Effect of Esomeprazole and Its Combined Action with Vancomycin on Staphylococcus aureus Biofilm Formation

Thesis

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A	**Aap**	Accumulation-associated protein
	ABC-1	Antibiofilm compound 1
	AHLs	Acylated homoserine lactones
	ATPase	Adenosine triphosphatase
B	**Bap**	Biofilm associated protein
	BM	Bone marrow
C	**°C**	Degrees Celsius
	CBD	Calgery biofilm device
	CFU	Colony forming unit
	CoNS	Coagulase negative *Staphylococci*
	CSF	Cerebrospinal fluid
D	**Dept**	Department
	Ddl	D-alanyl-D-alanine ligase
	DNA	Deoxyribonucleic acid
	DNAses	Deoxyribonucleases
E	**E**	Esomeprazole
	eDNA	Extracellular DNA
	EDTA	Ethylenediaminetetraacetic acid
	ENT	Ear, Nose and Throat
	EPS	Extracellular polymeric substances
	ER	Emergency room

F	**FnBPs**	Fibronectin-binding proteins
G	**GERD/ GORD**	Gastroesophageal reflux disease
	GISA	Glycopeptide-intermediate *Staphylococcus aureus*
	Gm	Gram
	GNB	Gram negative bacilli
H	**H^+**	Hydrogen
	H_2O_2	Hydrogen peroxide
	Hrs	Hours
	HSL	Homoserine lactone
I	**Ica**	Intracellular adhesion gene cluster
	ICUs	Intensive care units
	IgG	Immunoglobulin G
	IL	Interleukin
k	**K^+**	Potassium
M	**MDR**	Multi drug resistance
	Mg	Milligram
	µg	Microgram
	MIC	Minimum inhibitory concentration
	Min	Minute
	Ml	Milliliter
	µL	Microliter
	Mm	Millimolar

	MRSA	Methicillin resistant *Staphylococcus aureus*
	MRSE	Methicillin-resistant *Staphylococcus epidermidis*
	MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
	MTP	Microtitre plate
	MurF	UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D-alanine ligase
N	**NaCl**	Sodium chloride
	Nm	Nanometer
	NSAID	Non-steroidal anti-inflammatory drug
O	**OB&Gyn**	Obstetric and gynecology
	OD	Optical denisty
P	**PIA**	Polysaccharide intercellular adhesion
	PNAG	Poly- *N* – acetylglucosamine
	PPIs	Proton pump inhibitors
	PSMs	Phenol-soluble modulins
	PVC	Polyvinyl chloride
Q	**QS**	Quorum sensing
	QSIs	Quorum sensing inhibitors
R	RNA	Ribonucleic acid
S	***S. aureus***	*Staph aureus*
	SCVs	Small-colony variants

	SPSS	Statistical Package for Social Sciences
	SD	Standard deviation
T	**TSB**	Tryptic soy broth
	TSST-1	Toxic shock syndrome toxin-1
U	**UAE**	United arab emirates
	UDP	Uracil diphosphate
V	**VISA**	Vancomycin-intermediate *Staphylococcus aureus*
	VRSA	Vancomycin-resistant *Staphylococcus aureus*
	VRE	Vancomycin-resistant *Enterococci*
W	**WHO**	World health organization

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Abstract

Staphylococcus aureus is a common nosocomial pathogen responsible for biofilm-associated infections. Proton pump inhibitors (PPIs), such as esomeprazole, may have antimicrobial properties.The objective of this study was to assess whether esomeprazole prevents biofilm formation and whether it may have synergistic effect with vancomycin on biofilm formation. *Staphylococcus areus* isolates were tested for their ability to form biofilm using microtitre plate (MTP) based system. Then the effect of esomeprazole and its combined action with vancomycin was tested on the thirty isolates detected to be biofilm forming. There was significant difference (P value <0.01) between type of sample and biofilm formation. Among different types of samples, swabs have the highest rate of biofilm formation (60%). There was no significant difference (P value >0.05) in optical density (OD) of biofilm formation with and without esomeprazole after 24 hrs of incubation, while there was significant difference (P value < 0.001) in OD of biofilm formation after 48 hrs and 72 hrs of incubation with and without esomeprazole. Also there was significant difference (P value : < 0.001) in OD of biofilm formation after challenging the isolates with vancomycin in the presence of esomeprazole. In conclusion, esomeprazole demonstrated an antibiofim effect and synergistic effect with vancomycin on biofilm formation.

Key words

Biofilm, esomeprazole, proton pump inhibitors (PPIs), vancomycin, *Staphylococcus aureus (S. aureus)*, microtitre plate (MTP), optical density (O.D).

INTRODUCTION

Staphylococcus aureus (*S. aureus*) which is present as commensal microflora in many parts of the human body including the skin, external orifices, and the upper respiratory tract acts as one of the most common opportunistic pathogens and is the most common infectious agent encountered by human beings. Almost every individual will experience at least one skin infection due to this microorganism. It has been shown to form biofilms on variety of biotic and abiotic surfaces ***(Elston and Barlow, 2009)***.

Bacterial biofilm is the term used to describe the surface-attached bacterial lifestyle. Cells in biofilm grow as communities, surrounded by a self-produced thick layer of extracellular polymeric substances (EPS, also known as matrix or slime). Biofilm is structurally and also functionally different from single-cell (suspended or planktonic) bacteria ***(Sauer and Camper, 2001)***.

The presence of the EPS protects cells in biofilms from the detrimental effects of chemical insults and harsh environmental conditions. Moreover, in the complex tri-dimensional architecture of biofilms, subpopulations of bacterial cells (sessile) co-exist in all stages of growth, including a fraction of dormant cells that are not metabolically active. This combination of factors helps to explain why

biofilms possess much lower susceptibility to antimicrobial therapy or biocides, when compared to suspended cells ***(Landini et al., 2010)***.

Vancomycin is one of the oldest antibiotics that has been now in clinical use close to 60 years. It is effective against most Gram-positive cocci and bacilli with the exception of rare organisms as well as *Enterococci* that became vancomycin resistant, mostly *Enterococcus faecium*. The major use of vancomycin today is for infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and amoxicillin-resistant *Enterococci* ***(Moellering, 2006).***

Traditional antibiotics such as vancomycin were developed to kill planktonic bacteria and often have a limited effect on the killing of sessile bacteria encased within a biofilm. In addition, antimicrobial resistance development is common in sessile bacteria. For these reasons, there is an urgent need to develop non antimicrobial treatment strategies to prevent or treat biofilm-associated infections ***(Kuehn, 2011)***.

Proton pump inhibitors (PPIs) have been shown to have antibacterial properties ***(Mills et al., 2004)***. Against *Helicobacter pylori*, benzimadazoles PPI have been shown to have direct antimicrobial effects ***(Sjostrom et al., 1997)***. PPIs have also been shown to have other effects on microbiological activity, including inhibition of urease ***(Park et al., 1996)***. In

2005, an in vitro study investigated the antibiofilm properties of PPI benzimidazoles against oral *Streptococci* ***(Nguyen et al., 2005)***. The results showed that the addition of omeprazole and lansoprazole had a significant effect on *Streptococcus mutans* biofilms, a common organism found in the human oral flora. However, antibiofilm effects of PPI on other bacteria have not been well studied.

According to ***Singh et al. (2012)*** Esomeprazole exposed isolates compared to untreated controls demonstrated an antibiofilm effect against biofilm producing *S. aureus.*