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DETECTION OF THE ANTIBACTERIAL ACTIVITY OF BACTERIOCIN FROM LOCAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* MULTIPLE RESISTANT STRAINS AGAINST GRAM POSITIVE&GRAM NEGATIVE BACTERIA

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ABSTRACT

The antibacterial activity of local isolates frome isolated from Baghdad, Iraq samples of different sources urine and wounds, ear&eye swab (25) strain of *Pseudomonas aeruginosa* (P1) (from wound infaction) multiple resistant strains that produced largest inhibition zone against the indicator strain was chosen for further study, and identified by morphological, physiological properties and antibiotic sensivity test were the neutralized *Pseudomonas aeruginosa* (P1) cell free supernatant of inhibited growth of a number of standard strains of pathogenic microorganisms bacteria Gve + and Gve- These isolates were screened for bacteriocin production using the agar well diffusion method. The isolates of *P. aeruginosa* were producer of bacteriocin with a wide inhibition range on growth of gram positive and negative pathogenic bacteria. P1 inhibited the bacterial growth of *S. aureus*, *Bacillus subtilis*, *Streptococcus pyogens,* isolates with a range of inhibition zone (15-21) mm. while inhibited the bacterial growth gram negative *Klebsiella pneumonia*, *Salmonella typhai*, *Escherichia coli* with a range of inhibition zone was (13- 22) mm.

KEY WORDS: *Pseudomonas aeruginosa*, antibacterial activity, bacteriocin, pathogenic Bacteria.

INTRODUCTION

Bacteria of the genus *Pseudomonas aeruginosa* are gram negative aerobic rods with size of cells from 2 to 3 µm. They are usually occurred in the wild, in the waste water and pure water and in the intestinal tract of man and animals, which live as saprophytes. A healthy individual has in his digestive tract; these microorganisms are present and are not dangerous for him. They occur in contaminated environment with colonization, but no signs of disease.[1] This bacterium is a good environmental bioreporters and as a model organism for understanding bacterial colonization and transport, cells immobilization strategies, and the kinetics of cellular bioluminescent emission. *Pseudomonas aeruginosa* has the extensive range of applications in the monitoring of bioremediation processes and biosensing of environmental pollution.[2] pyocin, an antibiotic produced by *Pseudomonas aeruginosa*, showed a high level of activity against *staphylococci* and *streptococci* and against certain gram- negative bacteria, including *Haemophilus influenzae* and *Neisseria gonorrhoeae*, but was much less active against most gram negative bacilli and anaerobes. Nearly all clinical isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*, including multiple resistant strains, were susceptible to pyocin. There was no cross resistance between pyocin and clinically available antibiotics, and the selection of resistant.[3] *Pseudomonas aeruginosa* HV37a also inhibited growth of the fungus *Pythium ultimum* on potato dextrose agar PDA.[4] *Pseudomonas aeruginosa* strain AH2 was used against the fish-pathogenic bacterium *Vibrio anguillarum* as probiotics in fish farming.[5] The bacterial strain MM-B16, which showed strong antifungal and antioomycete activity against some plant pathogens, was isolated from a mountain forest soil in Korea.[6] Few studies mentioned the role of this bacterium in human infections ,an outbreak of *P. aeruginosa* in bacteremia among oncology patients, one in the oncology ward andthree in the chemotherapy room was recorded in 1997.[7]

Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s).[8] Bacteriocins are of interest in medicine because they are made by non- athogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibiotic use may allow opportunistic pathogenic bacteria to invade the human body. Bacteriocins have also been suggested as a cancer treatment. Bacteriocins were tested as AIDS drugs (around 1990) but not progressed beyond in-vitro tests on cell lines.[12]

The aim of this study was to test the production of bacteriocin from local isolates of *P. aeruginosa* multiple resistant strains from clinical isolates and its effect on some Gram positive and negative pathogenic bacteria because there is only one study in Iraq on environmental isolates Bacteriocins are extracellularly released peptides or proteinaceous antimicrobial compounds,. Several types of bacteriocins from food-associated lactic acid bacteria have been identified and characterized, of which the important ones are Nisin, Bacteriocin, Diplococcin, Acidophilin, Bulgaricin, Helveticins, pyocin and Plantaricins. Lactobacillus species are primarily used as probiotics, but can also be used as starter cultures in various fermented foods.[7]

MATERIAL AND METHODS

Sample collection

The exudates of twenty five different patients suffering from wound, urine,ear and eye and detection multibal resistins antibiotic , and submitted for aerobic culture on MacConkey agar plat.

Media

MacConkey agar and Kligler Iron agar that we used, were purchased from Sigma Company, both media were recom- mended for differentiation of Gram-negative bacilli from clinical specimens. Additional chemicals; Indole, Simmen Citrate and Urea test were purchased from Merck Millipore company. The samples of exudates were grow on MacConkey agar for 24 hours in 37°C, Every single colony of different samples was tested biochemically and inoculated in biochemical media tubes including (Kligler Iron agar, Indole, Simmen Citrate and Urea Test). Tubes incubated aerobically at 35 ± 2°C and examined for growth after 18-24 hours.

Twenty five samples screened on MacConkey agar and eighteen samples showed brown colonies color, four samples were showed as green colonies, Other bacterial types were confirmed and identified biochemically. All plates with green colonies color were diagnosed biochemically and confirmed genetically as *Pseudomonas aeruginosa*, figure (1).

Antibacterial Resistance

pattern of the isolates was studied by Kirby-Bauer disc diffusion technique. Susceptibility of the isolates were done and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations. The antibiotic concentration per disk was as following. Ampicillin (10 Mg), Amikacin(30 Mg), Gentamicin (10 Mg), Cephalexin (30 Mg) and Ceftriaxone (30 The Maximization of Bacteriocins Production Induction of pyocin in ,fluid media. The medium used was tryptone soya broth (Oxoid) (3). A 2 ml portion of a static over- night culture of the pyocinogenic strain was added to 200 ml of sterile broth and incubated with agitation for 2 to 3 h at 32 "C. The lysate was centrifuged at 2400g for 30 min to remove bacteria1 debris and the supernate treated with 5 % (v/v) chloroform. The supernatant fraction was designated crude pyocin.

Bacteriocin assay

The antibacterial spectrum of the bacteriocin from *Pseudomonas aeruginosa* (P1) was determined using the well diffusion method. The supernatant from a 48-h culture of *Pseudomonas aeruginosa* (P1) was filter sterilized by passage through a 0.45: m pore size membrane filter (PALL Corporation, Mumbai). Aliquots (50 :l) of the sterile supernatant were placed in 6-mm-diameter wells that had been cut in Mueller-Hinton agar plates and MaCconky agar previously seeded with the indicator bacteria. After 12-24 h of incubation, the diameters of the zones of growth inhibition were measured. Antimicrobial activity was expressed in arbitrary units (AU/ml). One AU was defined as the reciprocal of the highest level of diluteon resulting in a clear zone of growth inhibition.[11]

Inhibitory activity

bacteriocin has been tested the antimicrobial activity of against the test organisms following the method described in.[9] A. hydrophila inoculated into BHI broth and incubated at 37°C, without aeration until mid logarithmic phase of growth. Aliquot of 10 µ l cell-free culture supernatant has been spotted on the surface of agar plate seeded with actively growing cells of the test organism. Plates have been incubated at the optimal growth temperature of the test organism.

RESULTS AND DISCUSSION

Table (1) shows the categorization of cases showed antibiotic resistance of *Pseudomonas aeruginosa*. The results of the present study showed that (60%) were wound infaction (15%) from urine (25%) frome ear and eye infaction.

Table (1): Categorization of *Pseudomonas aeruginosa* cases.

Category	Numbers of neonates No. & %
Wound	(60)%
Urin	(15)%
ear and eye	(25)%

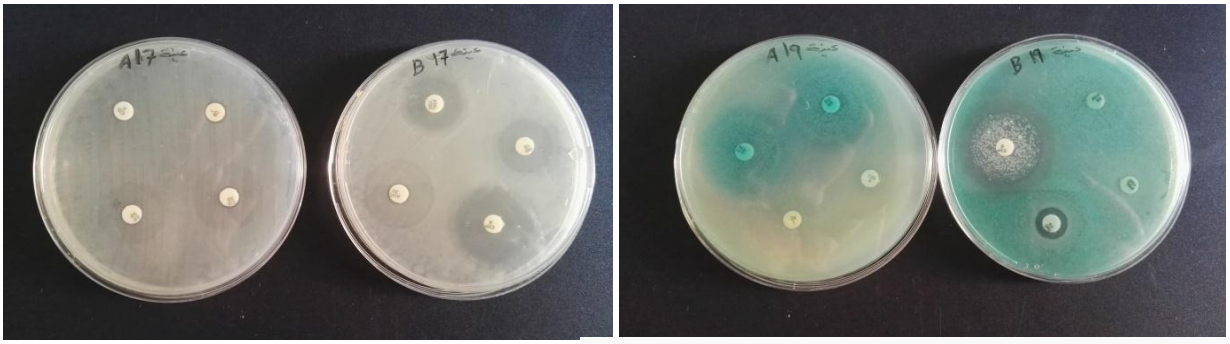
Table (2) showed antibiotic resistance of *E. coli, Pseudomonas* and *Klebsiella* sepsis to ampicillin, gentamicin, cephalexin and ceftraxone.

Antibiotic	Amikacin	Ampicillin	Cephalexin	Ceftriaxone	Gentamicin
P1	R100 S 0	R75 S 0	R100 S 25	R100 S 0	R75 S 0
P2	R 88 S 12	R 75 S 0	R 88 S 9	R 75 S 11	R 46 S 26
P3	R 72 S 28	R 75 S 25	R80 S 20	R82 S 18	R52 S 48
P4	R 66 S 34	R 74 S24	R80 S20	R80 S 20	R50 S 50
P5	R 66 S 34	R 70 S30	R 77 S 17	R 70 S 30	R 40 S 60
P5	R 60 S 40	R 55 S 45	R60 S 40	R55 S 45	R 82 S 18
P7	R 60 S 40	R 65 S 35	R 76 S 24	R 82 S 18	R 81 19
P8	R 55 S 45	R 60 S 40	R 77 S 23	R 80 S 20	R 75 S 25
P9	R 70 S30	R66 S34	R 66 S 34	R 71 S 29	R 80 S 20
P10	R 84 S 16	R 46 S 56	R 60 S 40	R 56 S 46	R 66 S 34
P11	R 88 S 12	R 73 S 27	R 88 S 12	R 76 S 24	R 85 S 15
P12	R62 S 38	R 77 S 23	R 54 S 46	R 45 S 54	R 16 S 84
P13	R 44 S 56	R 70 S 30	R 77 S 23	R 44 S 56	R 33 S 67
P14	R 55 S45	R 68 S 32	R 35 S 65	R 60 S 40	R 77 S 23
P15	R 66 S 34	R 81 S 19	R 77 S 23	R 81 S 19	R
P16	R 48 S 52	R 55 S 45	R 80 S 20	R 70 S 30	R 37 S 63
P17	R 44 S 36	R 77 S 23	90 10	R 50 S 50	R 86 S 14
P18	R 33 S 77	R 61 S 39	R 64 S 36	R80 S20	R 44 S 46
P19	R 56 S 44	R 70 S 30	R 90 S 10	R 89 S 11	R 77 S 23
P20	R 89 S 11	R61 S 39	R 52 S 48	R66 S 34	R 91 S 9
P21	R55 S45	R 27 S 73	R 66 S 33	R 80 S20	R 60 S 40
P22	R 33 S77	R60 S40	R80 S20	R 71 S 29	R60 S40
P23	R 56 S 44	R80 S 20	R71 S29	R88 S 12	R70 S30
P24	R 24 S 76	R 78 S 22	R90 S 10	R44 S45	R 33 S 77
P25	R 71 S 29	R54 S 45	F60 S40	R72 S 28	R39 S61

Table (4): Antibiotic sensitivity test of gram negative bacteria
S: Sensitive, R: Resistant

***Pseudomonas aeruginosa(*P):** Presumptive Pyocin production frome P1 most bacteriocin producers were obtained from the investigated sausage samples and other sources. isolates produced clear zones of inhibition against the indicator organisms. Only 22 isolates were production pyocin out of 1 were the strongest bacteriocin producers and were therefore selected for further tests. The one bacteriocin-producing were

***Pseudomonas aeruginosa* (P1):** identified as Gve + *Streptococcus pyogens*, *Bacillus cereus* and *Staphylococcus aureus* (Plates 1, 2, 3). And Gve- *Salmonella typhia*, *Pseudomonas aerugin* ,*Klebsella*, and *E.coli* (Plates 4, 5, 6) have the ability to produce bacteriocins that inhibit or kill gram-positive and gram-negative bacteria.[8], [11] and were found to be suitable for improving food safety.[12] have an attractive interest to be used as biopreservative in food industry.[7] Different types of.



Fig(1) Antibiotic resistics *Pseudomonas aeruginosa*The antibiogram of *P. aeruginosa* onMueller-Hinton agar

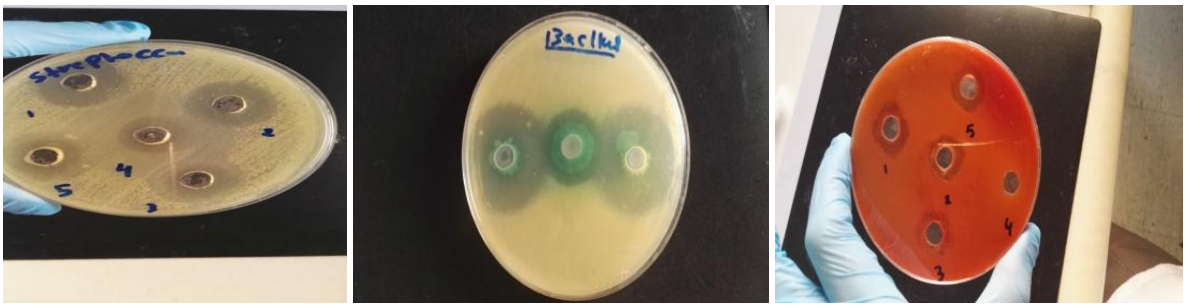


Figure 2 Antimicrobial activity CFCS *Pseudomonas sp,* Results of the well-diffusion assay of three bacterial strains(a) *Streptococcus pyogens* (b) *Bacillus subtilis* (c) *S. aureus*

Figure 2 Antimicrobial activity CFCS *Pseudomonas sp,* Results of the well-diffusion assay of three bacterial strains (a) *salmonella typha* (b) *E.coli* 157 O (c) *Klebsilla*

Table-2-The antimicrobial spectrum of crude bacteriocin produced by *Pseudomonas aeruginosa* (P1) isolate against sensitive strain Gve+&Gve-

Bacteriocin of *Pseudomonas aeruginosa* (P1)	**Indicator /sensitive strain**	**Average Zone of inhibition (mm)diameter**
	S. aureus	18
	Bacillus subtilis	27
	Streptococcus pyogens	23
	Klebsiella pneumoniae	26
	Salmonella typhia	20
	E.coli 157 O	22

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