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Antimicrobial Activity of Bacteriocins Extracted from Some Gram Negative Bacteria on *Candida albicans*

A thesis submitted to the College of Medicine,
Department of Microbiology, University of Diyala in Partial fulfillment
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صدق الله العظيم

سورة يوسف آية ٧٦

CERTIFICATION

We certify this thesis entitled (**Antimicrobial Activity of Bacteriocins Extracted from Some Gram Negative Bacteria on *Candida albicans***) prepared by **(Nayarah Samer Hussain)** has been conducted under my supervision at College of Medicine/ University of Diyala, as a partial requirement for Master Degree of Science in Medical Microbiology.

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We, the examining committee, certify that we have read this thesis entitled **(Antimicrobial Activity of Bacteriocins Extracted from Some Gram Negative Bacteria on *Candida albicans*)** was prepared by (**Nayarah Samer Hussain**) and as the examination committee examined the student in its content and in our opinion it adequate for award the Degree of Master of Science in Medical Microbiology.

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Dedication

I dedicate my thesis to;

My precious parents and my dearest brothers who supported me.

And to my friends.

Nayarah Samer Hussain

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I'm grateful to Allah, for inspiring and giving me the strength, patience and willingness to perform this work.

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Nayarah Samer Hussain

Summary

Urine specimens (250 specimens) were collected from urinary tract infection (UTI) out patients clinic in Baqubah teaching hospital and Al-Batool teaching hospital. UTI is more prevalent among women than men. In the case of women, the most infected ages between twenty-one to forty while men; UTI was most prevalent in the age category 1-10 years.

Sixty-six urine specimens had positive culture for gram-negative bacterial growth. Microscopic examination, culturing on MacConkey and EMB, and biochemical tests were dependent for identification of bacterial isolates and confirmed by Vitek-2 compact system as well as culturing on CHROMagar. *Escherichia coli* was more predominant than other isolates 41 (62.12%), while *Enterobacter aerogenes* was less prevalent at the value of 2 (3.22%). The prevalence of *Klebsiella pneumonia* was 13 (20.96%), *Proteus mirabilis* 7 (11.29%), and *Pseudomonas aeruginosa* was 3 (4.83%).

Oral swabs were used to isolate *Candida albicans* from patients with renal impairment. *C. albicans* were detected by using the germ tube technique and Vitek-2 compact system. From thirteen (43.33%) positive growth culture of oral swabs, eight (61.53%) isolates were identified being *C. albicans*.

Detection the ability of bacterial isolates to produce bacteriocins, a cup assay was used and the produced bacteria were detected by visualizing inhibition zone around the suspected bacteriocin producer's discs. Less than half of the bacterial isolates 31(46.96%) were bacteriocin producers. Twenty isolates out of 41 isolate of *E. coli* were colicin producers while all the isolates of *P. aeruginosa* and *E. aerogenes* were bacteriocin producers. The most efficient bacteriocin producers were chosen by determining the larger inhibition zone. After extraction of bacteriocins, protein concentrations of these bacteriocins were estimated by using the Lowery method and the

activity of bacteriocins was determined by using well method. The titer was determined by the detection of inhibition zone at the lowest concentration. Colicin 23 protein concentration was 2950µg/ml, activity was 40U/ml and inhibition zone was 15mm. Meanwhile, protein concentrations of klebicin 13 and pyocin 26 were (3050 and 2620)µg/ml, their activity were (80 and 320)U/ml and inhibition zones were (16 and 25)mm respectively.

The antimicrobial activity of bacteriocins on *C. albicans* was detected by using well method. This method didn't give inhibition of *C. albicans* growth, on another hand; there was an interesting inhibition of *C. albicans* growth by using cup assay. *P. aeruginosa* 26 (P26) was the most efficient isolate that inhibits most *C. albicans* isolates from other efficient bacteriocins producer bacterial isolates. P26 showed the highest inhibition zone (40mm) against isolate 3 of *C. albicans*. *C. albicans* isolate 2 was the most sensitive to all bacteriocins producers bacterial isolates.

Resazurin salt dye was used to detect the effect of bacteriocins. The effect of bacteriocins against *C. albicans* was considerable even at the lowest concentration used in this study. Isolate 1 was significantly ($P \leq 0.05$) less affected to bacteriocins when compared with other isolates. Whilst isolate 7 were significantly ($P \leq 0.05$) more affected by klebicin and pyocin with inhibitory rate 100% and 99% inhibited by colicin at a high concentration of 2500 µg/ml, while isolate 1 was significantly ($P \leq 0.05$) less affected isolate to bacteriocins with inhibitory rate (86, 94, and 85)% for colicin, klebicin and pyocin respectively .

The ability of bacteriocins to inhibit biofilm formation of *C. albicans* to form biofilm was studied. Colicin showed significantly ($P \leq 0.05$) an inhibition of biofilm formation even at low concentrations when compared to klebicin and pyocin, in which both of them had a significant ($P \leq 0.05$) activation of

biofilm at low concentration. Pyocin was the most used bacteriocin that causes activation of biofilm even at high concentration (1250 µg/ml) of isolate 4 with a value of 107%. At high concentration (2500 µg/ml), *C. albicans* isolate 5 was mostly sensitive to colicin with a value of 46%, while isolate 8 was sensitive to klebicin 31% and isolate 6 with value 49% to pyocin.

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Table of Abbreviations

Abbreviations	Meaning
ADPRT	ADPribosyltransferase
A/E	Attaching and Effacing Lesions
ALS	Agglutinin Like Sequence proteins
BFP	Bundle-Forming Pili
BRP	Bacteriocin Release Protein
BSA	Bovine Serum Albumin
BSI	Bloodstream Infection
CD	Crohn's Disease
CFA/I	Fimbrial Colonization Factor
cKP	Classic *K. pneumoniae*
CS	Coli Surface
DC	Dendritic Cells
ECs	Endothelial Stem Cells
EF2	Elongation Factor 2
EPS	Extracellular Polymeric Substances
GTPase	Nucleotide Guanosine Triphosphate
HNSCC	Head and Neck Squamous Cell Carcinoma
hvKP	Hypervirulent *K. pneumoniae*
IBD	Inflammatory Bowel Disease
IM	Inner Membrane
Las	Quorum sensing systems of *P. aeruginosa*
LifA	Lymphocyte Inhibitory factors

LT	Heat-labile enterotoxin
Man-PTS	Mannose phosphotransferase
MERSA	Methicillin-Resistant *Staphylococcus aureus*
OM	Outer Membrane
OmpF	Major outer membrane proteins of *Escherichia coli*
PAI	Pathogenicity islands
PEAF	Possess adherence factor plasmid
PLA	Pyogenic Liver Abscess
Pta	Proteus toxin agglutinin
QS	Quorum Sensing
Rhl	Quorum sensing systems of *P. aeruginosa*
ROS	Reactive Oxygen Species
SOS	Save Our Souls
SPATE	Serine Protease Autotransporters of Enterobacteriaceae
T3SS	Type III Secretion System
ST	Heat-stable enterotoxin
Stx	Shiga toxins
VRE	Vancomycin-Resistant Enterococci

Chapter One

Introduction

1.1 Introduction.

Urinary tract infections (UTIs) are a serious public health problem that caused by many pathogens, but mostly by *Escherichia coli*, *Klebsiella pneumonia, Proteus mirabilis*, *Staphylococcus saprophyticus* and *Enterococcus faecalis* (Flores-Mireles *et al,* 2015). These pathogens are transmitted from person-to-person contact by food or water, as well as, they might be obtained from hospitals. Uropathogenic bacteria has a specialized feature, for example, toxins, siderophores, and adhesins that used to colonize and invade the urinary tract (Foxman. 2010).

The family Enterobacteriaceae is prevalent in various ecological sources such as soil, water, vegetation and animals (Brenner, 2006). This family caused two types of infections: intestinal and extraintestinal diseases. The extraintestinal infections include: neurologic infections for example neonatal meningitis that caused by *E. coli*, pyogenic liver abscess caused by *K. pneumoniae*, and most of its members cause bacteremia and urinary tract infection (Jenkins, 2017). *Pseudomonas aeruginosa* cause urinary tract infection as a complication due to the presence of foreign bodies like stones, catheter or stent. Also, obstruction in the genitourinary system or frequent use of antibiotics can cause urinary infection by *P. aeruginosa* (Pier, 2012).

Due to the appearance of antibiotics resistance pathogens (Cooper and Shlaes, 2011), the commensal microbiota can be damaged by using antibiotics with abroad-spectrum activity, as well as, increasing in the incidence of allergy and autoimmune diseases with broad-spectrum antibiotics administration (Blaser, 2011). There will be necessary to develop a usable antimicrobial agents such as bacteriophages, probiotics, plant derived compounds, and antimicrobial peptides like bacteriocins (Nishie *et al.,* 2012). Bacteriocins are ribosomally synthesized antimicrobial peptides produced by gram positive and gram negative bacteria and archaea with inhibitory activity against a wide range of microorganisms. As well as, it has an anti-viral (Chikindas *et al.,*

2018) and anti-cancer activity (Kaur and Kaur, 2015). Moreover, bacteriocins can be used as food preservatives since they are easily digested by the human gastrointestinal tract (Mills *et al.,* 2011). Bacteriocins from gram-negative bacteria used nutrient uptake pathways to transport into the cell (Atanaskovic and Kleanthous, 2019). Genes of most bacteriocins are located either on plasmids or on chromosomes (Maan and Garcha, 2018).

Candida albicans is opportunistic yeast, which resides in the oral cavity, intestinal tract, and vagina (Nobile and Johnson, 2015) and has the ability to cause superficial infections as well as, life-threatening systemic infections (Tsui *et al*., 2016). Virulence factors of *C. albicans* help to evolve disease more than other species of candida. Germ tubes formation and presence of glycoproteins in the cell wall facilitate the adherence of yeast to the cell membrane. Some components (for example polysaccharides) of fungal wall prompt suppressor of T-lymphocytes. Besides, mannan can intervene in antigen presentation (Coronado-Castellote and Jiménez-Soriano, 2013). Moreover, one of the major virulence is biofilm formation; densely packed cells that attached to the solid surface and living tissue. Biofilm gives a resistant to antifungal therapies, the host immune system, and other factors (Gulati, M. and Nobile, C.J., 2016).

1.2 Aims of study.

Investigation antimicrobial effect of gram-negative bacteriocins on *C. albicans* by:

1- Isolation of gram negative bacteria from UTI patients.
2- Isolation of *C. albicans* from patients with renal impairment.
3- Detection of bacteriocins producing bacteria.
4- Extraction of bacteriocins from efficient isolates.
5- Study antimicrobial activity of bacteriocins on planktonic cells and biofilm formation of *C. albicans*.