

Original Research Article

Potentiality of Marine *Streptomyces* sp. AIAH-10 against Ciprofloxacin and Azithromycin Induced Resistant *Escherichia coli*

ABSTRACT

Rapidly emergence drug resistance among bacterial strains turns a critical health problem to the 21st century. To combat drug resistant, searching of new broad-spectrum antibiotics is urgent and marine microbial flora may be the potential source to look for new antibiotics. Regarding this mind, present study was designed to isolate 29 marine Actinomycetes strain (AIAH-1 to AIAH-29) from the soil sediments of different locations of 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 to its potential performance against a series of pathogenic bacterial strains. On the basis of morphological, cultural, biochemical and cell composition study, the strain AIAH-10 belongs to *Streptomyces* sp. Secondary metabolites of AIAH-10 exhibited a potential susceptibility (15 and 12mm) against ciprofloxacin and azythromycin induced resistant *Escherichia coli* (*E. coli*) respectively (50 µg/disc). Minimum inhibitory concentration (MIC) of extracts against ciprofloxacin and azythromycin 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 said that marine *Streptomyces* sp. AIAH-10isolated from mangrove forest, sundarbans, Bangladesh can have the ability to produce novel molecules that can be applied resistant and multi-drug resistant pathogens.

Keywords: Mangrove forest; Susceptibility; Marine *Streptomyces* sp. Antimicrobial activity; Multidrug resistant Susceptibility

1. INTRODUCTION

Recently the world health organization (WHO) identified antimicrobial resistance is one of the greatest threats to human health, jeopardizing patient safety and public health. In recent year, indiscriminate use of antibiotics and other unknown measure to the pathogenic organisms are gaining resistance of the existing in microbial agent [1]. Due to this high prevalence of antimicrobial resistance, WHO has themed 2011 year's World Health Day, action against antimicrobial resistance -“*Antimicrobial resistance: no action today, no cure tomorrow*”. The prevalence of drug resistance pathogenic strains becomes severe treat to human health and can lead to high mortality rate in immunocompromised patients basically who is suffering distressed with AIDS [2]. Usually gram-negative bacteria become more resistant against antibiotics than that of gram-positive bacteria. The reason for different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while poring constitute 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 is not an effective permeability barrier [4-7].

To combat antibiotic resistant, researchers currently engaged towards marine bacteria as a source of potent antimicrobial compounds that could be main target to resistant species. It is appraisal that not more than 1% of desired chemicals from marine derived environment have been screened. However actinomycetes flora that also find in salty marine environment, are sickly screened and only few researches are done pertaining to microorganism from mangroves forest [8, 9]. Due to

different environmental conditions of marine derived microorganisms from that of terrestrial ones, it is assumed that microorganisms isolated from marine locations have different nature from those of terrestrial compartment and hence marine derived microorganisms might produce different types of bioactive compounds [10]. Now days, the researchers point out that, marine oriented microorganism has become a super recognized source of discovering novel antibiotic, anti-inflammatory, antifungal, antiviral, anticoagulant, cardiovascular and anticancer agent with distinguish structure and properties. Moreover, marine oriented antibiotics are found to exhibit more efficient activity against pathological infections and yet have not any published data concerning drug resistant against them [11].

Focus of this study was to evaluate potential susceptibility of ethyl acetate extracts of marine *Streptomyces* sp. AIAH-10 against Ciprofloxacin and Azithromycin induced resistant *E. coli* isolated from mangrove forest, Sundarbans, Bangladesh.

2. MATERIAL AND METHODS

2.1 Collection of marine soil samples

Marine soil samples were collected from the different locations of mangrove forest (Sundarbans), like Kochikhali, Jamtoplapoint, Tigerpoint, Dublarchor, Koramjol of Bangladesh, from the layers beneath the upper surface to the 1.5cm depth. Sixteen soil samples were collected in sterile plastic bag with proper labeling. The samples were allowed to dry in hot air oven at 60-65°C for about 3 hours to remove of spores from the vegetative cells [12] and kept in 4°C up to further processing.

2.2 Isolation of soil actinomycetes

Actinomycetes isolation from marine soil sediments was performed by serial dilution and spread plate method [13]. One gram of previously processing soil sample was serially diluted in sterilized distilled water to get a concentration ranging from 10^{-1} to 10^{-6} . A volume of 80µl of each dilution was transferred aseptically to isolation medium (casein starch media), modified nutrient agar (MNA) and Kuster's medium (KA) which was supplemented with nystatine (25µg/ml). The sample was spread uniformly to the plate by rotating it clockwise and anticlockwise direction. The plates were incubated at room temperature for 7 days [14]. The actinomycetes isolates were picked up as they are dusky and powdery in nature as well as earthy odor. The isolated strains were further subcultured on the respective media in order to obtain pure culture. Pure isolates were maintained at 4°C in refrigerator for further studies.

2.3 Primary screening (cross streak method)

The initial screening of isolated actinomycetes strains was performed by cross streak method on MNA plates [15]. The actinomycetes isolates were inoculated in straight line on MNA plates and incubated for 7 days. Pathogenic bacterial strains were cross streak on the same plate in perpendicular manner. The plates were incubated at 37°C for 24 hours. The plates were examined for the zone 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 mediums (ISP 1 to ISP 7) described in "International *Streptomyces* Project" and incubated for 8 days at 37°C temperature. The colonies were observe under a high power magnifying lens and colony morphology was noted with respect to color, aerial mycelium, size, nature of colony, reverse side color and feeling the consistency with a sterile loop [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 studied following the method recommended by 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 such as amino acids analysis and whole cell sugars analysis.

2.5 Fermentation process

The antagonistic actinomycetes isolate's suspensions were prepared by suspending a loopful of pure colony 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 isolate suspension was inoculated in 100 ml of MN broth in Erlenmeyer flasks. Flasks were lodged on the flask shaker at a speed of 120 rpm at room temperature for 8 days. After fermentation, the medium was harvested and centrifuged to remove growth and debris. Filtrate was collected in a sterilized screw cap bottle and stored in freeze at 4°C for further use.

2.6 Isolation of antibacterial metabolites

The bioactive compounds were recovered from the harvested medium by solvent extraction method. The culture supernatant was extracted twice with equal volumes of ethyl acetate (1:1 v/v), shaken vigorously for 1 hour in a solvent extraction 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 where as pathogenic bacteria 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 (30µg/disc) and azithromycin (30 µg/disc). Drug sensitivity test was performed by disc diffusion method on nutrient agar (Difco) plates. The isolate was inoculated in to nutrient broth for 8 hours. Resistant *E. coli* was seeded on nutrient agar plates by using sterilize cotton swabs. The antibiotic discs were placed on the agar surface using a sterilize forceps. Plates were incubated at 37°C for 48 hours. Plates were observed for zone of inhibition [18].

2.9 Antimicrobial activity

The antimicrobial activity of secondary metabolites 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 100µl of suspension of each culture was uniformly distributed on nutrient agar (Difco) plates. The test sample impregnated discs (50 µg/disc) were plated gently on solidified agar plates seeded with the organism to ensure contact with the media, by means of a sterile forceps. The plates were prepared at 4°C for 4 hour. Then, the inoculated plates were incubated at 37°C for 24 hour. At the end of incubation period, the inhibition zones were measured.

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 counts. Eighty microliter (80 µl) of suspension of known cell density of selected test bacteria was added to 10 ml of Nutrient 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. Exactly 0.5 ml volume of the reaction mixture was withdrawn at the appropriate time and transferred to 4.5 ml of nutrient broth recovery medium containing 3% sodiumthioglycolate or 3% "Tween-80" to neutralize the effects of the extracts carry-overs from the test suspensions and diluted serially in sterile nutrient broth. 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 Log₁₀ 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 Log₁₀cfu/ml, and bactericidal activity was defined as a reduction of ≥ 3 Log₁₀cfu/ml at 3 and 6 h compared to that at 0 h [20]. McCartney bottles containing broth and test organism without extract was used as growth control in each experiment.

3. RESULTS

Total 29 microorganisms were isolated from different marine sediments and soil samples of Sundarbans (Bangladesh) surroundings. These were designed as AIAH-1 to AIAH-29. The isolates AIAH-1 to AIAH-12 were isolated from the soil of Dublarchor (Sundarbans) while the isolates AIAH-13 to AIAH-19 from the soils of Kochikhali (Sundarbans) and the isolates AIAH-20 to AIAH-29 from the soils of Koromjol (Sundarbans). 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	1.2×10^4	AIAH-1 to AIAH-12
Kochikhali	6 inches	0.7×10^4	AIAH-13 to AIAH-19
Koromjol,	1 feet	1.0×10^4	AIAH-20 to AIAH-29

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

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

ID number of the isolates	<i>Streptococcus agalactiae</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Shigelladysenteriae</i>	<i>Shigellasonnei</i>	<i>Agrobacterium</i>
AIAH-1	15	22	16	20	20	30	21
AIAH -2	2	5	4	2	-	-	4
AIAH -6	10	12	7	9	15	-	12
AIAH-7	-	-	-	-	-	-	-
AIAH-8	13	14	15	12	16	10	12
AIAH-10	32	37	30	30	35	28	25
AIAH-11	-	-	-	-	-	-	-
AIAH-12	6	-	-	-	3	6	2
AIAH-13	16	11	14	12	2	10	8
AIAH-15	13	15	10	5	6	4	10
AIAH-17	5	23	5	7	2	3	-
AIAH-20	-	4	6	2	3	6	8
AIAH-22	15	19	12	11	10	10	12
AIAH-23	-	-	-	-	-	-	-
AIAH-24	11	2	3	5	6	2	3
AIAH-25	10	5	5	2	-	6	7
AIAH-26	16	8	5	7	2	3	-
AIAH-27	-	2	1	2	-	2	-
AIAH-29	28	41	37	30	25	31	27

Potential strain AIAH-10 was identified by morphological and biochemical test. The complete data was reported in Table 3 and 4. Furthermore, generic investigation was made for the isolate. Results are listed in Table 5. Findings of this study conclude that the strain belongs as *Streptomyces* sp. by using nonomura key and designated as *Streptomyces* sp. 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 agar (ISP- 4)	+++	Grayish yellow	Yellowish white	+++
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 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 ciprofloxacin and azithromycin induced resistant and sensitive *E. coli* strain was recorded to be 12mm, 15mm and 24mm respectively. The result is shown in the Table 6.

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, 32, and 2µg/ml respectively. The result is presented in the Table 7.

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

<i>E. coli</i> strain	MIC (µg/ml)	MBC (µg/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 viable count of test bacteria was observed at each time interval. Results are presented as Log₁₀ cfu/ml.

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

Nine kill assay of metabolites against azithromycin and ciprofloxacin induced resistant <i>E. coli</i> .									
Name of the compound	<i>E. coli</i> strain	MIC		2×MIC		3×MIC		4×MIC	
		(Log ₁₀)		(Log ₁₀)		(Log ₁₀)		(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 Log₁₀, 0.62 Log₁₀, 0.86 Log₁₀ and 1.23 Log₁₀ cfu/ml after 3h interaction in MIC, 2×MIC, 3×MIC and 4×MIC, respectively and between 0.45 Log₁₀, 0.73 Log₁₀, 0.97 Log₁₀ and 1.36 Log₁₀cfu/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 Log₁₀, 0.89 Log₁₀, 1.21 Log₁₀ and 1.71 Log₁₀cfu/ml after 3h interaction in MIC, 2×MIC, 3×MIC and 4×MIC, respectively and between 0.72 Log₁₀, 1.04 Log₁₀, 1.44 Log₁₀ and 1.8 Log₁₀cfu/ml after 6h interaction in MIC, 2×MIC, 3×MIC and 4×MIC, respectively (against ciprofloxacin resistant *E. coli*).

4. DISCUSSION

The need for new, save and more effective antibiotics is a major challenge to the pharmaceutical industry, specially with the increase in antibiotic resistance. For this constant increasing of antibacterial resistance against known antibiotics in world wide public health, it is now essential need to search for new sources of antibiotics. By concerning this, 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 isolates were separated 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* sp. AIAH-10. Similar procedure was applied previously [21] for the identification of *Streptomyces*. Potentiality of the extracts of this species against ciprofloxacin and azithromycin induced resistant *E. coli* was found as 15 and 12mm respectively on 50 µg extracts per disc whereas MIC value was recorded as 16 and 32µg/ml respectively. Time-kill profile of the extracts suggests the potential activity against the antibiotic resistant *E. coli* strain. To our best knowledge no work has been carried out against ciprofloxacin and azithromycin induced resistant *E. coli*, although some researches were done against multi-drug resistant *Staphylococcus aureus* and some other pathogenic bacteria [22-24].

5. CONCLUSION

Marine microorganisms are natural laboratories which possess a series of secondary metabolites which 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. This study demonstrates that the diversity of the mangrove forest, Sundarbans, Bangladesh as a rich and interesting source of antibiotic molecules producing new and potential marine bacterial species.

REFERENCE

1. Haque MA, Sarker AK, Islam MS, Rahman MA, Chouduri MAU, Islam MA. In vitro Insecticidal and Time-Kill Profile of Ethyl Acetate Extract of Marine *Streptomyces* sp. Isolated from Sundarbans, Bangladesh. Bangladesh Pharm J. 2014; 17:151-156.
2. Valan AM, Duraipandiyan V, Agastian P, Ignacimuthu S. In vitro antimicrobial activity of Streptomyces spp. ERI-3 isolated from Western Ghats rock soil (India). J Medi Mycology. 2009; 19: 22-28.
3. Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. Microbiol Reviews. 1985; 1: 1–32.

4. Scherrer R, Gerhardt P. Molecular sieving by the *Bacillum megaterium* cell wall and protoplast. *J Bacteriol.*1971; 107: 718–735.
5. Poole K. Multidrug resistance in Gram negative bacteria. *Curr Opin in Microbiol.* 2001;4: 500-508.
6. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. *J Bio sci.* 2008; 33:593-603.
7. Kenneth SM, Walmsley MB, Adrian RW. Microbial and viral drug resistance mechanisms. *Trends in Microbiology.* 2002; 10:S8-S14.
8. Rathna K, Chandrika V. Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments. *Indian J Mar Sci.*1993; 22: 297 -299.
9. Vikineswary S, Nadaraj P, Wong WH, Balabaskaran S. Actinomycetes from a tropical mangrove ecosystem – Antifungal activity of selected strains. *Asia Pacific J Mol Biol Boitec.* 1987; 5: 81 – 86.
10. Imada C, Koseki N, Kamata M, Kobayashi T, Hamada-Sato N. 2007. Isolation and characterization of antibacterial substances produced by marine actinomycetes in the presence of seawater. *Actinomycetologica.* 2007; 21: 27-31.
11. Jensen PR, Gontang E, Mafnas C, Mincer TJ, Fenical W. Culturable marine Actinomycetes diversity from tropical Pacific Ocean sediments. *Appl Environ Microbiol.* 2005; 7: 1039-1048.
12. Karthik L, Gaurav K, Bhasakara KV. Diversity of marine Actinomycetes from Nicobar marine sediments and its anti fungal activity. *Int J Pharm and Pharma Sci.* 2010; 2:199-203.
13. Sivakumar K, Maloy KS, Kathiresan K. Isolation and characterization of Streptomycetes producing antibiotic from mangrove environment. *Asian J Microbiol Biotech Environ Sci.* 2005; 7:457-464.
14. Santina M, Luigi M, Consolazione, Matteo B, Viviva B, Renato F, Angelina LG. Antagonistic interaction between psychrotrophic cultivable bacteria isolated from Antarctic sponges: A priliminary analysis. *Res Microbiol.* 2009; 160:27-37.
15. Nonomura H. Key for classification and identification of 458 species of the Streptomycetes included in ISP. *J of Ferment Tech.* 1974; 52:78-92.
16. Shirling EB, Gottlieb D. Methods for characterization of Streptomycessp. *Int J Syst Bacteriol.* 1966; 16:313-340.
17. Iqbal M, Ali KM, Daraz B, Siddique U. Bacteriology of mastitic milk and in vitro antibiogram of the isolates. *Pak Vet J.* 2004; 24:161-164.
18. Hassan A, Rahman S, Deebe F, Mahmud S. Antimicrobial activity of some plant extracts having hepatoprotective effects. *J. Medicin Plants Res.* 2009; 3: 20-23.
19. Spangler SK, Jacobs MR, Appelbaum PC. Bactericidal activity of DU6859a compared to activities of three quinolones, three Beta-lactams clindamycin and metronidazole against anaerobes as time-kill methodology. *Antimicrob Agents Chemother.* 1997; 41: 847-849.
20. Marderosian AD. Marine pharmaceuticals. *J. Pharm Sci.* 1969; 58: 1-30.
21. Austin B. A review on pharmaceutical compounds from marine bacteria. *J Appl Bacteria.* 1989; 67: 461-470.
22. Sathish KSR, Kokati VBR. In-vitro antimicrobial activity of marine actinobacteria against multi drug resistance *Staphylococcus aureus*. *Asian Pacific J. Tropical Biomed.* 2012; 23: S1802-S1807.
23. Deepika S, Talwinder K, Chandha BS, Rajesh KM. Antimicrobial activity of actinomycetes against *Staphylococcus aureus* , *E. coli* and various other pathogens. *Tropical J. Pharm. Res.* 2011; 10: 801-808.
24. Benita MR, Kannabiran K. Identification of antibacterial secondary metabolite from marine Streptomyces sp. VITBRK4 and its activity against drug resistant gram positive bacteria. *Int J Drug Dev & Res.* 2013; 5: 224-232.
25. Aravamuthan N, Gaurav K, Karthik L, Bhaskara RKV. In-vitro antagonistic activity of soil actinobacteria against multidrug resistant bacteria. *Pharmacologyonline.* 2010; 2: 507-516.