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# Análisis bacteriológico de superficies y efectividad *in vitro* de desinfectantes en el área de neonatología y quirófano del Hospital Humanitario Fundación Pablo Jaramillo Crespo. Cuenca – Ecuador

*Bacteriological analysis of surfaces and in vitro effectiveness of disinfectants in the neonatology and operating room area of the Pablo Jaramillo Crespo Foundation Humanitarian Hospital. Cuenca - Ecuador*

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**Palabras claves:**
Neonatología, quirófano, susceptibilidad microbiana, antibióticos, desinfectantes.

**Resumen**

**Introducción:** La resistencia bacteriana hacia antibióticos y desinfectantes es un severo problema sanitario mediado por mecanismos de resistencia, afecta a los sistemas de salud mundial debido a las pocas alternativas de tratamiento y elevados costos. Por otro lado, la sensibilidad a desinfectantes se ha visto disminuida al evaluar su efectividad *in vitro* según concentraciones recomendadas por entidades sanitarias como la OMS y MSP. **Objetivo:** Valorar la efectividad in vitro de desinfectantes de uso hospitalario en bacterias aisladas en las áreas de neonatología y quirófano del Hospital Humanitario Fundación Pablo Jaramillo Crespo – Cuenca – Ecuador. **Metodología:** Se realizó un estudio de campo, de tipo descriptivo de corte transversal. Se obtuvo muestras de las áreas de neonatología y quirófano del hospital Humanitario Fundación Pablo Jaramillo Crespo. Cuenca – Ecuador. Identificación bacteriológica mediante métodos fenotípicos para su posterior evaluación de susceptibilidad y resistencia por medio del método de Kirby – Bauer. **Resultados:** En el 48% de muestras hubo crecimiento microbiano identificando cepas de *S. aureus, Enterococcus spp, Staphylococcus saprophyticus, Streptococcus agalactiae, Pseudomona spp, E. coli, Klebsiella spp*. Destacando resistencia hacia β-lactámicos, cefalosporinas. *Pseudomona* resistente a meropenen y *Enterococcus* resistente hacia linezolid. La sensibilidad hacia desinfectantes es muy escasa con resistencia total a etanol, hipoclorito, Monopersulfato de potasio, glutaraldehído, sensibilidad de media a elevada con yodopovidona, amonio cuaternario, peróxido de hidrógeno a concentraciones aprobadas por autoridades sanitarias. **Conclusión:** Se valoró la efectividad in vitro de antibióticos y desinfectantes de uso hospitalario en bacterias aisladas de las áreas: neonatología y quirófano, encontrando un alto porcentaje de muestras resistentes. **Área de estudio general:** Microbiología. **Área de estudio específico:** Bacteriología. **Tipo de estudio:** Articulo original

**Keywords:**
Neonatology, operating room,

**Abstract**

**Introduction:** Bacterial resistance to antibiotics and disinfectants is a severe health problem mediated by resistance

microbial susceptibility, antibiotics, disinfectants.

mechanisms, affecting global healthcare systems due to limited treatment alternatives and prohibitive costs. On the other hand, disinfectant sensitivity has decreased when evaluating in vitro effectiveness according to concentrations recommended by health entities such as the World Health Organization and the Ministry of Public Health. Objective: To assess the in vitro effectiveness of hospital disinfectants on bacteria isolated in the neonatology and operating room areas of the Humanitarian Hospital 'Pablo Jaramillo Crespo' - Cuenca - Ecuador. Methodology: A descriptive cross-sectional field study was conducted. Samples were obtained from the neonatology and operating room areas of the Humanitarian Hospital 'Pablo Jaramillo Crespo' in Cuenca - Ecuador. Bacteriological identification was performed using phenotypic methods for subsequent evaluation of susceptibility and resistance using the Kirby - Bauer method. Results: Microbial growth was observed in 48% of samples, identifying strains of S. aureus, Enterococcus spp, Staphylococcus saprophyticus, Streptococcus agalactiae, Pseudomonas spp, E. coli, and Klebsiella spp, highlighting resistance to β-lactams and cephalosporins, where Pseudomonas are resistant to meropenem, and Enterococcus is resistant to linezolid. Sensitivity to disinfectants is exceptionally low, with total resistance to ethanol, hypochlorite, potassium monopersulfate, glutaraldehyde, and medium to high sensitivity to iodopovidone, quaternary ammonium, hydrogen peroxide at concentrations approved by health authorities. Conclusion: The in vitro effectiveness of hospital antibiotics and disinfectants was evaluated in bacteria isolated from the neonatology and operating room areas, finding a high percentage of resistant samples.

## Introduction

Over the years, a severe public health problem has arisen worldwide due to infections acquired in hospitals, prolonging the patient's stay and with a high probability of long-term consequences.(1). Enterobacteria and Staphylococcus aureus have a high prevalence in the hospital environment, mainly on handles, tables, faucets, among other easily contaminated surfaces that pose a risk to the health of patients.(2).

The World Health Organization (WHO) indicates that antimicrobial resistance shows warning signs in health systems that are increasingly generating more infections with a tendency towards drug resistance.(3)This is related to the excessive use of antibiotics, which represents considerable economic costs, increased morbidity and mortality in patients susceptible to infections and the demand for new, more aggressive treatment schemes or alternatives to conventional therapies such as the use of antibodies, bacteriophages, probiotics or antimicrobial peptides through molecular biology.(4).

The complexity of the problem encourages the search for coordinated actions with innovations in the pharmaceutical industry in a sustainable way, research, patient support programs in pharmacological treatment, legislation programs, awareness and adherence to treatment mainly in multi-resistant bacteria such as Staphylococcus aureus, Enterococcus, Enterobacteria, Pseudomonas, among others.(5).

On the other hand, bacterial resistance to disinfectants is a frequent problem, and is often related to Health Care Associated Infections (HAIs).(6). The use of hospital-grade disinfectants helps prevent these infections by ensuring sterility and cleanliness of these areas; however, nosocomial infections have currently increased greatly.(7)In recent years, the emergence of resistance linked to biocides, particularly to disinfectants for hospital use, can be observed. This is a favorable factor in survival and multi-resistance, resulting in intra-hospital contamination.(8).

Disinfectants have a non-specific antibacterial activity, so their misuse triggers new resistance mechanisms, contributing to prolonging the length of hospital stay and generating additional costs to public health.(9). One of the mechanisms of microbial resistance is mediated by changes in the bacterial wall when exposed to antibiotics or disinfectants, forming biofilms that help survival on inert surfaces and medical devices.(10).

An example of this mechanism is Staphylococcus aureus, which represents more than 80% of biofilms on dry surfaces; of these, approximately 50% to 58% are highly pathogenic, so their susceptibility to hospital disinfectants must be evaluated.(11). Other mechanisms of resistance to disinfectants are: Membrane permeability, degradable enzymes, and flow pumps that are characterized by responding quickly to stress, in addition the resistance generated to disinfectants is transmitted through genes as inheritance patterns, which decreases the effectiveness of disinfectants towards pathogenic bacteria.(9).

Nosocomial infections, also known as hospital-acquired infections, are a major challenge worldwide due to the increase in mortality and morbidity and mortality.(2). A study carried out in Miami, United States, shows that premature newborns have a high susceptibility to nosocomial infections, associated with the lack of hygiene in the hospital

environment. The excessive and indiscriminate use of antibiotics, delays in the detection and treatment of bacterial infections, generate resistance to biocides, which is why the analysis of the bacterial load present on surfaces and the identification of susceptibility to disinfectants plays an important role in the prevention of infections transmitted during the hospital stay.(12).

At the Pablo Jaramillo Crespo Foundation Humanitarian Hospital in Cuenca, Ecuador, no studies have been conducted to determine the in vitro effectiveness of hospital disinfectants on bacteria such as Staphylococcus aureus and Enterobacteria. For this reason, this research is of great importance in order to identify the bacteria present in the neonatal and operating room areas, determine their antimicrobial susceptibility, and verify the effects of each disinfectant in 24 hours to establish effective disinfectants that reduce the microbial load in areas that require strict sterile conditions. This research seeks to analyze the in vitro effectiveness of disinfectants on bacteria found on surfaces in neonatal and operating room areas, ensuring their continuous, effective, and safe use.

Based on the above background, the objective of this research is: To evaluate the in vitro effectiveness of hospital disinfectants on bacteria isolated from the neonatal and operating room areas of the Pablo Jaramillo Crespo Foundation Humanitarian Hospital – Cuenca – Ecuador.

## Methodology

A descriptive, cross-sectional field study was conducted. Samples were obtained from the neonatal and operating room areas of the Pablo Jaramillo Crespo Foundation Humanitarian Hospital – Cuenca – Ecuador. Forty samples were obtained in the aforementioned areas. The samples were taken unexpectedly to avoid prior disinfection.

*Inclusion criteria*

- Surfaces with greater contact (stretchers, lamps, shelves, equipment, tables, chairs, containers, handles and faucets) in the operating room and neonatology areas.

*Exclusion criteria*

- Areas outside the operating room and neonatology

*Sampling:* In the neonatology and operating room area, 40 samples were obtained using the swab technique, which were enriched in trypticase soy broth in screw-cap tubes to maintain the conservation viability of the bacteria until sowing. The samples were transferred to the Microbiology Laboratory of the Faculty of Biochemistry and Pharmacy

of the Catholic University of Cuenca for incubation, sowing in CHROMagar orientation and subsequent microbial identification.

*Bacteriological Identification,throughphenotypic methods*

Identification of Staphylococcus aureus: Sowing was carried out on mannitol salt agar, until isolated colonies were obtained for subsequent observation of the biochemical characteristics and microbiological tests such as: Gram stain, catalase and coagulase.(13).

Identification ofEnterobacteria: CHROMagar Orientation plates were used to carry out the seeding to identify bacteria such as: (E. coli., Enterococcus spp., Klebsiella spp., Enterobacter spp., Proteus spp., Morganella spp., Pseudomona) allowing the colonial characteristics of each species to be observed.(14).

*Antimicrobial susceptibility and resistance*

Bacterial susceptibility tests with antibiotics and disinfectants were performed using the disk diffusion or Kirby-Bauer technique.

The antibiotics used to determine antimicrobial susceptibility were: Cefoxitin 30 µg, Erythromycin 15 µg, Penicillin G 10 units and Clindamycin µg. On the other hand, for enterobacteria the following were used: Ampicillin 10 µg, amoxicillin + clavulanic acid 20/10 µg, piperacillin + tazobactam 100/10 µg, cephalexin 30 µg, ceftazidime 30 µg, cefuroxime 30 µg, aztreonam 30 µg, ertapenem 10 µg, imipenem 10 µg, meropenem 10 µg, gentamicin 10 µg, amikacin 30 µg, ciprofloxacin 5 µg, levofloxacin 5 µg, trimethoprim-sulfa 1.25% 23.75 µg, fosfomycin 200 µg, erythromycin 15 µg, clindamycin 2µg, cefoxitin 30µg and ceftriaxone 30µg.

*In vitro evaluation of disinfectants.*

The Kirby-Bauer technique was used in Petri dishes with Mueller Hinton agar culture medium. A sowing was carried out on the entire surface and disks impregnated with disinfectants were placed at the concentration recommended by MSP and WHO, which are detailed in the following paragraph. 5ul of the disinfectant was inoculated and the inhibition halo formed after 24 hours of incubation was measured. The results were read and susceptibility was verified by means of the Duraffourd scale.(15).

The hospital disinfectants used were:

1% or 0.5% sodium hypochlorite: 0.5% concentration by diluting 250 ml of water with 25 ml of 5% sodium hypochlorite or so-called commercial chlorine(16). The mechanism of action of this disinfectant is based on the inactivation of nucleic acids, denaturation of proteins and inhibition of enzymatic reactions. In addition, its microbial spectrum is associated with bacteria, lipophilic viruses, hydrophilic viruses, Micobacterium

Tuberculosis and fungi. They are clearly corrosive and become inactivated by the presence of organic matter and produce instability against light. They can cause irritation of the skin and mucous membranes.(16).

2% Glutaraldehyde: Found in concentrations between 1% and 50%, as a surface disinfectant in hospital environments it is between 1 and 2%(17). Used as a bactericide, virucide, fungicide and sporicide, depending on its concentration the sterilization time is between 10 hours, it has an alkaline pH and a shelf life of at least 28 days.(18).

1.5% Chlorhexidine: One of the most important surgical antiseptics and oral antiseptics used today, this is due to its effectiveness, sustainability, low irritation and above all its broad spectrum of action, it has a pH between 5 and 8, and excellent stability at room temperature but very unstable in solution.(18)It is bactericidal against gram-negative and gram-positive bacteria, has variable antiviral activity including HIV, herpes simplex, cytomegalovirus and influenza, to achieve better efficacy it is advisable to combine it with alcohol. In Ecuador it is sold in combination with cetrimide for 1% reconstitution.(18).

70% Ethanol: Bactericidal due to its effectiveness against all vegetative forms of bacteria, it is also tuberculicidal, fungicidal and virucidal, its activity varies according to its concentration, for bactericidal action a range between 60% and 90% solution in water (volume/volume) is indicated, a range of concentrations between 60% and 80% is a powerful virucidal, considered to treat cases of lipophilic and hydrophilic viruses, in the present research the 70% alcohol concentration will be used(18).

10% Povidone-iodine: It is an iodophor, representative of antiseptics, its concentrations range from 2% to 10%, unstable complex of elemental iodine bound to a surfactant such as polyvinylpyrrolidone, it is active against gram-positive and gram-negative bacteria, fungi, viruses, mycobacteria and effective against S. aureus MRSA and Enterococcus species, in addition, it is used as an antiseptic and skin disinfectant.(18, 19).

Hydrogen peroxide 10%:Hydrogen peroxide is a colorless liquid chemical agent at room temperature, with a bitter taste, it has antiseptic properties and is the most used on the market in formulations from 5% to 20%, to obtain better results, 10% hydrogen peroxide will be used with 33 volumes(18, 20)It has oxidizing effects by producing OH and free radicals, which attack the essential components of microorganisms such as lipids, proteins and DNA.(18,20).

1% potassium monopersulfate:It is composed of a powder for reconstitution that contains surfactants favoring its activity, it lasts 24 hours, its commercial forms come in presentations to reconstitute in one, three and eight liters of water.(18)At a concentration of 1% it showed bactericidal activity against*Pseudomona aeruginosa, Escherichia coli*,

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*Staphylococcus aureus,Enterococcus and Mycobacterium smegmatis*In addition, its virucidal activity against poliovirus was demonstrated.(18).

0.4% Quaternary Ammonium:Generally colorless, odorless, non-irritating and deodorant compounds, they have a detergent action, soluble in water and alcohol, in addition, the presence of any protein residue nullifies their effectiveness, it can be found commercially with concentrations of 80%, and generate dilutions from this concentration, so it is recommended to use it at 0.4% as a sanitizer and disinfectant.(18,20).

## Results

From 40 isolated samples belonging to the operating room and neonatology areas of the Pablo Jaramillo Crespo Foundation Humanitarian Hospital – Cuenca – Ecuador, 19 samples were positive with colony growth in CHROMagar Orientation representing 48%, as shown in Figure 1.


**Figure 1.***Percentage of positive samples from sampling in hospital areas*

Six strains of S. aureus were isolated on Mannitol Salt Agar, as well as one strain of S. agalactiae, four strains of Enterococci, two strains of S. saprophyticus, two strains of Klebsiella, one strain of Pseudomona, and three strains of E. coli, n=19 strains identified.

As for Gram-positive cocci, the following were identified: 6 strains identified as S. aureus by different biochemical tests (32%), two strains of Staphylococcus saprophyticus (11%), one Gram-positive beta-hemolytic strain of Streptococcus agalactiae (5%) and 4 strains with a small and smooth appearance on blood agar of Enterococcus spp. (21%).

As for fermenting gram-negative cocci, the following were identified:two strains of Klebsiella spp. (10%) and three strains with a small, non-mucoid, pink appearance, E.

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coli (16%). As for non-fermenting gram-negative cocci, the following were identified: a strain of irregular colonies with a characteristic odor of Pseudomona spp. (5%),as shown in Figure 2.

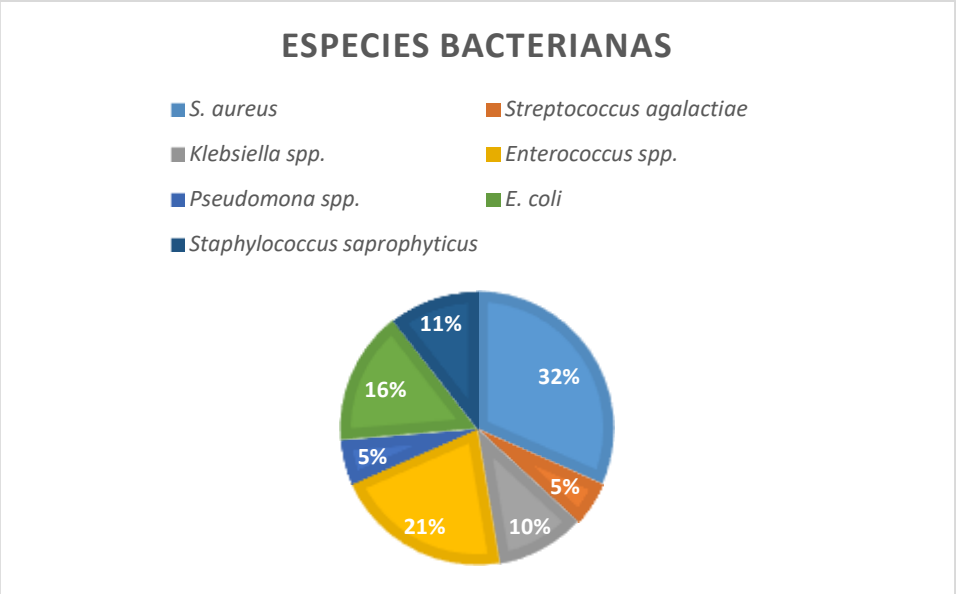


***Figure 2.****Percentage of bacterial species identified in the Neonatology and Operating Room area of the Pablo Jaramillo Crespo Foundation Humanitarian Hospital – Cuenca – Ecuador, 2023.*

Regarding the isolation of bacterial species in hospital areas, 11 bacterial strains isolated from the operating room area represent 58% and 8 bacterial strains were isolated in the neonatology area representing 42%, indicating a higher prevalence of contamination in the operating room area, as shown in Figure 3.

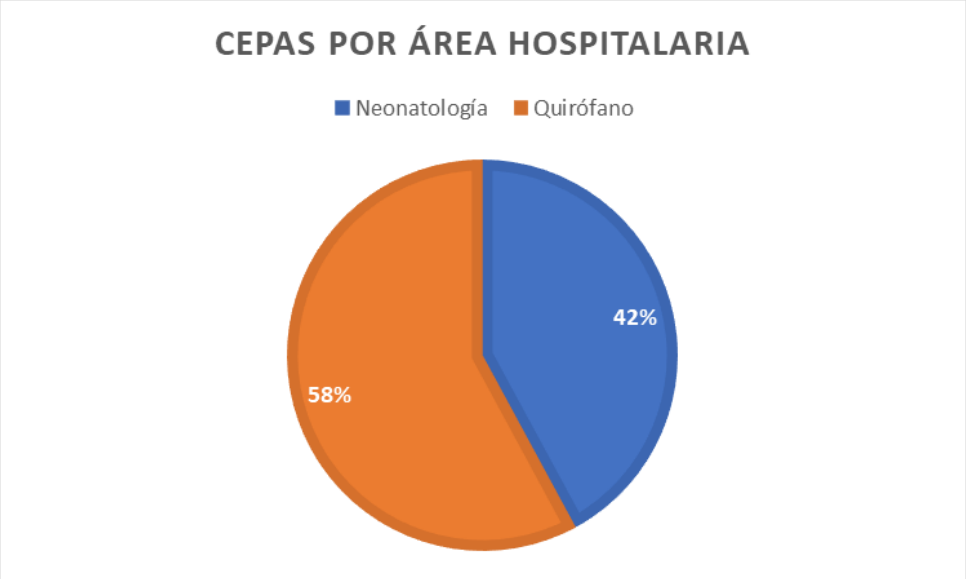


**Figure 3.**Percentage of bacterial strains identified by hospital area*at the Pablo Jaramillo Crespo Foundation Humanitarian Hospital – Cuenca – Ecuador, 2023.*

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Of the strains identified within the neonatology area, four strains are recognized:*S. aureus.*, *two*strains of Enterococcus spp., and two strains of Staphylococcus saprophyticus.On the other hand, in the operating room area where two strains of*S. aureus.*, *a strain of**Streptococcus agalactiae.*,two strains of Klebsiella spp., two strains of*Enterococci spp.*, *a strain of**Pseudomona*spp., and three strains of E. coli.

Antimicrobial susceptibility and resistance

Of the 6 strains identified as S. aureus, 50% were resistant to penicillin, 33% to erythromycin and clindamycin. In addition, 33% were intermediately sensitive to erythromycin, and 100% were sensitive to cefoxitin, as shown in Table 1.

To evaluate the sensitivity to methicillin, cefoxitin was used, according to the CLSI 2023 recommendations, resulting in total sensitivity, in addition the D-test was positive in 33.33% of the 100% of samples belonging to*S. aureus*as shown in Table 1 and Figure 4.

**Figure 4.** Positive D-test

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**Table 1.**Susceptibility of S. aureus to different antibiotics

| *Sample* | *Erythromycin* | *Clindamycin* | *Penicillin G* | *Cefoxitin* |
|---|---|---|---|---|
| *1.* | R | R | R | S |
| *2.* | Yo | S | S | S |
| *3.* | R | R | R | S |
| *4.* | S | S | S | S |
| *5.* | S | S | R | S |
| *6.* | Yo | S | S | S |

**Note:**Legend: S: sensitive; R: resistant; I: intermediate sensitivity.

Of the other 13 strains that were identified as E. coli., Klebsiella., Pseudomona, Staphylococcus saprophyticus., Enterococci., Streptococcus agalactiae. In Klebsiella, 100% resistance was observed to ampicillin and amoxicillin/clavulanic acid. In the case of E. coli, 66% resistance was observed to amoxicillin/clavulanic acid and 33% to cephalexin, gentamicin, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole, as shown in Table 2.

**Table 2.**Susceptibility of E. coli and Klebsiella to different antibiotics

| | *Klebsiella* | | *E. coli* | | |
|---|---|---|---|---|---|
| | Sample 2. | Sample 3. | Sample 9. | Sample 10. | Sample 11. |
| *AMP* | R | R | S | S | S |
| *AUG* | R | R | R | R | S |
| *TZP* | S | S | S | S | S |
| *CFL* | S | S | S | R | S |
| *HUNTING* | S | S | S | S | S |
| *CXM* | S | S | S | S | S |
| *ATM* | S | S | S | S | S |
| *ETP* | S | S | S | S | S |
| *IPM* | S | S | Yo | S | S |
| *MRP* | S | S | S | S | S |
| *CN* | S | S | S | S | R |
| *AMK* | S | S | S | S | S |
| *CIP* | S | S | S | S | R |
| *LEV* | S | S | S | S | R |
| *SXT* | S | S | S | S | R |
| *FF* | S | S | S | S | S |

**Note:**Legend: S: susceptible; R: resistant; I: intermediate susceptible. AMP: ampicillin; AUG: amoxicillin/clavulanic acid; TZP: piperacillin/tazobactam; CFL: cephalexin; CAZ: ceftazidime; CXM: cefuroxime; ATM: aztreonam; ETP: ertapenem; IPM: imipenem; MRP: meropenem; CN: gentamicin; AMK: amikacin; CIP: ciprofloxacin; LEV: levofloxacin; SXT: trimethoprim/sulfamethoxazole; FF: fosfomycin.

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In Pseudomona, 100% sensitivity was observed to piperacillin/tazobactam, aztreonam, gentamicin and ciprofloxacin. Additionally, resistance to ceftazidime and meropenem was noted, as shown in Table 3.

**Table 3.**Susceptibility of Pseudomona to different antibiotics

| *Sample* | *TZP* | *HUNTING* | *ATM* | *MRP* | *CN* | *CIP* |
|---|---|---|---|---|---|---|
| *6.* | S | R | S | R | S | S |

**Note:**Legend: S: susceptible; R: resistant; I: intermediate susceptible. TZP: piperacillin/tazobactam; CAZ: Ceftazidime; ATM: aztreonam; MRP: meropenem; CN: gentamicin; CIP: ciprofloxacin.

When observing the S. saprophyticus strains, 100% resistance to erythromycin and 100% susceptibility to clindamycin and cefoxitin were evident, as shown in Table 4.

**Table 4.**Susceptibility of Staphylococcus saprophyticus to different antibiotics

| *Sample* | *Erythromycin* | *Clindamycin* | *Penicillin G* | *Cefoxitin* |
|---|---|---|---|---|
| *12.* | R | S | S | S |
| *13.* | R | S | R | S |

**Note:**Legend: S: sensitive; R: resistant; I: intermediate sensitivity.

Regarding Enterococcus, 75% sensitivity to penicillin G, linezolid and ampicillin was observed, as shown in Table 5.

**Table 5.**Susceptibility of Enterococcus to different antibiotics.

| *Sample* | *Penicillin G* | *Linezolid* | *Ampicillin* |
|---|---|---|---|
| *4.* | S | S | S |
| *5.* | S | S | S |
| *7.* | S | S | S |
| *8.* | R | R | R |

**Note:**Legend: S: sensitive; R: resistant; I: intermediate sensitivity.

*Streptococcus agalactiae*It indicates 100% sensitivity to penicillin G and ampicillin, and also presents 33% intermediate sensitivity to ceftriaxone, as shown in Table 6.

**Table 6.**Susceptibility of Streptococcus agalactiae to different antibiotics.

| *Sample* | *Penicillin G* | *Ampicillin* | *Ceftriaxone* |
|---|---|---|---|
| *1.* | S | S | Yo |

**Note:**Legend: S: sensitive; R: resistant; I: intermediate sensitivity.

*In vitro evaluation of disinfectants*

Since there is no protocol to help us determine the sensitivity of bacteria to disinfectants, the Duraffourd scale was taken as a reference to determine the degree of sensitivity.(15).

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The reading was performed after a 24-hour incubation, to determine whether or not there was antimicrobial activity, the growth of inhibition halos was observed and the diameter of the inhibition halos was measured to be compared with the Duraffourd scale.(21). According to the Duraffourd scale, the following are considered: Null ≤ 8 mm; Sensitive ≥ 9 to 14 mm; Very sensitive ≥15 to 19 mm; Extremely sensitive ≥ 20 mm(21).

When observing the plates with the discs that have been impregnated with disinfectants such as sodium hypochlorite and ethanol, the existence of 100% resistance to these bacteria was determined, as shown in Table 7.

**Table 7.**In vitro assessment of sodium hypochlorite and ethanol in S. aureus., Streptococcus agalactiae., Enterococcus., S. saprophyticus., Klebsiella., Pseudomona., and E. coli.

| *Strains* | *Sodium hypochlorite* | *Ethanol* |
|---|---|---|
| *S. aureus* | Null (Resistant) | Null (Resistant) |
| *Streptococcus agalactiae* | Null (Resistant) | Null (Resistant) |
| *Enterococcus* | Null (Resistant) | Null (Resistant) |
| *S. saprophyticus* | Null (Resistant) | Null (Resistant) |

**Table 7.**In vitro assay of sodium hypochlorite and ethanol on S. aureus, Streptococcus agalactiae, Enterococcus, S. saprophyticus, Klebsiella, Pseudomona, and E. coli (continued)

| *Strains* | *Sodium hypochlorite* | *Ethanol* |
|---|---|---|
| *Klebsiella* | Null (Resistant) | Null (Resistant) |
| *Pseudomona* | Null (Resistant) | Null (Resistant) |
| *E. coli* | Null (Resistant) | Null (Resistant) |

**Note:**Legend:Null ≤ 8 mm; Sensitive ≥ 9 to 14 mm; Very sensitive ≥ 15 to 19 mm; Extremely sensitive ≥ 20 mm.

Of the plates with the discs that have been impregnated with iodinepovidone and potassium monopersulfate disinfectants, 86% of the bacteria were resistant and 14% were sensitive to potassium monopersulfate, in addition, 100% of the bacteria were sensitive to iodinepovidone, as shown in Table 8.

**Table 8.**In vitro assessment of potassium monopersulfate and povidone-iodine in S. aureus., Streptococcus agalactiae., Enterococcus., S. saprophyticus., Klebsiella., Pseudomona., and E. coli.

| *Strains* | *Potassium monopersulfate* | *Povidone iodine* |
|---|---|---|
| *S. aureus* | Null (Resistant) | Sensitive |
| *Streptococcus agalactiae* | Null (Resistant) | Sensitive |
| *S. saprophyticus* | Null (Resistant) | Sensitive |
| *Klebsiella* | Null (Resistant) | Sensitive |
| *Pseudomona* | Null (Resistant) | Sensitive |
| *E. coli* | Null (Resistant) | Sensitive |
| *Enterococcus* | Sensitive | Sensitive |

**Note:**Legend:Null ≤ 8 mm; Sensitive ≥ 9 to 14 mm; Very sensitive ≥ 15 to 19 mm; Extremely sensitive ≥ 20 mm.

Regarding the plates that had been impregnated with glutaraldehyde and chlorhexidine disinfectant discs, 43% of bacteria were found to be resistant to chlorhexidine and 100% to glutaraldehyde. Likewise, 43% of bacteria were sensitive and 14% were extremely sensitive to chlorhexidine, as shown in Table 9.

**Table 9.**In vitro assessment of chlorhexidine and glutaraldehyde in S. aureus., Streptococcus agalactiae., Enterococcus., S. saprophyticus., Klebsiella., Pseudomona., and E. coli.

| *Strains* | *Chlorhexidine* | *Glutaraldehyde* |
|---|---|---|
| *S. aureus* | Sensitive | Null (Resistant) |
| *Streptococcus agalactiae* | Extremely sensitive | Null (Resistant) |
| *S. saprophyticus* | Sensitive | Null (Resistant) |
| *Klebsiella* | Null (Resistant) | Null (Resistant) |
| *Pseudomona* | Null (Resistant) | Null (Resistant) |
| *E. coli* | Null (Resistant) | Null (Resistant) |
| *Enterococcus* | Sensitive | Null (Resistant) |

**Note:**Legend:Null ≤ 8 mm; Sensitive ≥ 9 to 14 mm; Very sensitive ≥ 15 to 19 mm; Extremely sensitive ≥ 20 mm.

When testing the plates impregnated with hydrogen peroxide and quaternary ammonium disinfectant discs, it was observed that 100% of the bacteria are sensitive to hydrogen peroxide and 14% to quaternary ammonium. In addition, 14% were found to be resistant, 14% were sensitive, and 58% were very sensitive to quaternary ammonium, as shown in Table 10.

**Table 10.**In vitro assessment of chlorhexidine and glutaraldehyde in S. aureus., Streptococcus agalactiae., Enterococcus., S. saprophyticus., Klebsiella., Pseudomona., and E. coli.

| *Strains* | *Hydrogen peroxide* | *Quaternary ammonium* |
|---|---|---|
| *S. aureus* | Extremely sensitive | Very sensitive |
| *Streptococcus agalactiae* | Extremely sensitive | Extremely sensitive |
| *S. saprophyticus* | Extremely sensitive | Very sensitive |
| *Klebsiella* | Extremely sensitive | Null (Resistant) |
| *Pseudomona* | Extremely sensitive | Very sensitive |
| *E. coli* | Extremely sensitive | Sensitive |
| *Enterococcus* | Extremely sensitive | Very sensitive |

**Note:**Legend:Null ≤ 8 mm; Sensitive ≥ 9 to 14 mm; Very sensitive ≥ 15 to 19 mm; Extremely sensitive ≥ 20 mm.

## Discussion

Bacterial contamination in the neonatal area is more prevalent than in the operating room area, the main bacterial species was S. aureus representing 32%, which is one of the main agents causing infections within the hospital environment and which requires monitoring and timely treatment especially in methicillin-resistant staphylococci described by Kalu et al. (22), in the American Journal of Perinatology in their manuscript “Knowledge,

Attitudes, and Perceptions about Antibiotic Stewardship Programs among Neonatology Trainees"(22).

Likewise, contamination in operating room areas is one of the most representative areas in the study, where reports of staphylococci colonization are indicated, especially in the respiratory tract.(23)Other studies show that this pathogen persists even after cleaning and disinfection of the operating room with positive samples taken during the perioperative period.(24). In addition, Sánchez, A., Rincón., et al., in the area of pediatrics and other previous research. In the area of neonatology, they have reported the presence of bacteria such as Escherichia coli, enterococcus spp, Klebsiella, pseudomona, Staphylococcus aureus and epidermidis, with high resistance to cephalosporins.(25).

*Antibiotic susceptibility*

Currently, the resistance of S. aureus is marked due to its versatility, which makes it resistant to conventional regimens, presenting inducible resistance mechanisms with macrolides, lincosamides and streptogramides.(26)According to Sharon and Gavin in a study carried out in the United Kingdom, by 2015 the use of penicillin turned out to be ineffective since the rate of bacterial resistance reached up to 90%.(27).

If we compare the resistance patterns of S. aureus in the current literature, we find that the first resistance mechanism of this bacteria was described in 1942 towards penicillin, a mechanism by which, through penicillinase enzymes or also called β-lactamases encoded by the blaZ gene, transmitted by plasmids, these enzymes hydrolyze the β-lactam rings of penicillin, inactivating it and making it useless for treating infections caused by this bacteria.(27).

After several studies, semi-synthetic β-lactams were developed to replace penicillin, and resistant β-lactamases called methicillin, however, after their successful use, the first strains of methicillin-resistant S. aureus (MRSA) were found as a nosocomial pathogen mediated by the mecA gene that encodes penicillin-binding proteins (PBP) giving resistance to all β-lactam drugs.(27). Later, vancomycin is used as an alternative (it also shows resistance factors due to the vanA gene) as the last treatment options in severe infections in addition to other new antibiotics such as linezolid and daptomycin.(27).

This research shows a 50% resistance to penicillin in the isolated S. aureus strains, which indicates the presence ofβ-lactamases, however, do not present other more complex resistance mechanisms such assynthesis of a new PBP (penicillin-binding protein) and β-lactam proteins of chromosomal nature (mec gene)(27).

Despite the favorable results, inducible resistance to Clindamycin should be clinically evaluated using the D-test to avoid treatment failures according to the literature

(27).Besides,In Enterobacteriaceae, the main resistance mechanisms revolve around the synthesis ofβ-lactamases which mainly encompasses 4 subdivisions of coding genes:

- BLEA: This gene encodes enzymes that confer plasmid-guided resistance to all natural and synthetic penicillins, but is sensitive to first- and second-generation cephalosporins.(28).
- ESBL: Extended spectrum beta-lactamases are mainly expressed in bacteria such as Klebsiella and E. coli and confer resistance to 1st, 2nd and 3rd generation cephalosporins, +/- to 4th generation cephalosporins.(29).

They are a surveillance tool since this gene can identify variants that modify microbial susceptibility. More than 300 variants such as TEM, SHV, CTX-M and OXA are described in the literature (28).

- AmpC: They belong to the Ambler molecular class C. They influence resistance to aztreonam, giving resistance to this and all the previous groups, which includes the subsequent creation of carbapenems.(30).
- Carbapenemases: These confer resistance to drugs used as a last resort in nosocomial infections such as meropenem, empinen, ertapenem. There are several types such as KPC, NDM, OXA-48 carbapenemases.(31).

The AmpC and carbapenemase phenotypes have a mechanism mediated by mutations in DNA gyrase and topoisomerase IV or those mediated by plasmids induced by modified enzymes.(30).

In the case of Klebsiella spp., resistance to two of 16 antibiotics was observed in the two strains isolated from this species, these are ampicillin and amoxicillin/clavulanic acid, indicating BLEA-type resistance, in the operating room area, for which reason the first-line treatment should be re-evaluated in nosocomial infections by this bacteria. Likewise, contamination in the operating room areas agrees with the bibliography studied as in the manuscript by: Herrera, Andrade and Reinoso, however, this research diverges from the resistance patterns found, since the study indicates more developed resistance mechanisms mediated by carbapenemases ofKPC-2, NDM and OXA-48, genes that were not reflected in microbial resistance in this research(32).

In the case of E. coli, resistance to penicillin, inhibitors, some first-generation cephalosporins and aminoglycosides was observed. In accordance with the resistance factors described for the bacteria, we also see non-advanced resistance mechanisms as those highlighted in other studies.(33).

A particular case presents a strain isolated from an operating room (sample 11), with resistance to 4 antibiotics including trimethoprim/sulfamethoxazole, gentamicin, ciprofloxacin and levofloxacin, drugs used for the treatment of urinary tract infections. If

we compare these results with other research such as that carried out by Betrán, et al., we find similarity in the patterns of resistance to these antibiotics.(34).

Pseudomona resistance patterns require strict hospital monitoring as it can present resistance naturally and acquired because its cell membrane has excellent impermeability properties and each strain can transmit genetic material that mediates resistance, which also occurs with gram-negative bacteria such as enterobacteria in its environment.(35).

These mechanisms include:β-lactamases and alterations in membrane permeability due to the presence of efflux pumps and mutations in transmembrane porins. The two classes ofβ-lactamases areAmp-C and ESBLs, the first has the ability to be induced by β-lactams themselves, which can lead to resistance to penicillins and cephalosporins (ceftazidime, cefepime). In addition, this enzyme is inducible before or during treatment with β-lactams and cephalosporins.(35). As for ESBLs, they also manifest with resistance to penicillins and cephalosporins and production of Carbapenems giving resistance to carbapenems, additionally the PER-1 polymorphism confers clear resistance to ceftazidime.(35).

Class A carbapenemases, class B or metallo-beta-lactamases (MBL) that hydrolyze and are not inhibited by inhibitors such as clavulanate, tazobactam, among others, and class D, oxacillinases (OXA) that hydrolyze imipenem and meropemen have been described.(36).

In Pseudomona, an interesting resistance to ceftazidime and meropenem was observed, which is worrying after previously describing the resistance mechanisms of pseudomonas. However, the fact that it is sensitive to piperacillin/tazobactam, which is a beta-lactamase inhibitor, indicates that in the case of nosocomial infection there is the possibility of an effective treatment despite being a carbapenemase.

When compared with other studies such as the documentary review by Barbecho Diana, who studied antimicrobial susceptibility in Pseudomona spp., at the Vicente Corral Moscoso Hospital, Cuenca, it coincides with increasing carbapenem resistance, however, it does not show greater resistance to third-generation cephalosporins as found in this research.(37). Adding that to have greater knowledge of the resistance mechanisms, it would be necessary to isolate a greater number of strains in different areas.(37).

*S. saprophyticus*showed resistance to erythromycin, which was not induced by clindamycin. In the literature, this type of resistance refers to the MSB phenotype.(38), encoded by the msrA gene that facilitates active expulsion(39), when there is resistance to erythromycin and sensitivity to clindamycin without alteration in the halo, the MLSB phenotype could also be presented in which resistance to erythromycin induces resistance to clindamycin.(38).

If we relate these results with another bacteriological characterization in the operating room carried out by Cáceres et al. It shows resistance patterns of coagulase-positive Staphylococcus towards erythromycin without inducing Clindamycin.(40)The susceptibility of Enterococcus to different antibiotics shows a relevant sample in which it presents an interesting pattern being resistant to penicillin, linezolid and ampicillin, a multi-resistance requiring extreme caution.

The American Society of Infectious Diseases recommends the use of linezolid or daptomycin when Enterococcus strains are resistant to ampicillin and vancomycin.(41). In the hospital environment, vancomycin or linezolid are used as last-line antibiotics, however, resistance in particular to this oxazolidinone, which is described in the literature as a low resistance among gram-positive bacteria (less than 0.5%) as it is bacteriostatic that inhibits protein synthesis by binding to the 50S subunit of the bacterial ribosome, but finding strains resistant to it becomes worrying and causes strict sanitary control within the hospital environment.(41,42). In addition, further study and characterization of Enterococcus strains is needed in order to reduce the risk of nosocomial infections and monitor in vitro susceptibility to vancomycin.(42).

Finally, the susceptibility to antibiotics of Streptococcus agalactiae is favorable, registering sensitivity to beta-lactams and an intermediate sensitivity to Ceftriaxone (third generation cephalosporin) according to the CLSI used as a guide.(43).

*Disinfectant susceptibility*

Disinfectant products play a fundamental role in managing the spread of infections in healthcare environments. Their main function is to reduce the presence of microorganisms on surfaces and equipment, in order to mitigate the risk of infections linked to healthcare.(44)These disinfectants can be classified according to their scope of action, ranging from those with a broad spectrum, effective against bacteria, viruses and fungi, to those with a more limited spectrum, specifically directed against certain types of microorganisms.(44).

The sensitivity of S. aureus, Streptococcus agalactiae, Enterococcus, S. saprophyticus, Klebsiella, Pseudomona and E. coli to disinfectants showed a marked resistance, which is worrying compared to the sensitivity to antibiotics. Chacón and Rojas in their scientific review indicate that the appearance of bacterial mechanisms is not only due to exposure to drugs, but to constant exposure to biocides favoring survival and generating new factors of microbial resistance.(8).

In this study the resistance of*S. aureus, Streptococcus agalactiae, Enterococcus, S. saprophyticus, Klebsiella, Pseudomona*and E. coli towards sodium hypochlorite and ethanol. McDonnell and Russell mention that alcohols have been shown to be effective

as antiseptics and disinfectants, due to the rapid antimicrobial activity towards bacteria, viruses and fungi.(45). Besides,A comparative study conducted by Galván et al. used 6% sodium hypochlorite concentrations, which gave a favorable result without bacterial growth, however, it is a highly oxidizing substance.(46).

Although potassium monopersulfate has been proven to be effective against some bacteria and viruses, even those that do not respond to other disinfectants, there is a possibility that bacteria may become resistant to this product over time, as several studies have detected bacteria that show resistance to potassium monopersulfate.(47)Another very important disinfectant is povidone-iodine, which has a broad antimicrobial spectrum, including gram-negative bacteria such as Klebsiella pneumoniae and gram-positive S. aureus and methicillin-resistant E. coli. This disinfectant has shown greater effectiveness than chlorhexidine.(48). Likewise, povidone iodine is a very promising alternative within hospital areas due to its wide antimicrobial spectrum and effectiveness.(48).

## Conclusions

- The in vitro effectiveness of hospital disinfectants on bacteria isolated from the areas: neonatology and operating room was evaluated, finding a high percentage of samples resistant to ethanol, potassium monopersulfate, sodium hypochlorite and glutaraldehyde,under the average concentrations established by the MSP and WHO, predominant against resistance to antibiotics that turned out to be low, resulting in resistance mainly to B lactams, some cephalosporins and an isolated case of Enterococcus resistant to Linezolid or a strain of Pseudomona resistant to meropenem.
- Antibiotic-disinfectant resistance is mainly related to the resistance capacity of bacteria to most disinfectants used in hospitals, which becomes a focus of infection that requires important control because the two areas studied must be a sterile environment free of pathogenic macroorganisms, in addition to especially considering the strains that have shown resistance to antibiotics considered to be the last line of treatment.
- The ideal concentration to inhibit microbial growth in these areas remains under study, observing the growth of Enterobacteria, possible resistance mechanisms already developed, residual action time after disinfection, control of sanitization processes and disinfection of surfaces and health personnel.(49).

## Conflict of interest

There is no conflict with the authors.

## Authors' contribution statement

The article was developed by all authors.

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