

## Effect of UV Rays and Plasmid Curing on the Bacteriocin Antimicrobial Activity of *Pseudomonas Aeruginosa*

Rohama Zahid<sup>1\*</sup>, Maliha J Butt<sup>2</sup>, Sabahat Gulzar<sup>3</sup>, Shahzaib Ahmed<sup>4</sup>

<sup>1,3,4</sup> School of Biological Sciences, University of the Punjab, Lahore, Pakistan

<sup>2</sup>Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan.

**\*Corresponding Author:** Rohama Zahid, School of Biological Sciences, University of the Punjab, Lahore, Pakistan

**Abstract:** Bacteriocins are described as ribosomally synthesized small polypeptides that exert antimicrobial effects against a wide range of bacteria. The major producer group for bacteriocins is lactic acid bacteria (LAB) that contain a great variety of microorganisms described as "generally recognized as safe (GRAS)" by the US Food and Drug Administration. This study is design to test the antimicrobial activity of the *Pseudomonas aeruginosa* because of its ability to produce bacteriocins. Also experiments were designed to compare the bacteriocins activity *E coli* vs *Pseudomonas aeruginosa*. All the bacterial strains used in this study were isolated form the Tap water and were identified by the biochemical testing methods. *Pseudomonas aeruginosa* and *Escherichia coli* were used for the bacteriocins antimicrobial activity assay on the test strains and the effect of UV rays exposure was checked on both of them. Plasmid isolation and plamid curing was also performed and then again bacteriocin antimicrobial activity assay was done. Out of *E coli* and *P aeruginosa*, only *P aeruginosa* holds the bacteriocin antimicrobial activity, secondly this activity remained unaffected after the UV rays exposure. After the UV rays exposure the antibiotic resistant strain started exhibiting the sensitivity. *P aeruginosa* possess a plasmid of 10Kbp. Plasmid curing confirmed that the bacteriocin encoding gene might be residing on the plasmid. Disinfection with UV rays can be used to damage the antibiotic resistant genes in bacterial population. Further purification of the bacteriocins can be done and can be used in the food industry for preservation, to treat the antibiotic resistant infections and cancers as well. Instead of this, the new bioengineered bacterial strains encoding the desired bacteriocin gene can be made, in order to increase the spectrum of application in the field of medicines.

**Keywords:** *Pseudomonas aeruginosa*, *E coli*, Bacteriocins, Antimicrobial, UV Rays, Plasmid curing, Resistant, Antibiotics.

**Abbreviations:** (LAB) lactic acid bacteria, (GRAS) generally recognized as safe, (*E coli*) *Escherichia coli*, (*P aeruginosa*) *pseudomonas aeruginosa*, (*S aureus*) *Staphylococcus aureus*, (*S epidermidis*) *Staphylococcus epidermidis*, (*M luteus*) *Micrococcus luteus*, (ARG) Antibiotic resistant genes, (UV rays) Ultraviolet rays

### 1. INTRODUCTION

*Pseudomonas* is considered as one of the metabolically multifaceted genus, it has the ability to grow in diverse range of environments and varies from being opportunistic to the pathogenic one [1] [2]. Some of the *Pseudomonas* species possess the antimicrobial molecules to face the competitors, On the basis of structure and mode of action, these molecules are called as Bacteriocins [3]. It has been reported that the numerous strains of *E coli* and *Pseudomonas aeruginosa* encodes the bacteriocin producing genes in their genomes [4].

Bacteriocins are of the protein nature and they are released in the form of peptides [5]. Bacteriocins weighed not so much that's why they are known to be the non sedimentable molecules or peptides, and it is the reason they are present in the supernatant, after centrifugation [6]. Bacteriocins encoding genes can be present on the chromosomes, plasmids or on the other mobile genetic elements in the bacteria [7]. Bacteriocins can be used to treat the antibacterial resistant infections because of its antagonistic activity against various bacterial strains [8]. It has been investigated that the bacteriocin activity of a bacterial strain get vanish after the plasmid curing [9]. On the other hand, plasmid curing also results in the conversion of antibiotic resistant bacteria into the sensitive one because the antibiotic resistant genes (ARG) mostly resides on the plasmid [10]. Various classes of bacteriocins exist in the *Pseudomonas* genus but the most diverse one is S type bacteriocin [3]. Bacteriocins also

show the analogy with the colicins that are present in *E coli* [11]. It is not a hard and fast rule that every *E coli* or *Pseudomonas* species possess bacteriocin [12]. Pyocins (Bacteriocin) produced from the *Pseudomonas* species owns different type of characters such as, DNase, tRNase, rRNase, nuclease activity and membrane pore forming activity [13].

It has been reported that bacteriocins production and its activity remain same, after exposing the bacterial strain to some mutagenic agents, such as the exposure of ultraviolet radiations [14]. On the other hand, UV rays exposure has the ability to convert the antibiotic resistant strain into the antibiotic sensitive strain [15]. It has been investigated that the UV rays have the potential to change the bacterial colony cultivability and growth characteristics as well [16].

This present work is basically focusing on determining the antimicrobial activity of *Pseudomonas aeruginosa* due to the presence of bacteriocins. Secondly, the effect of UV rays on its bacteriocins antimicrobial activity. After UV rays exposure the antibacterial activity of the streptomycin and chloramphenicol was checked on the wild type and mutant strains of *E coli* and *Pseudomonas aeruginosa*. In order to determine, whether the bacteriocin encoding gene is present on the chromosome or plasmid, plasmid curing was done.

## **2. MATERIAL AND METHODS**

### **2.1. Bacterial Strains, Culture Media and Growth Conditions**

Bacterial strains used in this study are *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Micrococcus luteus*, and *Bacillus cereus*, mentioned in the (Table 1), they were isolated from the tap water by the spreading plate method [17], that sample was taken from the Bagarian, military accounts, Lahore, Pakistan. Biochemical identification of the bacterial strains was done, [17] [18]. All the strains were grown in the (LB) Luria – Bertani (Difco) broth medium and L agar medium at 37°C under aerobic conditions. For the antimicrobial activity assay, Mueller Hinton (MH) agar (Difco) was used. Two antibiotics were used Streptomycin (50 ug/ml and 100 ug/ml on *Pseudomonas aeruginosa* and *E coli* strains after and before UV rays exposure) and chloramphenicol (50 ug/ml and 100 ug/ml on *Pseudomonas aeruginosa* and *E coli* strains after and before UV rays exposure). Ampicillin and Tetracycline (50ug/ml) was also employed in an experiment. All the antibiotics were purchased from the BIOANALYSE Ankara, Turkey. Plasmid isolation was done by the alkaline lysis method [19], for that purpose the stock solutions of EDTA, Tris-Cl, Glucose, NaOH, SDS and Potassium Acetate were made, all these chemicals including glacial acetic acid were purchased from the Sigma Aldrich, Thermofisher and Merck. To visualize the Plasmid, agarose gel electrophoresis was done [19], agarose, and bromphenol blue was purchased from the Sigma Aldrich. For sizing, 1Kb DNA (SM0311) ladder from the ThermoFisher SCIENTIFIC was used. For the plasmid curing, 50ug/ml and 100ug/ml of the Acridine orange and Ethidium bromide was used, from the Sigma Aldrich.

### **2.2. Experimental Procedure**

#### **Isolation and the Biochemical Identification of the Bacterial Strains**

10X dilution of the water sample was made and 50ul of it was spreaded on the L agar plate, 5 type of the colonies were selected and they were streaked further to get the pure colonies, and after that, the biochemical identification was done [17] [18].

#### **Bacteriocin Antimicrobial Activity Assay**

Bacterial cells free supernatant of *E coli* and *Pseudomonas aeruginosa* was taken, for that purpose, *E coli* and *Pseudomonas aeruginosa* strains were grown in Lb broth medium, by providing the incubation at 37°C for 24 hours, then the cultures were centrifuged at 4700 rpm for 20 minutes, the supernatants were heated at 70°C in oven for 25 to 30 minutes, further the denatured supernatant was added in each well [20]. Agar well diffusion method [21] was used in which the *Micrococcus luteus*, *Staphylococcus epidermidis* and *Bacillus cereus* were plated on the MH agar plates by spreading, after that, the denatured supernatants of *E coli* and *Pseudomonas aeruginosa* were added into the respective wells and additionally the 30ul of ampicillin (50ug/ml) and 30ul of tetracycline (50ug/ml) were also used. The plates were incubated for 24 hours at 37°C under aerobic conditions.

### UV Rays Exposure Assay

5ml Lb broth cultures of *E coli* and *Pseudomonas aeruginosa* were grown overnight, next day the 1% bacterial cultures of each strain was prepared by putting 100ul of incubated culture into the 10 ml of autoclaved Lb broth, replicates of each strain were made, one was labeled mutant type and other one as wild type. After that the tubes were given the incubation for 5 hours at 37°C. Then the mutant labelled test tubes of *E.coli* and *Pseudomonas aeruginosa* were exposed to UV rays under UV illuminator for 3 minutes, after that the mutant labelled tubes were wrapped in the foil paper. Both the mutant and wild type strains containing test tubes were given the incubation at 37°C for 24 hours. Next day, the bacteriocin activity of each strain (mutant *E coli* and *Pseudomonas aeruginosa*, wild type *E coli* and *Pseudomonas aeruginosa*) was tested on the *Micrococcus luteus*, *Staphylococcus epidermidis* and *Bacillus cereus* by the help of bacteriocin antimicrobial activity assay.

### Antibacterial Activity Testing on *E coli* and *P aeruginosa* after UV Exposure

Antibacterial activity of Streptomycin and chloramphenicol was checked on Mutant and wild type strains of *E coli* & *Pseudomonas aeruginosa*, by agar well diffusion method [21], Mutant and Wild type *E coli* and *Pseudomonas aeruginosa* were plated on the MH agar plates by spread plate technique [17], and then the 30ul of Streptomycin (50 ug/ml and 100 ug/ml) and Chloramphenicol (50 ug/ml and 100 ug/ml) was added into the respective wells on each spread plate, then the plates were incubated for 24hours at 37°C, next day the results were jotted down.

### Colony Morphology Testing after UV Rays Exposure

Mutant and wild type strains of *E coli* and *Pseudomonas aeruginosa* were streaked on the L agar and incubated for 24 hours at 37°C for 24hours and next day the observation was made.

### Plasmid Isolation and Agarose Gel Electrophoresis

Plasmid isolation was done by the minipreparation; Alkaline lysis method [19]. First of all, the stock solutions were prepared for the formation of alkaline lysis solution 1, 2 and 3. Solution 1 and 3 after preparation were stored at 4°C while the solution 3 was freshly prepared and placed at the room temperature. For the plasmid isolation the 16 hours incubated culture was used. After the plasmid isolation procedure, 0.5% agarose gel was prepared in TAE buffer, with the addition of ethidium bromide and after the solidification, gel apparatus was assembled and 7ul sample with 5ul tracking dye was loaded into the wells along with the 1Kb ladder. Gel was run at 100V for 40 minutes. After that the gel was observed under the UV illuminator.

### Plasmid Curing

50ug/ml and 100ug/ml of Ethidium bromide and 50ug/ml and 100ug/ml of acridine orange were used as the curing agents [22]. For that purpose the *Pseudomonas aeruginosa* strain was incubated overnight and next day 50ul of the incubated culture was added into the 5ml of the Lb broth test tubes containing the above mentioned concentrations of curing agents, and incubated for 24hours at 37°C. Next day the denatured supernatants of each culture was made. Then the 24 hours incubated culture of *Micrococcus luteus*, *Staphylococcus epidermidis* and *Bacillus cereus* were spreaded on the MH agar plates and the denatured supernatants were poured into the wells [21], then the plates were incubated for 24 hours at 37°C. Next day the results were noted down.

## 3. RESULTS

### 3.1. Biochemical Identification of Bacterial Strains

The colonies obtained from the spreading plate results were too much to count ( Figure 1), 5 type of colonies were selected and they were streaked further to get the pure colonies, biochemical identification was done by [17] [18], results of biochemical identification are showed in (Table 1). According to the biochemical identification results: P was identified as *Pseudomonas aeruginosa*, Q as *E coli*, R as *Bacillus cereus*, S as *Micrococcus luteus* and T as *Staphylococcus epidermidis*.



**Figure1.** Spread plate results of the water sample from Bagarian, Military accounts.

**Table1.** Biochemical identification results. Key: + indicates the positive result for a test, - indicates the negative result for a test, \* indicates that the test was not performed on that strain, LF means Lactose Fermenter, NLF means non lactose ferementer, A/A indicates alkaline slant and alkaline butt, K/K indicates acidic slant and acidic butt.

Tests	Unknown Bacterial Stains				
	P	Q	R	S	T
Gram Staining	-	-		+	+
Spore Staining	-	-	+	-	-
Shape	Rod	Rod	Rod	cocci	cocci
Catalase test	+	+	+	+	+
Oxidase Test	+	-	-	-	-
Mac Conkeys agar test	NLF	LF	*	*	*
Mannitol agar test	*	*	-	-	-
EMB agar test	+	+	*	*	*
Novobiocin sensitivity test	*	*	*	*	Sensitive
TSI agar test	A/A	K/K	*	A/A	*
Citrate Test	+	-	*	*	*
Methyle red test	*	-	*	*	*
Vogues proueuskar test	*	+	*	*	*
Nitrate Reduction Test	+	*	*	*	*
Indole Test	-	+	*	*	*

### 3.2. Bacteriocin Antimicrobial Activity Assay

Zones of inhibition were measured, according to them, *E coli* didn't show bacteriocin antimicrobial activity on any of the three test strains (*B cereus*, *S epidermidis*, *M luteus*), on the other hand *Pseudomonas aeruginosa* exhibited the bacteriocin antimicrobial activity against all the three strains (*B cereus*, *S epidermidis*, *M luteus*). Zone of inhibitions are shown (Figure 2) and in (Table 2). While the comparative results are depicted in the graphical form (Figure 3). *S epidermidis* exhibited the maximum sensitivity against the denatured supernatant of *P aeruginosa* while the *M luteus* exhibited

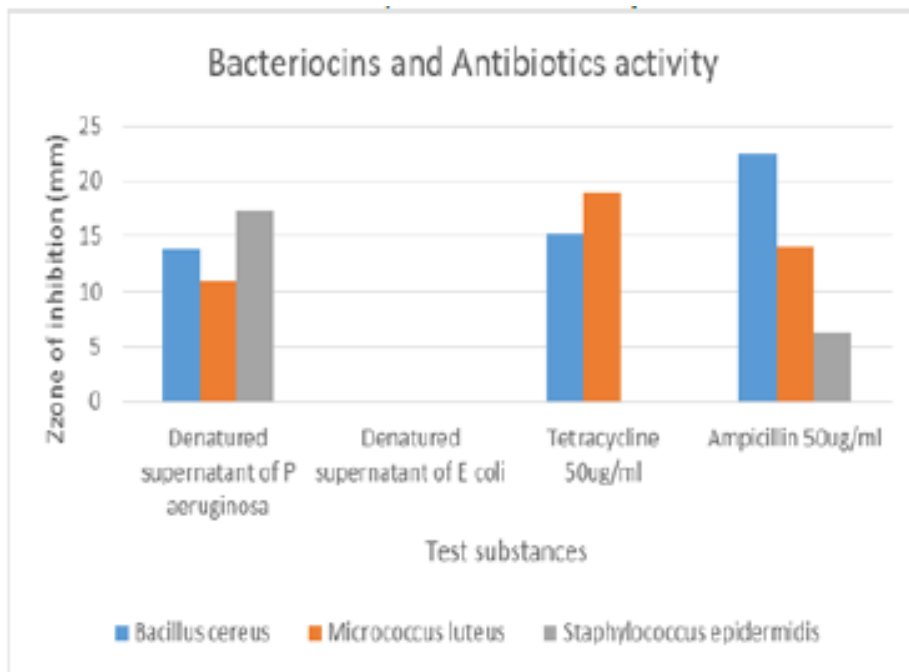


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the least. Secondly, all the three strains (*B cereus*, *S epidermidis*, *M luteus*) were sensitive to the Ampicillin (50ug/ml) and Tetracycline (50ug/ml) but *S epidermidis* was resistant to the Tetracycline.



**Figure2.** Bacteriocin antimicrobial activity assay. (A) Effect of bacteriocin and antibiotics on *S epidermidis* (B) Effect of Bacteriocin and antibiotics on *M luteus* (C) Effect of Bacteriocin and antibiotics on *B cereus*. Key: DS=Denatured supernatant.



**Figure3.** Comparative results of the effect of antibacterial agents (Bacteriocins & antibiotics) on the three bacterial strains.

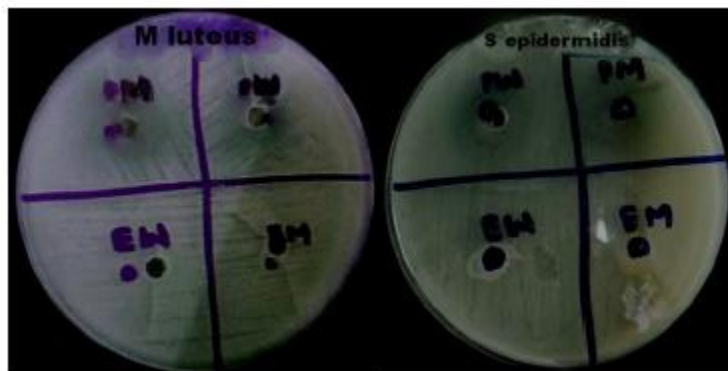
**Table2.** The bacterial strains used in the study.

Microrganisms	Strains
Bacteria	<i>Escherichia coli</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Staphylococcus epidermidis</i>
	<i>Micrococcus luteus</i>
	<i>Bacillus cereus</i>

**3.3. UV Rays Exposure Assay**

After the UV rays exposure, the bacteriocin antimicrobial activity of the *E coli* and *Pseudomonas aeruginosa* was determined, results indicated that the both mutant and wild type strain of *E coli* don't possess any Bacteriocin antimicrobial activity. On the other hand, the bacteriocins antimicrobial activity of the wild type and mutant denatured supernatant of the *P aeruginosa* on the *S epidermidis*,

*M luteus* and *Bacillus cereus* were approximately same. Results are shown in the (Figure 4). Zones of inhibition are mentioned in the (Table 3).



**Figure4.** Bacteriocin antimicrobial activity of the mutant (Em) and wild type *E coli* (Esw) and *Pseudomonas aeruginosa* (Pm) (Pw) on *M Luteus* and *S epidermidis*. (A) Bacteriocin antimicrobial activity on *M luteus* (B) Bacteriocin antimicrobial activity on *S epidermidis*.

**Table3.** Zone of inhibition against the antibacterial agents (Bacteriocins and antibiotics).

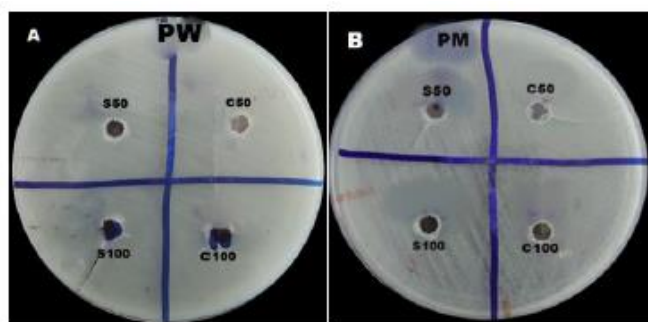
Antibacterial agent	Bacterial strains		
	<i>Micrococcus luteus</i>	<i>Bacillus cereus</i>	<i>S epidermidis</i>
	Zone of inhibition (mm)		
Tetracycline (50 ug/ml)	19	15.3	0
Ampicillin (50ug/ml)	14	22.5	6.3
Denatured supernatant of <i>E coli</i>	0	0	0
Denatured supernatant of <i>P aeruginosa</i>	11	13.7	17.2

### 3.4. Antibacterial Activity Testing on *E Coli* and *P aeruginosa* after UV exposure

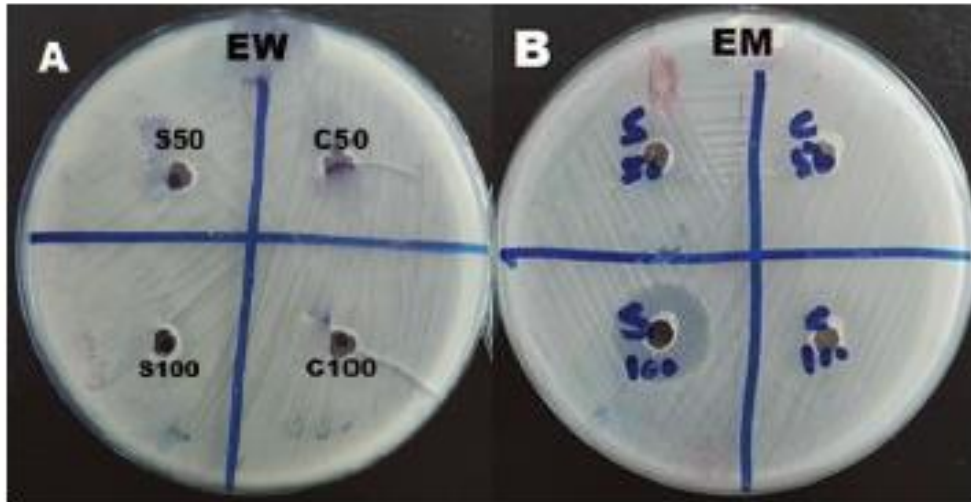
*E coli* wild type and *P aeruginosa* wild type were resistant to the both concentrations of chloramphenicol and streptomycin, but the mutant *E coli* was sensitive to 100ug/ml concentration of Streptomycin and mutant *P aeruginosa* was resistant to the 100ug/ml of chloramphenicol, as depicted in the (Figure 5 & 6).

**Table4.** Zone of inhibition against the denatured supernatant of wild type and mutant strains of *E coli* and *P aeruginosa*.

Antibacterial agent	Bacterial strains		
	<i>M luteus</i>	<i>S epidermidis</i>	<i>B cereus</i>
	Zone of inhibition (mm)		
Denatured supernatant of <i>E coli</i> (wild)	0	0	0
Denatured supernatant of <i>P aeruginosa</i> (wild))	15	15	11.5
Denatured supernatant of <i>E coli</i> (mutant)	0	0	0
Denatured supernatant of <i>P aeruginosa</i> (mutant)	15	16.5	12



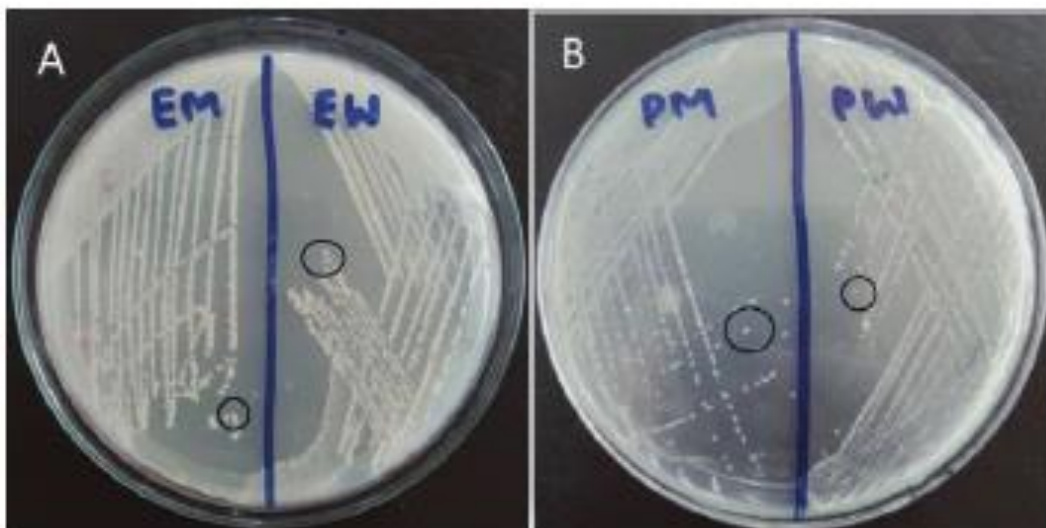
**Figure5.** Antibacterial activity of Streptomycin (50ug/ml & 100ug/ml) and Chloramphenicol (50ug/ml & 100ug/ml) on the *P aeruginosa* and *E coli* after and before UV rays exposure. (A) PW: *P aeruginosa* (Wild type), (B) PM: *P aeruginosa* (Mutant type)



**Figure 6.** Antibacterial activity of Streptomycin (50ug/ml & 100ug/ml) and Chloramphenicol (50ug/ml & 100ug/ml) on the *E coli* after and before UV rays exposure. (A) EW: *E coli* (Wild type), (B) EM: *E coli* (Mutant type). Key for figure 5 and 6: S50 = Streptomycin 50ug/ml, S100 = Streptomycin 100ug/ml, C50 = chloramphenicol 50ug/ml, C100 = Chloramphenicol 100ug/ml

### 3.5. Colony Morphology Testing

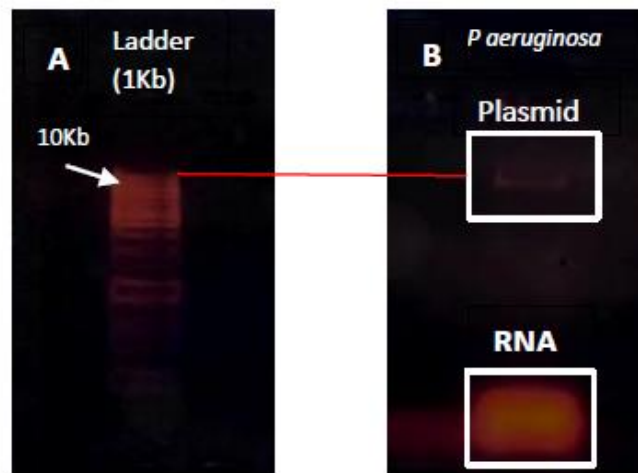
To check the effect of the UV rays on the colony morphology of *E coli* and *P aeruginosa*, both the mutant and wild type strains were streaked on the L agar plates. There was not any difference in the colony pigmentation and shape of both *E coli* mutant and *E coli* wild type. On the other hand the mutant *P aeruginosa* depicts difference in the shape of the colony, *P aeruginosa* mutant (*Pm*) colony was eye shaped while the wild type *P aeruginosa* exhibits the circular shaped colony. Results are shown in (Figure 7)



**Figure 7.** Streaking plate results of *P aeruginosa* (*Pm* & *Pw*) and *E coli* (*Em* & *Ew*) to determine the Colony morphology. (A) *E coli* (*Em* & *Ew*) (B) *P aeruginosa* (*Pm* & *Pw*). Key: , *Ew* = *E coli* wild, *Em* = *E coli* mutant, *Pm* = *P aeruginosa* mutant, *Pw* = *P aeruginosa* wild

### 3.6. Plasmid Isolation and Agarose Gel Electrophoresis

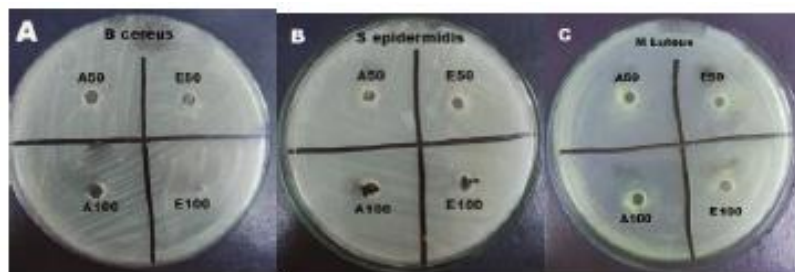
After the plasmid isolation of the *P aeruginosa*, the sample was run on the agarose gel along with the 1Kb ladder. The results indicated that the *P aeruginosa* has the mega plasmid of 10Kb, by comparing it with the ladder, secondly the RNA of *P aeruginosa* can be seen because the plasmid isolation was carried out without RNase treatment, and results on gel are exhibited in the (Figure 8).



**Figure8.** Plasmid isolation result of *P aeruginosa* on the agarose gel (A) DNA 1Kb Ladder (B) Plasmid and RNA of *P aeruginosa*.

### 3.7. Plasmid CURING

According to the obtained results, cured cells of *P aeruginosa*, by both the concentrations (50ug/ml and 100ug/ml) of Acridine orange and Ethidium bromide, didn't show any bacteriocin activity on the test strains (*M luteus*, *B cereus*, *S epidermidis*). Results are shown in (Figure 9).



**Figure9.** Bacteriocins antimicrobial activity of *Pseudomonas aeruginosa* after plasmid curing. (A) *B cereus* (B) *S epidermidis* (C) *M luteus*. Key: A50 = Acridine 50ug/ml Treated denatured supernatant of *P aeruginosa*; A100 = Acridine 50ug/ml Treated denatured supernatant of *P aeruginosa*; E50 = Ethidium bromide 50ug/ml Treated denatured supernatant of *E coli*; E100 = Ethidium bromide 50ug/ml Treated denatured supernatant of *E coli*.

### 4. DISCUSSION

One of the biggest problem the world facing today is the antibiotic resistance and their increasing toxicity, in this time, there is a need of some alternatives and at this time, bacteriocins are considered as one of the solution to this problem [23]. Strains of *Pseudomonas aeruginosa* has been used to isolate the variety of bacteriocins and these novel bacteriocins had shown the antimicrobial activity against the *Staphylococcus* species, *Bacillus* species and *E coli* [24]. Plenty of the bacteriocins (Colicins) are produced from the strains of *E coli* at much high frequency but not every strain of the *E coli* has the genes encoding bacteriocins [25]. In this present study, initially *E coli* and *Pseudomonas aeruginosa* was used to analyze their bacteriocins antimicrobial activity but results indicated that the *E coli* didnt possess any bacteriocins antimicrobial activity, on the other hand, *Pseudomonas aeruginosa* showed bacteriocin antimicrobial activity against the *Staphylococcus epidermidis*, *Bacillus cereus* and *Micrococcus luteus*. On comparing the zone of inhibition of each test bacteria, *S epidermidis* showed highest sensitivity against the denatured supernatant of *P aeuroginosa* and *M luteus* depicted the least sensitivity (*S epidermidis* > *B cereus* > *M luteus*).

As the multi drug resistance is emerging day by day, out of the millions of the bacterial species, *Staphylococcus epidermidis* has been reported resistant to the vancomycin and tetracycline [26]. In this study the three test strains were subjected to the two antibiotics (Ampicillin 50ug/ml and Tetracycline 50ug/ml), *Bacillus cereus* and *Micrococcus luteus* were sensitive to both the antibiotics but the *Staphylococcus epidermidis* was ressitant to the tetracycline.



Bacteriocins production and activity in *Bacillus cereus* didn't get effected after exposing them to the UV rays [14]. It has been reported that there was not any effect on the bacteriocin activity of the *Lactobacillus plantarum* after the UV rays exposure [27]. In this study, the zones of inhibition exhibited by the three test strains were of the approximately same size even before and after the UV Rays exposure, it is indicating that the UV rays exposure has not any effect on the *Pseudomonas aeruginosa's* bacteriocin production and its activity. On the other hand *E coli* didn't show any bacteriocin antimicrobial activity even before and after UV rays exposure.

Antibiotic resistance bacteria in the drinking water is an alarming situation and a lot of bacterial strains have been reported as the multiple antibiotic resistant (MAR) such as ciprofloxacin, sulfamethoxazole, quinolone, or tetracycline [28]. 29% of the *E coli* and 17% of the *Staphylococcus* from the public drinking water of Lahore, had the Kanamycin and Ampicillin resistant genes [29]. Both the strains of *Pseudomonas aeruginosa* and *E coli* that were isolated from the tap water, were subjected to the antibacterial activity testing. Both of the bacteria were resistant to both antibiotics (50ug/ml & 100ug/ml) Streptomycin and (50ug/ml & 100ug/ml) Ciprofloxacin.

UV rays are considered to have the disinfection properties, by restricting the spread of the antibiotic resistant genes (ARG), damage to the ARG has been reported in the *E coli*, *Pseudomonas aeruginosa* and *Enterococcus faecium* [30]. As the result of disinfection the surviving *E coli* had shown the change in the zones of inhibition against the antibiotics [15]. After exposing the *Pseudomonas aeruginosa* and *E coli*, *Pseudomonas aeruginosa* showed the resistant against the Ciprofloxacin streptomycin. On the other hand, *E coli* exhibited the sensitivity against the 100ug/ml of the Streptomycin but was still resistant to the Ciprofloxacin. It's depicting that the UV rays has a damaging effect on the antibiotic resistant genes (ARG) of *E coli*. After the UV rays exposure, the *Pseudomonas aeruginosa* showed variation in its shape of colony on the LB agar plate.

Plasmid presence in the *P aeruginosa* was confirmed by performing the plasmid isolation by the alkaline lysis method, results indicated that it contains a large plasmid of 10Kbp, plasmids of *P aeruginosa* plays a dominant role in the emergence of the multidrug resistance in the world [31].

Bacteriocin encoding genes are known to be reside on the plasmid, chromosomes or on the mobile genetic elements [11]. Genes that are encoded by the plasmid can be precluded by using the heterocyclic compounds. These heterocyclic compounds basically binds with the superhelical forms of the plasmid DNA with more affinity rather than that of the linearized one [32]. Many plasmid curing agents are there and the plasmid of the *E coli* has been reported to be cured by using various concentrations of Sodium dodecyl sulfate (SDS), Ethidium Bromide (Et-Br) and Acridine orange (AO) [22].

As out of the *Pseudomonas aeruginosa* and *E coli*, bacteriocins antimicrobial activity was exhibited by the *Pseudomonas aeruginosa*, so further to find whether the bacteriocins encoding gene is present on the chromosomal DNA or on the Plasmid, plasmid curing was done. After the plasmid curing, bacteriocins antimicrobial activity assay was performed on the 3 test bacterial strains. *Pseudomonas aeruginosa* didn't show any bacteriocins antimicrobial activity on all of the three strains after the plasmid curing.

## **5. CONCLUSION**

All in all, this study has concluded that the *Pseudomonas aeruginosa* possess the bacteriocins antimicrobial activity, and this activity was lost on the plasmid curing. Secondly, the *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *E coli* isolated from the tap water had shown resistance to the antibiotics (tetracycline, streptomycin and ciprofloxacin). UV rays don't have any effect on the bacteriocin antimicrobial activity of the bacterial strains. Exposure of the UV rays can convert the antibiotic resistant strains of *E coli* into the antibiotic sensitive one. Further the bacteriocins encoding genes can be cloned in the bacteria and can be used as the alternatives of the antibiotics, further, these bacteriocins can be used as the therapeutics in the field of medicines such as against the cancers. Bacteriocins can also be used in the animal feed for protecting them against the pathogens. Bioengineered bacteriocins can be made for the welfare of mankind and for the livestock as well. As the bacterial isolates from the tap water are exhibiting the antibiotic resistance, so there should be the proper disinfection of the water in the pipelines and further quality control surveys must be conducted.

REFERENCES

- [1] Silby, M. W., Winstanley, C., Godfrey, S. A., Levy, S. B., & Jackson, R. W. (2011). *Pseudomonas* genomes: diverse and adaptable. *FEMS microbiology reviews*, 35(4), 652-680. <https://doi.org/10.1111/j.1574-6976.2011.00269.x>
- [2] Ramos, J. L., Goldberg, J. B., & Filloux, A. (Eds.). (2014). *Pseudomonas: Volume 7: New Aspects of Pseudomonas Biology*. Springer. <https://doi.org/10.1007/978-94-017-9555-5>
- [3] Ghequire, M. G., & De Mot, R. (2014). Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas*. *FEMS microbiology reviews*, 38(4), 523-568. <https://doi.org/10.1099/mic.0.055624-0>
- [4] Barreteau, H., Tiouajni, M., Graille, M., Josseume, N., Bouhss, A., Patin, D., ... & Touzé, T. (2012). Functional and structural characterization of Paem, a colicin M-like bacteriocin produced by *Pseudomonas aeruginosa*. *Journal of Biological Chemistry*, 287(44), 37395-37405. <https://doi.org/10.1074/jbc.M112.406439>
- [5] Azevedo, P. O. D. S. D., Molinari, F., & Oliveira, R. P. D. S. (2018). Importance of the agar-media in the evaluation of bacteriocin activity against the same test-microorganisms. *Brazilian Journal of Pharmaceutical Sciences*, 54(1). <https://doi.org/10.1590/s2175-97902018000117533>
- [6] Wannun, P., Piwat, S., & Teanpaisan, R. (2014). Purification and characterization of bacteriocin produced by oral *Lactobacillus paracasei* SD1. *Anaerobe*, 27, 17-21. <https://doi.org/10.1016/j.anaerobe.2014.03.001>
- [7] Dimov, S., Ivanova, P., & Harizanova, N. (2005). Genetics of bacteriocins biosynthesis by lactic acid bacteria. *Biotechnology & Biotechnological Equipment*, 19(sup2), 4-10. <https://doi.org/10.1080/13102818.2005.10817270>
- [8] Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins—a viable alternative to antibiotics?. *Nature Reviews Microbiology*, 11(2), 95-105. <https://doi.org/10.1038/nrmicro2937>
- [9] Milioni, C., Martínez, B., Degl'Innocenti, S., Turchi, B., Fratini, F., Cerri, D., & Fischetti, R. (2015). A novel bacteriocin produced by *Lactobacillus plantarum* LpU4 as a valuable candidate for biopreservation in artisanal raw milk cheese. *Dairy science & technology*, 95(4), 479-494. <https://doi.org/10.1007/s13594-015-0230-9>
- [10] Osuntokun, O. T., Mayowa, A., Thonda, O. A., & Aladejana, O. M. (2019). Pre/Post Plasmid Curing and Killing Kinetic Reactivity of *Discorea Bulbifera* Linn Against Multiple Antibiotics Resistant Clinical Isolates, Using *Escherichia Coli* as A Case Study. *Int J cell Sci & mol biol*, 6, 555685. <http://dx.doi.org/10.19080/IJCSMB.2019.06.555685>
- [11] Cascales E, Buchanan SK, Duché D, Kleanthous C, Llobès R, Postle K, Riley M, Slatin S, Cavard D *Microbiol Mol Biol Rev*. 2007 Mar; 71(1):158-229. <http://dx.doi.org/10.1590/s2175-97902018000117533>
- [12] Al-daan, W. R. T., Ali, S. A. A. R., & kadhim Al-Saffar, A. (2017). Effect of Piper cubeba Fruits Extract on Bacteriocin Production of *E. coli* Isolated from Patient with Urinary Tract Infection. *Biomedical & Pharmacology Journal*, 10(1), 111. <https://dx.doi.org/10.13005/bpj/1088>
- [13] Ghequire, M. G., Kemland, L., Anoz-Carbonell, E., Buchanan, S. K., & De Mot, R. (2017). A natural chimeric *Pseudomonas* bacteriocin with novel pore-forming activity parasitizes the ferrichrome transporter. *MBio*, 8(1). <https://doi.org/10.1128/mBio.01961-16>
- [14] Sudha, S. S., & Aranganathan, V. (2021). Experimental elucidation of an antimycobacterial bacteriocin produced by ethnomedicinal plant-derived *Bacillus subtilis* (MK733983). *Archives of microbiology*, 1-12. <https://doi.org/10.1007/s00203-020-02173-7>
- [15] Zhang, C. M., Xu, L. M., Wang, X. C., Zhuang, K., & Liu, Q. Q. (2017). Effects of ultraviolet disinfection on antibiotic-resistant *Escherichia coli* from wastewater: inactivation, antibiotic resistance profiles and antibiotic resistance genes. *Journal of applied microbiology*, 123(1), 295-306. <https://doi.org/10.1111/jam.13480>
- [16] Said, M. B., Masahiro, O., & Hassen, A. (2010). Detection of viable but non cultivable *Escherichia coli* after UV irradiation using a lytic Q $\beta$  phage. *Annals of microbiology*, 60(1), 121-127. <https://doi.org/10.1007/s13213-010-0017-4>
- [17] James G. Cappuccino, N. S. (2014). *Microbiology a laboratory manual* (10th ed.). USA: PEARSON. [https://www.abebooks.com/products/isbn/9780321840226/30924411496&cm\\_sp=snippet-\\_-srp1-\\_-PLP1](https://www.abebooks.com/products/isbn/9780321840226/30924411496&cm_sp=snippet-_-srp1-_-PLP1)
- [18] Bergey, D. H., Buchanan, R. E., Gibbons, N. E., & American Society for Microbiology. (1974). *Bergey's manual of systematic bacteriology*. <https://doi.org/10.1007/0-387-28022-7>

- [19] Sambrook, Joseph. & Russell, David W. & Cold Spring Harbor Laboratory. (2001). *Molecular cloning : a laboratory manual*. Cold Spring Harbor, N.Y : Cold Spring Harbor Laboratory <https://nla.gov.au/nla.cavn2284148>
- [20] Azevedo, P. O. D. S. D., Molinari, F., & Oliveira, R. P. D. S. (2018). Importance of the agar-media in the evaluation of bacteriocin activity against the same test-microorganisms. *Brazilian Journal of Pharmaceutical Sciences*, 54(1).<https://doi.org/10.1590/s2175-97902018000117533>
- [21] Holder, I. A., & Boyce, S. T. (1994). Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns*, 20(5), 426-429.
- [22] Zaman, M. A., Pasha, M. H., & Akhter, M. Z. (2010). Plasmid curing of Escherichia coli cells with ethidium bromide, sodium dodecyl sulfate and acridine orange. *Bangladesh Journal of Microbiology*, 27(1), 28-31. <http://dx.doi.org/10.3329/bjm.v27i1.9165>
- [23] Ahmad, V., Khan, M. S., Jamal, Q. M. S., Alzohairy, M. A., Al Karaawi, M. A., & Siddiqui, M. U. (2017). Antimicrobial potential of bacteriocins: in therapy, agriculture and food preservation. *International Journal of Antimicrobial Agents*, 49(1), 1-11. <https://doi.org/10.1016/j.ijantimicag.2016.08.016>
- [24] Lakshmanan, R., Kalaimurugan, D., Sivasankar, P., Arokiyaraj, S., & Venkatesan, S. (2020). Identification and characterization of *Pseudomonas aeruginosa* derived bacteriocin for industrial applications. *International Journal of Biological Macromolecules*, 165, 2412-2418. <https://doi.org/10.1016/j.ijbiomac.2020.10.126>
- [25] Gordon, D. M., Oliver, E., & Littlefield-Wyer, J. (2007). The diversity of bacteriocins in Gram-negative bacteria. In *Bacteriocins* (pp. 5-18). Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-540-36604-1\\_2](https://doi.org/10.1007/978-3-540-36604-1_2)
- [26] Weiß, S., Kadlec, K., Feßler, A. T., & Schwarz, S. (2013). Identification and characterization of methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus pettenkoferi* from a small animal clinic. *Veterinary microbiology*, 167(3-4), 680-685. <https://doi.org/10.1016/j.vetmic.2013.07.036>
- [27] Ogunbanwo, S. T., Sanni, A. I., & Onilude, A. A. (2003). Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *African Journal of Biotechnology*, 2(8), 219-227. <https://doi.org/10.5897/AJB2003.000-1045>
- [28] Sharma, V. K., Johnson, N., Cizmas, L., McDonald, T. J., & Kim, H. (2016). A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. *Chemosphere*, 150, 702-714. <https://doi.org/10.1016/j.chemosphere.2015.12.084>
- [29] Samra, Z. Q., Naseem, M., Khan, S. J., Nadia, D. A. R., & Athar, M. A. (2009). PCR targeting of antibiotic resistant bacteria in public drinking water of Lahore metropolitan, Pakistan. *Biomedical and Environmental Sciences*, 22(6), 458-463. [https://doi.org/10.1016/S0895-3988\(10\)60002-5](https://doi.org/10.1016/S0895-3988(10)60002-5)
- [30] McKinney, C. W., & Pruden, A. (2012). Ultraviolet disinfection of antibiotic resistant bacteria and their antibiotic resistance genes in water and wastewater. *Environmental science & technology*, 46(24), 13393-13400. <https://doi.org/10.1021/es303652q>
- [31] Cazares, A., Hall, J. P., Wright, L. L., Grimes, M., Emond-Rhéault, J. G., Pongchaikul, P., ... & Winstanley, C. (2020). Characterisation of a new megaplasmid family associated with the spread of multidrug resistance in *Pseudomonas aeruginosa*. *Access Microbiology*, 2(7A), 646. <https://doi.org/10.1099/acmi.ac2020.po0545>
- [32] Spengler, G., Molnár, A., Schelz, Z., Amaral, L., Sharples, D., & Molnár, J. (2006). The mechanism of plasmid curing in bacteria. *Current drug targets*, 7(7), 823-841. <https://doi.org/10.2174/138945006777709601>.

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