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Der Pharmacia Lettre, 2015, 7 (6):116-124
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Anti-CTXM-ESBL (extended spectrum B-lactamase) activity of marine actinobacteria

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ABSTRACT

Urinary tract infection (UTI) is the most common and important clinical problem in millions of humans each year and drug resistant uropathogens like CTXM-extended spectrum β-lactamase (CTXM-ESBL) producers poses a catastrophic threat to healthcare community worldwide. ESBL pathogens were isolated from urine samples (90 nos) collected at Health center, VIT University, India. Phenotypic and genotypic characterization (CLSI-2010 guidelines) resulted inidentification 10 ESBLs isolates (Escherichiacoli(3 isolates)and Klebsiellapneumoniae (7 isolates).Uniplex PCR revealed CTXM-ESBLs (3/10). The Streptomyces species isolated from Cheyyur beach, Tamil Nadu, India, was screened for its inhibitory activity against clinically important MDR-ESBL producers (E.coli and K.pneumoniae). The ethyl acetate extract prepared fromStreptomycesspeciesexhibited anti-ESBL activity against CTXM-ESBL pathogens with the MIC value of 0.25 µg/mL against E. coli (S1), 2.0 µg/mL against K.pneumoniae (S2) and 0.5 µg/mL against E. coli (S3). Ertapenem was used as a positive control.The isolate was characterized by molecular taxonomy and identified to be belonged to the genus Streptomyces and designated as Streptomyces sp. VITSJK8.The results of our study shows that Streptomyces sp. VITSJK8 is the potential isolate for isolation anti-ESBL compounds.

Key words*: Streptomyces* sp. VITSJK8, CTXM-ESBL, anti-ESBL activity, urinary tract infection

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INTRODUCTION

Urinary tract infection continues to be the second most common and leading infection among human adults despite widespread availability of antibiotics. *E. coli* and *Klebsiella*speciesare the most frequent pathogens that cause UTI, bacteremia and intra abdominal infections [1]. In the recent past, there have been significant changes in the antimicrobial resistant pattern of uropathogens[2]. Physicians depend upon clinical expertise and published guidelines with choices in empirical therapy for system based infections. Emergence of drug resistant microbeslike extended spectrum β-lactamase pathogens are important cause of treatment failure in UTIs[3]. Main contributing factor for multi-drug resistance in ESBLs is the β-lactamase enzyme, this enzyme hydrolyze β-lactam ring present in the β-lactam antibiotic thereby inactivating the compound. They mediate resistance against first, second and third line cephalosporins and monobactams, but not to cephamycins[4].Molecular classification of ESBLs includes: TEM, SHV, CTX-M, OXA and AMP-C types [5]. There have been several cases of treatment failure due to UTI caused by CTX-M ESBLs. These enzymes are mostly found in the Enterobacteriaceae family and in few non-fermentors. Over 50 CTX-M types have been reported, they can be divided into 5 groups (CTX-M1, CTX-M2, CTX-M3, CTX-M4, CTX-M5) based on their amino acid sequence [6]. In Israel and Spain CTX-M *E. coli* is an important cause of community-onset blood stream infections [5]. High prevalence of CTX-M β-lactam resistance in Cambodia due to the intrinsic capacity of CTX-M–encoding genes was already reported[7]. Due to increasing multi-drug resistance among medically important pathogens against highline antibiotics, the necessity for safe and potent compounds are of current importance to combat drug resistant strains. Natural sources are prime resources for synthetic organic chemists and drug discovery researchers. Marine environment contains tremendous diversity and novelty that are prolific producers of thousands of biologically active secondary metabolites. In particular, marine actinomycetes are

one of the most efficient groups of secondary metabolite producers and are highly valued from an industrial point of view. Representative genera of actinobacteria include *Streptomyces, Micromonospora, Arthrobacteria, Corynebacteria, Actinomyces, Frankia,* and several others. Over 22,500 bioactive compounds from actinomycetes alone have been reported and majority of them belong to the genus *Streptomyces*[8]. Marine microbes had produced wide variety of secondary metabolites that are used for drug development and serves as a source of modern pharmaceutical companies [9]. Some of the hallmark of secondary metabolite production from actinomycetes includes non-ribosomal peptide synthetase (NRPS), and polyketide synthetase (PKS) biosynthetic pathways. In specific, genus *Streptomyces*is a major producer of enormous bioactive molecules. *Streptomyces*has yielded valuable therapeutics like broad spectrum antibiotics amino glycosides, chloramphenicol, macrolides, tetracycline, etc. Antitumor drugs exemplified by adriamycin, antifungals such as amphoteracin, immunosuppressant includes tacrolimus and other industrially important enzymes lipases, caseinase, amylase, cellulases etc.[10]. They hold a prominent position in screening due to their proven ability to produce pharmaceutically important novel metabolites. Considering the consequences of developing resistance among threatening diseases and in the view of potential marine actinomycete secondary metabolite as a source of novel bioactive compounds, we evaluated the anti–ESBL potency of *Streptomyces* species against CTXM- ESBLs. Multi drug resistance (MDR) is the key to the development of therapeutic and chemotherapeutic drugs.

MATERIALS AND METHODS

Collection of urine sample

Urine samples were collected in a sterile container from human volunteers at Health center, VIT University, Vellore, Tamilnadu, India and labeled. Volunteers were instructed to collect morning first voided, clean catch mid-stream urine after thorough cleansing of external genitalia to avoid urethral contamination and to target uropathogens[11]. Samples were collected in wide mouthed containers and transferred to the laboratory and processed immediately. Standard precautions were followed while processing the samples. Smear examination (Gram stain, 100X) is used as a guide for choice of the media. Direct sensitivity is done on Muller Hinton Agar (MHA) if the smear reveals one type of organism. If the staining observation shows more than one type of organism,0.1 mL of the sample was streaked upon nutrient agar and MacConkey agar media to obtain isolated colonies. All plates were incubated aerobically at 37ºC for 24 hours.

Screening for ESBL pathogens

Coliform bacteria were targeted and identified based on their morphological, cultural and biochemical characteristics with the help of Bergey's manual of systemic bacteriology. Sub-culture was made upon MacConkey agar, nutrient agar media and Gram staining was carried out to study their morphological characteristics. Sub-culture was made to obtain isolated pure colonies for further investigation. Recognized coliform bacteria were identified through biochemical tests such as methyl red, vogesproskauer, indole, citrate, urease, mannitol motility test and triple sugar iron (TSI), lysine iron agar (LIA). All the media were incubated for 24 hours at 37ºC [12]. Identified uropathogens (n=33) were screened for ESBL production. Phenotypic confirmation was done by disc diffusion (Kirby-Bauer) test and interpretations were compared to CLSI 2010 guidelines. Ampicillin (30 µg), amikacin (30 µg), piperacillin/tazobactam (10µg/100 µg) and third line cephalosporins like cefotaxime (30 µg), cefotaxime/clavulanic acid (30 µg/10 µg), ceftazidime (30 µg), ceftazidime/clavulanic acid (30 µg/10 µg) (Hi-media, Mumbai) discs were used. A lawn culture of ESBL bacterial suspension was spread using sterile swabs on Muller-Hinton agar media. Plates were made to stand for 3-4 minutes to allow absorption of excess moisture before application of the discs. The plates were incubated aerobically at 37ºC for 16-18 hours. For disc diffusion testing,>5 mm increase in a zone diameter for either antimicrobial agent tested in combination with inhibitor compared to its zone when tested alone confirms an ESBL-producing organism. Control was satisfactory in comparison to the standard chart. Zones of inhibition wereinterpreted by referring to Tables 2A through 2I (zone diameter interpretative standards and equivalent minimum inhibitory concentration breakpoints) of the NCCLS M100-S12: Performance standards for antimicrobial susceptibility testing: Twelfth informational supplement [13].

Molecular identification of ESBL pathogens

Molecular characterization to detect the presence of ESBL gene was carried out using uniplex polymerase chain reaction (PCR) [14](Singh et al., **2012**). To identify bla_{TEM} gene the following forward primer GTATCCGCTCATGGAGACAATAACCCTG and 5' CCAATGCTTAATCAGTGGAGGCACC 3' reverse primer was used. For bla_{SHV} gene the following forward primer 5' CGCCTGTGTATTATCTAAATGTTAGCC 3' and 5' TTGCCAGTGCTCGATAGAC 3'reverse primer was used. For bla_{CTXM}genethe following forward primer 5' CGCTTTGCGATGCGAG 3' and 5' ACCGCGATATCGTT 3'reverse primer was used. The following procedure was adapted for DNA template preparation; 500 µL of test culture and control strains were mixed well by vortexing in order to obtain a uniform suspension. Bacterial cells were heated at 100ºC for 10 minutes and the suspension was

then centrifuged at 10,000 rpm for 5 minutes. Heat lyses of the bacterial cell and centrifugation removes cell debris. Supernatant was used as a template. A total of 25µL [master mix (20µL) and DNA (5µL)] was dispensed to the tubes. Master mix contains the following: 2.5 µL of PCR buffer, 2.5 µL of 2 mM DNTPs, 2.5µL of 25 mM $MgCl_2$, 0.3 µLof Taq polymerase (Fermentas, Glen Brunie, MD, USA), 10.2 µLMilliQ H_2O their forward and the reverse primers 1µL each. The presence of PCR products were checked by running electrophoresis with 1.5% agarose gels in 0.5X TBE buffer. The gels were stained with 5µg/mL ethidium bromide. PCR products were visualized under Ultra-Violet (UV) light. *E. coli* A-2-23 was used as the control strain for the PCR [15].

Sampling and isolation of marine actinomycetes

Marine sediment samples were collected at Cheyyur beach, Kanchipuram district, (latitude-12º 66´, longitude-79º 54´), Bay of Bengal, India. Sample was collected at the depth of 1 meter in the sea. 15-20g soil was collected in a sterile airlock polythene bag along with sea water to maintain ambient temperature during transport.The sediment samples were transported to the laboratory immediately and stored at 4ºC in the refrigerator. Samples were aseptically transferred to sterile petri-dishes and air dried in the laminar hood for 10-15 hrs and pretreated at 45ºC for 20-30 days. Preheated samples were considered for isolation of actinomycetes. Serial dilution up to (10^{-6}) was carried out and 0.1mL of each dilution was spread plated on various media prepared with 50% of sterile sea water to mimic the sample source environment. Actinomycetes isolation agar (AIA), International Streptomyces Project-1 (ISP-1), Kuster's agar, Starch Casein Agar (SCA) media supplemented with nalidixic acid (25µg/mL), cyclohexamide (25µg/mL) (Hi-media, India) was used to target selective isolation of actinomycetes[16]. The plates were incubated at 28ºC for 7-14 days and the observations recorded. Actinomycetes were recognized based on their colony morphology and sub cultured for further investigations. Triplicate copies of isolates were stored and maintained on ISP-1 slants stored at 4ºC and 20% (v/v) glycerol stock at -80ºC.

Fermentation and extract preparation

Matured sporulating colonies of actinomycetes were Gram stained to check for pure culture and then inoculated in to ISP-1 broth (containing 50% sterile sea water at pH7.0) in 1000 mL Erlenmeyer flask and fermented under sterile conditions in an orbital shaker for 14 days at 28ºC [17]. After fermentation the broth was centrifuged at 4000 rpm for 15 minutes. Equal volume of various solvents was added to the cell free culture supernatant and the flasks were kept in an orbital shaker over night for thorough mixing. Aqueous phase was collected using a separating filter and the extract was then concentrated in rotary vacuum and lyophilized in a freeze dryer (Thermo, USA). Every step was performed under strict aseptic conditions. All the extracts were screened for anti-ESBL activity against ESBL pathogens including CTXM-ESBLs and the extract showing significant activity was chosen for further characterization.Stock solution was prepared by using lyophilized crude extract dissolved in DMSO (20 mg/mL).

Culturing conditions

The potential actinomycetesisolate was optimized for morphological, cultural, physiological, and biochemical characteristics as per International Streptomyces Project (ISP) protocol. Direct examination of spores, their branching pattern, spore bearing hyphae were observed under 100X(oil immersion). Different media including AIA, SCA, ISP-1 to ISP-7 and ISP-9, Modified Bennett's agar, Kuster's agar (Himedia, India) were inoculated and incubated at 28°C, 14 days [18]. Actinomycete spores (10^7/mL)was used to inoculate ISP-1 broth in 1000 mL Erlenmeyer flask; carbon sources 1% (w/v) (D-glucose, D-mannitol, fructose, xylose, sucrose, raffinose, inositol, D-galactose, arabinose and rhamnose), nitrogen sources 1% (w/v) leucine, histidine, tryptophan, serine, glutamic acid, lysine, arginine, methionine, tyrosine), pH range, sodium chloride concentration (%) andvaryingtemperature ranges were investigated to examine the influence of different sources over the growth of the isolate [19]. Growth was measured as dry weight of the mycelium. Diffused pigmentations were recorded on ISP-3, ISP-1 and ISP-7. The reverse side pigments of the colony, distinctive (+), non distinctive (-) was tested using peptone yeast extract iron agar (ISP-6) [20]

Taxonomical characterization

DNA from the isolate was isolated by DNA isolation and purification kit (Himedia, India) and 16SrDNA was amplified by PCR using a master mix kitMedoxmix (Medox, India). The forward and reverse primers for sequencing, PCR conditions and the steps followed for sequencing were adapted from previous reports [21-23]. The 16S rDNA sequence of the isolate was determined on both strands using dideoxy chain termination method. The nucleotides obtained were searched through NCBI's GenBank database using nucleotide BLAST to check the similarity and homology of the sequence. Phylogenetic tree was constructed using GeneBee software along with the boot strap values. Maximum-parsimony method based phylogenetic tree was constructed using Mega 4.0.2—biologist-centric software for evolutionary analysis of DNA and protein sequences (Mincer *et al*., 2002). For G + C content determination, the DNA was isolated by the method of Marmurand the G + C content was determined using the thermal denaturation method of Marmur and Doty [24].

Screening for anti-CTXM ESBL activity

Anti-ESBL activity of the potential actinomycete isolatewas investigatedagainst CTX-M ESBL pathogens*E. coli*(S1), *K.pneumoniae*(S2), *E. coli*(S3) by broth micro dilution method using the standard protocol (CLSI M38). The lyophilized EA extract was dissolved in DMSO (16 µg/mL) and it was serially diluted using sterile water to obtain varying concentration (0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 µg/mL) and 100 µL of each concentration was delivered into the well (8mm/dm).ESBL bacterial suspensions were made to 0.5 McFarland standards turbidity using 0.85% sterile saline.This results in a suspension containing approximately 2 X 10^8 CFU/mL [25]. Ertapenem was used as a positive control. Results were interpreted as mean ± standard deviation (SD) of three independent values for the tests. All tests were carried out in an identical condition.

RESULTS

Identification of CTX-M ESBLS from UTI patients

Collected urine samples (n=90) were directly plated on MacConkey agar and nutrient agar media. Culturing of urine samples resulted in the isolation of 150 coliform bacteria. Direct microscopic examination (Gram stain, 100X) revealing single type of bacteria was directly taken for biochemical identification and antimicrobial susceptibility tests. Other morphologically distinct coliforms, preferably isolated pure colonies were sub-cultured on MacConkey agar and Gram stained to study their morphological characteristics. Biochemical tests including methyl red, vogesproskauer, indole, citrate, urease, mannitol motility test, TSI, LIA was done to identify the uropathogens. All tubes for biochemical tests were incubated aerobically for 24 hours at 37ºC.A total of 33uropathogens (22%) were identified and confirmed, remaining 117 isolates (78%) were other Gram negative bacteria. Out of 33 uropathogens, 21 were *E.coli* (70%) and12 were *K.pneumoniae*(30%).All 33 uropathogens were screened for ESBL production as per CLSI-M38-A protocol. Ten ESBL producers (*K.pneumoniae*(n=7) and *E.coli* (n=3) isolates) showing characteristic resistance patterns against third line cephalosporins were identified. These strains were stored in 1% nutrient agar deeps at -20ºC until further characterization. Disc diffusion test was carried out with the following antibiotics,ampicillin (30 µg), amikacin (30 µg), œfotaxime (30 µg), cefotaxime/clavulanic acid (30 µg/10 µg), ceftazidime (30 µg) and ceftazidime/clavulanic acid (30 µg/10 µg).The interpretation chart of ESBL producers is shown in Figure 1.

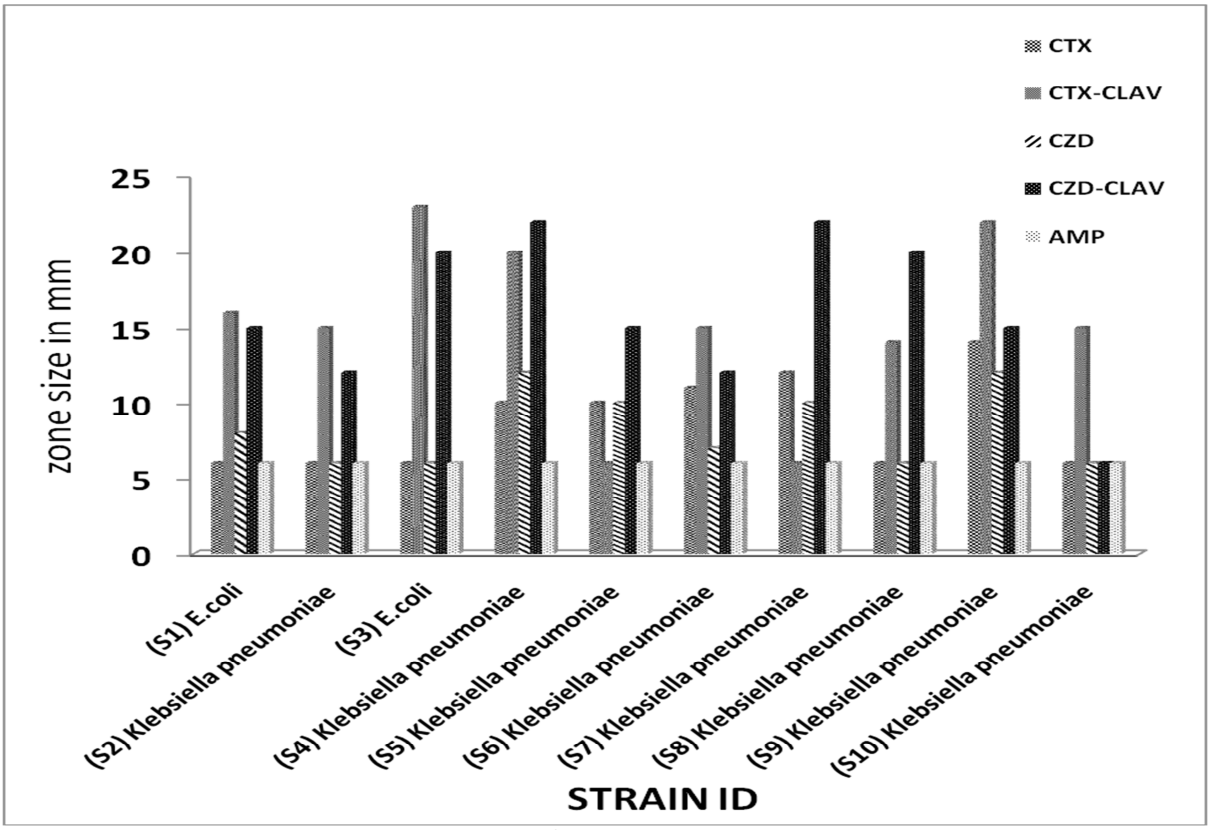

Figure1 Antibiotic susceptibility test of ESBL producing uropathogens against third line
Discs (Hi-media, Mumbai)AMP- Ampicillin 30 µg' CTX-Cefotaxime 30 µg; CTX/CLAV-Cefotaxime/Clavulanic acid 30/10 µg; CZD-Ceftazidime 30 µg; CZD/CLAV-Ceftazidime/Clavulanic acid 30/10 µg.

Molecular characterization was carried out using uniplex PCR method. Three CTXM-ESBL pathogens were identified. PCR products were electrophoresed in 1.5% agarose gel and visualized under ultra-violet (UV) light. The gel picture displays CTXM-ESBL isolates, lane-1 (S1) and lane-3 (S3) contains *E. coli*ESBL isolates while lane-2 (S2) has ESBL *K.pneumoniae*isolate. *E. coli* A-2-23 was used positive control(Figure2.).

Isolation and screening for anti-ESBL activity

A total of 54 actinomycete isolates were obtained from marine sediment samples. Colonies showing dry powdery appearance were selectively subcultured on ISP-1 media and incubated at 28°C for 14 days. Each actinomycete isolate was screened for anti-ESBL activity. Out of two potential isolates, VITSJK8 showed.

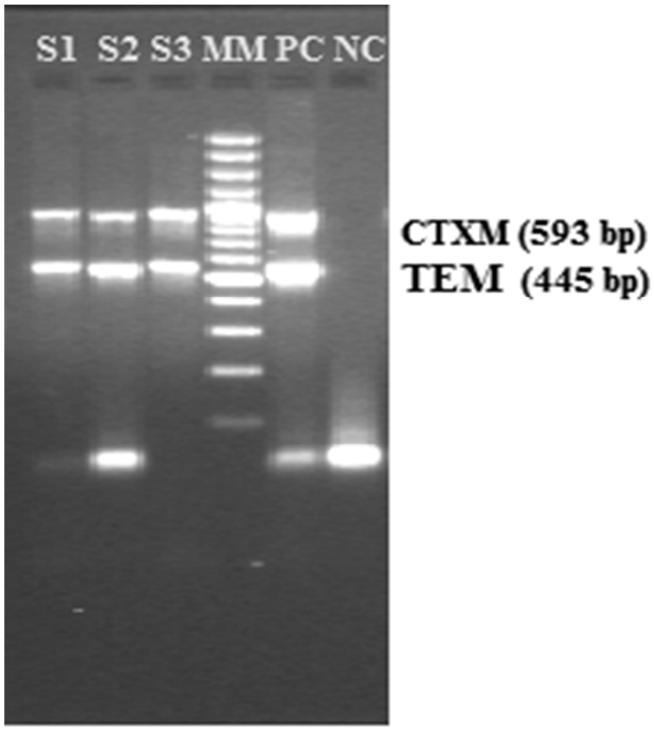

Figure 2 PCR detection of CTXM-ESBL gene for *E coli* and *Klebsiellapneumoniae*. Lane MM-100 base pair ladder molecular marker (Bio Rad). Lane S1, S3 are *E coli* isolates and Lane S2-*Klebsiella pneumoniae*, PC-Positive control, NC-Negative control

strong anti-ESBL activity and was chosen for further investigation. Cell free culture supernatant of VITSJK8 showed zones of inhibition for ESBL producing pathogens, *K. pneumoniae* (ATCC 700603) 18 mm, *E. coli* (VITEB1) 23 mm, *K.pneumoniae*(VITEB2) 16 mm,*K.pneumoniae*(VITEB5)15 mm, *K.pneumoniae*(VITEB6) 18 mm, *K.pneumoniae*(VITEB7) 20 mmand *K.pneumoniae*(VITEB8) 24 mm.

Characterization of potential marine actinomycetes

Culturing conditions were optimized with different parameters as per International Streptomyces Project (ISP) recommendations. Colonies were pink,powdery, dry, matured with branching hyphae (5-7 mm in dm). Spore arrangement under scanning electron imaging (SEM) shows long branching chains, each chain bearing 10-15 spores.Matured spores were wrinkled cylindrical, about 0.5-1.0 mm in diameter(Figure 3). Potential isolate *Streptomyces* sp. VITSJK8 was fermented with production media ISP-1 broth (Tryptone yeast extract broth) (Hi-media, Mumbai), an optimized medium consisting of carbon source1% (w/v) glucose, D-mannitol, nitrogen sources1% (w/v) soyabean meal and ammonium chloride, soil extract and sea water 25% each, distilled water 50% at pH 7.0. Phenylalanine and methionine were utilized as amino acid sources. Optimal growth was observed at 28°C after 14 days, incorporating 2% to 3% NaCl. The optimized cultural conditions for the growth of the isolate are shown in Figure 4. 16S rDNA analysis revealed that the isolate belonged to genus *Streptomyces* and designated as *Streptomyces* sp. VITSJK8. The organism showed excellent mycelial growth and secondary metabolite production at the optimized conditions.

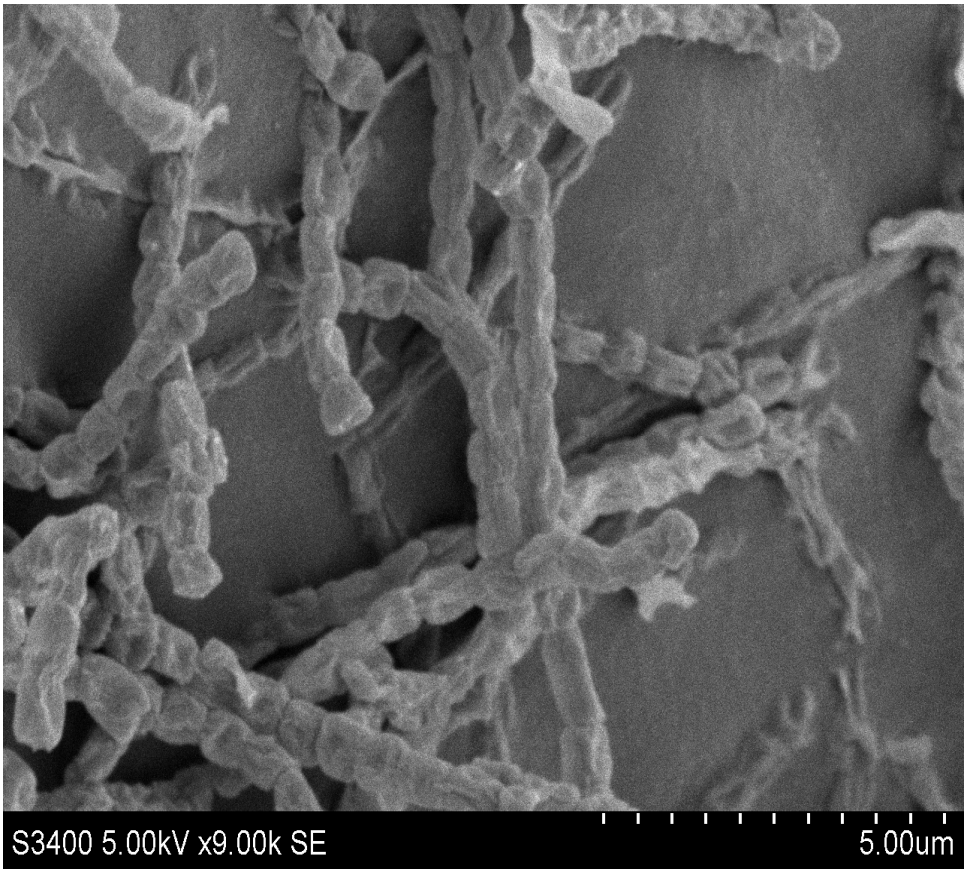

Figure 3 Scanning Electron Microscope (SEM) image of *Streptomyces* sp.VITSJK8 showing wrinkled matured spores arranged in chains. Each hypha bears approximately 10–15 spores

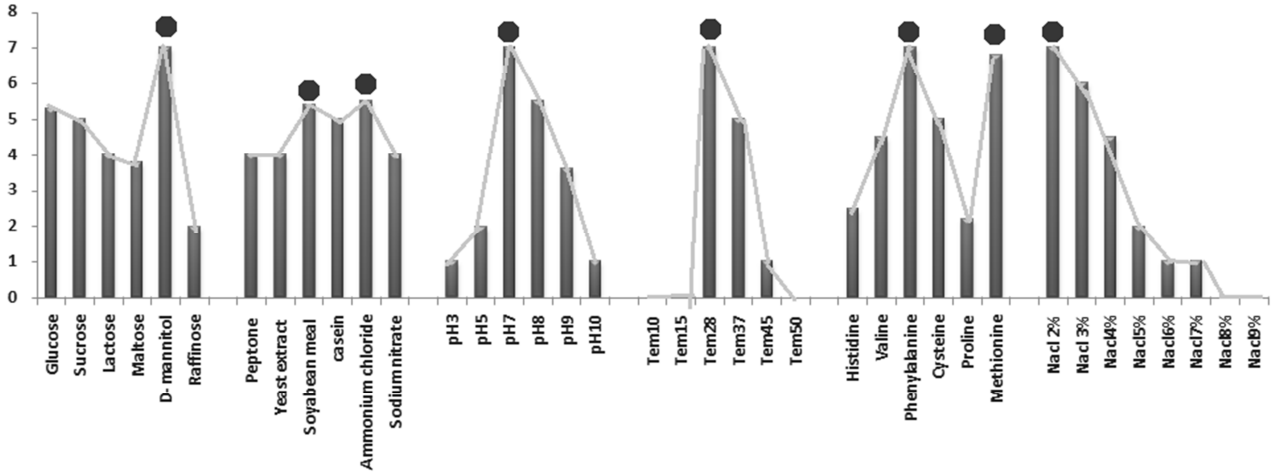

Figure 4 Optimization of culturing conditions of *Streptomyces* sp. VITSJK8

Taxonomical characterization of VITSJK8

16S rDNA sequencing of VITSJK8yielded837 nucleotideswhich has been searched through NCBI's GenBank database using nucleotide BLAST. The sequence was then submitted to GenBank (NCBI, USA) under the accession number KF289838. BLAST search showed 98% similarity to *Streptomyces* sp. M518 (DQ184649.1). The sequence was submitted in GeneBeeonline software to construct phylogenetic tree along with bootstrap values. The morphological, cultural and biochemical characteristics of the known actinomycetes species mentioned in Bergy's manual of systemic bacteriology resembling that of the isolated potential actinomycetes isolate strongly recommending that the isolate belongs to genus *Streptomyces* and designated as *Streptomyces* sp. VITSJK8. The isolate is facultatively aerobic, non-motile, non-acid fast, Gram positive, and produces distinct branching aerial and

substrate mycelium. Phylogenetic tree was constructed by neighbor joining method and the isolate is placed at the position within the wings including representatives of the *Streptomyces* family(Figure 5).

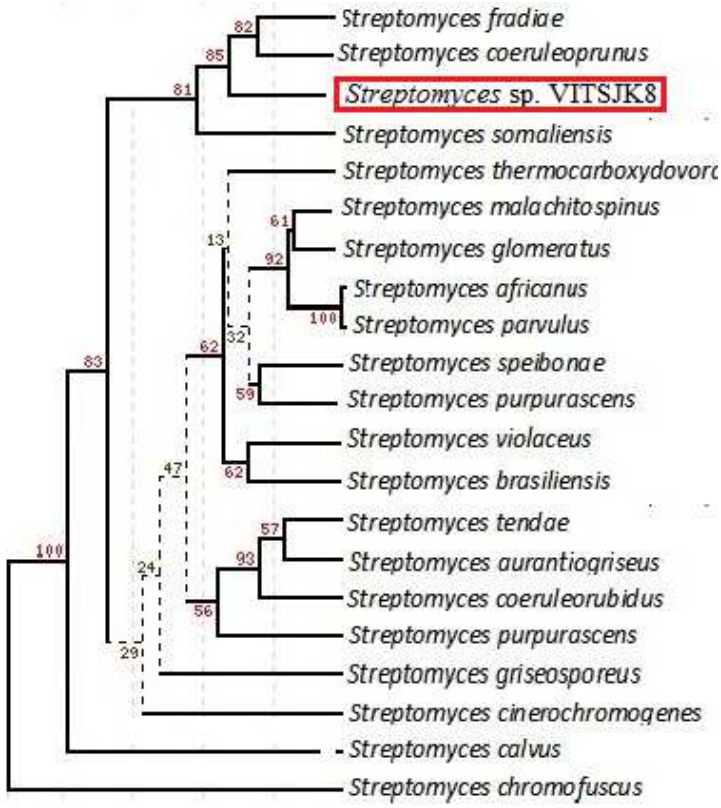

Figure 5 Phylogenetic tree of *Streptomyces* sp. VITSJK8 based on 16S rDNA constructed using neighbor joining method with bootstrap support. Score bar represents one nucleotide substitution per 100 nucleotides

Table 1. MIC values of *Streptomyces* sp. VITSJK8 ethyl acetate extract against ESBL producing pathogens

CTXM	AMP	CTX	CTX/CLAV	CZD	CZD/CLAV	Ertapenem (µg/mL)	Ethyl acetate extract of *Streptomyces* sp. VITSJK8 (20µg/ml DMSO)
	Inhibition zone size in mm						
S1-*E. coli*	6	6	16	10	16	4	0.25
S2- *Klebsiellapneumoniae*	6	10	15	6	15	3	2.0
S3- *E. coli*	6	6	20	8	14	4	0.125

Antibiotic Discs (Hi-media, India); AMP- Ampicillin (30 µg); CTX - Cefotaxime (30 µg); CTX/CLAV- Cefotaxime/Clavulanic acid (30/10 µg); CZD-Ceftazidime (30 µg); CZD/CLAV- Ceftazidime/Clavulanic acid (30/10 µg).

Assay of anti- CTXM ESBL activity

The anti-ESBL activity of EA extract of *Streptomyces* sp. VITSJK8 showed significant activity against CTXM-ESBL pathogens. The MIC value of EA extract against CTXM-ESBLs isolates is given in Table 1. EA extract of *Streptomyces* sp. VITSJK8 showed significant activity against CTXM-ESBL isolates with the MIC values S1-*E. coli* (0.25 µg/mL), S2-*K. pneumoniae* (2.0 µg/mL), S3-*E. coli* (0.5 µg/mL) and compared to standard drug ertapenem with the MIC value S1-*E.coli* (4 µg/mL), S2-*K. pneumoniae* (3 µg/mL), S3-*E. coli* (4.0µg/mL) for CTXM-ESBL pathogens. ESBL isolates showedmore susceptibility to the ethyl acetate extract with MICvalues ranging from 0.25–2.0 µg/mL.

DISCUSSION

In the present study, we evaluated the inhibitory activity of marine *Streptomyces* species against clinically important MDR CTXM-ESBL (*E.coli* and *K. pneumoniae*) uropathogens. Ethyl acetate extract (16µg/mL in DMSO) prepared

from *Streptomyces* sp. VITSJK8 exhibited significant activity against CTXM-ESBL uropathogens with the MIC value of 0.25 µg/L and standard antibiotic ertapenem showed a higher MIC value (3 µg/mL). In our investigation, detection of ESBL production and determination of antibiotic susceptibility standards was done using Clinical and Laboratory Standards Institute guidelines and 10 positive ESBL producers; *K pneumoniae* (n=7) and *E coli* (n=3) were identified. Further, uniplex PCR was carried out to determine ESBL subtypes.

Urinary tract infection is most widespread in both men and women. Recurrences in UTI are common and it is a serious threat in terms of antibiotic resistance by uropathogens[26] ESBL producers are one of the leading causes of UTI in the current scenario and they have an inbuilt ability to develop resistance to higher class of antibiotics available in clinical regimen at present [27]. This remains an important reason to support research on alternative novel anti-ESBL compounds from natural sources. ESBL related mortality is very high in spite of potent antimicrobials available commercially to treat ESBL infections. A yellow pigmented compound from *Streptomyces* had shown good anti-ESBL activity as reported [28].

It is evident from our study, that the isolate *Streptomyces* sp. VITSJK8 isolated from unexplored environment, possess significant activity against ESBL producing uropathogens. Although, carbapenems are active against ESBL pathogens, resistance to carbapenems reported recently in some strains of *Klebsiella* species and *E. coli* known as carbapenamses [*Klebsiella* producing carbapenamases(KPC) and New Delhi metallo-β-lactamases (ND-MBL)] have limited the treatment options and have been associated with high mortality rates [29]. In a recent study, the side effects of carbapenems have immediate, sometimes severe complicated reactions in humans. These enzymes are often found on mobile genetic elements which have the potential to be transferred to other pathogens widely [30]. Drugs from marine bacteria have been advancing through clinical trials and marine actinobacteria turns out to be an important contributor. Metabolites from actinobacteria posses novel chemistry and forms the basis of structurally diverse and biologically active secondary metabolites. Two-thirds of naturally occurring antibiotics and industrially important bioactive compounds have been isolated from the genus *Streptomyces.*

CONCLUSION

From this investigation, we can conclude that the secondary metabolites produced by the isolate *Streptomyces* sp. VITSJK8 can be used for treating MDR-ESBL uropathogens.

Acknowledgements
The authors thank the management of VIT University for providing facilities to carry out this study.

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