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¿Emergency ambulances potential source of infections? An assessment of cleaning and disinfection procedures

Ambulacias como potencial fuente de infección? Una evaluación de los procedimientos de limpieza y desinfección

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Abstract

Objective: Evaluate the cleaning and disinfection procedures (CDP) in six ambulances from three different entities in Pereira (Risaralda-Colombia).

Methods: Cross-sectional descriptive study frequencies of presence/absence were calculated from data obtained in the bacterial growth results before and after CDP, taking samples in three different places on the ambulances, being: back door, stretcher and wall next to the patient, before and after said processes. Additionally, surveys were carried out at the domicile of companies providing pre-hospital transport service.

Results: Were positive 77.8% of the samples. The most frequent morphology in the study was gram-positive cocci, which remained in a greater proportion after disinfection. At the microbiologic level, most of these suggested being *Staphylococcus aureus*-type. By implementing CDP, microbiological isolates were eliminated in 33.3%, being the door the ambulance area, which showed the greatest decrease (50.0%).

Conclusions: A high prevalence of potentially pathogenic microorganisms exists in non-critical points of emergency ambulances. With CDPs, a decrease in microorganisms is achieved, but not their elimination, leaving in evidence that different factors must be considered in order to improve these CDP.

Resumen

Objetivo: Evaluar los procedimientos de limpieza y desinfección (CDP, por sus siglas en inglés) en seis ambulancias de tres entidades diferentes en Pereira (Risaralda, Colombia).

Métodos: Se calcularon frecuencias descriptivas de presencia / ausencia de estudio de corte transversal a partir de los datos obtenidos en los resultados de crecimiento bacteriano antes y después de la CDP, tomando muestras en tres lugares diferentes en las ambulancias, siendo: puerta trasera, camilla y pared al lado del paciente, antes y después de dichos procesos. Además, se realizaron encuestas en el domicilio de las empresas que prestan servicios de transporte prehospitalarios.

Resultados: El 77.8% de las muestras fueron positivas. La morfología más frecuente en el estudio fueron los cocos grampositivos, que se mantuvieron en mayor proporción después de la desinfección. A nivel microbiológico, la mayoría de estos sugirió ser *Staphylococcus aureus*-type. Al implementar CDP, se eliminaron los aislamientos microbiológicos en 33.3%, siendo la puerta el área de ambulancia, que mostró la mayor disminución (50.0%).

Conclusiones: Existe una alta prevalencia de microorganismos potencialmente patógenos en puntos no críticos de ambulancias de emergencia. Con los CDP, se logra una disminución de los microorganismos, pero no su eliminación, lo que deja en evidencia que se deben considerar diferentes factores para mejorar estos CDP.

Key Study Facts

Objective	To evaluate the cleaning and disinfection procedures (CDP) in six ambulances from three different entities in Pereira (Risaralda-Colombia).
Study design	Qualitative-descriptive.
Fuente de información	Primary and secondary.
Population / sample statistical analysis	Simple statistical analysis. Descriptive frequencies of the physical ambulance space. A total of six ambulances were analyzed in the study, to collected samples and cultured contaminated microorganism in different culture media. Additionally, a qualitative component was implemented by interviews with the personnel in charge of each emergency ambulance, including questions being assessed, such as: types of products implemented, frequency of implementation of CDP, among others
Main finding	There is a high prevalence of potentially pathogenic microorganisms in non-critical points of emergency ambulances.

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Introduction

Emergency ambulances constitute an essential part of healthcare systems. However, given the role they play can be a potential source of infections, both for patients and health personnel (1-3). Health systems verify compliance of basic conditions for the proper functioning of primary transport vehicles (such as qualified human resources, adequate equipment, maintenance, among others). Nevertheless, usually more attention is paid to the sterility of surgical environments, but the environment existing in ambulances that transport patients is not usually taken into account. For this reason, the control over the proper cleaning and disinfection procedures (CDP) for ambulances is not considered as an essential part for healthcare providers and epidemiological surveillance systems (1, 4).

Cleaning and disinfection processes must be implemented and strictly monitored to ensure the complete elimination of potentially pathogenic microorganisms (2). Within these processes, the use of disinfectant substances has been implemented. The main objective of its use is the reduction of pathogens and/or toxins to minimum levels. Additionally, these must guarantee that microorganism not produce adverse health events, as well as their ease in everyday use and by the operator (5).

However, when disinfection processes fail, they can lead to infections associated with healthcare services. Which are a major worldwide impact issue, since they can put the patient's life at risk, as well as lead to economic overloads for the healthcare system (6, 7), making the need of identifying and eliminating the microorganisms present in pre-hospital environments a priority (7, 8).

In Colombia, there is little information about microorganisms prevalence in ambulances (one of the main pre-hospital environments), so the objective of this study was to describe the presence of microorganisms in the greatest risk stratification areas and assess the effectiveness of CDPs usually implemented by companies providing pre-hospital transportation services (EPSPH, per its acronym in Spanish) in the city of Pereira (Risaralda, Colombia).

Methods

Study design and information gathering

This is a cross-sectional descriptive study, in which a convenience sampling was carried out including emergency ambulances from the city of Pereira (Risaralda Department), belonging to three different EPSPH. Two ambulances were randomly selected by each EPSPH, for a total of six ambulances analyzed in the study.
A qualitative component was implemented by carrying out interviews with the personnel in charge of CPDs of each emergency ambulance, including questions being assessed, such as: types of products implemented, frequency of implementation of CDP, among others.

Collection of samples

Three sampling sites were selected in each ambulance, recognized as noncritical points according to the risk stratification (2), that is: rear door handle, stretcher and wall adjacent to the patient. At each point, samples were collected at two moments: i) during the initial contact with each ambulance, after their transportation shift, and ii) after the usual cleaning and disinfection procedure 'CDP' carried out by the personnel in charge of each ambulance. Sampling moments were named as pre-CPD and post-CDP, respectively. (Figure 1).

Sample collection and processing

Samples for each sampling point and at each assessment time were collected with a sterile swab and transported through Stuart media (Oxoid, CM 0111). Subsequently, each person in charge applied the CDP usually implemented in each ambulance. After a waiting period (15-20 min after completing the CDP), the second sample was collected in each of the three sampling points previously described. Samples were processed in the Microbiology Laboratory from Universidad Libre (Pereira branch).

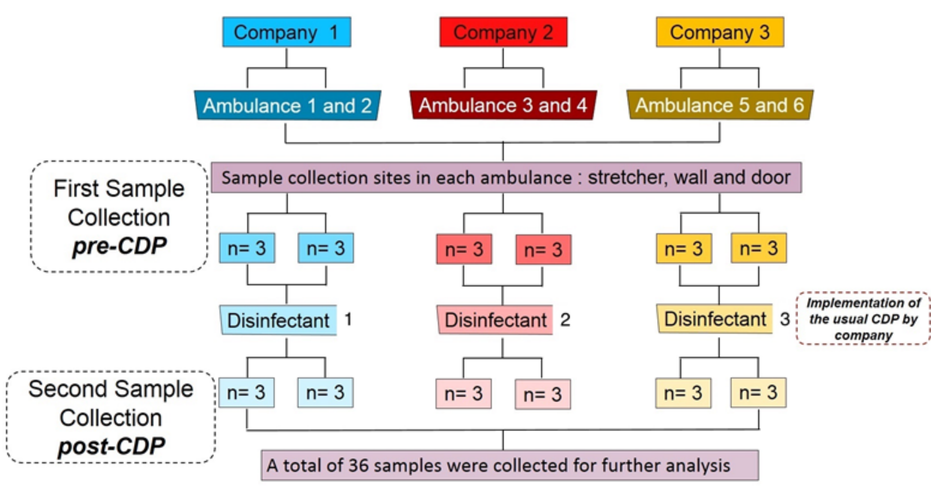

Figure 1. Flow diagram of the zones and the number of samples taken.

Microorganisms identification

The three samples pre-CDP and three post-CDP taken in the transportation services were carried to the laboratory and cultured through a streaking technique using a bacteriological loop in Blood Agar (BA; used as enrichment medium), Red Phenol Mannitol Saline Agar (RPMSA; for Gram-positive bacteria) and MacConkey Agar (MCK; for Gram-negative bacteria). In addition, the samples taken with saline solution were cultured in Tryptic Soy Agar (TSA) and cultured afterwards with Drigralskly loop massively on surface. All the cultured media were incubated for 48 hours at 37 °C.

After the count of colonies forming units (CFU) was performed, a macroscopic description of the colonies morphology was carried out in all the media implemented. Representative colonies from different morphologies were recovered, in a saline solution drop on slides. The microscopic morphology was described by routine inspection, after the implementation of Gram stain.

Statistical analysis

Taking the data obtained in the bacterial growth results before and after the disinfection process, presence or absence (percentages) / reduction, increase or evenness (percentages) of microbial load and site of higher microbial load, with their respective confidence intervals at a 95% reliability (95% CI).

Ethical responsibilities

This study was approved by the research center of the Faculty of Health Science of the Universidad Libre. This research did not use animal nor human material or data.

Results

Cleanings and disinfection procedures (CDP) description

Interviews with the personnel responsible for carrying out CDPs allowed identifying that EPSPH in the city of Pereira use between 2 and 5 different chemical products to carry out their routine CDP. The frequency of CDP implementation was very similar among EPSPH, being predominant the completion of a thorough ambulance cleaning once a week, a general cleaning a day at the shift end and a cleanup in case of fluid spills showing biological risk, the latter limited to the affected area. Although the objective in which CDPs are carried out are common to all EPSPH, the CDP implementation strategy varied according to the number of chemical products used. All the companies claimed to have disposable materials, certified suppliers, guaranteed by the national vigilance entity (Instituto de Vigilancia de medicamentos y alimentos – INVIMA-), as well as biosecurity elements for the operating team. CDP information for each EPSPH is described in Table 1.

Morphology of detected microorganisms

A total of 36 samples were analyzed in this study between pre-CDP and post-CDP from six ambulances (Figure 1). Results showed that 77.8% of the total samples collected presented microorganisms (n= 28; 95% CI: 60.8-89.8).

The macroscopic characteristics assessment on blood agar revealed two types of colonies; the first group corresponded to small, punctiform colonies with a beige-yellow coloration. The second

Table 1. Survey carried out to the personnel in charge of cleaning and disinfection procedures (CDPs) from the providing companies of healthcare services assessed in the study.

Question	Providing company of the emergency ambulance service		
	1	2	3
Which type of chemical products use for carrying out CDP procedures?	-Bactidin: Surfaces -Benzidine: Equipment	-Benzaldine: Surfaces and biological fluids -Deteridin: General cleaning -Enzidin: Metallic utensils -Bactidin: Equipment	-Deterganium: Surfaces -Sulfanium: Surfaces and fluids inactivation -Endozime: Instruments -Hydrogen peroxide: Fluids inactivation - Ambu: Plastic utensils
How frequently do you use CDP?	General cleaning per week Simple daily cleaning, at the end of the shift When fluids are spilled the affected area is cleaned	General cleaning per week Cleaning at the night when the shift change. A quick cleaning is done when fluids are spilled	A thorough cleaning at the end of each shift during the night A quick cleaning in the morning Between transfers, only if there is a spill of some type of fluid
What is the type of CDP that you carry out?	- Apply bactidin on all surfaces, and benzidine to clean equipment	Initially apply deterdine on all surfaces, followed by benzaldine, which is left on for about 5 minutes and removed with sterile towels	-Apply deterganium on all surfaces, leaving it to act for 5 minutes, then apply sulfanium and let dry. Sulfanium and hydrogen peroxide are used in case of a spill, such as vomiting or any biological fluid. Endozime is used to clean the equipment, at a concentration of 15 mL in 4 liters of water. For plastic material, ambu is used. If mud has entered the ambulance, it is washed with plenty of water. Operators use topical disinfectant gel
How do you carry out the CDP?	All companies carry out the process of disinfection from the inside out and top from down in a zigzag direction		

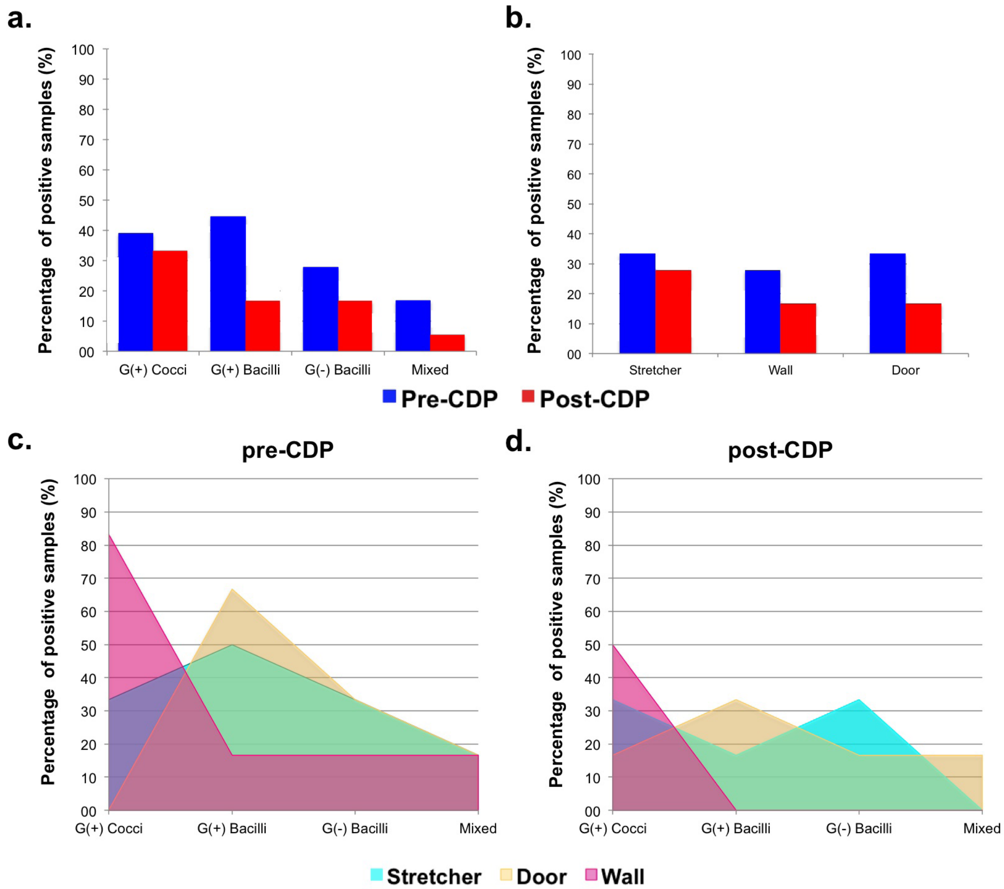

Figure 2. Percentage of isolates found. a. Percentage of positive samples according to the type of microorganism found. b. Distribution of positive samples according to the place sampled. c. Percentage of pre-CDP isolates. d. Percentage of post-CDP isolates. G(+) = Gram-positive; G(-) = Gram-negative. CDP = cleaning and disinfection procedures.

group of colonies identified showed greater size, dry appearance and irregular edges. Colonies showed beta-hemolysis in some cases.

The growth of colonies on MCK agar showed whitish colonies with large size and variable texture (some slimy and others dry), some of them lactose positive colonies, which could initially indicate the presence of enterobacteria species. When samples were grown on RPMSA agar colonies with punctate and mucous morphologies appeared, some of them mannitol positive which turns out to be presumptive for *Staphylococcus aureus*.

At the microscopic level, three groups of microorganisms were isolated corresponding to: Gram-positive G(+) cocci (n: 13, 36.1%), G(+) bacilli (n: 11, 30.6%) and Gram negative G(-) bacilli (n: 8, 22.2%). Additionally, a group of samples from which microorganisms with 'mixed' morphology were recovered (n: 4, 11.1%) were identified.

The percentage of positive samples was assessed according to the collection moment of the samples, finding that 94.4% (n: 17) of the samples collected pre-CDP were positive, while 61.1% (n: 11) were positive post-CDP. Pre-CDP the group of microorganisms most frequently found were G(+) bacilli (44.4%, n: 8), however, this group was the one, which showed the greatest reduction after CDP implementation (27.8%). G(+) cocci showed a smaller reduction after CDP implementation (post-CDP). The positivity frequency per group of microorganisms according to the collection time of samples is described in Figure 2a.

Microorganism detection by risk zones

A total of 12 samples were processed for each risk zone in the analyzed ambulances (pre-CDP and post-CDP, six ambulances). The area with the highest detection frequency was in the ambulance stretcher with 91.7% (n= 11, 95% CI: 61.5-99.7), followed by the ambulance door with 75.0% (n= 9; 95% CI: 42.8-94.5) and finally, the ambulance wall with 66.7% (n= 8; 95% CI: 34.8-90.0) of isolated microorganisms. The frequency of microorganisms' isolation in each risk zone is shown in Figure 2b.

The analysis according to the risk zones assessed showed that all the ambulance areas presented bacterial growth pre-CDP, being G(+) cocci frequently detected in the door (83.3%), followed by G(+) bacilli in the wall (66.7%). The detection frequency of pre-CDP microorganisms in the different risk areas sampled is described in Figure 2c. Although the frequency of all microorganisms was reduced post-CDP, an elimination of G(-) bacilli and mixed detections in the wall was observed; for the other groups of microorganisms and sampling areas, growth was detected after the usual CDP application (Figure 2d).

Diversity of detected microorganisms

Two categories were defined to identify microbial diversity. Being 'single' the microorganisms detection with unique microscopic morphology, while 'mixed' was defined for microorganisms recovery that show different morphologies from a sample, according to the previously established criterion.

Table 2. Bacterial detection (culture growing) percentage according to the sampling site and collection time (Pre-CDP and post-CDP).

		Ambulance risk zone		Stretcher (n=6)		Wall (n=6)		Door (n=6)	
		n	%	n	%	n	%	n	%
Pre-CPD (n=18)	Bacterial detection	17	94.4	6	100.0	5	83.3	6	100.0
	Sample with unique morphology	14	77.8	5	83.3	4	66.7	5	83.3
	Sample with mixed morphology	3	16.7	1	16.7	1	16.7	1	16.7
Post-CPD (n=18)	Bacterial detection	11	61.1	5	83.3	3	50.0	3	50.0
	Sample with unique morphology	10	55.6	5	83.3	2	33.3	3	50.0
	Sample with mixed morphology	1	5.6	0	0.0	1	16.7	0	0.0

Although most of the samples revealed growth with single morphology, the risk zone with the highest number of isolated microorganisms with mixed morphology was the wall (16.7%; 95% CI: 9.9-29.1) (Figure 3a). A decrease in the number of isolates was found with CDP implementation (94.4% vs. 61.1%), with the door being the ambulance area with the greatest decrease (100% vs. 50.0%) (Table 2). Although these findings reveal some efficiency of the CDPs usually implemented, it is evident that the reduction of the detection frequencies of post-CDP microorganisms was limited. Total elimination was presented in the case of mixed morphologies in the stretcher and door (Figure 3b).

CDP impact over microorganisms groups

For surface cleaning, each EPSPH used a specific disinfectant substance, Disinfectant 1 (Bactidin), Disinfectant 2 (Benzaldin) and Disinfectant 3 (Deterganium). In this context, the decrease in microorganisms observed post-CDP was assessed. On a general level, the results showed a relative decrease in bacteria percentage between 50.0% and 66.7% (Figure 4a). For disinfectant 1, a decrease was observed for all microscopic morphologies observed (Figure 4b), for disinfectant 2 and 3 the decrease was observed for Gram-positive bacilli (Figure 4c and 4d, respectively).

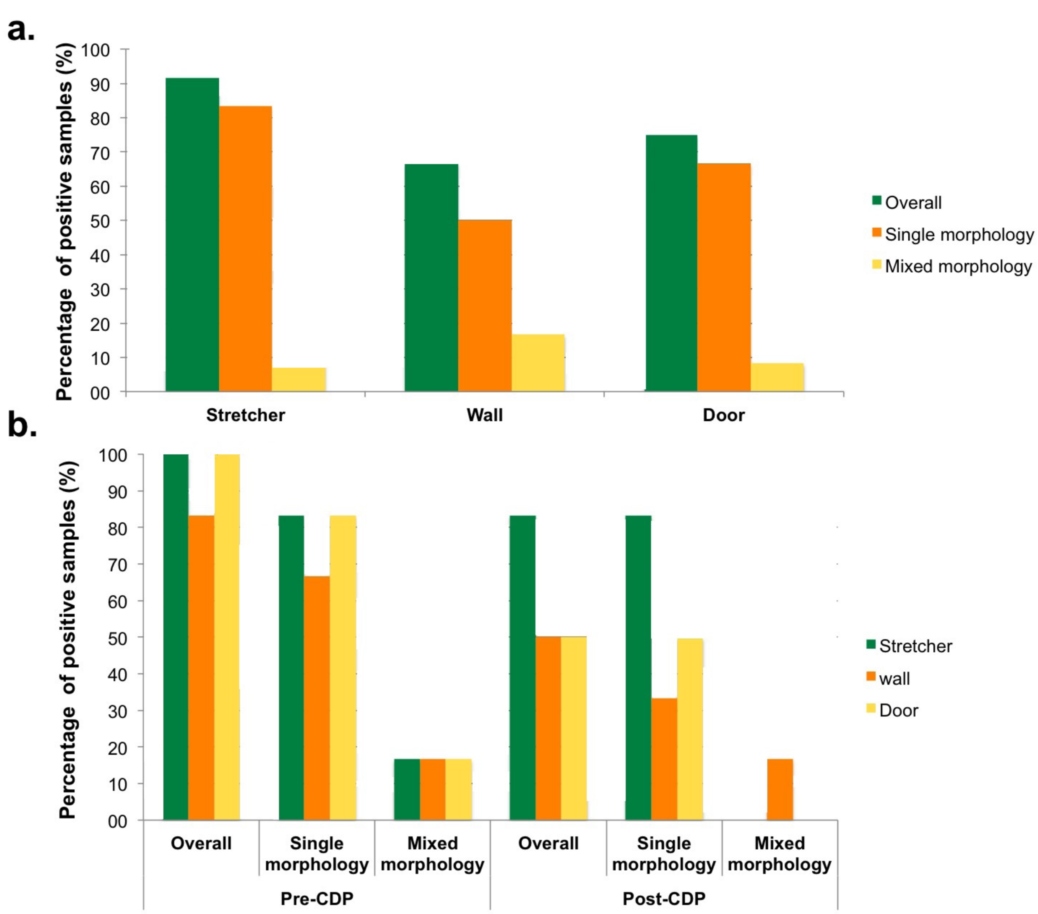

Figure 3. Distribution of the observed morphology according to place sampled. a. Samples with overall, single or mixed morphology according the risk zone b. Samples with overall, single or mixed morphology according the risk zone, pre-CDP and post-CDP. CDP= cleaning and disinfection procedures.

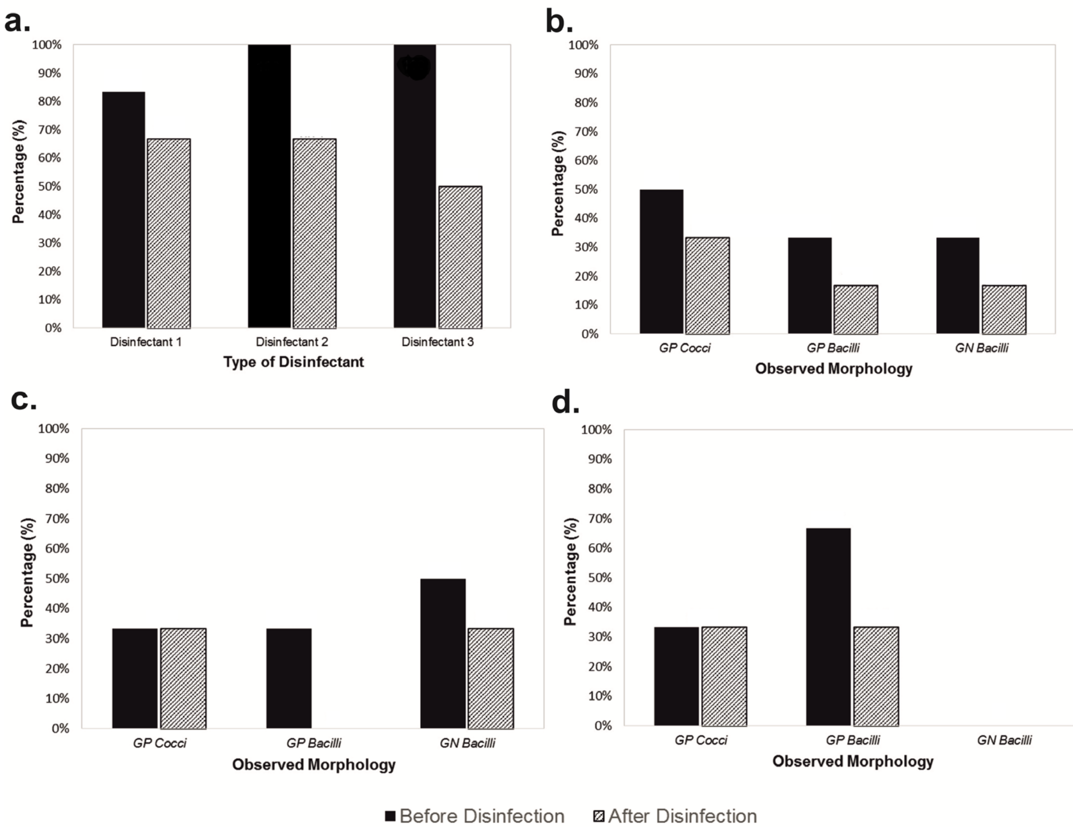

Figure 4. Percentage of isolations found according to the disinfectant used. a. Comparison of the percentage of pre and post-cleaning isolates according to the disinfectant used. b. Distribution of the morphology observed before and after cleaning for Disinfectant 1 (Bactidin). c. Distribution of the morphology observed before and after cleaning for Disinfectant 2 (Benzaldine). d. Distribution of the morphology observed before and after cleaning for Disinfectant 3 (Deterganium).

Discussion

For Colombia, there is no significant information about microorganisms present in emergency ambulances and the efficiency of their cleaning process. This study constitutes a preliminary analysis, which provides useful information for the improvement, control and adequate implementation of cleaning protocols in ambulances. Additionally, this type of study allows the generation of appropriate measurements that can facilitate the control of nosocomial infections derived from pre-hospital care due to exposure in ambulances (4, 9).

In this study, the isolates percentage was higher than 94% (Table 2). However, a decrease of these microorganisms was observed when carrying out the cleaning and disinfection processes. This latter highlights the importance of implementing adequate and routine protocols that allow the reduction of infectious agents that are present in primary emergency response vehicles (2, 10, 11).

The stretcher showed the highest contamination percentage between the sampled areas (Figure 2b and 3a), as observed in previous studies in ambulances (12, 13). The sample taken from this site includes part of the stretcher surface but also of the stretcher strap, which has greater contact with the patient; the material of this element facilitates microorganisms persistence, and because of its porous material it tends to store a greater amount of microorganisms (12). Therefore, despite its difficult disinfection, the strap should be washed and changed regularly, given its exposure and direct contact with the patient. The rear door handle and the ambulance wall were the surfaces with greater facility for applying CDP, but also that conserved microorganisms after these processes. The ambulance wall showed the highest percentage of isolated microorganisms with different morphology, despite being an area that does not come into contact with the patient.

These findings might be due to a failure in the disinfection process for these ambulance areas, facilitating the existence of these microorganisms. The effectiveness of the disinfection process will depend on several factors, among which are the surface material, the type of contaminant, the quality with which the cleaning process is carried out, the concentration in which the disinfectant is used, the disinfection protocol and the interaction of patients and operators with different devices and surfaces (5, 9, 14, 15).

The most frequent morphology in the study and that remained in greater proportion after disinfection was the Gram-positive cocci at the microscopic level and mannitol positives at a macroscopic level, suggesting that are presumptive *Staphylococcus aureus*-type. This microorganism is relevant in public health, since it has generated bacterial outbreaks carrying plasmids that are resistant to methicillin, which have expanded rapidly worldwide and has established itself as one of the major causes of infections associated with hospital care (16, 17). A significant *S. aureus* contamination has been previously reported in emergency vehicles (15, 17-19), considering that the surfaces around patients or healthcare personnel are more likely to be contaminated with multi-resistant Gram-positive pathogens, whose survival is favored both by biochemical, as well as molecular-genetic and cellular mechanisms (17, 19).

This study constitutes a first approach to the determination of microorganisms' prevalence in emergency ambulances in Colombia, as well as an assessment of the effectiveness of CDPs used. There was a high prevalence of microorganisms of different potentially pathogenic morphology, at non-critical points of ambulances, but which both patients and operators have contact, representing a potential source of nosocomial infections. This prevalence was reduced after using the CDPs, but differentially depending on the type of disinfectant used and the type of microorganism present, but also without completely eliminating the microorganisms, leaving evidence that these CDPs should implement improvements.

A limitation of the study is that bacterial species were not identified. Therefore, future studies aimed at the phenotypic and molecular characterization of suspicious colonies, as well as the increase in the number of samples, sampling points and use of antibiotic-supplemented cultured media, should be carried out in order to support the data obtained and the identification of pathogenic microorganisms and its susceptibility profiles. Disinfection is a process that seeks to eliminate most microorganisms, many of which have pathogenic potential (5, 13). Nevertheless, it is necessary to carry out studies aimed at determining the microorganisms present in specific areas, in order to establish successful and effective methodologies that allow eliminating most of these, as well as improving existing protocols and contributing to the generation of sterile environments that are beneficial for the patient and the personnel of emergency vehicles.

Conflict of interest
The authors declare that they have no potential conflicts of interest regarding this work.

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