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Analysis of antibiotic resistance of bacteria in the soil of Sundarbans mangrove forest, Bangladesh

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Abstract

An increasing antimicrobial resistance has resulted in mortality from treatment failures and has increased health care costs. Therefore, the development of accurate and simple methods to determine the antibiotic susceptibility of micro-organisms is of increasing importance. The most widely used method at present is the paper-disk sensitivity test that provides flexibility and possible cost savings. Other methods, such as the agar well-diffusion, plate-dilution and ditch-diffusion, are very demanding and are not frequently used. In the present study an investigation was carried out to study the antibiotic resistant properties of some of the soil bacteria from studied zones in Sundarbans mangroves forest in Bangladesh which showed that all bacteria were highly sensitive to the antibiotic CIP-5. The results indicate that all species are wild type and thus, the environment is free from any contamination occurred by indiscriminate use of antibiotics.

Keywords: Mangrove Forest; Antibiotics; Resistance; Sensitivity; Bacteria; Environment.

1. Introduction

Antibiotic resistance is far reaching threat to global health. Usually antibiotic causes the bacterial lyses by damage and distortion to the cell membrane that cause discharge of essential cell materials and death (John s 2007; Sykes R. 2001; Aleksandrov A, Schuldt L, Hinrichs W 2009; Nilsson J, Rüetschi U, Halim A, Hesse C, Carlsohn E, Brinkmalm G 2009; Marquez B. 2005). Antibiotic resistance takes place when bacteria change its response to the medicines.

Human deaths and suffering from severe diseases dramatically declined over the last 50 years after the invention and use of antibiotics (Nwosu VC 2001). According to global antibiotic statistics about 35% antibiotic consumption increases from 2000 to 2010. Major contributing countries where antibiotic utilization estimated to increase by 76% are China, India and South Africa (Van Boeckel T.P., Gandra S., Ashok A., Caudron Q., Grenfell B.T., Levin S.A. 2014).

However, nowadays antimicrobial resistance is emerging in microbes which has severely endangered its utilization and has originated the spread of first-line drugs resistant organisms (Jacoby GA 1991; Carattoli A, Filetici E, Villa L, Dionisi AM, Ricci A 2002; Hidalgo-Grass C 2004; Zhou Z, Raskin L 2009; Sorlozano A, Gutierrez J, Martinez T, Yuste ME, Perez-Lopez JA, Vindel A, Guillen J 2010; Livermore DM, Canton R, Gniadkowski M, Nordmann P,

Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L 2006; Miguet L, Zervosen A, Gerards T, Pasha FA, Luxen A, Distèche-Nguyen M 2009).

According to world health organization (WHO), by 2050 antibiotic resistance might increase death from 700,000to 10 million per year (Brogan D.M. 2016). The antibiotic resistance can be a cause of death from minor injuries and common infections in the 21st century (Viens A.M. 2015).

However, majority of resistance determinants have been found in clinical microbes, whereas antibiotic resistance from other sectors such as in the environment are not clearly identified (Séveno NA, Kallifidas D, Smalla K, van Elsas JD and Karagouni AD 2002; Nwosu VC 2001).

Over the past few years, several researches have recorded many antimicrobial resistant bacteria in food and in the environment (Jensen LB, Baloda S, Boye M 2001). Use of antibiotic has been increasing steadily in agriculture and land (Mudryk ZJ 2004). Nevertheless, our knowledge about the amount of the antibiotic releasing to the surrounding after use is very little (Hirsch P, Ludwig W, Hethke C, Sittig M, Hoffmann B 1998). The indiscriminate use of antibiotics indicate the increase in emergence of resistant bacteria in those areas where antibiotics were widely used and also in aquatic environments (Schwartz T, Kohenen W, Jansen B 2003).

Previous research have shown that bacteria from different group can transmit antibiotic resistance to the environment (Kruse H 1994). Bacteria modify themselves to develop resistance against these antibiotics. It is unquestionable that the utilization of antibiotic causes critical pressure that brings antibiotic resistant bacteria. Some resistant bacteria are normally found in the environment (Gaston 1988; Reinsenfeld CS, Goodman, RM 2004). According to some studies soil bacteria carry more genetically diverse antibiotic resistance gene.

Most prior research has not paid attention to possible source of antibiotic resistance genes in uncultured microbes. Most of the bacteria are not promptly cultivated on standard laboratory media (Szuki MT, Rappe MS, Haimberger ZW, Winfield H, Adair N and SJ 1997; Giovannoni SJ, Britschgi TB, Moyer CL 1990; Amann RI, Ludwig W 1995; Ward DM, Weller R 1990; Hugenholtz P, Goebel BM 1998) and the range of the uncultured bacteria is huge (Béjà O, Suzuki MT, Heidelberg JF, Nelson WC, Preston CM 2002; Head IM, Saunders JR 1998; Torsvik V, Daae FL, Sandaa RA 1998; Whitman WB, Coleman DC 1998).

More research on soil bacteria may help to identify antibiotic resistant bacteria and to develop new antibiotics (Rang HP, Dale MM, Ritter JM 2007). Mangrove soil has been considered as a source of incredible diversity of bacteria for decades. The determination of bacterial load and identification of bacteria from different soil depths from Sundarban have been done in previous studies (Afruza Begum 2020; Afruza Begum, Rasheda Yasmin Shilpi 2020). Present study is carried out to explore the antibiotic resistance of bacterial population in the soil subsurface of Sundarbans Mangrove Forest in Bangladesh towards antibiotic ciprofloxacin (CIP-5).

2. Materials and Methods

This discrete section includes the collection of factual knowledge about methodology that was utilized in execution of the experiment. It contains a short description of location of the experimental site, materials that used for the experiment, data collection procedure etc.

Study area and soil sampling

A total of 20 soil samples were collected carefully from four depths of soil from each of five areas of Sundarbans: Mrighamari, Kholisgonj, Sutarkhali, Sarbotkhai and Kalaboghi, and transported to the microbiology laboratory described in (Afruza Begum 2020; Afruza Begum, Rasheda Yasmin Shilpi 2020).

Sample preparation and identification

The isolation of bacteria colony from these mangrove soil samples was accomplished by using the spread plate technique (Timoney JF, Port J, Giles J 1978; Mudryk ZJ 2004). After incubation at 37°C for 24 hours, morphologically unique colonies were picked and then place on nutrient agar slant and incubated at 37°C for 24 h. Thus, a total of 46 colonies were isolated as pure culture (Afruza Begum 2020; Giraffa G 2004) (Table -01). The pure cultures were preserved at -4°C for further experiments and antibiotic sensitivity testing. Bacterial isolates have been identified based on selective media tests and biochemical tests as *Salmonella*, *Shigella*, *E. coli*,

Klebsiella, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas*, *Bacillus sp*. described in (Afruza Begum, Rasheda Yasmin Shilpi 2020) (Figure-01).

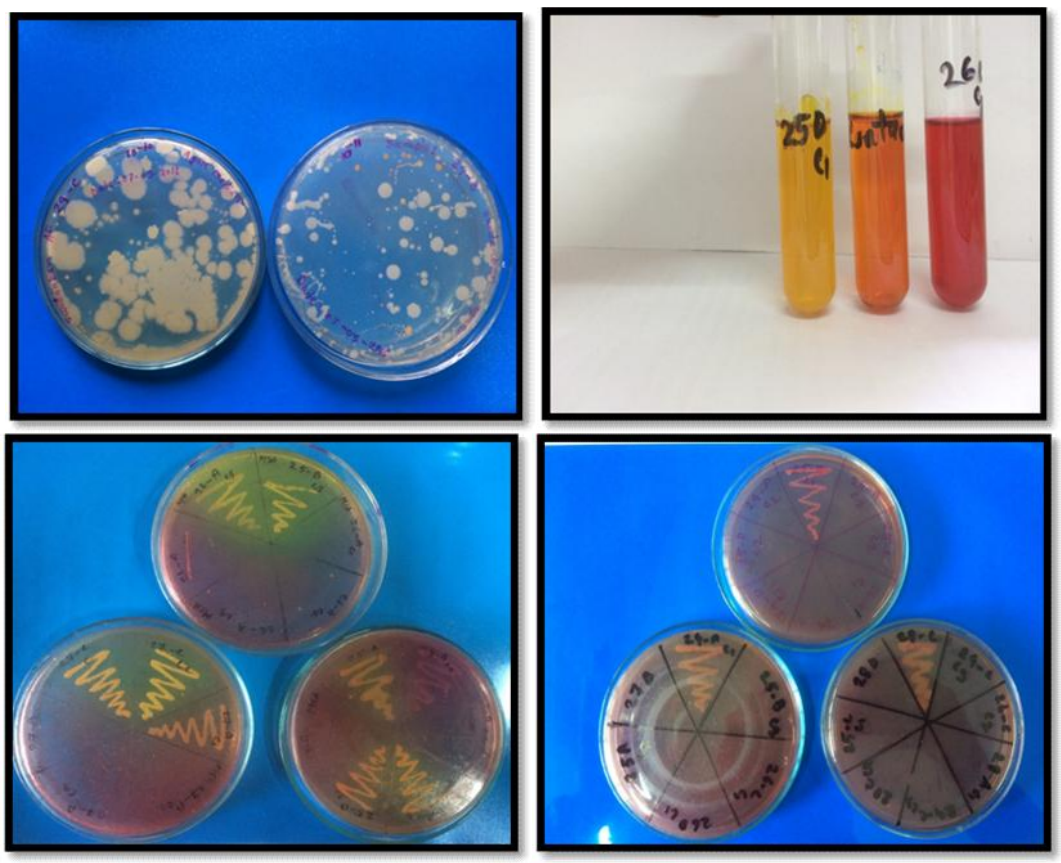

Figure 01: Photographic representation of bacterial load on nutrient agar media and selective media tests and biochemical tests

Antibiotic Susceptibility Testing

The performance of antimicrobial susceptibility testing is significant to detect resistance in bacterial isolates. The paper disk susceptibility testing is straightforward and efficient method. The test was performed by applying bacterial broth culture to the surface of agar plate. Paper antibiotic disk was then placed on the inoculated agar surface. Plates were incubated for 24 h at 37°C prior to the determination of results (Figure-02). The formation of zones of inhibition around each of the antibiotic disks were related to the susceptibility of isolates and were measured to the nearest millimeter (Table-01).

3. Interpretation of results of antibiotic sensitivity testing

Antibiotic-resistant organisms are used to develop productive antimicrobial drugs. In a bacterial population, resistance occurred bypasses of resistant genes between bacteria and transfer of bacteria between peoples (Rang HP, Dale MM, Ritter JM 2007).

Among 48 bacterial colonies, 11 strains were experimented to know their sensitivity to the antibiotic ciprofloxacin (CIP-5). All the bacterial isolates were susceptible to ciprofloxacin due to its broad-spectrum nature (Table-01). Ciprofloxacin act by inhibiting the topoisomerase II that causes negative supercoil in DNA and thus, permits transcription or replication. The highest zone of inhibition was found in 25A-C_1 from (0-25) cm depth of soil at Kholisgonj station and the inhibition zone was 34mm in diameter which indicated that they were highly sensitive to the antibiotic CIP-5. The lowest zone of inhibition was found in 26C-C_2 from (50-75) cm depth of soil at Sarbotkhalistation (Table-01, Figure -03).

The unsystematic utilization of antibiotics is one of the major reasons for the emergence of antibiotic resistance against some drugs and thus, reduce the efficacy of antibiotic treatment for human and other animal diseases (Asha K, Vinitha DA, Kiran SG, Manjusha W, Sukumaran N 2005)

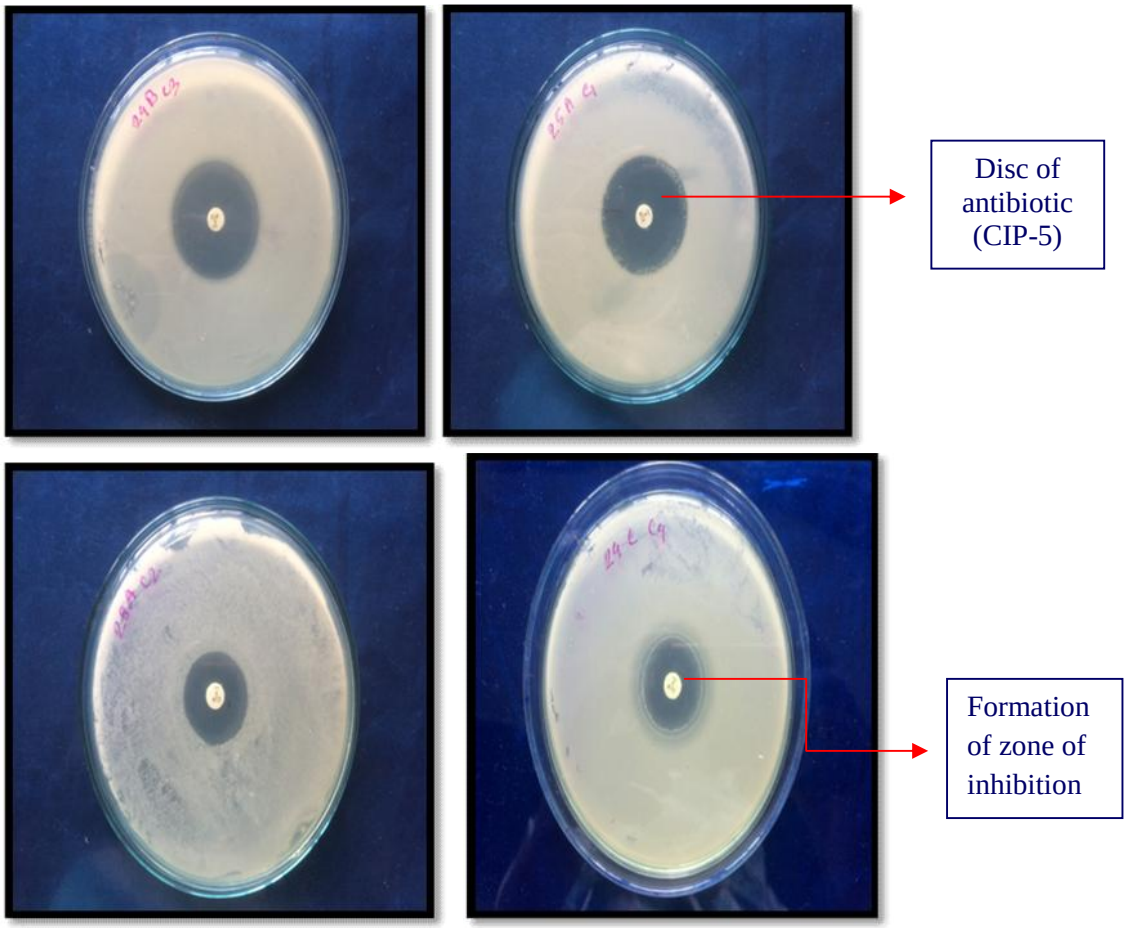

Figure 02: Disk diffusion tests of ciprofloxacin (CIP-5)with the isolates from mangrove soil.

Table 01: Antibiotic sensitivity pattern of bacterial isolates against ciprofloxacin (CIP-5)

Location	Depth(in cm)	Colony code number	Isolated colony	Zone of inhibition (mm)
Mrighamari	0-25	24A	C_1 C_3	29 22
	25-50	24B	C_3	32
	50-75	24C	C_1 C_4	25 22
	75-100	24D	C_2	30
Kholisgonj	0-25	25A	C_1	34
	25-50	25B	C_1	22
	50-75	26C	C_1 C_2	27 18
	25-50	27B	C_1	26
Kalaboghi	0-25	28A	C_2	23
	50-75	28C	C_1	26
	75-100	28D	C_1	23

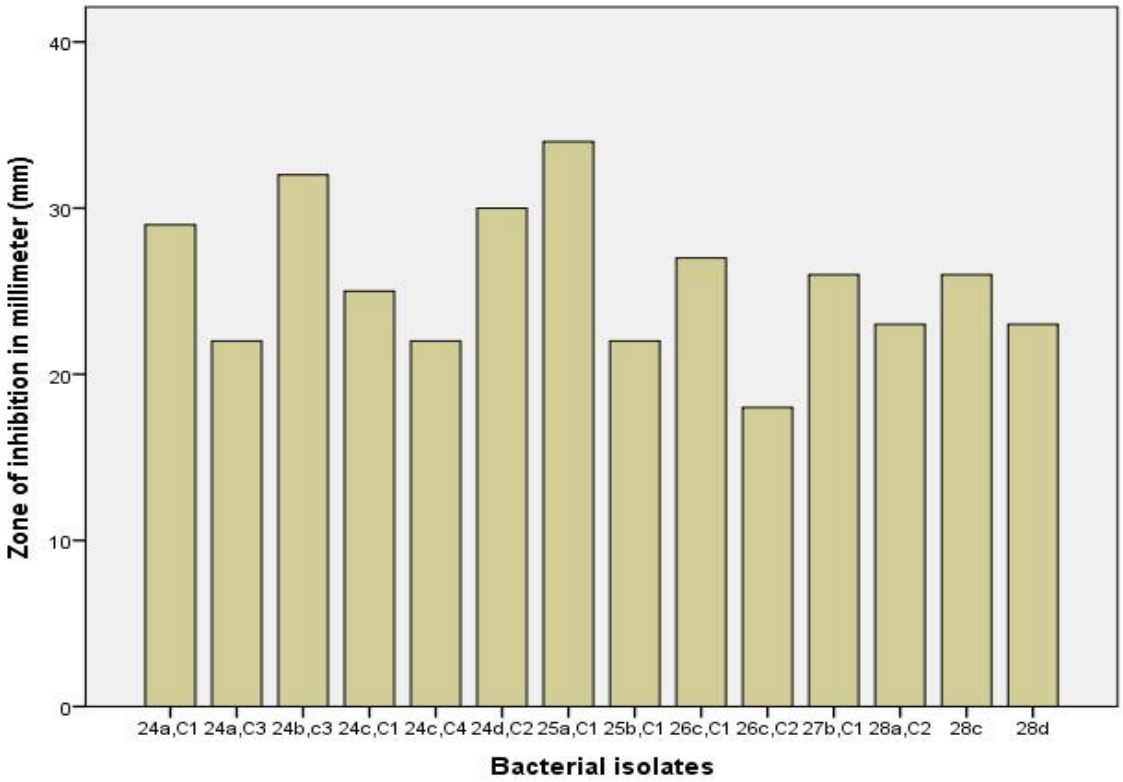

Figure 03: Antibiotic sensitivity of the isolates against ciprofloxacin (CIP-5).

Conclusion

Nowadays antibiotic resistance is a global concern as many disease-causing bacteria are becoming more resistant to the common antibiotics. Regarding the result, all the species are classified as wild-type (WT) population. This is the first report in studied areas that showed the original genetic constitution of the isolates has not been changed. From this observation, it can be stated that the soil in the studied areas is protected from any contamination or haphazard utilization of antibiotics by human beings that may induce antibiotic resistance by alternation of genetic properties which encourage mutation in species. It is clear from the experiment that microorganisms in Sundarbans still remained wild type. These microbes play a great role in improving soil texture, nutrients, and productivity (Bhattarai1 A. 2015).

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