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ANTIMICROBIAL ACTIVITY OF 'BACILLUS SPECIES' ISOLATES FROM AGRICULTURAL ENVIRONMENTS

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ABSTRACT

Antibiotic Resistant *Bacillus* species were isolated from the 3 different agricultural field areas i.e. field soil near industrial area (Musal gaon, MIDC, sinnar), Field soil near Construction area (Lokhande mala, nashik road, nashik), Field soil near hospital area (Sambhaji nagar, sinnar). The microscopically, biochemical test suggested it to be *Bacillus* species. Our major aim was to check the effect of isolated *Bacillus* species on the human by the hemolytic test, as it enters into the human life cycle by the crops eaten by human from agriculture fields.

Keywords: *Bacillus*, Antibiotic Drug Resistant, MDR, Hemolytic.

I. INTRODUCTION

Antibiotic use has been beneficial &, when prescribed & taken correctly, their value in patient care is enormous. Antibiotics are medicines that kill bacteria or slow the growth of bacteria. They are used to cure diseases. However, these drugs have been used so widely & for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making the drugs less effective. Many fungi, viruses, & parasites have done the same. Antibiotics were first produced in 1939. The term antibiotics were introduced by S.A Waksman in 1942. Antibiotic is very different from chemotherapeutic drugs, antibiotics are natural drugs that is produced by several fungi or bacteria but chemotherapeutics drugs are manmade substances. History of antibiotics began in 1932, (Nussbaum., et al, 2006) when the first drug sulfonamide was prepared. Sulfonamides are effective drugs. Sulfonamides have shown tremendous positive results on urinary tract infections, shigellosis & Pneumococcal pneumonia. The problems of infectious diseases suddenly increased when certain became antibiotic resistant. Some microorganisms may develop resistance to a single antimicrobial agent (or related class of agent), while others develop resistance to several antimicrobial agents or classes. These organisms are often referred to as multidrug-resistant or MDR strains. In some cases, the microorganisms have become so resistant that no available antibiotics are effective against them. Large amounts of antibiotics used for human therapy resulted in the selection of pathogenic bacteria resistant to multiple drugs. Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs. Due to the increase of resistance to antibiotics, there is a pressing need to develop new & innovative antimicrobial agents. Among the potential sources of new agents, plants have long been investigated. Because, they contain many bioactive compounds that can be of interest in therapeutic. Because of their low toxicity, there is a long tradition of using dietary plants in the treatment of infectious disease & to combat the MDRs successfully.

II. MATERIALS & METHODS

1. SOIL SAMPLE COLLECTION

1. The agricultural field soil sample was collected from 3 different locations from different field areas.
2. The first site corresponds to Field soil near industrial area (Musal gaon, MIDC, sinnar), Field soil near Construction area (Lokhande mala, nashik road, nashik), Field soil near farming area (Sambhaji nagar, sinnar) in Maharashtra, India.
3. From each site approx. 10 gram of soil sample were collected and placed in sterile plastic bags, then taken to the laboratory.
4. The debris from the soil sample were removed after collection (as in Figure no. 1).

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Figure 1: Soil Sample Collection

2. ISOLATION OF BACTERIA FROM SOIL SAMPLES

➢ Soil bacteria were isolated by the Standard Serial Dilution and Spread Plate method.

- **Requirement:-**

a. Soil sample
b. Saline solution
c. Sterile test-tubes
d. Sterile Nutrient Agar plates
e. Micropipette
f. L-shape glass rod
g. Ethanol

- **Protocol:-**

1. In which 1gm of each soil sample was weighed and mixed in 10 ml of Sterile Saline.
2. Then the 1ml from first tube is added to the next tube containing 9 ml Sterile Saline, in this way samples were then serially diluted 5 times. (as in figure no.2)
3. Out of the 6 dilutions, 100µl from the each dilution (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) of each sample were spreaded on sterilized Nutrient Agar Plates by sterile spreader under aseptic conditions.
4. This plates were incubated in incubator at 37^0C for 24 hrs. for bacterial colony. (as in figure no. 3)

Figure 2: Standard Serial Dilution

Figure 3: Spread Plate Method

3. PURE CULTURE MAINTAINANCE BY STREAK PLATE METHOD

- **Requirement:-**

a. Inoculating loop (wire loop)
b. Sterile nutrient agar plates / slants
c. Plates of serial dilutions

- **Protocol:-**

1. The inoculating loop was sterilized by putting it in flame till red hot.

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2. After cooling it down, the well isolated colonies were picked from the spread plates and were streaked on the Nutrient Agar plates.
3. Plated were incubated for 24hrs at 37^0C. Then stored at 4^0C for subsequent studies. (as in figure no.4)

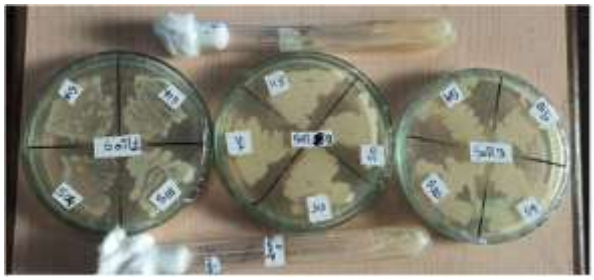

Figure 4: Pure Culture

4. MORPHOLOGICAL CHARACTERIZATION OF BACTERIA

➢ Morphological characterization of bacteria was performed by Gram's Staining.

- **Requirements:-**

a. Bacterial suspension
b. Wire-loop
c. Slides
d. Crystal violet
e. Decolorizer (ethanol)
f. Gram's iodine
g. Distilled water
h. Tap water
i. Burners

- **Protocol:-**

1. Bacterial suspension were made in sterile saline and were used.
2. Under aseptic conditions, loop full suspension was picked and smear was made on a clean grease free slide. Then they were air-dried and heat-fixed.
3. Stain the smear with Crystal Violet Stain for 1 minute & wash the slide in gentle and indirect stream of tap water for 2 sec.
4. Flood the smear with Gram's Iodine and wait for 1 minute & wash the slide in gentle and indirect stream of tap water for 2 sec.
5. Add drop by drop Gram's Decolorizing agent to smear till slide runs clear.
6. Flood slide with counterstain, Safranin and wait for 30 seconds & Wash the slide in gentle and indirect stream of tap water until no color appears and then blot dry with absorbent paper.
7. Smear was covered with Oil Immersion and observed under microscope.

(As in figure no. 5)

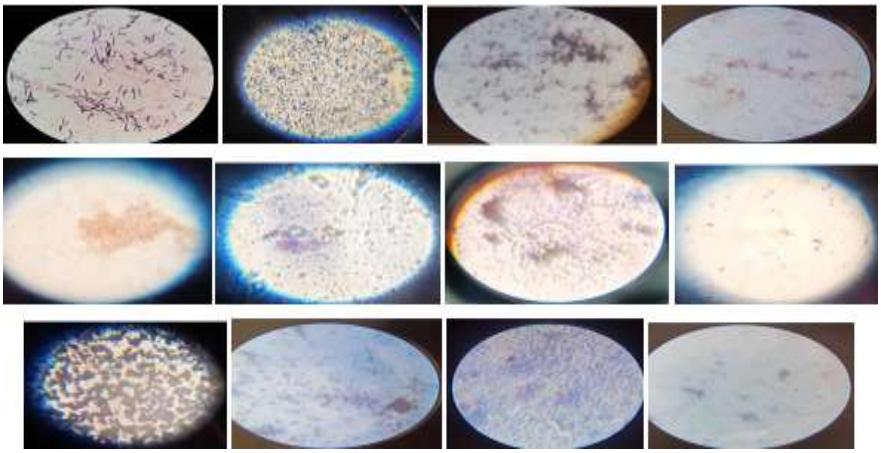

Figure 5: Gram's staining

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5. BIOCHEMICAL CHARACTERIZATION OF BACTEIA

5.1. CATALASE TEST

- **Requirement:-**

a. Wire loop
b. Ethanol
c. Slides
d. 3% H_2O_2 (Hydrogen Peroxide)
e. Pure bacterial suspension

- **Protocol:-**

1. Taken a loop full of bacterial suspension and make smear on an ethanol cleaned grease free slide.
2. Then add 1 drop of 3% H_2O_2 (Hydrogen Peroxide) (do not mix or cover the slide with cover slip) and place slide in Petri plate and observe instantly.

(results in figure no. 6)

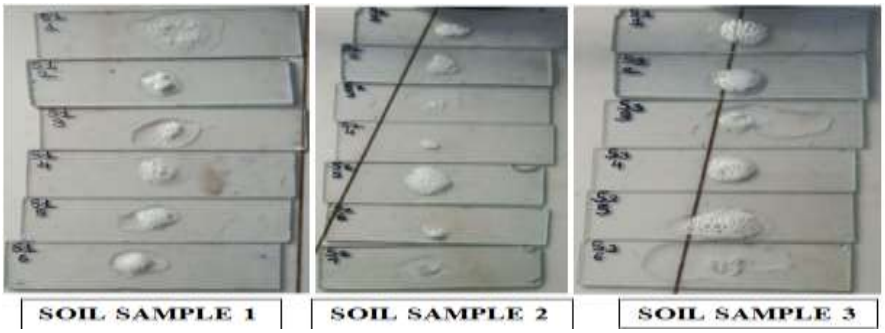

Figure 6: Catalase Test

5.2. GELATIN HYDROLYSIS TEST

- **Requirement:-**

a. Gelatin Hydrolysis media
Gelatin……120 g/L
Peptone……5 g/L
Beef extract……3 g/L
pH……6.8
b. Sterile test-tubes
c. Wire-loop
d. Pure bacterial suspension

- **Protocol:-**

1. Pick-up the colony with wire-loop from the pure culture and inoculate it by stabbing 4-5 times, half inch into Gelatin Hydrolysis Media.
2. Incubate tubes in incubator for 48 hrs. at 37^0C.
3. Remove tube from incubator and place in ice bath or refrigerator for 30 minutes or until control tube solidify.

(Results in figure no. 7)

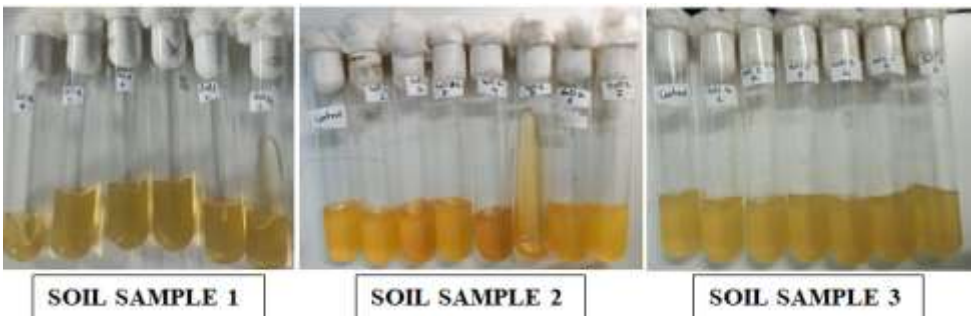

Figure 7: Gelatin Hydrolysis Test

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5.3. INDOLE TEST

- **Requirements:-**

a. Tryptone Broth
Tryptone……10 g/L
$NaCl_2$……5 g/L
b. Kovac's Reagent
Amyl Alcohol ……150 ml
DMAB (p-dimethylaminobenzyldehyde)…..10g
HCl……50 ml
c. Sterile test-tubes
d. Dropper
e. Wire loop
f. Pure Bacterial culture

- **Protocol:-**

1. Take 2ml Tryptone Broth and inoculate it with pure bacterial culture.
2. Incubate tubes in incubator at 35^0C for 24-48 hrs.
3. Add 5 drops of Kovac's Reagent to tubes.
(Results in figure no. 8)

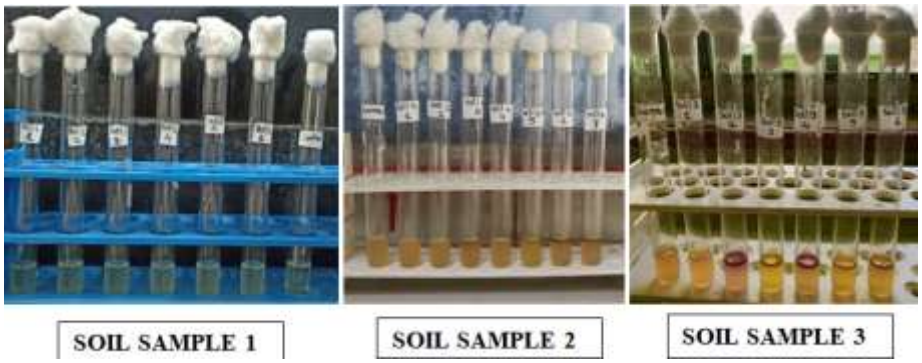

Figure 8: Indole Test

5.4. METHYL RED TEST

- **Requirement:-**

a. MRVP Broth
Buffered peptone……7 g/L
Glucose……5 g/L
Dipotassium phosphate……5 g/L
pH……6.9
b. Methyl Red Solution (0.02%)
Methyl red……0.1 g
Ethyl Alcohol……300 ml
Distilled Water……..makeup volume to 500 ml
c. Wire loop
d. Pure bacterial culture
e. Sterile test-tubes
f. dropper

- **Protocol:-**

1. Take 2 ml MRVP Broth in tubes and inoculate it with pure bacterial culture.
2. Incubate the tubes in incubator at 37^0C for 24 hrs.
3. Add 2-3 drops of Methyl red Indicator to tubes and observe it immediately.

(Results in figure no. 9)

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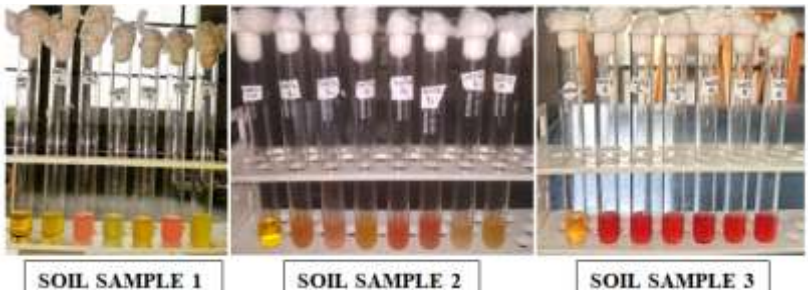

Figure 9: Methyl Red Test

5.5. MOTILITY TEST

- **Requirement:-**

a. SIM (Sulphur, Indole, Motility) Media
Pancreatic Digest of Casein…….20 g/L
Peptic Digest of Animal Tissue…..6.1 g/L
Agar……3.5 g/L
$Fe(NH_4)_2(SO_4)_2.6H_2O$ …… …0.2 g/L
$Na_2S_2O_3.H_2O$…… … …..0.2 g/L
Ph……7.3
b. Wire-loop
c. Pure bacterial culture
d. Sterile test-tubes

- **Protocol:-**

1. Take pure bacterial culture with wire loop and stab the colony in SIM Media to 1/3 inch in middle of tube and remove.
2. Incubate the tubes in incubator for 7 days at 35-37^{0}C.
(results in figure no. 10)

Figure 10: Motility Test

5.6. CITRATE UTILIZATION TEST

- **Requirement:-**

a. Simmons's Citrate Agar
$NaCl_2$……5 g
Sodium Citrate (dehydrate)……2 g
Ammonium dihydrogen phosphate…….1 g
Dipotasium phosphate……1 g
Magnesium Sulphate (heptahydrate)…0.2 g
Bromothymol Blue……..0.08 g
Agar……15 g
Deionized water……1000 ml
Ph……6.9
b. Pure bacterial culture
c. Sterile test-tubes
d. Wire-loop

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- **Protocol:-**

1. Make the Simmons's Citrate Agar slants.
2. Pickup pure bacterial culture with wire loop and streak from center.
3. Incubate the tubes in incubator for 4-7 days at 35-37^{0}C.

(Results in figure no. 11)

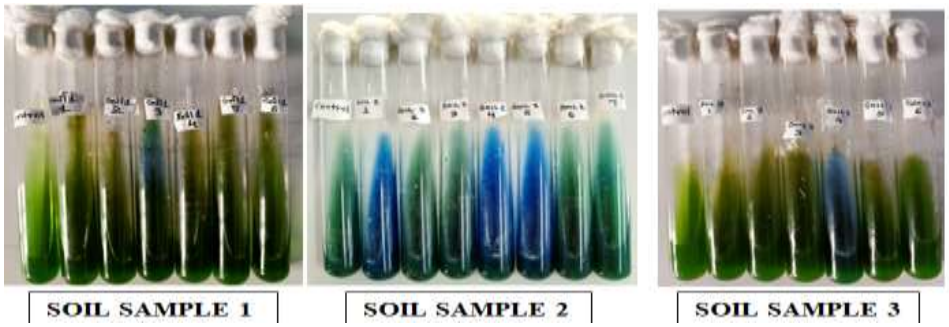

Figure 11: Citrate Utilization Test

5.7. VOGES-PROSKAUER TEST

- **Requirement:-**

a. MRVP Broth
Buffered peptone……..7 g/L
Glucose……5 g/L
Dipotassium phosphate……5 g/L
pH……6.9
b. Voges-Proskauer Reagent A
α-napthol 5%.....50 g
Absolute Ethanol……1000 ml
c. Voges-Proskauer Reagent B
Potassium hydroxide……400 g
Deionized Water……1000 ml
d. Pure bacterial culture
e. Sterile test-tubes
f. Wire-loop

- **Protocol:-**

1. Take 2 ml of MRVP Broth in test-tubes and inoculate tubes with pure bacterial culture. Incubate tubes in incubator at 37^{0}C for 24 hrs.
2. Add 6 drops of Voges-Proskauer Reagent A in test-tubes and mix well to aerate & Add 2 drops of Voges-Proskauer Reagent B in test-tubes and mix well to aerate.
3. Observe for pink-red color at surface within 30 minutes.
4. Shake the tubes vigorously during 30 min period.

(Results in figure no. 12)

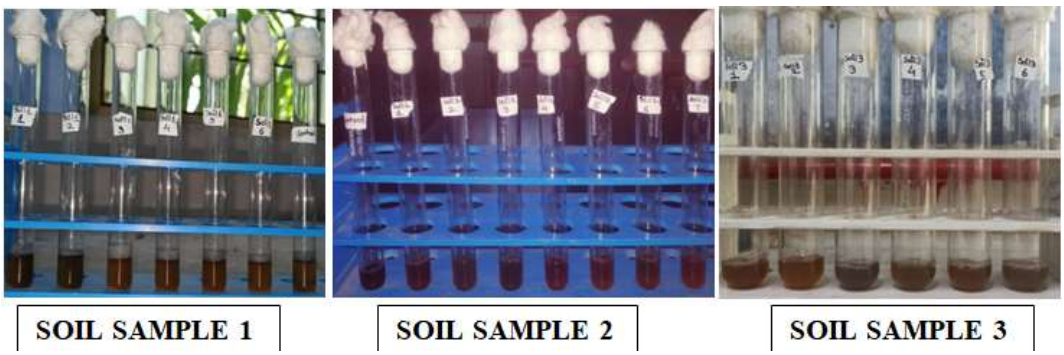

Figure 12: Voges-Proskauer Test

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➢ **All biochemical results in table no. 1, table no. 2 & table no. 3**

Table 1: Soil Sample 1

Sr. no.	Gram's staining	Catalase Test	Gelatin Hydrolysis Test	Indole Test	Methyl Red Test	Motility Test	Citrate Utilization Test	Voges-Proskauer Test
1	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
2	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
3	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve
4	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
5	+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve
6	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
Control	------	------	-ve	-ve	-ve	-ve	-ve	-ve

Table 2: Soil Sample 2

Sr. no.	Gram's staining	Catalase Test	Gelatin Hydrolysis Test	Indole Test	Methyl Red Test	Motility Test	Citrate Utilization Test	Voges-Proskauer Test
1	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
2	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
3	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
4	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
5	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve
7	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Control	------	------	-ve	-ve	-ve	-ve	-ve	-ve

Table 3: Soil Sample 3

Sr. no.	Gram's staining	Catalase Test	Gelatin Hydrolysis Test	Indole Test	Methyl Red Test	Motility Test	Citrate Utilization Test	Voges-Proskauer Test
1	+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve
2	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
3	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
4	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve
5	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
6	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve
Control	------	------	-ve	-ve	-ve	-ve	-ve	-ve

6. ANTIMICROBIAL RESISTANCE, SUSCEPTIBILITY PROFILING OF SELECTED *Bacillus* SPECIES USING ANTIBIOTICS DISCS

➢ **Concentrations Of Antibiotic Discs**

a. Ampicillin (10µg)
b. Penicillin (10µg)
c. Chloramphenicol (30µg)

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d. Levofloxacin (5µg)
e. Streptomycin (10µg)
f. Amoxicillin (30µg)
g. Doxycycline (30µg)
h. Ciprofloxacin (15µg)
i. Clindamycin (2µg)

➢ **Preparation Of Antibiotic Discs**

- **Requirement:-**

a. Whatman paper no. 1
b. Punching machine
c. Antibiotics solutions
d. Forceps
e. Petri plate
f. Burners
g. Sterile dropper
h. Hot plate

- **Protocol:-**

1. Make discs of Whatman filter paper number 1 with the punching machine and autoclave it.
2. Make the solution of antibiotics as per the concentration, which are purchased from medicals.
3. With the help of sterile forceps place a single discs on a Petri plate.
4. Add the antibiotic solutions dropwise on the discs.
5. Keep the petri-plate containing disc on hotplate.
6. After drying, the discs are ready for further use.

(Results in figure no. 13)

Figure 13: Antibiotics Discs Preparation

Kirby-Bauer Disc Diffusion Method

- **Requirement:-**

a. Sterile Muller Hinton Agar plates
b. Pure bacterial culture suspensions of selected *Bacillus* species
c. L-shape glass rod
d. Antibiotic discs
e. Forceps
f. Scale
g. HiMedia resistance chart
h. Sterile nutrient agar plates
i. Wire loop
j. Ethanol

- **Protocol:-**

1. Pure bacterial culture suspensions were made and spreaded on the MHA Plates.

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2. Antibiotics discs of the above mentioned concentrations were placed carefully on spreaded bacterial culture MHA plates and left for diffusion for some time.
3. Then plates were incubated at 37^0C for 24 hrs. in incubator.
4. Later the diameter of zone of inhibition was measured using ruler and noted & compared with the resistance chart of HiMedia.
5. The colonies which resist to more than 2 antibiotics are collected and stated as Multi-Drug resistance.
6. Selected colonies suspension were spreaded on the nutrient agar plates and incubated at 37^0C for 24 hrs. in incubator, for the pure culture and further studies.

(Results in figure no. 14 & table no.4)

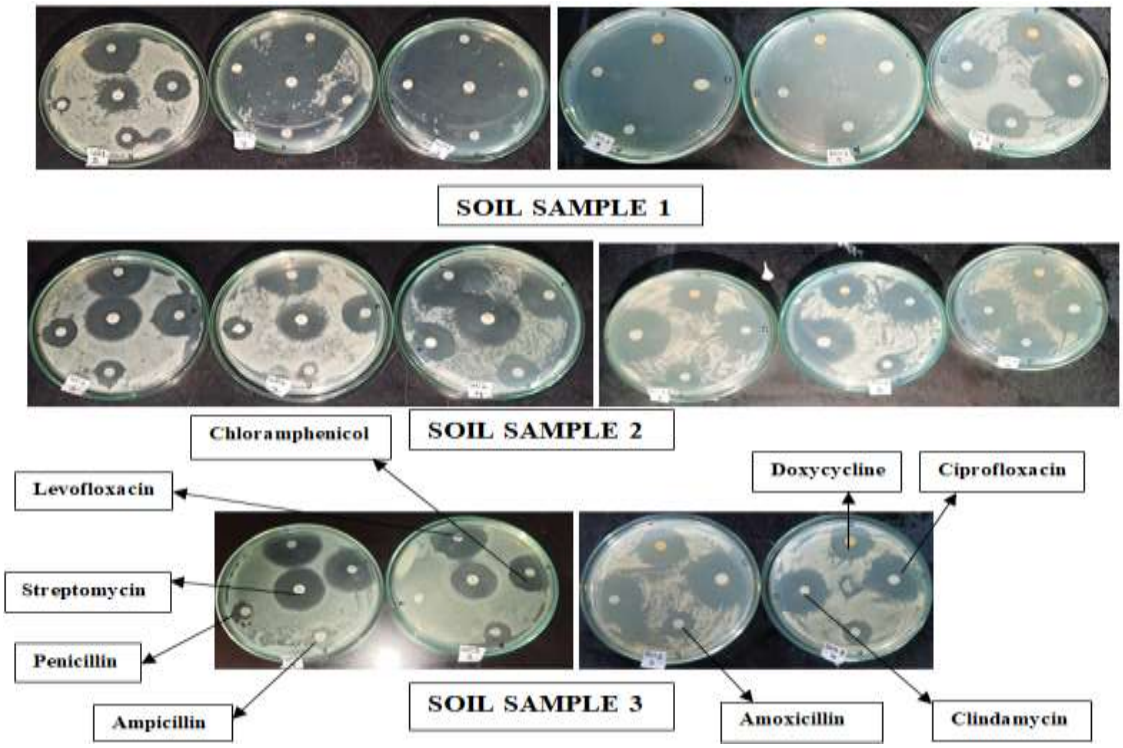

Figure 14: Disc Diffusion Method

Table 4: Multi-Drug Resistance

Sr. no.	Antibiotics	Resistance diameter	S 1(3) mm	S 1(4) mm	S 1(5) mm	S 2(1) mm	S 2(2) mm	S2(4) mm	S 3(3) mm	S 3(4) mm
1	Ampicillin (10µg)	≤13	-	30	12-R	14-R	8-R	22	16	10-R
2	Penicillin (10µg)	≤19	-	34	10-R	16	12-R	26	0-R	6-R
3	Chloramphenicol (30µg)	≤12	-	24	20	28	26	28	24	26
4	Levofloxacin (5µg)	≤15	-	44	34	36	32	40	32	32
5	Amoxicillin (30µg)	≤30	-	24	13-R	13-R	12-R	24	10-R	12-R
6	Ciprofloxacin (15µg)	≤45	-	42	42	28-R	24-R	36-R	36-R	28-R
7	Doxycycline (30µg)	≤12	-	24	26	26	22	24	24	22
8	Clindamycin (2µg)	≤14	-	34	24	36	32	30	32	26
9	Streptomycin (10µg)	≤11	-	30	20	32	26	32	26	30
MDR OR NOT			No	No	Yes	No	Yes	No	No	Yes

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7. CONFIRMATIONAL TEST OF *Bacillus* SPECIES BY GROWTH ON SOYABEAN CASEIN DIGEST AGAR OR TRYPTIC SOY AGAR

➢ **The Soybean Casein Digest Agar also known as Tryptic Soy Agar is a selective media for the *Bacillus* species.**

- **Requirement:-**

a. Sterile Soybean Casein Digest Agar plates
b. L-shape glass rod
c. Ethanol
d. Pure bacterial suspension of selected MDR species

- **Protocol:-**

1. Soybean Casein Digest Agar plates were prepared.
2. 100µl of selected MDR Pure bacterial culture suspension were spreaded on plates with L-shape glass rod.
3. Plates are incubated in incubator at 37^{0}C for 24 hrs.
4. If growth of bacteria is seen, then *Bacillus* species are confirmed.

(Results in figure no. 15)

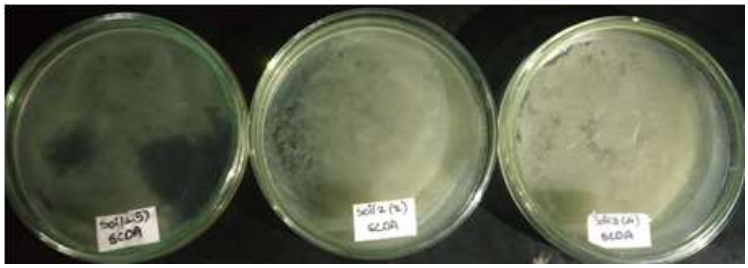

Figure 15: Confirmation of *Bacillus* species by growth on soyabean casein digest agar

8. PLASMID ISOLATION AND TO CHECK PURITY OF GENOMIC DNA OF MDR *Bacillus* SPECIES

➢ **Plasmid Isolation Of MDR *Bacillus* SPECIES**

- **Requirements:-**

a. Selected MDR *Bacillus* species suspension
b. LB (Luria- Bertani) Broth
c. TAE Buffer
d. Micro-centrifuge tubes
e. 100% Ethanol
f. 70% Ethanol
g. 1X TE (TRIS , EDTA) (ph-2.8)
h. RNase
i. Alkaline lysis solution 1

50mM Glucose……100µl
25mM Tris-Cl (ph-8)…….50µl
10mM EDTA (ph-8)……20µl
Sterile double distilled water……..final volume to 2ml

j. Alkaline lysis solution 2

0.2N NaOH……40µl
1% SDS……200µl
Sterile double distilled water……..final volume to 2ml

k. Alkaline lysis solution 3

5M Potassium acetate…….1.2ml
Glacial acetic acid……230µl
Sterile double distilled water……..final volume to 2ml

(Results in figure no. 16)

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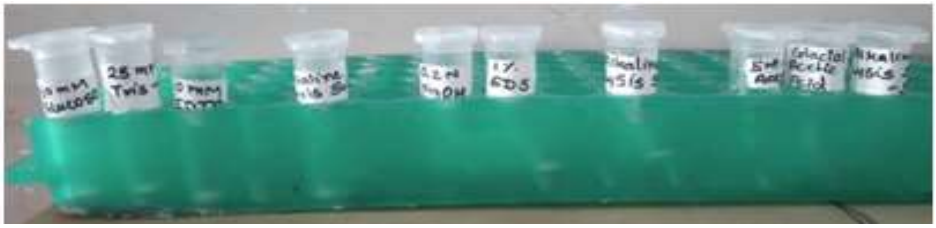

Figure 16: Alkaline Lysis Solutions

- **Protocol:-**

1. Inoculate single bacterial colony of all selected MDR species in 5ml LB Broth & Incubate the culture overnight at 37^0C by vigorous shaking.
2. Pipette 1.5ml of each culture into micro-centrifuge tubes & centrifuge at 8000rpm at 4^0C for 10 mins.
3. Discard supernatant and invert the tubes on blotting paper to drain out leftover supernatant. Place on ice.
4. Resuspend the pellet in 100µl of ice cold alkaline lysis solution 1 by vigorous vertexing. Place on ice for 5 mins and shift to room temperature.
5. Add 200µl of freshly prepared alkaline lysis solution 2 to each bacterial suspension at room temperature. Close the tubes and mix content by inverting the tubes rapidly by 5 times. Do not vortex. Store tubes on ice for 5-10 mins.
6. Add 150µl of ice cold alkaline lysis solution 3. Close the tubes and mix gently by inverting the tube several times. Store tubes on ice for 3-5 mins.
7. Centrifuge tubes at 8000rpm at 4^0C for 10 mins. & Transfer supernatant immediately to another fresh micro-centrifuge tubes.
8. Add 450µl of 100% ethanol to precipitate the DNA. Mix by inverting the tubes. Incubate at room temperature for 10-15 mins.
9. Centrifuge at 6000rpm for 30 mins at 4^0C & Discard supernatant and invert the tubes on blotting paper to drain out leftover supernatant.
10. Add 1ml of 70% ethanol to pellet. Close the tubes and mix the tubes by inverting several times.
11. Recover the DNA by centrifugation at maximum speed for 2min at 4^0C & remove supernatant by aspiration.
12. Dry the sample by storing open tubes at 37^0C for 10-15 min till there is no traces of ethanol or until ethanol has evaporated.
13. Resuspend the pellet (DNA) in 50µl of TE + 20µg/ml DNase free RNase & mix it by tapping the tubes so that DNA goes into the solution.
14. Store DNA solution at -20^0C.

(Results in figure no. 17)

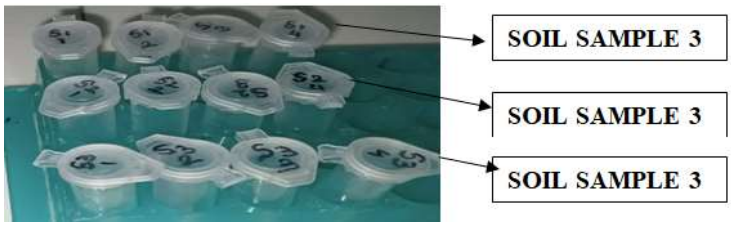

Figure 17: Plasmid Isolation

- **Preparation of 1% agarose gel**
- **Requirement:-**

a. Agarose
b. Ethidium Bromide
c. 1X TAE Buffer
d. Gel box
e. Well comb
f. Beaker
g. Pipette

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- **Protocol:-**

1. Take 100 ml of 1X TAE Buffer in beaker add 1gm agarose powder.
2. Heat for 1-3 min until the agarose is completely dissolved (do not over boil the solution).
3. Let agarose solution cool down to about 150^{0}C (about when you can comfortably keep your hand on beaker) about 5 min.
4. Add Ethidium bromide (EtBr) to solution about 2-3µl.
5. Pour the agarose into a gel tray with the well comb in place.
6. Let sit the gel at room temperature for 20-30min, until I has completely solidified.
7. Remove the combs. Gel is ready to check purity of genomic DNA.

(Results in figure no. 18)

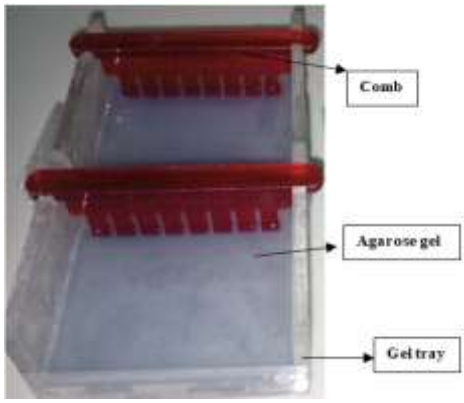

Figure 18: Gel preparation

➢ **To check the purity of genomic DNA**

- **Requirement:-**

a. Agarose gel with wells
b. DNA solution isolated from MDR *Bacillus* species
c. Loading dye
d. Micropipette
e. 1X TAE Buffer
f. Casting tray
g. Voltage source
h. Ice pack
i. UV Trasilluminator

- **Protocol:-**

1. Fill the casting tray with 1X TAE buffer.
2. Place the gel in such a position that it is near to negative electrode.
3. Load the well with 6µl loading dye and 12µl isolated DNA sample.

(As in figure no. 19)

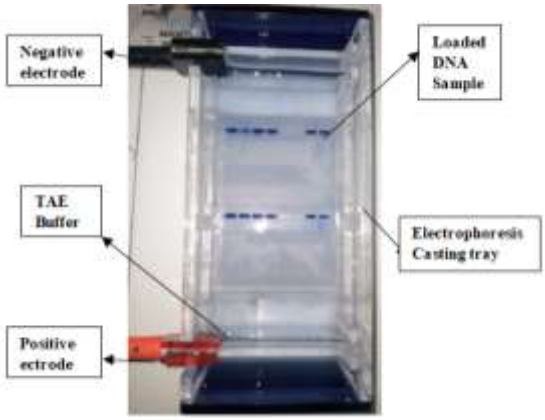

Figure 19: Purity Checkup of Genomic DNA

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4. Cover the electrophoresis chamber with ice pads.
5. Run the electrophoresis at 100V for 1-2 hrs. , till it runs 3/4th.
6. Remove the gel and observe the bands under the UV light.

(As in figure no. 20)

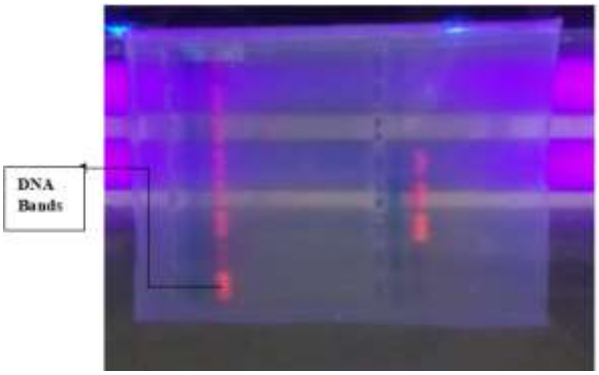

Figure 20: Purity of Plasmid DNA Under UV Light

9. HEMOLYTIC ANALYSIS

➢ **Hemolysis test to detect that , does the MDR *Bacillus* species rupture the RBC's**

- **Requirement:-**

a. Human blood sample
b. Sterile saline solution
c. Selected MDR *Bacillus* species culture
d. Syringe
e. Sterile test-tubes
f. Sterile pipettes

- **Protocol:-**

1. Take 3ml sterile saline in sterile test-tubes.
2. Inoculate it with bacterial culture.
3. Take one tube as control, in which no bacterial culture is present.
4. Add 0.5ml blood sample to each tube, leaving control tube.
5. Incubate the tubes at 35^0C for 24 hrs. (As in figure no. 21)

Figure 21: Hemolysis Test

III. CONCLUSION

The data derived from this study indicate that the soil sample may serve as numerous *Bacillus* species. These bacteria are considered as one of the most contaminants and infectious for human health. We hereby conclude that soil sample sever as source of *Bacillus* species resistant to multi-antibiotics. The antibiotic strains were identified as Ampicillin-resistant strains; Penicillin-resistant strains; ciprofloxacin-resistant strains and Amoxicillin-resistant strains. The result of this study highlighted the need to study antibiotic resistant data, as it is limited. The early detection and close monitoring of MDR, XDR, or even PDR bacterial strains must be started by all clinical microbiology laboratories to reduce the menace of antimicrobial resistance which is now a global problem. Gram negative bacteria cause plague, cholera and typhoid fever.

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