Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Studies on interactions between certain microbial species isolated from mixed infection

A Thesis

Submitted in Partial Fulfillment of the Requirements for the

PhD degree

In Pharmaceutical Sciences
(Microbiology and Immunology)

By

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والحمد لله رب ا لعالمين**.....**

Sarra Ebrahim Saleh

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List of Abbreviations

Acineto	*Acinetobacter*
AHLs	N-acyl-homoserine lactones
AI	Autoinducer
AQs	2-alkyl-4-quinolones
BLAST	Basic Local Alignment Search Tool
bp	base pair
CAP	Community acquired pneumonia
CF	Cystic fibrosis
cfu	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CV026	*Chromobacterium violaceum*
CSP	Competence signal peptides
DKPs	Diketopiperazines
E.coli	*Escherichia coli*
EDTA	Ethylene diaminetetraacetic acid
EMB	Eosin methylene blue
EPEC	Enteropathogenic *Escherichia coli*
HHL	Hexanoyl homoserine lactone
HHQ	2-heptyl-4-quinolone
HQNQ	4-hydroxy-2-heptyl quinolone N-oxide
HSI	Hemolysin co-regulated protein secretion island
HSL	Homoserine lactone
1-OH-PHZ	1-hydroxyphenazine
ICH	Intensive care unit
IDSA	Infectious Diseases Society of America
Kl	*Klebsiella*
LB	Luria Bertani
LEE	Locus of enterocyte effacement
LPS	lipopolysaccharide
MH	Mueller-Hinton
MRSA	methicillin resistant *Staphylococcus aureus*
MSSA	methicillin sensitive *Staphylococcus aureus*
NCBI	National Center for Biotechnology Information

NOR	Nitric oxide reductase
OMP	outer membrane protease
ORF	Open raeding frame
P. aeruginosa	*Pseudomonas aeruginosa*
PCA	Phenazine-1- carboxylic acid
PCN	Phenazine-1-carboxamide
PCR	Polymerase chain reaction
PQ/S	Pseudomonas quinolone signal (2-Heptyl-3-hydroxy-4-quinolone)
Ps	*Pseudomonas*
QS	Quinolone signaling
RL	Rhamnolipid
rpm	Round per minute
RNA	Ribosomal ribonucleic acid
RTI(s)	Respiratory tract infection(s)
S. aureus	*Staphylococcus aureus*
Staph	Staphylococci
SCVs	Small colony variants
SigB	Sigma factor B
SDS	Sodium dodecyl sulphate
SOB	Super optimal broth
SOC	Super optimal broth with catabolite repression
Ta	Annealing temperature
TAE	Tris-acetic acid-EDTA
TE	Tris-EDTA
Tris	Trishydroxymethylaminomethane
tRNA	Transfer ribonucleic acid
TSI	Triple sugar iron agar
T3SS	Type ШI secretion system
T6SS	Type VI secretion system
URTI(s)	Upper respiratory tract infection(s)
VLPs	Virus like particles
V. fischeri	*Vibrio fischeri*

Abstract

The present study focused basically on studying the interactions between certain microbial species isolated from mixed infection and how these interactions affect their coexistence. Therefore, isolates recovered from clinical specimens showing mixed infections were collected. Pus was the clinical specimen of the highest prevalence of mixed infections in comparison to other clinical specimens. Both *Staphylococcus* and *Pseudomonas* isolates coexisted in mixed infection at the highest prevalence. Different *Pseudomonas* isolates that coexisted in mixed infection with *Staphylococcus* isolates were assayed for their protease, lipase, and acylhomoserine lactone (AHL) productivities. Results obtained showed no significant difference in both protease and lipase productivities however, production of AHL showed significant variation among the respective isolates. Antibiogram analyses of the collected clinical isolates were determined and showed significant variation. From the previous findings, two models of coexisting *Pseudomonas* and *Staphylococcus* isolates (SP12 and SP14 models) were selected for studying their interactions using different physiological parameters. This selection was based on the results obtained where the two *Staphylococcus* isolates, S12 and S14 were methicillin-resistant (MRSA) and the two *Pseudomonas* isolates, P12 and P14 were identified as *Pseudomonas (P.) aeruginosa* however, they showed significant difference in their antibiogram analysis as well as presence or absence of endogenous plasmids. Results showed that there was significant reductions in the viable count of both *S. aureus* isolate S14 and *P. aeruginosa* isolate P14 when grown in co-culture as compared to their growth in monocultures. While, there was no significant difference in the growth of *P. aeruginosa* isolate P12 in monoculture and in co-culture with *S. aureus* isolate S12. Moreover, there was no significant effect of different physiological factors (incubation temperature and pH) on growth profile however, optimum reduction effect of P14 on its coexisted *S. aureus* S14 was observed at 37ºC and initial pH 7.2.

Furthermore, the culture supernatant of *P. aeruginosa* P14 (harboring no plasmids) exerted a significant reduction effect on the biofilm formation (about 57% reduction) of the co-existing MRSA isolate (S14) however, this effect was not observed upon using the culture supernatant of *P. aeruginosa* P12 (harboring plasmids) on the biofilm