



## Antimicrobial potential of routine disinfectants agents used in health care units against opportunistic pathogenic bacterial species

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### Abstract

Biofilms are surface attached, matrix embedded micro-organisms. Biofilms are difficult to eradicate since Biofilm structure prevents the penetration of chemical agents and makes more resistant to antimicrobials. This research evaluate the efficacy routine chemical agents in removing bacterial biofilms. Agar well diffusion, MIC and MBC evaluate the efficacy against planktonic bacteria. Post-exposure biofilm biomass was quantified using CV assay. All disinfectants tested effectively eradicate biofilms and planktonic bacteria, whereas 2% chlorhexidine digluconate and 2% sodium hypochlorite demonstrate highest efficacy.

### Key words:

HAI, Disinfectants, biofilms

### Introduction

Hospital acquired infections are common health care facility associated problem which cause serious health issues annually. Different precautions are taken to prevent and control HAI, including surface decontamination using synthetic chemicals.

Biofilms are complex ecosystem; consist of surface attached microbial cells embedded in exo-polymeric substances derived from cells and their environment. Biofilms are more resistant to external physical and chemical stresses due to their extracellular matrix. Therefore, the efficacy of disinfectants use in health care facilities in Piracicaba/SP is necessary to be researched in order to control the occurrence of HAI.

This study determines the efficacy of 77% ethyl alcohol, 1% and 2% chlorhexidine digluconate and 2% sodium hypochlorite against hospital acquired infection causing bacterial species by using *in vitro* biofilm models on 96-well sterile flat bottomed microtiter plates.

### Results and Discussion

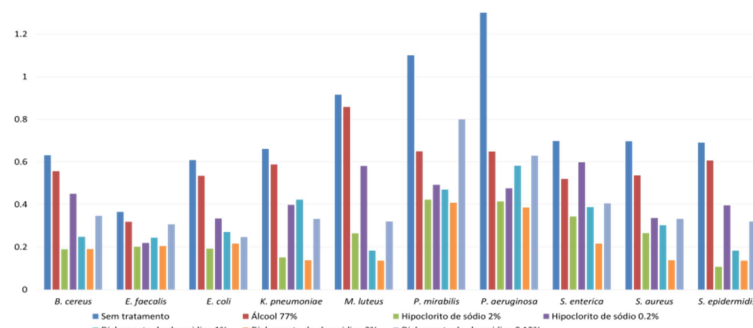
Synthetic chemical agents, namely, 77% ethanol, 2% sodium hypochloride, 1% chlorhexidine digluconate and 2% chlorhexidine digluconate were used to evaluate the antimicrobial effect on *Proteus mirabilis* (ATCC 25933), *Pseudomonas aeruginosa* (ATCC 25619), *Salmonella enterica* (ATCC 14028), *Escherichia coli* (ATCC 43845), *Klebsiella pneumoniae* (ATCC 13883), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 11778), *Enterococcus faecalis* (ATCC 29212), *Micrococcus luteus* (ATCC 14452) sessile and biofilm cells. Agar well diffusion was performed to determinate the effectivity against planktonic cells. Minimum inhibitory and minimum bactericidal concentrations were determined using CLSI M-27A broth microdilution method with modifications. Post exposure biomass reduction was determined using crystal violet assay on 24h mature *in vitro* bacterial biofilms.

All test strains in planktonic state exhibited sensitivity for all chemical agents. MIC and MBC values were significantly smaller than working concentrations of chemicals.

Chart 1. MIC & MBC of disinfectants against planktonic bacteria

Treatments	Alcohol 77%		Sodium hypochlorite 2%		Sodium hypochlorite 0,2%		Chlorhexidine digluconate 1%		Chlorhexidine digluconate 2%	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>B. cereus</i>	19,25%	19,25%	0,12%	0,12%	0,25%	0,25%	<0,00048%	0,00097%	<0,00048%	<0,00048%
<i>E. faecalis</i>	19,25%	38,50%	0,12%	0,12%	0,25%	0,25%	<0,00048%	0,0019%	<0,00048%	<0,00048%
<i>E. coli</i>	9,62%	19,25%	0,12%	0,12%	0,75%	0,75%	<0,00048%	0,0078%	<0,00048%	<0,00048%
<i>K. pneumoniae</i>	19,25%	19,25%	0,25%	0,25%	0,75%	0,75%	<0,00048%	0,03%	<0,00048%	<0,00048%
<i>M. luteus</i>	19,25%	19,25%	0,12%	0,12%	0,50%	0,50%	<0,00048%	0,0019%	<0,00048%	<0,00048%
<i>P. mirabilis</i>	9,62%	19,25%	0,06%	0,06%	0,25%	0,25%	<0,00048%	0,015%	<0,00048%	<0,00048%
<i>P. aeruginosa</i>	9,62%	9,62%	0,06%	0,06%	0,10%	0,10%	<0,00048%	0,03%	<0,00048%	<0,00048%
<i>S. enterica</i>	19,25%	9,62%	0,12%	0,12%	0,25%	0,25%	<0,00048%	0,03%	<0,00048%	<0,00048%
<i>S. aureus</i>	19,25%	38,5%	0,12%	0,12%	0,75%	0,75%	<0,00048%	<0,00048%	<0,00048%	<0,00048%
<i>S. epidermidis</i>	9,62%	9,62%	0,06%	0,06%	0,25%	0,25%	<0,00048%	<0,00048%	<0,00048%	<0,00048%

Graph 1. Post-exposure CV absorbance of bacterial biofilms at 595nm.



Biofilm biomasses of all test strains were significantly reduced (>50%) after 24h exposure to decontaminating agents.

### Conclusions

77% ethanol, sodium hypochlorite, 1% chlorhexidine digluconate and 2% chlorhexidine digluconate are effective for surface decontamination while 2% chlorhexidine and 2% sodium hypochlorite exhibits highest potency.

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