Ain Shams University

Faculty of Pharmacy

Microbiology and Immunology Department

Evaluation of Biocidal Activity of Antimicrobial Loaded Nanoparticles Against Staphylococcal Biofilms

A thesis submitted for partial fulfillment of the requirements for the

Master's Degree

In Pharmaceutical Sciences

(Microbiology and Immunology)

Submitted by:

Mennat-allah Alaa Mohamed Abd El-fatah

Bachelor of Pharmaceutical Sciences, 2012

Teaching Assistant, Department of Microbiology,

Faculty of Pharmacy, Misr International University

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List of abbreviations

Abbreviation	Definition
AHL	Acyl homoserine lactone
AI-2	Autoinducer-2
AIP	Autoinducing peptide
AMPs	Antimicrobial peptides
ASEM	Atmospheric scanning electron microscopy
ATCC	American type culture collection
CBD	Calgary biofilm device
CFU/ml	Colony forming unit per milli
CLSI	Clinical and laboratory standards institute
CLSM	Confocal laser scanning microscopy
CoNS	Coagulase-negative Staphylococci
DMSO	Dimethyl sulfoxide
Eos	Essential oils
EPS	Extracellular polymeric substance
ESEM	Environmental scanning electron microscopy
HEPES	(4-(2-hydroxyethyl)-1piperazineethanesulfonic acid)
IC_{50}	50% inhibitory concentration
LDH	Lactate dehydrogenase
LUV	Large unilamellar vesicles
MHB	Mueller Hinton broth
MIC	Minimum inhibitory concentration
MLV	Multilamellar vesicles
Mm	Micrometer
MRSA	Methicillin resistant *Staphylococcus aureus*

Abbreviation	Definition
MSA	Mannitol salt agar
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide)
Nm	Nanometer
NPs	Nanoparticles
O/W nanoemulsion	Oil in water nanoemulsion
PAE	Post-antibiotic effect
PBS	Phosphate buffered saline
PCM	Phase contrast microscopy
PDI	Polydispersity index
PIA	Polysaccharide intercellular adhesin
RPMI medium	Rosewell Park Memorial Institute medium
QS	Quorum sensing
QSI	Quorum sensing inhibitors
QSQ	Quorum sensing quenchers
ROS	Reactive oxygen species
S. aureus	*Staphylococcus aureus*
SEM	Scanning electron microscopy
S. epidermidis	*Staphylococcus epidermidis*
S. haemolyticus	*Staphylococcus haemolyticus*
S. intermedius	*Staphylococcus intermedius*
S. lugdunensis	*Staphylococcus lugdunensis*
Spp.	Species
SUV	Small unilamellar vesicles
TEM	Transmission electron microscopy
TSA	Tryptic soy agar
TSB	Tryptic soy broth
W/O nanoemulsion	Water in oil nanoemulsion

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Abstract

Multi-drug resistant and biofilm forming bacteria have surprisingly increased over recent years. On the contrary, the rate of development of new antibiotics to treat these emerging superbugs is very slow. Therefore, aim of the present study was to prepare novel nanobiotic formulations to enhance the antimicrobial activity of the three antibiotics; clindamycin, doxycycline, and linezolid against different species of Staphylococci. Antibiotics were conjugated with nanoemulsions and evaluated for their antimicrobial activities, cytotoxicity, as well as post-antibiotic effects.

Upon quantitative assessment of *Staphylococcus* biofilm formation, 84 isolates (66.14%) were biofilm forming. Minimum biofilm inhibitory concentrations displayed that 77.2%, 50.87%, and 5.3% of *Staphylococcus* isolates were sensitive to linezolid, doxycycline, and clindamycin nanobiotics, respectively. Doxycycline and linezolid nanobiotics exhibited promising enhanced antibacterial activities. On the contrary, clindamycin nanobiotic exhibited poor antibacterial activity.

Cytotoxicity of the conventional antibiotics and nanobiotics was analyzed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on rat hepatocytes. Half-maximal inhibitory concentration (IC_{50}) was estimated from an experimentally derived dose-response curve for each concentration using GraphPad Prism software. Post-antibiotic effects (PAEs) were determined by viable count technique. Clindamycin, doxycycline, and linezolid antibiotics as well as their nanobiotics were tested against two selected methicillin resistant *Staphylococcus aureus* (MRSA) isolates. The PAE values for MRSA-S1 were 2.5 h for the three conventional antibiotics. However, the PAEs for nanobiotic formulations were 4 h for both clindamycin and linezolid,