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Detección de *Enterococcus sp* en superficies inertes de servicios higiénicos en la Unidad Académica de Salud y Bienestar de la Universidad Católica de Cuenca

Detection of Enterococcus sp on inert surfaces of toilets in the Academic Unit of Health and Welfare of the Catholic University of Cuenca

[1] Erika Elizabeth Hurtado Jiménez https://orcid.org/0009-0004-6410-6012
Student of Biochemistry and Pharmacy, Catholic University of Cuenca, Ecuador.
erika.hurtado@est.ucacue.edu.ec

[2] Luis Alfredo Velez Zamora https://orcid.org/0000-0002-5427-6577
Professor of Biochemistry and Pharmacy, Catholic University of Cuenca, Ecuador.
lvelez@ucacue.edu.ec

[3] Jose Antonio Baculima Suarez https://orcid.org/0000-0002-6695-665X
Professor of Biochemistry and Pharmacy, Catholic University of Cuenca, Ecuador.
jbaculima@ucacue.edu.ec

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Palabras claves:
Enterococcus sp , inert surfaces, toilets.

Resumen

Introducción: *Enterococcus sp* es una bacteria que forma parte de la microbiota intestinal y también contamina el tracto genital femenino. Por lo general, no causa problemas, pero puede causar infección si ingresa al tracto urinario, al torrente sanguíneo, a heridas en la piel u otras áreas estériles. **Objetivo:** Determinar y cuantificar la presencia de *Enterococcus sp* en superficies inertes de servicios higiénicos (SH) en la Unidad Académica de Salud y Bienestar de la Universidad Católica de Cuenca. **Metodología:** Se realizó un estudio no experimental, cuantitativo de diseño transversal, en el que se tomó 424 muestras de 106 servicios higiénicos, las muestras fueron tomadas en las manijas de puertas y perillas de inodoro, usando la técnica del hisopado y su consecutivo aislamiento en medio de cultivo cromogénico, en los diferentes SH de la Unidad Académica de Salud y Bienestar, transportadas al Laboratorio de Microbiología de la Unidad Académica de Bioquímica y Farmacia de la Universidad Católica de Cuenca. **Resultados:** Se determinó mayor presencia de *Enterococcus sp* en los baños antes de la limpieza rutinaria que representa el 1,44% del total; mientras que luego de la limpieza 0.48%. **Conclusión:** El estudio indicó que los servicios higiénicos tienen mayor carga bacteriana de *Enterococcus sp* antes de la limpieza de los baños.

Keywords:
Enterococcus sp, inert surfaces, restrooms.

Abstract

Introduction: Enterococcus sp is a bacterium that is part of the intestinal microbiota and contaminates the female genital tract. Generally, it does not cause problems; However, it can develop infection if it enters the urinary tract, bloodstream, skin wounds, or other sterile areas. Objective: To determine and quantify the presence of Enterococcus sp on inert surfaces of restrooms in the Academic Department of Health and Wellness of the Catholic University of Cuenca. Methodology: A non-experimental, quantitative cross-sectional study was conducted; 424 samples from 106 restrooms were collected. The samples were gathered from door and toilet flush handles in the different restrooms of the Academic Department of Health and Wellness employing the swab technique and subsequent isolation in chromogenic culture medium. Then,

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the samples were taken to the Microbiology Laboratory of the Academic Department of Biochemistry and Pharmacy of the Catholic University of Cuenca. Results: A higher presence of Enterococcus sp was determined in the restrooms before routine cleaning, representing 1.44% of the total, while after cleaning, it was 0.48%. Conclusion: The study revealed that restrooms have a higher bacterial load of Enterococcus sp before bathroom cleaning.

Introduction

Over the years, human beings have coexisted with living conditions, which can be environment, climate, society and health, delving into health, there have always been microorganisms that can be harmless to human health, some capable of collaborating for different types of life cycles and harmful ones that can harm health and in many cases trigger the death of people (1)(2).

Bacteria cause millions of deaths worldwide, but there are also bacteria that do not cause death, but do trigger many problems when it comes to public health. Enterococcus sp that usually lives in the intestine and female genital tract (3). In most cases, it does not cause problems, but, it can cause infection if it enters the urinary tract, bloodstream, skin wounds or other sterile sites (4).

According to the World Health Organization (WHO), poor hygiene is one of the most important risk factors for contracting infectious diseases (5). If we put this in the context of public toilets, which are sensitive places, it is easy to understand that all kinds of contagious ideas occur to a person when he or she thinks that the cleaning conditions are not the most adequate and that the toilet is being used by a large number of individuals, even though they were previously disinfected (6) (7).

In 2018 at the University of Peru, Union, Peru. The study “Pathogenic bacteria in school hygiene services” was carried out, whose objective was to know the presence of pathogenic bacteria in fecal coliforms belonging to the Enterobacteriaceae family, such as Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli. and Salmonella typhi in fomites used by several users, whose microbiological study was carried out on a total of 48 samples collected from the door handles and toilets of the men's and women's bathrooms on the first floor of the Technical Academy and the University of Architecture of Peru. The samples were plated for subsequent isolation in selective media, characterization, identification and Gram staining tests to confirm bacterial identity. In the fomites analyzed, the four types of bacteria were present on the sampling days with

certain differences, so they can cause infections and be harmful to human health when coming into contact with the skin (8).

The toilets of the Catholic University of Cuenca in the academic unit of Health and Wellbeing could be a potential source of microorganisms that can harm the health of students, teachers or people who occupy them when the cleaning conditions are not adequate, as well as the cleaning staff and the people who use them. For the reasons stated above, in Ecuador there are not enough studies carried out on the determination of Enterococcus sp. on inert sanitary surfaces, it is for this reason that, with this study, it is intended to determine and quantify the presence of Enterococcus sp. on inert surfaces of toilets (SH) in the Academic Unit of Health and Wellbeing of the Catholic University of Cuenca.

Methodology

A non-experimental, quantitative, cross-sectional study was conducted, in which 424 samples were taken from 106 toilets.. The samples were taken from door handles and toilet knobs, using the swab technique and their subsequent isolation in the chromogenic culture medium, in the different SH of the Academic Unit of Health and Well-being, and transported to the Microbiology Laboratory of the Academic Unit of Biochemistry and Pharmacy of the Catholic University of Cuenca.(9).

Inclusion criteria: Surfaces such as door handles and toilet knobs were taken into consideration because the toilets are very frequented by students from the different Academic Units of the institution, in which the bathrooms of the Degree Courses of the Academic Unit of Health and Wellness located on the main campus were analyzed. Exclusion criteria: SH of the degrees outside the matrix of the Academic Unit of Health and Wellness and disabled toilets (10).

The samples were collected thanks to the authorization and support of the competent entities of the Catholic University of Cuenca, which sent a request for the collection of samples and presentation of the results obtained, which was accepted.

For the collection of samples, sterilized swabs were used, taking the sample uniformly (from side to side) by smearing the surface to be analyzed, using the swabbing technique and its subsequent isolation in the chromogenic culture medium (11), observing small colonies between greenish blue and turquoise blue and thus making the comparison in the different careers of the Academic Unit of Health and Well-being.

Isolation and identification by conventional biochemical assays

Biochemical tests are commonly used to identify and characterize bacteria, including those of the genus Enterococcus sp.

At the time of taking the sample with the swab on the inert surface, it was placed in a sterile tube with peptone water (1/10); and it was transported to the microbiology laboratory for its respective sowing in the Petri dish with the chromogenic agar, and it was placed in the oven (35°C - 37°C) for a period of 18 to 24 hours. Once the time had elapsed, the growth was observed and to identify the strain, the catalase test was performed. (12)

This test is used to determine the presence of the enzyme catalase, which catalyzes the decomposition of hydrogen peroxide. Enterococcus sp is generally catalase negative, meaning it does not produce the enzyme (13). Therefore, for identification, a strain was placed in 4% hydrogen peroxide and did not produce effervescence.

Finally, the culture was carried out on Bile esculin agar (BEA): Bile esculin agar is a selective culture medium containing bile salts and esculin. Enterococcus sp can grow in this medium and hydrolyze esculin, resulting in the formation of a dark complex (14) (15).

In data analysis, inferential statistics is used as a key tool to examine the relationships between specific variables. In particular, it was decided to use the Chi-square statistical test with a significance level set at $p<0.05$. This choice is based on the nature of the variables under study and the need to assess their interrelationship or dependence. This process involves comparing the observed frequencies with the expected frequencies under the null hypothesis of independence, allowing us to determine whether there are significant differences. This statistical approach provides a solid basis for decision-making and provides valuable results on the relationships between the variables considered in the study.

Results

Of the 424 samples analyzed in the different handles and knobs of the SH of the Academic Unit of Health and Well-being, 8 samples were positive for Enterococcus sp (1.9%), while the additional 416 samples were negative (as seen in Table 1).

Table 1. *Prevalence of Enterococcus sp before and after cleaning.*

Time period	Number of plates	UCACUE Health and Wellness Unit			Total number of contaminated plates
		Biochemistry and Pharmacy	Medicine	Dentistry	
Before cleaning	212	6 (20.0%)	0 (0%)	0 (0%)	6 (2.8%)
After cleaning	212	2 (6.7%)	0 (0%)	0 (0%)	2 (0.9%)
Total	**424**	**8 (13.3%)**	**0 (0%)**	**0 (0%)**	**8 (1.9%)**

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Findings from the analysis of Enterococcus sp on door handles prior to cleaning at the Health and Wellness Unit facilities reveal that the percentage of presence is 5.7% in the bathrooms designated for women, 0% in the bathrooms for men, and 0% in the shared bathrooms (see table 2). Despite these differences, no statistically significant relationship was observed between the various categories of users (Chi-square=2.308; p=0.129).

Table 2. Prevalence of Enterococcus sp on handles and knobs before and after cleaning.

Time period	**Number of plates**	UCACUE Health and Wellness Unit			**Total number of contaminated plates**
		Women's bathrooms	**Men's bathrooms**	**Shared bathrooms**	
Before cleaning	212	6 (5.7%)	0 (0%)	0 (0%)	6 (2.8%)
After cleaning	212	2 (1.9%)	0 (0%)	0 (0%)	2 (0.9%)
Total	**424**	**8 (3.8%)**	**0 (0%)**	**0 (0%)**	**8 (1.9%)**

Before cleaning, 5.7% of the plates in the women's restrooms were found to be contaminated, while 2.8% of the plates in total were contaminated. After cleaning, these values were significantly reduced to 1.9% and 0.9% respectively. Comparing the observed and expected frequencies under the null hypothesis of independence allowed us to determine whether cleaning had a significant effect on reducing restroom contamination. At the $p<0.05$ level of significance

Table 3.*Plates contaminated with Enterococcus sp and the type of user in total.*

Time period	**Number of plates**	UCACUE Health and Wellness Unit		**Total number of contaminated plates**
		Door handle	**toilet knob**	
Before cleaning	212	0 (0%)	6 (5.7%)	6 (2.8%)

Table 3.*Plates contaminated with Enterococcus sp and the type of user in total. (continued)*

Time period	**Number of plates**	UCACUE Health and Wellness Unit		**Total number of contaminated plates**
		Door handle	**toilet knob**	
After cleaning	212	0 (0%)	2 (1.9%)	2 (0.9%)
Total	**424**	**0 (0%)**	**8 (3.8%)**	**8 (1.9%)**

In the detailed analysis of the specific areas of contamination, focusing on door handles and toilet knobs, Chi-square statistical tests were used to assess the relationship between cleaning and the presence of contaminated plaques. Before cleaning, 5.7% of plaques on toilet knobs were found to be contaminated, while no contamination was recorded on door handles. In total, 2.8% of plaques were contaminated. After cleaning, these values decreased, with 1.9% of contamination on toilet knobs and no contamination on door

handles, reaching a total of 0.9%. The application of Chi-square tests with a significance level of $p<0.05$ revealed a statistically significant relationship between cleaning and the reduction of contaminated plaques in both areas, supporting the effectiveness of the measures taken in the decontamination of these specific surfaces.

Regarding the distribution of the areas where the Enterococcus sp samples were taken, we have that, of the 8 positive samples, 6 (2.8%) were found when the cleaning was not carried out in the SH, while when the cleaning was carried out, 2 (0.9%) samples were positive (as seen in Table 1).

The results presented show the presence of Enterococcus sp in the toilets of the Academic Unit of Health and Wellbeing of the Catholic University of Cuenca, with a total of 8 samples being considered positive, representing 1.98%. It is not an alarming figure, but the presence of microorganisms exists, so cleaning the toilets is essential to minimize the risk.

The presence of Enterococcus sp in bathrooms may indicate bacterial contamination and unhygienic conditions. These bacteria can spread through direct contact with contaminated surfaces or from contaminated hands to the mouth, nose or other points of entry into the body (16).

Certain species of Enterococcus sp are known to be resistant to antibiotics, which could represent a public health problem if these bacteria spread and cause infections in susceptible individuals. However, it should be noted that not all strains of Enterococcus sp are pathogenic and some may be part of the normal microbiota of the human gut. To prevent the spread of Enterococcus sp or other bacteria in bathrooms, it is recommended to follow good hygiene practices, such as washing hands thoroughly with soap and water, especially after using the bathroom.

It is also important to periodically clean bathrooms and surfaces with appropriate disinfectants to reduce bacterial load. In public places, such as restaurants, hospitals or educational centers, it is the responsibility of the owners or administrators to ensure the proper cleaning and disinfection of toilets to protect the health of users (17).

Discussion:

The presence of microorganisms in the bathrooms of the Academic Unit of Health and Wellbeing of the Catholic University of Cuenca, as in any other place, is a relevant issue for public health and health safety. Bathrooms are environments conducive to the proliferation of various microorganisms, some of which may be pathogenic and pose risks to people's health.

Bathrooms can harbor a variety of bacteria, including those associated with gastrointestinal and respiratory diseases. The presence of Escherichia coli, Salmonella sp, Staphylococcus aureus or other pathogenic bacteria could be a cause for concern.

Humidity and lack of proper ventilation in bathrooms can encourage the growth of fungi, such as mold and yeast. Some of these fungi can release spores that could affect indoor air quality.

The results obtained from the analysis of the handles and knobs of the toilets (SH) of the Academic Unit of Health and Wellbeing present a significant perspective on the hygienic quality of these areas. In terms of the presence of Enterococcus sp, it is observed that 1.98% of the samples were positive, indicating a relatively low proportion of contamination. The vast majority of the samples, 98.02%, were negative, suggesting effective hygiene management in the Academic Unit.

In turn, a study carried out in the same place in 2023, where the presence of the microorganism E. coli was detected, highlights that before routine cleaning, 13.67% of the samples were contaminated with this bacterial indicator, while after cleaning, this percentage decreased to 7.78% (18). These findings suggest that routine cleaning has been effective in reducing the presence of E. coli on the surfaces evaluated.

It is crucial to discuss the prevention and control measures that are implemented to reduce the presence of pathogenic microorganisms in bathrooms. This could include regular cleaning and disinfection practices, the installation of efficient ventilation systems, and the promotion of hygiene habits among users.

Promoting awareness and education about the importance of personal hygiene and cleaning shared spaces is essential. This can help prevent the spread of disease and promote a healthier environment.

There are few studies conducted in Ecuador regarding the identification of microorganisms in specific school and university areas. The discussion could benefit from specific studies conducted in Ecuadorian universities to evaluate the presence of microorganisms in bathrooms, identify areas of concern and develop strategies adapted to local conditions.

Microorganisms in university bathrooms in Ecuador should be addressed from a multidisciplinary perspective that includes aspects of microbiology, public health, environmental engineering and health education to ensure the safety and well-being of the university community (19).

In summary, the results point to the effectiveness of routine cleaning measures in reducing bacterial contamination, specifically in decreasing Enterococcus sp and E. coli.

Furthermore, the identification of areas more prone to contamination, such as toilet handles, suggests the need for special attention and more rigorous cleaning protocols in these locations to maintain a healthy and safe environment in the Academic Health and Wellness Unit.

Conclusions

- The presence of Enterococcus sp. on inert surfaces in the toilets of the Academic Unit of Health and Wellbeing at the Catholic University of Cuenca was determined and quantified; the results also indicated that there was a decreased microbial quantity after cleaning by the cleaning staff.
- The importance of hygiene on the inert surfaces of SH is essential since approximately hundreds of students use it to perform their biological needs, and if there is a considerable microbial load this could generate a large number of infections in people.

Conflict of interest

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

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