

Microbiological characterization and antimicrobial resistance of two surfaces of Veterinarians' stethoscopes in Trujillo, Perú

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Abstract: The stethoscope is a medical instrument used daily for physical examinations, whose capacity as a fomite remains underexplored in veterinary medicine. This study aims to identify the main bacteria isolated from the stethoscopes of veterinarians in Trujillo (Peru) and to identify the factors associated with the presence of these pathogens through microbiological analysis and the application of a questionnaire. Fifty-nine stethoscopes were sampled using sterile swabs soaked in BHI broth (Brain Heart Infusion) and rubbed 4 times at different locations opposite the previous one. The surfaces sampled were chest pieces and ear tips. After being incubated for 24 hours at 37°C, the sample were cultured on blood agar, and after the bacterial morphology was identified, biochemical tests were performed. Results showed that 88% of stethoscopes were contaminated, yielding 100 isolates (8 genera). *Staphylococcus aureus* (35%) was the most frequently isolated species, followed by Coagulase-negative *Staphylococcus* (34%) and *Pseudomonas* spp. (18%). Notably, high levels of antimicrobial resistance were observed, with *S. aureus* exhibiting 94.3% resistance to penicillin and 80% to tetracycline. The chest piece was the most contaminated surface (95%; 56/59). A statistical association was found between disinfection practices and bacterial presence ($p<0.05$), with non-disinfection identified as a risk factor (OR=1.636; 95% CI=1.132-2.366). The above shows that veterinary stethoscopes, often contaminated with bacteria linked to nosocomial infections, require effective disinfection protocols and robust antimicrobial stewardship programs to mitigate the spread of multidrug-resistant bacteria, protect patient and user health, and preserve antibiotic efficacy in the long term.

Keywords: Antimicrobial Resistance; Veterinary Stethoscopes; Nosocomial Infections; fomites; *Staphylococcus aureus*.

1. Introduction

The stethoscope is a medical instrument used in different clinical areas, such as cardiology, pneumology, and gastroenterology; its correct use supports the veterinarian in performing an accurate physical examination of the patient, which, together with other diagnostic tools, helps to make a proper diagnosis and treatment (Pace, 2017).

Several authors have conducted studies to determine the prevalence of potentially pathogenic bacteria on the surface of stethoscopes in human hospitals, demonstrating that bacteria such as Coagulase-negative *Staphylococcus*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. are incident on this medical instrument, making the stethoscope a fomite for the spread of bacteria with pathogenic potential (Menacho et al., 2016; Charca, 2019; Bustamante, 2021). In the field of veterinary medicine, few studies have been conducted, of which a high incidence of the genre *Staphylococcus* spp. has been reported (Fujita et al., 2013; Souza et al., 2022; Kiraly et al., 2023). This high incidence of *Staphylococcus* spp. on inert surfaces in pet veterinary clinics, including stethoscopes, has prompted studies on the virulence potential of these bacteria. These studies have identified genes associated with biofilm formation, a characteristic that significantly enhances bacterial resilience and facilitates horizontal gene transfer. Consequently, the formation of biofilms enhances bacterial resistance to antibiotics, disinfectants, and the host immune response (Clutterbuck, 2007; Meroni et al., 2019; Chen et al., 2020; de Souto et al., 2024).

Likewise, several studies have demonstrated that these surfaces exhibit a high level of contamination, predominantly by methicillin-resistant *Staphylococcus* (MRS). Consequently, such surfaces can serve as reservoirs for the transmission of MRS, posing a risk not only to medical personnel but also to pet owners (Aklilu et al., 2012; de Souto et al., 2024; Leite et al., 2023; Perkins et al., 2020; Rojas et al., 2017). This bacterial contamination, which is not often considered, is associated with the indiscriminate use of the stethoscope, factors such as inefficient disinfection, shared use among staff, and the rotation between areas, which converts this medical instrument into a vector of bacteria with pathogenic potential. Therefore, it is essential to raise awareness of the role of the stethoscope as a vector of etiological agents (Ali et al., 2016).

It is important to consider this medical instrument as a vector for patient infections, given that the bacteria most frequently associated with nosocomial infections in veterinary medicine include *Staphylococcus aureus* and methicillin-resistant staphylococci (MRS), both of which have been reported to be highly prevalent on these surfaces (Peton and Le Loir, 2014; Stull and Weese, 2015).

Considering the virulence factors and antimicrobial resistance of bacteria on veterinary stethoscopes, their presence represents a notable risk to patients. These microorganisms are opportunistic, meaning that while they are often part of the host's natural microbiota and do not cause harm under normal circumstances, they can trigger severe infections in individuals with weakened immune systems. Additionally, veterinary staff are not exempt from this risk, as they may acquire these bacteria through contact with infected or colonized patients. This highlights the critical need for robust infection prevention practices (Madar and Baska, 2005; Walther et al., 2017).

It is also necessary to maintain a correct level of hygiene in this tool, trying to minimize the microbiological contamination to a level that does not affect the patient's health as the patient's skin comes into direct contact with this instrument and is exposed to different pathogens. Although some bacteria do not cause infection, others have pathogenic potential and can lead to multiple complications in patients (Saloojee and Steenhoff, 2001; Traverse, 2015).

Likewise, at the national level in Peru, there are no studies of this type in the area of veterinary medicine. This study was conducted to identify bacterial contamination of stethoscopes used by veterinarians, its associated factors, and the antimicrobial profile of these isolated bacteria, highlighting the potential risk of transmission of multidrug-resistant pathogens through stethoscopes.

2. Materials and Methods

The present study is descriptive and qualitative. Since the number of pet veterinary clinics in the district of Trujillo is still unknown, the formula for an infinite population was used, resulting in a sample size of 59 stethoscopes, considering that in these veterinary centers, access to stethoscopes and veterinarian users would be available (Aguilar-Barojas, 2005).

2.1. Inclusion and exclusion criteria

Only pet veterinary clinics with a licensed veterinarian, a usable stethoscope, an agreement to participate, and a signed confidential agreement were included ($n = 59$). One stethoscope per pet veterinary clinic was considered, as most pet veterinary clinics had only one stethoscope that was shared among the clinic's staff.

2.2. Sample collection

After collecting the sample, a questionnaire, which consisted of three binomial questions, was carried out. The aspects considered were disinfection (Yes/No), veterinarian use (Personal use/Shared among staff), and use on patients (Only for the appointment area/Rotation between areas). Two parts of the stethoscope were swabbed: the chest piece and the ear tips. The method used for sample collection, preservation, and transport was adapted from the Technical Guide on Criteria and Procedures for the Microbiological Examination of Inert Surfaces, written by the General Directorate of Health (DIGESA, Lima, Perú). First, the sterile swab was soaked in the Brain Heart Infusion broth (BHI) and then rubbed 4 times, in different locations in opposite directions to the previous one at a 30° angle, followed by placing the swab inside the tube with the broth, breaking the edge of the swab that came into contact with the hand. This procedure was repeated with both surfaces per sample of the stethoscope. Once the samples were collected, the tubes were labeled and stored inside a Styrofoam Cool Box with cooling gel on the side walls and underneath the test tube racks to prevent any chemical or biological changes in the sample until arrival at the laboratory (DIGESA, 2006).

2.3. Microbiological processing

In the laboratory, swab samples were cultured using calibrated inoculating loops, using the quadrant streak plate technique on blood agar with 5% sheep blood, and then incubated at 37°C for 24 to 48 hours. After Gram staining and morphological identification of colonies by microscopy, biochemical tests were performed, and all bacteriological diagnostic procedures were adapted from the National Institute of Health (2005). In this aspect, the tests performed for Gram-positive cocci were catalase, Mannitol salt agar, and coagulase. Meanwhile, the tests conducted for the Gram-negative bacilli were catalase, oxidase, MacConkey agar, Triple Sugar Iron Agar, Lysine Iron Agar, and Simmons Citrate Agar. All the biochemical tests and agar blood were from the Valtek laboratory (INS, 2005).

2.4. Antimicrobial efficacy test

The disk diffusion method (Kirby-Bauer) was performed following standard protocols (Lubbers, 2018). The inoculum was prepared using a sterile inoculating loop to collect 4 to 5 fresh colonies from nutrient agar plates (Merck Millipore, Burlington, United States). The colonies were transferred into a sterile test tube containing saline solution (B. Braun) and homogenized to achieve turbidity equivalent to 0.5% on the McFarland scale (Hardy Diagnostics, Santa Maria, United States), as confirmed visually. This preparation corresponded to an approximate bacterial concentration of $1\text{--}2 \times 10^8 \text{ CFU/mL}$ (National Institute of Health, 2002).

The inoculum was subsequently spread onto Mueller-Hinton agar plates (Valtek, Shanghai, China) using sterile swabs (Alkhofar, Lima, Perú) and the mass inoculation technique, ensuring complete coverage of the medium's surface. Afterward, eleven antibiotic sensitivity discs (Bioanalyse, Ankara, Turkey) were symmetrically placed on the agar. The tested antibiotics included doxycycline (DOX), streptomycin (STRP), cephalexin (CEF), clindamycin (CLIN), oxytetracycline (OXI), amikacin (AMI), tetracycline (TET), enrofloxacin (ENR), penicillin (PEN), trimethoprim sulfamethoxazole (SXT), amoxicillin with clavulanic acid (AAC). The drugs were chosen as these are the most frequently used in pet veterinary clinics in Perú (Cachicatari and Palomino, 2017; Trujillo, 2021). The plates were incubated at 35°C for 16–24 hours. The zones of growth inhibition were measured using a calibrator, and the results were interpreted based on the critical inhibition diameters specific to each antibiotic and microorganism, following established guidelines from the National Institute of Health (2002) and the Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals (Lubbers, 2018). Minimum Inhibitory Concentration (MIC) testing was not performed due to budget constraints; instead, antimicrobial susceptibility was assessed using the disk diffusion method mentioned above.

2.5. Statistical methods

The data corresponding to the study variables were compiled in frequency tables in Microsoft Excel. Statistical data analysis was performed by applying Fisher's exact test in SPSS version 25 software, Armonk, United States, to determine the association between the independent variables and pathogenic bacteria in the sampled stethoscopes. Likewise, the odds ratio statistical test was used to determine if any variables are bacterial presentation risk factors.

3. Results

3.1. Prevalence of bacteria with pathogenic potential on the surfaces of stethoscopes in pet veterinary clinics in Trujillo

It was determined that 88% (52/59) of stethoscopes had one or more bacteria with pathogenic potential on any of their surfaces, with only 7 (12%) stethoscopes in the study being free of this type of contamination. From these 52 stethoscopes, 100 bacterial isolates were obtained from both surfaces sampled. Table 1 shows the frequency of isolated bacterial agents, with *Staphylococcus aureus* (35%), being the most frequently isolated bacterial species, followed by Coagulase-negative *Staphylococcus* (34%), *Pseudomonas* spp. (18%), *Shigella* spp. (6%), *Klebsiella* spp. (3%), *Enterobacter* spp. (2%), Gram-negative coccobacilli (1%) and *Escherichia coli* (1%).

On the other hand, regarding the prevalence of these bacteria by stethoscope surface, Table 2 shows the frequency, observing that the surface of the chest piece is the one with the highest frequency of bacteria with pathogenic potential, with 95% (56/59) of the total number of positive surfaces. Ear tips represented 75% (44/59). The frequency of bacterial isolation by stethoscope surface was also analyzed, showing that the surface of the chest piece the one with the highest frequency of bacteria of Coagulase-negative *Staphylococcus* (21/100). At the same time, the most frequently isolated bacteria in the ear tips were *S. aureus* (19/100), as shown in Table 3.

| Bacterium | Total | |
|--|---------|----|
| | (n=100) | % |
| Gram-positive cocci | | |
| <i>Staphylococcus aureus</i> | 35 | 35 |
| Coagulase-negative <i>Staphylococcus</i> | 34 | 34 |
| Gram-negative bacilli | | |
| <i>Pseudomonas</i> spp. | 18 | 18 |
| <i>Shigella</i> spp. | 6 | 6 |
| <i>Escherichia coli</i> | 1 | 1 |
| <i>Enterobacter</i> spp. | 2 | 2 |
| <i>Klebsiella</i> spp. | 3 | 3 |
| Gram-negative coccobacilli | 1 | 1 |
| Positives (n=59) | 52 | 88 |
| Negatives (n=59) | 7 | 12 |

Table 1 – Distribution of aerobic microorganisms isolated from stethoscopes in this study.

| Contamination status | Chest Piece | | Ear Tips | |
|----------------------|-------------|------|----------|------|
| | (n=59) | % | (n=59) | % |
| Positive | 56 | 94.9 | 44 | 74.6 |
| Negative | 3 | 5.1 | 15 | 25.4 |

Table 2 – Contamination status according to the surface where it was isolated.

| Bacterium | Chest piece (n=56) | % | Ear tips (n=44) | % |
|--|-----------------------|------------|--------------------|------------|
| Gram-positive cocci | | | | |
| <i>Staphylococcus aureus</i> | 16 | 28.6 | 19 | 43.2 |
| Coagulase-negative <i>Staphylococcus</i> | 21 | 37.5 | 13 | 29.5 |
| Gram-negative bacilli | | | | |
| <i>Pseudomonas</i> spp. | 10 | 17.9 | 8 | 18.2 |
| <i>Shigella</i> spp. | 5 | 8.9 | 1 | 2.3 |
| <i>Escherichia coli</i> | 0 | 0.0 | 1 | 2.3 |
| <i>Enterobacter</i> spp. | 2 | 3.6 | 0 | 0.0 |
| <i>Klebsiella</i> spp. | 2 | 3.6 | 1 | 2.3 |
| Gram-negative coccobacilli | 0 | 0.0 | 1 | 2.3 |
| Total positive isolates | 56 | 100 | 44 | 100 |

Table 3 – Distribution of aerobic microorganisms according to the surface where it was isolated.

3.2. Risk factors associated with the prevalence of bacteria in stethoscopes

The possible risk factors associated with the presence of bacteria in the stethoscopes of veterinarians in Trujillo were evaluated as shown in Table 4, determining the statistical association between the variable disinfection and the presence of bacteria using Fisher's exact test ($p<0.05$); likewise, the odds ratio (OR) was determined with a confidence interval of 95% (OR=1.636; 95% CI=1.132-2.366) showing that non-disinfection is a risk factor for the presence of bacteria with pathogenic potential.

Table 5 shows the disinfectants used by users who stated that they perform disinfection before and/or after patient care. Only 18 stethoscopes were reported to receive disinfection, of which 83% (15/18) used 70° alcohol as the only disinfectant, followed by quaternary ammonium 11% (2/18) and hypochlorous acid 6% (1/18). Likewise, the frequency of isolations by type of disinfectant used according to the surface of the stethoscope shows that in those where 70° alcohol was used, there was negativity for the isolation of bacteria with pathogenic potential on both or one of the surfaces. On the other hand, there was no harmful data for those who stated the use of quaternary ammonium and hypochlorous acid. Furthermore, the frequency of isolations was evaluated by the type of disinfectant used according to the surface of the stethoscope, showing that in those where 70° alcohol was used, there was negativity for the isolation of bacteria on both or one of the surfaces. On the other hand, no data were available for those who reported using of quaternary ammonium and hypochlorous acid.

| Factors | Bacterium | | | | | | p* | OR | CI 95% |
|-------------------------------|---------------|------|---------------|------|------------|------|-------|-------|-----------------|
| | Positive n | % | Negative n | % | Total n | % | | | |
| Disinfection | | | | | | | | | |
| No | 41 | 100 | 0 | 0 | 41 | 69.5 | 0.00 | 1.636 | 1.132- 2.366 |
| Yes | 11 | 61.1 | 7 | 38.9 | 18 | 30.5 | | | |
| Veterinarian use | | | | | | | | | |
| Shared among staff | 28 | 82.4 | 6 | 17.6 | 34 | 57.6 | 0.221 | 0.194 | 0.022- 1.731 |
| Personal use | 24 | 96 | 1 | 4 | 25 | 42.4 | | | |
| Use on patients | | | | | | | | | |
| Rotation between areas | 49 | 87.5 | 7 | 12.5 | 56 | 94.9 | 1 | 0.875 | 0.793- 0.966 |
| Only for the appointment area | 3 | 100 | 0 | 0 | 3 | 5.1 | | | |

Table 4 – Risk factors associated with the prevalence of bacteria with pathogenic potential on the surface of stethoscopes in pet veterinary centers in the district of Trujillo, Perú.

| Disinfectant | Negative stethoscopes n | | Positive stethoscopes n | | Total n=18 | % | Stethoscope surface | Bacterium | | |
|------------------------|-------------------------|-----------------------------------|-------------------------|-----------------------|------------|----|-------------------------|-----------------------------------|------------------|---|
| | Staphylococcus aureus | Coagulase Negative Staphylococcus | Pseudomonas spp. | Staphylococcus aureus | | | | Coagulase Negative Staphylococcus | Pseudomonas spp. | |
| 70 % Isopropyl alcohol | 7 | 6 | 0 | 8 | 15 | 83 | Ear tips Chest piece | 3 | 0 | 0 |
| Quaternary ammonium | 0 | 0 | 0 | 2 | 2 | 11 | Ear tips Chest piece | 0 | 1 | 0 |
| Hypochlorous acid | 0 | 0 | 0 | 1 | 1 | 6 | Ear tips Chest piece | 0 | 0 | 1 |

Table 5 – Frequency of bacterial isolates with pathogenic potential by type of disinfectant used according to stethoscope surface.

3.3. Antimicrobial susceptibility of bacteria on the surfaces of stethoscopes in pet veterinary clinics in Trujillo

The antimicrobial tests identified significant resistance levels among bacteria isolated from veterinary stethoscopes in Trujillo. Among gram-positive cocci, *S. aureus* exhibited high resistance rates to penicillin (PEN) (94.3%), tetracycline (TET) (80.0%), and oxytetracycline (OXI) (74.3%), while showing no resistance to amikacin (AMI). Similarly, coagulase-negative staphylococci demonstrated complete resistance (100%) to penicillin (PEN), alongside moderate resistance to trimethoprim sulfamethoxazole (SXT) (58.8%), tetracycline (TET) (44.1%) and cephalaxin (CEF) (32.4%).

For gram-negative bacteria, *Shigella* spp. displayed complete resistance (100%) to trimethoprim sulfamethoxazole (SXT) and amoxicillin with clavulanic acid (AAC). In contrast, *P. spp.* exhibited 33.3% resistance to enrofloxacin (ENR), *Klebsiella* spp. showed 66.7% resistance to enrofloxacin (ENR) and *Enterobacter* spp. demonstrated 50.0% resistance to amoxicillin with clavulanic acid (AAC). Additionally, *E. coli* presented 100% resistance to oxytetracycline (OXI). These findings highlight a worrying prevalence of antimicrobial resistance among bacterial isolates (Table 6).

| Antibiotics | n | DOX | | STRP | | CEF | | CLIN | | OXI | | AMI | | TET | | ENR | | PEN | | SXT | | AAC | | | |
|--|-----|-----|------|------|------|-----|------|------|------|-----|------|-----|---|-----|------|-----|------|-----|------|-----|------|-----|-----|---|------|
| | | R | % | R | % | R | % | R | % | R | % | R | % | R | % | R | % | R | % | R | % | R | % | R | % |
| Gram-positive cocci | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Staphylococcus aureus</i> | 35 | 9 | 25.7 | 21 | 60.0 | 15 | 42.9 | 11 | 31.4 | 26 | 74.3 | 0 | | 28 | 80.0 | 1 | 2.9 | 33 | 94.3 | 18 | 51.4 | - | - | - | - |
| Coagulase-negative <i>Staphylococcus</i> | 34 | 5 | 14.7 | 8 | 23.5 | 11 | 32.4 | 4 | 11.8 | 9 | 26.5 | 0 | | 15 | 44.1 | 0 | 0.0 | 34 | 100 | 20 | 58.8 | - | - | - | - |
| Gram-negative cocci | | | | | | | | | | | | | | | | | | | | | | | | | |
| <i>Pseudomonas</i> spp. | 18 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 6 | 33.3 | - | - | - | - | - | - | - | - |
| <i>Shigella</i> spp. | 6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 6 | 100 | 6 | 100 | | |
| <i>Escherichia coli</i> | 1 | - | - | - | - | - | - | - | - | 1 | 100 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Enterobacter</i> spp. | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 50.0 |
| <i>Klebsiella</i> spp. | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 | 66.7 | - | - | - | - | - | - | - | - |
| Total | 117 | 14 | 12 | 29 | 24.8 | 26 | 22.2 | 15 | 12.8 | 36 | 30.8 | 0 | 0 | 43 | 36.8 | 9 | 7.7 | 67 | 57.3 | 44 | 37.6 | 7 | 6.0 | | |

Table 6 – Antimicrobial Resistance Profiles of Bacteria Isolated from Veterinary Stethoscopes in Trujillo, Peru.

4. Discussion

The results found in the present study determined that the stethoscopes, instruments used for the physical evaluation of patients, are contaminated with potentially pathogenic bacteria. Of the 59 stethoscopes, 100 bacteria were isolated being the isolated bacteria in order of prevalence: Coagulase-negative *Staphylococcus*, *S. aureus*, *P. spp.*, *Shigella* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp. and Gram-negative cocobacilli. Likewise, within the list of isolated bacteria, those of the genus *Bacillus* spp. were excluded because they are considered agents of environment contamination. Most nosocomial infections are due to pathogens known by the acronym ESKAPE: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., of which species have been reported in this study (Zohra et al., 2021).

Chest pieces are the most contaminated surfaces of stethoscopes due to their constant contact with the patients, which is why a contamination positivity of 95% was reported, with a higher prevalence of *Staphylococcus* spp. being present on the skin and surfaces. Likewise, the chest piece is also usually associated with *Micrococcus* spp. because they commonly colonize the skin of humans and animals; however, this genus was not reported in the present study (Souza et al., 2022). The genus *Staphylococcus* spp. is mainly isolated from surfaces within pet veterinary clinics (Hamilton et al., 2012). Coagulase-positive *Staphylococcus* is the bacteria with the highest incidence of clinical infections in animals and humans. Conversely, Coagulase-negative *Staphylococcus* presents a higher prevalence in mucous membranes and skin (Gemma et al., 2019). The genus *Staphylococcus aureus* is the leading cause of nosocomial bacteremia in Latin America and North America due to the resistance it generates, the pathogenicity factors it

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possesses, the virulence, and the proteins they have on the surface that create the formation of biofilm and help in the colonization and invasion process at the cellular level within the host (Pasachova et al., 2019). Regarding *Pseudomonas aeruginosa*, the importance of its isolation lies in the fact that, in canines and felines, it is the genus that causes systemic, urinary tract, cutaneous, and ear infections; it also produces hemorrhagic crusts, erythematous papules, dental abscesses, and periodontal disease (Plókarz et al., 2022).

The high prevalence of *Staphylococcus* spp. on the surface of veterinary stethoscopes is also reported to be the most isolated species: *Staphylococcus epidermidis* (56.7%), *Staphylococcus hominis* (33.3%), *Staphylococcus pasteuri*, *Staphylococcus capitis* and *Staphylococcus schleiferi* (3.3%). Unlike the present study, *S. aureus* was a non-isolated pathogen because it is a genus that has a limited ability to survive on dry surfaces for more than 24 hours, contrary to *S. epidermidis*, which is usually isolated from domestic animals, which would agree that it is isolated more frequently in the chest piece of the stethoscopes (Kiraly et al., 2023). Previous studies also proved the low prevalence of *S. aureus* on inert surfaces in pet veterinary facilities (Leite et al., 2023). However, the high prevalence of *S. aureus* found in the present study similar to that reported in studies from Peru in human medicine facilities (Menacho et al., 2016; Charca, 2019; Bustamante, 2021).

The present study reported a statistical association between bacterial prevalence and disinfection, as shown in other studies. Likewise, it was found that, of the 59 stethoscopes sampled, only 18 disinfected this medical instrument, the most commonly used disinfection method being 70° alcohol. Regarding this method, it is proven that cleaning the chest piece of the stethoscope for 10 seconds with a 70° isopropyl alcohol wipe and letting it evaporate for 10 more seconds is a quick and effective protocol to reduce the contamination of the medical instrument (Marcos et al., 2020; Souza et al., 2022). Nevertheless, most veterinarians do not have a disinfection protocol for the stethoscope after auscultating each patient.

The present study relates the problem mentioned above to the lack of awareness about disinfection and the absence of studies that report on the consequences; as more studies exist that demonstrate the capacity of stethoscopes as a fomite, the creation of policies and disinfection products only for stethoscopes will be allowed (Uneke et al., 2012). However, it should be considered that the best way to reduce bacterial contamination is to adopt a multimodal approach to disinfection, including the protocol applied and promoting good habits and hand hygiene (Fernandez et al., 2023). Regarding the use factor, there are currently no studies that relate it to a specific use, so studies relating the form of use with the prevalence of the bacteria found should be considered. The analysis of antimicrobial resistance in bacteria isolated from veterinary stethoscopes underscores the critical role of fomites in the spread of multidrug-resistant pathogens within veterinary practice. These findings align with global trends, identifying medical tools like stethoscopes as significant reservoirs for resistant bacteria due to inadequate disinfection and improper antibiotic use (Uneke et al., 2014; Souza et al., 2022).

The study revealed notable variability in resistance across bacterial species, reflecting the complexity of managing antimicrobial resistance in clinical settings. Gram-positive bacteria, such as *Staphylococcus aureus* and coagulase-negative staphylococci, were reported to be prominent contributors to nosocomial infections due to their biofilm-forming ability and resistance to common antibiotics. Concurrently, gram-negative bacteria like *P. spp.* and *Klebsiella spp.* posed additional challenges due to their intrinsic and acquired resistance mechanisms, some of which have zoonotic implications, posing risks to veterinary staff and pet owners (Leite et al., 2023). Resistance to multiple antibiotic classes, including penicillins, tetracyclines, and fluoroquinolones, highlights the urgency of implementing antimicrobial stewardship programs tailored explicitly to veterinary medicine. Such programs must prioritize the rational use of antibiotics while considering local resistance patterns and minimizing reliance on broad-spectrum antibiotics (Hamilton et al., 2012). Alarmingly, pathogens like *Shigella spp.* with 100% resistance to erythromycin and amoxicillin point to gaps in existing infection control practices, particularly for gram-negative bacteria. These results contribute to the growing body of evidence highlighting the role of veterinary equipment in the epidemiology of antimicrobial resistance. The high resistance rates observed and the zoonotic potential of specific pathogens underscores the need for a comprehensive approach to infection control. This approach should integrate effective disinfection, hand hygiene, and judicious antibiotic use to limit the spread of resistant bacteria within pet veterinary clinics and beyond.

While this study provides valuable insights into the bacterial contamination and antimicrobial resistance patterns on veterinary stethoscopes, certain limitations should be acknowledged. One key limitation was the inability to perform Minimum Inhibitory Concentration (MIC) tests, which would have offered more precise information on resistance levels. This constraint was primarily due to financial limitations, which restricted the scope of laboratory analyses. Addressing this limitation in future studies will enable a more comprehensive understanding of the resistance mechanisms and their clinical implications. Future research should investigate alternative disinfectants or combined cleaning strategies as well as the molecular mechanisms underlying resistance driving resistance among bacterial isolates. Addressing these gaps will facilitate the development of targeted interventions to protect both animal and human health.

5. Conclusion

In conclusion, the stethoscopes of veterinarians contaminated with bacteria that have already been reported as agents that identified as agent that cause nosocomial infections. Hence, this information contributes to promoting awareness among veterinarians about the contamination of the stethoscopes used and encourages the implementation of correct aseptic measures that are efficient for their disinfection thereby preserving the health of the patient and the user. Additionally, the high levels of antimicrobial resistance observed, particularly among *Staphylococcus aureus* and gram-negative bacteria, emphasize the urgent need for infection control protocols and antimicrobial stewardship programs in pet veterinary clinics.

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