Effectiveness of 0.35% Diluted Povidone-Iodine Irrigation on Bacterial Cultures: An In Vitro Study

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ABSTRACT

Introduction: Hip and knee arthroplasties are increasingly performed due to their excellent outcomes in pain relief and quality of life improvement. However, they are not free from complications, with periprosthetic infection being one of the most challenging. This study aimed to evaluate the effectiveness of 0.35% diluted povidone-iodine irrigation against various microorganisms as a prophylactic measure against periprosthetic infections. **Materials and Methods:** A prospective study was conducted using *in vitro* irrigation of 0.35% diluted povidone-iodine on bacterial cultures. Gram-positive cocci (*Staphylococcus aureus*, coagulase-negative *Staphylococcus*, and *Enterococcus faecalis*) and gram-negative bacilli (*Pseudomonas aeruginosa*, *Acinetobacter* sp., and *Klebsiella pneumoniae*) were studied to simulate intraoperative contamination. The bacterial inoculum was quantified using the McFarland scale, reflecting concentrations similar to those expected in *in vivo* periprosthetic infections. **Results:** Growth inhibition of *Staphylococcus* sp. (*S. aureus* and coagulase-negative *Staphylococcus*) was observed in the presence of diluted povidone-iodine. However, there was no significant reduction in the colony-forming units of gram-negative bacilli treated with povidone-iodine. **Conclusions:** Povidone-iodine diluted to 0.35% significantly inhibits the growth of *Staphylococcus* sp. However, gram-negative bacilli and *Enterococcus* sp. (*E. faecalis*) exhibited substantial colony growth, highlighting the limited efficacy of this dilution against these pathogens *in vitro*.

Keywords: Periprosthetic infection; McFarland scale; povidone-iodine; gram-negative microorganisms.

Level of Evidence: IV

Efectividad de la irrigación de povidona yodada diluida al 0,35% en cultivos bacterianos. Estudio *in vitro*

RESUMEN

Introducción: Las artroplastias de cadera y rodilla siguen en aumento debido a sus excelentes resultados en cuanto al alivio del dolor y la mejoría de la calidad de vida; sin embargo, no están exentas de complicaciones y una de las más desafiantes es la infección periprotésica. El objetivo de este estudio fue evaluar la efectividad de la irrigación de povidona yodada diluida contra distintos microorganismos como profilaxis contra infecciones periprotésicas. **Materiales y Métodos:** Se realizó un estudio prospectivo que consistió en la irrigación de povidona yodada diluida al 0,35% a cultivos bacterianos *in vitro*. Se estudiaron cocos grampositivos (*Staphylococcus aureus, Staphylococcus* coagulasa negativo y *Enterococcus faecalis*) y bacilos gramnegativos (*Pseudomonas aeruginosa, Acinetobacter* sp., *Klebsiella pneumoniae*), simulando una contaminación intraquirúrgica. Usando la escala de McFarland se cuantificó el inóculo bacteriano infectante, de manera similar a las concentraciones esperadas en infecciones periprotésicas *in vivo*. **Resultados:** Se evidenció inhibición del crecimiento de *Staphylococcus* sp. (*S. aureus* y *Staphylococcus* coagulasa negativo) en presencia de povidona yodada diluida. Sin embargo, no se observó un descenso significativo en la cantidad de unidades formadoras de colonias de bacilos gramnegativos tratados con povidona yodada. **Conclusiones**: La povidona yodada diluida al 0,35% inhibe significativamente el crecimiento de *Staphylococcus* sp. Sin embargo, los bacilos gramnegativos y *Enterococcus* sp. (*E. faecalis*) muestran un gran crecimiento de colonias, lo que pone de manifiesto la baja efectividad de la dilución contra estos patógenos *in vitro*.

Palabras clave: Infección periprotésica; escala de McFarland; povidona yodada; microorganismos gramnegativos.

Nivel de Evidencia: IV

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INTRODUCTION

As hip and knee arthroplasties continue to evolve and grow steadily, there has been an exponential increase in complications, such as periprosthetic joint infections (PJI).[1] Efforts to reduce the risk of infection have focused on numerous patient-related factors, including bacterial skin decolonization, optimization of nutritional status, metabolic diseases, obesity, and smoking. Additionally, surgical factors such as prophylactic antibiotics, operating room environment, and surgical duration play a crucial role. Among these measures, surgical site irrigation is highly cost-effective in preventing PJI, as it minimizes bacterial contamination. This is often performed using saline alone or with the addition of chlorhexidine or povidone-iodine (PI). Thorough irrigation is essential to reduce the risk of infection in arthroplasty procedures.[2] While various irrigation methods have been described,[3] the World Health Organization Clinical Practice Guidelines recommend the use of PI for wound irrigation during surgical procedures.[4-6]

The Second Philadelphia International Consensus on Musculoskeletal Infections (2018) recommended diluted PI irrigation for PJI prophylaxis;[7] however, there are no clear guidelines regarding the optimal type, volume, or irrigation protocol for PJI management.

PI is highly soluble in water, allowing its gradual release in an aqueous medium with a broad spectrum of antimicrobial activity against bacteria, protozoa, fungi, and viruses. It achieves this through the iodination of lipids and the oxidation of cytoplasmic and membrane components. Additionally, PI inhibits the formation of staphylococcal biofilms, and no acquired resistance has been reported. However, it has been found to cause histological damage in its pure form due to cytotoxicity. Therefore, a dilution of 17.5 cc of PI in 500 cc of saline (0.35%) is recommended as an antiseptic agent for tissue irrigation.[8,9]

The objective of this study was to evaluate the effectiveness of 0.35% diluted PI irrigation in reducing bacterial growth and preventing complications related to PJI, thereby decreasing the economic costs associated with managing this complication.

MATERIALS AND METHODS

A prospective *in vitro* study was conducted to evaluate the bactericidal effect of 0.35% diluted PI against selected bacteria. The study was carried out in the bacteriology unit of Hospital Central de San Isidro "Melchor Á. Posse," using tryptic soy agar as the culture medium.

The microorganisms analyzed included:

- Gram-positive bacteria: Coagulase-negative *Staphylococcus*, *S. aureus*, *E. faecalis*.
- Gram-negative bacteria: *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Klebsiella pneumoniae*.
- *In vitro* bacteria obtained from clinical samples and cultured for 24 hours at 37 °C.

The selection of these microorganisms was based on the local institutional epidemiology of Hospital Central de San Isidro "Melchor Á. Posse," as they are the most prevalent in PJI cases.

The McFarland scale[10] was used as a reference for the number of colony-forming units (CFU) seeded and cultured, in order to subsequently make *in vitro* suspensions of the microorganisms, with 0.5 McFarland corresponding to approximately 1×10^8 colony-forming units per milliliter (CFU/ml). Serial dilutions were then performed to achieve lower bacterial concentrations (1×10^4 and 1×10^2 CFU/ml), representing intraoperative contamination levels.

Each bacterial strain was cultured at concentrations of 1×10^8, 1×10^4, and 1×10^2 CFU/ml on tryptic soy agar and incubated in Petri dishes at 37 °C for 4 hours (estimated duration of a joint arthroplasty surgery).

For each bacterial concentration, three additional Petri dishes were prepared to assess the effectiveness of irrigation. The effect of the 0.35% diluted PI was evaluated by irrigating the plates post-incubation (Figure 1) for 3 minutes (Figure 2).

The plates were then washed with sterile saline to remove excess antiseptic, halting its activity, and incubated at 37 °C for 24 hours for subsequent analysis. In parallel, 2 control groups were included; the first was the growth control, plates without any added solution and the second, a wash control, in which the plates were rinsed with sterile saline for the same duration as the PI.

Figure 1. Irrigation of Petri dishes with 0.35% povidone-iodine.

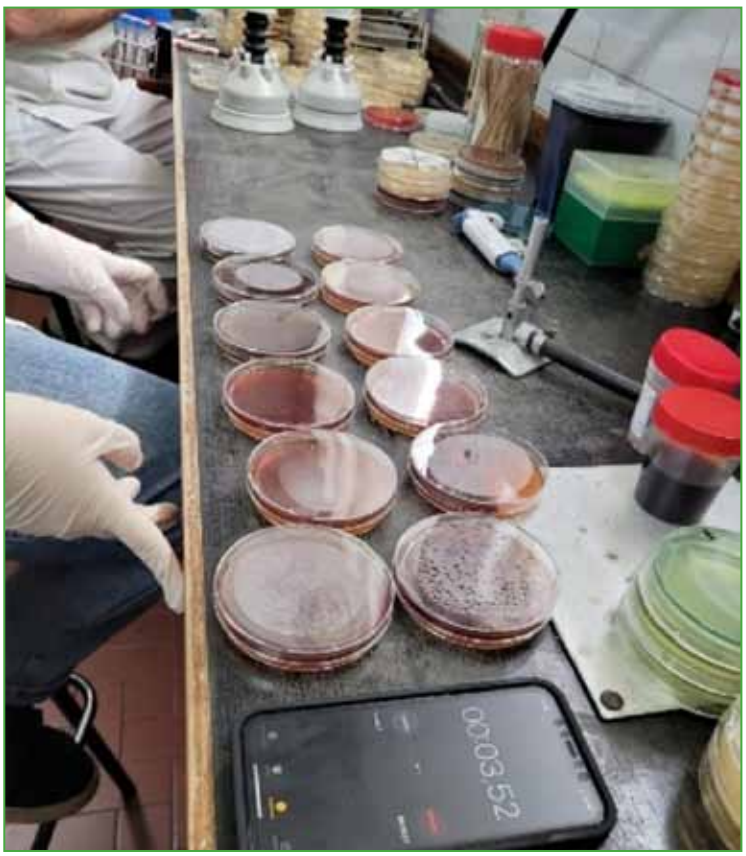

Figure 2. Timed control of povidone-iodine application to Petri dishes.

After 24 hours of incubation, the plates were analyzed, and CFU/ml counts were recorded for each dilution and bacterial strain.

RESULTS

A significant reduction in CFU/ml was observed in *Staphylococcus* sp. cultures treated with 0.35% diluted PI compared to both the growth control and the saline-treated group. However, no reduction in CFU/ml was noted for the gram-negative bacilli or *Enterococcus* sp. in any of the growth controls (Tables 1-3)

Table 1. *Pseudomonas aeruginosa* culture at 24 hours.

Bacteriological CFU concentration of P. aeruginosa	Povidone-iodine diluted at 0.35%.	Washing control (saline)	Growth control (without irrigation)
1 x 10^8	Develops	Develops	Develops
1 x 10^4	Develops	Develops	Develops
1 x 10^2	Develops	Develops	Develops

CFU = colony forming units.

Table 2. Culture of *Acinetobacter* sp. at 24 hours.

Bacteriological CFU concentration of Acinetobacter sp.	Povidone-iodine diluted at 0.35%.	Washing control (saline)	Growth control (without irrigation)
1 x 10^8	Develops	Develops	Develops
1 x 10^4	Develops	Develops	Develops
1 x 10^2	Develops	Develops	Develops

CFU = colony forming units.

Table 3. Culture of *Klebsiella pneumoniae* at 24 hours.

Bacteriological CFU concentration of K. pneumoniae	Povidone-iodine diluted at 0.35%.	Washing control (saline)	Growth control (without irrigation)
1 x 10^8	Develops	Develops	Develops
1 x 10^4	Develops	Develops	Develops
1 x 10^2	Develops	Develops	Develops

CFU = colony forming units.

Specifically, growth inhibition of gram-positive cocci (Figures 3 and 4) (coagulase-negative *Staphylococcus* and *S. aureus*) was detected in the presence of the antiseptic (Tables 4 and 5). In contrast, gram-negative bacilli strains (*P. aeruginosa*, *K. pneumoniae* and *Acinetobacter* sp.) (Figures 5-7) and a culture of *E. faecalis* (Figure 8, Table 6), developed bacterial growth in 100% of the samples (Tables 1-3) across all experimental conditions, including PI-treated cultures, saline-treated controls, and the untreated control. These findings indicate differential susceptibility to diluted PI, with greater sensitivity observed in *Staphylococcus* sp. compared to gram-negative bacilli and *Enterococcus* sp.

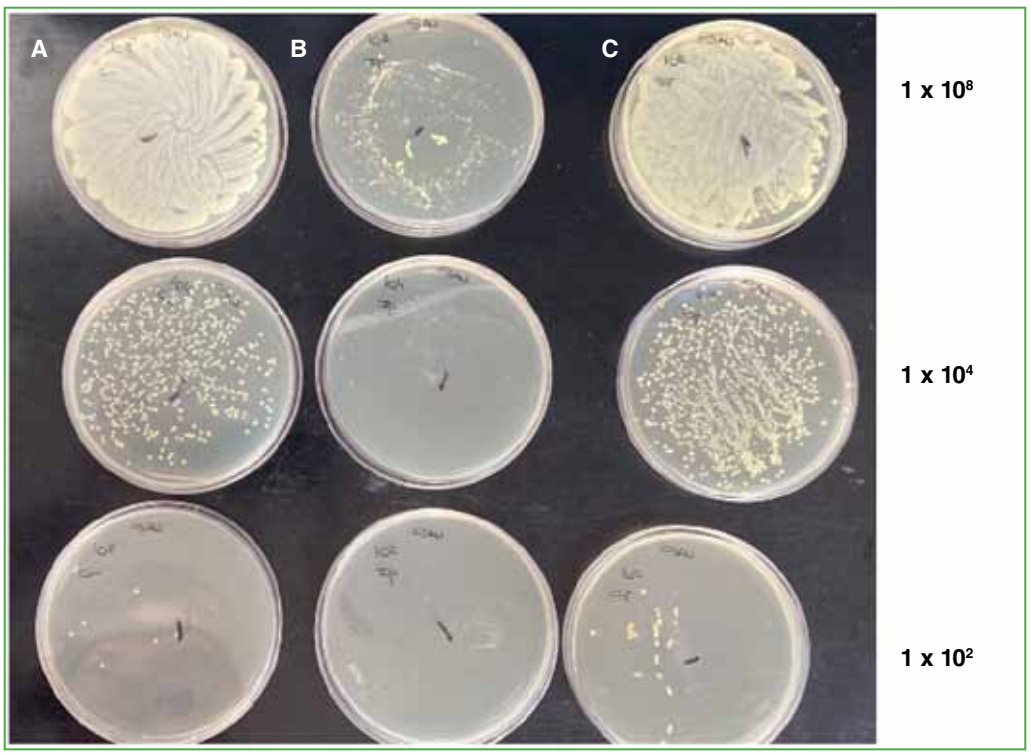

Figure 3. *In vitro* cultures of *S. aureus*. Column **A**, Control cultures without irrigation, showing extensive colony growth. Column **B**, Cultures treated with povidone-iodine, showing clear inhibition of CFU growth. Column **C**, Cultures exposed to saline irrigation, with a reduction in CFU count but preserved colony-forming capacity.

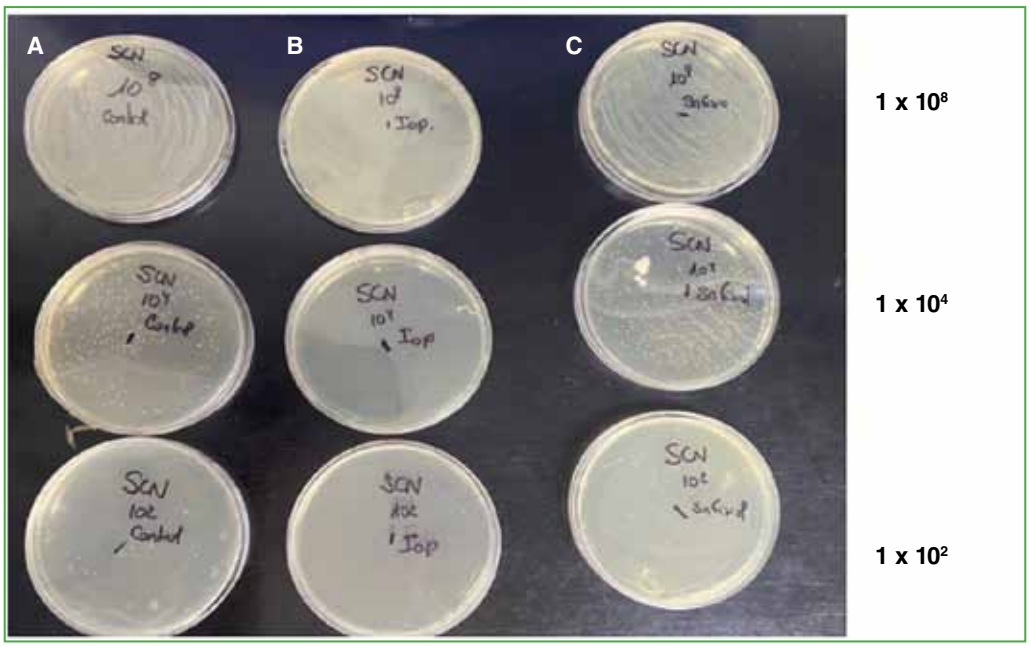

Figure 4. *In vitro* cultures of coagulase-negative *Staphylococcus*. Column **A**, control cultures without irrigation. Column **B**, Cultures treated with povidone-iodine, showing clear inhibition of CFU growth. Column **C**, Cultures exposed to saline irrigation, with a reduction in CFU count but preserved replication capacity.

Table 4. *Staphylococcus aureus* culture at 24 hours

Bacteriological CFU concentration of S. aureus	Povidone-iodine diluted at 0.35%.	Washing control (physiological solution)	Growth control (without irrigation)
1 x 10^8	Does not develop	Develops	Develops
1 x 10^4	Does not develop	Develops	Develops
1 x 10^2	Does not develop	Develops	Develops

CFU = colony forming units.

Table 5. Coagulase negative *Staphylococcus* culture at 24 hours

Bacteriological CFU concentration of coagulase negative Staphylococcus	Povidone-iodine diluted at 0.35%.	Washing control (saline)	Growth control (without irrigation)
1 x 10^8	Does not develop	Develops	Develops
1 x 10^4	Does not develop	Develops	Develops
1 x 10^2	Does not develop	Develops	Develops

UFC = unidades formadoras de colonias.

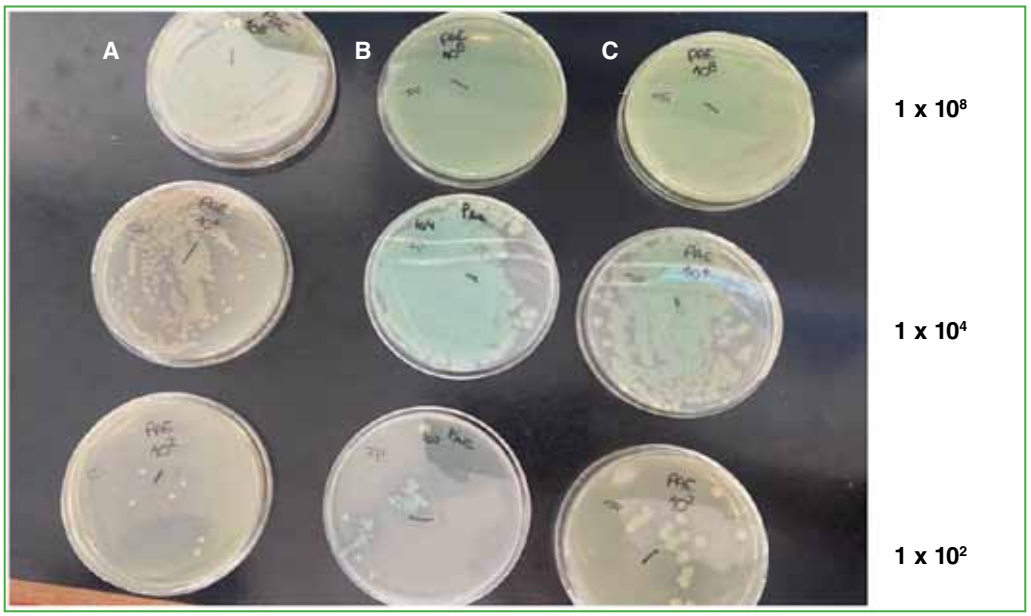

Figure 5. *In vitro* cultures of *Pseudomonas aeruginosa*. Column **A**, control cultures without irrigation. Column **B**, cultures exposed to povidone-iodine. Column **C,** Cultures irrigated with saline. CFU formation remains evident in all conditions.

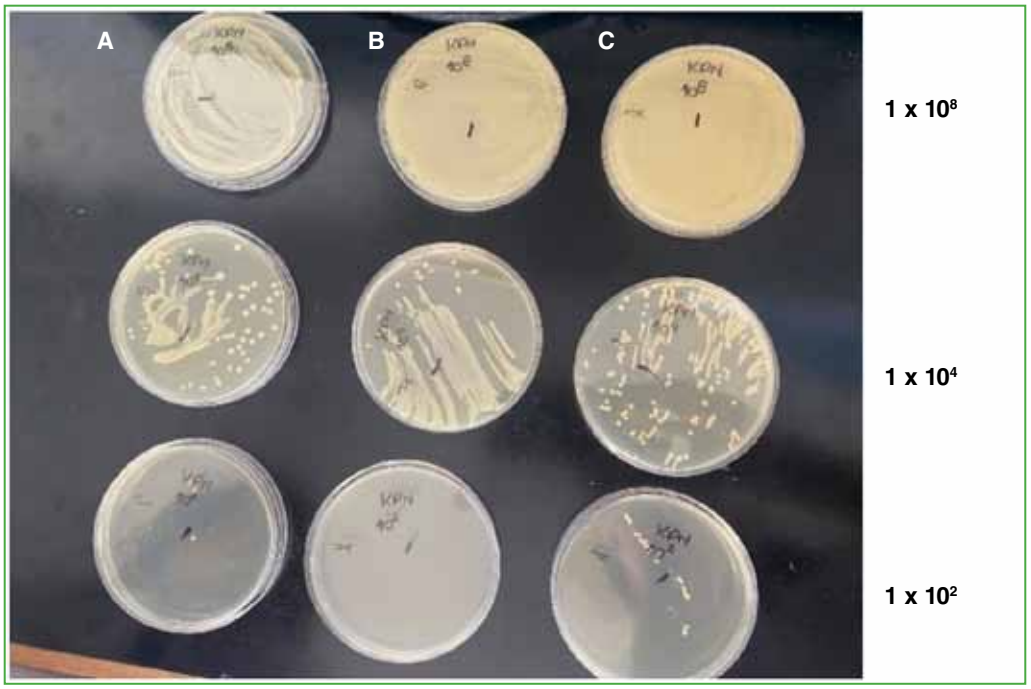

Figure 6. *In vitro* cultures of *Klebsiella pneumoniae*. Column **A**, control cultures without irrigation. Column **B**, cultures exposed to povidone-iodine. Column **C,** cultures irrigated with saline. A reduction in CFUs is observed; however, colonies retain their replication capacity across all conditions.

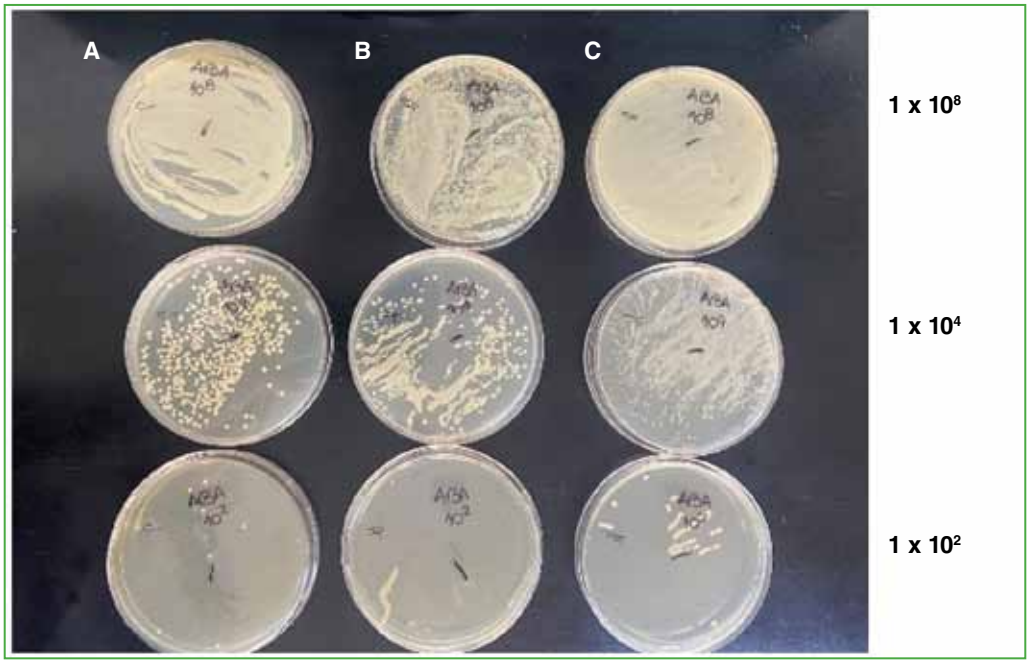

Figure 7. *In vitro* cultures of *Acinetobacter baumanni*. Column **A**, control cultures without irrigation. Column **B**, cultures exposed to povidone-iodine. Column **C,** cultures irrigated with saline. CFU formation remains evident in all controls.

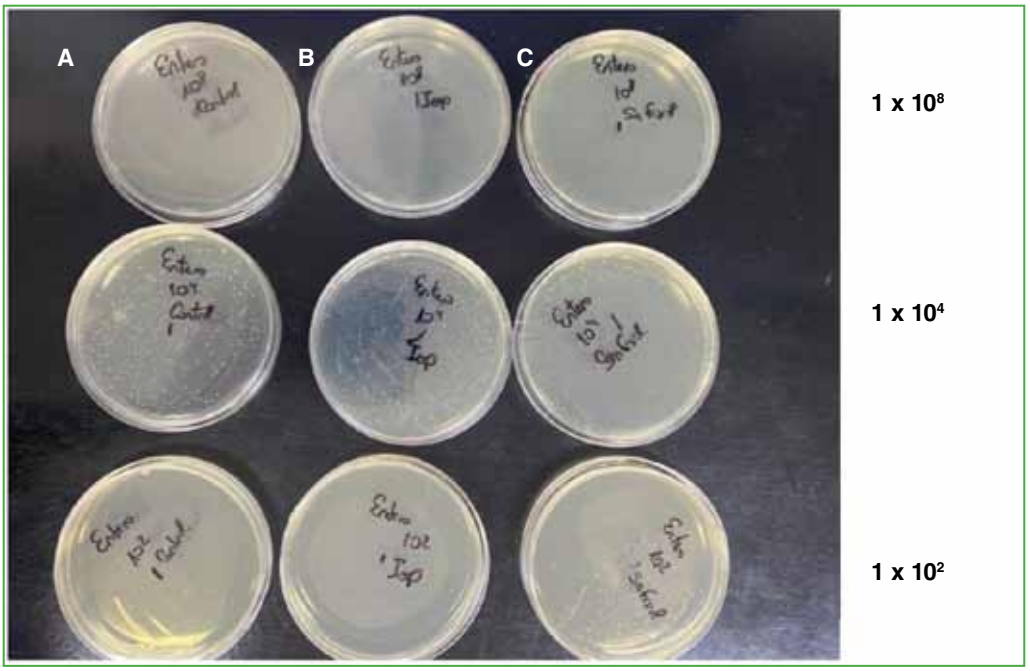

Figure 8. *In vitro* cultures of *Enterococcus faecalis*. Column **A**, control cultures without irrigation. Column **B**, cultures irrigated with povidone-iodine, showing partial inhibition of CFU growth. Column **C**, Cultures exposed to saline irrigation, showing a slight CFU reduction.

Table 6. *Enterococcus faecalis* culture at 24 hours

Bacteriological CFU concentration of E. faecalis	Povidone-iodine diluted at 0.35%.	Washing control (saline)	Growth control (without irrigation)
1 x 10^8	Develops	Develops	Develops
1 x 10^4	Develops	Develops	Develops
1 x 10^2	Does not develop	Develops	Develops

CFU = colony forming units.

DISCUSSION

The effectiveness of PI against polymicrobial flora in vitro has been documented in several studies, highlighting its efficacy against a range of bacteria, including *S. epidermidis*, *H. influenzae*, *Burkholderia cepacia*, and *Escherichia coli*.[11]

According to Cichos et al., PI irrigation has been shown to eradicate common bacteria associated with prosthetic joint infections, such as methicillin-resistant *S. aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), *S. epidermidis*, *H. influenzae*, *P. aeruginosa*, and *E. coli*, on a variety of orthopedic materials, including stainless steel screws, titanium discs, and polyethylene washers in *in vitro* studies.[12]

In 2010, Brown et al. demonstrated a decrease in the rate of PJI with the use of 0.35% diluted PI. They reported 18 cases (0.97%) of infection within the first 90 days before the use of the antiseptic and only one (0.15%) after its implementation. As a result, its use began to expand to other institutions.[13]

However, despite its antimicrobial efficacy, PI also poses potential risks to patient health. According to studies by Driesman et al. and Von Keudell et al., this antiseptic, in its undiluted form, can be highly toxic and cause tissue damage, potentially delaying the healing process. Therefore, it is crucial to address this issue and implement measures to mitigate its adverse effects. Its potential toxicity raises significant concerns for patient safety. Dilution to 0.35% with saline emerges as a key strategy to reduce these risks while preserving the antimicrobial benefits, minimizing its negative impact on wound healing and overall patient health.[14,15]

Several studies have shown that PI diluted with saline helps reduce its toxicity while maintaining antimicrobial effectiveness. This practice is particularly relevant in primary arthroplasty surgeries, where minimizing the risk of postoperative infection is critical. By diluting PI, a balance can be achieved between antimicrobial efficacy and patient safety, significantly reducing complications associated with antiseptic toxicity. Furthermore, dilution may help preserve surrounding tissue and promote faster and more effective wound healing.[16]

In our study, PI demonstrated efficacy as an antimicrobial agent against gram-positive bacteria (*S. aureus*, coagulase-negative *Staphylococcus*) for the prevention of prosthetic joint infections. However, its effectiveness was found to be limited against gram-negative bacilli and *Enterococcus* sp., as bacterial growth was observed following exposure to PI.

A strength of this research is that *in vitro* studies were conducted to recreate and simulate the typical duration of a joint prosthesis surgery, mimicking theoretical intraoperative contamination and subsequent irrigation with diluted PI.

This study also has limitations: the small number of strains and species analyzed, as well as the lack of a thorough assessment of gram-negative bacilli resistance to PI dilution. Prospective multicenter studies are needed to determine whether the observed lack of susceptibility is an institutional finding or a more generalized phenomenon. Another limitation is the absence of other antiseptic solutions as a control group.

CONCLUSIONS

0.35% PI is an effective intraoperative irrigation solution for inhibiting *Staphylococcus* sp. bacterial growth. However, its efficacy against gram-negative bacilli was shown to be limited, making it an unreliable option for reducing the overall risk of PJI. This is an emerging area of research, and further studies are needed to elucidate the resistance mechanisms of gram-negative bacteria to this antiseptic agent and to improve our understanding of the role of intraoperative irrigation in PJI prevention.

Conflict of interest: The authors declare no conflicts of interest.

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