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Handscape

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Infection control in a veterinary hospital by improving adherence to hand hygiene protocols through personal feedback on plate counts and with special attention to resistant flora and their origin (microbial resistance genes).

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Abstract

An important measure in hygiene protocols in health care settings is hand hygiene, as this reduces the number of hospital acquired infections, both in human hospitals, and also in a veterinary environment. Here, a new method to improve adherence to hand hygiene protocols in a veterinary hospital will be proposed.

The aims of this project were, firstly, to determine the loading of viable bacteria on hands of people working in veterinary clinical (SATH group) and non-clinical environments (Leahurst group), and who have contact with small animals. Results showed a significant difference ($p<0.0005$) in bacterial loading between groups, with higher bacterial loading on hands of participants in the Leahurst group, suggesting better hand hygiene in the veterinary clinical environment. This was expected, considering the many measures already in place in that environment to ensure good compliance with hand hygiene protocols.

The second aim was to determine the types of bacteria and antimicrobial resistance patterns on hands of people working in these environments. *S. aureus* carriage was quite common in both groups, 18.2% and 16.1% for Leahurst and SATH group respectively, but *S. pseudintermedius* was only isolated from hands of participants in the clinical group. *MecA* carriage was common in both groups (35%). ESBL carrying *E. coli* were only isolated from hands of participants in the Leahurst group and displayed worrying levels of resistance to other antibiotic classes as well. Presence of *E. coli* suggests faecal contamination, but the origin of this could not be determined. Antimicrobial susceptibility testing showed more frequent resistance to antibiotics in staphylococci isolated from the SATH group than Leahurst group.

The last aim was to determine the acceptability of hand plates as a sampling method and assessment of the feedback system. Our intervention method did not have the desired effect of lower plate counts during a second round of sampling, but since most intervention methods aimed at improving hand hygiene need a multimodal approach in order to be effective, this was not very surprising.

Our method is timesaving and useful for determining bacterial loading on hands of people and screening for common bacteria. Although the feedback did not have the desired effect of lowered plate counts, further research is needed to determine whether it could have the expected effect if combined with other strategies to improve hand hygiene compliance.

Abbreviations:

AMH – alcohol-based antimicrobial hand rub
AMR – antimicrobial resistance
CFU – colony forming unit
CI – confidence interval
ESBL – extended-spectrum β-lactamase
HAI – hospital acquired infection
MRSA – methicillin-resistant *Staphylococcus aureus*
MRSP – methicillin-resistant *Staphylococcus pseudintermedius*
SATH – Small Animal Teaching Hospital

1. Introduction

Infection control is important not only in human hospitals, but also veterinary hospitals in order to prevent spread of nosocomial diseases or infectious diseases.[1,2] There are many different methods to prevent cross-contamination of pathogens or opportunistic bacteria, which may be a source for infections. Measures that prevent the physical transmission of microbes between patients, staff and the environment are called contact measures.[1] Hand hygiene protocols, glove use, use of disposable gowns are examples of contact measures.[2,3] Adequate hand hygiene measures can significantly reduce the number of hospital associated infections (HAI's) (also called nosocomial infections), as was first observed by Ignaz Semmelweis, who observed a difference in mortality rates in mothers between two clinics where babies were delivered and attributed it to lack of hygienic measures such as hand washing in between patients.[4] There are now numerous examples of the role of hand hygiene in HAI's.[5] Ideally, hand hygiene reduces or eliminates the transient microbiota of the hand, prevents rebound growth and transmission of pathogenic or opportunistic microbes from hands to individuals. This may be done either by the use of antimicrobial compounds or physical removal of these microbes, for example with water and soap, preferably without irritating or damaging the skin.[2] Nowadays, soap has widely been replaced by alcohol-based hand rubs for in between patient contact, as these have proven just as effective in reducing bacterial load, but need significantly shorter contact time to be effective.[1] Current recommendations from the WHO include the five moments for hand hygiene: before and after touching a patient, before a clean or aseptic procedure, after exposure to bodily fluids, after touching a patient surrounding.[6]

The importance of hand hygiene and infection control has also been recognized in veterinary medicine.[2] Although the number of reported HAI's in veterinary medicine is not as high as in human hospitals there have been reports of outbreaks, such as *Salmonella* in veterinary horse clinics and methicillin-resistant *Staphylococcus aureus* (MRSA) in small animal hospitals.[7–9] Urinary tract infections, surgical site infections, blood stream infections are the most common, as well infectious diarrhoea.[10] In addition, some pathogens have zoonotic potential and are therefore a risk for personnel.[11]

A threat in treating these HAI's is the emergence of antibiotic resistance among bacteria involved in HAI's.[12] This subject has received a lot of attention in human medicine, but HAI's with resistant bacteria occur in animals as well.[3] MRSA and methicillin-resistant *S. pseudintermedius* (MRSP) are staphylococci that carry the *mecA* gene, resulting in resistance to β-lactam antibiotics, e.g. penicillin, amoxicillin, cephalosporins, and there may be additional resistance to other antibiotics, such as lincosamides (clindamycin), trimethoprim-sulfonamides, tetracyclines, macrolides and fluoroquinolones due to chromosomal mutations or acquired resistance genes with these resistant bacteria.[12,13] *S. aureus* is frequently carried by people and occasionally by dogs and cats, whereas *S. pseudintermedius* is mainly a coloniser of dogs and cats.[14–17] Although both non-resistant and resistant strains of these staphylococci are capable of causing nosocomial infections,

treatment of MRSP and MRSA is much more challenging.[10] For example, Nienhoff et al. screened 814 dogs admitted to a small animal hospital for MRSP. 60 of them (7.4%) were reported positive. Factors associated with MRSP carriage were previous hospitalisation, antibiotic treatment, and contact with a veterinarian within the last four weeks.[18] Another study demonstrated that transmission from MRSP positive animals to their owners or personnel is rare, but that contact animals and the environment are at risk of being colonised or contaminated.[19] Like MRSP, MRSA is an opportunistic pathogen and can be found in clinically healthy animals, but may also be the cause of post-operative infections, wound infections, skin infections, urinary tract infections and others. It is likely that these MRSA isolates originate from humans and were transmitted to their pets, but pets may also act as a source for human infections.[16] In a study from Loeffler et al. a MRSA prevalence of 18% among staff in a veterinary was reported, suggesting that veterinary staff may be more at risk for MRSA carriage.[20] Walther et al. reported a sudden rise in colonisation in a veterinary hospital that occurred simultaneously with a rise in MRSA infections, supporting this suggestion.[8] In a recent case-control study the following risk factors for dogs and cats have been identified: number of treatments with antibiotics, number of days at a veterinary clinic, having received implants.[21]
Other pathogens of concern with regard to antibiotic resistance are *Escherichia coli* (*E. coli*) and bacteria belonging to the *Enterobacteriaceae*. *Enterobacteriaceae* are commensals of the intestinal microflora and especially *E. coli* is also associated with HAI's, e.g. urinary tract infections and surgical site infections.[10] The role of other Enterobacteriaceae such as *Klebsiella* and *Actinobacter* in veterinary HAI's has not been well explored, but they are important sources of HAI's in humans.[10] Hamilton et al. studied risk factors for acquiring multi-drug resistant *E. coli*, as well as MRSA, and found extended hospital stay (> 3 days) a risk factor in dogs.[22] Other bacteria that are less frequently involved in small animal HAI's but can also develop resistance are *Salmonella, Acinetobacter* and *Pseudomonas* species. The latter is especially known for its ability to form biofilms and its ability to quickly become multi-drug resistant. This makes HAI's where *Pseudomonas* species are involved hard to treat.[10]

Despite the fact that the role of hand hygiene in preventing in controlling HAI's has been well established[1], adherence to hand hygiene protocols is often low.[23] There are only few reports on compliance in veterinary medicine, but these suggest even lower compliance rates than in human hospitals.[2,24] In a study of Anderson et al. an overall compliance rate of 14% was reported.[25] Wright et al. reported rates of 48% in small animal veterinarians for hand hygiene in between patients, and 18 % in equine and large animal veterinarians.[11]
Various actions to improve adherence to these protocols or hand hygiene in general have been proposed. Some are very effective, for example the 4-year hospital wide program in Geneva by Pittet et al., while others were not at all effective or only modestly effective.[25–27] The reason for success of the Geneva program was attributed to the introduction of antimicrobial handrubs (AMH's) among other things. Antimicrobial

handrubs (AMH's) have shown equal efficiency in reducing the counts of bacteria as washing with water and soap.[1] Advantages of AMH's are numerous and include easy placement and access, limited skin irritation after repeated use and less time needed to complete the cleaning process compared to soap and water.[2] However, introduction of AMH's alone is usually not sufficient to improve hand hygiene. In a report from Harbarth et al. an alcohol-based hand gel was introduced, but this lead to only moderate improvement in compliance rates, after a significant drop at the start of the intervention period.[27] In Geneva they not only introduced an AMH solution, but also provided educational posters at strategic places and, most importantly, encouraged senior staff to become involved in the program through meetings and public support of the program.[26]
As successful hand hygiene compliance adherence programmes have shown, improving hand hygiene should be multi-faceted, e.g. not only placing posters, but also providing health-care workers with easily accessible alcohol-dispensers, providing additional education, and creating motivation from within the institution.[24,28]
Outside the hospital environment, the importance of hand hygiene has also been recognised. In home and community settings, handwashing probably leads to a reduction in the number of gastrointestinal infections and to a lesser extent respiratory infections.[29–31] In addition, it is can reduce transmission of skin and wound pathogens, such as MRSA.[30]

The aims of this project were, firstly, to determine the loading of viable bacteria on hands of people working in veterinary clinical and non-clinical environments, and who have contact with small animals.
The second aim was to determine the types of bacteria and antimicrobial resistance patterns on hands of people working in these environments.
The last aim was to determine the acceptability of hand plates as a sampling method and assessment of the feedback system. It is expected that personal feedback with photographs of plates will lead to increased adherence to hand hygiene protocol and reduced plate counts.

2. Materials and methods

Sampling & feedback

The first round sampling in the non-clinical area took place on the following dates; 7th, 8th and 12th of December. In total 71 participants were sampled in a non-clinical setting (hereafter referred to as Leahurst group), at Leahurst campus, mostly in the main building's hallways and offices. Other people sampled in this group were in Leahurst House, the farm animal building, and the zoonosis centre. In the farm animal building, only people working in offices, with no clinical contact with animals, were recruited.
Sampling at the Small Animal Teaching Hospital (SATH) was carried out on 4th and 9th of January. A total of 59 participants were sampled, including a number of students, clinicians, nurses, clerical staff, and supporting staff. Both people in the clinical areas,

namely exam rooms, kennels, and non-clinical areas, i.e. reception desk area, computer area, were sampled. All will be counted among the clinical group (hereafter referred to as SATH group).

Participants were informed about the project by an information sheet and invited to take part in the study, after which a questionnaire was given (see Appendix I). Since participation was anonymous, subjects were given a study code, which they could find on the information sheet. After filling in the first questionnaire, participants were asked to give a hand sample by pressing all fingertips including the thumb of the dominant hand on a blood agar plate.

All participants were provided with feedback on their counts. This was presented as a photo of the blood agar plate after overnight incubation, total counts of colonies on the plate, and a graph showing the count distribution in different categories for the corresponding group. The count categories included in the graph were 0-50 colonies, 51-100, 101-200, 201-300 and >301 colonies. An arrow was drawn indicating the category of the participant and some general comments about hand washing and hand hygiene were given (see Appendix II for an example of a feedback form). Because participation was anonymous, with only participants knowing their study code, feedback had to be picked up personally. Feedback forms were stored in envelopes containing the study code. Also enclosed was a second questionnaire (see Appendix II) containing questions about impact of feedback perceived by the participants and their view on whether this would affect future hand washing behaviour. These questionnaires could be left in the same box. Encouraging people to pick up their feedback was done via word of mouth and news updates via a local site email newsletter.

Repeated sampling of hands was carried out on multiple dates in December (14 and 19th) and January (12th) for Leahurst group. For the SATH group a repeat sampling round took place on the 22nd February. During the second round, participants were asked to give another hand sample and provide their study code. In that way, results could be paired with their previous counts, in order to determine whether providing feedback influenced hand hygiene and thus loading of bacteria on hands of participants.

Processing samples

Plates were incubated overnight (±18 hours) at 37°C. Next day, colony forming units (CFU) were counted using a colony counter and all plates were photographed. If a plate contained >300 CFU, counting was stopped and >300 was written down, as further counting was impossible due to the colonies being almost confluent.

For plates with >80 CFU all colonies were harvested with a swab and suspended in 400µL of sterile water in a 1.5ml eppendorf (see figure 1). Swabs were pressed to the side of the 1.5ml eppendorf to release as many bacteria as possible. 200µL of the suspension was added to 300 µL of glycerol broth in eppendorfs, vortexed and stored at −80° C. The rest of the suspended sample (±200µL) was used for DNA extraction. DNA extraction was carried out using the Qjagen QiaAmp DNA mini kit (Kit Cat no. 51306), following a standard protocol (see Appendix III) with some slight alterations, because it was expected that this would lead to higher DNA yield, especially from Gram positive

bacteria which may be more prevalent on handplate samples.[32] All DNA samples were stored at −20°C and upon the end of the three month research period at -80°C.
For plates with <80 CFU, bacteria were not harvested, but two to four representative colonies picked off and plated out on a fresh blood agar plate (see figure 1). Plates were incubated aerobically overnight at 37°C (18-24h). Simple Gram staining was performed with fresh colonies and each characterised (cocci or rods, Gram positive or Gram negative). For the Gram positive cocci additional tests (catalase, coagulase and staphylase – see Appendix III) were carried out at a later date using fresh cultures. Antimicrobial susceptibility testing was carried out for Gram positive isolates that were catalase positive, coagulase positive and/or staphylase positive (see *Antimicrobial susceptibility testing*).
All plates were stored at 4°C after counting or use and binned after susceptibilities were noted and photo's taken.
For the second and third sampling round, plates were incubated aerobically at 37°C overnight (18-24h), before counting CFU next day. There was no formal feedback about this sampling round, but if people wanted to know the results, they were given their counts.

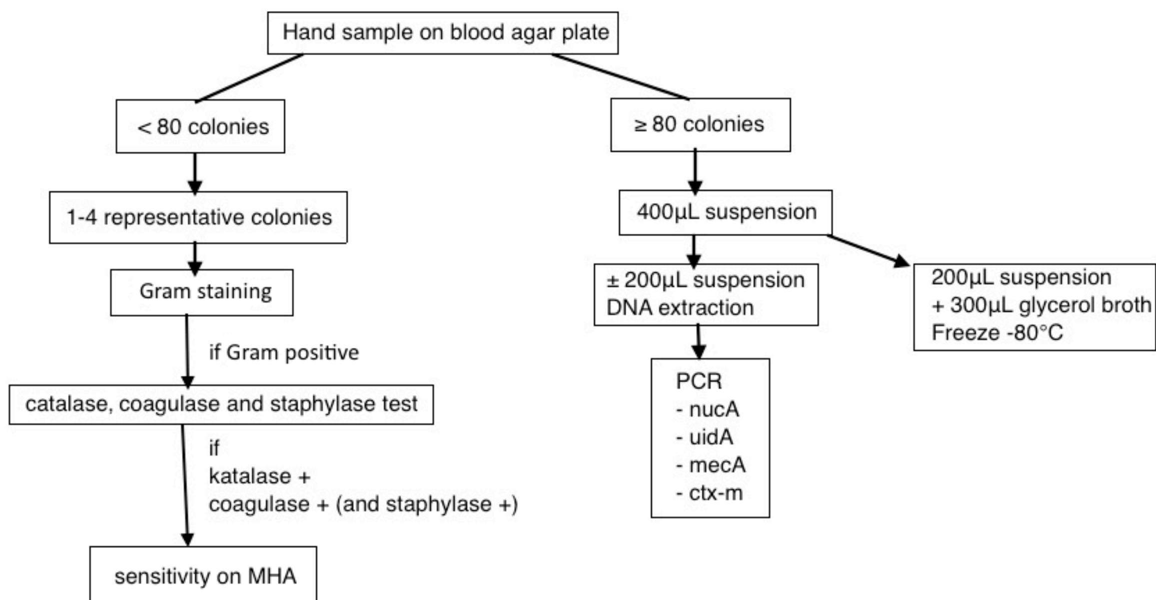

Figure 1. Overview of the protocol for processing of hand plates after counting. Two different ways of processing were carried out depending on the count.

PCR assays

DNA samples were screened by PCR for common bacteria, namely *S. aureus* and *E. coli*. For *S. aureus*, primers were used that target the *nuc* gene, which encodes information for a thermostable nuclease (TNase) enzyme, and which is both specific and sensitive for *S. aureus*.[33] For *E. coli*, *uidA* gene was used for screening, which encodes for the β-D-glucuronidase and detects 97 to 100% of *E. coli* isolates.[34]
Likewise, PCR was used to screen for common resistance genes of staphylococci and extended-spectrum β-lactamase (ESBL) carrying *E. coli*. For staphylococci, the *mecA*

gene was used, which encodes information for the production of PBP 2a, a penicillin binding protein that is present in methicillin resistant *S. aureus* and coagulase negative staphylococci.[35] For *E. coli*, there are many different types of ESBLs, but in this project bla_{CTX-M} gene was used, since this is currently the prevalent ESBL type.[36,37] This gene contains information about a class of ESBLs, the cefotaximases (CTX-M), which exhibit greater activity against cefotaxime than ceftadizime.[36]

A 96 microwell plate was used and each well filled with 24 μL of the following mixture: 0.5 μl of each primer (100pmol/μl), 18.4μl water (ultrapure), and 4.6μl part of FIREPol® (5x Master Mix Ready to Load, with 12.5 mM $MgCl_2$, all PCR reagents were obtained from Solis Biodyne). 1 μL of DNA was used to make up a total of 25 μL per well.

The following settings and primers were used for the different PCR's.

Screening for *S. aureus* with *nuc* gene.[33]

- nuc1 GCGATTGATGGTGATACGGTT
- nuc2 AFCCAAGCCTTGACGAACTAAAGC

PCR conditions: 94°C – 4 min (94 – 60s; 55 – 60s; 72 – 60s x 37 cycles) 72 – 4 min

Product size 279bp.

Screening for *E. coli uidA* gene.[34]

- uidAF – CCAAAAGCCAGACACAGT
- uidAR – GCACAGCACATCAAAGAG

PCR conditions: 94°C – 4 min (94 – 60s; 58 – 60s; 72-60s x 25 cycles) 72 – 7 min

Product size 623 bp.

Screening for methicillin resistance *mecA* gene.[35]

- mecA F – TGGCTATCGTGTCACAATCG
- mecA R – CTGGAACTTGTTGAGCAGAG

PCR conditions: 94°C – 4 min (94 – 60s; 55 – 60s; 72 – 30s x 30 cycles) 72 – 4 min

Product size 310bp.

Screening for bla_{CTX-M} β-lactamase gene.[36]

- CTXMU F – ATGTGCAGYACCAGTAARGTKATGGC
- CTXMU R – TGGGTRAARTARGTSACCAGAAYCAGCGG

PCR conditions: 94°C – 5 min (94 – 60s; 58 – 60s; 72 – 60s x 30 cycles) 72 – 7 min

Product size 585bp

Amplicons were visualised by running in a 1% agarose gel containing Peq green DNA/RNA dye (Peqlab) in TAE x 1 (tris-acetate-EDTA) buffer at 120V (400mA) for ± 1 hour. UV light was used to visualise the amplicons in the gel. For each assay, these were compared to the 100-bp molecular size ladder (100 bp DNA Ladder Ready to Load, Solis Biodyne), a positive control and a negative control consisting of water.

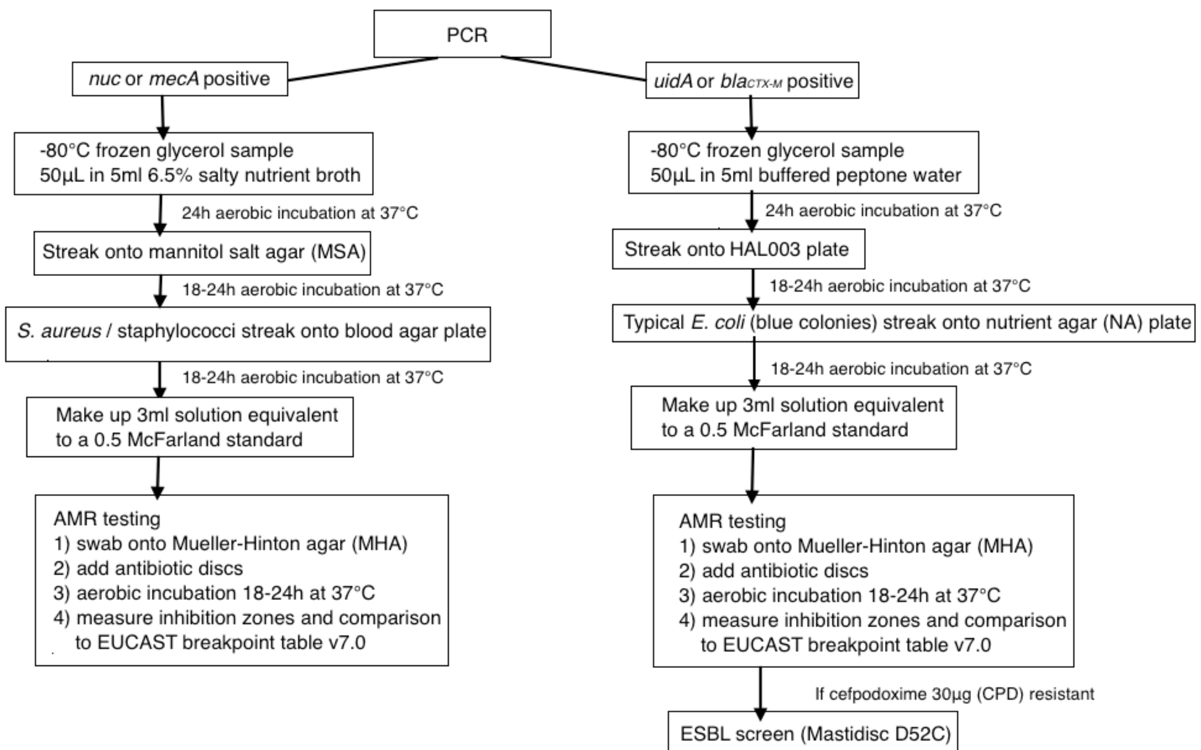

Figure 2. Protocol for samples after PCR testing.

For either *mecA* positive samples, *nuc* positive samples or both, deep frozen (-80°C) colony homogenate samples were removed from the freezer and 50µL of the glycerol/water suspension added to 3ml of a 6.5% NaCl nutrient broth (see figure 2). This was incubated aerobically for 24h at 37°C and then swabbed onto a Mannitol Salt Agar (MSA) plate using the method shown in figure 3. A cefoxitin disc (Mast discs, 30 µg) was put on the plate to check whether the *mecA* positive samples showed resistance phenotypically (see figure 3), but sensitivity testing was also conducted using the standardised EUCAST protocol (see susceptibilities) on isolated colonies.

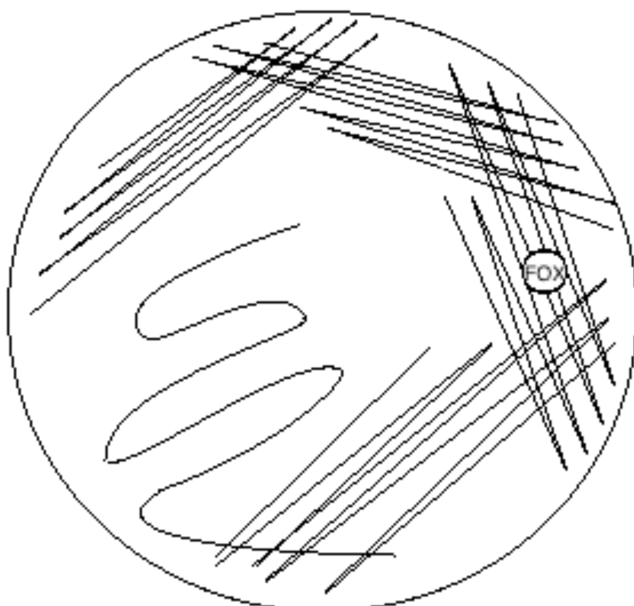

Figure 3. Streak pattern on the Mannitol Salt Agar (MSA) with Cefoxitin (FOX) disc 30μg

MSA plates were incubated aerobically at 37°C overnight, visually examined for characteristic bacteria and resistance to cefoxitin. *S. aureus* was recognised by the colour change to yellow due to fermentation of mannitol and type of colonies, greyish to white, medium size.[38] Of the *S. aureus* positive MSA plates, a colony was picked off and plated out on a blood agar plate that was aerobically incubated at 37°C 18-24h. *S. aureus* was further confirmed by staphylase test carried out on these samples (see Appendix III).
On the MSA plates with no colour change but recognisable staphylococci (lightgrey, small to medium size) the same process was carried out. These staphylococci were also tested for coagulase (see Appendix III). If coagulase positive but staphylase negative samples were presumptively *S. pseudintermedius* and susceptibility testing carried out (see figure 2).
For *uidA* positive samples, 50μL of frozen colonial homogenate was cultured for 24h in 5ml buffered peptone water at 37°C. Enriched broth was plated out on Harlequin plates (type 003 – Harlequin Tryptone Bile Glucuronide agar M-LAB[38]). Typical blue *E. coli* colonies were tested for oxidase (negative) and catalase (positive) (see Appendix III) and subsequently put on nutrient agar (NA plate) for susceptibility testing next day (see figure 3).

Antimicrobial susceptibility testing

For staphylococci isolates, 2 to 3 colonies were picked off the fresh culture on blood agar plate and, in a 5ml sample container, suspended in 3ml sterile water to give an inoculum equivalent to a 0.5 McFarland standard. With a swab and rotary plater the inoculum plated out on a Mueller-Hinton agar (MHA) plate and antibiotic discs added.
Susceptibilities were determined using the EUCAST standardised disk diffusion method and comparison of inhibition zones to the EUCAST breakpoint table[39] (see figure 2). For staphylococci including *S. aureus* and *S. pseudintermedius,* seven different antibiotic discs (Mast Group) were used: cefoxitin 30 μg (FOX30), ciprofloxacin 5 μg (CIP5), gentamicin 10 μg (GM10), erythromycin 15 μg (E15), tetracycline 30 μg (T30), trimethoprim 1.25 μg/ sulfamethoxazole 23.75 μg (TS25), clindamycin 2 μg (CD2). After 24h aerobic incubation at 37°C inhibition zones were measured against a dark background. If there was too little growth, plates were incubated for another 6 hours.
For *E. coli* isolates the same protocol was used, the only difference being that the fresh culture was taken from a nutrient agar (NA) plate and different antibiotic discs were used: amoxicillin 20μg / clavulanic acid 10μg (AUG30), trimethoprim 1.25 μg/ sulfamethoxazole 23.75 μg (TS25), ciprofloxacin 5 μg (CIP5), tetracycline 30 μg (T30), ampicillin 10μg (AP10), gentamicin 10 μg (GM10), nalidixic acid 30μg (NA30), cefpodoxime 10μg (CPD10). Isolates resistant to CPD, which was used to screen for potential ESBL producers, were subjected to the combination double disk method to identify ESBL positive isolates, using the Mastidiscs™ D52C Extended Spectrum β Lactamase Set[40] with the following discs: 30μg of ceftazidime (CAZ), 30μg cefotaxime (CTX), 30μg of cefpodoxime (CPD), and each of these in combination with 10μg of

clavulanic acid (CV). ESBL production was confirmed if inhibition zone increased by 5mm or more for the antimicrobial agent in combination with CV versus the antimicrobial agent alone.

Statistical analysis

All statistical analyses and tests were carried out using SPSS Statistics 24. Level of significance was set at 0.05, confidence intervals (CI) at 95%.
For statistical testing of counts assumptions had to be made regarding the number of colonies for counts >300. Therefore, counts over 300 were set at 350 colonies.
For comparisons of continuous data between groups Mann Whitney U-test was used. For categorical data comparisons Kruskal-Wallis test was used. Kruskal-Wallis test was also used for within group comparisons, namely comparison in counts and time between different roles. Pearson Chi square was used to discover whether there were correlations between roles, farm animal contact, hospital visit or previous use of antibiotics and carriage of genes (*nuc*, mecA, uidA and $bla_{CTX\text{-}M}$) screened by PCR within groups. Wilcoxon signed rank test was used for pairwise comparison of the first and second count. Finally, correlation testing and regression analysis was carried out to determine correlation between time since last hand hygiene event (washing or application of alcohol rub) and colony count.

3. Results

A total of 71 people were sampled at Leahurst campus in non-clinical areas (referred to as Leahurst group). Within the SATH 59 subjects were sampled (referred to as SATH group). Response rates for the first questionnaire were 93.0% (66/71) for the Leahurst group and 98.3% (58/59) for the SATH group. Two additional participants in Leahurst group and one in the SATH group filled in part of the questionnaire. For the second questionnaire that was provided with the feedback form, a response rate of 46.5% (33/71) was seen in the Leahurst group. However, only two people (3.4%) returned the second questionnaire in the SATH group.
A second round of sampling yielded 38 (53.5%) hand samples for Leahurst group and 12 (20.3%) hand samples for SATH group. The Leahurst group was sampled a third time with 12 respondents. No third sampling attempt was made in the SATH as response rates were already low the second round.

Questionnaire results

Of the participants in the Leahurst group, 35.8% was male (24/67), 64.2% female (36/67), versus 22.0% (13/59) and 78.0% (46/59) in the SATH group, respectively (see also Table 1). Figure 4 shows the age distribution in both groups. In the Leahurst group most participants belonged in middle categories, 26-55. In SATH most respondents were in the 18-25 and 26-35 category (74.6%).

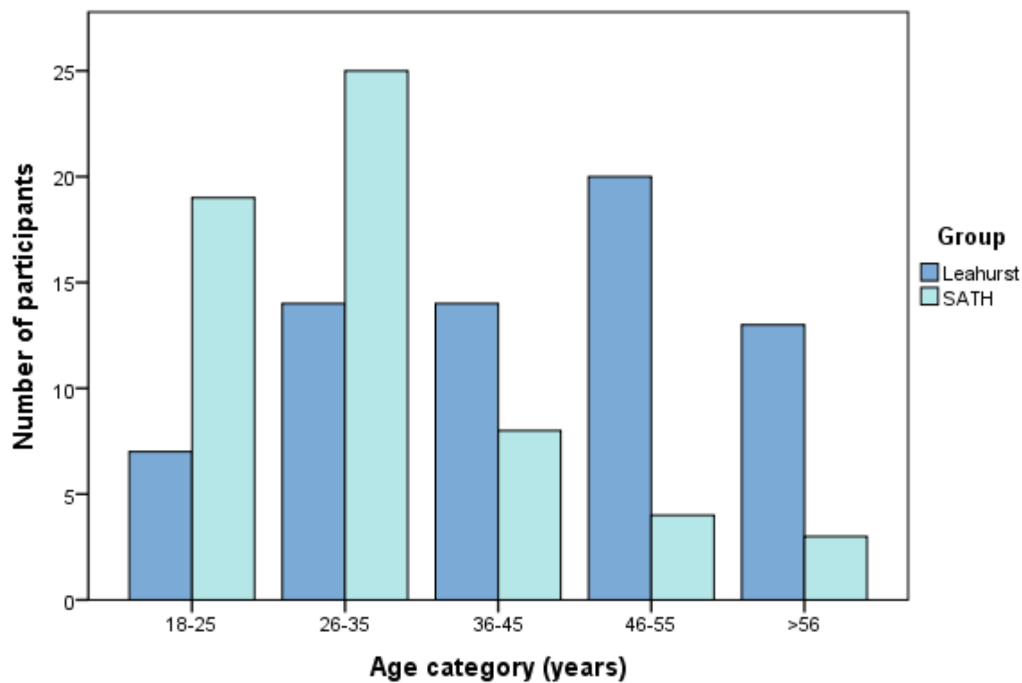

Figure 4. Age distribution across age categories in Leahurst and SATH group.

Within SATH 16 clinicians (27.1%) were sampled, 16 nurses (27.1%), 17 students (28.8%), 7 clerical staff (11.9%), and 3 auxiliary staff (8.5%). For Leahurst group there were no specific roles, as questionnaires were aimed at people in clinical environments. Pet possession was more common in the SATH group: 81.3% in this group had pets, versus 52.1% in the Leahurst group (see table 1 for an overview of questionnaire results).

		Group			
		Leahurst		SATH	
		N	%	N	%
Gender	Female	43	64.2%	46	78.0%
	Male	24	35.8%	13	22.0%
Age	18-25	7	10.3%	19	32.2%
	26-35	14	20.6%	25	42.4%
	36-45	14	20.6%	8	13.6%
	46-55	20	29.4%	4	6.8%
	>56	13	19.1%	3	5.1%
Pets at home	No	34	51.5%	11	18.6%
	Dog	14	21.2%	12	20.3%
	Cat	8	12.1%	13	22.0%
	Other	10	15.2%	23	39.0%
Farm animal contact	No	54	81.8%	41	69.5%
	Yes	12	18.2%	18	30.5%
Hospital visit (last month)	No	47	71.2%	49	83.1%

	Yes	19	28.8%	10	16.9%
Antibiotics in past month	No	61	92.4%	54	91.5%
	Yes	5	7.6%	5	8.5%

Table 1. Results from the first questionnaire from both groups. Leahurst is the non-clinical group, whereas people in SATH work in a clinical environment.

With regard to hand washing practice, all participants indicated they washed their hands after visiting the toilet (100%). Fewer people in Leahurst (47.7%) than in the SATH group (74.1%) indicated they washed their hands after handling a pet (see table 2). 53.4% used sanitiser more often than hand washing. In the Leahurst group the use of hand sanitiser was very uncommon (1.5%).

		Group			
		Leahurst		SATH	
		N	%	N	%
I wash my hands after I visit the toilet	No	0	0.0%	0	0.0%
	Yes	65	100.0%	58	100.0%
I wash my hands before eating	No	20	30.8%	13	22.4%
	Yes	45	69.2%	45	77.6%
I wash my hands after handling an animal or pet	No	34	52.3%	15	25.9%
	Yes	31	47.7%	43	74.1%
I wash my hands between each patient	No	NA	NA	21	36.2%
	Yes	NA	NA	37	63.8%
I wash my hand when leaving a clinical area	No	NA	NA	30	51.7%
	Yes	NA	NA	28	48.3%
I use sanitiser more often than I wash my hands	No	60	92.3%	27	46.6%
	Yes	5	7.7%	31	53.4%
I use sanitiser between patients	No	NA	NA	25	43.1%
	Yes	NA	NA	33	56.9%
I use sanitiser between patients but wash my hands periodically	No	NA	NA	32	55.2%
	Yes	NA	NA	26	44.8%

Table 2. Overview of hand washing practice among staff at Leahurst (non-clinical) and SATH (clinical). Questions were aimed at people in a clinical environment.
NA = non applicable

Count results

Figure 5 gives an overview of the different count categories and number of people in each category for both SATH group and Leahurst group. In the Leahurst group, most participants, 24 out of 71 (33.8%), belonged to the highest count category, whereas in the SATH group most participants, 23 out of 59 (39.0%) belonged in the lowest (0-50 CFU) category (figure 5).

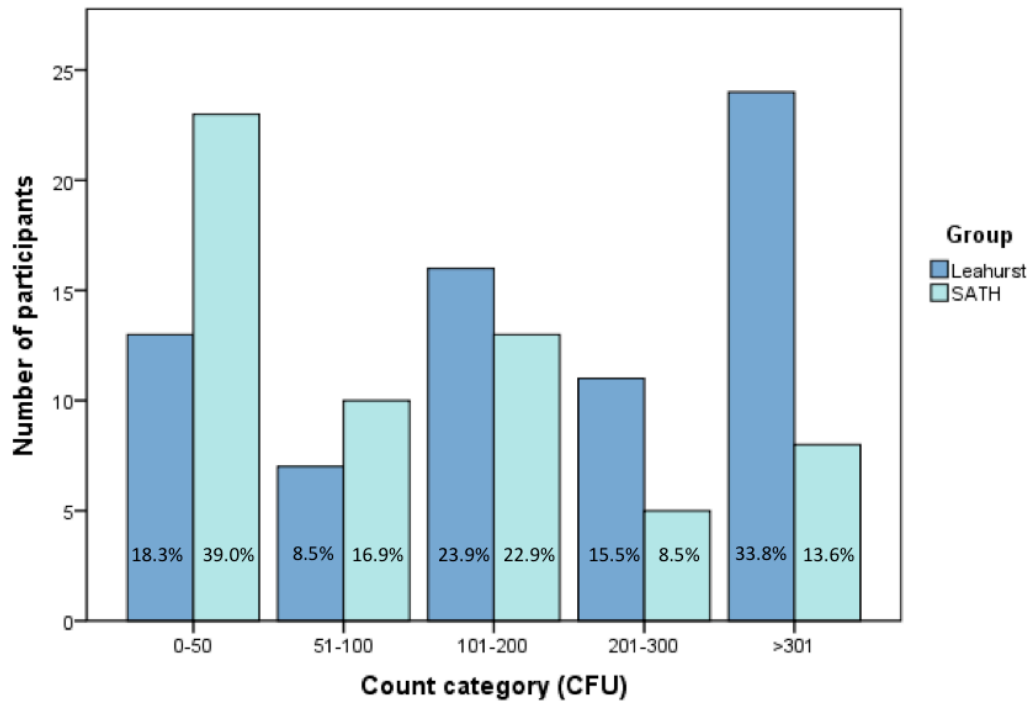

Figure 5. Overview of distribution of CFU count on hand plates from Leahurst and SATH group. The numbers in the bars display the percentage (%) of participants in each category per group.

38 Leahurst participants were sampled a second time. When compared to their previous count category, 10 participants (26.3%) belonged to a lower count category, 11 (28.9%) to a higher category and 17 (44.7%) belonged to the same count category. When looking at count number, 18 participants had a lower CFU count, 13 higher and 7 participants had the same CFU count (see table 5). In SATH 14 people were sampled a second time, but only 8 were able to provide their study code and their result paired with their previous result. Six participants belonged to a lower count category, one to a higher category, and one had the same count category.

Of the 12 participants in the Leahurst group that were sampled a third time, five participants (41.7%) had a lower CFU count, six people had a higher count (50.0%) and 1 had the same count (8.3%).

Group	Number of participants sampled a 2nd time N (%)	Lower count N (%)	Higher count N (%)	No change in count N (%)
Leahurst	38 (53.5)	18 (47.4)	13 (34.2)	7 (18.4)
SATH	14 (23.7)	6 (75.0)*	1 (12.5)*	1 (12.5)*
Total	52 (40.0)	24 (52.2)	14 (30.4)	8 (17.4)

*using 8 as total

Table 5. Overview of count in the second sampling round compared to the first.

In total 33 respondents at Leahurst main building filled in the second questionnaire. 18 (54.5%) said they were surprised by their own hand count, and that this would

influence their future hand hygiene behaviour. 8 respondents (44.4%) said the photograph of their plate had most impact. One person (5.6%) found the graph most influential and 9 respondents (50.0%) found both the graph and the count having the most impact. Of the 2 people from the SATH group who handed in the second questionnaire, both indicated they were surprised by their counts and found both the count and comparison having most impact.

PCR assay results

Of the DNA extracts from Leahurst group 18.2% (10/55) was positive for *nuc* gene, and 16.1% (5/31) of SATH DNA extracts (see figure 6 for an example of a PCR result). Positive screening for *mecA* was found in 34.5% of DNA extracts (19/55) at Leahurst and 35.5% (11/31) at SATH. In the SATH samples no *E. coli* was found by *uidA* gene screening, but in Leahurst group samples 7.3% (4/55) was positive. For $bla_{CTX\text{-}M}$ gene all SATH samples were negative, and in the Leahurst group 10.9% of the DNA extracts tested positive for $bla_{CTX\text{-}M}$ (see table 6).

Group	*nuc* gene N (%)	*mecA* gene N (%)	*uidA* gene N (%)	$bla_{CTX\text{-}M}$ N (%)
Leahurst	10 (18.2)	19 (34.5)	4 (7.3)	6 (10.9)
SATH	5 (16.1)	11 (35.5)	0 (0)	0 (0)
Total	15 (17.4)	30 (34.9)	4 (4.7)	6 (7.0)

Table 6. Overview of PCR results for the different genes tested.

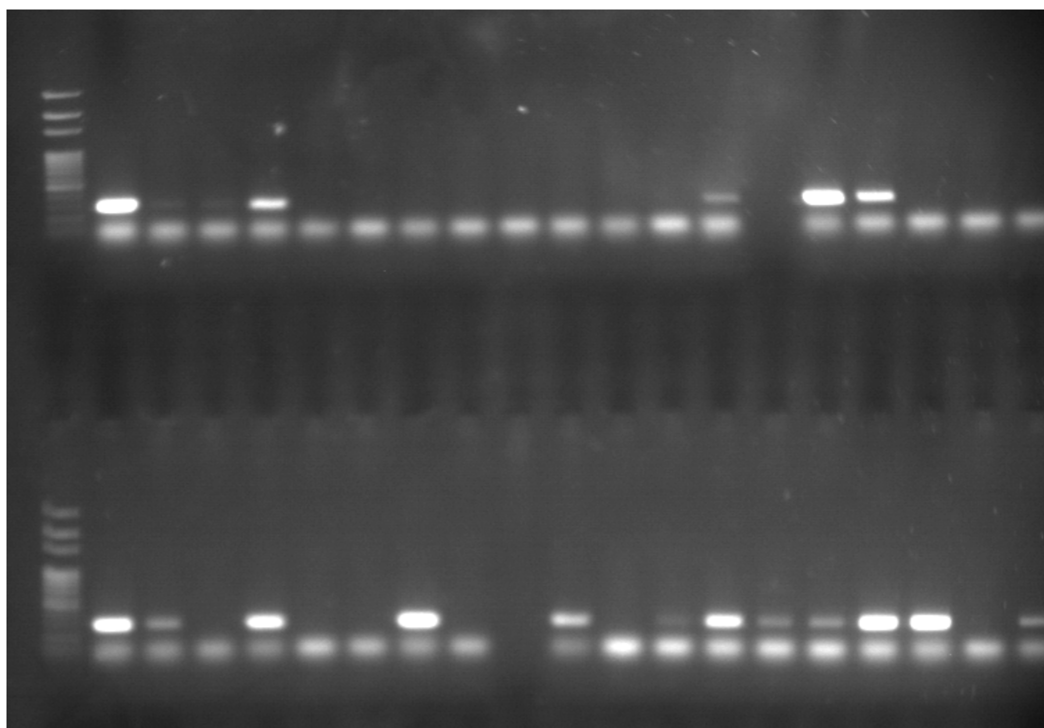

Figure 6. PCR for *mecA*; left to right; 1st 100bp ladder, 2nd positive control, 3rd and on DNA samples from SATH group and Leahurst group. After every row in the 96well plate, one well in the gel was left empty.

In all but one *nuc* gene positive samples *S. aureus* was isolated. In two other samples, that were *mecA* positive but *nuc* negative on PCR screen, *S. aureus* was isolated as well. None of the staphylococci isolated from *mecA* positive samples, showed resistance to cefoxitin on MSA plate screen phenotypically.

E. coli was isolated from 4 deep-frozen samples that were positive on the *uidA* screen. All samples were from participants in the Leahurst group that were sampled in the Leahurst main building. In addition two samples positive for bla_{CTX-M}, but negative for *uidA* were screened for colonies, but no *E. coli* was isolated. These samples were also from participants in the Leahurst group.

Individual isolates results

In total, 16 hand samples from Leahurst group and 28 from the SATH group had counts <80 CFU and colonies were individually picked off and further characterised (see figure 1). Most often isolated were coagulase negative staphylococci (CNS), found in 100% of samples. In addition, *S. aureus* was found on 2/16 (12.5%) hand samples at Leahurst and 3/28 (10.7%) hand samples from SATH. *S. pseudintermedius* was not isolated in the Leahurst group, but was present in 5/28 (17.8%) samples from SATH (see table 7). Other bacteria were not further characterised other than Gram staining and catalase testing.

	Coagulase negative staphylococci (CNS) N (%)	Coagulase positive staphylococci N (%)		Gram negative rods N (%)	Gram positive rods N (%)
		S. aureus	*S. pseudintermedius*		
Leahurst	16 (100.0)	2 (12.5)	0 (0.0)	0 (0.0)	2 (12.5)
SATH	28 (100.0	3 (10.7)	5 (17.8)	6 (21.4)	7 (25.0)
Total	42 (100.0)	5 (11.4	5 (11.4)	6 (13.6)	9 (20.5)

Table 7. Overview of different bacteria isolated from hand samples with counts <80, both number of hand samples (N) and percentage (%) in both groups.

Combined results

In total *S. aureus* was isolated from 13 (18.3%) hands of Leahurst participants and 8 hands (13.6%) of SATH participants. *S. pseudintermedius* was not isolated in Leahurst group but present on hands of 7 (11.3%) of SATH participants. Overall carriage rate of *S. aureus* was 16% and *S. pseudintermedius* 5.3%.

Antimicrobial susceptibility testing results

Antimicrobial resistance (AMR) testing was carried out on all coagulase positive staphylococci isolates from hand plates with count <80 and on staphylococci isolated from deep-frozen samples that were either *mecA* or *nuc* positive samples during PCR screening.

25 staphylococci isolates were tested from Leahurst hand samples, 23 from deep-frozen samples and 2 from hand plates with count <80. 12 isolates were coagulase negative staphylococci (CNS) and 13 *S. aureus.* Resistance to ciprofloxacin was present in one *S.*

aureus isolate. There was no resistance to gentamicin or cefoxitin, but resistance to tetrycycline was present in two CNS isolates and two *S. aureus* isolates. One CNS isolate was resistant to trimethoprim/ sulfamethoxazole and five CNS isolates were resistant to erythromycin. One *S. aureus* isolate was also resistant to erythromycin (see table 8).
23 staphylococci isolates from SATH were tested, 15 from deep-frozen samples and 8 from hand plates with count <80. 15 isolates were coagulase positive, eight were *S. aureus* and seven were *S. pseudintermedius.* Resistance to ciprofloxacin was present in three CNS isolates and two *S. aureus* isolates. Two CNS isolates were resistant to gentamicin, three to cefoxitin, and four to tetracycline (see table 8). In addition 3 *S. pseudintermedius* were resistant to tetracylin. Four CNS isolates were resistant to erythromycin, and one *S. pseudintermedius* and one *S. aureus*. The same was true for clindamycin, although these were not always the same isolates. Combined resistance against both erythromycin and clindamycin was present in one *S. pseudintermedius* and four CNS isolates.

			CIP5	GM10	FOX30	T30	TS25	E15	CD2	Total N isolates
Leahurst	A	*S. aureus*	0	0	0	0	0	0	0	2
		S. pseud.	NA	NA	NA	NA	NA	NA	NA	0
	B	CNS	0	0	0	2	1	5	1	12
		S.aureus	1	0	0	2	0	1	1	11
		S. pseud.	NA	NA	NA	NA	NA	NA	NA	0
SATH	A	*S. aureus*	1	0	0	0	0	0	0	3
		S. pseud.	0	0	0	3	0	1	1	5
	B	CNS	3	2	3	4	2	4	4	8
		S.aureus	1	0	0	0	0	1	1	5
		S. pseud.	0	0	0	0	0	0	0	2
Total		CNS	3	2	3	6	3	9	5	20
		S. aureus	3	0	0	2	0	2	2	21
		S. pseud.	0	0	0	3	0	1	1	7

Table 8. Overview of number of colonies that showed resistance against one or more of the 7 antibiotic discs used. The last column shows the total number of isolates tested. NA = non applicable (because *S. pseudintermedius* was not isolated from samples in Leahurst group).
A: coagulase positive isolates from plates with CFU count <80
B: staphylococci isolated from deep-frozen samples that were either *mecA* or *nuc* positive
Abbreviations:

- CIP5 – ciprofloxacin 5µg
- GM10 – gentamicin 10µg
- FOX30 – cefoxitin 30µg
- T30 – tetracycline 30 µg
- TS25 – trimethoprim/sulfamethoxazole
- E15 – erythromycin 15 µg
- CD2 – clindamycin 2 µg
- NI – not isolated

Apart from staphylococci, *E. coli* isolates were also tested for presence of AMR and an ESBL screen was carried out with cefpodoxime CPD) 10 µg. All four *E. coli* isolates (100%) were resistant to CPD and on subsequent testing via ESBL double disk test, were

found to be carriers of ESBL. In addition all isolates were resistant to trimethoprim/sulfamethoxazole, tetracylcine, ampicillin and gentamicin. One isolate was resistant to both ciprofloxacin and nalidixic acid as well. For the other three isolates sensitivity to nalidixic acid could not be determined, because no breakpoint was available. All isolates were sensitive to amoxicillin and clavulanic acid.

Statistical analysis

Average hand counts differed between SATH and Leahurst groups. Average count at Leahurst was 201 CFU (95% CI 172 – 231) versus 123 CFU (95% CI 94 – 153) in the SATH group (see table 9). Difference in count was statistically significant ($p<0.0005$). Median count category was 101-200 for Leahurst group and 51-100 for the SATH group (see table 4 and figure 5).

Group	Number of participants	Mean count	Median	Std. Deviation	Std. Error of Mean	% of Total Sum
Leahurst	71	201.8	196.0	124.5	14.8	65.7%
SATH	59	123.3	83.0	113.1	14.7	34.3%
Total	130	165.4	132.0	124.3	10.9	100.0%

Table 9. Comparison of counts between Leahurst and SATH.

There was a significant age difference between the two groups ($p<0.0005$). At Leahurst respondents were older, median 36-45 versus 26-35 in SATH. In addition, mean time since the last hand hygiene event differed ($p<0.0005$) between the two groups. The average time since participants last washed their hands was 53 (95% CI 34 – 71) minutes in the SATH group versus 87 (95% CI 71 – 104) minutes in the Leahurst group. Overall the average time was 71 minutes (95% CI 58 – 83).

There were no significant differences between groups regarding contact with farm animals, hospital visits, or antibiotic use in the past month. Comparison of roles was not possible between groups, as the roles were only applicable for people in the SATH group and could not be extrapolated to people in a non-clinical environment.

Within the SATH group no significant difference in CFU count was found between age groups, gender or role. However, there was a significant difference in time since last hand hygiene event between different roles ($p=0.003$), with a mean average time of 48 minutes for clinicians, 21 minutes for nurses, 50 minutes for students, 19 minutes for auxiliary staff and 160 minutes for clerical staff. In Leahurst group, roles were not specific enough to test for differences, as questionnaires were aimed at people working in a clinical setting.

There were no correlations between farm animal contact, hospital visit, or previous antibiotic use and carriage of *mecA*, *nuc*, *uidA*, or $bla_{CTX\text{-}M}$ genes by bacteria on hands.

Average CFU count in the Leahurst group was lower in the second and third round compared to the first, 187 CFU (95% CI 147 – 228) in the second round and 183 CFU (95% CI 133 – 234) in the third, but this difference was not statistically significant ($p=0.868$). In contrast, average CFU count in SATH group was higher during the second

round, 161 CFU (95% CI 83 – 239). Whether this was a significant difference could not be tested, as data could not be paired due to the fact that most respondents did not remember their study code.

There was a moderate positive correlation between time since last hand hygiene event and count in the SATH group, which was statistically significant (P=0.0004) (see table 10). A simple linear regression was calculated to predict count based on time since last hand and hygiene event. A significant regression equation was found (F(1,56)=10.048, p=0.002), with an R^2 of 0.152. The scatterplot in figure 7 gives an overview of the relationship between and count.

			Count	Time
Spearman's rho	Count	Correlation Coefficient	1.000	.425**
		Sig. (1-tailed)	.	.000
		N	59	58
	Time	Correlation Coefficient	.425**	1.000
		Sig. (1-tailed)	.000	.
		N	58	58

**. Correlation is significant at the 0.01 level (1-tailed).

Table 10. Overview of correlation between time since last hand hygiene event and colony count using Spearman's rho test.

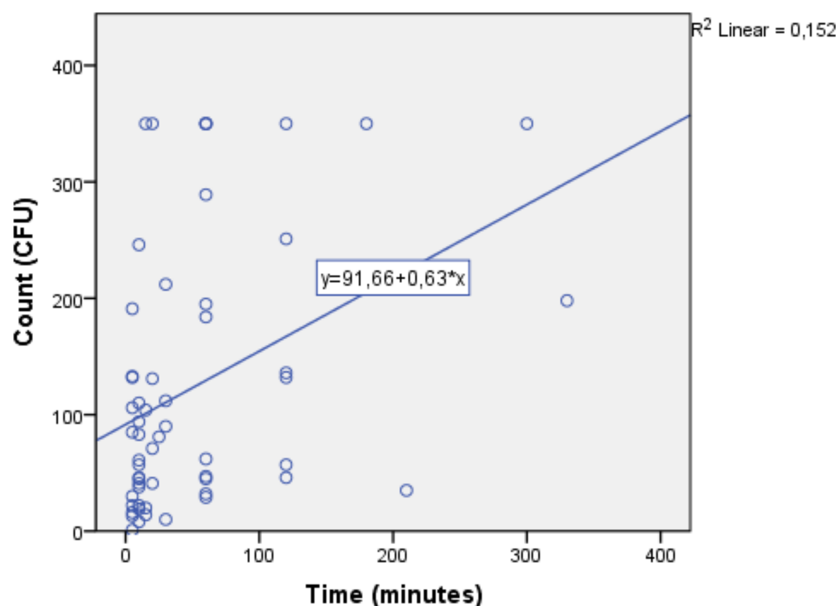

Figure 7. Scatterplot showing the relation between time since last hand hygiene event (x-axis) and count (y-axis).

4. Discussion

In this project the first aim was to determine bacterial loading on hand of participants in both clinical and non-clinical environments. A significant difference was found in bacterial counts between the clinical and non-clinical group ($p<0.0005$). Previous studies have also compared bacterial loading on hands, before or after patient contact[32,41,42], between different hand hygiene methods or agents[43–45] or to the assess effectiveness of an intervention program[27,46,47]. However, few determined the bacterial loading on hands of people from a veterinary profession, outside a hospital environment, or made comparisons between a hospital and non-hospital environment. Pittet et al. determined the bacterial loading on hands of hospital staff, also using the fingertip method.[32] They found lower average loading of 100 CFU, compared to our average of 123 CFU in the SATH group. In another studies, the average count of health care workers was 85 CFU[44], and 81 CFU[43] before hand washing, but they did not provide information about time since last hand hygiene event. All the studies mentioned measured hand hygiene in a clinical environment, where there is usually some form of education about the importance of hand hygiene.[1] The same is true for the SATH, where both clinicians, nurses and other staff get regular updates about the importance of hand hygiene and results of environmental sampling that is carried out regularly within the hospital. In addition, before entering the clinic and working with animals, new staff and students are given lectures about hand hygiene. These educational campaigns and measures help to ensure good hand hygiene compliance.[2] This might explain why lower counts were found in the SATH group compared to the Leahurst group, where no such measures are present and HAI's are not an issue. In addition, the difference in time since last hygiene event between both groups illustrate that hand washing is much less frequent in non-clinical environment. This is supported by the fact that within the SATH group, clerical staff, which are not strictly clinical, also had the longest time since handwashing, compared to other roles. Still, average time since last hand hygiene event was quite high in the SATH group, when compared to the study by Pittet et al., 53 minutes versus 5 minutes.[32] This suggests that compliance to hand hygiene protocols may be improved, as this leads to shorter time interval between hand hygiene events.[26]

The second aim of this project was to determine the types of bacteria and presence of antimicrobial resistance among bacteria on hands. As *S. epidermidis* is the most common CNS in people, it is not surprising that coagulase negative staphylococci were most often isolated.[48] *S. aureus* was present on 16.0% of hands of participants, 13.6% in SATH and 18.3% in Leahurst. This corresponds to prevalence found in an older study by Cespedes et al., who reported presence of *S. aureus* on 18.1% of hands of non-medical personnel and 10.2% of medical personnel in a hospital[49], but is lower than the national prevalence in the UK of 25.8%.[17] The national prevalence was measured using nasal swabs[17], however, not hand samples and carriage rates on hands may different from nasal carriage rates.[50,51] Furthermore, prevalence differs between communities, sex, and age groups.[52] *S. pseudintermedius* was only isolated from staff in the SATH. This was unsurprising, as *S. pseudintermedius* rarely colonises humans, but might be transiently

present as a result of treating and handling dogs or cats, as happens in a veterinary hospital.[19] Carriage rates from 11% to 69% in dogs have been reported, so S. *pesudintermedius* is frequently present in dogs.[15,53,54] Carriage rates in humans vary; in pet owners a carriage rate of 4.1% has been reported by Hanselman et al.[54] Paul et al. reported a nasal carriage rate of MRSP 3.9% in small animal vets, but did not find evidence for methicillin sensitive *Staphylococcus pseudintermedius* (MSSP) nasal carriage.[14] In contrast, our study found a MSSP hand carriage rate of 5.3% among veterinary staff. This difference may be due to the type of samples, nasal swabs versus hand samples. In addition, transient carriage cannot be excluded in our study as typing of bacteria was only carried out at one time point and not necessarily after hand washing, which may protect against *S. pseudintermedius* carriage.[54]

The overall carriage rate of *E. coli* was 4.7%, based on the PCR results. This is similar to carriage rate found in commuters in the UK (4.5%)[55] or doctors (4.5%)[42], and health care workers (2%)[44]. *E. coli* is a transient coloniser of skin, and an indicator of faecal contamination, which may be the result of inadequate hand hygiene, environmental contamination or petting of animals.[10,55] Therefore, it is surprising that in none of the DNA extracts from SATH *E. coli* was identified. On the other hand, Gram negative rods were isolated in SATH group, so presence of *E. coli* or *Enterobacteriacea* cannot be excluded in this group. Interestingly, all four *E. coli* isolates were carriers of ESBL and multi-drug resistant. ESBL carriage is becoming more frequent in *E. coli* of people, but also of pets,[56] and, as opportunistic pathogen, *E. coli* frequently causes infection.[57] Although the exact origin of contamination with *E. coli* is unknown, the level of resistance is worrying, especially since the ESBL *E. coli* were isolated from hands of people in the non-clinical group and all four isolates were multi-drug resistant. Several participants in the Leahurst group indicated they had been in contact with forms that came from farms or students who had been on farms, which might explain the faecal contamination on hand of these participants. Inadequate hand hygiene measures on leaving the laboratory where clinical samples are being processed might be another explanation for the presence of these MDR *E. coli*. Unfortunately, it could not be determined from the questionnaire whether the isolates came from lab workers. Two other samples that were positive on the *bla*-$_{CTX\text{-}M}$ screen were not further determined, but could have been other types bacteria that may also carry CTX-M-type ESBLs, such as *Pseudomonas* or *Klebsiella pneumoniae*.[58] Again, these samples were from the Leahurst group.

MecA screening was positive in 34.5% of PCR samples in the Leahurst group and 35.5% of samples in SATH group. In our study there was little difference observed between the two groups, but a study by Klingenberg et al. found a *mecA* positive rate of 45% on hands of medical personnel versus a rate of 16% on hands of non-medical personnel.[59] An explanation for this disparity might be that in this study the group labelled as non-clinical also had participants working with clinical samples, e.g. in laboratories, and vice versa, e.g. clerical staff sampled in SATH. Although sampling of staff at Leahurst was not done in laboratories and hand washing is compulsory after leaving the lab, it cannot be ruled out that this influenced carriage rate of *mecA*.

Despite frequent *mecA* gene carriage, no MRSA was isolated from hand samples. Considering the community carriage rate of 0.4% in the UK[17], this was to be expected. On the other hand, higher carriage rates of MRSA have been reported in veterinary personnel, ranging from 1.6% to 17.9%.[14,20,60,61] Apart from coagulase positive staphylococci, CNS are also frequent carriers of *mecA*[62], but only in three isolates resistance against cefoxitin was observed. An explanation for this fact may be that our method was not specific for the isolation of methicillin resistant coagulase negative staphylococci.
With regard to other resistance patterns, resistance to ciprofloxacin was more common in SATH *S. aureus* isolates (25.0%) than Leahurst isolates (9.1%), but there was no difference between groups for other antibiotics. Antibiotic resistance to tetracycline in *S. pseudintermedius* was common (43%), which is a little higher than observed in dog population (30%).[53] CNS were more often resistant to the antibiotics tested than coagulase positive staphylococci. Especially in the SATH isolates, resistance to all antibiotics tested was observed, ranging for 25% to 50%. Although there are not many reports about antibiotic resistance in CNS in non-clinical samples, these correspond to our results insofar as resistance was more common in hospital personnel than non-medical personnel.[63] As not many CNS isolates were tested for antimicrobial susceptibilities, prevalence of resistance should be interpreted with caution.

The third aim was to assess the acceptability of hand plates as a sampling method and to assess the effect of feedback on plate counts. Overall, response rate to the first sampling round and questionnaires was very good in both Leahurst group and SATH group, and hand plate sampling was easy and fast. No significant effect of feedback was observed, however, but the average count was a little lower in the second and third sampling round in the Leahurst group, but not in SATH. The effect of feedback in SATH cannot be determined, however, as response rates to the second questionnaires were very low. There may a number of reasons why this feedback method did not have an effect on plate counts. Firstly, plate counts might not be an adequate reflection of hand hygiene, unless measured at a certain activity or after a certain time. As a study by Devamani et al. showed, bacterial loading quickly increases after handwashing, and may return to baseline levels within one hour.[64] Secondly, effect of our intervention (feedback form and questionnaire) might be short-lived. A review on hand washing interventions by Naikoba et al. showed that in health care workers one-off interventions have limited effect and that a multimodal approach works better to change hand hygiene behaviour.[65] The same may be true for non-medical personnel.

There are a number of limitations to this study and methods. Firstly, two methods were chosen to process the hand plates, which may not have had the same sensitivity for isolation of certain bacteria. For example, it is possible that presence *S. aureus* or *S. pseudintermedius* in the hand samples with count <80 CFU was missed, because only a few colonies were subcultured. PCR testing may have been more sensitive for detection of *S. aureus*, but not for detection of other coagulase positive staphylococci, as no PCR

specific for *S. pseudintermedius* detection was carried out. Therefore, in samples >80 CFU *S. pseudintermedius* may have been underreported. Secondly, apart from staphylococci, types of bacteria in hand samples were not fully determined due to time restrictions. For example, it would have been interesting to see how many of the Gram negative rods were *E. coli* and susceptibility testing of these samples carried out. In addition, Gram positive rods could have been determined.

Thirdly, although the Leahurst group was classified as non-clinical and the SATH group as clinical, this may not be true for all participants. As people in Leahurst building mainly work in laboratories, it cannot be excluded that they have been in contact with animal samples. Furthermore, some of the staff sampled worked in post mortem rooms, with dead animals or animal tissues. To avoid contact with clinical samples, sampling was only carried out outside laboratories. In the SATH staff were sampled who only worked in clerical offices, with no animal contact. In addition, students and staff were sampled who were not at that time working in clinical areas, but behind computers.

Finally, response rate to the second questionnaire and feedback form was low in the SATH group. There may be a number of reasons why participants in the SATH did not pick up their feedback or filled in the second questionnaire. At SATH less time was available to explain about the project, especially regarding the feedback process, as sampling took place in areas, such as the anaesthesiology department, where people were also busy with patients. This might explain why so few participants in SATH came to claim their feedback and fill in the second questionnaire. Furthermore, for Leahurst group most people were sampled in their offices and several attempts were made at a second sampling round. In the SATH, only one additional sampling round was attempted due to time restrictions. Moreover, staff varies more in the SATH than in other Leahurst buildings. For example, students rotate between different department and nurses work at different departments on different days as well. This made the repeat sampling round challenging.

Conclusion

To sum up, current hand plate method is a simple and useful way to determine bacterial loading on hands of people and could easily be applied to test a larger sample population. Bacterial loading was lower in the clinical group compared to the non-clinical group, suggesting better hand hygiene practices in the veterinary clinical environment. PCR screening of samples instead of individual determination of isolates is timesaving, and a relatively easy way to gather information about types of bacteria and resistance patterns. On the other hand, in order to gather information about hand hygiene practices, questionnaires may be more valuable. Use of plate counts could be a supplemental source of information, provided that sampling takes place at a certain time point (e.g. before eating) instead of random. However, this may more difficult to achieve when sampling a large number of people.

Our feedback method did not have the expected effect of reduced plate counts. Further research is needed to determine whether it could have the expected effect if combined with other strategies to improve hand hygiene compliance.

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