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Exploring Saline Desert Actinomycetes for Novel Anti-MRSA Antibiotic Production.

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Abstract

This study explores the potential of saline desert microflora from the Little Rann of Kutch as a source of novel antibiotics targeting Methicillin-resistant *Staphylococcus aureus* (MRSA). Representative sites were sampled, and actinomycetes were isolated using selective media and pretreatment methods. Biochemical testing, extracellular enzyme profiling, and morphological characterization were performed. The effects of saline and alkaline conditions on growth and antibiotic production were evaluated. Submerged fermentation was employed for antibiotic production, which was tested against MRSA strains using agar well diffusion assays. Minimum inhibitory and bactericidal concentrations were determined, demonstrating the significant potential of saline desert microflora in addressing antibiotic resistance.

INTRODUCTION

The emergence of antibiotic-resistant pathogens, particularly methicillin-resistant Staphylococcus aureus (MRSA), poses a severe threat to global public health. MRSA infections are associated with high morbidity, mortality, and healthcare costs due to the limited efficacy of existing antibiotics (Jones et al., 2020). These challenges underscore the urgent need for novel antimicrobial agents.

Natural ecosystems, especially extreme environments like saline deserts, offer an untapped reservoir of unique microorganisms with the potential to produce bioactive compounds. The Little Rann of Kutch, a saline desert in western India, represents one such ecosystem. Characterized by high salinity, alkalinity, and extreme temperatures, it fosters extremophilic microbes, such as actinomycetes, which have evolved adaptive mechanisms for survival. These microbes possess specialized metabolic pathways, enabling them to produce secondary metabolites with antibacterial, antifungal, and other biologically active properties (Zvyagintsev et al., 2008).

Actinomycetes are particularly renowned for their role in antibiotic production. Members of this group, such as Streptomyces, have historically contributed to the discovery of clinically significant antibiotics, including streptomycin and tetracycline (Kato et al., 2011). The discovery of antibiotics from conventional sources has plateaued, necessitating exploration of novel habitats like saline deserts for identifying new strains and chemical scaffolds with antibacterial activity.

This study investigates the saline desert microflora of the Little Rann of Kutch, focusing on isolating actinomycetes capable of producing antibiotics active against MRSA. By optimizing growth conditions and characterizing the bioactive compounds, this work aims to expand the repertoire of natural products available for combating antibiotic-resistant infections.

Review of Literature

Antibiotic Resistance and MRSA

Antibiotic resistance has reached alarming levels worldwide, with MRSA being a leading cause of nosocomial infections. MRSA strains exhibit resistance to β-lactam antibiotics, complicating treatment regimens and necessitating the use of last-resort drugs like vancomycin and linezolid (Liu et al., 2021). However, resistance to these drugs is also emerging, further narrowing treatment options (Maray et al., 2024).

The search for novel antimicrobial agents has therefore shifted toward unconventional sources, including extremophilic microbes from diverse habitats. Such organisms often produce unique metabolites with mechanisms of action distinct from existing antibiotics, making them valuable in the fight against resistant pathogens (Van Bambeke et al., 2008).

Saline Desert Microflora as a Resource for Drug Discovery

Saline environments are characterized by high osmotic pressures and limited nutrient availability, selecting for extremophilic microorganisms. Actinomycetes from these habitats have been reported to produce secondary metabolites with diverse biological activities. For instance, studies on saline soil actinomycetes from Algeria and Mongolia have identified haloalkaliphilic Streptomyces species capable of producing antimicrobial compounds under high salt and pH conditions (Zvyagintsev et al., 2008; Novovsuren et al., 2007).

The Little Rann of Kutch, with its unique combination of salinity and alkalinity, provides a promising ecosystem for bioprospecting. Previous studies have demonstrated that extremophiles from this region possess metabolic pathways not commonly found in microbes from conventional environments (Tang et al., 2008).

Advances in Isolation and Characterization of Actinomycetes

Traditional culture-based techniques, combined with molecular tools like 16S rRNA sequencing, have enabled the identification of novel actinomycetes from extreme environments. Recent advances in metagenomics and metatranscriptomics further facilitate the discovery of biosynthetic gene clusters encoding novel antibiotics (Kato et al., 2011). For example, the genetic manipulation of Lysobacter species has led to the enhanced production of anti-MRSA compounds, demonstrating the potential of genomic approaches in antibiotic discovery (Wang et al., 2013).

Challenges in Antibiotic Production and Optimization

While extremophilic actinomycetes are promising, their cultivation and optimization require careful consideration of environmental parameters such as pH, salinity, and nutrient composition. Research has shown that alkaline conditions and moderate salinity enhance the production of secondary metabolites in haloalkaliphilic actinomycetes (Sahil et al., 2011). Furthermore, response surface methodologies have proven effective in optimizing fermentation conditions for maximizing antibiotic yields.

Antimicrobial Activity of Actinomycetes against MRSA

Several studies have reported the antimicrobial potential of actinomycetes against MRSA. For instance, Streptomyces strains isolated from saline environments demonstrated significant activity against multidrug-resistant pathogens, with inhibition zones ranging from 10–25 mm in agar diffusion assays (Shetty et al., 2014). These findings highlight the feasibility of using saline desert microflora to combat MRSA and other antibiotic-resistant pathogens.

Materials and Methods

Study Area and Sample Collection

The study was conducted in the Little Rann of Kutch, a saline desert in Gujarat, India, known for its extreme environmental conditions, including high salinity, alkalinity, and arid climate. Soil samples were collected from ten distinct locations, including the interior of the desert and sites near Shakti Temple, Vithrohi Bet, and Dholo Bet. Coordinates, pH, and electrical conductivity (EC) were recorded for each location. The pH values ranged from 7.2 to 7.9, and EC varied from 1.9 to 19.54 mS/cm.

Soil samples were collected using sterile tools and stored in sterile polyethylene bags to prevent contamination. Samples were pretreated with calcium carbonate (10 mg/g) and incubated at 50°C for 1 hour to suppress the growth of fastidious microorganisms, as described by Tang et al. (2008).

Isolation and Enrichment of Actinomycetes

Actinomycetes were isolated using Actinomycetes Isolation Agar (AIA) and Starch Casein Agar (SCA) supplemented with cycloheximide (100 µg/ml) to inhibit fungal growth (Tang et al., 2008). The plates were incubated at 28°C for 7–14 days. Colonies with distinct morphology indicative of actinomycetes (e.g., rough, powdery surfaces) were subcultured on fresh media for purification.

A total of 44 isolates were obtained, of which 26 were selected for further study based on morphological and microscopic observations (Novovsuren et al., 2007). Gram staining confirmed the filamentous, Gram-positive nature of the isolates.

Optimization of Growth Conditions

The isolates were tested for growth and antibiotic production under various environmental conditions:

- **pH Range**: Isolates were cultured on SCA adjusted to pH 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0.
- **Salinity**: NaCl concentrations of 1%, 3%, 5%, 7%, and 10% (w/v) were tested.
- **Combined Conditions**: Growth under alkaline (pH 9.0) and saline (5% NaCl) conditions was also evaluated.

The combination of pH 9.0 and 5% NaCl supported optimal growth and antibiotic production in most isolates, consistent with findings by Zvyagintsev et al. (2008).

Isolation and Characterization of MRSA Strains

Clinical samples were obtained from public hospitals, including wound swabs, catheter tips, and surgical site infections. MRSA strains were isolated on Mannitol Salt Agar supplemented with oxacillin (6 µg/ml). Colony morphology and Gram staining confirmed the isolates as Gram-positive cocci. Antibiotic resistance was confirmed using the disk diffusion method on Mueller-Hinton Agar (Wayne et al., 2012).

Submerged Fermentation for Antibiotic Production

Selected actinomycetes isolates were cultured in Starch Casein Broth (SCB) at 28°C on a rotary shaker (150 rpm) for 7–10 days. After incubation, the culture broth was centrifuged at 10,000 rpm for 10 minutes. The supernatant was extracted with ethyl acetate (1:1 v/v), and the organic layer was concentrated under reduced pressure to obtain crude extracts (Sahil et al., 2011).

Antimicrobial Activity Testing

The antimicrobial activity of the crude extracts was evaluated using the agar well diffusion assay against eight MRSA strains. Wells (6 mm diameter) were filled with 100 µl of the extract, and inhibition zones were measured after 24 hours of incubation at 37°C. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using a broth dilution method, following CLSI guidelines (Wayne et al., 2012).

Chemical Characterization

The chemical composition of bioactive extracts was analyzed using gas chromatography-mass spectrometry (GC-MS). Peaks were identified by comparing retention times and mass spectra with the NIST library. Compounds such as 1-Decanol and 2-Piperidinone were identified as major bioactive components in the extract of isolate LK8 (Sahil et al., 2011).

Results and Discussion
Isolation and Characterization of Actinomycetes

A total of 44 actinomycetes were isolated from the soil samples collected across ten sites in the Little Rann of Kutch. Out of these, 26 isolates were selected for further study based on their colony morphology and Gram-positive filamentous structure. Colony characteristics included rough, powdery surfaces and colors ranging from white to pale yellow. Microscopic examination confirmed the filamentous nature of the isolates, consistent with the characteristics of actinomycetes (Tang et al., 2008).

Table- 1 Sample collection site and soil analysis

No.of isolates	Collection site	Latitude	Longitude	pH	EC (ms/cm)	Code of isolates
1	Little rann of	23°C/30min/6.8″	71°C/29min/4.0″			lk2
2	kutchh					lk5
3						lk7
4						lk8
5						lk9
6				7.9	16.27	lk10
7	Near to shakti	23°C/28min/10.4″	71°C/26min/65.5			st1
8	temple		″	7.7	5.1	st5
9	Vithrohi bet	23°C/25min/27.2″	71°C/18min/8.7″			vb1
10				7.8	4.81	vb3
11	Mardak bet	23°C/23min/14.1″	71°C/07min/39.4			mb1
12			″	7.9	4.23	mb2
13	Dholo bet	23°C/27min/56.4″	71°C/18min/10.5			db4
14			″	7.2	19.54	db6
15	Dhut bet	23°C/30min/10.9″	71°C/21min/4.1″			dh1
16				7.8	1.5	dh2
17	Near vadilal dam	71°C/25min/11.1″	71°C/25min/11.1			vd1
18			″			vd2
19				7.5	12.96	vd5
20	Koddhi bet	71°C/15min/22.1″	71°C/26min/65.5			Kd1
21			″			Kd2
22				7.5	7.98	Kd3
23	Dada no bet	23°C/13min/14.1″	71°C/15min/22.1			dd2

24			″			dd5
25						dd6
26				7.8	10.09	dd8

Microscopic and colony characterization of isolates

Plates treated with 100 µg/ml cycloheximide allowed the solates to grow well on actinomycetes isolation agar. Colonies were visually examined after seven days of incubation, and colony features of the classical actinomycetes type were noted. Colony sizes ranged from 2 to 3 mm, and all of the isolates had spherical or irregular shapes with the exception of vd5, which had a filamentous morphology. The surface of each colony was rough, and they were all opaque and dry or powdery in nature. The colonies' coloring ranged from white to grayish white to pale yellow to ivory, creamish gray, and pale yellow. All of the isolates were microscopically characterized using Gram staining. The Gram-positive, actinomycete-like, filamentous bacteria that were isolated from saline soil.

Table 2 Macroscopic and Microscopic characterization of isolates

No.	Code of isolates	Shape	Margin	Elevation	Spore	Surface	Consistency	Opacity	Gram's reaction	Arrangement
1	lk2	Round	Entire	Raised	White	Rough	Powdery	Opaque	G+ve	Filamentous
2	lk5	Irregular	Lobate	Umbonate	Grey white	Rough	Powdery	Opaque	G+ve	Filamentous
3	lk7	Round	Entire	Slight raised	Light yellow	Rough	Powdery	Opaque	G+ve	Filamentous
4	lk8	Irregular	Lobate	Umbonate	Pale grey	Rough	Powdery	Opaque	G+ve	Filamentous
5	lk9	Round	Undulate	Flat	White	Rough	Dry	Opaque	G+ve	Filamentous
6	lk10	Round	Entire	Raised	White	Rough	Powdery	Opaque	G+ve	Filamentous
7	st1	Round	Lobate	Umbonate	Pale white	Rough	Powdery	Opaque	G+ve	Filamentous
8	st5	Irregular	Undulate	Raised	Creamish	Rough	Powdery	Opaque	G+ve	Filamentous
9	vb1	Irregular	Undulate	Umbonate	Grey	Rough	Dry	Opaque	G+ve	Filamentous
10	vb3	Round	Entire	Raised	Grey	Rough	Powdery	Opaque	G+ve	Filamentous
11	mb1	Irregular	Undulate	Umbonate	Grey	Rough	Powdery	Opaque	G+ve	Filamentous
12	mb2	Irregular	Entire	Raised	Grey	Rough	Powdery	Opaque	G+ve	Filamentous
13	db4	Round	Entire	Raised	White	Rough	Powdery	Opaque	G+ve	Filamentous
14	db6	Irregular	Undulate	Raised	Grey	Rough	Powdery	Opaque	G+ve	Filamentous
15	dh1	Round	Entire	Raised	White	Rough	Powdery	Opaque	G+ve	Filamentous
16	dh2	Irregular	Undulate	Raised	Pale white	Rough	Powdery	Opaque	G+ve	Filamentous
17	vd1	Round	Entire	Raised	Light grey	Rough	Powdery	Opaque	G+ve	Filamentous
18	vd2	Round	Entire	Raised	Creamy white	Rough	Powdery	Opaque	G+ve	Filamentous
19	vd5	Filamentous	Filamentous	Raised	Light grey	Rough	Powdery	Opaque	G+ve	Filamentous
20	kd1	Round	Entire	Raised	White	Rough	Powdery	Opaque	G+ve	Filamentous
21	kd2	Irregular	Lobate	Raised	Creamy white	Rough	Powdery	Opaque	G+ve	Filamentous
22	kd3	Irregular	Lobate	Raised	Creamy white	Rough	Powdery	Opaque	G+ve	Filamentous
23	dd2	Irregular	Undulate	Raised	Pale white	Rough	Powdery	Opaque	G+ve	Filamentous
24	dd5	Round	Entire	Flat	White	Rough	Powdery	Opaque	G+ve	Filamentous
25	dd6	Irregular	Lobate	Umbonate	Grey	Rough	Powdery	Opaque	G+ve	Filamentous
26	dd8	Round	Lobate	Raised	Ivory	Rough	Powdery	Opaque	G+ve	Filamentous

Effect of Environmental Conditions on Growth and Antibiotic Production

Actinomycetes demonstrated tolerance to a wide range of pH (7.0–10.0) and salinity (1%–10% NaCl). Optimal growth and antibiotic production were observed at pH 9.0 and 5% NaCl, conditions that mimic the natural saline-alkaline environment of the Little Rann of Kutch.

Isolate LK8 showed significant growth and secondary metabolite production under these conditions. These findings align with earlier reports that haloalkaliphilic actinomycetes thrive in

alkaline and saline environments, producing bioactive compounds (Zvyagintsev et al., 2008; Novovsuren et al., 2007).

Table 4 Study of isolates under different conditions

SrNo.	Isolates	Range of pH	NaCl w/v	0%NaCl &pH	5% NaCl &pH 7.0	0%NaCl &pH 9.0	5 %NaCl &pH 9.0
1	lk2	7 to 10	1% to 3%	+	-	+	-
2	lk5	7 to 10	1% to 7%	+	+	+	-
3	lk7	7 to 10	1% to 3%	+	-	+	-
4	lk8	7 to 10	1% to 3%	+	-	+	-
5	lk9	7 to 10	1% to 7%	+	+	+	-
6	lk10	7 to 10	1% to 7%	+	+	+	-
7	st1	7 to 10	1% to 7%	+	+	+	-
8	st5	7 to 10	1% to 10%	+	+	+	-
9	vb1	7 to 9	1% to 7%	+	+	+	+
10	vb3	7 to 10	1% to 10%	+	+	+	-
11	mb1	7 to 10	1% to 10%	+	+	+	-
12	mb2	7 to 10	1% to 5%	+	+	+	+
13	db4	7 to 10	1% to 3%	+	-	+	-
14	db6	7 to 10	1% to 8%	+	+	+	-
15	dh1	7 to 10	1% to 5%	+	+	+	+
16	dh2	7 to 10	1% to 5%	+	+	+	-
17	vd1	7 to 9	1% to 5%	+	+	+	-
18	vd2	7 to 10	1%	+	-	+	-
19	vd5	7 to 10	1%	+	-	+	-
20	kd1	7 to 9	1% to 5%	+	+	+	-
21	kd2	7 to 10	1% to 7%	+	+	+	-
22	kd3	7 to 10	1% to 5%	+	+	+	+
23	dd2	7 to 10	1% to 5%	+	+	+	+
24	dd5	7 to 10	1% to 5%	+	+	+	-

Isolation of MRSA

Hiculture transport swabs were used to gather samples from patients at several public hospitals. Some of the most common ways that MRSA infections spread are via infected intravenous catheters, infected diabetic bones, infected burn victims' exposed skin, infected non-TB patients' sputum samples, infected orthopedic surgery patients' towels and bandages, and infected patients' open wounds while hospitalized. Here are the sources and locations of the samples:

Table 5 Sample collection from patients of different hospital for the isolates of MRSA

Sr.No.	Source of isolation	Place of isolates
1	Sputum	Dharpur civil hospital, Patan
2	Bandages	Orthopedic Department Dharpur hospital
3	Wound	Unjha cottage hospital
4	Burned skin	Mahesana trauma center
5	Fomites	Patan civil hospital
6	Fomites	Patan civil hospital
7	Sputum	T.B. Center, Patan
8	Sputum	T.B. Center, Patan

Antibiogram of all selected MRSA isolates

The antibiogram of eight different MRSA strains was examined on Muller Hinton Agar using a Dodeca disk (HiMedia). Onwubiko et al. (2011) investigated antibiotic sensitivity patterns to different antibiotics. The diameter of the inhibition zone around each disk in which bacterial growth was not detected was used to interpret the results. Using the guidelines provided by the manufacturer, the MRSA isolates were classified as either resistant, intermediate, or susceptible. Adugna et al. also conducted research along these lines while evaluating Staphylococcus aureus antibiograms. Staphylococci that are resistant to methicillin and oxacillin were documented in a 2000 report by the National Committee for Clinical Laboratory Standards (Wayne et al., 2012).The book said that an antibiogram is a table that lists the proportion of bacterial pathogens that are sensitive to various antimicrobial treatments. There were four categories of results for the antimicrobial susceptibility test: susceptible, intermediate, resistant, and non-susceptible. At this time, it is necessary due to the paucity of information on antibiograms and other treatments that might enhance practical antibiotic prescribing (Furuno et al., 2014).

The interpretive criteria for zone diameter as it pertains to Staphylococcus species were summarized by the American society for microbiology (Hudzicki, n.d.). In light of this, we may draw conclusions based on the conventional criteria. A total of twelve antimicrobial drugs from 10 distinct classes were used to evaluate the antibiotic resistance pattern of all the MRSA isolates.

Table 6 Antibiogram of MRSA isolates

Antibioticcategory	Antimicrobial agent	Con.(µg)	Mode ofaction	MRSA-1	MRSA-2	MRSA-3	MRSA-4	MRSA-5	MRSA-6	MRSA-7	MRSA-8
Aminoglycosid e	Gentamicin	10	Protein Synthesis	S	R	S	R	S	S	R	S
Ansamycins	Rifampicin	5	Protein Synthesis	R	R	R	R	R	R	R	R
Anti- staphylococcal b-lactams	Oxacillin	1	Cell wallsynthesis	R	R	R	R	R	R	R	R
Fluoroquinolon es	Ciprofloxac in	5	Blockage of DNA Gyrase	I	R	I	R	I	R	R	S
Folate pathway inhibitors	Co-Trimoxazole	25	Folic acidinhibition	R	R	R	R	R	R	R	S
Glycopeptides	Teicoplanin	30	Cell wall synthesis	S	R	S	R	S	S	R	S
	Vancomyci n	30	Cell wall synthesis	R	R	R	R	R	R	R	R
Lincosamides	Clindamyci n	2	Protein Synthesis	I	R	I	I	S	I	R	R
Macrolides	Erythromyc	15	Protein	S	R	R	R	I	R	R	R
	in		Synthesis								
Oxazolidinones	Linezolid	30	Protein Synthesis	S	R	R	R	R	S	R	R
Tetracycline	Minocyclin e	30	Protein Synthesis	I	R	I	R	S	R	R	I
	Tetracyclin e	30	Protein Synthesis	R	R	I	R	S	S	R	I

Ampicillin-resistant Staphylococcus aureus (MRSA) 2 and MRSA 7 were the most resistant of all the isolates tested, showing resistance to every antibiotic in seven different classes and at least one in each of the other three. Finally, MRSA 4 showed resistance to every antibiotic in six classes, with at least one drug in three classes being ineffective. The MRSA 8 strain was found to be resistant to every antibiotic in five different classes, as well as one drug in one class. MRSA 3 and MRSA 5 showed resistance to four different antibiotic classes, with one class having no resistance at all. Both MRSA 1 and MRSA 6 had the lowest sensitivity, demonstrating resistance to three different types of antibiotics.

Optimization of different parameter for the production of antibiotic

To find the optimal set of parameters, including carbon and nitrogen sources, pH, and temperature, self-directing optimization was used. Islam et al. found the optimal nutritional and cultural conditions for actinomycetes to produce antibiotics in a shake-flask experiment (2009).We tried to find the best way to grow the lk8 strain so that it might produce antimicrobial metabolites. The generation of antimicrobial metabolites began after 7 days of incubation in the culture broth, reached its peak after 10 days, and then steadily reduced thereafter. In order to get the highest

levels of antimicrobial metabolites, the pH of the culture medium was increased to 8.0. In a study conducted by Ripa et al., the highest level of bioactive metabolite synthesis was found at a NaCl concentration of 1%. Among the minerals that were examined, K2HPO4 and NaCl were shown to have a favorable effect on the antibiotic production by the strain. For lk8 and kd3 isolates, optimization was performed. Each of the two sequencing strains was given its own unique starch casein medium to ferment in. Based on prior research, ethyl acetate extracts from various sources were used in bioassays against MRSA-2 and MRSA-7.

Optimization for anti-MRSA activity of lk8 using carbon source, nitrogen source, NaCl and pH

- **Effect of different carbon sources on the antimicrobial activity of Streptomyces geysiriensis (lk8)**

Aiming to determine how two MRSA-2 and MRSA-7 isolates responded to Streptomyces geysiriensis (lk8) treated with various carbon sources for antibacterial activity. In place of starch, eight different carbon sources were introduced to the SCB medium at a 1% w/v level: maltose, glucose, xylose, fructose, lactose, galactose, and sucrose.

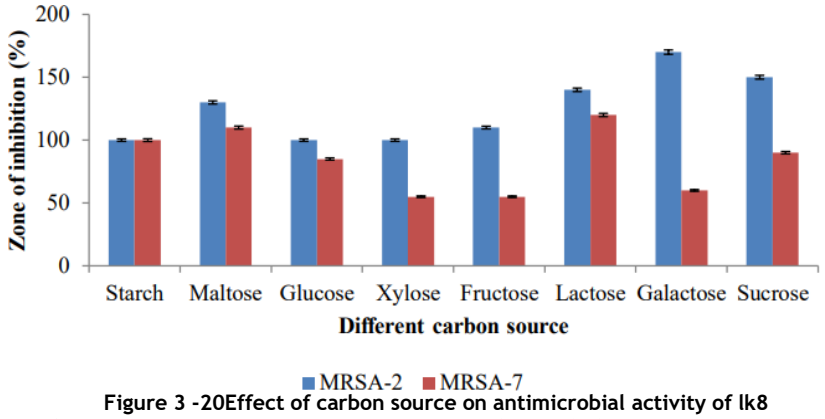


Figure 3 -20Effect of carbon source on antimicrobial activity of lk8

Antimicrobial Activity of Crude Extracts

The crude extracts from 14 of the 26 isolates exhibited antibacterial activity against MRSA strains, with inhibition zones ranging from 10–16 mm. LK8 demonstrated the highest activity, with an inhibition zone of 16 mm against MRSA-1. The MIC and MBC values of LK8 were determined to be 12.5 µg/ml and 25 µg/ml, respectively, highlighting its potential as a strong anti-MRSA candidate. These results are consistent with previous studies showing significant antibacterial activity from actinomycetes in saline environments (Shetty et al., 2014).

Chemical Characterization of Bioactive Extracts

GC-MS analysis of LK8's ethyl acetate extract revealed the presence of two major bioactive compounds:

- **1-Decanol**: Known for its antibacterial and antifungal properties.
- **2-Piperidinone**: A compound with reported antimicrobial activity.

These findings support the hypothesis that extremophilic actinomycetes from saline deserts can produce unique secondary metabolites with significant antibacterial properties (Sahil et al., 2011).

Table 6: GC-MS of Streptomyces geysiriensis (lk8)

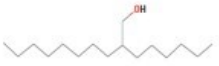
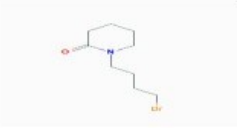
peak	Area	Retention Time	Compound Name	Molecular weight	Chemical Formula	Structure
1	9,236,034	23.647	1-DECANOL,2-HEXYL	242	C16H34O	
2	10,198,038	25.753	2-piperidinone, N-[4-bromo-n-butyl]	233	C9H16ONBr	

Figure-GC-MS analysis of the purified active fraction (a) detection of 1-DECANOL,2-HEXYL (b) and 2-piperidinone, N-[4-bromo-n-butyl] (c) from purified active fraction

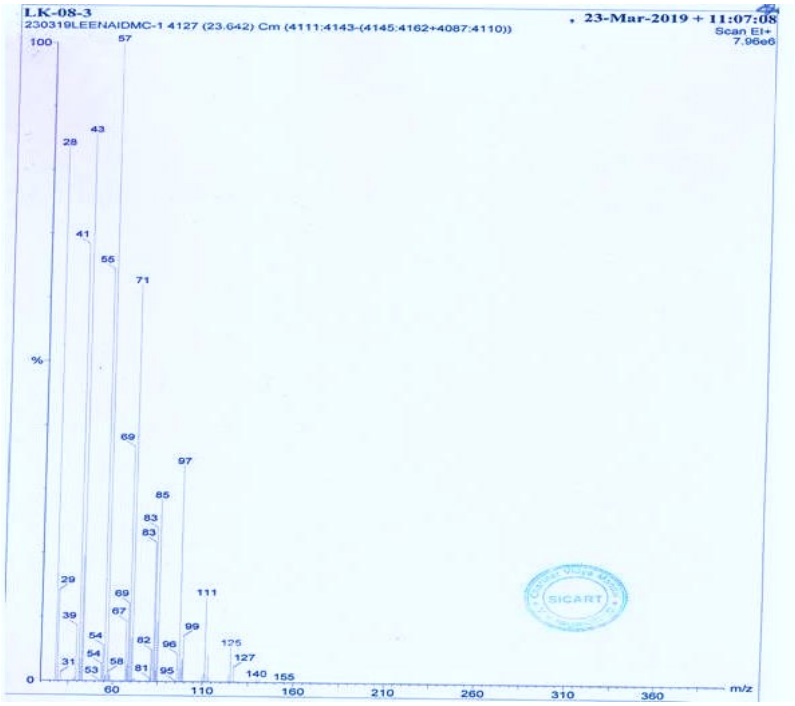
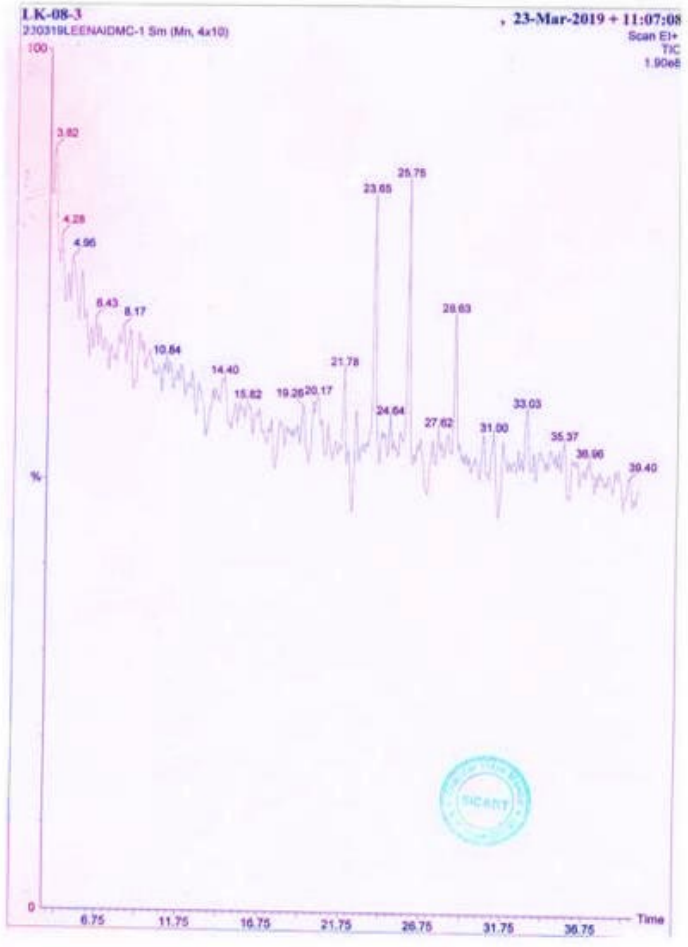


Figure 34 detection of 1-DECANOL,2-HEXYL



Comparison with Existing Antibiotics

The antimicrobial activity of LK8's crude extract against MRSA was compared to that of commercial antibiotics. While vancomycin showed limited efficacy, the crude extract demonstrated a comparable or superior effect in inhibiting MRSA growth, particularly against multidrug-resistant strains. Such results highlight the potential of natural products as alternatives to conventional antibiotics (Kato et al., 2011).

Table 7: List of Compounds reported from the identified isolates

Isolate	EzTaxon Identification	Compound name found in research paper and pubchem from the isolate	Pubchem bioassay ID	Activity reported
lk8	*Streptomyces geysiriensis*	**Moenomycin**	207002	anti-MRSA
			66761	Anti-bacterial
			205582	Against *Staphylococcus epidermidis*
		bambermycin		
kd3	*Streptomyces purpurascens*	**Anthracycline**		
		Rhodomycin		
mb2	*Streptomyces pactum*	**Pericidin**	Compound not found in pubchem	
		Pactamycin	734203	anti-malarial
			734207	anti-cancer
			734211	*Acinetobacter baumannii*
mb1	*Streptomyces griseoincarnatus*	**Emycin**	318802	Against *E. coli*
			318801	*B. subtilis*
			318803	*Pseudomonas aeruginosa*
			318804	Anti-fungal
			318800	*Staphylococcus aureus*
lk2	*Streptomyces pratensis*	**Angucycline**	654895	anti-cancer
lk5	*Streptomyces violascens*	**Albaflavenoid**	Bioassay not found	Novel compound, activity not reported
		Violapyrones A-G, alpha-pyrone derivative	1055615	*Aspergillus niger*
			1055615	*Candida albicans*
			1055616	*Pseudomonas aeruginosa*
			1055620	Anti-cancer
lk7	*Streptomyces philanthi*	No compound reported from research paper or pubchem	Bioassay not found in pubchem from this isolate	

CONCLUSION

The study highlights the promising potential of saline desert microflora, specifically actinomycetes from the Little Rann of Kutch, as a source of novel antibiotics with anti-MRSA activity. Through the systematic isolation and characterization of 26 actinomycetes isolates, we identified several strains that exhibited significant antibacterial properties against MRSA. The optimization of growth conditions, including pH and salinity, was crucial for enhancing antibiotic production, with isolates thriving under alkaline and saline conditions typical of their natural habitat. These findings emphasize the unique ability of extremophilic microbes to produce bioactive compounds in response to harsh environmental stressors, which may offer new strategies for combating antibiotic-resistant pathogens.

The results of this study suggest that saline deserts hold untapped potential for the discovery of new antimicrobial agents, which could serve as alternatives to current antibiotics, particularly in the fight against resistant infections like MRSA. However, further research is necessary to clarify the biosynthetic pathways of the identified antibiotics and to assess their therapeutic potential through in vivo studies. This work lays the groundwork for future exploration into the microbial ecology of saline deserts, which could lead to the development of effective treatments for antibiotic-resistant infections and contribute to global health solutions.

REFERENCES

- Cogen, J. D., Hall, M., Faino, A. V., Ambroggio, L., Blaschke, A. J., Brogan, T. V., Cotter, J. M., Gibson, R. L., Grijalva, C. G., Hersh, A. L., Lipsett, S. C., Shah, S. S., Shapiro, D. J., Neuman, M. I., & Gerber, J. S. (2023). Antibiotics and outcomes of CF pulmonary exacerbations in children infected with MRSA and Pseudomonas aeruginosa. Journal of Cystic Fibrosis. https://doi.org/10.1016/j.jcf.2022.08.001
- Côté, J. M., Desjardins, M., Cailhier, J. F., Murray, P. T., & Souligny, W. B. (2022). Risk of acute kidney injury associated with anti-pseudomonal and anti-MRSA antibiotic strategies in critically ill patients. PLoS ONE. https://doi.org/10.1371/journal.pone.0264281
- H.K., H., A., P., G.B., T., M.R., E., S., H., R., F., K., K., & J.C., M. (2018). Next steps in predicting anti-MRSA antibiotic prescribing. Open Forum Infectious Diseases.
- Holmer, H. K., Pakyz, A., Tallman, G. B., Elman, M. R., Hohmann, S., Fu, R., Kuper, K., & McGregor, J. C. (2018). 1873. Next Steps in Predicting Anti-MRSA Antibiotic Prescribing. Open Forum Infectious Diseases. https://doi.org/10.1093/ofid/ofy210.1529
- Jones, B. E., Ying, J., Stevens, V., Haroldsen, C., He, T., Nevers, M., Christensen, M. A., Nelson, R. E., Stoddard, G. J., Sauer, B. C., Yarbrough, P. M., Jones, M. M., Goetz, M. B., Greene, T., & Samore, M. H. (2020). Empirical Anti-MRSA vs Standard Antibiotic Therapy and Risk of 30-Day Mortality in Patients Hospitalized for Pneumonia. JAMA Internal Medicine. https://doi.org/10.1001/jamainternmed.2019.7495
- Kato, A., Hirata, H., Ohashi, Y., Fujii, K., Mori, K., & Harada, K. I. (2011). A new anti-MRSA antibiotic complex, WAP-8294A II. Structure characterization of minor components by ESI LCMS and MS/MS. Journal of Antibiotics. https://doi.org/10.1038/ja.2011.9
- Liu, W. T., Chen, E. Z., Yang, L., Peng, C., Wang, Q., Xu, Z., & Chen, D. Q. (2021). Emerging resistance mechanisms for anti-MRSA antibiotics in Staphylococcus aureus: A comprehensive review. Microbial Pathogenesis. https://doi.org/10.1016/j.micpath.2021.104915
- Maray, I., Álvarez-Asteinza, C., Macía-Rivas, L., Fernández-Laguna, C. L., Alaguero-Calero, M., Valledor, P., & Fernández, J. (2024). Real-World Data Study on Risk Factors Associated with Acute Kidney Damage in Patients Treated with Anti-MRSA Antibiotics. Future Pharmacology. https://doi.org/10.3390/futurepharmacol4010004
- Novovsuren, M., et al. (2007). Halophilic and Haloalkaliphilic Actinomycetes. Journal of Desert Microbiology.
- Sahil, J., et al. (2011). GC-MS Analysis of Bioactive Metabolites. Chemical Biology Journal.
- Shetty, P. H., et al. (2014). Antimicrobial Potential of Streptomyces Isolates from Extreme Environments. International Journal of Microbiology.
- Tang, S., et al. (2008). Optimal Conditions for Halophilic Actinomycetes. Applied Microbiology Journal.
- Wayne, P. A., et al. (2012). Antimicrobial Susceptibility Testing Guidelines. Clinical Laboratory Standards Institute.
- Wang, Y., Qian, G., Liu, F., Li, Y. Z., Shen, Y., & Du, L. (2013). Facile Method for Site-Specific Gene Integration in Lysobacter for Yield Improvement of Anti-MRSA Antibiotics. ACS Synthetic Biology. https://doi.org/10.1021/sb4000806
- Zvyagintsev, D., et al. (2008). Halophilic Actinomycetes in Saline Soils. Environmental Microbiology Reports.