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ISOLATION AND IDENTIFICATION OF ANTI-MRSA COMPOUND FROM TERRESTRIAL ACTINOMYCETES SPECIES VITBKA3

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ABSTRACT

Drug resistant pathogens are a major threat to human health and well-being. Methicillin resistant *Staphylococcus aureus* infection and their resistance to third generation antibiotics are increasing worldwide. Screening of tterrestrial actinomycetes isolate VITBKA3 for anti-MRSA activity showed 20mm zone of inhibition against MRSA strain (ATCC 43300). The potential isolate was subjected to morphological, biochemical and molecular taxonomic characterization andidentified as *Streptomyces* species. Molecular taxonomy and phylogenic studies showed that the isolate was similar to *Streptomyces ganicidicus* sp. and hence designated as *Streptomyces ganicidicus* sp. VITBKA3. Submerged fermentation followed by preparation of ethyl acetate extract and bioactivity guided purification of the active compound by silica gel column chromatography and characterization by FT-IR, NMR and GC-MS yielded an aromatic anhydrous compound. The structure of the pure compound was identified as 2-amino-3-phenylpropanoic (E)-3-cyclohexyl-2-methylacrylic anhydride (APCMA) having the chemical formula of $C_{19}H_{25}NO_3$ and the molecular weight of 315.18 g/mol. The lead compound showed 18mm zone of inhibition against MRSA strain with the MIC value of 6.25 µ/ml. The outcome of this study suggests that the lead compound APCMA from *Streptomyces ganicidicus* sp. VITBKA3 can effectively inhibit MRSA strain.

Keywords: Antimicrobial resistance; Methicilin resistant *Staphylococcus aureus; Streptomycesganicidicus* sp. VITBKA3; 2-amino-3-phenylpropanoic (E) -3-cyclohexyl-2-methylacrylic anhydride

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a Gram-positive opportunistic pathogen found in hospital settings affecting the patients being hospitalized for a longer period of time. The morbidity and mortality associated with MRSA infections are increasing every year due to its resistance towards most of the antibiotics (**Rasko and Sperandio, 2010**). Vancomycin is the only choice of antibiotics available for the treatment of MRSA infections which also has its own side effects and moreover vancomycin resistance *Staphylococcus aureus* (VRE) has alsoalready been reported. Among all superbugs MRSA found to be more lethal and it can cause severe bacteremia or septicaemia which leads to the death of the patient. Due to the emergence of vancomycin resistance *Staphylococcus aureus* (VRSA) daptomycin and other anti-MRSA drugs were used. Many these strains develop drug resistance due to overuse or abuse of antibiotics. To overcome this problem researchers are searching for novel compounds and new chemical entities from natural sources (Cascioferro et al., 2021). As the incidence of drug resistance is increasing worldwide, there is an urgent need to discover new antibiotics for effective control of drug resistant pathogens. *S. aureus* become extreme drug resistant (XDR) and total drug resistant (TDR) superbug resistant to even third line of antibiotics (**Singh *et al.*, 2022**).

MATERIALS AND METHODS

Isolation, identification and characterization of the isolate *Streptomyces ganicidicus* sp. VITBKA3 was already reported (**Bhakyashree and Kannabiran, 2020**).

Preparation of ethyl acetate (EA) extract from the potential isolate

Fermentation was carried out in a 1L Erlenmeyer flask by following the procedure as described by Liu et al (2011). ISP1 medium was prepared and the isolate VITBKA3 was inoculated onto the flasks and kept for incubation at room temperature in rotatory shaker for 7 days. After incubation the cells were separated using centrifugation method and the supernatant was collected and checked for its antagonistic activity. The crude extract was extracted using ethyl acetate. Seed culture was prepared using Tryptone yeast extract broth and after 7 days of incubation in a1L Erlenmeyer flask, supernatant was separated and extracted with ethyl acetate using separating funnel. The supernatant was concentrated in rotatory shaker and the crude collected was checked for its antibacterial activity as reported earlier (**Bhakyashree and Kannabiran, 2020**).

Purification of the lead compound

The compounds present in EA extract was separated by TLC using the optimal solvent system petroleum ether and ethyl acetate in the ratio of 8:2 as a solvent system. The EA extract was loaded on silica gel in a column chromatography and eluted with petroleum ether and ethyl acetate in the ratio of 8:2 as a solvent system. The flow rate was maintained as 1ml/min and all the fractions collected were analysed for anti-MRSA activity, Active fractions were pooled and concentrated, the purity of the concentrated fractions was checked and stored for further processing.

Antibacterial activity of the pure compound

The antibacterial activity of the pure compound was measured by diluting to 100 µg/mL in DMSO. Different concentrations of pure compound was added to the wells made in the plate seeded with MRSA ATCC 43300 and kept for incubation at 37 ºC for 24 h. After incubation the plates were observed for the zone of inhibition.

Minimal inhibitory concentration (Microtitre plate method)

The test organism was prepared freshly in a nutrient broth and adjusted the turbidity by comparing 0.5 MacFarland turbidity. 96 well plates were used for the testing of MIC, 100 µL of sterile nutrient broth were added into the first eight rows of plate. Then100 µL of test organisms was added in the first well and serially diluted to eighth well which gives the concentration of 50 µg, 25 µg, 12.5 µg, 6.25 µg, 3.125 µg, 1.56 µg,0.78 µg/mL. The last well was used as negative control and ciprofloxacin antibiotic solution was used as positive controlTo each of the well 5µL of bacterial inoculum was added and the plate was kept for incubation at 37 ºC for 24 h. After incubation the clearing of turbidity in the well can be observed in the ELISA plate reader and the corresponding readings were recorded and the MIC was determined. The wells containing higher bacterial growth show high absorbance and the wells containing lower bacterial growth shows less absorbance. The final lowest concentration up to which the absorbance was very low was considered to be the MIC.

Identification of the Anti-MRSA compound

The anti-MRSA compound was subjected to Fourier-transform infrared spectroscopy (FTIR) (Schimadzu IR affinity-1 FTIR Spectrometer, Japan) analysis for identification of functional groups present. The ^{1}H and ^{13}C NMR spectra for the anti-MRSA compound were recorded on Bruker Ascent 400MHz spectrometer using dimethyl sulfoxide-d_6 (DMSO- d_6) as the solvent to identify the pure compound. Chemical shift values are reported in δ notation of parts per million using trimethylsilane (TMS) as the standard. The pure compound of the isolate VITBKA3 was analyzed in GC-MS JEOL (GCMATE II GC-MS, Agilent Technologies 6890N Network GC system for GC). The column (HP5) used was fused silica 50 m × 0.25 mm I.D. The column temperature was maintained at 100 °C for 20 min, 235 °C for 3 min and injector temperature was maintained at 240 °C. The carrier gas used was helium and the split ratio is 5:4. The sample (1 µL) was evaporated in a split less injector at 300 °C. The approximate run time was fixed as 30 min and the mass spectra (MS) obtained was matched with the reference compounds listed in the National Institute of Standards and Technology (NIST) library. The molecular mass of the compound was calculated based on MS spectra.

Mechanism of anti-MRSA activity of APCMA

The mechanism of anti-MRSA activity of APCMA was studied by *In silico* molecular docking studies. The lead compound APCMA was docked with five different (MRSA) bacterial target proteins, dihydrofolate reductase, penicillin-binding protein 2A, penicillin-binding Protein 2', panton-valentine leucocidin F and pyruvate kinase.

RESULTS

Anti-MRSA activity of the purified compound from *Streptomyces gancidicus* sp. VITBKA3

The purified compound was subjected for antibacterial activity against ATCC 43300MRSA by well diffusion method. After incubation the purified compound yielded 18mm of zone of inhibition against ATCC 43300 MRSA strain (Tab 1).

Table 1 Anti-MRSA activity of the purified compound from *Streptomyces gancidicus* sp. VITBKA3

Organism	#Ciprofloxacin (10mcg/disc)	Pure compound Zone of inhibition (mm)	MIC (µg/mL)
Methicillin Resistant *Staphylococcus aureus* (ATCC43300)	25.0 ± 0.5	18 ± 0.5	6.25

Positive control

Identification of the Anti-MRSA compound

The FT-IR spectrum of the anti-MRSA compound is shown in figure 1. The important peaks corresponding to the different functional groups present in the compound are given below. IR (KBr, cm^{-1}) 3226, 2954, 2992, 2852, 1718, 1462, 1377, 1049, 966, 788, 455 cm^{-1}. N-H group is refers to be unknown compound, C-H refers to be alkane, C-H refers to be alkane, R-CO-O-CO-R is refers to be anhydride, C=C is refers to be alkane. The ^{1}H NMR spectrum of the anti-MRSA compound is shown in figure 2. The pure compound was found to be anhydrous aromatic compound since it contains aromatic rings in their structure. The molecular formula of the pure compound was found to be $C_{19}H_{25}NO_3$. The purified compound from *Streptomyces* sp. VITBKA3 is subjected to GC-MS analysis to calculate the molecular mass of the pure compound. The GC-MS spectrumof the pure compound is shown in figure 3. The molecular weight of the pure compound was calculated as 315.18 g/mol. Based on spectroscopic data the pure compound was identified as 2-amino-3-phenylpropanoic (E) -3-cyclohexyl-2- methylacrylic anhydride (APCMA) with molecular formula of $C_{19}H_{25}NO_3$ (figure 4). The compound was found to be anhydrous aromatic compound due to the presence of aromatic rings in the structure. The lead compound APCMA is a novel anti-MRSA secondary metabolite produced by *Streptomyces gancidicus* sp. VITBKA3.

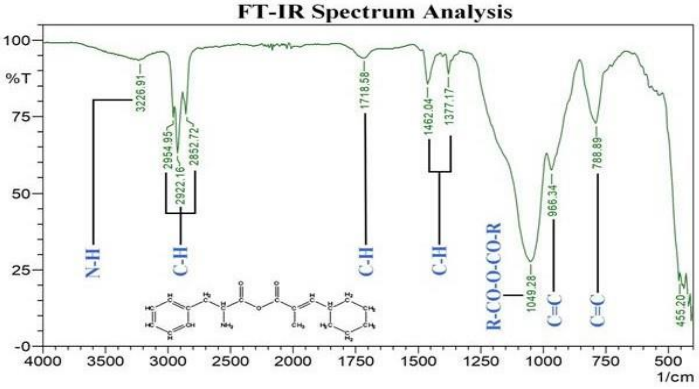

Figure 1 FT-IR spectrum of the anti-MRSA compound from *Streptomyces gancidicus* sp.VITBKA3.

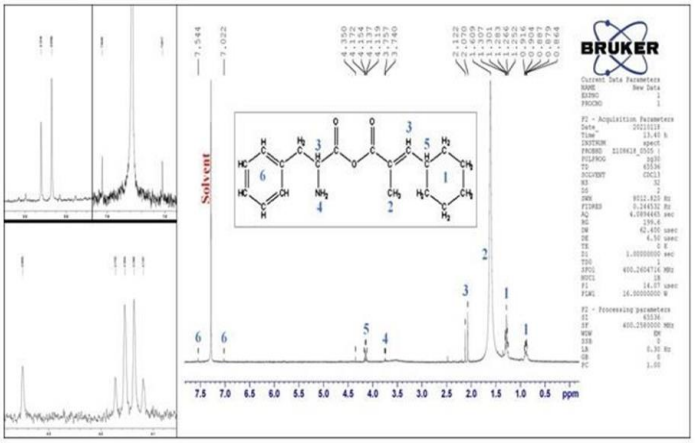

Figure 2 NMR spectrum of the purified compound from *Streptomyces gancidicus* sp. VITBKA3.

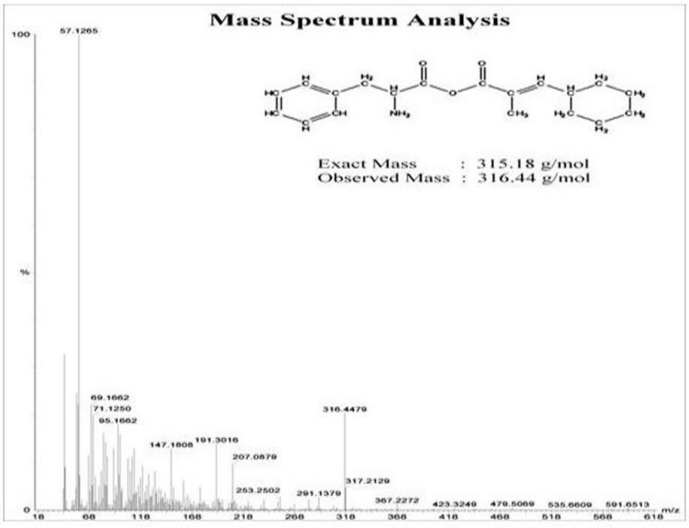

Figure 3 GC-MS spectrum of the pure compound from *Streptomyces gancidicus* sp. VITBKA3.

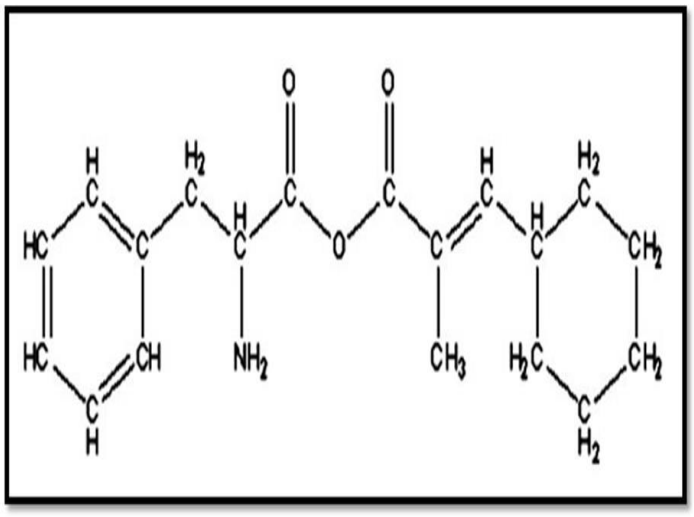

Figure 4 The structure of the pure compound 2-amino-3-phenylpropanoic (E) -3-cyclohexyl-2-methylacrylic anhydride (APCMA).

ADMET ANALYSIS

ADMET stands for Adsorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) of any molecule to determine its drugability. ADMET analysis was done using online software *Swissadme*. Based on the *Swissadme* analysis the compound possesses drug-gable properties and was found to be suitable to consider as a drug based on several pharmacokinetics properties. The ADME properties ofAPCMA are listed in Table 2.

Mechanism of anti-MRSA activity of APCMA

The interaction of APCMA with MRSA target proteins and their binding energy and the number of hydrogen bonds formed are given in Table 3. Among the bacterial target proteins tested dihydrofolate reductase showed the least binding energy -7.8 Kcal/mol (figure 5).

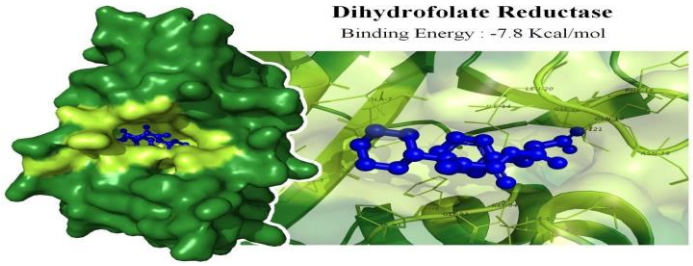

Figure 5 Interactions of APCMA with MRSA drug target protein dihydrofolate reductase.

Table 2 ADME properties of the lead compound APCMA

ADME Properties	
Molecular weight	**315.41g/mol**
Heavy atoms	3
Aromatic heavy atoms	6
Fraction Csp3	0.47
Rotatable bonds	7
H-bond acceptors	4
H-bond donors	1
Molar Refractivity TPSA	90.70
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-5.14 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability score	0.55

Table 3 Molecular docking studies of APCMA with MRSA target proteins

Protein	**PDB ID**	**Organism**	**Binding energy (K cal/mol)**	**No.of H-bonds**
Dihydrofolate Reductase	3FYW	MRSA	-7.8	0
Penicillin-binding protein 2A	1MWT	MRSA	-6.6	0
Penicillin-binding protein 2'	4DKI	MRSA	-6.4	0
Panton-Valentine leucocidin F	6U3F	MRSA	-5.8	3
Pyruvate kinase	3T07	MRSA	-5.5	0

DISCUSSION

Screening of terrestrial actinomycetes for anti-MRSA activity resulted in identification of *Streptomyces gancidicus* sp. VITBKA3. Bioactivity guided extraction, purification and chemical characterization lead to identification of anti-MRSA compound APCMA. The mechanism of anti-MRSA activity of APCMA was mediated through the inhibition of MRSA bacterial target protein dihydrofolate reductase. It is required for the synthesis of folic acid and also failure of conversion of the dihydrofolate to tetrahydrofolate (cofactor) which is responsible for the DNA and protein synthesis in bacteria.

Not many reports are available on Anti-MRSA activity of actinomycetes derived fromterrestrial soil samples, since most of the natural compounds were isolated from marine sources. The increase in the antibiotic resistance forced us to discover newer agents effective against MRSA to control the spread of disease. Actinomycetes play a major role in producing various bioactive compounds with diverse biological activities including anti-MRSA activity. The cell free supernatant and the ethyl acetate extract obtained by liquid-to-liquid extraction from actinomycetes were often used to assess their antibacterial activity. Marinomycins A to D was isolated from newly discovered marine actinomycete *Marinispora* which consists of series of bioactive polyene-polyols. Marinomycin A showed significant antibacterial activity with the MIC of 0.13 µM against MRSA and VRE whereas marinomycins B to D were found to be active against only MRSA with the MIC value of 0.25 µM. Bioassay-guided fractionation of the extracts from the culture broth of another *Marinispora* species (NPS12745) results in identification five halogenated bisindole pyrroles namely lynamicins A to E showed good antibacterial activity against MRSA and VRE srtrains. The MIC value ranges from 1-3 µg/mL and 2-8 µg/mL for lynamicins A-D against MRSA and VRE, lynamicin E was less active when compared to others (**Rahman *et al*., 2010**).

Moenomycin A, nosokomycins A- D and angumicynone B isolated from marine *Streptomyces* species reported to be potential marine drugs against MRSA (**Haste et al., 2012**). The anti-MRSA compound 8-O-methyltetrangomycin produced by the strain SBRK2 *Streptomyces longispororuber* inhibits the biofilm formation. It also exhibited haemolytic activity on red blood cells. **Sun *et al*.** (2015) isolated anti-MRSA compound polybrominated diphenyl ethers, such as 2-(20, 40- dibromophenoxy)-3, 4, 5-tribromophenol and 4, 5- tribromophenol from two marine sponges, *Dysidea granulosa* and *Dysidea* sp. from United States. The compound 4, 5- tribromophenol was found to be the most potent and exhibited broad spectrum activity against Gram positive and Gram-negative bacteria including MRSA with the MIC range of 0.1mg/ml. This compound has the potential to be developed as a drug for the treatment of MRSA in the future.

Cadiolides J-M, 1, 3–5, and cadiolide H exhibited significant anti- MRSA activity when compared to the commercial drugs like vancomycin and linezolid with the MIC valueof 1–8 µg/mL. The new sarcoplasmic calcium-binding protein J2-C4 from Chinese Sea *Arca inflat* showed inhibitory activity against MRSA (MIC-750µg/ml) (**Wang *et al*., 2018**). The *Streptomyces* sp. MN41 isolated from Caspian Sea sediment produced a bioactive compound with pyrrole-like structure showed significant anti-MRSA and anti-tumor activities (**Norouzi *et al*., 2019**). The novel compound C23 isolated from *Streptomyces rubrolavendulae* ICN3 showed promising activity against MRSA in zebrafish embryo model (**Kannan *et al*., 2014**). Siddharth, (2019) reported the isolation of two bioactive compounds, 4-bromophenol (a bromophenol derivative) and bis (2-ethylhexyl) phthalate, (a phthalate ester) from the actinomycetes strain SCA21 isolated from Andaman and Nicobar Islands. Both the compounds showed broad spectrum of activity against MRSA ATCC NR-46171, MRSA ATCC-46071, *Klebsiella pneumoniae* ATCC 13883, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 12600 (**Siddharth, 2019**). The anti-MRSA compounds produced by *Streptomyces* sp. MUSC 125possess anti-MRSA and anti- biofilm activities (**Kemung *et al*., 2020**). Several studies reported that actinomycetes especially*Streptomyces* species are found to be the best producer of anti-MRSA compounds. Many anti-MRSA molecules like Bogorol A, Abyssomicin C, 2,4-Diacetylphloroglucinol, Loloatins A–D, Fijimycin A, Marinopyrroles A, Lipoxazolidinone A–C have been isolated from marine microorganisms (**Ansari et al., 2020**). *Streptomyces* sp.AN100483 isolated from the soil sample collected from *Colocasia esculenta* field, Baekam Mountain, Gyeongsangbuk-do, Korea yielded a new antibiotic antharacenone.

After extraction the purification process were carried out and the antibacterial activity of the compound were checked for every compounds. Compounds extracted showed good antibacterial activity against *S. aureus* RN4220, MRSA, *S. aureus* CCARM 3167 and *S. aureus* CCARM 3506 and quinolone-resistant *S. aureus* (*S. aureus* CCARM 3505 and *S. aureus* CCARM 3519) with the MIC value of 32 μg mL^{-1}. Ciprofloxacin showed the MIC value of 0.125, 4 and 128 μg ml^{-1} respectively. It also showed activity agaainst *Enterococcus faecalis* KCTC 5191 and *Bacillus cereus* KCTC 1661 with the MIC value of 32 μg mL^{-1}. This new chlorinated anthracenone produced by *Streptomyces* sp. AN100483 is rare metabolite having activity against MRSA and QRSA (**Kwon *et al.*, 2016**).

Streptomyces sp. DSD011 isolated from marine sediments collected near the coast of Islasde Gigantes, Iloilo exhibited potent activity against the multidrug-resistant MRSA strain carrying the Staphylococcal Cassette Chromosome mec (SCCmec) type 1 gene which is considered to be biomarker for drug resistance. Based on its 16s rRNA and protein-coding genes (atpD, recA, rpoB, and trpB) sequences and it was found to be a new species ofsalt-tolerant marine *Streptomyces*. It harbours both non-ribosomal peptide synthetase (NRPS) and type II polyketide synthase (PKS) in itsgenome. Two polycyclic aromatic polyketide angucycline glycosides, fridamycin A and fridamycin D (products of type II PKS biosynthesis) was derived from the isolate. It showed good antibacterial activity against MRSA with the MIC value of 500 g/mL and 62.5 g/mL (**Sabido *et al.*, 2020**).

Marine-derived actinomycete *Streptomyces* sp. MBTI36 produced new chromomycin A9, along with chromomycin Ap, chromomycin A2, and chromomycin A3 showed antibacterial activities against Gram-positive bacteria which includes MRSA. The strain was identified using 16S rDNA sequence and found to be closely related to *Streptomyces microflavus* (**Cho *et al.*, 2020**). Four new napyradiomycin (1-3,5) and napyradiomycin SC (Napyradiomycins B2, B4, and B5 and the new napyradiomycin D1) isolated from the culture broth of marine *Streptomyces* sp.CA-271078 from Medina's microbial collection showed antibacterial activities against MRSA and *M. tuberculosis* H37Ra (**Molina *et al.*, 2019**). A novel Actinomycetes species named *Streptomyces* sp. A1-08 isolated from the volcanic soils in Mount Mayon showed promising anti-MRSA activity with the MIC values same as Vancomycin MIC which is been widely used for the treatment of MRSA infections (**Oliveros *et al.*, 2021**). Lactoquinomycin A (LQM-A) (compound 1) and its derivatives (2–4) isolated from the marine-derived *Streptomyces bacillaris* strain MBTC38 exhibited inhibitory activity against MRSA with the MIC value of 0.25–0.5 g/mL. The mode of inhibition of LQM-A- reported to induce DNA damage and not inhibited protein synthesis (**Chung *et al.*, 2021**). *Streptomyces rubrolavendulae* ICN3 strain yielded a compound C23 showed maximum anti-MRSA activity and the MIC value of pure compound was found to be 2.5μg/mL in *in vitro* assay (Kannan *et al*., 2014). Polyketide antibiotic SBR-22 isolated from *Streptomyces psammoticus* BT-408 showed maximum anti-bacterial activity against MRSA pathogen which can be a better source for the treatment of MRSA infection (**Al-Shabani *et al.*, 2021**).

Antibiotics alboflavusins (AFNs) isolated from *Streptomycesalboflavus* sp.313 are a novel cyclic hexapeptide with a chlorine atom reported to have higher activity against MRSA. The proposed mechanism was that L-tryptophan (Trp) 6-halogenase AfnX is responsible for halogenation of L- Trp to generate 6-Cl-L-Trp as a precursor of AFN biosynthesis, Trp halogenases like AfnX are flavin-dependent enzymes usually possessing high region specificity towards Trp substrate while it may have higher substrate promiscuity towards the halide ions and can able to take $Br^-$and I^-, which further can be used for the generationof congeners with different halogen substituents. In this study they replaced NaCl by NaBr or NaI in the fermentation broth and two novel brominated AFN congeners were produced. The analysis of the gene *afnX* inactivated mutant *S. alboflavus 1afnX* giving three dechlorinated AFNs with two new AFN congeners. The AFNs were tested for its antibacterial activities against several MRSA strains halogenated AFNs showed good activity than the dechlorinated AFNs. Brominated compounds showed good antibacterial activities against the tested MRSA strains. Halogen substitution plays a key role to AFNs for their anti- MRSA activities (**Li *et al.*, 2021**). Another angucycline antibiotic 8-*O*-metyltetrangomycin from *Streptomyces* sp. SBRK2 isolated from marine sponge in Gulf of Mannar, Rameshwaram, India. Based on molecular characterization the isolate found to be closely related species to *Streptomyces longispororuber* NBRC 13488^T. Agar plate fermentation and solvent extraction of crude followed by purification process results in identification of bioactive molecule 8-*O*- metyl tetrangomycin and it showed anti- MRSA activity with the MIC value 2.0μg/mL. It also inhibits the biofilm formation of *S.aureus* ATCC25923 and also increased the cell surface hydrophobicity index. SEM analysis revealed cell damage property of the compound. *In-vivo* studies on Zebrafish embryo model showed that the compound is safe up to 100 μg/mL. This study suggests that angucycline antibiotic 8-*O*-metyltetrangomycin cn be used as anti-biofilm agent to treat drug resistant pathogens (**Mary *et al.*, 2021**).

Streptomyces pluripotens MUSC 135^T isolated from the mangrove forest in Malyasia showed anti-MRSA activity. Genome miming to identify the BCGs for the production of various secondary metabolites and followed by anti-SMASH pipeline to annotate the BCGs showed the presence of putative gene clusters in *Streptomyces pluripotens.* The methanloic extract yielded a purified protein135 which showed a MIC of 3 μM against MRSA ATCC 33591 in 24 h incubation. It showed comparatively higher activity than vancomycin with MIC value of 0.25μM against MRSA ATCC 33591 (**Lee *et al.*, 2021**). The bioactive compound from *Streptomyces* sp.KB1 TISTR2304 showed activity against *Bacillus subtilis* ATCC 6633 (spores), *Staphylococcus aureus* TISTR 517, clinical methicillin-resistant *S. aureus*, *Escherichia coli* TISTR 887, *Pseudomonas aeruginosa* TISTR 1467, and extended spectrum beta-lactamase (ESBL)-*Klebsiella pneumoniae* 342 and *Candida albicans* TISTR 5779. *Streptomyces* sp.KB1 TISTR2304 is biocidal to TISTR strains and clinical isolate MRSA, ESBL. The results of this study showed that the bioactive compounds from *Streptomyces* sp.KB1 TISTR2304 showed higher activity against clinical MRSA strain when compared to oxacillin which makes them worthy enough to use for treating MRSA infections (Lertcanawanichakul and **Chawawisit, 2021**). A polyketide type of antibiotic extracted from *Streptomyces* sp. JRG-02 has been reported to very effective against MRSA strains (**Givindarajan *et al.*, 2021**). This study also suggests touse the bioactive compounds from microorganism as disinfectants against pathogens to reduce the chemical wastes caused by commercially available antibiotics (**Lertcanawanichakul and Chawawisit, 2021**). Seven aromatic polyketides 6-Deoxy-13-hydroxy-8,11-dionedihydrogranaticin A, Granaticin A, Granaticin B, BSM2, BSM1, Fogacin, and dihydroxy GTRI-02 extracted from *Streptomyces* sp. QHH-9511 was reported recently (**Feng *et al.*, 2023**) Recently a compound derived from *Streptomyces* sp. SP5 has been shown to be effective against MRSA and (vancomycin resistant *Enterococci*) VRE strains (**Devi *et al.*, 2023**).

CONCLUSIONS

The benefits of APCMA can be realized by large scale synthesis by combinatorial organic synthesis and investigating the in vitro, in vivo toxicity and extensive drug trails for their efficacy and suitability. The results of the study suggest that the bioactive compounds from actinomycetes are effective in inhibiting the multidrug resistant pathogens. Isolation and screening of actinomycetes from unexplored and under explored habitats would provide novel chemical compounds / antibiotics against ever growing drug resistant pathogens.

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Conflict of interest: The authors declare that there is no conflict of interest.

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