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Environmental ESKAPE Organisms and Other Environmental Equine-Specific Indicator Pathogens—Are They Relevant in Equine Nosocomial Wound Infections?

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Abstract

Background: Multidrug-resistant organisms increasingly challenge infection prevention and control (IPC) in equine referral hospitals. **Objectives**: Assessment of selected environmental indicator pathogens in an equine teaching hospital and evaluation of their relevance for nosocomial wound infections. **Study design**: Observational study with repeated environmental sampling and retrospective evaluation of wound culture submissions. **Methods**: During three sampling periods (SPs), surface samples were collected across functional areas before and after routine cleaning/disinfection. Revision of the cleaning/disinfection protocols and environmental support staff feedback and training was performed between SP 1 and 2. Indicator pathogens were defined as an equine-adapted ESKAPEE-derived panel. Samples from surgical wounds were considered nosocomial if infection developed ≥3 days after hospitalisation. **Results**: Indicator pathogens were detected in all SPs, with the greatest frequency and diversity in SP 1. SP 1 yielded indicator pathogens in 25.7% of the 152 samples. After IPC optimisation, detection decreased in SP 2 to 11.2% of the 152 samples. In SP 3 with fewer samples ($n = 42$) sporadic recovery (7.1%) was observed. Contamination involved in particular high-touch surfaces and floors, and several pathogens persisted after cleaning/disinfection. Of 125 wounds, 38 met the criteria for nosocomial infection. Isolated from both, the environment and infected wounds were *Streptococcus equi* ssp. *zooepidemicus*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter cloacae*. Molecular typing was unavailable. **Conclusions**: Environmental equine-relevant indicator pathogens were detected across clinical areas. Some persisted after routine cleaning and/or were detected in nosocomial wound infections. Revision of cleaning/disinfection protocols with staff training was temporarily associated with reduced environmental contamination.

Keywords: environmental pathogens; nosocomial infection; hygiene

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1. Introduction

Nosocomial infections and the emergence of multidrug-resistant organisms (MDROs) represent an increasing challenge in equine referral hospitals. In these referral hospitals, the treatment of critically ill horses, frequent invasive procedures, as well as intensive handling of the patients and possibly shared equipment contribute to the promotion and distribution of resistant bacteria. The frequently necessary antibiotic use in equine intensive care patients, both medical and surgical, promotes the emergence and persistence of MDROs as well. Therefore, for equine hospital biosecurity, structured infection prevention programmes as well as targeted environmental microbiological surveillance are essential [1–13].

Surveillance strategies in human medicine frequently focus on the so-called ESKAPE indicator pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.)—pathogens that are commonly involved in nosocomial infections and that commonly show a high grade of antimicrobial resistance and therefore are able to "escape" antimicrobial therapy and infection control efforts [14]. Many of these organisms have also been increasingly identified in equine hospitals, both as causes of clinical disease and as colonisers of horses and the hospital environment [15,16]. Antimicrobial resistance surveillance data from equine clinical submissions in the United Kingdom have demonstrated the regular occurrence of resistant *Enterobacterales* and *staphylococci*, including organisms encompassed by the ESKAPE framework [13,17]. However, since beta-Lactamase-producing *E. coli* are commonly encountered in equine hospitals and are a well-recognised cause of equine hospital-acquired wound infections [6,9,18–21], the ESKAPE framework has been expanded to ESKAPEE [22].

In equine medicine, surgical site infections as well as infection of traumatic wounds are commonly caused by MDROs, often including pathogens of the ESKAPEE spectrum [18,19,21]. In addition, equine-adapted pathogens such as *Streptococcus equi* ssp. *zooepidemicus* are well-known causes of infections in equines. This organism may survive on environmental surfaces for variable periods depending on conditions, supporting the plausibility of indirect transmission routes and highlighting the importance of adequate biosecurity protocols in a clinical setting [23]. Therefore, these organisms typically causing hospital-acquired infections in horses should be included in an equine-clinic-specific indicator pathogen panel to optimise surveillance in these environments. Furthermore, equine patients may be colonised with indicator pathogens upon hospital admission or after antimicrobial therapy, which highlights the necessity of appropriate biosecurity protocols [18,21].

The combination of structured hygiene management and systematic evaluation of infection control protocols and resistance surveillance initiatives such as the Equine Veterinary Surveillance Network (EVSNET) provide the basis for a risk-based environmental surveillance specifically tailored to equine hospitals [3,17].

For this reason, this study aimed at the systematic assessment of the occurrence of selected environmental indicator pathogens as well as their occurrence in nosocomial wound infections in an equine hospital.

2. Material and Methods

2.1. Environmental Samples

During 3 sampling periods, impression smears of surfaces (DIN 10113-3) with agar plates provided by Laboklin GmbH & Co. KG (Bad Kissingen, Germany) were collected throughout the functional areas (surgery, treatment areas, stables) of the clinic. The samples were collected before and after cleaning and disinfection of the areas, regardless of macroscopic defilement. Samples collected before and after cleaning and disinfection were obtained from the same predefined sampling locations but not from the exact same spots. Adjacent surface areas were sampled to avoid potential interference caused by the removal of microorganisms during the initial contact-plate sampling.

Samples were collected in November 2021, February 2022 and June 2022. In June 2022, only sensitive surfaces were sampled, which led to a smaller sample size. Sensitive surfaces were defined as particularly relevant from a hygiene and infection prevention perspective due to their potential involvement in the transmission of microorganisms. In between November 2021 and February 2022, the cleaning and disinfection schedule was revised and optimised. Both the old and the new cleaning schedule are available in Supplementary File S1. Additionally, the environmental services staff responsible for

cleaning and disinfection received substantial feedback and further training. Additional surfaces were included in the cleaning schedule, whereas others were not repeatedly sampled. Furthermore, the contact time of the disinfectants was increased.

Sampling period 2 was performed immediately after the revision of the cleaning schedule; sampling period 3, with a reduced sample size, was performed four months after the intervention.

Due to the highly variable detection of indicator pathogens during the sampling periods and in the different functional areas, statistical analysis was omitted, and a descriptive approach chosen.

2.2. Wound Infections

To identify potentially hospital-acquired infections with indicator pathogens, bacteriological samples of infected wounds were evaluated. Exclusively included in this study were samples of surgically created wounds in which the skin had been intact and surgically incised since traumatic injuries or older wounds may have been infected with indicator pathogens previously. As postoperative nosocomial wound infections usually manifest on the third day of hospitalisation at the earliest [24], only samples of wound infections occurring three days after surgery at the earliest were considered.

Samples of infected wounds were collected routinely and as clinically indicated. For this study, samples collected between October 2021 and June 2022 were considered. Therefore, considered wound samples were collected up to one month prior to the first sampling period until the end of the third sampling period. Since only clinically indicated samples were collected, an animal ethics approval application was not required. Upon admission, horse owners consented to the use of their horses' anonymised data for scientific purposes.

2.3. Definition of Indicator Pathogens

In human medicine, nosocomial infections are often caused by the so-called ESKAPE pathogens, which comprise *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.

To adjust this selection of commonly multi-drug-resistant bacteria to an equine clinic, we added bacteria typically causing nosocomial (wound) infections in horses presented to our hospital. The equine-adapted ESKAPE-derived indicator pathogen panel was developed to investigate the occurrence of selected bacterial species of clinical and hygienic relevance in our equine hospital environment. The selection of microorganisms was based on their known or potential clinical relevance in horses, their role as opportunistic or wound-associated pathogens, and their relevance to environmental hygiene monitoring. Furthermore, the selection considered the clinical experience and pathogen spectrum encountered in our equine hospital and was designed as a targeted approach to monitoring selected bacterial species rather than as a comprehensive screening tool for all potential nosocomial pathogens. Accordingly, the detection of the selected microorganisms was performed at the species level, without specific characterisation of antimicrobial-resistance phenotypes. The panel focused on bacterial species considered relevant to equine wound infections and the hospital environment. Although *Salmonella enterica* is recognised as an important nosocomial pathogen in equine hospitals, it was not included as a specific target in the panel. Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) was not investigated separately, as *S. aureus* detection was performed at the species level without antimicrobial susceptibility testing. The final selection of bacterial indicator pathogens included *Streptococcus equi* ssp. *zooepidemicus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, *Enterococcus faecium*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

2.4. Bacteriological Examinations

After sample collection, the samples were shipped cooled and overnight to Laboklin GmbH & Co. KG (Bad Kissingen, Germany). Upon arrival at the laboratory, the agar plates were incubated at +37 °C in an incubator. The incubation period was typically 48 h, and the plates were examined at both 24 and 48 h of incubation.

For identification of the bacteria, Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS, Bruker, Bremen, Germany) was used.

3. Results

3.1. Detection of Indicator Pathogens in Surface Samples

Overall, indicator pathogens were detected in all sampling periods, with the highest number and diversity observed during sampling period 1. *Acinetobacter* spp. were the most frequently detected organisms in sampling period 1 (18/152), followed by *Staphylococcus* spp. (6/152), *Enterococcus faecium* (5/152), and *Escherichia coli* (5/152). Additionally, *Pseudomonas* spp. (4/152), *Staphylococcus aureus* (2/152), *Enterococcus* spp. (1/152), and *Acinetobacter baumannii* (1/152) were identified. In total, 39 (25.7%) samples tested positive for indicator pathogens (one sample 2 indicator pathogens, one sample 3 indicator pathogens). In sampling period 2, the overall detection frequency decreased, with *Klebsiella pneumoniae* emerging as the most frequently isolated pathogen (4/152), followed by *Acinetobacter* spp. (3/152) and *Enterococcus faecium* (3/152). Furthermore, *Staphylococcus aureus* (2/152), *Escherichia coli* (1/152), *Pseudomonas* spp. (1/152), *Enterococcus faecalis* (1/152), *Enterobacter cloacae* (1/152), and *Streptococcus equi* ssp. *zooepidemicus* (1/152) were detected. In total, 11.2% of the environmental samples tested positive for indicator pathogens in sampling period 2. In sampling period 3, fewer pathogens were identified overall in absolute numbers; however, 7.1% of the environmental samples tested positive for indicator pathogens. *Pseudomonas* spp. was the most frequently (2/42) isolated organism, followed by single detections of *Staphylococcus aureus* (1/42; Table 1).

Table 1. Overview of the detected indicator pathogens during the sampling periods (samples before and after cleaning and disinfection included).

Indicator Pathogen	1	2	3
Acinetobacter baumannii	1/152		
Acinetobacter spp.	18/152	3/152	
Enterobacter cloacae		1/152	
Enterococcus faecalis		1/152	
Enterococcus faecium	5/152	3/152	
Enterococcus spp.	1/152		
Escherichia coli	5/152	1/152	
Klebsiella pneumoniae		4/152	
Pseudomonas spp.	4/152	1/152	2/42
Staphylococcus aureus	2/152	2/152	1/42
Staphylococcus spp.	6/152		
Streptococcus equi spp. *zooepidemicus*		1/152	

1: sampling period 1; 2: sampling period 2; 3: sampling period 3.

Spatial distribution analysis showed that indicator pathogens were detected across multiple clinical areas, including isolation stables, intensive care stables, surgical theatres, induction and recovery areas, orthopaedic examination rooms, and medicine examination

areas. Frequently contaminated sites included floors, stable door handles, mangers, walls, and medical equipment such as keyboards, ultrasound probes, endoscopes, and radiology equipment. Notably, several pathogens were detected both before and after cleaning and disinfection procedures. For example, *Acinetobacter* spp. were identified after disinfection on stable door handles, floors, and mangers in intensive care and isolation stables, as well as before disinfection on floors, equipment, and door handles in surgical and examination areas. Similarly, *Enterococcus faecium*, *Escherichia coli*, and *Pseudomonas* spp. were detected both before and after cleaning, indicating incomplete removal through routine hygiene measures. *Staphylococcus aureus* was detected after disinfection on examination tables and surgical door handles and before disinfection on thermometers and stable door handles. *Klebsiella pneumoniae* was exclusively detected before cleaning on surgical theatre floors, surgical equipment, and cleaning devices, suggesting potential reservoirs in the surgical environment. In sampling period 1, three surfaces tested positive for the same indicator pathogen before and after, 20 before and 13 after cleaning and disinfection. In sampling periods 2 and 3, no indicator pathogens were detected on the same surfaces before and after cleaning and disinfection. In sampling period 2, 12 surfaces tested positive before and five after cleaning and disinfection in sampling period three, the indicator pathogens on three surfaces were recovered after cleaning and disinfection. Overall, high-touch surfaces and floor areas represented the most frequent sites of contamination, and the persistence of indicator pathogens after cleaning highlights the potential for ongoing environmental reservoirs and the optimisation of cleaning and disinfection regimens within the clinical setting (Table 2).

Table 2. Location and relation to cleaning and disinfection of the detected indicator pathogens.

Indicator Pathogen	Cleaning/ Disinfection Status	Sampling Site	1	2	3
Acinetobacter baumannii	Before	Manger (Isolation stable)	X		
	Before	Endotracheal tube	X		
	After	Endotracheal tube	X		
Acinetobacter spp.	Before	Floor 1 (Intensive care stable)		X	
	Before	Floor (Orthopaedic examination area)	X		
	Before	Floor 1 (Induction stable)	X		
	After	Floor 1 (Intensive care stable)	X		
	Before	Floor 1 (Intensive care stable)	X		
	Before	Floor 1 (Surgical theatre)	X		
	After	Floor 2 (Intensive care stable)	X		
	Before	Floor 2 (Intensive care stable)	X		
	Before	Floor 2 (Recovery stable)	X		
	Before	Hoof loops (Surgical area)		X	
	Before	Keyboard (Orthopaedic examination area)	X		
	After	Manger (Intensive care stable)	X		
	After	Stable door handle (Increased hygiene stable)		X	
	Before	Stable door handle (Intensive care stable)	X		
	After	Stable door handle (Isolation stable)	X		
	Before	Table (Surgical theatre)	X		
	Before	Table (Orthopaedic examination area)	X		
	Before	Ultrasound Probe (Orthopaedic examination area)	X		

Table 2. *Cont.*

Indicator Pathogen	Cleaning/ Disinfection Status	Sampling Site	1	2	3
Enterobacter cloacae	Before	Manger (Isolation stable)		X	
Enterococcus faecalis	Before	Keyboard (Orthopaedic examination area)		X	
Enterococcus faecium	Before	Computer mouse (Orthopaedic examination area)	X		
	After	Floor 2 (Isolation stable)		X	
	Before	Stable door handle (Increased hygiene stable)		X	
	After	Stable door handle (Intensive care stable)	X		
	After	Stable door handle (Medicine examination area)		X	
	After	Wall (Orthopaedic examination area)	X		
	Before	Wall (Orthopaedic examination area)	X		
	After	Wall 2 (Induction area, surgical area)	X		
Enterococcus spp.	After	Door handle (Medicine examination area)	X		
Escherichia coli	After	Floor 1 (Increased hygiene stable)	X		
	After	Floor 1 (Isolation stable)	X		
	Before	Floor 2 (Increased hygiene stable)	X		
	After	Floor 2 (Intensive care stable)	X		
	Before	Stable door handle (Intensive care stable)		X	
	Before	Wall 2 (Isolation stable)	X		
Klebsiella pneumoniae	Before	Clipper (Surgical area)		X	
	Before	Floor 1 (Surgical theatre)		X	
	Before	Pillow (Surgical theatre)		X	
	Before	Vacuum cleaner (Surgical area)		X	
Pseudomonas spp.	After	Ceiling supply units (Surgical theatre)			X
	Before	Cupboard handles (Orthopaedic examination area)	X		
	After	Floor 1 (Surgical theatre)			X
	After	Floor 2 (Recovery stable)	X		
	After	Floor 2 (Surgical theatre)		X	
	After	Stocks, hindside (Medicine examination area)	X		
	After	X-ray apron (Orthopaedic examination area)	X		
Staphylococcus aureus	After	Door handle (Surgical theatre)			X
	Before	Stable door handle (Isolation stable)		X	
	After	Stocks, hindside (Medicine examination area)		X	
	After	Table (Orthopaedic examination area)	X		
	Before	Thermometer (Medicine examination area)	X		
Staphylococcus spp.	Before	Footrest (Surgical theatre)	X		
	Before	Hoof loops (Surgical area)	X		
	Before	Lamp (Surgical theatre)	X		
	After	Manger (Intensive care stable)	X		
	Before	Wall 2 (Intensive care stable)	X		
	After	X-ray apron (Orthopaedic examination area)	X		

Table 2. *Cont.*

Indicator Pathogen	Cleaning/ Disinfection Status	Sampling Site	1	2	3
Streptococcus equi ssp. spp. *zooepidemicus*	Before	Endoscope (Medicine examination area)		X	

1: sampling period 1; 2: sampling period 2; 3: sampling period 3. X: Pathogen detected at the respective location and examination period.

3.2. Detection of Indicator Pathogens in Wound Infections

In total, 125 samples of infected wounds were submitted. Of these, 38 were classified as nosocomial infections with indicator pathogens (mixed infections occurred). Of these 125 samples, 38 were classified as nosocomial, as they either originated from previously clean wounds or were taken during a timely close follow-up visit due to a wound infection associated with the performed surgery. Some of the submitted samples were taken as a precaution, while other wounds already showed signs of infection.

In the nosocomial wound infections, most frequently identified were *Streptococcus equi* ssp. *zooepidemicus* ($n = 11$), followed by *Staphylococcus aureus* ($n = 8$), and *Escherichia coli* ($n = 8$). Less frequently detected was *Enterobacter cloacae* (four samples). Neither *Acinetobacter* spp. nor *Pseudomonas aeruginosa* were detected in any of the collected wound samples. *Streptococcus equi* spp. *zooepidemicus*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter cloacae* were detected in both wound and surface samples, but a direct epidemiological link between individual cases was not possible, as no molecular typing was performed (Table 3).

Table 3. Overview of detected indicator pathogens in the 38 wounds with nosocomial infection (one mixed infection).

Indicator Pathogen	Positive Samples (n)
Streptococcus equi ssp. spp. *zooepidemicus*	11
Staphylococcus aureus	8
Escherichia coli	8
Enterobacter cloacae	4
Enterococcus faecium	2
Pseudomonas aeruginosa	0
Acinetobacter baumannii	0

4. Discussion

This study evaluated environmental contamination with indicator pathogens in an equine teaching hospital across three sampling periods and assessed the impact of the revision of the cleaning and disinfection protocol and staff training after sampling period 1. The results demonstrate a progressive reduction in both the frequency and diversity of recovered pathogens over time, supporting the effectiveness of targeted infection prevention and control (IPC) interventions in the equine hospital environment. As antimicrobial susceptibility testing was not performed, no conclusions can be drawn regarding the antimicrobial resistance profiles of the detected isolates. The use of an equine-adapted ESKAPE-derived indicator pathogen panel was intended to assess the occurrence of selected bacterial species rather than to identify multidrug-resistant organisms.

During the first sampling period, multiple indicator organisms were detected across all tested functional areas of the clinic. Detected indicator pathogens included *Acinetobacter* spp., *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas* spp. and *Staphylococcus aureus*.

High-touch surfaces such as door handles, keyboards, and medical equipment were identified as contaminated; furthermore, indicator pathogens were recovered both before and after cleaning and disinfection procedures.

These findings are consistent with previous reports describing widespread environmental contamination with diverse indicator pathogens in both equine and small animal hospitals, including intensive care units, surgical theatres, and isolation areas [25–30]. The detection of organisms following cleaning indicates that the original cleaning and disinfection procedures were insufficient to consistently eliminate environmental contamination.

After revision of the cleaning and disinfection schedule, a clear reduction in environmental contamination was observed during the second sampling period. However, the indicator pathogens were still identified after cleaning and disinfection but in fewer areas than after sampling period 1. This emphasises the previously reviewed possibility of survival or persistence of nosocomial pathogens on surfaces for months if inappropriate cleaning and disinfection procedures are employed [31].

In sampling period 2, fewer sampling sites yielded indicator pathogens, and some organisms identified previously were no longer detected. By the third sampling period, contamination with indicator pathogens had decreased further in absolute numbers, with only sporadic recovery of organisms such as *Pseudomonas* spp. and *Staphylococcus aureus*, but in looking at the relative numbers, an increase in their detection was observed. Notably, pathogens such as *Acinetobacter baumannii* and *Klebsiella pneumoniae*, detected earlier in the study, were not identified in the final sampling period. However, this observation must be interpreted carefully, since fewer environmental samples were collected in this sampling period. This reduction suggests that the revised cleaning and disinfection protocol as well as the staff training contributed to the observed reduction in detected indicator pathogens. Although direct causality cannot be definitively established, the temporal association strongly suggests that the revised cleaning and disinfection measures as well as the staff training contributed to the observed reduction as shown in sampling periods 2 and 3. The relative increase in indicator pathogens in sampling period 3 highlights the need for repeated staff training and ongoing optimisation of the cleaning and disinfection schedules in equine clinical environments.

It is worrying that some indicator pathogens were only detected after cleaning and disinfection. On the one hand, this may be due to a slightly different location being sampled than the one tested before cleaning and disinfection, but it may also indicate that the bacteria were present in the cleaning utensils, just like *K. pneumoniae* on the vacuum cleaner in this study, or there was bacterial resistance to the disinfection agent. Therefore, the selection of appropriate disinfectants, as mentioned as crucial in literature [32], was performed.

Studies in human medicine have shown that approximately 50% of the environment surrounding patients and intensive care surfaces were satisfactorily cleaned at baseline. After employment of a targeted control method (fluorescent targeting method) and providing substantial feedback to environmental services personnel, approximately 80% of the surfaces were cleaned adequately [33,34]—an effect that was also seen in this study though different indicators (fluorescence vs. presence of indicator pathogens) for adequate cleaning were used.

In the present study, high-touch surfaces and frequently handled equipment represented important contamination points, particularly during the first sampling period. This highlights the need to prioritise such sites within cleaning protocols—not only in equine but also in human clinics, these frequently handled, easily forgotten contact points are often insufficiently cleaned [33].

Effective environmental hygiene requires not only appropriate disinfectant selection but also correct dilution, adequate contact time, thorough mechanical cleaning and consistent

adherence to the protocols [32]. Protocol revision and staff training likely improved one or more of these components in this study, which contributed to the reduction in detected indicator pathogens observed in later sampling periods.

Education and behavioural factors are also critical. Increased awareness among clinical and support staff may have improved compliance with hand hygiene, cleaning technique and biosecurity measures. For hand hygiene in human healthcare, for example, the World Health Organization recommends multimodal IPC strategies incorporating training, reminders and monitoring [35] since the combination of these strategies has proven to be more effective than protocol changes alone [36–38].

Despite encouraging results, some contamination persisted in surgical and examination areas during later sampling periods.

In addition to environmental contamination, several of the detected environmental indicator pathogens were also isolated from hospital-acquired wound infections, highlighting their clinical relevance and potential role in nosocomial transmission. The most frequently recovered organism from wound samples was *Streptococcus equi* ssp. *zooepidemicus* ($n = 11$), followed by *Staphylococcus aureus* and *Escherichia coli* (each $n = 8$), and *Enterobacter cloacae* ($n = 4$). The inclusion of horses with uncomplicated wound healing as a control group would have provided further insights into the occurrence of bacterial species in wounds without clinical signs of infection and helped to better understand the clinical relevance of the detected microorganisms. However, the findings of this study regarding bacteria involved in wound infection are consistent with the known epidemiology of equine wound infections, where streptococci, staphylococci and Gram-negative enteric organisms are common opportunistic pathogens [18,21,39,40]. The detection of *Staphylococcus aureus*, *Escherichia coli* and *enterococci* in both the hospital environment and clinical wound samples is of particular importance, as it suggests a potential link between environmental reservoirs and patient infections—however, horses may be colonised with these pathogens upon admission or during hospitalisation with and without antibiotic treatment [9,18,21,41].

Streptococcus equi ssp. *zooepidemicus*, the most frequently isolated wound pathogen in this study, is an opportunistic organism of the equine upper respiratory tract but is also an important opportunistic pathogen especially associated with respiratory disease [42–44].

Although it was only detected once in the environmental sampling, its high prevalence in wound infections suggests that endogenous flora and/or horse-to-horse transmission as well as adherence to a strict biosecurity protocol during bandage changes may also play a major role. Furthermore, it must be acknowledged that only a limited number of surfaces were sampled throughout the clinic, so that possible contamination with *Streptococcus equi* ssp. *zooepidemicus* might have been missed, which may have contributed to persistence and transmission within the hospital, particularly in high-risk areas.

Similarly, *Staphylococcus aureus*, isolated from both environmental samples and wounds, is a well-established cause of surgical-site infections in both humans, small animals, and horses [45–48]. Its ability to colonise skin, survive on dry surfaces and resist adverse environmental conditions enhances its transmission potential [49]. Environmental contamination with staphylococci therefore represents only one out of several significant risk factors for post-operative wound infections, particularly in surgical settings where invasive procedures compromise natural barriers [49].

The isolation of enteric organisms such as *Escherichia coli* and *Enterobacter cloacae* from wound infections further emphasises the importance of environmental hygiene, particularly in equine hospitals where faecal contamination is common since horses defaecate in their stables.

These organisms may be introduced into wounds directly from the patient's own microbiota or indirectly via contaminated surfaces, equipment or personnel. Their presence

in nosocomial wound infections highlights the need for strict adherence to cleaning, hand hygiene and aseptic techniques [5,15,50,51]. The use of culture-based methods and an incubation period of up to 48 h may have limited the detection of slow-growing or fastidious bacteria, which may have resulted in false-negative findings. Furthermore, the absence of molecular characterisation prevented a more detailed assessment of the genetic relatedness and potential transmission pathways of the bacterial isolates.

Importantly, some organisms detected in environmental samples during earlier sampling periods, such as *Acinetobacter baumannii*, were not isolated from wound infections in this study. This may reflect limited clinical transmission, effective infection prevention measures, or the opportunistic nature of these pathogens. Nevertheless, their presence in the hospital environment remains concerning due to their known ability to cause severe healthcare-associated infections [52–54].

Overall, the overlap between environmental and clinical isolates supports the hypothesis that the hospital environment may serve as a reservoir for opportunistic pathogens capable of causing wound infections. The reduction in environmental contamination observed after revision of the cleaning and disinfection protocol may therefore contribute not only to improved environmental hygiene but also to a reduced risk of nosocomial wound infections. These findings emphasise the importance of integrated infection prevention and control strategies addressing both environmental and patient-associated risk factors in equine hospitals.

5. Conclusions

This study shows that selected indicator pathogens, including equine-associated bacteria and members of the ESKAPEE group, can be found in the equine hospital environment and may play a role in nosocomial wound infections. Following improvements in cleaning and disinfection procedures and additional staff training, fewer bacterial detections were observed. However, some pathogens were still detected after cleaning and disinfection, highlighting the challenges of maintaining hygiene in a busy equine hospital. Although some bacterial species were found in both environmental and wound samples, a direct link between these findings could not be established. Overall, our results underline the importance of regular environmental monitoring, effective cleaning and disinfection practices, as well as continuous staff training. Further studies using molecular typing would help to better understand possible transmission routes between the hospital environment and equine patients.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/antibiotics15111091/s1, Supplementary File S1: Original and revised cleaning schedule.

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