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SPECIAL ARTICLE

The growth of bacteria in infusion drugs: propofol 2% supports growth when remifentanil and pantoprazole do not

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Abstract
Background and objectives: Contamination risks of propofol 2%, remifentanil, and pantoprazole; and in vitro effects of these drugs on the growth of common infective agents in intensive care units were evaluated.
Methods: For detection of contamination risk, drugs were prepared ready to use under intensive care unit conditions, were tested. Effects of these three drugs on bacterial growth were also investigated. Drugs were prepared at the concentrations used in the intensive care unit and inoculated with common pathogens after which they were incubated at 4°C, 22°C and 36°C. Subcultures were made at 0, 2, 4 and 8 h and colony counts were evaluated. Minimum inhibitory concentration values were determined for all drugs at 4°C, 22°C and 36°C.
Results: No growth was observed in the drugs prepared in the intensive care unit. Propofol tended to support while remifentanil inhibited bacterial growth. Effect of pantoprozole differed according to the bacteria tested. None of the drugs showed antibacterial activity at the maximum concentrations which may be achieved in blood of the patients.
Conclusion: Propofol strongly supports the growth of the microorganisms tested, although remifentanil and pantoprazole do not. Therefore, it is important to follow the strict aseptic techniques for the preparation of propofol.

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Introduction

Nosocomial infections in the intensive care units (ICU) significantly increase morbidity, mortality rates and financial cost.[1,2] Although, ICUs account for approximately 10% or less of hospital beds, more than 20% of all nosocomial infections occur in patients who are in the ICU.[3] Drugs used in ICU may influence nosocomial infections by their effect on bacterial growth.[4] Used ampoules and syringes may be contaminated in a busy environment.[5,6] There have been sporadic reports of bacteremia caused by the distribution of infected drugs. Simple infection control protocols are shown to be effective in different hospital settings.[7,8] Type of drug and duration of usage may also be an important factor. Knowing the drugs which have a greater tendency to create an infection risk, especially the ones used by long infusion, would be important for setting up regulations and minimizing the risk. Three commonly used drugs in critically ill patients and ICU were chosen in this study: Propofol, remifentanil and pantoprazole. Propofol is known as a good growth medium for bacteria.[9] Remifentanil and pantoprazole have antibacterial properties.[9,10] All these drugs are given by long infusions.[9,10] Antibacterial effects of propofol 1%, remifentanil 1, 10 and 100 $\mu g \bullet mL^{-1}$ has been studied.[4,9] However, the antibacterial effectiveness of propofol 2%, remifentanil (40 $\mu g \bullet mL^{-1}$) and pantoprazole remains to be determined.

The aim of this study was to evaluate the contamination risks of propofol 2%, remifentanil, and pantoprazole, and to investigate the in vitro effects of these drugs on the growth of microorganisms known to be frequent causes of infection in intensive care units.

Material and methods

The antimicrobial effect of three anesthetic drugs, propofol 2% (1.g.50.mL^{-1} Fresenius Kabi, Germany), remifentanil (2 mg, GlaxoSmithKline, Italy) and pantoprazole (40 mg, Altana Pharma, Germany) were evaluated. All experiments were performed in duplicate.

Investigation of contamination risk

All three drugs were prepared for usage in ICU conditions according to the protocols used in the ICU to prepare i.v. drugs for patients and placed in two separate injectors as described.[11] As a control, 0.85% NaCl solution was also placed in two injectors. One of the injectors was incubated at room temperature (22 ± 2ºC) and the other in the refrigerator (4 ± 2ºC) in the ICU and 100 µl of the incubated drugs were cultured onto Columbia sheep blood agar (Becton Dickinson, Germany) at 0, 2, 4 and 8 h. Plates were evaluated after overnight incubation at 36 ± 2ºC. In case of any bacterial growth, colony counts were detected.

Effect on bacterial growth

Bacteria which are frequent causes of nosocomial infections and which belong to the normal flora of the skin were selected for the study. *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and a clinical isolate of a multidrug resistant *Acinetobacter* spp. were chosen.

Effect of drugs at the concentrations used in the ICU on bacterial growth

The method used in this part of the study is modified from the studies by Batai et al.[12] and Wu et al.[13] All three drugs were prepared for usage in ICU conditions and distributed into three sets of sterile tubes, 1 mL per tube. Three sets of sterile 0.85% NaCl solution were also prepared. A set of tubes consisted of 7 tubes, including all bacteria to be tested plus one tube for control. Bacterial solutions were prepared at 0.5 MacFarland and diluted by 1/1000.[14] All tubes except the control tubes were inoculated with 50 µL of bacterial solutions. No bacteria were added to the control tubes. The first set of tubes was incubated at 4 ± 2ºC, the second at 22 ± 2ºC and the third at 36 ± 2ºC. The incubated drugs were diluted by 1/100 and 100 µL of the dilutions were subcultured onto Columbia sheep blood agar at the 0, 2, 4 and 8 h. Plates were evaluated after overnight incubation at 36 ± 2ºC. In case of any bacterial growth, colony counts were detected.

Determination of minimum inhibitory concentrations of drugs

Minimum inhibitory concentration (MIC) values of all three drugs and 0.85% NaCl solution were studied by microdilution method.[14] Microdilution was performed at three different temperatures, 4 ± 2ºC, 22 ± 2ºC and 36 ± 2ºC. Cation adjusted Mueller Hinton broth (Oxoid Ltd., England) was used for all the bacteria. The concentrations to be tested were selected according to the maximum concentrations of the drugs in blood of the patients when administered.

Statistical analysis

Statistical analysis was carried out using SPSS 11.5 (SPSS Inc., Chicago, IL). The one-sample Kolmogorow-Simirnov test was used for determining whether the data were normally distributed. For colony counts, ANOVA test was used to compare four groups of drugs. A t-test on two independent samples was used to compare the drug studied with normal saline or two different drugs with each other. The colony counts at different time points studied was analyzed using repeated measures ANOVA. Unless noted otherwise, data were presented as mean with standard deviation (SD).

Results

Investigation of contamination risk

In the first part of the study no growth was observed in samples prepared ready to use in the ICU and incubated in both temperatures.

Effect of drugs at the concentrations used in the intensive care units on bacterial growth

The mean colony counts of *S. aureus*, *E. faecalis*, *S. epidermidis*, *E. coli*, *P. aeruginosa* and *Acinetobacter* spp. after exposure to test solutions are shown in Figs. 1 to 6, respectively.

Propofol supported the growth of bacteria. The bacterial growth increased or stayed the same for all bacteria at all temperatures (Figs. 1-6). Growth of S.aureus in propofol at room temperature is shown at Fig. 7.

Remifentanil inhibited bacterial growth and the decrease in bacterial counts was more evident at 36 ± 2ºC (Figs. 1-6).

Pantoprazole, did not support bacterial growth and when compared to 0th hour, significantly ($p < 0.05$) reduced the bacterial counts of *S. epidermidis* and *Acinetobacter* spp. in 8 hours at 36 ± 2ºC (Figs. 1-6).

Determination of minimum inhibitory concentrations of drugs

The MIC values were above the tested concentrations for all the drug, microorganism and temperature combinations. MICs were > 5 $\mu g \cdot mL^{-1}$ for propofol 2%, > 500 $\mu g \cdot mL^{-1}$ for remifentanil and > 10 $mg \cdot mL^{-1}$ for pantoprazole.

Discussion

Although propofol is a rich growth medium for bacteria,[15] if propofol was drawn into sterile syringes immediately after the ampoules had been opened, no growth was detected after 24 hours. Our data are comparable with those from other investigations. Warwick et al.[16] suggested that propofol might be used safely up to 24 hours when drawn into sterile syringes. Others have suggested 72 hours.[17] Webb et al. reported contamination of propofol in syringes although none caused clinical infection.[18] However, in our study, colony counts in contaminated syringes reached significant difference in 8 hours for *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa* and *Acinetobacter* spp. at 36 ± 2ºC. Bacterial counts increased in time even at room temperature (Fig. 7). Our results were similar with previous studies which show that propofol supports the rapid growth of *E. coli*, *P. aeruginosa*, *Enterobacter cloacae*, *Moraxella osloensis*, *Acinetobacter* spp., *S. aureus*, *S. epidermidis*, *E. faecalis* and *Candida albicans*, when inoculated in vitro.[19,20] These findings support the importance of strict aseptic techniques. Fluids and drugs may become contaminated by microorganisms during production and/or preparation for infusion. Poor aseptic technique may be common among health care workers especially in a busy work environment.[21,22] Bacterial contamination of propofol may occur during opening of the glass ampoules, and there is poor compliance with data sheet recommendations for the use of propofol. To escape from bacterial contamination, the neck of the ampoule should be wiped with alcohol; hands should be washed before any manipulation; syringes and pumps should be prepared in aseptic conditions immediately before the use of propofol; ampoules and syringes should be labeled with the date and hour of preparation; propofol should be drawn into syringes in amounts that can be used at one time and the residual, if any, should be discarded; and finally, disposable devices such as syringes, infusion sets and triple manifolds should be used for a single patient only.[23-25] However, in our study, the necks of the ampoules were not wiped with any disinfectant to reproduce usual daily working conditions but other recommendations were followed. Our results are in consistence with the manufacturer's recommendation that propofol should be used within six hours of its handling

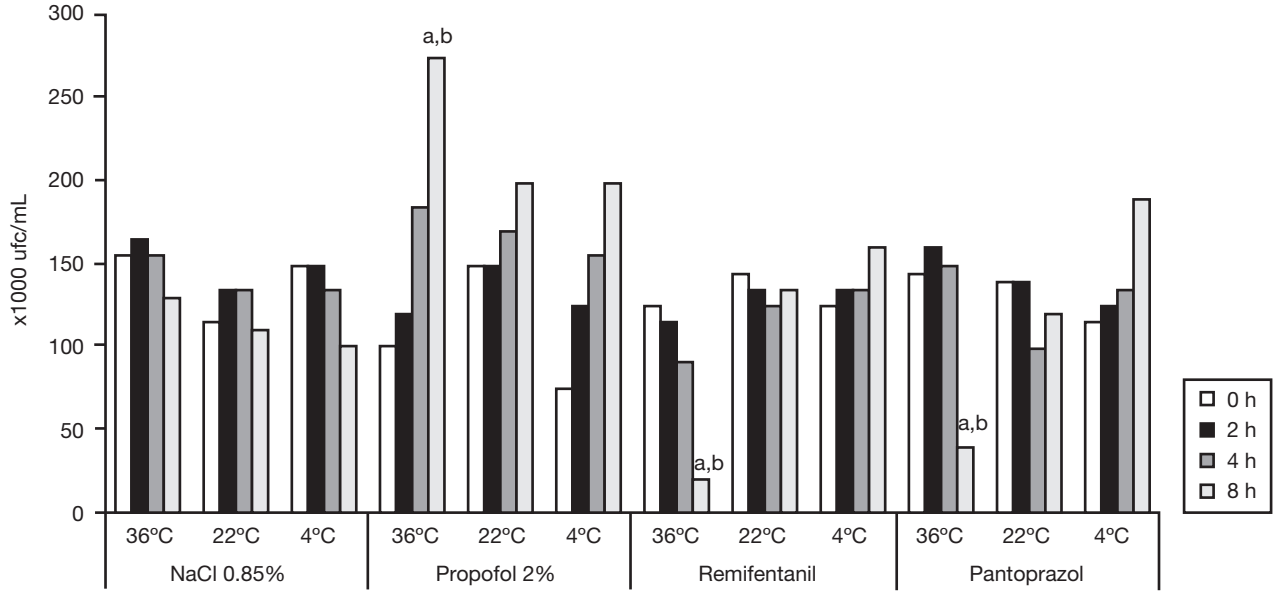

Figure 1 The colony counts of *Staphylococcus aureus* in the solutions tested. [a] Result is significantly different from the beginning (0 h), $p < 0.05$. [b] Result is significantly different ($p < 0.05$) when compared to 0.85% NaCl.

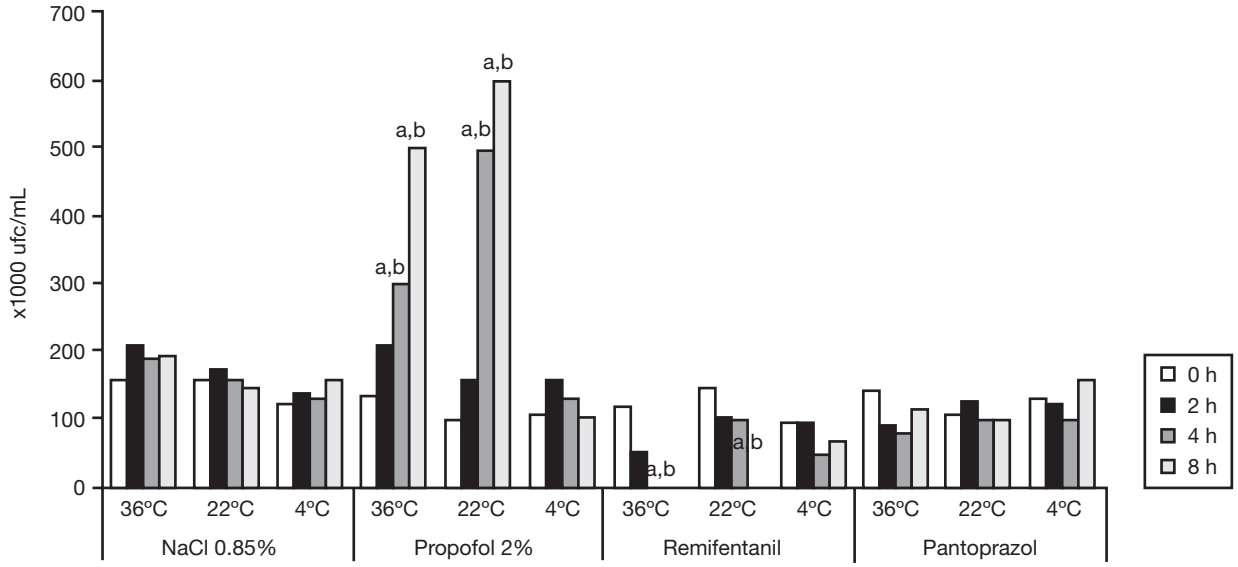

Figure 2 The colony counts of *Enterococcus faecalis* in the solutions tested.[a] Result is significantly different from the beginning (0 h), $p < 0.05$. [b] Result is significantly different ($p < 0.05$) when compared to 0.85% NaCl.

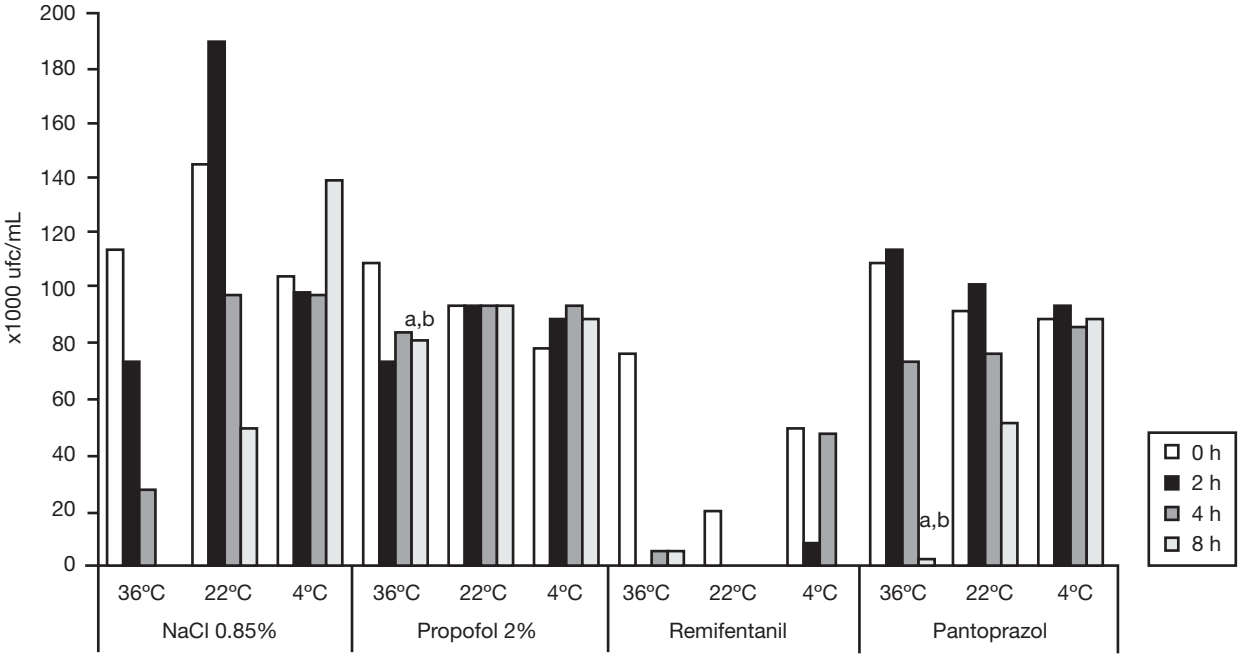

Figure 3 The colony counts of *Staphylococcus epidermidis* in the solutions tested. [a] Result is significantly different from the beginning (0 h), $p < 0.05$. [b] Result is significantly different ($p < 0.05$) when compared to 0.85% NaCl.

and, aseptic techniques should be used in the handling and administration of propofol. Even trace contamination of propofol is a risk of a significant bacterial load to the patient if the drug is not used within the recommended time interval.[26]

On the other hand, temperature had impact on growth rates of contaminated propofol. Crowther et al.[25] reported that the lower temperature may reduce the growth of *S. aureus*. Similarly, our results showed increased growth of *S. aureus*, *E. faecalis*, *E. coli* and *Acinetobacter* spp. at higher temperature. However, colony counts increased even at 4 ± 2ºC, which pointed out that temperature does not guarantee safety in case contamination occurs.

When remifentanil was tested, antimicrobial activity was more distinctive for *S. aureus* and *Acinetobacter* spp.. Strains of *E. coli* seemed to be more resistant to

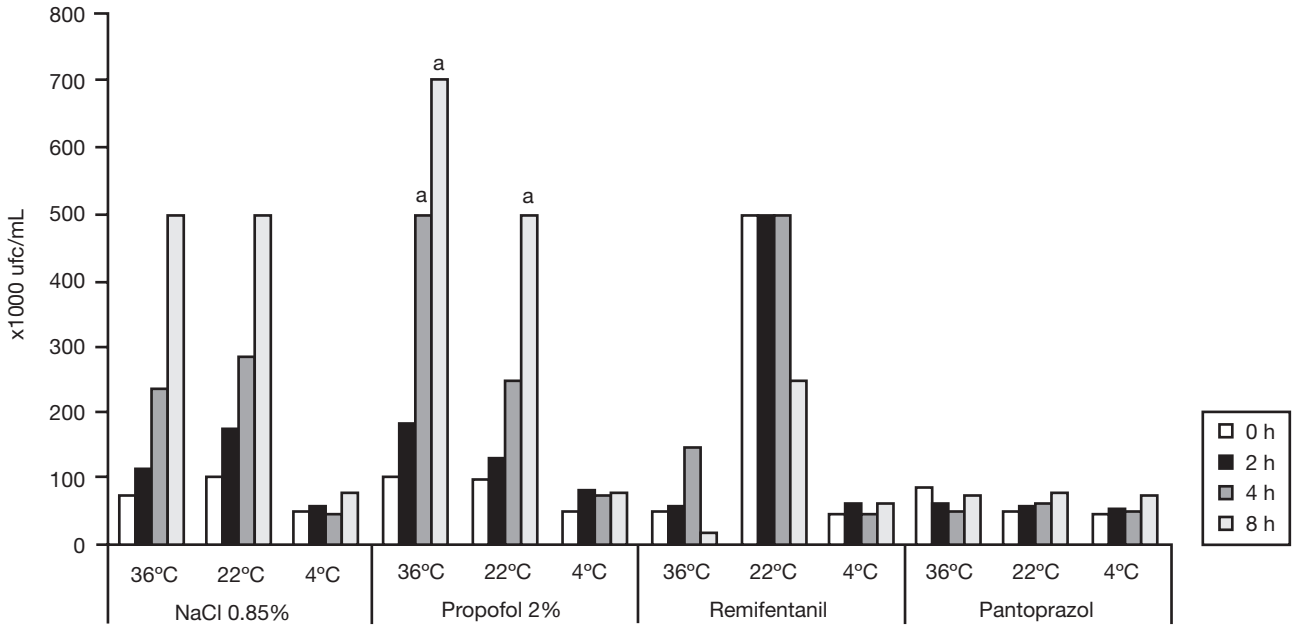

Figure 4 The colony counts of *Escherichia coli* in the solutions tested. [a] Result is significantly different from the beginning (0 h), $p < 0.05$.

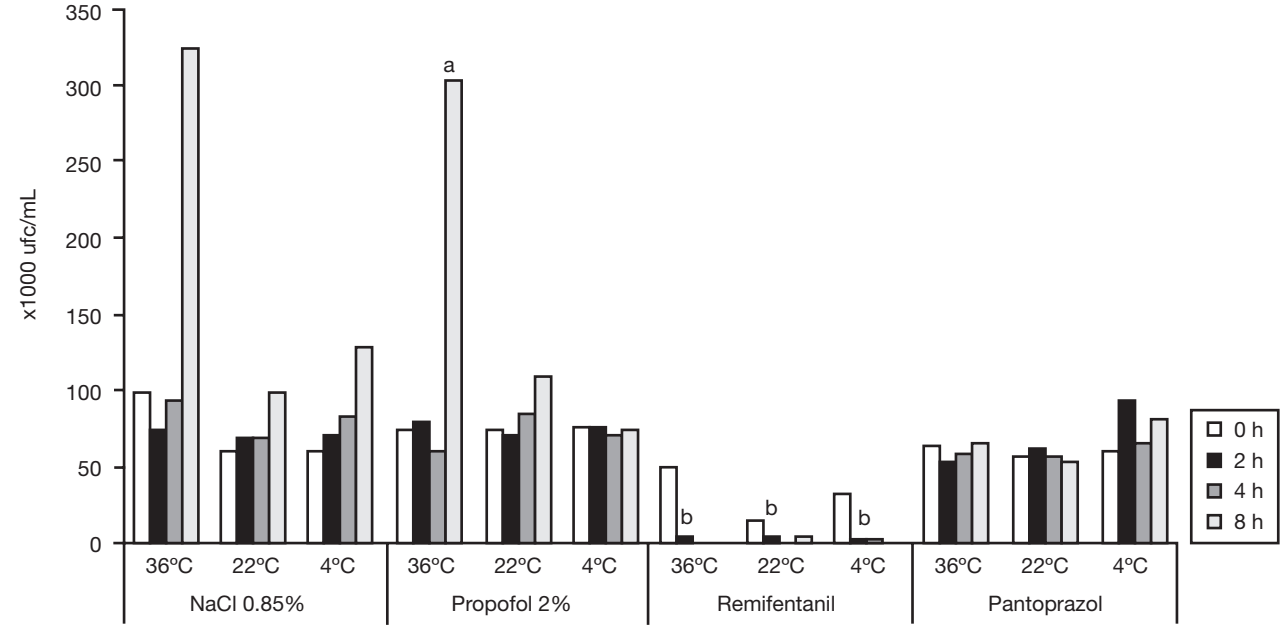

Figure 5 The colony counts of *Pseudomonas aeruginosa* in the solutions tested. [a] Result is significantly different from the beginning (0 h), $p < 0.05$. [b] Result is significantly different ($p < 0.05$) when compared to 0.85% NaCl.

antimicrobial effect of remifentanil, supporting Apan et al.'s results.[9] They reported that the antibacterial effect of remifentanil was concentration-dependent. The concentrations they used were 1, 10 and 100 $\mu g \bullet mL^{-1}$, where ours was 40 $\mu g \bullet mL^{-1}$. The concentration of remifentanil we studied was the clinically used concentration in our ICU.

Because bacteria are effected by drug pH and most pathogenic bacteria prefer a narrow pH range of 6.0-8.0,[25] the bactericidal property of remifentanil might be secondary to its low pH. The pH of remifentanil was 2.1 which is much lower than propofol (pH = 6.35) and pantoprozol (pH = 7.68). The growth patterns of *S. aureus* ATCC 25923, *E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853

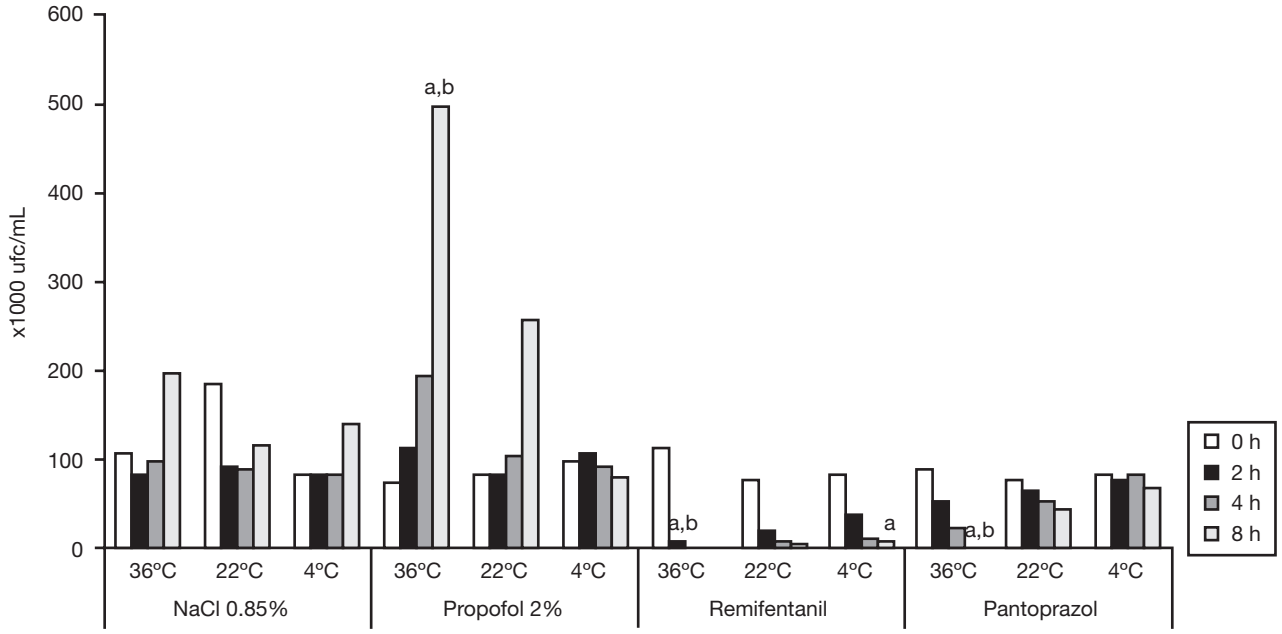

Figure 6 The colony counts of *Acinetobacter spp.* in the solutions tested. [a] Result is significantly different from the beginning (0 h), $p < 0.05$. [b] Result is significantly different ($p < 0.05$) when compared to 0.85% NaCl.

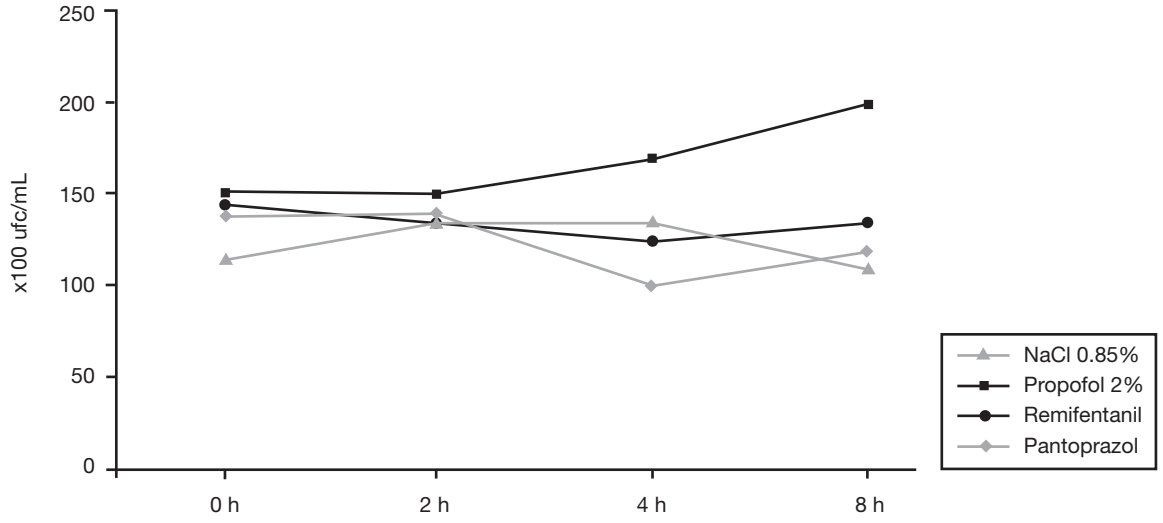

Figure 7 Growth of *S. aureus* in contaminated syringes at room temperature.

were not affected by pH between 5.0-8.0.[27] In addition, remifentanil contains glycine as a preservative which increases the duration of antimicrobial activity.[28] Presence of glycine might contribute to antibacterial activity of remifentanil.

Pantoprazole has a widespread use for the treatment of a range of upper gastrointestinal diseases in ICU. Suerbaum et al.[29] reported that pantoprazole has potent in vitro antibacterial activity against *Helicobacter pylori*. The mechanism of the antibacterial effect against *H. pylori* was propounded to be the interaction between the bacterial proteins via sulfonamide formation. This mechanism might be the explanation of pantoprazole's antibacterial effect against *S. epidermidis* and *Acinetobacter* spp. in our study, however it is yet to be determined.

The main finding of our study is, while remifentanil and pantoprazole do not, propofol strongly supports the growth of the microorganisms tested. To avoid life threatening

complications due to bacterial growth in contaminated propofol, it is important to follow the strict aseptic techniques for the preparation of propofol. Further studies should also evaluate the effects of contaminated drugs given by infusion on the development of bacteremia in patients.

Conflicts of interest

The authors declare no conflicts of interest.

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