/h in passive air samples from settle plates placed on the ultrasound machine and the computer table in an ultrasound room. During H2-H6 patients were present in the room. Number of settle plates on the ultrasound machine and the computer table are presented within brackets. The x marks the mean, and the line marks the median at each time slot (60 ± 10 min for all time slots but lunch break which varied between 35 and 60 min) and sampling locations

and staphylococci known to carry resistance of particular interest, such as methicillin resistance (e.g. *Staphylococcus aureus*, cefoxitin MIC > 4 mg/L and *S. pseudintermedius*, oxacillin MIC > 0.5 mg/L) were selected for antimicrobial susceptibility testing. No suspected methicillin-resistant (MR) isolates were detected in the 51 analysed staphylococci isolates. Thirty-six of the 51 isolates were allocated to ten phenotypes based on the antibiotic susceptibility pattern and selected for sequencing. Of the 36 staphylococci isolates, 35 were successfully analysed by whole genome sequencing. There was no indication of clonal relationship among these isolates. Multilocus sequence types and allelic profiles are presented in Additional file 5. Identification of resistance genes was not the main purpose of the study. However, the sequencing revealed genes conveying resistance to disinfectants, e.g. quaternary ammonium compounds (QACs) and chlorhexidine in 12 of 35 isolates: *qacA* was found in *S. epidermidis* (n = 1) and *S. hominis* (n = 4), *qacB* was found in *S. capitis* (n = 3) and *S. hominis* (n = 3), and *qacJ* was found in *S. epidermidis* (n = 1). A majority of the isolates with genes conveying resistance to biocides were collected in the OR (10/24) and only a few in the UR (2/11). Genes conveying resistance identified by sequencing are presented in Additional file 6.

Table 5 Observations and data during surgery

	Median (25th-75th percentile)	Min	Max
Surgery time (min)	49 (37.5–68)	16	135
Opening of door (n[a])	5 (2–9)	0	25
Staff walking in or out (n)	3.5 (2–7)	0	23
Staff movement (n)	57 (40.8–92)	4	139
Staff (n)	4 (3–4.5)	3	6
Temperature[b] (°C)	23.0 (22.0–25.6)	21.4	28.1
Relative humidity[c] (%)	32.5 (24.4–41.7)	13.3	58.4

[a] Number of occasions. [b] Median temperature based on lowest and highest temperature for each surgery. [c] Median humidity based on lowest and highest humidity for each surgery

Factors potentially related to the bacterial load in the OR
Table 5 shows median, min, max, 25th and 75th percentile of surgery time, opening of door, staff walking in or out of the door, staff movement, number of staff, temperature, and relative humidity in OR. During most (74%) surgeries, an increase in temperature and a decrease in humidity were observed. The air change was ~21 changes/h which meets the suggested ventilation rate of 17–20

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changes/h to decrease the microbial air contamination [25].

The number of movements in the OR, described in spaghetti diagrams (Fig. 6), varied considerably between the surgeries, from only four movements to 139 movements. Correlation analysis showed a strong correlation between the number of times the door was opened and the number of persons going in and out of the room, therefore only one of these factors was evaluated as potentially associated with the outcome (door opening). None of the explanatory factors were significantly associated with the outcomes at the set P-value of 0.0033.

Discussion

This study shows that it is possible for small animal hospital ORs to achieve bacterial loads below the threshold values suggested for use in human healthcare facilities thus posing a reduced risk for post-operative infections. The low bacterial load prior to and between surgeries implies that the hospital's hygiene routines were sufficient and reduced the risk of environmental transmission of bacteria between patients. Similar conclusions can be drawn for the UR where 84–100% of the samples were below the threshold values suggested for use in human healthcare facilities.

In this study, bacterial loads prior to surgery were considerably lower than the values reported in a study of veterinary operating rooms [8], where 83% of the active air samples were above the suggested alert value for active air samples in human healthcare facilities [17]. Active air sampling is assumed to capture more bacteria, compared to passive air sampling. Results from passive and active air sampling have been shown to correlate in ORs with a turbulent mixed airflow, both in empty ORs and during surgery [26]. In a recent study, including ORs with different ventilation systems (~75% with unidirectional airflow), a correlation between passive and active air sampling was also shown [27]. The study also showed that the EU GGMP relationship between passive and active air 1:8 could be considered valid for operating rooms [27]. For the suggested target and alert values, the relationship is 1:12 respectively 1:11 indicating the suggested target values for active air sampling could be a bit easier to meet [17]. Based on this the difference between the result in the present study and the other study investigating the bacterial load prior to surgery [8] can be assumed to be accurate. Similar results as those presented in that study [8] were presented in another study [9]. In that study [9] the reported geometrical mean bacterial loads per operation room was however below the suggested threshold value for clean surgical procedures without increased susceptibility for infections [16], although type of surgery was not described. The number of surgeries included for sampling were fewer than in our study and they only sampled one time for 10 min per surgery, thus comparison is difficult [9]. According to the Swedish guidelines [16] it is recommended to do repeated active air samplings during surgery, where 3 to 4 samplings is preferable. The higher bacterial load found during the procedures in our study is in line with that human and animal presence may be one of the greatest sources of the airborne microbial load in the indoor environment [7].

Compliance with the hygiene routines during clinical procedures is likely one of the most important factors for limiting transmission of infectious agents. Hygiene routines include among other things adequate environmental cleaning and, when needed, disinfection between patients as well as pre-operative skin cleaning and
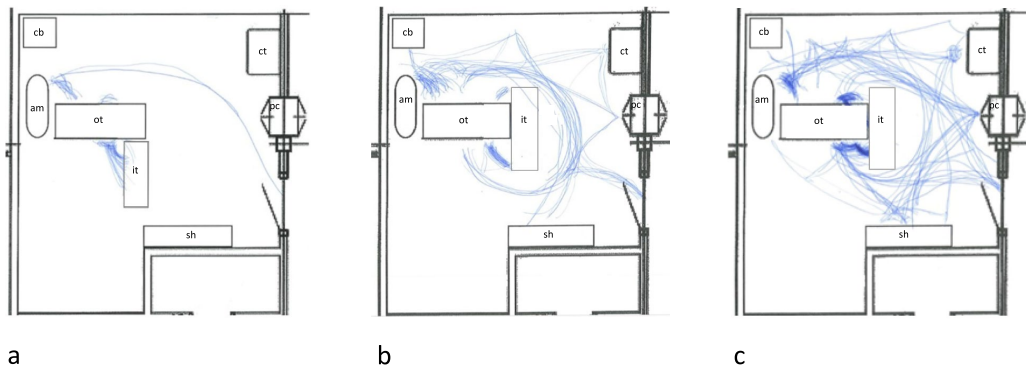

a   b   c

Fig. 6 Spaghetti diagrams of movements in the operating room. Spaghetti diagrams showing examples of **a** low-grade movement **b** moderate grade movement, and **c** high-grade movement during surgery. Equipment (size not according to scale): *am* anaesthesia machine, *cb* cupboard, *ct* computer table, *it* instrument table, *ot* operating table, *pc* pass-through cabinet, *sh* shelf

disinfection. The importance of adequate hygiene routines in veterinary OR was shown in a study identifying chlorhexidine solution, in which gauze was pre-impregnated, used for pre-operative skin disinfection as the source of a serratia-outbreak of HAI [5]. The compliance with correct wearing of cap and mask was high (> 90%, data not shown) in the studied hospital compared to the results in a study in human healthcare facilities where the proportion of correctly worn face masks were only 65% [28].

It is likely that adequate air change and the observed high compliance with the small animal hospital's OR hygiene routines (data not shown) are contributing reasons for the overall low bacterial load reported in our study. As expected, the bacterial load on settle plates and dip slides in the UR was considerably higher compared to the OR, as UR hygiene was adapted to non-invasive procedures. Nevertheless, the risk of spreading bacteria in a diagnostic imaging department should not be neglected. To reduce the transmission risk, ultrasound examinations of suspected or known infectious patients could be performed in separate rooms, or as the last patient of the day in the regular UR and followed by sanitation.

The bacterial flora was dominated by *Staphylococcus* spp. and *Micrococcus* spp.*,* especially in the OR and on high-touch surfaces. *Bacillus* spp. was also frequently detected. These findings are comparable to results from other studies performed in animal healthcare facilities [9–11]. *Micrococcus luteus, S. capitis, S. epidermidis, S. haemolyticus, S. hominis, S. warneri,* and *Kocuria* spp. are all common bacteria in the human skin microbiome [29, 30]. Findings of such bacteria might pose a lesser risk to patients. *Kocuria* spp., *Macrococcus* spp., *Micrococcus* spp., and *Staphylococcus* spp. including *S. pseudintermedius* are common in the canine skin flora [30–33]. Similarly, *Micrococcus* spp., and *Staphylococcus* spp. including *S. pseudintermedius* are common in the feline skin flora [34, 35]. *Bacillus* spp. has been reported to contaminate canine fur [32]. Hence, some of the most commonly detected *Staphylococcus* spp. in this study probably originated from staff (OR and UR) and/or animal owners (UR), while *S. pseudintermedius, Macrococcus* spp. more likely originated from the patients*. Kocuria* spp. and *Micrococcus* spp. could be of either human or animal origin. *Bacillus* spp. likely originated from the hospital environment.

There was no indication of clonal spread of the sequenced *Staphylococcus* spp., indicating that hygiene routines may have had the desired effect, i.e. old bacteria vanished with cleaning and new ones were introduced by staff, animal owners, and patients. Resistance to chlorhexidine has previously been reported in bacteria found in animal healthcare facilities [5]. The finding

of genes conveying resistance to chlorhexidine as well as QACs in the present study is interesting since such disinfectants are often used in animal healthcare facilities. If chlorhexidine and/or QACs are used in the clinic, pathogenic or opportunistic bacteria with such resistance traits may persist in the environment entailing an increased risk of HAI [36]. However, a newly published systematic review and meta-analysis showed there is no evidence of reduced susceptibility to chlorhexidine in staphylococci or streptococci of human origin [37]. In vitro studies have demonstrated multiple mechanisms for the development of resistance to QACs [38] although resistance in human clinical settings seems uncommon. Our finding of genes coding for resistance to QACs indicates that use of disinfectants may select for resistant bacteria in the veterinary clinical environment.

A limitation of the study was that the settle plates were produced under aseptic, but not sterile, conditions and several negative controls were found to be contaminated. Thus, the reported bacterial load might be slightly higher than the actual bacterial load, but as the negative controls had only a few contaminating colonies it can be assumed that this had limited impact on the overall results.

There is a need for evidence-based threshold values for animal healthcare facilities, but due to a lack of such, the present study used threshold values suggested for human healthcare facilities. In our study, most of the bacterial loads were below these values which can be assumed to reduce the risk for HAI. However, the threshold level to prevent HAI is still unknown. Future studies may investigate threshold levels for animal healthcare facilities, to ensure relevant and safe bacterial loads for the patients.

Conclusions

This study presents a generally low bacterial load in both the OR and UR, indicating a low risk of transmission of bacteria from the clinical environment. The results show that it is possible to achieve bacterial loads in the OR in small animal hospitals below the threshold values suggested for use in human healthcare facilities and thus posing a reduced risk of HAI. Bacteria carrying genes conveying resistance to disinfectants indicate that resistant bacteria can persist in the clinical environment, with increased risk for HAI.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13028-024-00768-4.

Additional file 1. Program versions and parameters for bioinformatic analysis. Sample reads were trimmed with Trimmomatic [1] and checked for contamination with Kraken2 [2]. For whole genome assembly the

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reads were normalized with the BBNorm tool from the BBTools suite [3] and assembled with Unicycler [4]. The assembly was then used for multi-locus sequence typing (MLST) using PubMLST schemes [5–9] and resistance gene identification with ResFinder [10–12]. Samples with the same sequence type or allele combination were compared with single nucleotide polymorphism (SNP)-analysis. SNP-analysis was performed for all S. capitis samples since there is no MLST scheme available for this species. For the SNP-analysis reads were downsampled using the reformat tool from BBTools [3] and mapped to reference genomes (Accession nrs. GCA_020740065.1, GCF_006094375.1, GCF_003812505.1, GCF_016126715.1) with Bowtie2 [13] and SAMTools [14]. SNPs were called and filtered with BCFTools [14] and an in-house python script [15].

Additional file 2. Bacterial flora identified on settle plates and dip slides in the operating room and the ultrasound room prior to procedures (including prior to the first procedure of the day), during procedures and after procedures. OR Operating room UR Ultrasound room.

Additional file 3. Additional data from bacterial air sampling with settle plates in the operating room and the ultrasound room. Description of data: CI confidence interval OR operating room UR ultrasound room.

Additional file 4. Additional data from bacterial surface sampling with dip slides in the operating room and the ultrasound room. a. confidence interval b. operation room c. surfaces that are frequently touched by healthcare workers and patients d. CASO (tryptic soy) agar e. CASO agar containing neutralizers that neutralize hexachlorophene, mercurial compounds, halogen compounds, chlorhexidines, aldehydes and phenolic compounds f. the area close to the incision covered by surgical drapes, instrument table and sterile surgical light handles g. ultrasound room h. surface in direct contact with or near the patient.

Additional file 5. Identified multilocus sequence types and allelic profiles. Description of data: OR Operating room UR Ultrasound room SP Settle plate DS Dip slide ST Sequence type. ~ n Denotes novel allele similar to a known allele n.

Additional file 6. Resistance genes identified by sequencing. Description of data: OR Operating room UR Ultrasound room SP Settle plate DS Dip slide blaZ Beta-lactam resistance qacA Disinfectant resistance qacB Disinfectant resistance qacJ Disinfectant resistance fosB Fosfomycin resistance vga (A) Streptogramin B resistance vga (A) V Streptogramin B resistance (Vga-A variant) fusB Fusidic acid resistance msr (A) Macrolide, Lincosamide and Streptogramin B resistance mph (C) Macrolide resistance.

Author contributions
TAJ, KB, SSL, AB and JP designed the study. TAJ collected samples. TAJ, KB and EÖ processed and analysed the samples. TAJ and JP analysed the data. TAJ and JP drafted the manuscript. All authors participated in reading and modifying drafts and have read and approved the final version of the manuscript.

Availability of data and materials
All raw sequencing data have been submitted to the European Nucleotide Archive and are available under accession number PRJEB52615. The datasets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Ethics approval and to participate
This study did not require official or institutional ethical approval. As only environmental samples were collected and no human or animal data were collected, seeking ethical permit was not relevant and could, under Swedish legislation, not be obtained.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

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Article

Bacterial Contamination of Equine Dentistry Equipment—Effect of Cleaning and Disinfection

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Simple Summary: Some of the equipment used in equine dentistry is difficult to clean and disinfect. Since it is vital to avoid the spread of infections in equine healthcare it is important to develop practical and easy-to-follow methods for cleaning and disinfecting dental equipment. The aim of this study was to investigate hygiene in equine dentistry. Dental equipment and the head support, where horses rest their head during dental care, were sampled for the amount of bacteria between each patient before and after dental care as well as after cleaning and/or disinfecting. The amount of bacteria was, in general, high on dental equipment and the head support after dental procedures. Bacteria were found in different amounts on most of the dental equipment after cleaning or disinfecting, which indicates a risk for spreading infections when using the equipment. For the head support, cleaning and/or disinfecting generally resulted in a reduced amount of bacteria, indicating a lowered risk for spreading infections. There is a great need for evidence-based guidelines on hygiene in equine dentistry to decrease the risk of transmitting infections between patients, facilities, and stables.

Abstract: Equine dentistry has developed immensely and human dental equipment, such as handpieces, are often used. Measures to avoid the spread of infectious microorganisms are important, but this is challenging since handpieces are difficult to decontaminate. Thus, it is necessary to develop effective IPC measures in equine dentistry. The aim of this study was to contribute to the evidence needed for future evidence-based guidelines on IPC by investigating hygiene in equine dentistry. Used handpieces and dummies (i.e., handpieces not used during dental procedure, reflecting environmental bacterial contamination) and the head support were sampled each day before the first patient, for each patient after treatment, and after decontamination. All equipment was sampled with 3M [TM] Swab Samplers and the head support additionally sampled with dip slides. After dental procedures, the detected bacterial load was often high on used handpieces, dummies, and the head support. After decontamination, handpieces did not meet the criteria for high-level disinfected equipment. In all but one case decontamination of the head support resulted in a lowered bacterial load. There is a great need for evidence-based guidelines on hygiene in equine dentistry, including IPC measures, to decrease the risk of spreading infectious microorganisms between patients, facilities, and stables.

Keywords: infection prevention and control; biosecurity; contamination; dental handpiece

1. Introduction

Equine dental care is carried out at veterinary hospitals, clinics, and mobile practices. Equine dental health is a rapidly growing area in veterinary clinical practice. The frequency of treatments and types of dental procedures have increased during the last decades. The advancement of equine dental care in Sweden has made it common to use human

dental equipment both for routine and advanced procedures. Handpieces, e.g., low-speed handpieces (LSH), surgical low-speed handpieces (SH), and high-speed handpieces (HSH), are used for both simple procedures, such as decreasing enamel ridges, and more advanced procedures, such as endodontic treatments. Even though protocols for cleaning, disinfecting, and sterilizing handpieces are in place for use in human dentistry there is a lack of knowledge regarding the cleaning, disinfecting, and sterilizing routines needed to ensure low risk for spreading infectious microorganisms between equine patients.

Measures to avoid the spread of infectious microorganisms between patients, and to staff, is common clinical practice in both human and veterinary medicine. This aspect of equine dentistry is challenging as handpieces are difficult to decontaminate, especially in mobile practices. Thus, there is a great need for effective infection prevention and control (IPC) measures in equine dentistry. The advanced dental procedures also entail a risk for transmission of infectious microorganisms between equine patients. For example, in a North American study, respiratory pathogens such as Equine herpes virus 1 and 4 (EHV-1, EHV-4), Equine influenza virus (EIV), Equine rhinitis B virus (ERBV), and/or methicillin-resistant *Staphylococcus aureus* (MRSA) were detected in 22% of healthy horses presented for routine dental care [1]. Furthermore, bacterial contamination of external and internal surfaces of handpieces has been shown after human dental procedures [2–5]. In addition, a higher degree of contamination of the environment and, thereby, an increased risk for patients when using high-speed devices, compared to using low-speed devices, has been shown [6,7]. There are, to our knowledge, no studies on contamination of handpieces or the surrounding environment in equine dentistry.

Based on use and hygiene, requirements for medical equipment, including dental handpieces, are categorized as non-critical, semi-critical, and critical [8]. The different categories require different levels of cleaning, disinfecting, and sterilizing (see Table 1). Handpieces are classified as semi-critical equipment, as long as they are used for non-sterile procedures, under Swedish and US hygiene guidelines for human dentistry as well as Swedish guidelines on IPC in equine healthcare and in small animal dentistry literature [8–11]. There is, however, no international consensus on criteria for the expected cleanliness for high-level disinfected equipment. Guidelines in human dentistry on how to clean, disinfect, and sterilize handpieces differ between countries. Several studies on human dental equipment demonstrate the challenges to meet criteria for both high-level disinfected and sterile equipment for handpieces. Surface disinfection of the external surface of handpieces resulted in failure to meet the criteria for high-level disinfection [12]. In another study, one of four cleaning devices intended for handpieces, the washer-disinfector (WD), provided an acceptable test result [13]. In one study, type N steam sterilizers failed to provide sterile handpieces whilst type B steam sterilizers provided sterile handpieces [14].

Table 1. Presentation of categorization of dental equipment in human dentistry, area of use, microbial definition according to Swedish guidelines and standards, and the level of cleaning, disinfecting, and sterilizing needed, according to Swedish guidelines.

Category	Use	Example of Equipment Used in Human Dentistry	Example of Equipment Used in Equine Dentistry	Microbial Definition of Category in Swedish Guidelines in Human Dentistry	Level of Cleaning, Disinfecting, and Sterilizing in Swedish Guidelines in Human Dentistry
Non-critical	In contact with intact skin	Spatulas	Mouth specula	Visibly clean [9]	Cleaning if not contaminated; if contaminated, cleaning and disinfecting in a washer-disinfector (WD) or manual cleaning followed by chemical disinfecting [9]

Table 1. *Cont.*

Category	Use	Example of Equipment Used in Human Dentistry	Example of Equipment Used in Equine Dentistry	Microbial Definition of Category in Swedish Guidelines in Human Dentistry	Level of Cleaning, Disinfecting, and Sterilizing in Swedish Guidelines in Human Dentistry
Semi-critical	In contact with mucus membranes, but not penetrating sterile tissue	Handpieces	Drills, burrs and handpieces used when not penetrating pulp, e.g., decreasing enamel ridges	Free from pathogenic micro-organisms and less than one microorganism on 1000 handpieces [9]	Cleaning and disinfecting in a WD [9]
Semi-critical	In contact with mucus membranes, but not penetrating sterile tissue	Compresses	Compresses	Free from pathogenic micro-organisms and occurrence of occasional vital micro-organisms [15]	Cleaning and disinfecting if the equipment is made for reuse
Critical	In contact with sterile tissue	Surgical instruments, like extraction forceps	Drills, burrs, handpieces, and other instruments used when penetrating pulp, e.g., endodontic treatments	Free from living micro-organisms/less than one microorganism on 1,000,000 handpieces [15,16]	Cleaning and disinfecting in a WD followed by sterilizing in a B-autoclave [9]

According to Pusterla et al. [1], equine dental equipment used for routine dental procedures, not expected to expose the pulp (i.e., semi-critical equipment), is rarely cleaned and disinfected between patients. Even though the equipment used for routine dental procedures can differ between countries, the conditions for cleaning procedures and risk of spreading infections can be assumed to be comparable. Moreover, IPC routines for equipment used in equine dentistry are not listed in the syllabus of equine dentistry courses in Sweden and in the European specialist program in equine dentistry [17,18]. In our experience, a commonly used decontamination method for handpieces in equine dentistry is surface disinfection with an intermediate disinfectant (a disinfectant with effect on most vegetative bacteria, some mycobacteria, some fungi, some enveloped and non-enveloped viruses [19]) without previous cleaning. However, no decontamination procedure in equine dentistry has yet been evaluated or published. Guidelines for IPC procedures in equine dental practice, based on solid data, are needed.

The overall aim of this study was to contribute to the evidence needed for future guidelines on IPC procedures for equine dentistry by investigating hygiene in equine dentistry, specifically by:

(1) assessing the bacterial load on handpieces and the patient environment during equine dental care;

(2) assessing if manual cleaning of handpieces with detergent or disinfection with surface disinfection is sufficient to meet the Swedish criteria for high-level disinfected equipment; and

(3) determining the bacterial load on the immediate surroundings after surface disinfection, or cleaning followed by surface disinfection.

2. Materials and Methods

The study was carried out in the dental practice of a veterinary hospital during the autumn of 2020. The veterinary hospital's patient load consists of approximately 9000 patients per year and approximately 500 of them are dental patients. Sampling was carried out during two working days, with a total of 11 horses submitted for dental care.

2.1. Sampling

Equipment and surfaces to be sampled for bacterial load were selected based on a pilot study carried out in the dental practice of another veterinary hospital (for details from the pilot study see Table S1: Bacterial load pilot study). The veterinary hospital's patient load consists of approximately 5000 patients per year and approximately 500 of them are dental patients. Based on the pilot results, sampling of handpieces and the head support was standardized (for details about sampling methods tested, see Table S2: Sampling methods tested). The sampling surfaces of the equipment are illustrated in Figure 1 and the sampling protocol is illustrated in Figure 2. Make and model of used handpieces can be found in the figure text in Figure 2.

Handpieces were sampled with 3M [TM] Swab Samplers (3M [TM] Swab Sampler, Saint Paul, Minnesota, USA) with letheen broth, an already established method in the food industry for control of hygiene [20]. The 3M [TM] Swab Samplers Method was modified to enable sampling of the different surfaces LSH, SH, and HSH, see Table 2 [21]. All samplings with 3M [TM] Swab Samplers were carried out by the second author and the first author held the handpieces, wearing non-sterile nitrile gloves, during sampling. The samples were then analyzed for total aerobic colony count (ACC).

The samples from the head support were also analyzed for total ACC, using two sampling methods; 3M [TM] Swab Samplers with letheen broth (both study days) and dip slides (Envirocheck [®] Dip Slide Disinfection Control (DC), 9,4 cm^2, Orange, USA) with TSA agar/TSA agar with a neutralizer, neutralizing several disinfectants (one of the study days). A particular 10×10 cm surface of the head support, a cushioned device supporting part of the horse's lower jaw, was repeatedly swabbed at each sampling with the 3M [TM] Swab Samplers Method, see Table 2 [21]. Also, an adjacent specified surface of the head support was sampled with dip slides. The surface chosen for sampling can be contaminated by fluid from the mouth during dental examination and treatment. The dip slide was pressed firmly to the surface for 15 s, then turned over and the opposite side of the slide was pressed against the adjacent surface for another 15 s [22]. All sampling with dip slides was carried out by the first author.

For each sampling day and sampling method, one or two negative controls (unexposed dip slide and swab sampler) were applied. The controls were put in the dental care room just before sampling started for the day, approximately 10 min before the day's first patient, and the controls were stored in the room until the gathering of sampling material after the last sampling of the day.
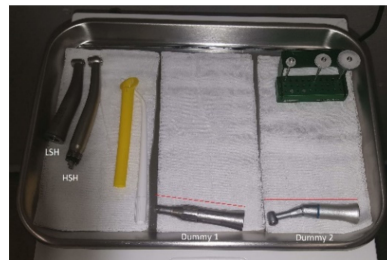
Figure 1. (**a**) Handpieces used for dental procedures in the study. LSH = Low-speed handpiece; SH = Surgical low-speed handpiece; and HSH = High-speed handpiece. Red lines indicate the external surface sampled of LSH, SH, and HSH. The red rectangle indicates the coupling surface sampled on HSH. (**b**) Handpieces used for dental procedures in the study. The red circle indicates the coupling of which the first 0.5 cm was sampled in LSH. (**c**) Shaft of SH; the red line indicates the surface sampled. (**d**) Some of the dental devices used in the study. The red lines indicate the external surface sampled on dummy 1 and 2.

Figure 2. Sampling schedule for the equipment. For assessment of environmental contamination one or two extra LSH (called dummy 1 and 2) and the head support were sampled. The dummies were not us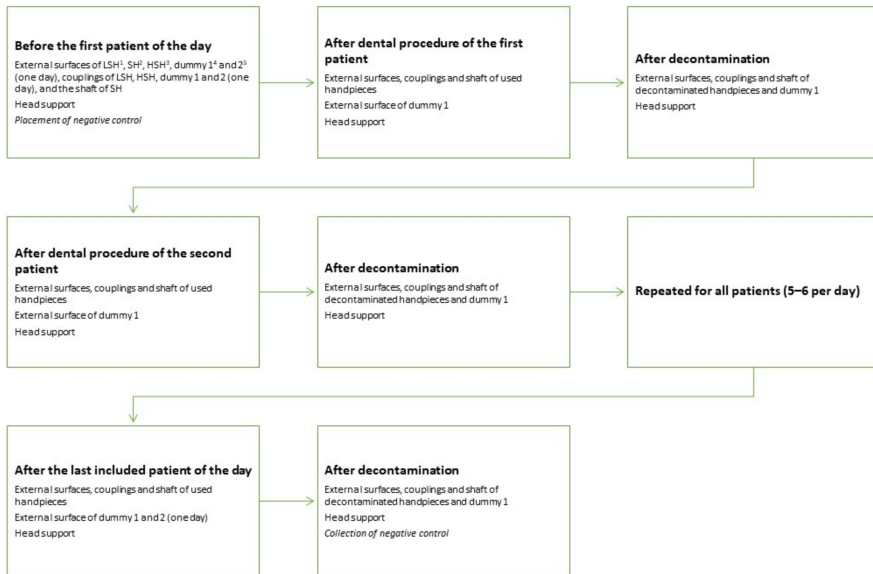ed during dental procedure, but consisted of one or two extra handpieces placed close by if needed during the procedure (approximately 70 cm from the patient's oral cavity). Sampling after dental procedure was carried out within 5 min after the dental procedure was finished and no equipment was rinsed or wiped off before sampling. [1] NSK Ti-Max X25, [2] W&H S-15, [3] Pferdefit-Dental Eco high speed SEA-F4-1-P DEN 1101, [4] NSK FX 65, and [5] NSK NAC-EC.

Table 2. Description of sampling methods for handpieces and the head support.

Sampling Surface	Sampling Method	Description of Method	Parallel Sampling Method	Description of Parallel Method
External surface LSH [1]/SH [2]/HSH [3]/ dummies	Modified swab sampler method (MSS)	Swabbed once, specification of surfaces (see Figure 1)	NA[4]	NA
Coupling LSH/ dummies	MSS	Swab rotated 360° three times, swabbing possible 0,5 cm into the tunnel (see Figure 1)	NA	NA
Shaft SH	MSS	Shaft swabbed once (see Figure 1)	NA	NA
Coupling HSH	MSS	Swab rotated 360° three times (see Figure 1)	NA	NA
Head support	Swab sampler method [21]	Specified, 10 × 10 cm surface. Swab rubbed three times over area, changing direction, first by 90° then by 45°.	Dip slide	Pressed against surface 15 s, turned and pressed 15 s against an adjacent surface

[1] Low-speed handpiece. [2] Surgical low-speed handpiece. [3] High-speed handpiece. [4] Not applicable. The swab was not put back in the tube between the three swabbings.

2.2. Cleaning and Disinfecting Methods

Five protocols for cleaning and disinfecting handpieces and the head support were used (for details see Table 3). Protocols 1 and 2 consisted of the veterinary hospital's own protocol for surface disinfection with an intermediate disinfectant of handpieces and the head support. Protocol 3 consisted of manual cleaning of LSH and HSH and protocol 4 consisted of manual cleaning of SH. Protocol 5 consisted of manual cleaning of the head support followed by surface disinfecting with an intermediate disinfectant. LSH and SH were lubricated after every cleaning or disinfecting with a lubricant (PANA SPRAY Plus, NSK / Nakanishi inc., Kanuma, Japan) containing ester oil, ethanol butane, and propane. The HSH was lubricated using lubricating oil (MD-30 Advantage Dental Handpiece Oil MD-30, iM3, Sidney, Australia) containing synthetic hydrocarbon oil and ester oil after the last patient of the day. All handpieces were put back, out in the open, on a metal tray adjacent to the patient after cleaning or disinfecting and lubricating. The veterinary technician and the first author carried out every other cleaning or disinfection and lubrication of handpieces and the head support. During the period of sampling the dental care room and dental equipment were used three days a week. When protocols 1 and 2 were used, the dental care room and equipment had been unused for four days and when protocols 3–5 were used the dental care room and the dental equipment were used the day before.

Table 3. Protocols used for cleaning and disinfection of handpieces and the head support.

Protocol	Equipment	Cleaning and Disinfecting Substance	Description of Cleaning and Disinfecting Methods
1	LSH [1], SH [2], and HSH [3]	LD [4]	Surface disinfection of external surfaces, including external surface of couplings, by rubbing with disinfection wipes (Wet Wipe Triamin Disinfection, Wet Wipes A/S, Vallensbæk, Denmark) until visibly clean for ≥12 s.
2	Head support	LD+EPT [5]/LD+EIT [6]	Surface disinfection by rubbing with disinfection wipes until visibly clean for ≥15-s, followed by spraying a surface disinfectant (Dax 75+, KiiltoClean AB, Täby, Sweden or LiV72+, Clemondo, Helsingborg, Sweden) and rubbing the surface for ≥25 s until dry and thereafter spraying surface disinfectant on the surface to air-dry.
3	LSH and HSH	SL-11C+L-10 [7]	Manual cleaning of external surfaces, including external surface of couplings, by rubbing with cleaning wipes (ICA Städservett, ICA, Solna, Sweden) until visibly clean for ≥12 s.
4	SH	Standard washing liquid+ SL-11C+L-10	Dismantling of SH, manual cleaning with a brush (below the water surface) of SH shell and shaft in warm water until visibly clean for ≥30 s. Rinsing in lukewarm-to-warm water and rubbing with a cleaning wipe until visibly clean for ≥12 s.
5	Head support	SL-11C+L-10 + EPT/EIT	Rubbing with cleaning wipes until visibly clean, for ≥15 s. Followed by spraying a surface disinfectant (Dax 75+, KiiltoClean AB, Täby, Sweden or LiV72+, Clemondo, Helsingborg, Sweden) and rubbing the surface for ≥25 s until dry and thereafter spraying surface disinfectant and left to air-dry.

[1.] Low-speed handpiece, [2.] Surgical low-speed handpiece, [3.] High-speed handpiece, [4.] Laurylamine Dipropylenediamine, [5.] Ethanol, propanol, and tensed, [6.] Ethanol, isopropanol, and tensed, and [7.] Sodium Laureth-11 Carboxylate and Laureth-10.

2.3. Bacteriological Analyses

All 3M Swab Samplers and dip slides were taken to the laboratory at the Swedish University of Agricultural Sciences on the day of sampling. 3M™ Swab Samplers were vortexed and 1 mL broth was drawn from the sampling tube and put onto a 3M Petrifilm™ aerobic count (AC) Plate (3M Petrifilm™ Aerobic Count Plate, Saint Paul, Minnesota,

USA), see Figure S1: Petrifilm [TM] negative control, as further described in the 3M [TM] Swab Sampler Method [21]. Samples were incubated aerobically in 30 ± 1 °C for 48 ± 2 h. Dip slides were incubated in 37 ± 1 °C for 48 ± 2 h. The colonies were counted manually by the first author, as described in the interpretation guide [23]. All 3M Petrifilm [TM] AC Plates and dip slides were photographed for documentation.

2.4. Data Management

Microsoft [®] Excel [®] 2016 (16.0.5134.1000) (Microsoft Corporation, Redmond, Washington, USA) was used for data management and descriptive statistics.

3. Results

The study included 80 samples from handpieces, 42 samples from dummies, 24 samples from the head support, and two negative controls using 3M [TM] Swab Samplers. In addition, dip slides were used in 14 samples from the head support and for two negative controls. No bacterial growth was detected in the samples from negative controls.

3.1. Handpieces

Bacterial growth was detected in all samples from HSH, both after dental procedures and after cleaning or disinfecting (see Table 4). In all samples from external surfaces from LSH and SH, bacterial growth was found after dental procedures, and after cleaning or disinfecting bacterial growth was still detected in all but one sample. After dental procedures, bacterial growth was detected in all samples from the coupling of LSH and in 6 of 10 samples from the shaft in SH. After cleaning or disinfecting, no bacterial growth was found in couplings from LSH while bacterial growth was found in 2 of 10 samples from the shaft of SH.

Table 4. Bacterial load of external surfaces, couplings, and the shaft of handpieces after dental procedures and after cleaning or disinfecting. Colony forming units (CFU) is given in total CFU/external surface, CFU/coupling and inner piece, respectively. Pulp exposure is divided into the categories: no exposure, risk for exposure, exposure, and not applicable. The difference between risk for exposure and exposure is that risk for exposure includes dental procedures when the pulp exposure is unintended and exposure includes dental procedures when pulp exposure is intended.

Cleaning or Disinfecting Substance	Type of Handpiece	Pulp Exposure	CFU after Dental Procedure, External Surface	CFU after Cleaning or Disinfecting, External Surface	CFU after Dental Procedure, Coupling or Shaft	CFU after Cleaning or Disinfecting, Coupling or Shaft
LD [1]	SH [3]	NA	NA	4 [7]	NA	0 [7]
LD	LSH [4]	NA	NA	8 [7]	NA	0 [7]
LD	HSH [5]	NA	NA	3 [7]	NA	3 [7]
LD	SH	No exposure	TNTC [6]	1	0	1
LD	SH	No exposure	TNTC	28	0	0
LD	SH	Risk for exposure	620	0	2	0
LD	LSH	Risk for exposure	510	1	26	0
LD	HSH	Risk for exposure	820	1420	TNTC	10
LD	SH	Risk for exposure	TNTC	8	1	0
LD	LSH	Risk for exposure	420	1260	20	0
LD	HSH	Risk for exposure	720	70	TNTC	520
LD	SH	No exposure	TNTC	5	1	0

Table 4. *Cont.*

Cleaning or Disinfecting Substance	Type of Handpiece	Pulp Exposure	CFU after Dental Procedure, External Surface	CFU after Cleaning or Disinfecting, External Surface	CFU after Dental Procedure, Coupling or Shaft	CFU after Cleaning or Disinfecting, Coupling or Shaft
SL-11C+L-10 [2]	SH	NA	NA	69 [7]	NA	0 [7]
SL-11C+L-10	LSH	NA	NA	TNTC [7]	NA	0 [7]
SL-11C+L-10	HSH	NA	NA	10 [7]	NA	8 [7]
SL-11C+L-10	SH	Risk for exposure	TNTC	TNTC	5	0
SL-11C+L-10	LSH	Risk for exposure	TNTC	231	88	0
SL-11C+L-10	HSH	Risk for exposure	TNTC	TNTC	TNTC	TNTC
SL-11C+L-10	SH	No exposure	TNTC	160	14	30
SL-11C+L-10	SH	No exposure	TNTC	TNTC	4	0
SL-11C+L-10	SH	Risk for exposure	TNTC	25	0	0
SL-11C+L-10	LSH	Risk for exposure	TNTC	900	520	0
SL-11C+L-10	SH	No exposure	TNTC	1350	0	0

[1.] Laurylamine Dipropylenediamine, [2.] Sodium Laureth-11 Carboxylate and Laureth-10, [3.] Surgical low-speed handpiece, [4.] Low-speed handpiece, [5.] High-speed handpiece, [6.] Too numerous to count, and [7.] Samples taken before the first patient of the day.

3.2. Dummies

Bacterial growth was detected in all samples from external surfaces of dummies before the first patient of the day and in 11 of 12 samples from the external surface of dummies after dental procedures compared to 5 of 11 after cleaning or disinfection (see Table 5). In 2 of 3 samples from couplings of dummies, bacterial growth was detected before the first patient of the day. In addition, bacterial growth was detected in 2 of 11 samples from the coupling of dummies after cleaning or disinfecting.

Table 5. Bacterial load of dummies (handpieces not used during dental procedure, illustrating extra handpieces placed close by (approximately 70 cm from the patient's oral cavity) if needed during the procedure. Day 1 dummy 1 was put in place approximately 10 min before the first patient of the day, day 2 dummies 1 and 2 were put in place approximately 10 min before the first patient of the day. Dummy 1 was sampled and cleaned or disinfected after each patient whilst dummy 2 was not cleaned or disinfected, and sampled only after the last patient included for the day. CFU is given in total CFU/external surface and CFU/coupling respectively.

Dummy	Cleaning or Disinfection Substance	CFU after Dental Procedure, External Surface	CFU after Cleaning or Disinfection, External Surface	CFU after Cleaning or Disinfection, Couplings
1	NA	NA	183 [3]	22 [3]
1	LD [1]	TNTC	1	0
1	LD	1340	18	3
1	LD	0	0	0
1	LD	67	0	0
1	LD	82	0	0
1	LD	1100	8	0
1	LD	NA	440 [3]	1 [3]
2	NA	NA	267 [3]	0 [3]
1	SL-11C+L-10 [2]	TNTC	0	0
1	SL-11C+L-10	330	2	1
1	SL-11C+L-10	87	1	0
1	SL-11C+L-10	18	0	0
1	SL-11C+L-10	35	0	0
2	NA	TNTC	NA	NA

[1.] Laurylamine Dipropylenediamine, [2.] Sodium Laureth-11 Carboxylate and Laureth-10, and [3.] Samples taken before the first patient of the day.

3.3. Head Support

In both samples from the head support before the first patient of the day bacterial growth was detected, and in one of them the CFU/cm^2 were too numerous to count. The bacterial load detected on the head support was high (usually too numerous to count) after dental procedures. Both cleaning and/or disinfection reduced the bacterial load as seen in most of the samples (see Table 6).

Table 6. Bacterial load detected on the head support after dental procedures, and after cleaning and/or disinfecting. Colony forming units (CFU) are given in CFU/cm^2. For bacteriological analyses 3M Petrifilm ^{TM} aerobic count (AC) Plates and dip slides with TSA agar/TSA agar with a neutralizer, neutralizing several disinfectants.

Cleaning and Disinfecting Substances	Bacterial Load after Dental Procedure			Bacterial Load after Cleaning and/or Disinfecting		
	Petrifilm ^{TM}	Dip Slide TSA + Neutralizer	Dip Slide TSA	Petrifilm ^{TM}	Dip Slide TSA + Neutralizer	Dip Slide TSA
LD[1]+EPT[2]/LD+EIT [3]	NA	NA	NA	0.03 [5]	0.11 [5]	0 [5]
LD+EPT/LD+EIT	TNTC	7.23	TNTC	3	0.11	0.96
LD+EPT/LD+EIT	TNTC	TNTC	TNTC	0.04	0.11	0
LD+EPT/LD+EIT	TNTC	TNTC	TNTC	0.07	0	0
LD+EPT/LD+EIT	TNTC	5.32	9.89	0.06	0	0.11
LD+EPT/LD+EIT	TNTC	1.49	6.38	0	0	0
LD+EPT/LD+EIT	TNTC	TNTC	TNTC	0.01	0	0.85
SL-11C+L-10 [4] + EPT/EIT	NA	NA	NA	TNTC [5]	NA	NA
SL-11C+L-10 + EPT/EIT	TNTC	NA	NA	1.45	NA	NA
SL-11C+L-10 + EPT/EIT	TNTC	NA	NA	0.10	NA	NA
SL-11C+L-10 + EPT/EIT	TNTC	NA	NA	TNTC	NA	NA
SL-11C+L-10 + EPT/EIT	TNTC	NA	NA	0	NA	NA
SL-11C+L-10 + EPT/EIT	TNTC	NA	NA	0.73	NA	NA

[1.] Laurylamine Dipropylenediamine, [2.] Ethanol, propanol and tensid, [3.] Ethanol, isopropanol and tensid, [4.] Sodium Laureth-11 Carboxylate and Laureth-10, and [5.] Samples taken before the first patient of the day.

4. Discussion

To the best of our knowledge, this is the first study of hygiene in equine dentistry. According to Swedish guidelines in human dentistry, all handpieces should meet criteria for high-level disinfected equipment, and handpieces used for surgical procedures should be sterile [9,15,16]. In equine dentistry, the risks of spreading blood-borne diseases, such as those that are important in human dentistry [8,24], are not seen as a major risk. In equine dentistry there are, however, risks of spreading various pathogenic and resistant microorganisms [1]. According to the Swedish guidelines on IPC in equine healthcare, dental equipment that comes in contact with mucus membranes, but not penetrating sterile tissue, shall meet criteria for high-level disinfected equipment and surgical dental equipment shall be sterile [10]. According to the American Animal Hospital Associations' dental care guidelines for dogs and cats, all dental instruments shall be cleaned and sterilized after each use [25] whilst World Small Animal Veterinary Association guidelines states all dental equipment shall be cleaned, disinfected, and/or sterilized based on the equipment's intended use [26] Also small animal dentistry literature recommends semi-critical and critical instruments to be sterilized after each use [11]. All the recommendations are brief and reflect hygiene recommendations in human dentistry and the differences in guidelines on how to clean, disinfect, and sterilize dental equipment reflects the differences found between countries in human dentistry. All these recommendations are very brief and no clear criteria is given for handpieces. It is important to take into account both the differences and the similarities in risks between human and equine dentistry and to

have knowledge of, e.g., the microbial contamination in equine dentistry when developing guidelines on IPC measures in equine dentistry. In this study, equipment and the close patient environment were highly contaminated after dental procedures. The bacterial load in the immediate environment was lower after cleaning and/or disinfecting, but handpieces did not meet the criteria for high-level disinfected equipment after cleaning or disinfecting.

It can be assumed that a high bacterial load is an indicator of the presence of potentially pathogenic microorganisms. In a study by Adams et al. [27] investigating the occurrence of *Staphylococcus aureus* in a human intensive care unit, most *Staphylococcus aureus* were detected on heavily contaminated hand-touch sites. This study has shown that dental procedures, using handpieces, contaminate the equipment and the surrounding environment, with a potentially increased risk of transmission of pathogens between patients, staff, and facilities. As HSH is used for endodontic treatment, transmission of microorganisms can have severe consequences since the pulp is exposed, i.e., there is an increased risk for infection. To classify HSH as critical equipment could emphasize its importance to improve IPC measures in equine dentistry. For semi-critical equipment as LSH and SH, when not used for endodontic treatment, it can be discussed whether the strictest definition of high-level disinfected equipment is needed in equine dentistry. An important factor for considering to use the less strict definition of high-level disinfected equipment (i.e., free from pathogens and occurrence of occasional vital microorganisms, see Table 1) for semi-critical LSH and SH is that it is important to identify an achievable, and measurable, threshold value for manual cleaning and disinfection. It is difficult to estimate the risk of infection transmission if the less strict Swedish definition of high-level disinfected equipment would be applied for handpieces. The results of our study indicate that even the less strict criterion may require more meticulous routines for cleaning and disinfection.

In this study, the effect of surface disinfection of handpieces was similar to the results reported by Pinto et al. [12] in a study in human dentistry, where handpieces did not meet even the less strict criteria for high-level disinfected equipment in human dentistry [9]. The couplings in both LSH and HSH can be regarded as a bridge between external and internal surfaces. The couplings can, if the IPC measures are ineffective, serve as a vector spreading infectious agents between patients. Infectious agents may occur in horses without clinical symptoms of infection, for example, Pusterla et al. [1] reported respiratory pathogens in 22% of healthy horses submitted for routine dental care.

If the upper limit for bacterial load on external contact surfaces of 2.5 CFU/cm^2, as suggested in studies on human hospital cleanliness [28–31], is used as the limit of acceptance for the head support (a non-critical piece of equipment), most samples in this study meet the criteria after cleaning and/or disinfecting.

Limits of the study: The bacterial load on the shaft of the SH after dental procedures in this study diverges from results reported by Smith et al. [3], in median 1000 CFU/surgical gear compared to up to 30 CFU/shaft in this study. Smith et al. [3] sampled the surgical gear which can be assumed to be more highly contaminated compared to the shaft sampled in this study. Smith et al. [3] also used a better sampling method and a culturing method enabling identification of a wider range of bacteria. If the surgical gear would have been sampled in this study using the same method as Smith et al. [3], it can be assumed the bacterial load would have been considerably higher. Petrifilms ^{TM} were incubated in 30 ± 1 °C which means environmental flora is probably dominating and potential pathogenic bacteria may have been overgrown. The amount of potential pathogen bacteria would probably have been more accurate if Petrifilms ^{TM} had been incubated in 37 ± °C, which is optimal for most mammalian pathogens [32]. Data on length of time in contact with dental tissue was not collected; this data could possibly have provided important information about the degree of contamination after different lengths of time in contact with dental tissue.

More research will be needed to evaluate how manual cleaning followed by chemical disinfecting of external and internal surfaces of handpieces can result in the less strict Swedish definition of high-level disinfected handpieces, i.e., free from pathogens and occurrence of occasional vital microorganisms. Other topics for future research should be to develop evidence-based guidelines by (1) design and test IPC routines for equine dental procedures and (2) investigate what level of hygiene is needed to minimize the risk of transmission of infectious agents between patients in equine dentistry.

High contamination of the equipment and the close patient environment, combined with handpieces not meeting criteria for high-level disinfected equipment after manual cleaning or disinfecting, indicates an urgent need for evidence-based guidelines on hygiene in equine dentistry. Based on the study results, protocols for assessment of contamination level could be developed. Larger series of data from several clinics as well as mobile practice should be collected as a basis for such guidelines. Categorization of dental equipment as critical, semi-critical, and non-critical equipment, and a clear definition of hygiene criteria for such equipment are also needed. In addition, guidelines on how to clean, disinfect, and, in some cases, sterilize dental equipment are necessary. Methods for monitoring each step are also needed.

5. Conclusions

The detected bacterial load on the equipment and in the close patient environment was often high after dental procedures. Handpieces did not meet the criteria for high-level disinfected equipment after cleaning or disinfecting. In most cases cleaning and/or disinfecting of the head support resulted in a lowered bacterial load. This implies there is a need for evidence-based guidelines on IPC procedures for equine dentistry. In addition, data to support appropriate threshold levels are needed.

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Microorganisms on surfaces and equipment in animal healthcare facilities may constitute a risk of healthcare-associated infections (HAIs). This thesis aimed to improve infection prevention and control in animal healthcare by studying the effect of cleaning and disinfection. The result showed a generally low bacterial load, except after microfibre cleaning of the floor in dog cages and after decontamination of dental handpieces. There is a need for evidence-based cleaning and disinfection routines, to reduce the risk of HAIs.

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