



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

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