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Microbiological evaluation of stethoscopes used in the clinical routine of Veterinarians in the Metropolitan Region of Recife, Pernambuco, Brazil

Avaliação microbiológica de estetoscópios utilizados na rotina clínica de Médicos Veterinários da Região Metropolitana do Recife, Pernambuco, Brasil

Evaluación microbiológica de estetoscopios utilizados en la rutina clínica de Veterinarios de la Región Metropolitana de Recife, Pernambuco, Brasil

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Abstract
We evaluated the level of contamination of stethoscopes used in the clinical routine of veterinarians. Specimens from 15 stethoscopes were cultured and analyzed before (M0) and after (M1) disinfecting the stethoscope diaphragm with alcohol at 70ºGL. At moment M0, bacterial growth was observed in 73.33% (11/15) of the stethoscopes. Seventeen strains were isolated, and the majority (41.2%, 7/17) were *Bacillus* spp. followed by *Staphylococcus* spp. (35.3%, 6/17) and *Micrococcus* spp. (23.5%, 4/17). In samples collected after disinfection with alcohol (M1), only *Bacillus* spp. were isolated in 13.3% (2/15) of the stethoscopes. Coagulase phenotypic testing of the *Staphylococcus* spp. revealed that all the strains were negative. Polymerase chain reaction was performed for the *mec*A resistance gene in coagulase-negative *Staphylococcus* strains. Amplification of *mec*A gene was observed in only 16.66% (1/6) of the samples. Stethoscopes are instruments that transfer pathogens, especially bacteria that may carry resistance genes. However, simple cleaning and disinfection measures in the veterinary medical clinic routine reduce contamination and minimize the risk of spread of resistant microorganisms to animals and the environment.
Keywords: Contamination; Disinfection; Instrument; *mec*A; Microorganisms; Molecular analysis.

Resumo

Foram avaliados o nível de contaminação de estetoscópios utilizados na rotina clínica de médicos veterinários. Amostras de 15 estetoscópios foram cultivadas e analisadas antes (M0) e após (M1) desinfecção do diafragma dos estetoscópios com álcool à 70ºGL. No momento M0, observou-se crescimento bacteriano em 73.33% (11/15) dos estetoscópios. Dezessete cepas foram isoladas, sendo a maioria (41.2%, 7/17) de *Bacillus* spp., seguido por *Staphylococcus* spp. (35.3%, 6/17) e *Micrococcus* spp. (23.5%, 4/17). Nas amostras coletadas após a desinfecção com álcool (M1), apenas *Bacillus* spp. foram isolados em 13.3% (2/15) dos estetoscópios. Teste fenotípico coagulase do *Staphylococcus* spp. revelou que todas as cepas foram negativas. A reação em cadeira da polimerase foi realizada para o gene de resistência *mec*A em cepas de*Staphylococcus* coagulase-negativa. A amplificação do gene *mec*A foi observada em apenas 16.66% (1/6) das amostras. Os estetoscópios são instrumentos que transferem patógenos, especialmente bactérias que podem carregar genes de resistência. No entanto, medidas simples de limpeza e desinfecção na rotina clínica médica veterinária reduzem a contaminação e minimizam o risco de disseminação de microrganismos resistentes aos animais e ao meio ambiente.

Palavras-chave: Contaminação; Desinfecção; Instrumento; *mec*A; Microrganismos; Análise molecular.

Resumen

Se evaluó el nivel de contaminación de los estetoscopios utilizados en la rutina clínica de los médicos veterinarios. Se cultivaron muestras de 15 estetoscopios y se analizaron antes (M0) y después (M1) de la desinfección del diafragma de los estetoscopios con alcohol a 70ºGL. En el momento M0, se observó crecimiento bacteriano en el 73,33% (11/15) de los estetoscopios. Se aislaron diecisiete cepas, con la mayoría (41,2%, 7/17) *Bacillus* spp. seguido de *Staphylococcus* spp. (35,3%, 6/17) y *Micrococcus* spp. (23.5%, 4/17). En las muestras recogidas después de la desinfección con alcohol (M1), solo *Bacillus* spp. se aislaron en el 13,3% (2/15) de los estetoscopios. Prueba fenotípica de coagulasa de *Staphylococcus* spp. reveló que todas las cepas eran negativas. La reacción de silla de la polimerasa se realizó para el gen de resistencia *mec*A en cepas de *Staphylococcus* coagulasa negativas. La amplificación del gen *mec*A se observó en sólo 16,66% (1/6) de las muestras. Los estetoscopios son instrumentos que transfieren patógenos, especialmente bacterias que pueden portar genes de resistencia. Sin embargo, las simples medidas de limpieza y desinfección en la rutina clínica médica veterinaria reducen la contaminación y minimizan el riesgo de diseminación de microorganismos resistentes a los animales y al medio ambiente.

Palabras clave: Contaminación; Desinfección; Instrumento; *mec*A; Microorganismos; Análisis molecular.

1. Introduction

Hospitals are important places for the maintenance and dissemination of pathogens, especially the contamination of inanimate surfaces and equipment (Dutra et al., 2013; Oliveira & Damasceno, 2010). Among the instruments that may harbor microorganisms, stethoscopes are noteworthy (Dutra et al., 2013). Despite being essential for a proper clinical examination, a stethoscope can be a source of contamination and dissemination of many types of pathogenic microorganisms (Dantas et al., 2014).

The microorganisms that can be transmitted by stethoscopes include coagulase-negative *Staphylococcus* (CoNS). These are frequently reported, are commonly associated with nosocomial infections. Health professionals play an important role in maintaining and spreading these agents in a hospital environment (Becker et al., 2015; Rosa et al., 2009). In addition to the transmission of biological agents, microbial resistance has proven to be a persistent challenge that reflects the indiscriminate use of antimicrobials selects for resistant bacterial strains, which can interfere with future therapies (Monteiro et al., 2020; Pereira & Cunha, 2009; Rosa et al., 2009).

CoNS can be resistant to numerous antimicrobials, especially to those reserved for hospital use. The resistance mechanism for CoNS is similar to that presented by *Staphylococcus aureus*, involving the PBP2a protein encoded by the *mec*A gene (Rosa et al., 2009; Santos et al., 2007). The prevalence of methicillin-resistant *Staphylococcus* spp. strains is considered high in most Brazilian hospitals, and in veterinary medicine. The abuse of antimicrobials has favored the emergence of these resistant strains (Pereira & Cunha, 2009; Rosa et al., 2009; Sexton et al., 2006).

Based on this perspective, the aim of the present study was evaluated the level of contamination of stethoscopes, before and after disinfecting, used in the clinical routine of veterinarians.

2. Methodology

2.1 Sample Collection

Samples from 15 stethoscopes were collected using a moist sterile swab, by rubbing across the diaphragm surface before (M0) and after (M1) the instrument was disinfected with alcohol at 70ºGL. The first sampling was performed before using the stethoscope for the first time on the particular day. After disinfection and complete evaporation of alcohol, the second sampling was performed. All specimens were sent to a microbiology laboratory for processing.

2.2 Microbiological processing

Swabs were incubated at 37ºC for up to 48h on blood agar (base) with 5% (v/v) sheep blood. Colonies were morphologically identified by gram staining and visualized at 100x magnification using an optical microscope.

For isolates showing staphylococcal morphology, the tube coagulase test was performed. A 0.05mL volume of the isolate inoculated in Brain Heart Infusion broth(Merck KGaA, Darmstadt, Hesse, Germany)and 0.5mL of rabbit plasma (BBL™ Coagulase Plasma, 2BD®, Sparks, MD, USA)was homogenized in test tubes. The samples were incubated at 37°C for 24h, according to the manufacturer's recommendations.

2.3 DNA extraction of *Staphylococcus* spp.

Isolates of *Staphylococcus* spp. were subsequently used for DNA extraction using the thermal method (Fan, Kleven & Jackwood, 1995). After extraction, quantification was performed using a spectrophotometer (NanoDrop ™ Lite Spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) at an absorbance of 260nm.

2.4 Molecular Analysis

PCR for the *mec*A gene was performed (Nakagawa et al., 2005). Reactions were prepared to contain a final volume of 12.5µL containing 100ng of DNA from the isolate, 0.5µL of each primer at 10pmol (2WTGGTATGTGGAAGTTAGATTGGGAT-3' 'and 2× 5'-CTAATCTCATATGTGTTCCTGTATTGGC-3'), 6,25µL of Go Taq Green Master Mix (Promega Corporation, Madison, WI, USA)and 2.5µL of Milli-Q ultrapure water. PCR products were subjected to electrophoresis on 2% agarose gel for 01h at 100V. Gels were stained with Blue Green (LGC Biotecnologia, Cotia, SP, Brazil) and were visualized under ultraviolet light and photographed. *S. aureus* N315 was used as a positive control in all reactions performed. After all analysis, the absolute and relative frequencies were calculated.

3. Results

Of the total stethoscopes evaluated in the first collection (M0), 73.3% (11/15) showed pure bacterial growth or associations. After disinfection (M1) with alcohol at 70°GL, only 13.3% (2/15) of the stethoscopes remained contaminated. There was an 86.7% reduction in bacterial growth after disinfection of the diaphragm.

At M0, we obtained 17 isolates pure or in combination. Of these, 41.2% (7/17) were morphologically compatible with *Bacillus* spp., 35.3% (6/17) with *Staphylococcus* spp., and 23.5% (4/17) with *Micrococcus* spp. In one sample, two distinct isolates of *Staphylococcus* spp. were obtained, showing different morphological characteristics. Relating bacterial growth to the number of stethoscopes analyzed, *Bacillus* spp. was isolated in 46.6% (7/15) of the stethoscopes, *Staphylococcus* spp. in 40% (6/15), and *Micrococcus* spp. in 26.6% (4/15).

At M1, *Bacillus* spp. was isolated in only 13.3% (2/15) of stethoscopes, representing reduced contamination of 86.6% when compared to the total contamination rate observed at M0 (Table 1).

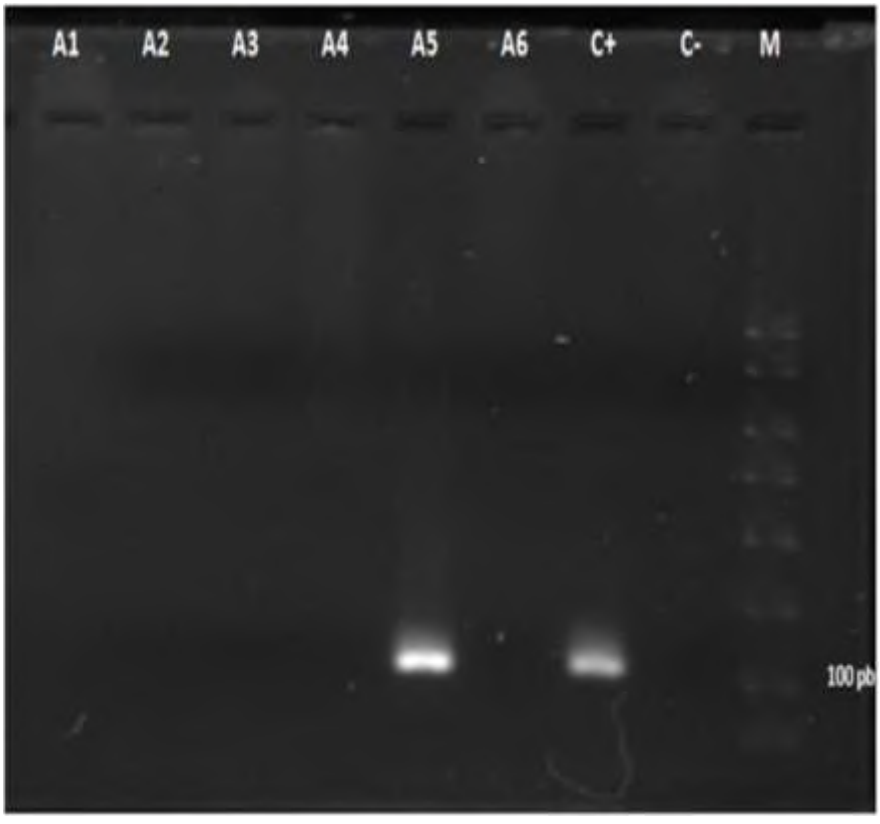
Table 1. Microbiological examination of stethoscopes used by veterinarians before and after disinfection.M0: before disinfection; M1: after disinfection. ** Two distinct isolates with different morphological characteristics.

Sample	Isolated microorganism	
	M0	M1
01	*Micrococcus* spp.	Negative
02	*Bacillus* spp.	Negative
03	*Staphylococcus* spp.+ *Micrococcus* spp.	Negative
04	*Staphylococcus* spp.** + *Bacillus* spp.	Negative
05	*Staphylococcus* spp. + *Bacillus* spp.	Negative
06	Negative	Negative
07	*Bacillus* spp.	*Bacillus*spp.
08	*Bacillus* spp.	*Bacillus*spp.
09	*Bacillus* spp.	Negative
10	Negative	Negative
11	*Micrococcus* spp.+ *Bacillus* spp.	Negative
12	*Staphylococcus* spp.	Negative
13	Negative	Negative
14	*Staphylococcus* spp.+ *Micrococcus* spp.	Negative
15	Negative	Negative

Source: Authors.

Coagulase testing of six staphylococcal isolates was negative in all cases. It was possible to classify the strains as CoNS. From these specific isolates, amplification of the *mec*A gene was observed in only 16.66% (1/6) (Figure 1).

Figure 1. PCR results for *mec*A gene in coagulase-negative *Staphylococcus* isolated from stethoscopes used by veterinarians. Samples A1-A4 and A6: negative; Sample A5: positive (155 bp); C +: positive control; C-: negative control; M: molecular weight marker.



Source: Authors.

4. Discussion

Microbiological analysis of the stethoscopes in this study showed high bacterial colonization on the surface of the diaphragm (73.3%). This was expected because it is an instrument continuously used in veterinary medical clinics. Similarly, considering the number of stethoscopes analyzed, the results obtained are consistent proportionally, with those observed in two other studies (Dutra et al., 2013; Teixeira et al, 2015), where bacterial contamination occurred in 96.2% (78/81), and 82.9% (87/105) of the analyzed stethoscopes, indicating that these instruments can transmit agents between patients and from the patient to the physician.

Considering the isolates obtained in the first set of samples, *Bacillus* spp. showed the highest prevalence (41.2%), followed by *Staphylococcus* spp. and *Micrococcus* spp. The presence of this agent may indicate environmental contamination, since bacteria of the genus *Bacillus* are widely found in nature, commonly isolated from numerous surfaces, and considered resistant to disinfectants and ultraviolet radiation (Logan & DeVos, 2015). Their presence in a sample is usually neglected, but they can be pathogenic under specific conditions.

Staphylococcus spp. is present on the skin and surfaces of all warm-blooded animals and is therefore commonly isolated on stethoscope diaphragms. In this study, all *Staphylococcus* spp. isolates were coagulase-negative and represented 40% (6/15) of the isolates in stethoscopes. This finding is superior to that observed by two others authors (Dutra et al., 2013; Teixeira et al, 2015), who isolated CoNS in 22.2% (18/81) and 22.9% (24/105) of stethoscopes, respectively.These microorganisms are frequently associated with infections and can form biofilms, enabling their perpetuation in a hospital environment (Rosa et al., 2009). However, this ability was not analyzed in this study, and it requires future analysis since studies on this topic in veterinary medicine are scarce.

Micrococcus spp. were also isolated from the diaphragms (23.5%). These bacteria colonize the skin of humans and animals and are also found in the environment (Becker, Skov & Von Eiff, 2015). Clinical reports associate the presence of *Micrococcus luteus* with cases of endocarditis and pneumonia in humans(Khan, Aung & Chaudhuri, 2019). However, in this study, these agents were not classified. Some studies indicate that *Micrococcus* spp. should not be considered as contaminants, but rather as pathogenic agents, which can favor the occurrence of systemic disorders (Hirata et al., 2009). In this study, isolation of *Micrococcus* spp. occurred in three stethoscopes simultaneously with other pathogens. However, in one, a pure isolate was obtained. Further studies are warranted to determine the real importance of this microorganism.

The presence of microorganisms in veterinary equipment probably occurs due to the lack of proper disinfection. This was evident in this study at M1 since there was a considerable reduction in contamination (86.6%) after disinfection, but *Bacillus* spp. could still be isolated in 13.3% (2/15) of stethoscopes. Despite this, *Staphylococcus* spp. and *Micrococcus* spp. were negative in 100% of the samples. These results are similar to those presented in a study carried out on 32 stethoscopes used by the medical staff of a public hospital (Dantas et al., 2014). The authors reported a 100% reduction in contamination of stethoscopes by *S. aureus* after disinfection. Isolation of *Bacillus* spp. in M1 can be justified by the resistance of these microorganisms to disinfection processes.

According to the National Health Surveillance Agency (ANVISA, 2012), 70ºGL alcohol has bactericidal, viricidal, fungicidal, and tuberculocidal properties, in addition to being an affordable and accessible product. However, it does not have a sporicidal potential. Under the conditions of this study, disinfection with alcohol at 70ºGL was effective in eliminating most of the bacterial contamination of the diaphragms, including bacteria such as CoNS, which carry antimicrobial resistance genes.

On PCR, one isolate of CoNS was positive for the detection of the *mec*A gene, which is related to high resistance to beta-lactam antimicrobials. The *mec*A gene has a chromosomal origin and encodes enzymes that have a low affinity for methicillin or oxacillin (Ghoshal et al., 2004). These drugs are used in human medicine to treat infections by *Staphylococcus* spp. (Pereira & Cunha, 2005; Velázquez-Meza, 2005).

Most studies on the detection of methicillin resistance in stethoscopes have focused on *S. aureus* (Dantas et al., 2014; Teixeira et al., 2016). In addition, the presence of the *mec*A gene in CoNS samples was also demonstrated. In two studies (Hira et al., 2007; Pereira & Cunha, 2009), the presence of the gene was detected in 87.8% (58/66) and 72.5% (79/109) of the CoNS isolates, respectively, which were higher than those obtained in this study. However, the origin of the samples must be considered, as their samples were obtained directly from patients.

The CoNS that carries the *mec*A gene may have a human origin, as these agents are known to colonize nasal fossae and human skin, and were probably transferred by the veterinarian's contact with the stethoscope. However, this hypothesis could not be confirmed. Nonetheless, this finding shows the importance of disinfecting instruments and surfaces in medical-veterinary settings.

5. Conclusion

Stethoscopes are instruments that can harbor pathogens, with special attention to bacteria carrying the *mec*A resistance gene. However, simple cleaning and disinfection measures in the veterinary medical clinic routine are effective in reducing contamination and minimizing the risk of spreading resistant microorganisms to animals and the environment.

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