



Phenotypic Characterization of Antimicrobial Resistance in Kerala *Bacillus Spp* Isolated from Paddy Field

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Abstract: Rice paddy environment is an exceptional reservoir for different microorganisms (Pathogenic and Non-Pathogenic). Fertilizers, Fungicides, Antibiotics and Insecticides were used for boosting rice paddy growth and preventing agronomy diseases. Enormous use of fertilizers, Fungicides, Antibiotics and Insecticides will develop drug resistance in the microorganisms present in the crop [figure-1]. Ninety dried rice paddy samples were collected from different places around regions of Kerala. Forty rod-shaped gram-stain-Positive endospore forming obligate aerobic bacteria were isolated from the samples. All the forty strains were subjected to for antibiotic susceptibility by using the standard antibiotic disc (Cefoxitin). Significant drug resistance and massive growth observed in the strain (SCOP-2) among other isolated bacteria. SCOP-2 was subjected for sensitivity to differ different Antimicrobial disc by using Kirby-Bauer disc diffusion method. The results show that SCOP-2 drug resistant against all the five Antibiotics. The Minimum Inhibitory Concentration (MIC) was determined by two fold serial dilution against Ciprofloxacin (CIP) and Linezolid (LNZ). From the results it is concluded that CIP and LNZ shows resistance against SCOP-2.

Keywords: Drug Resistant, Cefoxitin, Herbivorous, Agrochemicals

1. Introduction

Antibiotic resistance Bacteria have been reported due to the irrational use of Fertilizers, Fungicides, Antibiotics and Insecticides. Irrational use of biocides disturbs the environment and harm to public human health. Antibacterial resistance is largely developed by exposure to the commercial formulation rather than the active ingredient. Poor people, especially from developing countries who are exposed to unhygienic conditions in their daily activities, are the worst sufferers of infectious diseases. Treatment of infections caused by multidrug-resistant (MDR) Enterobacteriaceae represents a continuous challenge. Pathogens are frequently resistant to extended-spectrum

cepha-losporins owing to the production of extended-spectrum b-lactamases (ESBLs) and/or plasmid-mediated AmpC b-lactamases (pAmpCs) [6, 9, 11]. Their sufferings have been increased many-fold due to prolonged illness caused by widespread drug resistant pathogens. Antimicrobial resistance testing can be used for Discovery of new Antimicrobial agents, Epidemiology and prediction of therapeutic compounds. After the revolution in the “golden era”, when almost all groups of important antibiotics (Tetracyclines, Cephalosporins, Aminoglycosides and Macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s [5]

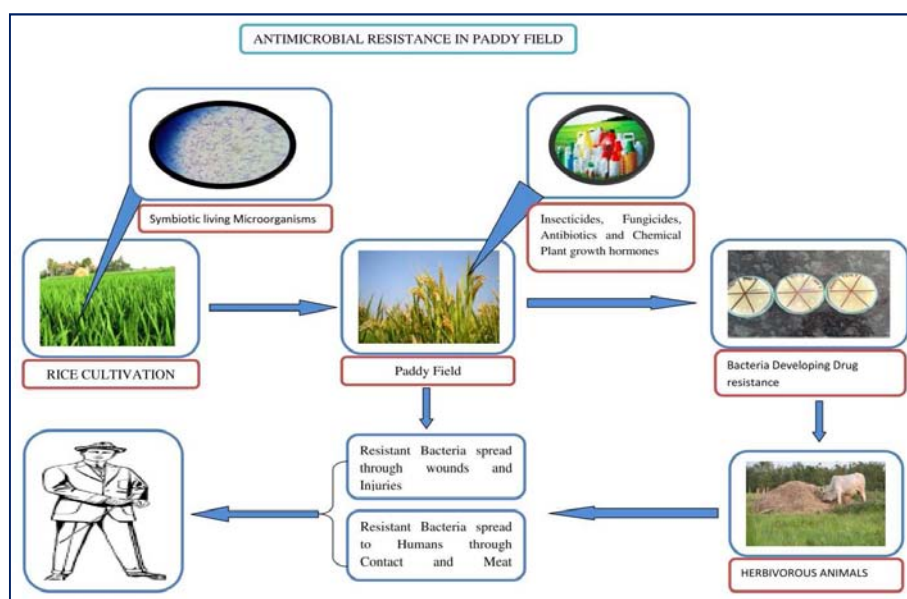


Figure 1. Development of Antimicrobial resistance.

2. Material and Methods

2.1. Collection of Paddy Samples

Ninety dried paddy samples are collected from different places from Kerala. In this 70% sampling were collected from stored paddy straws and 30% were collected from paddy field.

2.2. Enrichment

The sample was pasteurized for 1 h at 80°C and put for enrichment into nutrient broth Medium, incubated under aerobic conditions at room temperature for one week. After one week the enrichment was plated on Nutrient agar slants through crowded plating technique and incubated under aerobic conditions.

2.3. Study Drug Resistance by Disc Diffusion Method [2, 3, 8, 9]

All the isolates of microorganisms were screened with Cefoxitin by disc diffusion method according to NCCLS guidelines. The break point of less than ≤ 21 mm for Cefoxitin was indicative of resistance. All isolates of were showing Drug Resistant. [Figure 2]

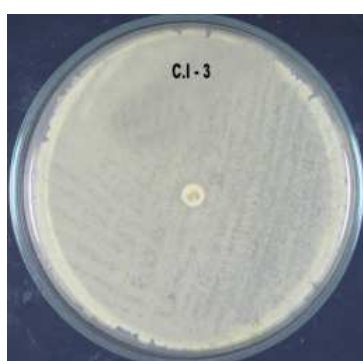


Figure 2. Study drug resistance by Cefoxitin disc diffusion method.

2.4. Culture Purification (Pour Plate Method)

Mixed enrichment bacterial culture is diluted in the test tubes containing melted agar in the liquid state at a temperature of 42-45°C (Agar solidification will occur below 42°C). The bacteria and the melted agar medium are mixed properly. Each test tube is poured into separate Petri plate, allowed to solidify aseptically, and then incubated inverted position at incubator (37°C). After 24 hrs, bacterial colonies were observed on the surface of the agar medium (surface colonies) and inside the agar medium (subsurface colonies). Bacterial colonies are picked up by inoculation loop and streaked into another petri plate to ensure the purity of bacterial colonies.

2.5. Inoculum

24 hrs old pure culture (Inoculum) of test organisms as mentioned above were used to make lawn over MHA plates. Antibiotic Discs were placed onto lawn and kept in refrigerator for 30 mins for dispersion of test solution. After 30 mins plates were incubated in inverted position for 24 -48 hrs at 35-37°C.

2.6. Antimicrobial Agents

Standard laboratory antibiotic discs of Cefoxitin-Cloxacillin (30-200) mcg (CXX), Amoxicillin/Clavulanic Acid, Augmentin, AMC-30, Penicillin-G 10mcg, Cefixime CFM-5mcg, Oxacillin OXL-1mcg, used in this study.

2.7. MIC Determination

The MIC of each antibiotic was determined by broth macrodilution using sterile MIC test tubes containing double strength Mueller Hinton broth. The inoculum contained 56105 c.f.u. ml⁻¹. The concentration ranges tested were: 0.3125 – 40 µg/ml LIZ ml⁻¹, 0.3125 - 40 µg/ml CIP ml⁻¹. Antimicrobial solutions were freshly prepared and diluted on

the day of testing. Each test was performed in duplicate.

3. Result

3.1. Morphology Characters

Size: Medium to big, Form: Round, Margin: Entire, Elevation: Convex, Texture: Smooth, Glistening, Pigmentation: Cream, Opacity: Opaque (Figure 3)



Figure 3. Morphology characters.

3.2. Characterization of Culturable Bacteria

Morphological characterization studied based on classical macroscopic techniques of shape, Size and elevation of pure colonies. Colonies were able to grow 1-2 days of incubation at room temperature. Cells do not disperse into medium even after mixing. Growth found to be very sticky when picked with a loop. The colony morphology of the isolates is Medium to big and endospore forming. They were smooth or rough and the colour ranged from white to cream and opaque. Microorganism studied by different biochemical test [Table 1]

Table 1. Biochemical Test.

Character	SCOP-2
Optimum (range) pH	7-8 (6-8)
Optimum NaCl (range) (%w/v)	0-1 (0-2)
Optimum (range) temperature (°C)	30-37(25-40)
H ₂ S production	-
Indole production from L-tryptophan	-
NO ₃ ⁻ reduction	+
MR Test	-
VP Test	-
Citrate utilization	-
Catalase test	-
Oxidase	-
Hydrolysis of	
Starch	+
Gelatin	+
Lipase	-
Casein	-
Phosphate solubilisation	-
Urease test	-

3.3. Screening of the Antimicrobial Resistance Using Cefoxitin (30µg) by Disc Diffusion Method [2, 3, 10]

All the isolates of microorganisms were screened with Cefoxitin by disc diffusion method according to NCCLS guidelines. The break point of less than ≤ 21 mm for Cefoxitin was indicative of resistance. All isolates of were showing Drug Resistant.

3.4. Antimicrobial Agents

Standard laboratory antibiotic discs of Cefoxitin-Cloxacillin (30-200) mcg (CXX), Amoxicillin/Clavulanic Acid, Augmentin, AMC-30, Penicillin-G 10mcg, Cefixime CFM-5mcg, Oxacillin OXL-1mcg, used in this study.

3.5. Study of Antimicrobial Resistance of the Paddy Field Isolates of *Bacillus sp* [3, 7, 8]

Antibiotic susceptibility testing was done on Mueller–Hinton (MH) agar using standard antibiotics by Kirby–Bauer disc diffusion method with CXX, AMC, PG, OXL and CFM. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute. The susceptible break points applied were ≤ 20 . Isolate of SCOP-2 resistant break point ≥ 14 . (Table-1), (Figure – 4)

Table 2. Study of Antimicrobial resistance by disc diffusion method.

Antibiotic Disc (mcg)	Zone of Inhibition (n=3)
Cefoxitin-Cloxacillin (CXX 30-200) mcg	R
Amoxicillin/Clavulanic Acid, Augmentin, (AMC-30 mcg)	R
Penicillin-G 10mcg	R
Cefixime CFM-5mcg	R
Oxacillin OXL-1mcg	R
R= Resistance	





Figure 4. Study of Antimicrobial resistance by disc diffusion method.

3.6. Determination of Minimum Inhibitory Concentration of Antibacterial Agents Against to SCOP-2

Table 3. Determination of Minimum inhibitory concentration of Antimicrobial agents against to SCOP – 2 (n=3).

S.NO	Marketed Antimicrobial agents	SCOP-2 (MIC values)
2	Ciprofloxacin µg/ml	20
3	Linezolid µg/ml	10
4	Conc. range used	0.3125-40

4. Conclusion

Antimicrobial resistance caused by inherited mutations. Mutations are associated with molecular mechanisms. Antimicrobial tolerance is the ability of a bacterial population to survive a transient exposure to antimicrobial agents, even at concentrations that far higher the MIC. Development of bacterial resistance decreases the effectiveness of the antimicrobial agents; that is, a higher concentration of the antimicrobial agents is required to produce the therapeutic effect in a resistant strain. Resistance is quantified by the Minimum Inhibitory Concentration (MIC). It can be defined as the minimum concentration of an antimicrobial agent that is required to prevent net growth of the microorganisms. In practice, the MIC is measured by exposing a bacterial population to increasing concentrations of the antimicrobial agents in a specified growth medium. Measurements of the MIC indicates the total insusceptibility to antimicrobial agents. Irrational, Combination use of pesticides and antibiotics in agriculture can develop Multiple-antibiotic resistance in pathogen microorganisms [1, 4, and 11]. The study of MIC involving CIP and LNZ against SCOP-2 was carried out. From the results of MIC Study it has been concluded that anti microbial resistance was observed in CIP and LNZ against SCOP-2. Development of multiple-antimicrobial resistance occurs on simultaneous exposure to antibiotics and is greater than the lethal effect of Biocides. To identify the exact cause of developing antibiotic resistance require wider our view of environmental conditions to the

estimation of Antibiotic resistance.

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