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**Original Research Article**  
**Antibacterial Potential of Ethyl-Acetate Extracts of Marin**  
***Streptomyces* spp. AIAH-10 against Drug Resistant**  
***Escherichia coli***

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8 | **ABSTRACT**  
9

The rapid increase in drug resistance among bacteria ~~is a public health challenge in strains turns a critical health problem to~~ the 21<sup>st</sup> century. To combat drug ~~resistant~~resistance, searching ~~of for~~ new broad-spectrum antibiotics is urgent and marine microbial flora may be ~~the a~~ potential source ~~to look for new antibiotics~~. ~~The P~~present study was designed to isolate marine actinomycetes strains (AIAH-01 to AIAH-29) from the soil sediments of different locations of ~~the Sundarbans~~ mangrove forest ~~Sundarbans~~, Bangladesh by serial dilution method. Primary screening of antimicrobial activity was performed by cross plate technique and AIAH-10 was undertaken for further ~~study owing analysis dye~~ to its potential activity against a ~~series number~~ of pathogenic bacterial ~~strains~~. On the basis of morphological, cultural, biochemical and cell composition analysis, ~~the strain~~ AIAH-10 belongeds to *Streptomyces* spp. Secondary metabolites of AIAH-10 exhibited ~~a significance significant~~ activity (~~with zone sizes of 15mm and 12mm~~) against ciprofloxacin and ~~azythromycin~~azithromycin induced resistant *Escherichia coli* (*E. coli*) respectively (~~50 µg/disc~~). Minimum inhibitory concentration (MIC) of extracts against ciprofloxacin and azithromycin induced resistant *E. coli* was found to be 16 and 32µg/ml respectively. Time-kill profile of the extracts also revealed its considerable antimicrobial activity. Therefore it may be concluded that ~~the~~ marine *Streptomyces* spp. AIAH-10 isolated from ~~the sundarbans~~ mangrove forest, ~~sundarbans~~, Bangladesh ~~can have the ability to produce~~ bioactive compounds that can be applied against resistant and multi-drug resistant pathogens.

10 | **Keywords:** Mangrove forest; Susceptibility; Marine *Streptomyces* spp. Antimicrobial activity; Multidrug resistant  
11 | Susceptibility  
12  
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14 | **1. INTRODUCTION**

15 | Recently the world health organization (WHO) identified antimicrobial resistance ~~ai~~is one of the greatest threats to human  
16 | health, jeopardizing patient safety and public health. In recent years, indiscriminate ~~using~~ of antibiotics and other  
17 | unknown ~~measured to the~~ pathogenic organisms are gaining resistance to the existing microbial agent [1]. Due to this  
18 | high prevalence of antimicrobial resistance, WHO ~~has~~ themed ~~the~~ 2011 year's world health's day "Antimicrobial  
19 | resistance: no action today, no cure tomorrow". The prevalence of drug ~~resistance resistant~~ pathogenic strains ~~become is~~  
20 | ~~a severe threat~~ to human health and can lead to high mortality rates ~~especially~~ patients ~~basically~~  
21 | ~~who is from suffering distressed with~~ AIDS [2]. Usually, gram-negative bacteria ~~become are~~ more resistant ~~against~~  
22 | antibiotics than ~~that of~~ gram-positive bacteria. The reason for different responses ~~between these microorganisms~~ between Gram-positive and Gram-  
23 | negative bacteria ~~is~~ due to their morphological differences. ~~Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This factor makes the cell wall impermeable to lipophilic solutes, while poring constitutes a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da [3]. The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which has not considerable permeability barrier [4-7].~~  
24 | To combat antibiotic resistancet, researchers ~~are~~ currently engaged ~~in studying toward~~ marine bacteria as a source of  
25 | potent antimicrobial compounds that could be ~~main target biocidal~~ to resistant species. It ~~has been is~~ appraisedal that not  
26 | more than 1% of desired chemicals from marine ~~derived~~ environments have been screened. However actinomycetes flora  
27 | of marine environment, are sickly screened and only few research are done pertaining to microorganism from mangroves  
28 | forest [8, 9]. Due to different environmental conditions of marine derived microorganisms from that of terrestrial ones, it is  
29 | assumed that microorganisms isolated from marine locations may have different ~~nature~~ from those ~~that of~~ terrestrial  
30 | compartment and hence marine derived microorganisms might produce different types of bioactive compounds [10]. Now  
31 | days, the researchers point out that, marine oriented microorganism may become a super recognized source of  
32 | discovering novel antibiotic, anti-inflammatory, antifungal, antiviral, anticoagulant, cardiovascular and anticancer agent  
33 | with distinguish structure and properties. Moreover, marine oriented antibiotics are found to exhibit more efficient activity  
34 | against pathogenic bacterial strains and yet have not any published data concerning drug resistant against them [11].  
35 | This study was performed to find out the potential susceptibility of ethyl acetate extracts of marine *Streptomyces* spp.  
36 | AIAH-10 against ciprofloxacin and azithromycin induced resistant *E. coli* isolated from mangrove forest, Sundarbans,  
37 | Bangladesh.  
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43 | **2. MATERIAL AND METHODS**

44 | **2.1 Collection of marine soil samples**

45 | ~~From the layers of the upper surface to the 1.5cm depth, s~~Soil samples were collected from the different locations of  
46 | mangrove forest (Sundarbans), like Kochikhali, Jamtoplapoint, Tigerpoint, Dublarchor, Koramjol of Bangladesh ~~to a depth~~  
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Comment [B1]: ???

Comment [B2]: What do you want to say?

Comment [B3]: See B2 above: sentence doesnt make sense.

Comment [B4]: Poor sentence structures and grammar. Revise to increase comprehension

Comment [B5]: Restructure. Poor grammar and syntax. Not comprehensible.

Comment [B6]: ?? Change

Comment [B7]: ??change

Comment [B8]: Poor grammar, syntax and sentencing. Change all.

of 15cm. Sixteen soil samples were collected in properly labeled sterile plastic bags with proper labeling. The samples were dried in hot air oven at 121°C for about 3 hours to remove spores from the vegetative cells [12] and kept stored in at 4°C up to till further processing.

## 2.2 Isolation of soil actinomycetes

Actinomycetes isolation from marine soil sediments was done by serial dilution and spread plate method [13]. One gram of processed soil sample was serially diluted in sterilized distilled water to obtain a concentration ranging from  $10^{-1}$  to  $10^{-6}$ . A volume of 100 µl of each dilution was transferred aseptically into an isolation medium (casein starch media), modified nutrient agar (MNA) and Kuster's medium (KA) which was supplemented with nystatine (25 µg/ml). The sample was spread uniformly on the plate by rotating it clockwise and anticlockwise direction. The plates were incubated at room temperature for 7 days [14]. The isolated actinomycetes were picked up using their organoleptic properties; as they are dusky and powdery in nature with anas well as earthy odor. The isolated strains were further subcultured (Yeast Extract Glucose Agar media) to obtain pure cultures. Pure isolates were kept at 4°C in refrigerator for further studies.

## 2.3 Primary screening (cross streak method)

The primary screening of isolated actinomycetes strains was performed by single cross streak method on plates containing MNA media [15]. Isolated strains were inoculated in straight line on MNA plates and incubated for 7 days. Test bacteria were cross streak on the same plate in perpendicular manner. The plates were incubated at 37°C for 24 hours. The plates were checked for the length of inhibition.

## 2.4 Taxonomic investigation of the potential strain

### 2.4.1 Morphological characteristics

The isolated strain AIAH-10 having potent antibacterial activity was inoculated in different media stated in "International Streptomyces Project" (ISP) (reference!!) and cultured for 8 days at 28°C temperature. The colonies were observed under microscope and morphology was identified in respect to the color, size, nature of colony both aerial and reverse side [16].

### 2.4.2 Biochemical characterization

The ability of isolated strain to assimilate of various carbon sources viz., xylose, inositol, mannitol, fructose, rhamnose, sucrose and raffinose as sources of energy was done following the method recommended by the International Streptomyces Project [17].

### 2.4.3 Generic investigation

The strain (AIAH-10) having tremendous antagonistic activity against the test bacteria was identified by using cell wall composition analysis like amino acids and cell sugars analysis [12].

## 2.5 Fermentation process

The antagonistic actinomycetes isolate's suspensions were prepared by suspending a loopful of pure colony of AIAH-10 in 2 ml sterile normal saline, vortexed to homogenize and stored at 4°C until ready for use. This suspension was used as Streptomyces inoculants in all cultivations. Fermentation for production of bioactive metabolites was done as described by Umasankar (2010) with modification. 100 µl suspension was cultured in 100 ml of modified nutrient (MN) broth in Erlenmeyer flasks. Flasks were kept on the shaker at a speed of 120 rpm at room temperature for 8 days. After successful fermentation, the medium was harvested and centrifuged to remove debris. Filtrate was preserved for further use.

## 2.6 Isolation of metabolites

The metabolites were recovered from the harvested medium by solvent extraction technique. The cultured supernatant was extracted twice with equal volumes of ethyl acetate, shaken sufficiently for 1 hour in extracting funnel, vaporized to dryness in a rotary evaporator at 50°C and dried in lyophilizer.

## 2.7 Collection of resistant species

Ciprofloxacin and azithromycin induced resistant *E. coli* was collected from Department of Pharmacy, University of Rajshahi, Bangladesh while pathogenic bacterial strains were collected from the "International Centre for Diarrheal Disease Research" Bangladesh (ICDDR'B).

## 2.8 Antibiogram

Induced resistant *E. coli* was screened for their sensitivity towards ciprofloxacin (30 µg/disc) and azithromycin (30 µg/disc) by disc diffusion method. Freshly cultured resistant *E. coli* was seeded onto plates (yeast extract glucose agar media) by using sterilized cotton swabs. The antibiotic discs were placed on the agar surface using a sterilized forceps. Plates were incubated at 37°C for 24 hours. Plates were checked for zones of inhibition [18].

## 2.9 Antimicrobial activity

The antimicrobial activity of secondary metabolites of AIAH-10 against ciprofloxacin and azithromycin resistant *E. coli* was carried out by the disc diffusion method [18]. The bacterial culture was maintained in nutrient broth. From this 100 µl suspension of each culture was uniformly distributed on plates containing yeast extract glucose agar media. The test sample impregnated discs (50 µg/disc) were placed gently on solidified agar plates which were seeded with the organism to ensure contact with the media, by means of a sterile forceps. The plates were kept at 4°C for 4 hours for proper diffusion of sample to the surrounding area. Then, the plates were incubated at 37°C for 24 hours. At the end of the incubation period, the zones of inhibition were measured by scale.

## 2.10 Determination of Minimum Inhibitory Concentration (MIC)

Sterile nutrient broth tubes containing different dilutions of extract (0.125 to 512 µg/ml) were specifically inoculated with 60 µl of ciprofloxacin and azithromycin resistant *E. coli* inoculums ( $10^7$  cfu/ml). The tubes were incubated aerobically at 37°C for 18-24 h. Two control (tube containing the growth medium, saline and the inoculums) tubes for each organism were maintained. The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC [19].

## 2.11 Determination of Time-kill profile

The method of Spangler [20] was adopted for the time-kill assay against ciprofloxacin and azithromycin resistant *E. coli*. Viable counts of the test bacteria were first determined. Initial inocula of  $10^5$  to  $10^6$  cfu/ml were prepared from 100 µl aliquots of strain in normal saline and this was verified by performing colony count. 80 µl suspension of selected test bacteria was added to 10 ml of MN broth in McCartney bottles containing known concentration (relative to MIC) of each of the extracts. Time kill assay of extracts against ciprofloxacin and azithromycin resistant *E. coli* bacteria were determined using the following extract concentrations: MIC, 2 × MIC, 3 × MIC and 4 × MIC and the reactants bottles were incubated in a rotary incubator at 37°C. The time kill kinetics were determined at 0, 3 and 6 h. Approximately 80 µl of *E. coli* suspension was withdrawn at the appropriate time interval and transferred to 4.5 ml of MN broth medium containing 2-4% sodium thioglycolate to minimize the action of the extracts carry-overs. Approximately 100 µl aliquots of each dilution were plated out for viable counts by pour plate technique. Each experiment was done in duplicate, and the mean of two almost identical results was calculated. Only plates yielding 30 to 300 colonies were selected for counting. Data were analyzed by expressing the reduction of growth as the Log<sub>10</sub> colony forming unit per milliliter (cfu/ml). For the Time-kill end point determination; bacteriostatic activity was defined as a reduction of 0 to 3 Log<sub>10</sub> cfu/ml, and bactericidal activity was defined as a reduction of ≥ 3 Log<sub>10</sub> cfu/ml at 3 and 6 h compared to that at 0 h [20]. McCartney bottles containing broth and test organism without extract were used as control in each experiment.

## 3. RESULTS

A total of 29 actinobacterial colonies were isolated from different marine sediments and soil samples of Sundarbans (Bangladesh) surroundings. They were designated as AIAH-01 to AIAH-29. The isolates AIAH-01 to AIAH-12 were isolated from the soil of Dublarchor while the isolates AIAH-13 to AIAH-19 from the soils of Kochikhali and the isolates AIAH-20 to AIAH-29 from the soils of Koromjol. It is shown in the Table 1.

**Table. 1** Collection site, depth and number of actinomycetes colonies per gram of soil.

| Collection site | Depth of the soil | Number of colony/gm of soil | Isolate strains    |
|-----------------|-------------------|-----------------------------|--------------------|
| Dublarchor      | 8 inches          | $1.2 \times 10^4$           | AIAH-01 to AIAH-12 |
| Kochikhali      | 6 inches          | $0.7 \times 10^4$           | AIAH-13 to AIAH-19 |
| Koromjol,       | 1 feet            | $1.0 \times 10^4$           | AIAH-20 to AIAH-29 |

Among the 29 isolates, 73.53% exhibited different ranges of antimicrobial activity during primary screening (Table 2). The isolated strain, AIAH-10 showing promising broad spectrum activity against different pathogenic organisms was selected for further study.

**Table. 2** Diameter of length of inhibition (in mm) of the isolates against a wide range of test pathogenic bacteria.

**Comment [B9]:** What concentration of *E. coli* was used?

| ID number<br>of the isolates | <i>Streptococcus<br/>agalactiae</i> | <i>Bacillus cereus</i> | <i>Pseudomonas<br/>aeruginosa</i> | <i>Escherichia coli</i> | <i>Shigella<br/>dysenteriae</i> | <i>Shigella sonnei</i> | <i>Agrobacterium</i> |
|------------------------------|-------------------------------------|------------------------|-----------------------------------|-------------------------|---------------------------------|------------------------|----------------------|
| AIAH-01                      | 15±0.12                             | 22±0.13                | 16±0.17                           | 20±0.27                 | 20±0.32                         | 30±0.16                | 21±0.14              |
| AIAH-02                      | 02±0.31                             | 05±0.26                | 04±0.14                           | 02±0.16                 | -                               | -                      | 04±0.37              |
| AIAH-03                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-04                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-05                      | 21±0.34                             | 28±0.21                | 19±0.17                           | 27±0.34                 | 30±0.11                         | 22±0.23                | 20±0.41              |
| AIAH-06                      | 10±0.24                             | 12±0.41                | 07±0.23                           | 09±0.34                 | 15±0.27                         | -                      | 12±0.24              |
| AIAH-07                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-08                      | 13±0.14                             | 14±0.32                | 15±0.38                           | 12±0.17                 | 16±0.52                         | 10±0.27                | 12±0.51              |
| AIAH-09                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-10                      | 32±0.23                             | 37±0.29                | 30±0.15                           | 30±0.53                 | 35±0.17                         | 28±0.33                | 25±0.27              |
| AIAH-11                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-12                      | 06±0.37                             | -                      | -                                 | -                       | 03±0.19                         | 06±0.15                | 02±0.31              |
| AIAH-13                      | 16±0.18                             | 11±0.48                | 14±0.19                           | 12±0.41                 | 02±0.23                         | 10±0.29                | 08±0.54              |
| AIAH-14                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-15                      | 13±0.14                             | 15±0.21                | 10±0.23                           | 05±0.17                 | 06±0.39                         | 04±0.52                | 10±0.41              |
| AIAH-16                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-17                      | 05±0.41                             | 23±0.14                | 05±0.39                           | 07±0.62                 | 02±0.69                         | 03±0.49                | -                    |
| AIAH-18                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-19                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-20                      | -                                   | 04±0.31                | 06±0.11                           | 02±0.21                 | 03±0.25                         | 06±0.17                | 08±0.29              |
| AIAH-21                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-22                      | 15±0.37                             | 19±0.33                | 12±0.51                           | 11±0.32                 | 10±0.14                         | 10±0.28                | 12±0.16              |
| AIAH-23                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-24                      | 11±0.43                             | 02±0.51                | 03±0.37                           | 05±0.12                 | 06±0.34                         | 02±0.44                | 03±0.18              |
| AIAH-25                      | 10±0.16                             | 05±0.57                | 05±0.42                           | 02±0.26                 | -                               | 06±0.31                | 07±0.31              |
| AIAH-26                      | 16±0.25                             | 08±0.32                | 05±0.68                           | 07±0.43                 | 02±0.28                         | 03±0.11                | -                    |
| AIAH-27                      | -                                   | 02±0.21                | 01±0.29                           | 02±0.19                 | -                               | 02±0.16                | -                    |
| AIAH-28                      | -                                   | -                      | -                                 | -                       | -                               | -                      | -                    |
| AIAH-29                      | 28±0.19                             | 41±0.42                | 37±0.41                           | 30±0.33                 | 25±0.43                         | 31±0.28                | 27±0.53              |

Legend, The values expressed as mean ± SEM of 3 -4 experiments. " - " indicates no inhibition.

The strain AIAH-10 was identified by morphological and biochemical test. The entire report was shown in Table 3 and 4. Furthermore, generic investigation was done for the isolate. Results are shown in Table 5. Findings of this study suggested that the strain belongs to *Streptomyces* spp. and designated as *Streptomyces* spp. AIAH-10.

**Table. 3** Growth and cultural characteristics of strain AIAH-10 on different media.

| Medium                                   | Growth | Aerial mycelium | Reverse mycelium      | Pigmentation |
|------------------------------------------|--------|-----------------|-----------------------|--------------|
| Trypton –yeast extract agar(ISP-1)       | +      | Light brown     | Light yellow          | +            |
| Yeast-extract-malt extract agar (ISP- 2) | ++     | Yellowish brown | Darkish pink          | ++           |
| Oatmeal agar (ISP -3)                    | ++     | Yellowish gray  | Dark yellowish orange | ++           |
| Inorganic salt-starch                    | +++    | Grayish yellow  | Yellowish white       | +++          |

|                                   |     |                  |               |     |
|-----------------------------------|-----|------------------|---------------|-----|
| agar (ISP- 4)                     |     |                  |               |     |
| Glycerol-asparagine agar (ISP-5)  | ++  | Grayish brown    | Pinkish white | ++  |
| Tyrosine agar(ISP-7)              | ++  | Light gray       | Dusky yellow  | +   |
| Yeast-extract glucose agar (YEGA) | +++ | Yellowish orange | Dark gray     | +++ |

Legend, '+++'=High growth, '++'=Moderate growth and '+'=Low growth.

**Table. 4** Assimilation of carbon sources by the strain AIAH-10.

| Carbon source                | Growth | Pigments production |
|------------------------------|--------|---------------------|
| D-Glucose(Positive control)  | +++    | ++                  |
| D-fructose                   | +      | +                   |
| Ethyl Cellulose              | +      | -                   |
| D-Xylose                     | -      | -                   |
| Lactose                      | +      | +                   |
| Sucrose                      | +      | +                   |
| Mannitol                     | +++    | ++                  |
| Inositol                     | ++     | +                   |
| L-Rhamnose                   | ++     | +                   |
| No carbon (Negative control) | -      | -                   |

Legend, '+++'= strongly positive assimilation, '++'= moderately positive assimilation, '+'= low assimilation and '-'= negative assimilation

**Table. 5** Cell wall amino acids and whole sugars content of AIAH-10 isolate.

| Cell wall composition | Possible results |
|-----------------------|------------------|
| LL-DAP                | Present          |
| Meso-DAP              | Absent           |
| Glycine               | Present          |
| Whole cell sugars     | Absent           |
| Wall type             | I                |

The metabolites were tested for susceptibility against ciprofloxacin and azithromycin induced resistant and sensitive *E. coli* strain. Resistance of the strain was confirmed by antibiogram test with ciprofloxacin and azithromycin and negligible zone of inhibition was observed. Antimicrobial potentiality of the crude extracts (50 µg/disc) against azithromycin and ciprofloxacin induced resistant and sensitive *E. coli* strain was recorded to be 12 mm (for azithromycin resistant *E. coli*), 15 mm (for ciprofloxacin resistant *E. coli*) and 24 mm (sensitive *E. coli*) respectively. The result is shown in the Table 6 and Figure 1.

**Table. 6** Antimicrobial activity of the extracts against azithromycin and ciprofloxacin induced resistant *E. coli*.

| Name of Antibiotic | Zone of inhibition(in mm)             |                                        |                          |
|--------------------|---------------------------------------|----------------------------------------|--------------------------|
|                    | Azithromycin resistant <i>E. coli</i> | Ciprofloxacin resistant <i>E. coli</i> | Sensitive <i>E. coli</i> |
| Metabolites        | 12 ± 0.15                             | 15 ± 0.23                              | 24 ± 0.51                |
| Ciprofloxacin      | 8 ± 0.31                              | 7 ± 0.26                               | 22 ± 0.18                |
| Azithromycin       | 8 ± 0.57                              | 10 ± 0.21                              | 25 ± 0.57                |

Legend, The values expressed as mean ± SEM of 3-4 experiments.

Minimum inhibitory concentration was determined by broth dilution method. The MIC values of the extracts against ciprofloxacin and azithromycin induced resistant and sensitive *E. coli* were found to be 16 µg/ml (for ciprofloxacin resistant *E. coli*), 32 µg/ml (for azithromycin resistant *E. coli*) and 2 µg/ml (sensitive *E. coli*) respectively. The result is presented in the Table 7.



**Figure. 1** Antimicrobial activity of the extracts against ciprofloxacin and azithromycin induced resistant *E. coli* respectively.

**Table. 7** MIC of the extracts against ciprofloxacin and azithromycin resistant and sensitive *E. coli*.

| <i>E. coli</i> strain   | MIC ( $\mu\text{g/ml}$ ) | MBC ( $\mu\text{g/ml}$ ) |
|-------------------------|--------------------------|--------------------------|
| Ciprofloxacin resistant | 16                       | 32                       |
| Azithromycin resistant  | 32                       | 128                      |
| Sensitive               | 2                        | 8                        |

Time-kill profile of the metabolites against azithromycin and ciprofloxacin induced resistant *E. coli* strain is shown in Table 8. The metabolites exhibited varying degrees of bacteriostatic activities depending on their potency. A significant decrease in mean of viable count of test bacteria was observed at each time interval. Results are presented as  $\text{Log}_{10}$  cfu/ml.

**Table. 8** Time-kill assay of metabolites against azithromycin and ciprofloxacin induced resistant *E. coli*.

| Name of the <i>E. coli</i> strain compound | MIC ( $\text{Log}_{10}$ ) |      | 2×MIC ( $\text{Log}_{10}$ ) |      | 3×MIC ( $\text{Log}_{10}$ ) |      | 4×MIC ( $\text{Log}_{10}$ ) |      |
|--------------------------------------------|---------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|------|
|                                            | 3h                        | 6h   | 3h                          | 6h   | 3h                          | 6h   | 3h                          | 6h   |
| Extracts                                   |                           |      |                             |      |                             |      |                             |      |
| Azithromycin resistant                     | 0.31                      | 0.45 | 0.62                        | 0.73 | 0.86                        | 0.97 | 1.23                        | 1.36 |
| Ciprofloxacin resistant                    | 0.63                      | 0.72 | 0.89                        | 1.04 | 1.21                        | 1.44 | 1.71                        | 1.8  |

Mean Log reduction in viable cell count for the metabolites ranges between 0.31  $\text{Log}_{10}$ , 0.62  $\text{Log}_{10}$ , 0.86  $\text{Log}_{10}$  and 1.23  $\text{Log}_{10}$  cfu/ml after 3h interaction in MIC, 2×MIC, 3×MIC and 4×MIC, respectively and between 0.45  $\text{Log}_{10}$ , 0.73  $\text{Log}_{10}$ , 0.97  $\text{Log}_{10}$  and 1.36  $\text{Log}_{10}$  cfu/ml after 6h interaction in MIC, 2×MIC, 3×MIC and 4×MIC, respectively (against azithromycin resistant *E. coli*).

Mean Log reduction in viable cell count for the metabolites ranges between 0.63  $\text{Log}_{10}$ , 0.89  $\text{Log}_{10}$ , 1.21  $\text{Log}_{10}$  and 1.71  $\text{Log}_{10}$  cfu/ml after 3h interaction in MIC, 2×MIC, 3×MIC and 4×MIC, respectively and between 0.72  $\text{Log}_{10}$ , 1.04  $\text{Log}_{10}$ , 1.44  $\text{Log}_{10}$  and 1.8  $\text{Log}_{10}$  cfu/ml after 6h interaction in MIC, 2×MIC, 3×MIC and 4×MIC, respectively (against ciprofloxacin resistant *E. coli*).

#### 4. DISCUSSION

In this study, we explored the presence of antimicrobial compounds producing microbes in soils of Sundarbans Bangladesh, the largest tidal halophytic mangrove forest in the world. The soils samples were collected from the regions of mangrove forest that is frequently inundated with moderate saline water. From seven soil samples, 29 pure strains were isolated and screened for antibacterial activity. Among them 79.31% isolates inhibited the test bacteria with variable activity-low, moderate to high, when the strains were undergone in primary antibacterial screening by cross streaking method. Due to tremendous broad-spectrum activity, AIAH-10 was picked up for further study. Based on experimental records of morphological, cultural, biochemical and cell-wall composition analysis as well as matching the keys given for classification of 458 species of actinomycetes included in International Streptomyces Project, the isolate was identified as *Streptomyces* species and designated as *Streptomyces* spp. AIAH-10. Similar procedure was applied previously [21] for the identification of *Streptomyces*. Potentiality-Inhibition zones of the extracts of this species against ciprofloxacin and azithromycin induced resistant *E. coli* was found as 15 and 12mm respectively on 50  $\mu\text{g}$  extracts per disc whereas MIC value was recorded as 16 and 32 $\mu\text{g/ml}$  respectively. Time-kill profile of the extracts suggests the potential activity of the

**Comment [B10]:** Revise sentence to improve comprehensibility.

extracts against the antibiotic resistant *E. coli* strain. To ~~our the best of our~~ knowledge no work has been carried out against ciprofloxacin and azithromycin induced resistant *E. coli*, although some research were done against multi-drug resistant *Staphylococcus aureus* and some other pathogenic bacteria [22-24].

~~We effort a lot to isolate the crude extracts and were able to collect approximately 200 mg only in 8 liters fermentation medium. Besides so many strengths we have exercised in this research work still it lacks few things such as we could use more solvent system during extraction process, in order to get fine elution. Secondly, sequencing and phylogenetic analysis of the two strains were incomplete. But by using controlled models and a variety of methods we hope to exclude confounding effects.~~

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## 5. CONCLUSION

~~Marine microorganisms are natural laboratories which possess a series of secondary metabolites that are indispensable for drug discovery. Bioactive compounds from different microorganisms have diverse applications. Hence these compounds are serves as basic platform to find out new and potential natural products. The *Streptomyces* AIAH-10 isolated from Sunderbans mangrove forest is a This study demonstrates the diversity of the mangrove forest, Sunderbans, Bangladesh as a rich and interesting potential source of antibiotic molecules producing new and potential marine bacterial species~~

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