Evaluating isolated microorganisms present on the surface of stethoscope's diaphragm used by medical students

Avaliação da presença de microrganismos isolados da superfície do diafragma de estetoscópios usados por alunos do curso de medicina

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ABSTRACT: Infections associated with healthcare (IAHC) represent a problem in Brazil that requires care, being among the first six causes of death in the country, which is strongly linked to the imbalance of the microbiota and the host's immunity. Among the instruments that can contribute to the appearance of IAHCs, medical devices, such as stethoscopes, can be an important vector of cross-infection, if not properly sanitized. The objective of this work was to identify the presence of bacterial biological agents contained in the diaphragms of stethoscopes of Medical students at the Centro Universitário de Patos de Minas/ UNIPAM. An observational, cross-sectional, and descriptive study was carried out by collecting microbiological material from 52 stethoscopes. The samples were collected with sterile swabs, rubbed on the stethoscopes' diaphragm surface and then they were transferred in BHI broth (Brain Heart Infusion), standard count agar (PCA), Salt Mannitol agar (SM) and methylene blue eosin agar (EMB). For the characterization of cultures, techniques of bacterial identification by inoculation and traditional biochemical series were used, after that Gram staining was performed and the characteristics were analyzed. Of the stethoscopes' diaphragms analyzed, 50% (n = 26) showed contamination with positive growth for at least one of the surveyed microorganisms: enterobacteria, total bacteria, Staphylococci aureus, and enteric gram-negative bacilli. The results show that Gram-negative bacteria prevailed in the sampled stethoscopes, which are important pathogens. In this sense, it can be concluded that the practice of cleaning the diaphragm of stethoscopes should be better disseminated in educational institutions for spreading diseases and putting the patient's health at risk.

Keywords: Stethoscopes; Biological contamination; Microbiological phenomena; Microorganisms; Bacteria; Students, medical.

RESUMO: As infecções associadas aos cuidados de saúde (IACS) representam no Brasil um problema que requer cuidado, estando entre as seis primeiras causas de óbito no país, de modo que a referida causa está fortemente ligada ao desequilíbrio da microbiota e da imunidade do hospedeiro. Dentre os instrumentos que podem contribuir com o aparecimento das IACS, destacam-se os dispositivos médicos, como o estetoscópio, que pode ser um importante vetor de infecção cruzada, caso não seja higienizado corretamente. O objetivo desse trabalho foi identificar a presença de agentes biológicos bacterianos contidos nos diafragmas dos estetoscópios de alunos do curso de Medicina do Centro Universitário de Patos de Minas/ UNIPAM. Realizou-se um estudo observacional, transversal e descritivo através da coleta de material microbiológico de 52 estetoscópios. As amostras foram coletadas com *swabs* estéreis, friccionados na superfície do diafragma de estetoscópios e depois foram repicadas em caldo BHI (Brain Heart Infusion), ágar padrão para contagem (PCA), ágar Sal Manitol (SM) e ágar eosina azul de metileno (EMB). Para a caracterização das culturas foram utilizados técnicas de identificação bacteriana por inoculação e série bioquímica tradicional, após isso foi realizada a coloração Gram e as características morfo-tintoriais foram analisadas. Dos diafragmas de estetoscópios avaliados, 50% (n=26) apresentaram contaminação com crescimento positivo para pelo menos um dos microorganismos pesquisados: enterobactérias, bactérias totais, *Staphylococcus aureus* e bacilos entéricos gram negativos. Os resultados permitem observar que as bactérias Gram-negativas prevaleceram nos estetoscópios amostrados, as quais são importantes agentes patogênicos. Nesse sentido, pode-se concluir que a prática de higienização do diafragma dos estetoscópios deve ser melhor difundida nas instituições de ensino por veicular doenças e colocar em risco a saúde do paciente.

Descritores: Estetoscópios; Contaminação biológica; Fenômenos microbiológicos; Bactérias; Estudantes de medicina.

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INTRODUCTION

Infections associated with health care represent (IAHC) a problem that needs attention in Brazil, because it is among the primary causes of death in the country, so the referred cause is strongly connected to the imbalance of microbiota and immunity of the host[1].

Among the instruments which can contribute to the presence of IAHC, there are medical devices such as stethoscopes, which can be an important vector of cross-infection if not properly sanitized. Related to it, which increases the importance of cleaning and disinfecting, is the fact that it can be commonly used by doctors and nurses, hence, come in direct contact with various patients.

It is known that knowledge about stethoscope's care is not widespread in the health system. Due to the low number of researches related to the theme, more studies are needed to determine the epidemiological characteristics of crucial clinical microorganisms to the health system. Since the profile of infections varies between institutions, when presented on surfaces, solutions, equipment, and possible similar positive results of cultures in patients[2]. Single hand contact with contaminated surface results in a degree of variable pathogen transference. A contaminated hand can also be a source of recontamination of the surface[2].

Bacteria can attack the human organism by producing endotoxins (lipopolysaccharide - LPS) or by producing exotoxins (proteins). The first group of toxins, in low concentration in the human body, generates alarm reactions (e.g. fever), while in high concentration causes chock and death. Exotoxins are responsible for actions in the nervous system, loss of fluid to intestinal level, inhibition of protein synthesis, and cell lysis[3].

Exotoxins are usually produced by Gram-positive bacteria, its effects in the organism are specific for a structure or cell function affected. These bacteria are unstable to heat (can be destroyed in 60ºC to 80ºC), have high toxicity (capacity of producing disease), do not generate fever, are neutralized by antitoxins, and have a low lethal dose. Endotoxins are mainly produced by Gram-negative bacteria, having general effects on the organism (fever, weakness, pain, and chock), are stable to heat, can undergo autoclave (121ºC for an hour), have low toxicity, are not easily neutralized by antitoxins and have a considerable high lethal dose[3].

Analyzing the prevalence of bacteria contamination in stethoscopes, Dutra[4] claims to have found 96.2% of instruments analyzed contaminated, in which there was a prevalence of *S. aureus* (78%), followed by *Corynebacterium* spp. (26%). Concerning organisms Gram-negative, it was found 17.3% of *Acinetobacter* spp. Dutra stresses that the last group of microorganisms (Gram-negative) causes potentially grave infections since they usually cause contamination of the urinary tract, gastrointestinal tract, respiratory tract, that can even reach septicemia, in addition to offering high resistance to multiple drugs[4].

The study by Neves[5] analyzed 40 lab coats, in which it was not observed the growth of Gram-negative bacteria, with a prevalence of *Bacillli* spp. (38%), *Staphylococci* spp. (20%) and *Streptococci* spp. (42%).

In the study, "*Quantitativo microbiano em jalecos de estudantes da área da saúde em instituição de ensino superior*" (Microbial quantity in lab coats of health students at a higher education institution), it was found a bigger colony of *Staphylococci* Gram-positive (68%), followed by Cocci Gram-positive (16.8%) and Bacilli Gram-negative (5.2%)[6].

Lastly, in the research done by Sales et al.[2], it was observed the presence of bacteria in inanimate surfaces in an intensive care unit, where it was found a prevalence of Gram-negative germ, more specifically *Acinetobacter baumannii* multi-resistant, which is related with the occurrence of nosocomial pneumonia.

Having in mind the evidence found in the studies mentioned it is possible to establish a connection with what is reported in the literature[3]. As the researches show a bigger presence of Gram-positive bacteria in human infections, which has high toxicity. However, despite the smaller number of Gram-negative associated with infections, it is notable that even though with lower toxicity, they show a higher resistance to antitoxins[2].

For that reason, the relevance of this research in recognizing microorganisms isolated in the stethoscope diaphragm was proven, considering that when the material is not properly sanitized and comes in contact with several patients, they end up becoming carriers of an important way of cross-infection and potential originators of IAHC.

It is expected that the results obtained in the present study help in the awareness of the importance of proper sanitation of stethoscopes and the dissemination of this practice. So, the present work has as objective identifying the presence of biological bacterial agents in the surface of the stethoscope diaphragm used by the Medical students in *Centro Universitário de Patos de Minas (UNIPAM)*.

METHOD

This is an observational, transversal, analytical, and quantitative study was done by collecting microbiological material from the diaphragm of stethoscopes submitted voluntarily by medical students from the years three to six of UNIPAM. They come in contact with basic health units (BHU), emergency care unit (EHU), and, mainly, high complexity hospitals, all in Patos de Minas/MG. The analysis was made in the General Microbiology lab of UNIPAM, block D, 1st floor, room 105.

The samples were collected by sterile swabs, rubbed in the stethoscope's diaphragm, put into a saline solution, and transported to the microbiology lab. Later,

the samples were inoculated in BHI broth (Brain Heart Infusion) incubated for 24 hours in an aerobic incubator at 35ºC ±1. After the period of incubation, the samples were replicated to Standard Methods Agar (SMA), Salt Mannitol agar (SM), and Eosin methylene blue (EMB), incubated for 24-48 hours, in aerobic at 35ºC ±1 and performed biochemical tests for bacterial identification[7,8].

To identify *Staphylococci aureus*, we used proof of fermentation of mannitol, catalase, and coagulase. To identify the total of bacteria we performed gram staining to classify the bacterias with basis of the cellular morphology, arrangement, and affinity for dyes, according to the chemical composition of its cellular wall, proof of catalase, and coagulase. To identify the Gram-negative rods, we used standard medium for bacterial identification, oxidation-fermentation of glucose, Simmons citrate, arginine broth, lysine, and ornithine decarboxylase and motility[7,8].

The period of sampling occurred between February and April 2018. The analysis of the data was made in an explanatory manner and presented in tables.

RESULTS

We analyzed a total of 52 stethoscopes diaphragms of Medical students from *Centro Universitário de Patos de Minas*, in which 26 (50%) were contaminated. Of the samples contaminated, none showed growth in an EMB, only SMA, and SM.

To analyze the bacteria we used the Gram staining technique, to characterize the bacteria with positive growth in SMA, and this presented the following results: 6 slides showed growth of Cocci Gram-negative and positive; 7 slides showed growth of Cocci Gram-negative; 6 slides showed growth of Cocci Gram-positive; 3 slides showed growth of Bacilli Grams positive; and 4 slides showed growth of Bacilli Gram-negative. The data is represented in Table 1.
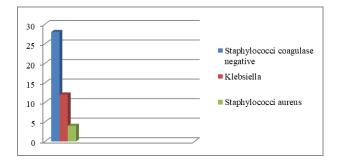
Among the contaminated stethoscopes, 65% (n=17) showed Gram negative bacteria, while 35% (n=9) showed Gram positive. In relation to the morphological characteristics, 73% (n=19) were cocci and 27% (n=7) were bacilli.

Table 1: Gram staining method

Number of stethoscopes contaminated	Characteristics
6	Cocci Gram – and +
7	Cocci Gram -
6	Cocci Gram +
3	Bacilli Gram +
4	Bacilli Gram -
Total: 26	

Source: Research data, 2018.

In both samples, there was a growth of more than one species of bacteria. We isolated (54%) of *Stapfylococci sp* coagulase-negative, (23%) strains of *Klebsiella sp* and (7.6%) of *Staphylococci aureus* in the diaphragms sampled (Figure 1).

Source: Research data, 2018.

Figure 1. Presence and identification of bacteria in the surface os stethoscopes of Medical students of UNIPAM

DISCUSSION

Results showed that 50% (n=26) of stethoscopes sampled were contaminated by microorganisms, the ratio was inferior to the results found in the study by Dutra et al.[4], in which 96.2% (n=78) were contaminated. The difference can be justified due to the fact that in the study by Dutra et al.[4] doctors' and nurses' stethoscopes were analyzed, in which 79% (n=64) were in the responsibility of nurses, and 21% (n=21) were in the responsibility of doctors, while in the present study it was only analyzed the stethoscopes of medical students.

In a study by Xavier and Ueno[9], done in a pediatrics unit, also had superior results: 86.8% (n=33) justified by the fact that the doctors do less disinfection than medical interns, according to a questionnaire done in the study mentioned.

The number of contaminations in the present study was inferior to the other two because the stethoscopes were in ICU environments, which is due to the fact that the medical students of UNIPAM do not have direct contact with ICUs.

Concerning the staining characteristics, we must stress that there was a finding of a great number of Gram-negative bacteria. The isolation of Gram-negative microorganisms represents a real risk of potentially grave infections, even though they have low toxicity, they are not easily neutralized by antitoxins[3,4].

This fact can be found among the studies by Neves[5] and Sales et al.[6], as both works found a prevalence of Gram-positive germs. One of the possible reasons for this discrepancy of results is the fact that these studies were done in lab coats that have a lot of skin contact, which

has a microbiota composed mainly of Gram-positive microorganisms.

Yet Sales et al.[2], who analyzed the presence of bacteria in surfaces of an intensive care unit, also found a great prevalence of Gram-negative germs, which agrees to what was found in this study.

In a hospital unit in Juiz de Fora/MG 22 stethoscopes were analyzed, of which 72.7% showed microbial growth. In this study, done by Garcia et al.[10] *Staphylococci* sp coagulase-negative were isolated, strains of *Acinetobacter baumannii*, *Enterococci* sp., and *Staohylococci aureus*, which corroborates with the current study.

CONCLUSION

The results allow us to conclude that the presence of pathogenic microorganisms contaminating the surface of the stethoscopes analyzed could represent a source of pathogenic bacterial dissemination contributing to IAHC.

In this sense, we stress the practice of good sanitation of stethoscopes diaphragms should be better disseminated in teaching institutions, having in mind that students are in constant contact with patients, and this instrument represents an important fomite of contamination.

Authors participation: *Lyza Alencar Siqueira*: main author. Responsible for the idea of the project, writing, collecting, and analyzing the samples. *Liliane Silva Anjos*: co-author. Responsible for helping write the project, collecting and analyzing the samples, and analyzing them. *Thainá Pereira Nascimento*: co-author. Responsible for helping write the project, collecting and analyzing the samples, and analyzing them. *Valeska Balen Ronsoni*: co-author. Responsible for helping write the project, collecting and analyzing the samples, and analyzing them. *Marilene Rivany Nunes*: co-supervisor. Responsible for guiding the written part of this project. *Ma. Bethânia Christine De Araújo*: Supervisor. Responsible for the idea, guiding the sampling and analyzing the material, as well as the writing.

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