

Caracterización bacteriana de superficies inertes de unidades móviles de atención pre hospitalaria

Bacterial Characterization of Inert Surfaces of Mobile Pre-Hospital Care Units

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Palabras claves:

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Resumen

Introducción. Las unidades de atención prehospitalaria permiten el traslado de pacientes vulnerables a diferentes instituciones de salud para su atención oportuna por parte del profesional médico. Sin embargo, pueden ser fuente de contaminación por la transmisión indirecta de microorganismos, principalmente bacterias que puedan alterar la salud e integridad de las víctimas, representando un riesgo en pacientes y personal de atención médica. **Objetivo.** Fue caracterizar las bacterias presentes en superficies inertes de unidades móviles de atención prehospitalaria de la ciudad de Cuenca en las áreas de mayor manipulación por parte de los primeros respondientes y otras áreas como ventilación, zona de desechos bio-peligrosos, manijas, entre otros. **Metodología.** La presente investigación es de tipo no experimental, de corte transversal y de campo. Para la selección del universo, se tomaron a las unidades móviles de atención pre hospitalaria públicas de la ciudad de Cuenca. A conveniencia del estudio se consideraron las tres unidades móviles de mayor uso, el muestreo fue de tipo no probabilístico por conveniencia, considerándose 30 sitios de mayor manipulación por parte del personal sanitario para obtener la muestra por cada ambulancia. **Resultados.** Se identificó principalmente el género *Staphylococcus*, y se verificó resistencia a la Oxacilina y Vancomicina, mientras que las demás presentaron sensibilidad a los demás antibióticos que son Claritromicina, Amoxicilina + Ácido Clavulánico y Ceftriaxona. **Conclusión.** El estudio permitió identificar los agentes bacterianos presentes en las superficies inertes de tres de las doce unidades móviles de atención prehospitalaria, observándose que en su mayoría eran cocos Grampositivos. **Área de estudio general:** Bioquímica y Farmacia. **Área de estudio específica:** Microbiología. **Tipo de estudio:** Artículos originales / Original articles,

Abstract

Introduction. Pre-hospital care units facilitate the transfer of vulnerable patients to different health institutions for timely care by medical professionals. However, it can be a source of contamination due to the indirect transmission of

microorganisms, mainly bacteria, that can alter the victims' health and integrity, representing a risk for patients and healthcare personnel. objective. To characterize the bacteria, present on inert surfaces of mobile pre-hospital care units in Cuenca in the areas of greatest manipulation by first responders and other areas such as ventilation, biohazardous waste area, and handles, among others. Methodology. This is a non-experimental, cross-sectional field study. For the selection of the universe, the mobile units of public pre-hospital care in the city of Cuenca were used. For the convenience of the study, the three most frequently used mobile units were considered. The sampling was non-probabilistic by convenience, considering 30 sites of greatest manipulation by health personnel to obtain the sample for each ambulance. Results. The genus *Staphylococcus* was mainly identified, and resistance to Oxacillin and Vancomycin was verified, while the others showed sensitivity to the other antibiotics Clarithromycin, Amoxicillin + Clavulanic Acid, and Ceftriaxone. Conclusion. It was possible to identify the bacterial agents present on the inert surfaces of three of the twelve mobile pre-hospital care units, and it was observed that most of them were Gram-positive cocci.

Introduction

Pre-hospital care vehicles known as ambulances and the personnel who circulate in them are essential when verifying the health status and physical condition of a patient (1) and are intended to care for patients and transfer patients until they arrive at hospitals for specialized care if required. Therefore, they are subject to contact with a variety of potentially pathogenic microorganisms on surfaces that can be harmful to health and can be transmitted mainly by air, parenteral, ocular, digestive and dermal routes. The most frequently found microbial agents are bacteria and their presence could be due to the lack of hygiene of patients, infections present in both patients and personnel who care for them, application of poor cleaning and disinfection techniques, generating cross contamination in their system (2).

When we talk about bacteria, we refer to unicellular organisms that are present almost everywhere in the world and are essential for the ecosystem. Certain species are able to

live in adverse conditions of temperature and stress. Most of the bacteria that inhabit the human body do not cause harm and some are beneficial. However, a relatively small number of species are pathogenic, mainly when a patient's immune system is depressed, and can cause so-called nosocomial infections that in some cases lead to stronger treatments, longer hospital stays or death (3).

On the other hand, the ambulance or mobile pre-hospital care unit is an important part of the patient's care environment during their transfer; therefore, the universal principles of biosecurity must be applied in the same way, both for patients and professionals. It must be remembered that the inert surfaces of an ambulance are still susceptible to microbiological contamination and are considered a possible potential source for the transmission of different potentially pathogenic microorganisms due to the transfer of patients from one place to another or to hospitals. These patients sometimes present diseases of bacterial origin that are transmissible by their source of infection, which can be airborne, by contact or mixed (4).

According to the World Health Organization (WHO), around 1.4 million patients treated in hospitals each year acquire infections of various origins through direct or indirect (cross) transmission as a result of contact with contaminated surfaces or poor handling by health personnel (5). These data demonstrate the serious problem and reality of the situation in the health area and the importance of carrying out adequate treatments, mainly identifying the agents causing infections.

According to Salazar (6), infections known as intrahospital or nosocomial are considered as infectious processes that can be transmitted between patients or health personnel and that occur 48 to 72 hours after admission to the hospital. These infections were not present in the incubation period at the time of admission or manifest up to 72 hours after discharge. Likewise, they are defined as those that were acquired at the time the patient has entered the hospital areas and represent a serious problem at a local, national and global scale, since they are directly related to an increase in morbidity and mortality rates, as well as hospital costs for patients, family in general and society (7).

Based on the above, the importance of research carried out that highlights the importance of preventing indirect transmission and characterizing microbial agents is pointed out. Thus, we have the study carried out by Silva et al., which showed that in Brazil around 20% of the population infected with COVID-19 was made up of health professionals, including personnel in charge of transferring infected patients. 18% of health care personnel in the emergency area and pre-hospital care in ambulatory medical units tested positive (8), thus demonstrating that microorganisms can be transmitted directly through the respiratory tract and cause infections to patients or health personnel in general. Although it is true that the article mentions viruses, the transmission mechanisms are the same for all microorganisms.

The research developed by Acosta on healthcare-associated infections (HAIs) can also be detailed, showing that they pose a threat to health personnel and mainly to immunologically compromised patients, since it is estimated that, out of every 20 hospitalized patients, one will be infected simply by entering the hospital facilities, mainly by bacterial agents. These data are aggravated by the resistance to antibiotics that bacteria have developed and cause higher mortality rates every year than HIV/AIDS or influenza (9).

The above is demonstrated by the research carried out by Plasencia et al., where they found high contamination with gram-negative bacilli in hospital areas, reaching up to 50.87% of the total samples obtained in a third level Social Security hospital in Chiclayo, Peru. The authors stated that *Acinetobacter baumannii* was the microorganism with the highest percentage of isolation. Additionally, other species such as *Pseudomonas*, *Klebsiella* and Gram-positive bacteria such as *Staphylococcus* were also found (10).

Similar data were found in the research of Masó et al., where they verified a reduction in contamination due to the use of 20% potassium monopersulfate, a strong disinfectant, in relation to multi-resistant microorganisms in different environments and equipment used in hospitals, managing to identify mainly the *Acinetobacter* genus. The authors emphasized the importance of taking universal precautions to avoid cross contamination, as well as the importance of cleaning and disinfection processes (11).

The aforementioned research demonstrates the serious public health problem at the hospital level, however, in particular, in ambulances, bacterial contamination is also observed. This is demonstrated by Rivera et al., in their research, where they identified the presence of microorganisms present in ambulances in Colombia, finding genera such as *Micrococcus* and *Staphylococcus* mainly. Additionally, the samples that resulted with growth were mainly in ventilation areas, door handles, patient stretchers and medicine drawers (12). Likewise, the research demonstrates the problem that health personnel face in maintaining spaces free of microorganisms and preventing infections.

Therefore, it is necessary to identify the possible cause or source that could generate the infection in order to establish the appropriate treatments for the patient or to establish preventive measures and a possible infection whose consequences could be fatal. In the case of a bacterial infection, it is necessary to correctly identify the etiological agent in order to establish the necessary treatments with antimicrobials.

The research carried out by Matute (13) can be mentioned, where he points out that infections of bacterial origin are a major problem for health professionals, patients and the community in general. The causes of these infections are due to several factors, such as failure in sterilization and disinfection protocols, indiscriminate use of antibiotics and

an inadequate process by health personnel in the protocols for handling patients or medical supplies in surgical procedures.

Bacteria are the protagonists due to their characteristic of resistance to antibiotics. Therefore, it is essential not only to know but to acquire skills in biosecurity and asepsis protocols in the hospital area to maintain good patient care. Similarly, in the research carried out by Díaz et al. (14) the state of cleaning, disinfection and sterilization of mobile pre-hospital care units was analyzed and concluded that they turn out to be reservoirs of microorganisms and inadequate disinfection would facilitate the development of infections. Likewise, various studies have shown that the disinfection procedures carried out to reduce the microbial load have allowed it to be reduced by up to 90% of ambulances.

Similarly, in the study conducted by Alves & Bissell (15), the bacterial pathogens found in the vehicles used by the medical emergency service were qualitatively evaluated. The main conclusion obtained from this research was that, of the seven genera mainly isolated such as *Stenotrophomonas maltophilia*, *Pseudomonas*, *Bacillus*, *Staphylococcus epidermidis*, *Klebsiella*, *Streptococcus viridans*, *Acinetobacter*, four were nosocomial pathogens of importance to health while three of these four presented resistance to antibiotics, even though the antibiotics used were not specified.

Given the concern about the potential number of infectious agents in healthcare settings, microbiologists and infectious disease experts recommend the use of disposable personal protective equipment that significantly reduces risks to patients and healthcare personnel. However, inert surfaces in ambulances remain susceptible to bacterial contamination. On the other hand, the nature of emergency medical services imposes a number of pressures through often standardized procedures on prehospital care personnel (16).

According to the Pan American Health Organization (PAHO), nosocomial infections are mostly caused by multi-resistant bacteria that have survived the hospital environment and can cause death or leave serious sequelae in patients hospitalized in different areas. Hence the importance of characterizing the microorganisms present to verify their resistance to antimicrobials, with which appropriate treatments can be proposed and following standardized procedures and care is essential to prevent their spread, as well as safety standards to prevent the transmission of microorganisms (17).

A cross-sectional study carried out by Quindós (18) determined that after the analysis of the different samples, the team from the Public University of the Basque Country has indeed detected the existence of *Staphylococcus aureus* and *Staphylococcus epidermidis* and other gram-positive cocci, in addition to gram-negative bacilli which, although it is true, did not reach alarm levels, they do generate an alert in view of the possibility of

causing cross-contamination between the inside and outside of hospital areas through transfers made by ambulances.

No local sources were found detailing bacterial infections acquired through contamination in pre-hospital care units.

Methodology

The focus of this research is a quantitative, non-experimental, cross-sectional, analytical and field study. To determine the universe, the public mobile pre-hospital care units of the city of Cuenca were selected. For the convenience of the study, the three mobile units with the highest turnover in the city were considered: station 1 (E1), three (E3) and five (E5), with station three being the alpha ambulance for advanced life support. The sites of greatest manipulation by the staff were considered for each mobile unit, for which, the sampling was of a non-probabilistic type for convenience, considering 30 sites of greatest manipulation by the health personnel to obtain the sample for each ambulance, including door handles, handrails of stretchers and those used by health personnel, hospital waste site, ventilation sites, among others.

Samples were collected from the mobile units using the surface swab technique and stored in Stuart medium. For the identification of bacterial agents, the bacteria were seeded in Petri dishes using blood agar (BA) and eosin methylene blue agar (EMB) and left to incubate at 37° C for 24 hours using exhaustion streaking. After this period of time, the colonies and the presence of hemolysis in BA were analyzed. Subsequently, with each sample that showed growth on blood agar, the resulting bacteria were isolated in volumetric test tubes with nutrient agar and incubated again under similar conditions as the first incubation.

Subsequently, the culture was carried out on mannitol agar and a Gram stain was performed. Additionally, with the sample that resulted in growth on EMB agar, biochemical tests were performed for bacterial identification, which were TSI (Triple-Sugar-Iron-Agar or Triple Sugar Iron Agar), SIM (Sulfide Motility Indole Medium), Citrate and Urea, on the other hand, coagulase and catalase tests were also performed for Gram-positive bacteria to continue with the identification.

Finally, antimicrobial susceptibility tests were performed using the Kirby Bauer technique, for which Mueller-Hinton agar was used. A bacterial suspension was prepared and compared in the spectrophotometer until an absorbance of 0.08 - 0.10 at 600 nm was obtained. The bacteria were seeded with the suspension and antimicrobials that inhibit rapid growth such as *Staphylococcus* spp or the Enterobacteriaceae family, such as Oxacillin (OX), Clarithromycin (CLR), Vancomycin (VA), Amoxicillin + Clavulanic Acid (AMC) and Ceftriaxone (CRO) were used (30).

Results

The results of the research show the sites where there is the greatest manipulation by healthcare personnel within each of the mobile pre-hospital care units. Of a total of 90 sites analyzed, growth was observed in 34 sites, which are shown in Figure 1.

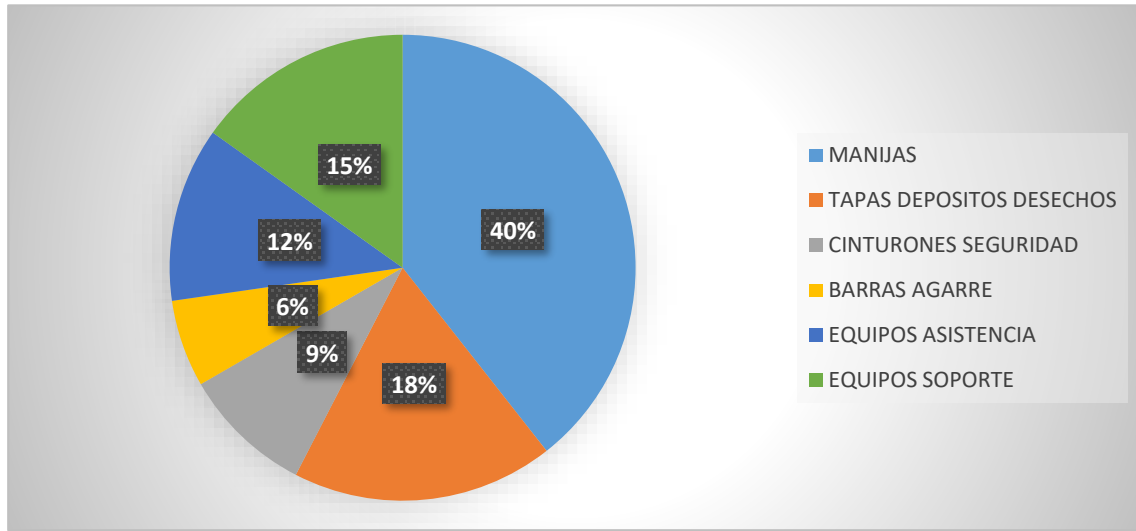


Figure 1.Percentage of bacterial growth based on surfaces

Figure 1 shows the percentage of bacterial growth on blood agar. In general, from the sampling carried out previously, the surface with the greatest growth was mainly on door or gate handles, due to direct dermal contact by pre-hospital care personnel. Despite handling the patient and using gloves, which constitute a primary protection barrier for reducing the transmission of microorganisms, contamination is evident on these surfaces.

On the other hand, the growth observed on EMB agar was in a single season (Table 1). Gram staining was performed, which showed Gram-negative bacilli. The biochemical tests performed were negative and are detailed in Table 2, while Table 3 shows the results of growth on Mannitol Agar, which was also subjected to Gram staining, showing Gram-positive cocci.

Growth on EMB agar is obtained from buttons from oxygen assistance equipment.

Table 1.Bacterial growth on EMB agar

| No. | Season | Sample | Sampling location |
|-----|--------|--------|-------------------|
| 1 | E5 | 21 | Airway buttons |

Table 2. Biochemical tests of colonies with growth on EMB agar

| No. Sample | Season | Biochemical tests | Result |
|------------|--------|-------------------|--------|
| 21 | E5 | Saccharose | - |
| | | Glucose | - |
| | | Lactose | - |
| | | Mobility | - |
| | | H2S | - |
| | | Indole | - |
| | | Citrate | - |
| | | Urea | - |
| | | Gas | - |

As an interpretation of the results obtained in the biochemical tests, glucose fermentation was evident, but not lactose or saccharose, gas production was observed. Urea was negative. Similarly, no hydrogen sulfide production was found in TSI. According to the bacterial identification table adapted by Elmer W. Koneman (31) and Jean F. Mc. Faddin (32), a similarity in the results with *Escherichia coli* can be seen based on the results obtained in relation to the table below.

Table 3. Biochemical Identification of Enterobacteriaceae

| Identificación bioquímica de Enterobacterias * | | | | | | | | | | | | | | | | | | | |
|--|-------------------------|-----------------|-------------------|-----------------------------|---------------------------|---------------------|------------------------------|---------------------------|-------------------------------|-----------------------------|---------------|-----------------|-------------------------|--------------------------|----------------------------|--------------------|--------------------------------|---------------------------------|-----|
| | <i>Escherichia coli</i> | <i>Shigella</i> | <i>Salmonella</i> | <i>Citrobacter freundii</i> | <i>Citrobacter koseri</i> | <i>Edwardsiella</i> | <i>Klebsiella pneumoniae</i> | <i>Klebsiella oxytoca</i> | <i>Enterobacter aerogenes</i> | <i>Enterobacter cloacae</i> | <i>Hafnia</i> | <i>Serratia</i> | <i>Proteus vulgaris</i> | <i>Proteus mirabilis</i> | <i>Morganella morganii</i> | <i>Providencia</i> | <i>Yersinia enterocolitica</i> | <i>Plesiomonas shigelloides</i> | |
| Indol | + | -/+ | - | - | + | + | - | + | - | - | - | - | + | - | + | + | + | +/- | + |
| Rojo de Meflo | + | + | + | + | + | + | +/- | +/- | - | - | +/- | -/+ | + | + | + | + | + | + | +/- |
| Voges-Proskauer | - | - | - | - | - | - | + | + | + | + | +/- | + | - | -/+ | - | - | - | +/- | - |
| Citrato de Simmons | - | - | + | + | + | - | + | + | + | + | - | + | +/- | +/- | - | + | - | - | |
| H ₂ S(TSI) | - | - | + | + | - | +/- | - | - | - | - | - | - | + | + | -/+ | - | - | - | |
| Ureasa | - | - | - | -/+ | +/- | - | +/- | +/- | - | +/- | - | +/- | + | + | + | -/+ | +/- | - | |
| Movilidad | +/- | - | + | + | + | + | - | - | + | + | + | + | + | + | + | -/+ | +/- | + | |
| Omitina | +/- | -/+ | + | - | + | +/- | - | - | + | + | + | + | + | + | + | - | + | + | |
| Lisina | + | - | + | - | - | + | +/- | + | + | - | + | + | - | - | +/- | - | - | + | |
| Fenilalanina | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | |
| Glucosa | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Gas de glucosa | + | - | + | -/+ | + | +/- | + | + | + | + | + | +/- | +/- | + | + | + | - | + | |
| Lactosa | +/- | - | - | +/- | +/- | - | + | + | + | + | - | - | - | - | - | - | - | + | |
| Sacarosa | - | - | - | +/- | +/- | +/- | +/- | + | + | + | - | + | + | +/- | - | +/- | + | - | |
| Malonato | - | - | +/- | -/+ | + | - | -/+ | +/- | + | +/- | +/- | - | - | - | - | - | - | - | |
| Pigmento rojo | - | - | - | - | - | - | - | - | - | - | - | +/- | - | - | - | - | - | - | |
| Oxidasa | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | |

* Géneros comunes en infecciones en humanos.

Adaptado por MC. Conna Hernández Mirón Macfadden, 2003 y Koronka, 2006

Familia Enterobacteriaceae
14 *Shigella*, *Salmonella*, *Escherichia*, *Citrobacter*, *Edwardsiella*, *Klebsiella*, *Enterobacter*, *Hafnia*, *Serratia*, *Proteus*, *Morganella*, *Providencia*, *Yersinia*, *Plesiomonas*

23 *Arsenophonus*, *Buchnera*, *Burkholderia*, *Burkholderia*, *Calymmatobacterium*, *Cedecea*, *Cluveria*, *Erwinia*, *Empedea*, *Lectera*, *Lemnorella*, *Moraxella*, *Oesombacterium*, *Pantoea*, *Photobacterium*, *Pragia*, *Rahnella*, *Saccharobacter*, *Tatumella*, *Trabucsisella*, *Wigglesworthia*, *Xenorhabdus*, *Yokenella*

Within the Mannitol agar culture medium, a change in the medium was observed, which allows us to suspect the presence of *S. aureus*, which changes the medium from pink to yellow, and *S. epidermidis*, which only turns pink.

Table 4. Growth on mannitol salt agar and catalase and coagulase tests

| Season | Sample | Catalase | Coagulase | Sampling location | Turn |
|--------|--------|----------|-----------|------------------------------|------|
| E1 | 4 | + | - | Biohazardous trash can lid 2 | Pink |

Table 4. Growth on Mannitol Salt Agar and Catalase and Coagulase Tests (continued)

| Season | Sample | Catalase | Coagulase | Sampling location | Turn |
|--------|--------|----------|-----------|-------------------|------|
|--------|--------|----------|-----------|-------------------|------|

| | | | | | |
|----|----|---|---|------------------------------|--------|
| E1 | 8 | + | + | Defibrillator handle 1 right | Yellow |
| E1 | 13 | + | + | Left stretcher handle | Yellow |
| E3 | 30 | + | - | Side suction cup | Pink |
| E5 | 4 | + | + | Defibrillator Handle 2 | Yellow |
| E5 | 19 | + | + | Top bar | Yellow |

Based on the tables described above, it can be seen that the highest number of bacterial growth in general occurred in the units of stations 1 and 5, while the unit of station 3 showed a lower number of bacterial growth.

On the other hand, for susceptibility tests and according to what was indicated by Picazo J. in the manual of procedures in Clinical Microbiology (29), the deduction of resistance or sensitivity to an antibiotic is based on the type of bacteria and antibiotic to which it is exposed, resulting in growth and the formation of inhibition halos, which are read in millimeters based on the standard ranges for each antibiotic (6). The results of the antibiotic susceptibility test to verify its susceptibility can be seen in the following table:

Table 5.Antibiotic susceptibility testing

| Sample | Season | Antibiotic | Halo inhibition | of | Result |
|--------|--------|--------------------|-----------------|----|-----------|
| 4 | E1 | OX (<10- >13 mm) | 5 mm | | Resistant |
| | | AMC (<18- >22 mm) | 14 mm | | Sensitive |
| | | CRO (<10 - >13 mm) | 15 mm | | Sensitive |
| | | CLR (<13 - >18 mm) | 20 mm | | Sensitive |
| | | VA (<12 - >15 mm) | 20 mm | | Sensitive |
| 8 | E1 | OX (<10- >13 mm) | 6 mm | | Resistant |
| | | AMC (<18- >22 mm) | 23 mm | | Sensitive |
| | | CRO (<10 - >13 mm) | 18 mm | | Sensitive |
| | | CLR (<13 - >18 mm) | 18 mm | | Sensitive |
| | | VA (<12 - >15 mm) | 7 mm | | Resistant |

Table 5.Antibiotic Susceptibility Testing (continued)

| Sample | Season | Antibiotic | Halo of inhibition | Result |
|--------|--------|--------------------|--------------------|-----------|
| 13 | E1 | OX (<10- >13 mm) | 14mm | Sensitive |
| | | AMC (<18- >22 mm) | 23mm | Sensitive |
| | | CRO (<10 - >13 mm) | 14mm | Sensitive |
| | | CLR (<13 - >18 mm) | 19mm | Sensitive |
| | | VA (<12 - >15 mm) | 17mm | Sensitive |
| 30 | E3 | OX (<10- >13 mm) | 1 mm | Resistant |
| | | AMC (<18- >22 mm) | 30 mm | Sensitive |
| | | CRO (<10 - >13 mm) | 14 mm | Sensitive |
| | | CLR (<13 - >18 mm) | 20 mm | Sensitive |
| | | VA (<12 - >15 mm) | 18 mm | Sensitive |
| 4 | E5 | OX (<10- >13 mm) | 15mm | Sensitive |
| | | AMC (<18- >22 mm) | 23mm | Sensitive |
| | | CRO (<10 - >13 mm) | 18mm | Sensitive |
| | | CLR (<13 - >18 mm) | 19mm | Sensitive |
| | | VA (<12 - >15 mm) | 17mm | Sensitive |
| 19 | E5 | OX (<10- >13 mm) | 16mm | Sensitive |
| | | AMC (<18- >22 mm) | 24mm | Sensitive |
| | | CRO (<10 - >13 mm) | 16mm | Sensitive |
| | | CLR (<13 - >18 mm) | 20mm | Sensitive |
| | | VA (<12 - >15 mm) | 17mm | Sensitive |

The following table shows the standard measurement ranges based on the clinical microbiology procedures manual.

Table 6.Resistance or sensitivity in the presence of an antibiotic

| Antibiotic | Standard range |
|----------------------|---------------------------------------|
| Oxacillin (OX) | ≤10mm (Heavy Duty); >13mm (Sensitive) |
| Clarithromycin (CLR) | ≤13mm (Heavy Duty); >18mm (Sensitive) |

Table 6.Resistance or sensitivity in the presence of an antibiotic (continued)

| Antibiotic | Standard range |
|-------------------------------------|---------------------------------------|
| Vancomycin (VA) | ≤12mm (Heavy Duty); >15mm (Sensitive) |
| Amoxicillin + Clavulanic Acid (AMC) | ≤18mm (Heavy Duty); >22mm (Sensitive) |
| Ceftriaxone (CRO) | ≤10mm (Heavy Duty); >13mm (Sensitive) |

According to the data obtained, it can be observed that all the bacteria found that turned out to be catalase and coagulase positive do not generate inhibition halos against the antimicrobials used, which is striking that some of the bacteria found also presented resistance to certain antibiotics, which represents a serious problem for the health of patients, since their state of vulnerability would favor the development of an infection in them, generating what are called intra-hospital infections by a cross-transmission mechanism with first responders.

Additionally, mobile units are used as a means to transport victims who in most cases are in critical condition, hence the importance of keeping them under aseptic conditions to avoid direct or indirect transmission (8), consequently it should be noted that in the research the Kirby Bauer technique was used to obtain results related to susceptibility tests. However, no disinfectants, antiseptics or biocides in general were used, which allow observing the formation of inhibition halos or including other disinfectant products to analyze their behavior against this type of bacteria.

The possible interaction between disinfectants and the culture medium was also not considered. Finally, it is necessary to mention that the results showed growth in 33 of the samples analyzed. However, only six were considered for further analysis, leaving aside the other samples with growth on blood agar (9).

The results obtained confirmed the presence of bacteria that could be pathogenic to health, which in turn are resistant to disinfectants, which suggests that they are multi-resistant bacteria even with the ability to survive on inert surfaces where environmental conditions are variable. Additionally, it can be observed that the concentrations of the disinfectants used according to the Disinfection Guide do not allow a real disinfection process, which is why it is suggested to expand research to use other types of disinfectants or a combination of these that allow a synergy between them. It is also recommended to train and educate the personnel who perform the cleaning and disinfection of inert surfaces (9).

Discussion

The objective of the research is mainly focused on bacterial identification, using laboratory methods and techniques for identification, in the same way using reagents and materials that facilitate obtaining results, within which as a result of the study, the presence of bacterial agents such as gram-positive cocci was identified on the inert surfaces investigated in the mobile pre-hospital care units, which were resistant to Oxacillin (OX), Clarithromycin (CLR), Vancomycin (VA), Amoxicillin + Clavulanic Acid (AMC) and Ceftriaxone (CRO).

Similar results to those found in this study have been confirmed by other studies that reveal that ambulance surfaces are subject to a high source of contamination with the possibility of infection or colonization. Among the investigations is the systematic review carried out by Obenza et al. who verified that in 16 studies in 8 different countries: Denmark, Egypt, Germany, Iran, Saudi Arabia, South Korea, Spain, and the USA, the presence of pathogenic bacteria was found in the patient care compartment and on a variety of surfaces in ambulances, reporting a high prevalence of organisms associated with acute respiratory infections. The most frequently contaminated sampled surfaces were located on the stretcher and its components such as handles and the organisms found corresponded to infections caused by methicillin-resistant *S. aureus* (MRSA), methicillin-resistant coagulase-negative Staphylococci (MRCoNS), coagulase-negative Staphylococci (CoNS), extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* spp. and extended-spectrum beta-lactamase (ESBL)-producing *E. coli* (19).

In a similar way to our study and an important finding of the cited work, is that the collection and analysis methods showed consistency between the studies, revealing that culture analysis was used in all studies, most used blood agar to help the growth of organisms or mannitol salt agar for the selection of Gram positive bacteria and the swab method was used for sample collection, which allows us to assert that these methods seem to be sufficient to recover microorganisms in this environment.

Regarding the importance of vehicle cleaning protocols, the need to ensure complete decontamination of all exposed surfaces, equipment and contact areas before and between each patient transport is mentioned (20). In this regard, experts point out that it is described that the disinfection of high-contact areas reduces the bacterial load of inanimate surfaces (21), and they also agree that infections caused by these drug-resistant bacteria not only result in greater morbidity and mortality, but also lead to more complex hospitalizations (22).

A limitation of this work is that it was not possible to find evidence of similar studies in the context of the country. In this regard, Muñoz et al. report that in Ecuador the expansion of prehospital care has occurred very quickly, but with poor control of the service they

provide (23). Burbano and Carrasco state that in the Ecuadorian National Health System there is a separation between prehospital and hospital care services and they attribute to the complexity of the system the segmentation that characterizes it and to which they attribute all its defects; they also highlight the lack of a provision by the health authority for public and private ambulances to be considered another health service and therefore be subject to control by it (24).

In accordance with this, Peñafiel supports the above and confirms that pre-hospital care in Ecuador in relation to the ambulance service is assumed by the country's fire departments, in order to cover the urgent needs of the community in the face of the inefficiency of the national health system, most of the time without complying with the essential standards for the development of this vital activity (25). As part of this investigation, it was possible to confirm the existence of the Cleaning and Maintenance Procedure for Pre-Hospital Care Equipment of the Specialized Ambulance Division of the Benemérito Cuerpo de Bomberos de Guayaquil, which incorporates the cleaning and disinfection instructions for medical equipment and surfaces of ambulances, which must be complied with by the Paramedic and Driver personnel that comprise it (26).

However, other studies recognize that the existence of pathogenic organisms in ambulances is due to total or partial non-compliance with cleaning procedures and strongly recommend the implementation of other procedures through regular and scheduled decontamination programs for cleaning, decontamination and disinfection of vehicles by personnel specialized in infection control through the use of appropriate disinfectants (27, 28).

This research revealed the presence of bacterial agents at the sampling sites of mobile pre-hospital care units, indicating that disinfectants have not been used appropriately or that they have been cleaned incorrectly.

Conclusions

- The study identified the bacterial agents present on the inert surfaces of three of the twelve mobile pre-hospital care units, observing that the majority were Gram-positive cocci.
- The bacteria identified in the mobile units studied that were catalase and coagulase positive when sown on Mannitol salt agar after the incubation period were used for susceptibility tests to antimicrobials and disinfectants. It was observed as a characteristic that they did not generate inhibition halos against the concentrations

of the disinfectant products used for disinfection and part of them showed resistance to antimicrobials.

- The verification of the bacteria to antimicrobial resistance was confirmed, resistance was evidenced to 2 (Oxacillin and Vancomycin) of the 5 antibiotics used in the sensitivity test to verify their susceptibility to antimicrobials. In addition, a possible resistance to disinfectants such as ethanol and sodium hypochlorite, which allowed us to assume that they are multi-resistant bacteria with the ability to survive on inert surfaces under variable environmental conditions.

Conflict of interest

The authors declare that they have no conflict of interest in relation to the submitted article.

Authors' contribution statement

Author 1: Participated in the interpretation of the researched content, as well as with the design corrections of the article itself.

Author 2: Contributed to the analysis and development of the research to create a clear and concrete discussion, and carried out surface sampling for the research.

Author 3: Provided ideas and opinions for the correction of the format, conducted a complete review of the research so that it contains accurate information. Participated in the completion of results and conclusions.

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