USE OF PROBIOTIC ACID BACTERIA FOR THE CONTROL OF MULTIDRUG RESISTANT BACTERIA ISOLATED FROM CLINICAL INFECTIONS

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Abstract

This study was carried out to evaluate the microbial infections in natural way and reduce the antibiotic resistance. One hundred bacterial isolates were collected from clinical specimens of patients suffering from bacterial infection (55 females and 45 males). The bacterial isolates were obtained from 8 different specimens with the following percentage representations: urine (30.58 %), blood (20%), abscess (20%), wound (9.41%), throat and tube swab (5.88%), ear discharges (5.88%), vaginal swabs (5.88%) and eye swab (2.35%).The results showed that (29.41%) *Escherichia coli*, (23.53%) *Klebsiella pneumoniae*, (17.65%) *Staphylococcus aureus*, (15.29%) *Pseudomonas aeruginosa*, (8.24%) *Proteus vulgaris*, (5.88%) *Acinetobacter baumannii*. Among 17 antibiotics tested, meropenem, a carbapenem antibiotic, was the most effective drug against most of the gram-negative and gram-positive bacteria. Detection of biofilm by two different methods show that most of isolates were multidrug resistant. Three species of lactic acid bacteria namely *L.acidophilus* (DSM20079), *L.plantarum* (DSM20174) and *L.salivarus* (DSM20555) were used for reducing the microbial infections. Bacteriocins were isolated from MRS broth culture of these lactic acid bacteria through the precipitation method using 1N HCL and were tested against different pathogenic bacteria. Antibacterial activity of bacteriocins extracted from *L. acidophilus* (DSM20079) showed maximum activity against most isolated bacteria as compare to others. Also, *L.salivarus* (DSM20555) have the highest activity against *Methicillin-resistant Staph aureus*. The present study has showed the antibacterial role of bacteriocin isolated from lactic acid bacteria can be used for treatment variety of human diseases.

Keywords: Multidrug resistance, Role of Meropenem, Lactic acid bacteria (LAB) and bacteriocins.

Introduction

Multidrug resistant bacteria are defined as abroad category of bacteria resistant to common antibiotics **(Abigail, 2010)**. Antimicrobial resistance (AMR) in bacteria is a great concern to the health and welfare of both human and animals **(McDermott *et al*., 2016 and Zawack *et al*., 2016).**

The increase and spread of multidrug resistant (MDR) bacteria have become a major concern worldwide. The hospital acquired infections caused by MDR bacteria have led not only to an increase in mortality, morbidity, and cost of treatment, but also continue to endanger the life of patients **(Martin and Yost, 2011 and Delle Rose *et al*., 2015)**. MDR bacteria can cause a wide range of infections, including bacteremia, pneumonia, urinary tract infection, peritonitis etc., which can lead to substantial morbidity and mortality, particularly in the ICU settings **(Chen *et al*., 2001)**.

Microbes become antibiotic resistant due to partial exposure to one or more antibiotics. Gene mutations as well as vertical and horizontal gene transfer among bacteria are also important factors for development of resistance **(Levy, 1997 and Salyers, 1995)**. Also, it has been reported that AMR kills around 50.000 people a year in US and Europe, and is estimated to kill more than 700.000 people globally **(O'Neill, 2016)**. If no action was made to reduce AMR, probably, 10 million people would die every year from drug-resistant infections by the year of 2050 **(O'Neill, 2016).**

The increase of multidrug-resistant bacteria and the restriction on the use antibiotics due to its side effects have drawn attention to search for possible alternatives. Probiotic LAB can act synergistically or have an additive effect in the antimicrobial activity when combined with other antimicrobials **(Viedma *et al*., 2010 and Gómez *et al*., 2012)**. Lactic acid bacteria (LAB) constitute part of the autochthonous microbiota of many types of food. They are defined as non- spore forming, gram- positive rods cocci as well as catalase- negative bacteria which share many biochemical, physiological, and genetic properties **(Abriouel *et al*., 2012)**. Interestingly, LAB may simultaneously secrete organic acids, bacteriocins and biosurfactants **(Kanmani *et al*., 2013)**. Bacteriocins are small antimicrobial peptides produced by numerous lactic acid bacteria. Much interest has been focused on bacteriocins because they exhibit inhibitory activity against pathogens. So, LAB is helpful in treatment without raising the antibiotic resistance level (**Stiles, 1996).**

Bacteriocins are antimicrobial peptides produced by many lactic acid bacteria, which are directed mainly to inhibit the growth of related species or species with the same nutritive requirements **(DeVuyst, 1995, Jack *et al*., 1995 and Todorov and Dicks, 2005)**. Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases

in the human digestive tract. They are ribosomally synthesized peptides, and this fact creates the possibility of improving their characteristics to enhance their activity and spectra of action **(Saavedra *et al.*, 2004)**. Thus, the aim of study was focused on extraction of bacteriocins from LAB and determination of its antibacterial effect against different antibiotic resistant pathogenic bacterial strains.

Materials and Methods

Collection and isolation of pathogenic bacteria

One hundred clinical samples were collected from different patients suffering from bacterial infection. All clinical samples were collected by standard microbiological technique **(Cheensbourgh, 2006)**. The sources of specimens were pus/swab from wound, urine, ear discharge, blood, throat/tube swab, abscess, vaginal swab and eye swab. Depending on the source of samples, each specimen were platted on to Nutrient agar, MacConkey agar, Blood agar and CLED agar media (Oxoid, UK) and then incubated aerobically at 37° C for 24-48h.

Identification of pathogenic bacteria isolated from different specimens

All the bacteria were identified by using cell morphology, Gram staining and conventional biochemical methods according to standard microbiological techniques **(Forbes *et al*., 1998 and Cheesbourgh, 2006).** Also, to confirm identification of selected bacteria, specific gene using specific primers was performed using PCR technique by using agarose gel used for separation of amplified genes in the selected isolates.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was done on Muller-Hinton agar (Oxoid, England) using standard disk diffusion technique according to Kirby-Bauer method **(Muller-Hinton 1941), (Bauer et al., 1966)** and **(Raja and Singh 2007)**.The antimicrobial agents tested were: Amoxicillin/clavulanic acid (30µg), piperacillin (100µg), cefadroxil (30µg), oxacillin (1µg), cefotaxime (30µg), ceftazidime(30µg), amikacin (30µg), gentamycin (10µg) trimethoprim/sulphamethoxazole (25µg), doxycycline (30µg), vancomycin(30µg), nitrofurantion (300µg), linezolid (30µg) and ciprofloxacin (5µg) (Oxoid, England). The antibiotic susceptibility profiles were interpreted based on Clinical and Laboratory Standards Institute **(CLSI, 2006)** guidelines. Moreover, MDR profile was determined against different classes of antimicrobials: Cephalosporin class (cefadroxil, cefotaxime, ceftazidime), Penicillin class (amoxicillin/clavulanic acid, piperacillin, oxacillin), Aminoglycosides class (gentamycin, amikacin), Quinolone class (ciprofloxacin), Glycopeptides class (vancomycin), Tetracycline class (doxycycline), Macrolides class (erythromycin, azithromycin) and

Carbapenem (meropenem). The zones of inhibition of bacterial isolates for these antibiotics were measured in mm by applying ordinary ruler.

Detection of biofilm of multi-drug resistant bacteria

After isolation and identification of bacterial isolates, detection of biofilm formation was done by two different methods (Tube method and Congo red agar method) to detect bacterial resistance of isolates **(Hassan, *et al.*, 2011).**

Tube method

Trypticase soy broth with 1% glucose (TSB) (10mL) was inoculated with a loopful of microorganism from overnight culture plates and incubated for 24 h at 37˚C. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Dried tubes were then stained with crystal violet (0.1%). Excess stain was removed, and tubes were washed with deionized water. Tubes were then dried in inverted position and observed for biofilm formation.

Congo red agar method (CRA)

This method requires the use of a specially prepared solid medium brain heart infusion broth (BHI) supplemented with 5% sucrose and Congo red. The medium was composed of BHI (Oxoid, UK) 37 g/L, sucrose 50 g/L, agar No.1 (Oxoid, UK) 10 g/L and Congo Red stain 0.8 g/L. Congo Red was prepared as a concentrated aqueous solution and autoclaved at 121˚C for 15 minutes, separately from other medium constituents and was then added when the agar had cooled to 55˚C. Plates were inoculated with test organisms and incubated aerobically for 24 to 48 h at 37˚C. Positive result was indicated by black colonies with a dry crystalline consistency**.**

Activation of lactobacillus strains

Lyophilized strains of *Lactobacillus acidophilus* (DSM20079), *Lactobacillus plantarum* (DSM20174) and *Lactobacillus salivarius* (DSM20555) were obtained from Cairo, MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. All strains were activated on MRS broth (De Man, Rogosa and Sharp which obtained from Biolife, Italy) at 37˚C for 24 h. Then three culture transfers performed to activate each culture.

Extraction of bacteriocin

Ten ml of activated culture of each strain were separately inoculated into one liter of MRS broth under aseptic conditions and incubated at 37˚C/16 h. as described by **(Hurst, 1966 and Abd El.Fattah, 1999)**. All bacteriocin producing cultures were adjusted to pH 2.0 by adding HCl 1N then cultures were heated in water bath at 100˚C for 5 min. The cells were harvested by centrifugation at 10.000 rpm for 20 min. at 4˚C and recentrifugated under the same conditions. The supernatants containing bacteriocin extracts were

collected for every strain **(Savadogo *et al.*, 2004)**. Then, bacteriocin extracts were sterilized by using Seitz filter with single sheet to eliminate the possible presence of viable bacterial cells (**Simova *et al.*, 2009)**.

Determination of antibacterial activity

Bacteriocin activity was assayed by disk diffusion assay (**Tagg *et al.*, 1976**). This method is described as follows:

Serial Dilutions were prepared from isolated pathogenic bacteria till obtain concentration of $1x10^6$ CFU/ml **(Abd El-Fattah, 1999)**. One ml quantities from approximately $1x10^6$ CFU/ml from each pathogenic were inoculated on Muller-Hinton agar. Sterile filter papers were saturated with 100µL of sterilized bacteriocin then plates were allowed prior to incubation at 37° C/24 h. and then examined for clear circular inhibition zone around the disk. The titer of inhibition was defined as the reciprocal of the highest dilution showing definite inhibition zone. Bacteriocins activity was recorded as positive if width of clear inhibition zone around the colonies of the producer was 2mm or larger **(Chumcholova *et al.*, 2004 and Anastasiadou *et al.*, 2008)**.

Results

Among the 100 samples were collected from different patients suffering from bacterial infections, 85 samples were positive (85% of total sample) and 15 samples were negative (15% of total samples). The total females samples were 55 and males samples were 45 (Table 1).

The bacterial isolates were obtained from 8 different specimens with the following percentage representations: Urine (30.58 %), blood (20%), abscess (20%), wound (9.41%), throat and tube swab (5.88%), ear discharges (5.88%), vaginal swabs (5.88%) and eye swab (2.35%) as shown in Table 2.

The commonest organisms isolated from all samples were *E.coli* 25 (29.41%), *Klebsiella pneumoniae* 20 (23.52%), *Staph. aureus* 15 (17.64%), *Pseudomonas aeruginosa* 13 (15.29%), *Proteus vulgaris* 7 (8.23%) and *Acinetobacter baumannii* 5 (5.88%) (Table 2). *E.coli* ranked first overall among patients 25 (29.41% of total sample) in which was isolated from urine 15 (57.69%), blood 1 (5.88%), abscess 3 (17.64%), wound 2 (25%), throat and tube 2 (40%) and vaginal 2 (40%). Also, *Klebsiella pneumoniae* ranked second overall among patients 20 (23.52% of total samples) in which was isolated from urine 5 (19.2%), blood 7 (41.17%), abscess 4 (23.5%), wound 2 (25%) and vaginal 2 (40%) (Table 2). *E.coli* was the most organisms isolated from urine samples (57.69%), while *Staph aureus* the most organisms isolated from blood stream (47.05%) and eye swabs (50%). Also, *Pseudomonas aeruginosa* the most organisms isolated from abscess swabs (35.29%) and ear swab (40%) as recorded in Table 2.

Methicillin resistant *Staph.aureus* (MRSA) comprised 40% of all *Staph.aureus* isolates (6/15) in which 4 isolates from blood stream, one isolated from abscess and one isolated from ear discharge. Also, among Gram negative isolates there are ESBL isolates in which 3 (15%) cases from all *Klebsiella pneumoniae* isolates (3/20), two cases (28.57%) from all *Proteus vulgaris* (3/7) and two cases (15.38%) from all *pseudomonas aeruginosa* (2/13).

All isolates were identified by agarose gel PCR technique depending on specific gene of each organism whereas, (16SrDNA) specific gene of *P.aeruginosa*, (atpD) specific gene of *P. vulgaris*, (recA) specific gene of (*A.baumannii*), (16S-23S ITS) specific gene of *K.pneumoniae*, (clfA) specific gene of *Staph.aureus* and (phoA) specific gene of *E.coli* (Fig.1).

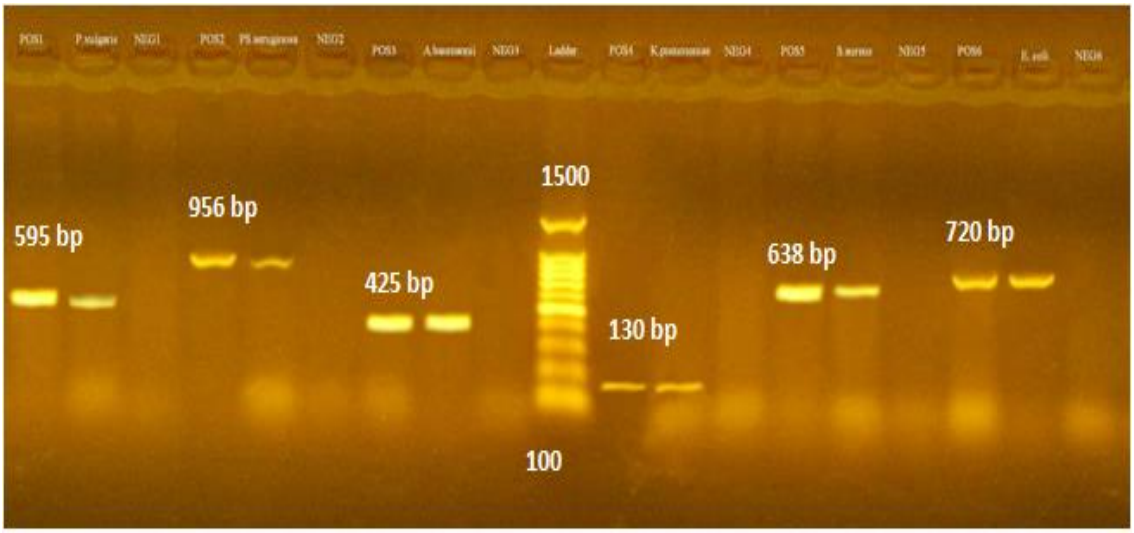

Figure (1): A photograph of agarose gel showing PCR products of partially amplified of S1 (*P. vulgaris* at atpD gene at 595 bp), S2 (*P. aeruginosae* at 16SrDNA gene at 956bp), S3 (*A. baumannii* at recA gene at 425 bp), S4 (*K. pneumoniae* at 16S-23S ITS gene at 130 bp), S5 (*Staph. aureus* at clfA gene at 638 bp), S6 (*E.coli* at phoA gene at 720 bp).

N.B: Pos.: Positive control strains from reference lab. of veterinary quality control on poultry production, Dokki, Giza.
Neg.: Negative control strains from reference lab. of veterinary quality control on poultry production Dokki, Giza.

Table (1): Age and sex distribution of common bacterial isolates from various sites of infections among patients

Type of isolates	Sex				Total n=100
	Female n=55		Male n=45		
	No.	%	No.	%	
E. coli	16	29.09	9	20	25
K. pneumonia	11	20	9	20	20
Staph.aureus	8	14.54	7	15.55	15
P.aeruginosa	7	12.72	6	13.3	13
Proteus Vulgaris	4	7.27	3	6.66	7
A.baumannii	2	3.63	3	6.66	5
NG	7	12.7	8	17.77	15

NG= no growth of organisms; n=total number of patients in each sex.

Table (2): Occurrence rates of bacteria in clinical samples collected from different site of infections occurred among patients

Organism	Type of clinical specimens																	
	Urine n=26		Blood n=24		Abscess n=22		Wound n=8		Throat n=6		Ear n=5		Vaginal n=5		eye n=4		*Total n=100*	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
E. coli	15	57.69	1	5.88	3	17.64	2	25	2	40	0	0	2	40	0	0	25	29.41
Klebsiella Pneumonia	5	19.2	7	41.17	4	23.5	2	25	0	0	0	0	2	40	0	0	20	23.52
Staph.aureus	0	0	8	47.05	2	11.76	0	0	2	40	1	20	0	0	2	50	15	17.64
P.aeruginosa	2	7.69	0	0	6	35.29	2	25	1	20	2	40	0	0	0	0	13	15.29
Proteus vulgaris	2	7.69	0	0	1	5.88	1	12.5	0	0	2	40	1	20	0	0	7	8.23
A.baumannii	2	7.69	1	5.88	1	5.88	1	12.5	0	0	0	0	0	0	0	0	5	5.88
Total isolation rate	26	30.58	17	20	17	20	8	9.41	5	5.88	5	5.88	5	5.88	2	2.35	85	100

n= total number of clinical specimen.

Antimicrobial susceptibility profiles of bacterial isolates

Among 17 antibiotics, meropenem was the most effective drug against most of the gram-negative and gram-positive bacteria 25 *E.coli* (92.3%), 20 *K.pneumoniae* (85%), 13*P.aeruginosa* (76.9%), 7*P.vulgaris* (85.7%), 5 *A.baumannii* (80%) and 15 *Staph.aureus***(**73.33%) as written in Table 3. *E.coli* was more resistant to cefadroxil (96%), doxycycline (90%), piperacillin (88%), erythromycin (88%) and trimethoprim/sulphamethoxazole (80%). All *K.pneumoniae* was resistant to cefadroxil (100%) and more resistant to doxycycline (95%), piperacillin (90%), erythromycin (85%), cefotaxime (75%) and ceftazidime (75%). In other hand, all *P.aeruginosa* was resistant to

Cefadroxil (100%) and more resistant to Doxycycline, Cefotaxime, erythromycin and Piperacillin (92.3%, 84.61%, 69.23%, and 69.23% respectively). *P.vulgaris* was more resistant to cefadroxil, doxycycline, erythromycin, piperacillin and trimethoprim/sulphamethoxazole (85.7%, 85.7%, 85.7%, 57.14% and 42.85% respectively). Also, *A.baumannii* similar other gram negative bacteria in which were resistant to cefadroxil, piperacillin, erythromycin, ciprofloxacin and trimethoprim/sulphamethoxazole (Table 4).

Vancomycin and linezolid have effect on *Staph.aureus* (80% and 60%) but cefadroxil, cefotaxime and ceftazidime were more resistant (93.3%, 66.6% and 66.6%). MRSA were resistant to all penicillin, cephalosporin and carbapenem.

Table (3): Antibiotics susceptibility patterns against 85 different bacterial isolates collected from one hundred different patients suffering from bacterial infection.

Antibiotics	***E.coli*** **n=25**		***Klebsiella pneumaniae*** **n=20**		***Staph. aureus*** **n=15**		***Pseudomonas aeruginosa*** **n=13**		***Proteus vulgaris*** **n=7**		***Acinetobacter baumanii*** **n= 5**	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Amoxicillin/Clavulanic acid (AMC)	9	36	5	25	7	46.66	2	15.38	3	42.85	0	0
Piperacillin (PRL)	2	8	0	0	2	13.3	3	23.07	2	28.57	0	0
Cefadroxil (CFR)	0	0	0	0	0	0	0	0	0	0	0	0
Oxacillin (OX)	--	--	--	--	8	53.3	--	--	--	--	--	--
Cefotaxime (CTX)	3	12	2	10	3	20	0	0	3	42.85	1	20
Ceftazidime (CAZ)	7	28	1	5	3	20	3	23.07	3	42.85	1	20
Amikacin (AK)	15	60	10	50	6	40	9	69.23	5	71.4	3	60
Gentamycin (CN)	16	64	8	40	10	66.66	8	61.53	4	57.14	2	40
Meropenem (MEM)	23	92	17	85	11	73.33	10	76.9	6	85.7	4	80
Azithromycin (AZM)	0	0	4	20	9	60	5	38.46	1	14.28	1	20
Erythromycin (E)	2	8	2	10	8	53.3	3	23.07	1	14.28	0	0
Trimethoprim/ Sulphamethoxazole (SKT)	4	16	3	15	6	40	1	7.69	4	57.14	2	40
Doxycycline (DO)	2	8	0	0	10	66.66	0	0	1	14.28	0	0
Vancomycin (VA)	--	--	--	--	12	80	--	--	--	--	--	--
Nitrofurantion (F)	13	52	2	10	6	40	2	15.38	2	28.57	1	20
Linezolid (LZD)	11	44	2	10	9	60	7	53.84	3	42.85	2	40
Ciprofloxacin (CIP)	5	20	3	15	6	40	5	38.46	2	28.57	2	40

n= number of isolates, % = percentage of antibiotics susceptibility

Table (4): Antibiotics resistance patterns against 85 different bacterial isolates collected from one hundred different patients suffering from bacterial infection.

Antibiotics	*E.coli* n=25		*Klebsiella pneumaniae* n=20		*Staph. aureus* n=15		*Pseudomonas aeruginosa* n=13		*Proteus vulgaris* n=7		*Acinetobacter baumanii* n=5	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Amoxicillin/ Clavulanic acid (AMC)	11	44	12	60	6	40	10	76.9	2	28.57	3	60
Piperacillin (PRL)	22	88	18	90	12	80	9	69.23	4	57.14	4	80
Cefadroxil (CFR)	24	96	20	100	14	93.33	13	100	6	85.7	5	100
Oxacillin (OX)	--	--	--	--	5	33.3	--	--	--	--	--	--
Cefotaxime(CTX)	21	84	15	75	10	66.66	11	84.61	4	57.14	2	40
Ceftazidime (CAZ)	15	60	15	75	10	66.66	8	61.53	3	42.85	3	60
Amikacin (AK)	4	16	5	25	7	46.66	3	23.07	1	14.28	1	20
Gentamycin (CN)	7	28	8	40	3	20	3	23.07	1	14.28	1	20
Meropenem (MEM)	0	0	0	0	2	13.3	2	15.38	0	0	0	0
Azithromycin (AZM)	22	88	15	75	5	33.3	7	53.84	5	71.4	4	80
Erythromycin (E)	22	88	17	85	6	40	9	69.23	6	85.7	4	80
Trimethoprim/ Sulphamethoxazole (SKT)	20	80	15	75	9	60	10	76.9	3	42.85	2	40
Doxycycline (DO)	23	92	19	95	3	20	12	92.3	6	85.7	4	80
Vancomycin (VA)	--	--	--	--	2	13.3	--	--	--	--	--	--
Nitrofurantion (F)	7	28	13	65	7	46.66	10	76.9	3	42.85	2	40
Linezolid (LZD)	12	48	16	80	3	20	5	38.46	2	28.57	2	40
Ciprofloxacin (CIP)	16	64	13	65	7	46.66	5	38.46	2	28.57	33	60

n= number of isolates, % = percentage of antibiotics resista

3.2. Detection of biofilm

Tube method and Congo red agar were to detect biofilm in pathogenic bacteria and show that most isolates were multi-drug resistant. From two methods, it was obtained that most Gram negative bacteria have strong biofilm but Staph. aureus have weak biofilm except cases of MRSA have strong biofilm.

Table (5): Correlation of biofilm production of different isolates with different clinical specimens.

No.	Organism	Biofilm production
1	*A. baumannii*	Strong
2	*K. pneumonia*	Strong
3	*P. aeruginosa*	Strong
4	*Staph.aureus*	Weak
5	*MRSA	Strong
6	**ESBLE	Strong
7	*E.coli*	Moderate
7	*P.vulgaris*	Strong

*MRSA=Methicillin resistant *Staph.aureus* **ESBL= Extended Spectrum B-lactam

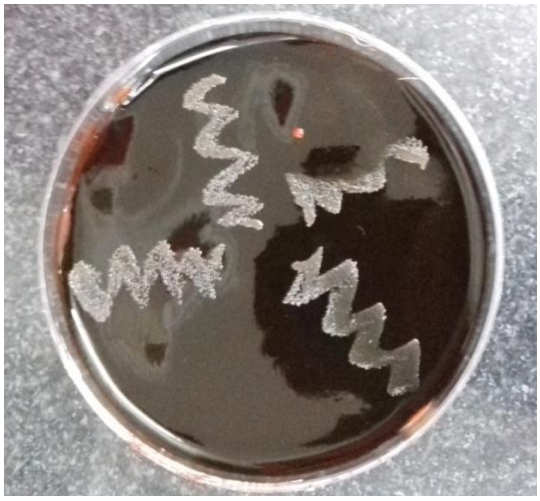

Figure (3): Positive biofilm by CRA method (Black color).

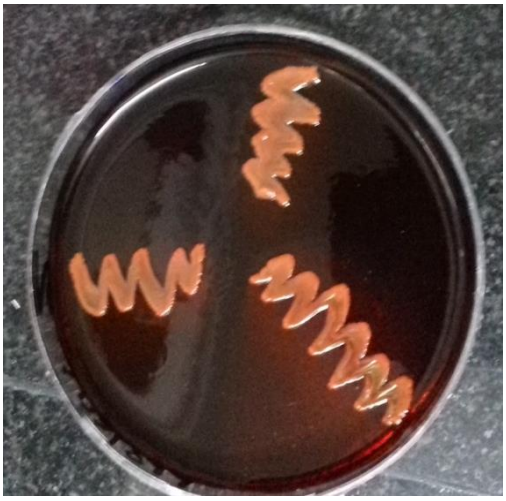

Figure (4): Negative biofilm by CRA method (Pink color).

Figure (5): Biofilm of different isolates by the tube method (blue color)

Effect of bacteriocin extracted from *Lactobacillus* species on isolated pathogenic bacteria

In this study, the production of bacteriocin from *Lactobacillus acidophilus (*DSM20079**)**, *Lactobacillus plantarum* (DSM20174) and *Lactobacillus salivarius* (DSM20555) have effect on the growth of Gram- negative (*E.coli, K. pneumaniae, P.aeruginosa, P.vulgaris* and *A.baumannii)* and Gram-positive bacteria *Staph.aureus*). The largest inhibition zone were obtained by bacteriocin extracted from *Lactobacillus acidophilus (*DSM20079**)** and the lowest inhibition zone of most Gram-negative pathogenic bacteria were obtained by *Lactobacillus salivarius* (DSM20555).

Results show that the affect of bacteriocin produced from *Lactobacillus acidophilus(*DSM20079**)** were on *P.vulgaris, P.aeruginosa, K.pneumoniae, E.coli* and *A.baumannii*(30,24,23,19 and 17 mm).Also, the affect of bacteriocin produced from *Lactobacillus plantarum* (DSM20174) on *P.vulgaris, P.aeruginosa, K.pneumoniae, E.coli* and *A.baumannii* were (24,20,12,15 and15mm ±1). On other hand, bacteriocin produced from *Lactobacillus salivarius* (DSM20555) have effect on *P.vulgaris, P.aeruginosa, A.baumannii, K.pneumoniae* and *E.coli*(24, 18, 16,15 and 12 mm) (Table 6).

Also, bacteriocin have effect on MRSA in which bacteriocin of *Lactobacillus salivarius* (DSM20555) were the only one have effect on most cases isolated from blood stream (20mm), but bacteriocin produced from *Lactobacillus acidophilus(*DSM20079**)** effect on other cases (23mm).

Table (6): Zones of inhibition of LAB against different multidrug resistant bacteria.

Pathogenic bacteria	Diameter zone of inhibition of bacteriocins(mm)		
Isolated bacterial	Lactobacillus acidophilus bacteriocin (DSM20079)	Lactobacillus planterum bacteriocin (DSM20174	Lactobacillus salivarius bacteriocin (DSM20555)
E.coli	19	15	12
K. pneumonia	23	12	15
P. aeruginosa	24	20	18
P. vulgaris	30	24	24
A. baumannii	17	15	16
Staph. aureus	10	9	11
MRSA (blood sample)	0	0	20
MRSA (urine sample)	23	13	8
ESBL	13	11	10

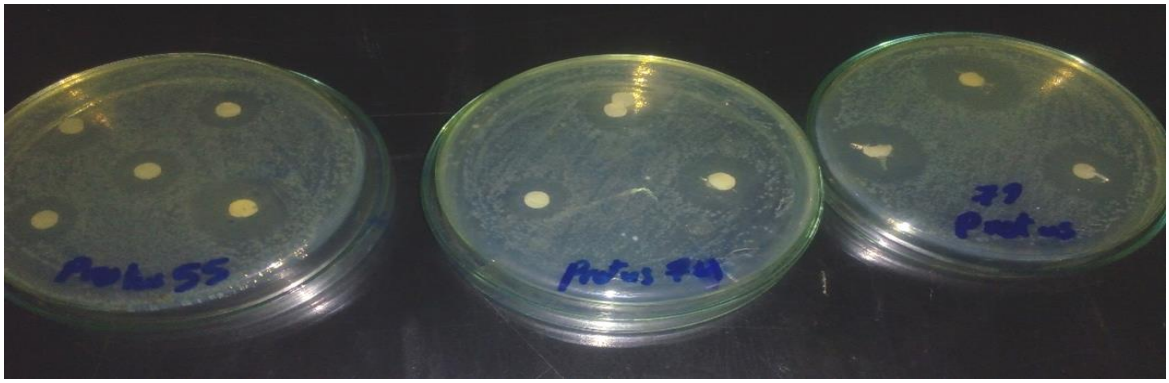

Figure (7): Effect of bacteriocin produced by *Lactobacillus acidophilus* (DSM20079), *Lactobacillus planterum* (DSM20174) and *Lactobacillus Salivarus* (DSM20555) on some pathogenic bacteria.

Effect of Lactic acid bacteria on biofilm

It was found that lactic acid bacteria also have an effect on the formation of biofilm, where they were grown on the biofilm of the pathogenic bacteria in the case of tube method and found that the blue color disappears (figure 7).

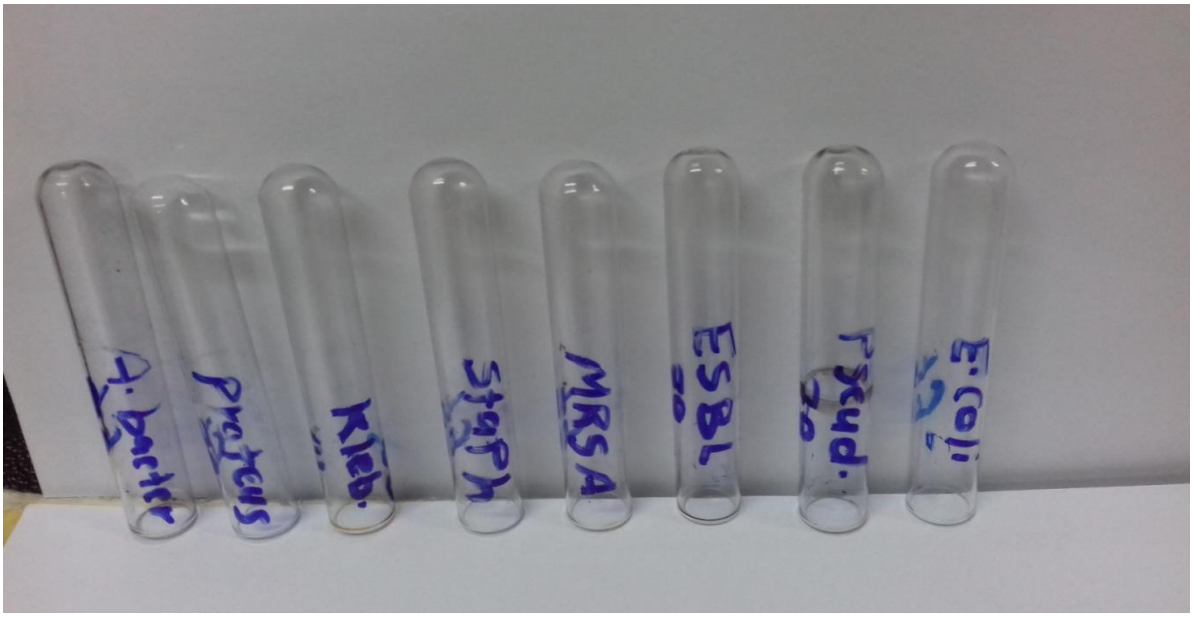

Figure (8): Effect of *Lactobacillus acidophilus* (DSM20079), *Lactobacillus planterum* (DSM20174) and *Lactobacillus Salivarus* (DSM20555) on biofilm of some pathogenic bacteria

Discussion

In the present study, a total of one hundred bacterial isolates were collected from many patients suffering from bacterial infection (55 females and 45 males) the results showed that (29.41%) *Escherichia coli*, (23.53%) *Klebsiella Pneumoniae,* (17.65%) *Staphylococcus aureus*, (15.29%) *Pseudomonas*

aeruginosa, (8.24%) *Proteus vulgaris*, (5.88%) *Acinetobacter baumannii*. The bacterial isolates were obtained from 8 different specimens with the following percentage representations: urine (30.58 %), blood (20%), abscess (20%), wound (9.41%), throat and tube swab (5.88%), ear discharges (5.88%), vaginal swabs (5.88%) and eye swab (2.35%).Our results show that most isolates were recovered from urinary tract and *E.coli* was the most common agent isolated followed by *K.pneumoniae*. **(Santigo *et al*., 2016)** reported that *E.coli* was the most common agent isolated from UTI. Also**, (Cornejo-Juárez *et al*., 2015)** reported that (20%) *E. coli*, (12%) *Staph.aureus*, (12%) *Enterococcus faecium* and (6%) *Acinetobacter baumannii* (all were MDR). **(Mulu *et al*., 2017)** reported that *E.coli* followed by *K.pneumoniae* was isolated from urinary tract infection and *P.aeruginosa* was the most frequent isolate from ear infection. While, **(Tabatabaei *et al.* 2015)** reported that the most common site of infection was the respiratory tract (67.9%) followed by the urinary tract (13.6%). Among the pathogens isolated, *Acinetobacter* and *Enterobacte*r were the most common (17.6%) followed by *E.coli* (11%). On other hand, **(Hecini-Hannachi *et al*., 2016)** were reported that most isolates were recovered from blood stream specimens (47.05%). The study showed that *Staph.aureus* (47.05%) was the most commonly isolated gram positive from blood stream followed to *K.pneumoniae* (41.17%) that represent the most commonly of Enterobacteriaceae. These results is similar to the study, which was carried out on infection of bloodstream by **(Latif *et al*., 2009).**

The identification of bacteria in the clinical microbiology laboratory was performed by isolation the organism and studying it phenotypically by means of Gram staining, culture, and biochemical method, which were once the gold standard of bacterial identification (**Wood *et al*., 2000).** Recently, more precise and accurate identification requires DNA- based methods which are increasingly used. Moreover, this study used the PCR technique as an accurate tool for identification depending on specific type of genes. Antibiotic-resistant bacteria continue to be a major health concern worldwide. In this study, the 85% isolated pathogenic bacteria were examined against 17 different antibiotics. The results showed that Meropenem have a broad spectrum and high activity against all Gram negative and gram positive clinical bacterial isolates. The susceptibility rates of Meropenem were 76.9%, 92%, 85.7%, 85%, 80% and 73.3% against *P.aeruginosa, E.coli, Proteus vulgaris, Klebsiella pneumonia, Acinetobacter baumannii* and *Staph.aureus* respectively**.** While, in this study, Cefadroxil and Piperacillin showed the lowest activity against all tested Gram- negative and Gram- positive bacterial isolates. The susceptibility patterns of Cefadroxil were 0% for all examined strains. The susceptibility patterns of Piperacillin were 28.57%, 23.07%,

13.3%, 8%, 0% and 0% against *Proteus vulgaris, Pseudomonas aeruginosa, Staph.aureus, E.coli, Klebsiella Pneumoniae and Acinetobacter baumannii.*
Most isolates of *Staph.aureus* were susceptible to Vancomycin and Linezolid (80% and 60%) respectively **(Bessa *et al.*, 2013)**. The resistance to oxacillin is particularly important because it can give us the percentage of *methicillin-resistant Staphylococcusaureus* (MRSA); in our study, a relevant percentage (40%) of *Staph. aureus* was oxacillin resistant. While **(Bessa *et al.*, 2013)** reported that the percentage of MRSA (21.8%) of all *Staph.aureus P.aeruginosa* was resistant to erythromycin, amoxicillin/clavulanic acid and Cefotaxime. The resistance of *P.aeruginosa* to antibiotics was reported previously by other authors **(Harvey *et al.*, 2010).** It was due to the ability of *P.aeruginosa* to form biofilm in patients. Also, *E.coli, P.aeruginosa, K.pneumoniae and A.baumannii* show high resistant rate to penicillin group (Amoxicillin/Clavulanic acid and Piperacillin) and cephalosporin group (Cefadroxil, Cefotaxime and Ceftazidime) and this similar to the observations of **(Gelaw *et al.*, 2013).** Also, in this study most isolates of Gram negative bacteria have high resistance to macrolides class erythromycin and Azithromycin.
Biofilm producing bacteria are responsible for many nosocomial infections, inflammation and increasing the resistance of antibiotics. In our study, we detect biofilm of many isolates by two different methods (Tube and Congo red agar) and this was agreed with **(Hassan *et al.*, 2011).** It was obtained that *Acinetobacter baumannii; Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the most isolates having biofilm and more resistance to selected antibiotics. But **(Omar *et al.*, 2017)** showed that *Staph.aureus* and *Pseudomonas aeruginosa* were pathogens having biofilm that associated mainly to wound burns. **(Nepal *et al.*, 2017)** showed that *Klebsiella pneumoniae* have the ability to adhere, multiply and persist on inanimate surface in the hospital environment causing nosocomial infections and it was ound that (73.3%) *Klebsiella Pneumoniae* strains form biofilm.
Because of the increase of multidrug-resistant bacteria and the restriction on the use antibiotics due to its side effects there is need to some alternative techniques for treatment. Lactic acid bacteria have the ability to synthesize antimicrobial compounds (like bacteriocins) during their growth **(Savadogo *et al*, 2004)**. The purpose of this study was to isolate bacteriocins from lactic acid bacteria for treatment/control multidrug-resistant bacteria.
Bacteriocins were isolated from cell free supernatant of LAB in MRS broth through precipitation method by adding 1N HCL. **(Abd El.Fattah, 1999, Savadogo *et al.*, 2004** and **Abdelsamei et *al.*, 2015)** also preferred this technique on the basis of short time and bacteriocin quantity. Inhibitory activity of bacteriocins against antibiotics resistant bacteria were checked

through agar diffusion disk method and zone of inhibition were measured. The diameters of inhibition zone against pathogenic bacteria were different**. (Schved *et al.*, 1993)** described that the inhibition zone can determine the degree of sensitivity and resistance bacteria.

It was obtained from our results that the antimicrobial activity of *L.acidophilus* (DSM20079) recorded by the diameters of inhibition zone (mm) with mean values was 30, 24, 23, 19, 17 and 10 mm. against *P. vulgaris, P. aeruginosa, K.pneumoniae, E. coli, A. baumannii* and *Staph.aureus* respectively. Also, it has activity on *Methicillin resistant Staph. aureus* with inhibition zone 23mm and ESBL with inhibition zone 13 mm. Bacteriocin produced by *L.salivarius* (DSM20555) has inhibitory activity against pathogenic bacteria. It was obtained from our study that inhibition zone (mm) of *L. salivarius* (DSM2055) was 24, 18, 16, 15, 12 and 11 mm. against *P. vulgaris, P. pseudomonas, A.baumannii, K.pneumoniae, E.coli and Staph.aureus* respectively. Also, *L. plantarum* (DSM20174) have antimicrobial activity against pathogenic bacteria with inhibition zone 24, 20, 15, 15, 12 and 9 mm against P. *vulgaris, P. aeruginosa, A. baumannii, E- coli, K. pneumoniae and Staph.aureus* respectively. From the previous results it was obtained that *L. acidophilus* have the highest activity against most isolated strains. Any researchers have highlighted the role of bacteriocins against pathogenic bacteria. **(Zahid *et al.*, 2015)** were recorded that the antimicrobial activity of bacteriocins extracted from *L. acidophilus* have maximum against pathogenic bacteria such as *Methicillin resistant Staphylococcus aureus, E.coli, Salmonella* and *Staphylococcus aureus* by using well diffusion agar method. It was found that the average inhibition zone (mm) of *L. acidophilus* were 14.5, 12.5 and 10.0 mm against *Bacillus subtilis, Staph.aureus* and *E.coli* respectively. Also, **(Sankaran, 2016)** showed that cell free supernatant (bacteriocins) isolated from Lactic acid bacteria have antimicrobial activity against gram positive bacteria such as (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria such as (*E.coli* and *K.pneumoniae*). **(Mahrous *et al.*, 2013)** also, found that bacteriocins isolated from *L. acidophilus* and *L. plantarum* have antimicrobial activity against food borne pathogenic as well as spoilage bacteria. It was found that the average diameter of inhibition zones measured ranged from 2-20 mm in size. More over in our study *L. plantarum* have high antimicrobial activity against tested pathogenic bacteria and these agreed with **(Sikorska and Smoragiewicz, 2013)** that observed that *L. plantarum* have inhibition activity against *Staph.aureus* and *P. aeruginosa*.

Conclusions

The study revealed that most common isolates in clinical samples causing infection were (29.41%) *E.coli*, (23.5%) *Klebsiella pneumoniae*, (17.65%) *Staph.aureus*, (15.29%) *Pseudomonas aeruginosa*, (8.24%) *Proteus vulgaris* and (5.88%) *Acinetobacter baumannii*. Isolates showed high levels of resistance to amoxicillin/clavulanic acid, cefadroxil, erythromycin, cefotaxime. Majority of gram negative and gram positive isolates showed high susceptibility to meropenem. The rise of multidrug resistant strains problem may be solved by using alternative therapies such as lactic acid bacteria that would decrease our reliance on antibiotic use. The study also suggest that the production of bacteriocins from lactic acid bacteria such as *L.acidophilus, L .plantarum* and *L .salivarus* can be used as antimicrobial agent to decrease the infection caused by pathogenic bacteria. Therefore, LAB may be helpful in the treatment of antibiotic resistance.

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استخدام بكتيريا حمض البروبيوتيك للسيطرة على البكتيريا المقاومة للمضادات الحيوية المتعددة المعزولة من العدوى السريرية

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الملخص العربى

اتجهت الأبحاث الان لاستخدام بكتريا البروبيوتك كبديل امن للمضادات الحيوية بسبب زيادة البكتريا المقاومة لها ولان البروبيوتك تعتبر بكتريا نافعة وتثبط نمو الكائنات الضارة بافرازها للمركبات الحيوية النشطة مثل البكتريوسين وفى هذه الدراسة تم أخذ 100 عينة من مرضى مختلفين فى الاعمار والجنس انثى(55 و 45 ذكر) والذين يعانون من العدوى البكترية :26عينة بول و24 عينة دم و22 عينة من خراج و8 عينة من جروح و5 مسحات من الاذن و5 مسحات من المهبل و6 مسحات من الحلق و4 مسحات من العين ومن خلال الزرع وجد أن منهم 85 عينة مسببة للعدوى والمرض وقد تم استخدام طرق بيولوجية وبيوكيميائية لتعريف وتحديد أنواع البكترية الممرضة وقد تم التأكيد على هذه الطرق باستخدام PCR.

وقد أظهرت هذه الدراسة أن أكثر العزلات شيوعا كانت بكتريا ايشيريشيا كولاى بنسبة (29.41%) يليها بكتريا كليبسيلا نيمويى بنسبة (23.53%) يليها الاستافيلوكوكاس اورياس بنسبة (15.29%) يليها بكتريا سودوموناس أوريجينوزا بنسبة (17.65%) يليها بكتريا بروتياس فولجاريس بنسبة (8.24%) وبكتريا الأسينتوباكتر باومنى بنسبة (5.88%). كما أظهرت أنه من بين 17 مضاد حيوى وجد ان الميرونام هو أكثر المضادات الحيوية تأثيرا على كل من البكتريا السالبه الجرام والموجبة الجرام. كما أنه تم تحديد البيوفيلم بطريقتين مختلفتين أتضح أن معظم العزلات كانت مقاومة للمضادات الحيوية.

تم عزل البكتريوسين من ثلاثة أنواع من بكتريا حمض اللاكتيك وهما لاكتوباسيللس اسيدوفيللس و لاكتوباسيللس بلانتارام ولاكتوباسيللس سيلفارس حيث كانوا لهم تأثير على كل من البكتريا السالبة الجرام والموجبة الجرام حيث أظهرت لاكتوباسيللس اسيدوفيللس كان أكثرهم تأثيرا على العزلات الممرضة ، كما بينت الدراسة أن اللاكتوباسيللس سيلفارس لها تأثير كبير على المرسا.