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Research Article

Novel class of antimicrobials from the marine isolates of actinomycetes and their potential screening against multidrug-resistant bacterial strains

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Abstract
Microbial pathogenesis contributes a significant proportion to the global human mortality rate. Further, the outbreak of antimicrobial-resistant strains represents an alarming threat to human and animal healthcare, which drives scientific research on searching for novel antimicrobials. The present study is one such initiative to isolate new classes of antibiotics from the marine actinomycetes to combat the perpetual increase of multidrug-resistant strains. The soil samples from Tamil Nadu, India's coastal regions, were collected, and eight isolates of the actinomycete species (S1, S1b, S2, S3, S4b, S4W, S4R, S5) were recovered. From their 16S rRNA sequencing, the isolates belonged to *Streptomyces sp.*; the phylogenetic tree was constructed through the neighbour-joining method. Further, the secondary metabolites of all the isolates were screened against ATCC strains, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25927) and *Acinetobacter baumanni* (ATCC 19606) and multidrug-resistant (MDR) strains, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), carbapenem-resistant *Klebsiella pneumonia* (OXA) and colistin-cephalosporin resistant *Escherichia coli* (MCR). Of the eight isolates, S1b and S3 showed good inhibition for all the strains tested and their genomic sequences were sequenced and submitted to Genbank, MK641472 (S1b) & MK641473 (S3). Conclusively, their metabolites were purified using LC-MS and no resemblances were found with standard classes of antimicrobials such as nitrofurans, sulfonamides, fluoroquinolones, tetracyclines, chloramphenicols or ivermectins, which suggests that these metabolites are novel and could be exploited for the prospective antimicrobial research.

Keywords: Liquid chromatography-mass spectrometry, Marine actinomycetes, Multi-Drug Resistance, Novel antimicrobials, 16S rRNA

INTRODUCTION

Owing to the improper and unregulated usage of antibiotics, the rapid outbreak of Multi-Drug Resistance (MDR) bacterial strains across the globe is highly inevitable. It is quite an alarming threat that greatly threatens the human community as it spreads exponentially (Ventola, 2015). Further, the discharges from the industrial sectors, aquaculture farms and agronomic practices might lead to the distribution and acquisition of a wide variety of antibiotic-resistant traits in the bacterial population (Serwecińska, 2020). Alternatively, the evolutionary changes due to the random/site-specific mutations can cause the organisms to acquire resistant properties at their genomic level (Arber, 2000; Durão *et al.,* 2018; Hadjadj *et al.,* 2019; Souque *et al.,* 2021; Baquero *et al.,* 2021 and Feng *et al.,* 2022). Owing to all these, even many of the non-pathogenic

bacteria belonging to the gram-positive and gram-negative classes are now included as a potent threat to the well-being of the human population. The most common MDR bacterial strains are methicillin-resistant *Staphylococcus aureus* (MRSA), colistin-cephalosporin-resistant *Escherichia coli* (MCR), carbapenem-resistant *Klebsiella pneumonia* (OXA) and vancomycin-resistant *Enterococcus faecalis* (VRE). The high incidence of such pathogens and a lack of combinatorial chemistry-based therapeutic agents have led to searching for novel and potent antimicrobials (Lam, 2007). Such bioactive compounds can be produced by varied classes of bacteria and fungi as a part of their secondary metabolism (Rana *et al.,* 2019; Roy *et al.,* 2022; Lázár *et al.,* 2018; Windels *et al.,* 2019 and Maeda *et al.,* 2020).

Actinomycetes are one of the most important producers of antimicrobials, which can be isolated from soil samples, and their metabolites can be explored for potential use in pharmaceutical chemistry (Selim *et al.,* 2021). About 2/3rd of the naturally existing antibiotics are derived from these actinomycetes species, contributing significant welfare to the human community (Okami and Hotta, 1988). The species such as Streptomyces, Actinoplanes and Nocardia from the soil samples were identified as potent inhibitors of gram-negative and -positive bacteria and fungi (Dhanasekaran *et al.,* 2009). The coastal regions of Tamil Nadu, India, are identified as one of the richest pools of actinomycetes as it has a diversified community with varying degrees of metabolite secretion potentials.

Secondary screening using MDR is used to identify and characterize microorganisms that exhibit resistance to multiple antimicrobial agents, including antibiotics (Sapkota et al., 2020). This type of screening is particularly relevant in the context of Actinomyces or any other microorganism with potential antibiotic-producing capabilities. MDR screening helps identify microorganisms that can withstand the effects of multiple antibiotics. These resilient strains are of particular interest because they may contain novel resistance mechanisms or produce potent antimicrobial compounds. Actinomyces and other antibiotic-producing microorganisms are valuable resources for drug development. MDR screening helps identify strains that produce antibiotics effective against multidrug-resistant pathogens, providing potential leads for new drug candidates. Studying MDR microorganisms, including Actinomyces, contributes to the One Health approach, which recognizes the interconnectedness of human, animal, and environmental health. Understanding MDR in soil microorganisms can shed light on the spread of antibiotic resistance through environmental reservoirs. Actinomyces and other MDR microorganisms can be used in biotechnological applications, such as bioremediation or enzyme production. Understanding their resistance profiles is essential for optimizing their use in various industries. Actinomyces and other antibiotic-producing microorganisms are valuable resources for drug development. MDR screening helps identify strains that produce antibiotics effective against multidrug-resistant pathogens, providing potential leads for new drug candidates.

With this fact, the present study has isolated the actinomycetes species from those soil samples, characterized their morphological features and sequenced their genomes. Further, a series of primary screening using the standard ATCC strains and secondary screening using screened MDR strains were examined to evaluate the antimicrobial ability of the isolates. Also, metabolites of the isolates were assessed to facilitate the development of novel antimicrobials against the wide range of MDR strains.

MATERIALS AND METHODS

Methodology

Collection of samples

Soil samples were collected from the seashore along the coastline of Tamil Nadu in aseptic uricol bottles which includes Thiruvanmiyur Beach (12.9736°N, 80.2665°E), Kottivakam Beach (12.9661°N, 80.2651° E), Edward Elliot's Beach(12.9989°N, 80.2719°E), Marina Beach (13.0550°N, 80.2824°E), Kovalam Beach (13.0827°N, 80.2707°E). The samples were kept in sterile air-tight poly bags during the transportation and preserved at room temperature until further use.

Isolation of actinomycetes

Actinomycetes were isolated from the collected marine soil samples using Actinomycete Isolation Agar (AIA) and Yeast Malt Extract Agar (YMEA), which were dissolved in filtered sea water augmented with the group of antibiotics viz. rifampicin (4.5µg/ml), fluconazole (150µg/ml) and amphotericin B (25µg/ml) as described by Chaudhary *et al.* (2013) and Hanshew *et al.* (2015). To mimic the native environment of marine actinomycetes, the sterilised sea water was used for the media preparation. Serially diluted and titrated soil samples were streaked on YMEA and AIA plates and incubated at 37°C for 14-28 days to observe the growth. The obtained colonies were gram-stained and visualized under a microscope (oil immersion lens, 100X). Colonies showing typical actinomycete-like appearances were sub-cultured.

Antimicrobial screening

Primary screening

The primary screening was done to screen the antimicrobial action of actinomycetes using Muller-Hinton

(MH) agar plates on the screened ATCC strains such as *Acinetobacter baumanni* (ATCC 19606), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25927) and *Staphylococcus aureus* (ATCC 25923). As per the modified Cross-streak method, the plates were inoculated with a single streak of actinomycete culture in the centre of the petri plate. The plates were incubated at 37°C for about 7 days to allow the substantial growing ability of the colonies and to produce and diffusion of antibiotics in the agar medium. After incubation, the ATCC strains were streaked perpendicular to the actinomycete and incubated to observe their antibacterial potential.

Secondary screening

The actinomycetes screened from the primary screening were subjected to investigate the antimicrobial potency against the multidrug-resistant bacterial strains such as Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus faecalis* (VRE), Carbapenem resistant *Klebsiella pneumonia* (OXA) and Colistin-cephalosporin resistant *Escherichia coli* (MCR) using the cross streak method as described in the previous section.

Biofilm disruption potential

The secondary metabolites from the isolates were tested for their activity against biofilm formation. *Staphylococcus aureus* was inoculated in MH broth supplemented with 2 mg/mL of glucose. The supernatant from the broth containing actinomycetes was added to each plate. The plates were left undisturbed for 48 hours in an incubator at 37°C. Following biofilm formation, the plates were washed twice with sterile water. The washed plates were stained with 0.1% crystal violet and the coverslips were visualized under the light microscope.

16S rRNA sequencing of actinomycetes

The genomic DNA of the isolates was extracted using the Qiagen's genomic DNA extraction kit as per manufacturer's instructions (Catalogue no: 69504). A 16S rRNA gene which was PCR-targeted was performed for the bacterial isolate with 25µL reaction volume consisting of broad-range 16S rRNA primers as described by Weisburg *et al.* (1991): 16S Forward primer -5'-AGAGTTTGATCCTGGCTCAG-3' and 16S Reverse primer -5' ACGGCTACCTTGTTACGACTT-3' (10 pM of each primer) (Table 1), 10× PCR buffer ,10 mM of dNTP mix, 1 unit Taq DNA, DNA template (0.1-1 µg of DNA) and PCR grade water (Table 2). The PCR amplification was performed in Mastercycler (Eppendorf, Germany) with initial denaturation at 95°C for 3 min followed by 37 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 1 min, and with final extension at 72°C for 7 min. The amplicons were resolved along with DNA markers in 0.8% agarose with ethidium bromide (10 mg/mL) by gel electrophoresis for ~25min at 135 V using Mupid-exU system (Takara, Japan) and the gel was analysed by BioGlow UV Transilluminators (Crystal Technology, USA). The resultant product size is 1500bp.

16S rRNA gene sequencing and BLAST analysis

The 16S rRNA PCR product was sequenced at Macrogen Inc. (Seoul, Korea) using ABI PRISM® BigDye™ Terminator and ABI 3730XL sequencer (Applied Biosystem, USA) using primers mentioned. The low quality ends of 16S rRNA sequences were trimmed by using Bio-Edit version 7.0.9 (Isis Pharmaceuticals) or Codon Code Aligner version 4.0 software (Codon Code Corporation). The species identification of the bacteria was achieved by comparing the nucleotide sequence of 16S rRNA gene against known sequences available in the GenBank microbial genomes database using the Basic Local Alignment Search Tool (BLAST), (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequences of the present study bacterial isolate were deposited in the National Center for Biotechnology Information (NCBI) GenBank database.

Phylogenetic analysis of 16S rRNA gene

Multiple nucleotide alignment of the 16S rRNA gene sequences was performed using CLUSTALW. The phylogenetic tree was constructed using the neighbourhood joining algorithm with a 500 bootstrap in the MEGA (molecular evolutionary genetic analysis) version 7.0 software. A total of 45 16S rRNA sequences of various *Streptomyces* sp. were used to build the evolutionary tree.

Extraction of bioactive secondary metabolites

The potent antibacterial compound-producing strains were grown in ISP-5 broth and incubated in a shaking incubator at 150 rpm, 30°C for 7 days (Singh *et al.,* 2017). Then the medium was centrifuged at 10,000 rpm for 10 min; the supernatant was subjected for

Table 1. Primers used in 16S rRNA PCR

Primer Name	Sequence	Product size (bp)
16S forward - fD1	5′-AGAGTTTGATCCTGGCTCAG-3′	1500bp
16S reverse - rP2	5′-ACGGCTACCTTGTTACGACTT-3′	

solvent extraction using an equal volume of ethyl acetate. After the vigorous shaking, the solvent phase was collected and dried. The dried residues were dissolved in dimethyl sulfoxide (DMSO) and used for further
analysis (Wei *et al.,* 2019).

RESULTS

Sample collection and isolation of actinomycetes

Actinomycetes are a group of filamentous, gram-positive bacteria commonly found in soil. They are known for their ability to produce spores and form branching hyphae, giving them a filamentous appearance like fungi. Actinomycetes play crucial roles in soil ecosystems and are considered essential contributors to soil health and fertility.

The isolation of Actinomyces from soil involves a series of steps, including culture and identification of these microorganisms. Actinomyces are a type of bacteria commonly found in soil and play an essential role in decomposing organic matter.

Eight isolates of actinomycetes were cultured from the five different soil samples and cultured on YMEA and AIA plates to enable maximal growth with an incubation period of 14-28 days as described (Janaki *et al.,* 2016 and Harir *et al.,* 2017) and their morphological observations are listed in Table 3. All the isolates were found to be gram-positive and showed distinct actinomycete-like morphological structures under the microscope (Fig. 1). The colonies were observed in 2 days of incubation period in YMEA media and 4 days in AIA media, resulting in the former media better suited for the rapid culturing of actinomycetes isolates.

Primary screening

Primary screening using plate culture is a widely used method in microbiology to identify and isolate microorganisms from a sample. This technique is particularly valuable when dealing with complex samples, such as soil, with diverse microbial populations. The primary screening aimed to select and isolate individual colonies of interest for further study or characterization.

The isolated actinomycetes species were studied for their antibacterial action against the screened ATCC bacterial strains viz. ATCC 25923, ATCC 29212, ATCC 25927 and ATCC 19606. All eight isolates showed good inhibitory potential against the ATCC 19606, and S1b and S3 showed the highest inhibitory potential against all the ATCC strains (Table 4). These strains were selected for further investigation.

Table 2. PCR Master mix composition

PCR components	Master Mix (25 µl reaction)
Sterile Milli Q water	18.4 µl
10x PCR Buffer (15mM$MgCl_2$) (10X)	2.5 µl
dNTP mix (10 mM of each dNTP)	1 µl
16S Forward primer (10 pmol)	1 µl
16S Reverse primer (10 pmol)	1 µl
Taq polymerase enzyme (5U)	0.1 µl
DNA template	1 µl
Total Volume	25 µl

Secondary screening

Secondary screening of Actinomyces colonies involves further characterization and evaluation of the isolated colonies from the primary screening. This step is essential for identifying specific Actinomyces strains and assessing their potential for various applications, such as antibiotic production, enzyme production, or other biotechnological purposes.

To further demonstrate the antimicrobial action of the selected isolates against the standard MDR strains such as MRSA, VRE, OXA and MCR, the secondary screening was performed and their observations are listed in Table 5. All the isolates showed good inhibitory action against MRSA strain, whereas the isolates S1b and S3 showed a remarkable inhibitory action against all the screened MDR strains (Table 5, Fig. 2). To screen for the antimicrobial potential of the actinomycetes, a modified Cross streak method (Promnuan *et al.,* 2020) was employed. Comparison with control plates showed that select species of actinomycetes had a good inhibitory potential against ATCC 25923, ATCC 29212, ATCC 25927, ATCC 19606, MRSA and VRE on actinomycete with a culture duration of 7 days. This suggested that a threshold time was required for the growth of actinomycetes and secretion of their bioactive

Table 3. Obtained Colonies' morphology and BLAST results

S.No	Isolates	Morphology	Identified Species
1	S1	White, powdery colonies	*Streptomyces sp.*
2	S1b	Dry, opaque colonies	*Streptomyces sp.*
3	S2	Milky white, chalky colonies	*Streptomyces sp.*
4	S3	Grey pigment producing colonies	*Streptomyces sp.*
5	S4b	Distinct white colonies	*Streptomyces sp.*
6	S4R	Grey pigment producing colonies	*Streptomyces sp.*
7	S4W	Dry, white colonies	*Streptomyces sp.*
8	S5	White, opaque colonies	*Streptomyces sp.*

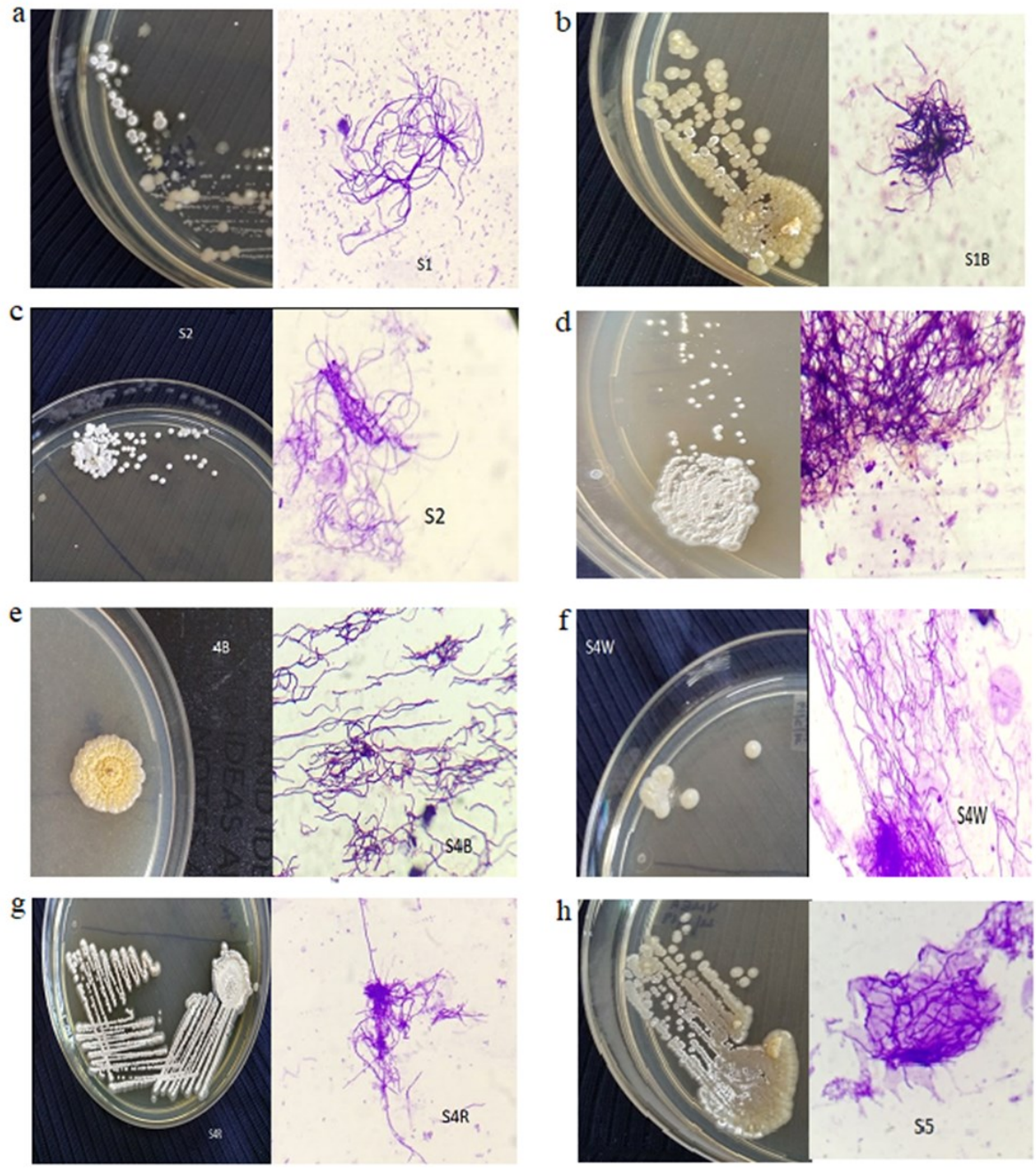


Fig 1. *Colonies morphology and its respective morphology of the eight actinomycetes isolates(Streptomyces sp.) (a) S1, (b) S1b, (c) S2, (d) S3, (e) S4b, (f) S4W, (g) S4R, (h) S5*

Table 4. Inhibitory potential of actinomycete isolates against the screened ATCC strains

Isolates	Standard ATCC bacterial culture			
	25923	**29212**	**19606**	**25927**
S1	+++	-	++++	-
S1b	++++	+	++++	+
S2	+	-	+++	-
S3	++++	++	++++	+++
S4R	++	-	++++	-
S4W	+	-	++++	-
S4b	+++	+	++++	-
S	+	-	++++	-

++++: Complete inhibition, +++: Moderate inhibition, ++: Partial inhibition, +: Low inhibition, -: No inhibition

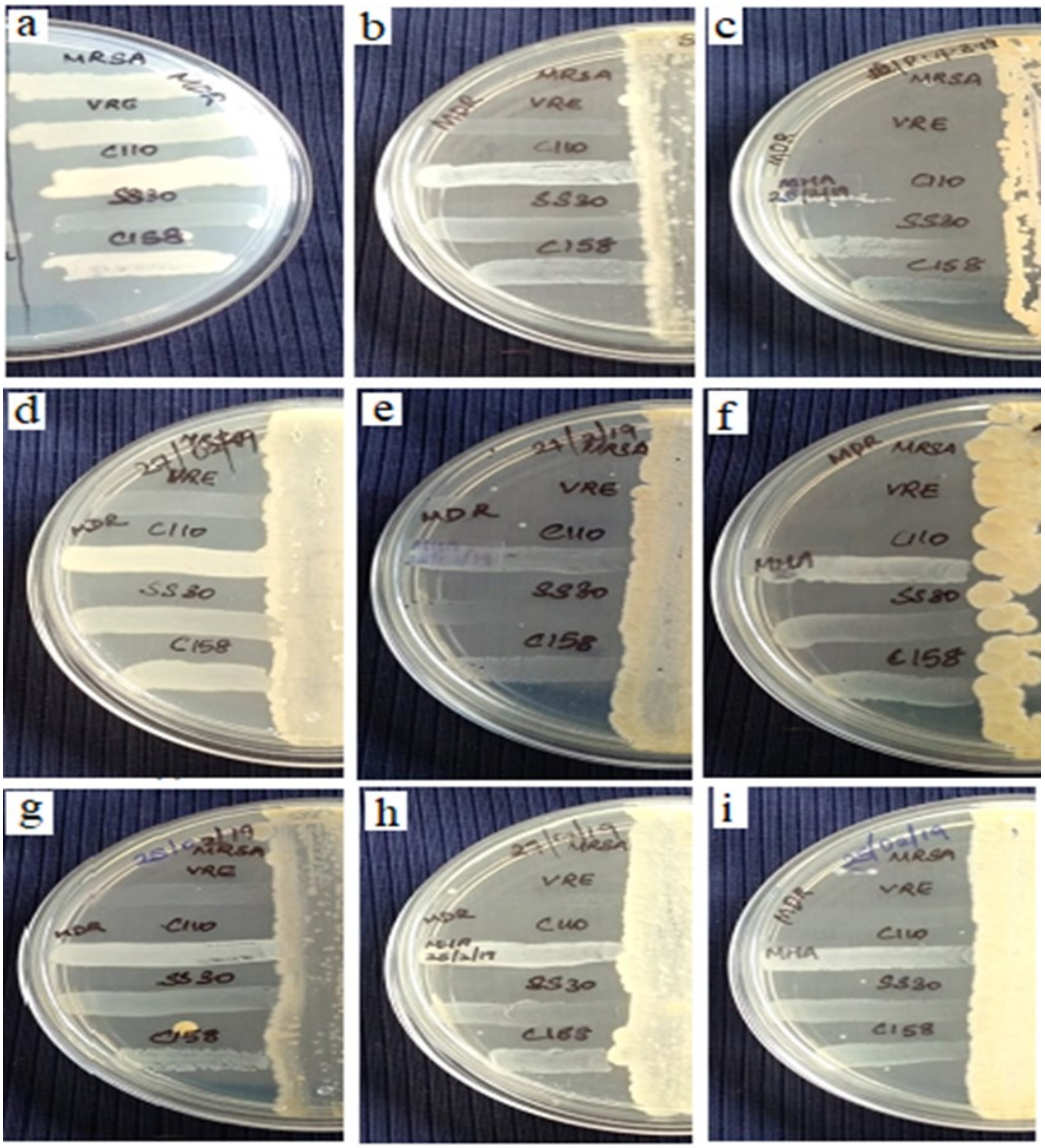


Fig 2. *Inhibitory action against the MDR bacterial strain samples (MRSA, VRE, OXA, MCR, NDM) a) control, b) S1, c) S1B, d) S2, e) S3, f) S4b, g) S4R, h) S4W and i) S5*

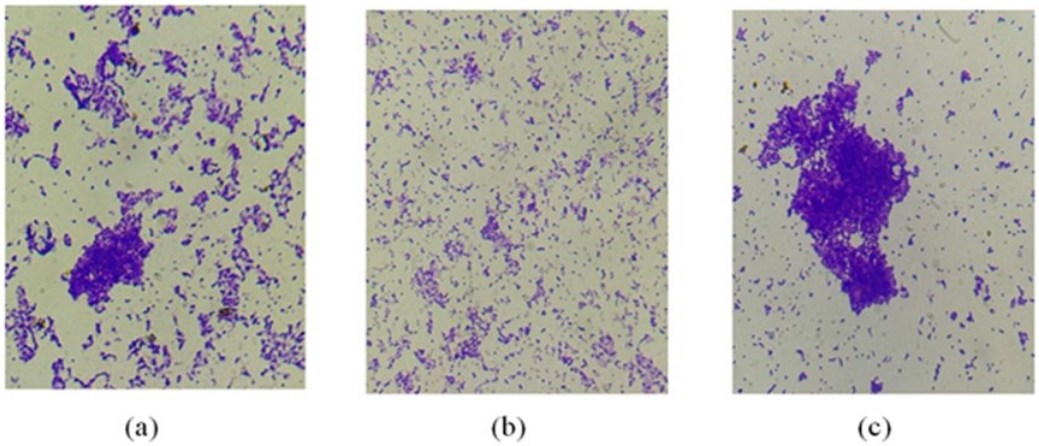


Fig 3. *Biofilm formation (a) control, (b) S1B and (c) S3 (Control showed instances of biofilm formation, observed as dense mass of bacterial growth under the microscope. The samples that were inoculated with* S1B and S3 in actinomycete broth did not show any significant inhibition of the biofilms of *Staphylococcus aureus)*.

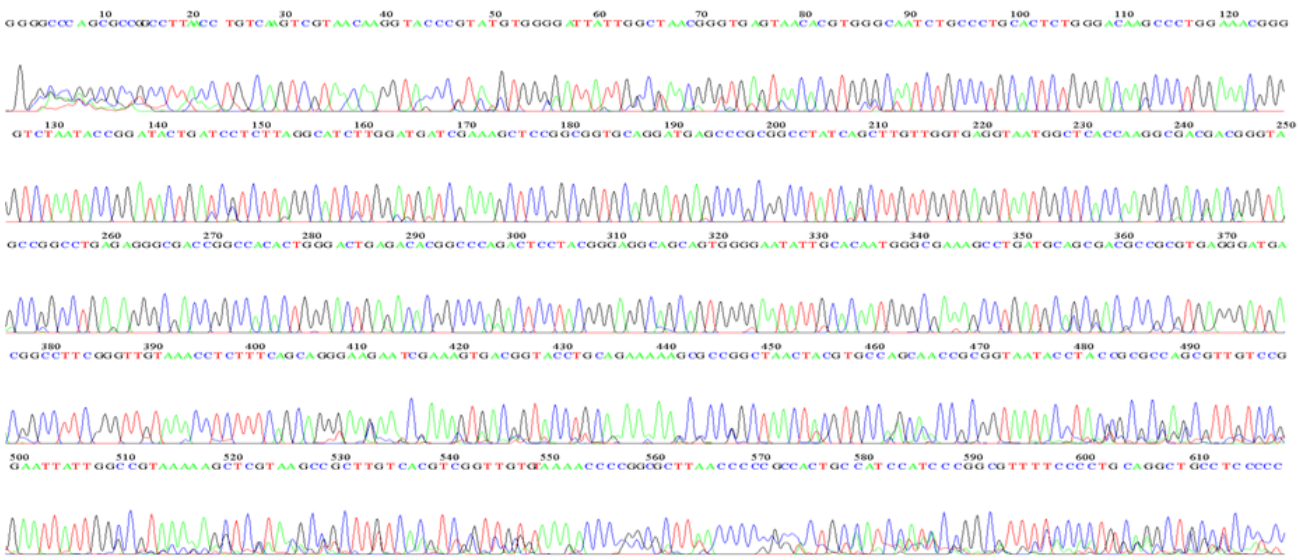


Fig. 4. *Electropherogram of 16srRNA gene of isolated Streptomyces sp.*

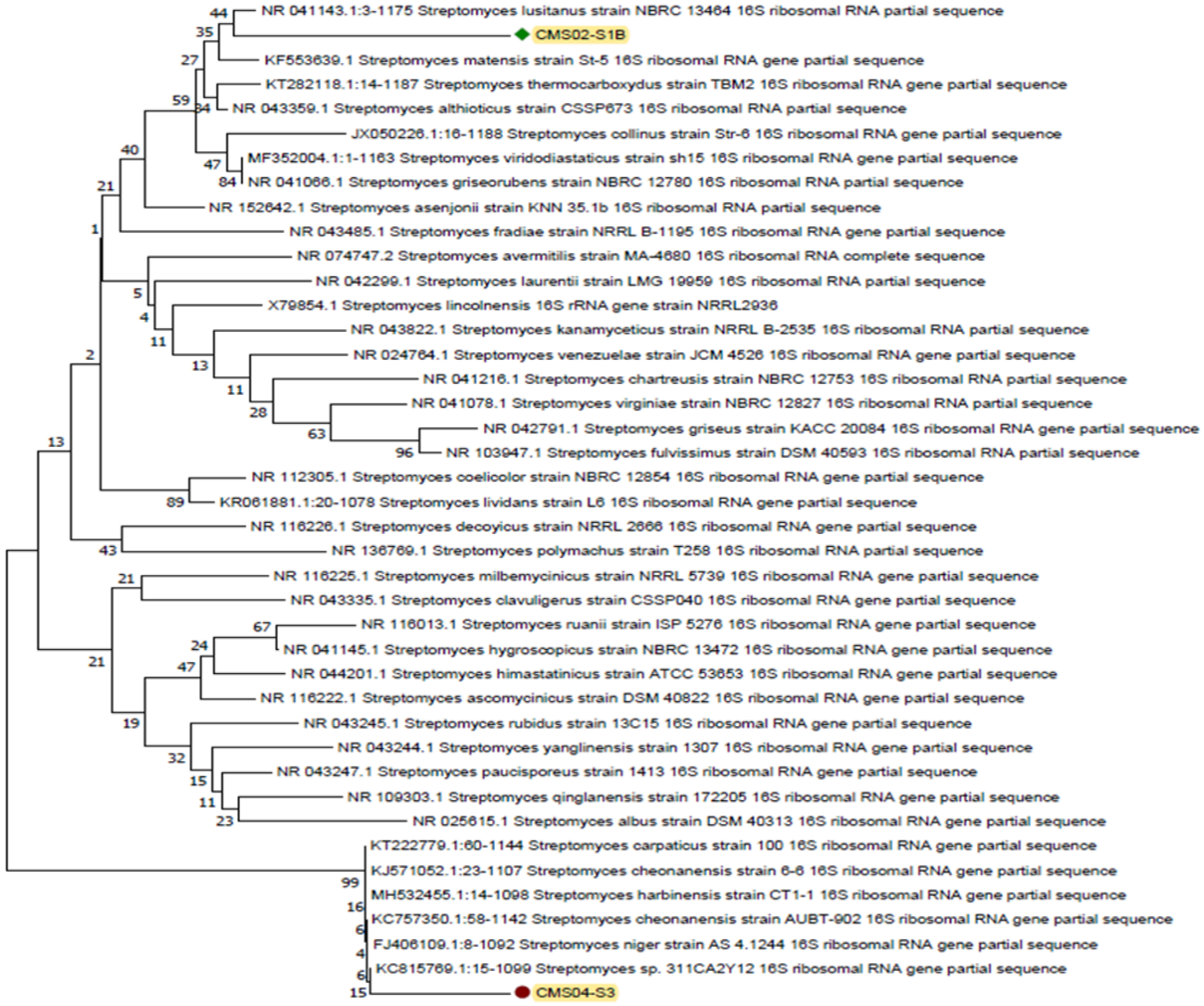
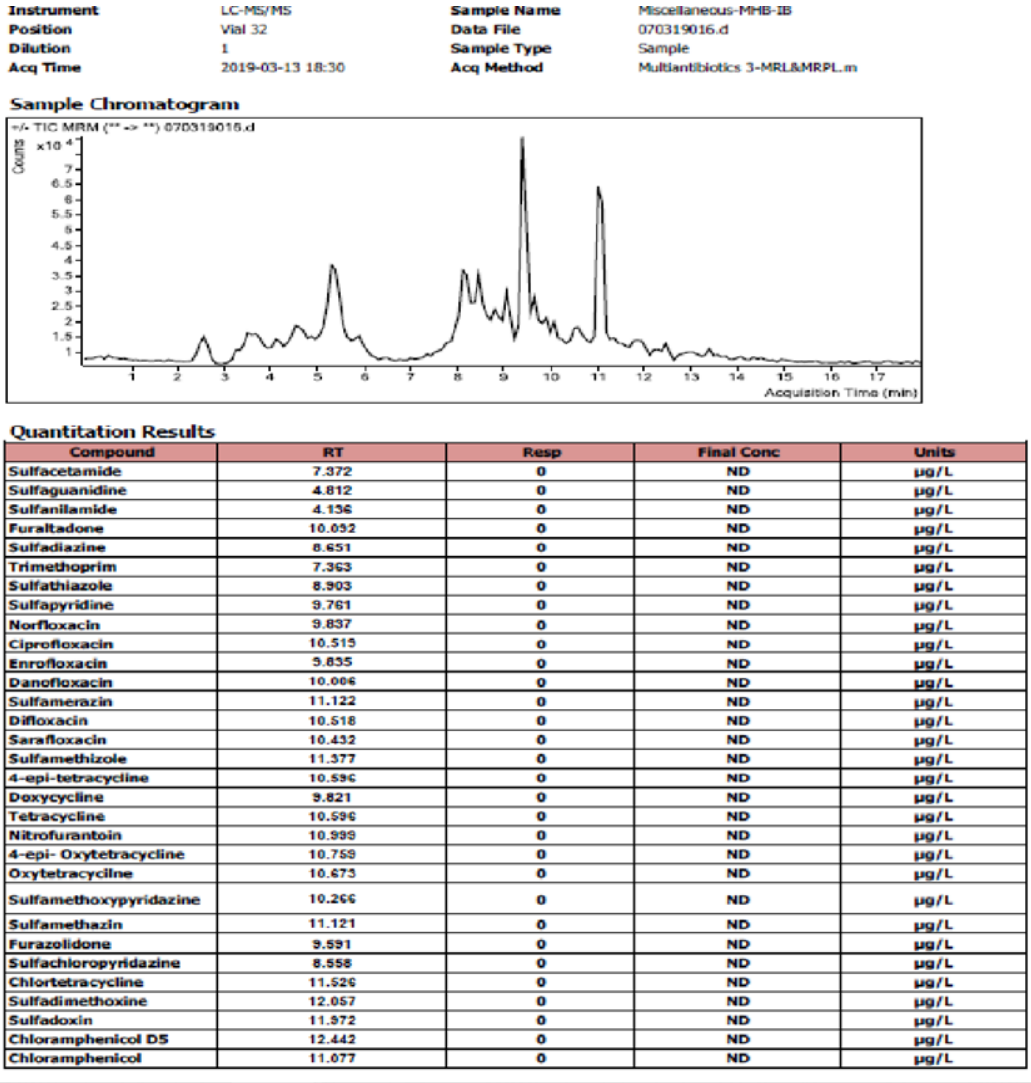


Fig 5. *Dendrogram of evolutionary origin relationship S1b (MK641472) and S3 (MK641473) Phylogenetic Tree formed by neighbour-joining method)*

Table 5. Inhibitory potential of the isolates against the screened MDR strains

Isolates	Standard multidrug-resistant (MDR) strains			
	MRSA	VRE	OXA	MCR
S1	++++	-	-	-
S1b	++++	+++	+++	++
S2	++++	-	-	-
S3	++++	+++	-	+
S4R	+++	-	-	-
S4W	++++	++	-	-
S4b	++++	+++	-	-
S5	+++	-	-	-

++++: Complete inhibition, +++: Moderate inhibition, ++: Partial inhibition, +: Low inhibition, -: No inhibition



Instrument	LC-MS/MS	**Sample Name**	Miscellaneous-MHB-IB
Position	Vial 32	**Data File**	070319016.d
Dilution	1	**Sample Type**	Sample
Acq Time	2019-03-13 18:30	**Acq Method**	Multiantibiotics 3-MRL&MRPL.m

Sample Chromatogram

Quantitation Results

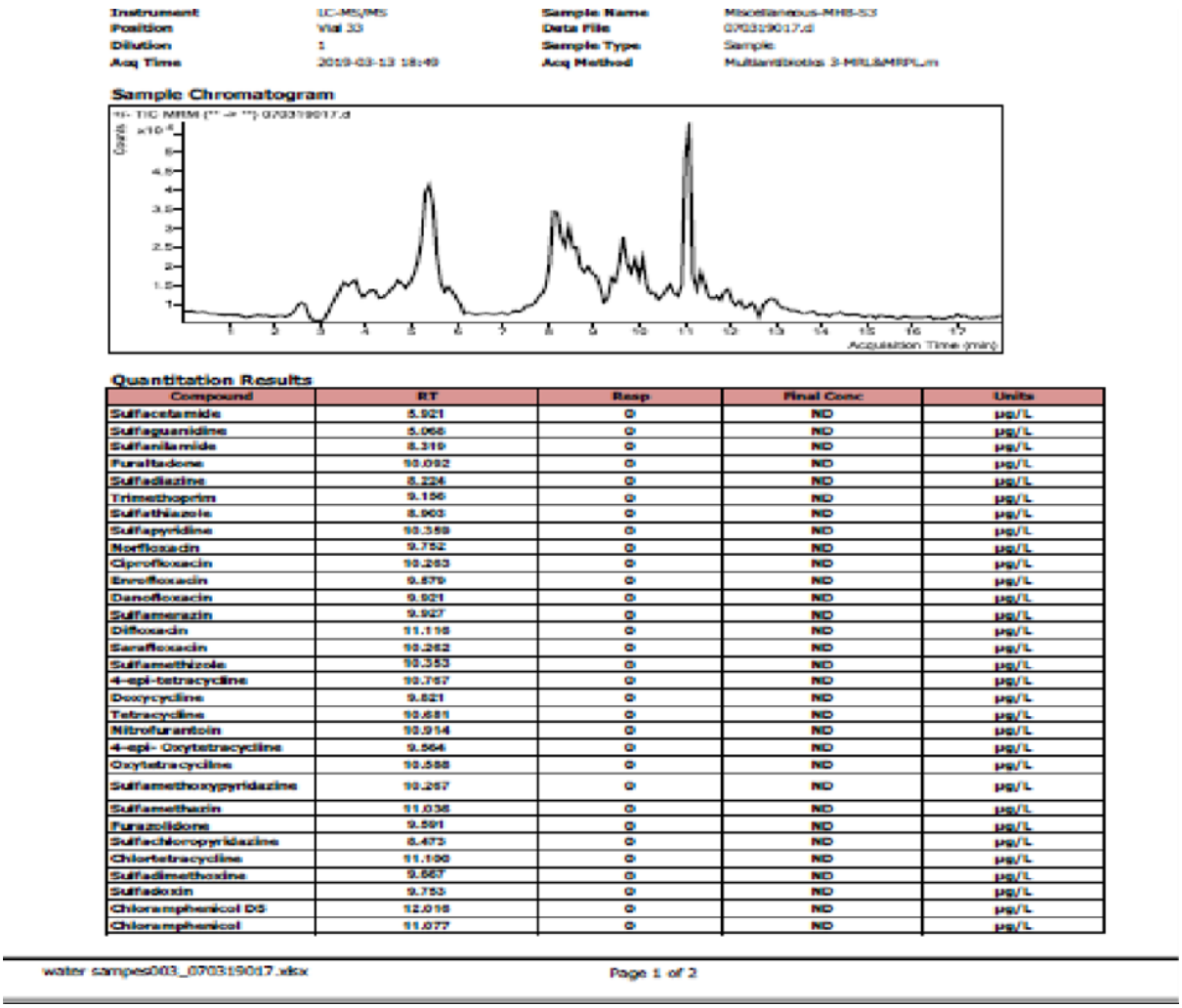
Compound	RT	Resp	Final Conc	Units
Sulfacetamide	7.372	0	ND	µg/L
Sulfaguanidine	4.812	0	ND	µg/L
Sulfanilamide	4.136	0	ND	µg/L
Furaltadone	10.052	0	ND	µg/L
Sulfadiazine	8.651	0	ND	µg/L
Trimethoprim	7.363	0	ND	µg/L
Sulfathiazole	8.903	0	ND	µg/L
Sulfapyridine	9.761	0	ND	µg/L
Norfloxacin	9.837	0	ND	µg/L
Ciprofloxacin	10.515	0	ND	µg/L
Enrofloxacin	9.835	0	ND	µg/L
Danofloxacin	10.006	0	ND	µg/L
Sulfamerazin	11.122	0	ND	µg/L
Difloxacin	10.518	0	ND	µg/L
Sarafloxacin	10.432	0	ND	µg/L
Sulfamethizole	11.377	0	ND	µg/L
4-epi-tetracycline	10.596	0	ND	µg/L
Doxycycline	9.821	0	ND	µg/L
Tetracycline	10.596	0	ND	µg/L
Nitrofurantoin	10.999	0	ND	µg/L
4-epi- Oxytetracycline	10.759	0	ND	µg/L
Oxytetracycline	10.673	0	ND	µg/L
Sulfamethoxypyridazine	10.266	0	ND	µg/L
Sulfamethazin	11.121	0	ND	µg/L
Furazolidone	9.591	0	ND	µg/L
Sulfachloropyridazine	8.558	0	ND	µg/L
Chlortetracycline	11.526	0	ND	µg/L
Sulfadimethoxine	12.057	0	ND	µg/L
Sulfadoxin	11.972	0	ND	µg/L
Chloramphenicol D5	12.442	0	ND	µg/L
Chloramphenicol	11.077	0	ND	µg/L

Fig 6. *LC-MS analysis of S1b showing that the isolated metabolite did not resemble any standard indicating that it could be a novel compound*

metabolites.

Biofilm formation

The biofilms formed upon the coverslips were observed under a light microscope. The control showed biofilm formation, which was observed as thick adhesive regions under the microscope. The samples that were inoculated with actinomycete broth did not show any significant inhibition of the biofilms. The sample inoculated with isolate S1B recorded lower instances of planktonic cells of *S. aureus,* whereas biofilm formation had not reduced (Fig. 3). The sample inoculated with the secretions of S3 showed neither a decreased planktonic cell count nor a reduction in the biomass



Instrument	LC-MS/MS	Sample Name	Miscellaneous-MHB-S3
Position	Vial 30	Data File	0703190017.d
Dilution	1	Sample Type	Sample
Acq Time	2019-03-13 18:49	Acq Method	Multiantibiotics 3-MRL&MRPL.m

Sample Chromatogram

Quantitation Results

Compound	RT	Resp	Final Conc	Units
Sulfacetamide	5.921	0	ND	µg/L
Sulfaguanidine	5.068	0	ND	µg/L
Sulfanilamide	8.319	0	ND	µg/L
Furaltadone	10.092	0	ND	µg/L
Sulfadiazine	8.224	0	ND	µg/L
Trimethoprim	9.156	0	ND	µg/L
Sulfathiazole	8.903	0	ND	µg/L
Sulfapyridine	10.359	0	ND	µg/L
Norfloxacin	9.752	0	ND	µg/L
Ciprofloxacin	10.283	0	ND	µg/L
Enrofloxacin	9.879	0	ND	µg/L
Danofloxacin	9.921	0	ND	µg/L
Sulfamerazin	9.927	0	ND	µg/L
Difloxacin	11.116	0	ND	µg/L
Sarafloxacin	10.262	0	ND	µg/L
Sulfamethizole	10.353	0	ND	µg/L
4-epi-tetracycline	10.767	0	ND	µg/L
Doxycycline	9.821	0	ND	µg/L
Tetracycline	10.681	0	ND	µg/L
Nitrofurantoin	10.914	0	ND	µg/L
4-epi- Oxytetracycline	9.564	0	ND	µg/L
Oxytetracycline	10.588	0	ND	µg/L
Sulfamethoxypyridazine	10.267	0	ND	µg/L
Sulfamethazin	11.036	0	ND	µg/L
Furazolidone	9.591	0	ND	µg/L
Sulfachloropyridazine	8.473	0	ND	µg/L
Chlortetracycline	11.196	0	ND	µg/L
Sulfadimethoxine	9.667	0	ND	µg/L
Sulfadoxin	9.753	0	ND	µg/L
Chloramphenicol DS	12.016	0	ND	µg/L
Chloramphenicol	11.077	0	ND	µg/L

Fig 7. *LC-MS analysis of S3 showing that the isolated metabolite did not resemble any standard indicating that it could be a novel compound*

formation of *S. aureus* as observed by Zheng *et al.* (2022).

16S rRNA Amplification and sequencing

DNA samples were extracted from all the isolates and were directed to PCR using the specified forward and reverse primers. The electrophoresis (1% agarose gel) results indicated that the band size of 1500 bp corresponds to 16S rRNA of actinomycetes. Further, the sequences of the colonies were deduced through Sanger's dideoxy sequencing to facilitate the phylogenetic analysis. The low-quality sequences were trimmed using Bioedit v.7.0.9 (Isis pharmaceuticals) or Codon code aligner version 4.0 (Codon code corporation) and used as the primary reference for BLAST search and from their results. The isolated actinomycete species were identified as *Streptomyces sp.* as shown (Fig. 4 & Table 6). The sequences of S1b and S3 were submitted to Genbank, and accession numbers were obtained, such as MK641472 for S1b and MK641473 for S3. The evolutionary origins of the isolated species were constructed using the neighbour-joining method and depicted in the dendrogram (Fig. 5).

LC-MS analysis

Fig. 6 and 7 illustrate the LC-MS chromatogram of the secondary metabolites derived from the isolates S1b and S3, respectively. Further, their absorption peaks were compared against a wide range of standard antibiotic standards, viz. nitrofurans, sulfonamides, pyridazines, fluoroquinolones, tetracyclines, chloramphenicols and ivermectins. As none of the observed peaks corresponded to any of the standards, the metabolites of the isolates could belong to a new class of antimicrobials. Since the coastal regions of Tamil Nadu are rich in actinomycete diversity, there might be a greater chance of indigenous antibiotic secretors in the local habitat.

Conclusion

The present study isolated 8 Streptomyces sp. (S1, S1b, S2, S3, S4b, S4W, S4R, S5) marine actinomycetes

from the Thiruvanmiyur Beach (12.9736°N, 80.2665°E), Kottivakam Beach (12.9661°N, 80.2651°E), Edward Elliot's Beach(12.9989°N, 80.2719°E), Marina Beach (13.0550°N, 80.2824°E), Kovalam Beach (13.0827°N, 80.2707°E) and of these two isolates, S1b and S3 showed excellent inhibitory action against ATCC 25923, ATCC 29212, ATCC 25927, ATCC 19606, MRSA, VRE, OXA and MCR. This indicated that the metabolites of the S1b and S3 were potent antimicrobial agents against MDR strains ((Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococcus faecalis* (VRE), Carbapenem-Resistant (OXA) *Klebseilla pneumoniae* and Colistin-Cephalosporin Resistant *Escherichia coli* (MCR). Sanger's dideoxy sequencing of the isolates was performed, and the sequences were submitted to Genbank with the accession numbers MK641472 (S1b) and MK641473 (S3). The BLAST research and the phylogenetic tree analysis using the N-J method indicated that the isolates were identified as *Streptomyces* sp. Eventually, the metabolites were purified using LC-MS and analysed for resemblances with the standard classes of antimicrobials such as nitrofurans, sulfonamides, fluoroquinolones, tetracyclines, chloramphenicols or ivermectins and found to be negative. Hence, from the observations of the present study, the secondary metabolites of the isolates, S1b and S3 were found to be novel and could be explored for the futuristic development of the antimicrobial compounds.

Conflict of interest

The authors declare that they have no conflict of interest.

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