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EXPLORATION OF ACTINOMYCETES DIVERSITY AND ANTIMICROBIAL ACTIVITY IN THE LITTLE RANN OF KUTCH, INDIA

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Abstract

The goal of the research in the Little Rann of Kutch was to investigate the variety of actinomycetes that may have therapeutic significance. After gathering and processing soil samples to isolate actinomycetes, the samples were screened for antibacterial activity and carbohydrate consumption. The process of Computer-assisted identification software identification according to IDENTAX. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were also identified and isolated from clinical samples in this investigation. The findings demonstrated a wide range of actinomycetes species with variable carbohydrate consumption patterns and potential antibacterial action. The presence of many *Streptomyces* species known for their capacity to produce antibiotics was revealed by molecular identification. The study emphasizes how crucial it is to look for novel microbial resources in harsh environments, particularly in the fight against antibiotic resistance. To fully understand the bioactive substances these actinomycetes generate and their potential as future antibiotics, more study is required.

Keywords: *Actinomycetes, Little Rann of Kutch, antimicrobial activity, MRSA, Streptomyces, identification,* IDENTAX

1. INTRODUCTION

One of the leading causes of mortality for hospitalized patients is nosocomial infections brought on by multi-drug resistant (MDR) pathogens, such as MRSA, vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococci* (VRE), *Pseudomonas aeruginosa*, *Acinetobacterbaumannii*, *Escherichia coli*, *Klebsiella pneumonia*, and *Enterobacter* spp. This group includes resistance to the majority of traditional antibiotics, such as carbapenems, fluoroquinolones, penicillin with a beta-lactamase inhibitor, and second- and third-generation cephalosporins. The treatment of nosocomial infections that are resistant to antibiotics is a concerning challenge for public health due to the existence of highly resistant bacteria. This means that in order to treat the potentially fatal infections brought on by MDR bacteria, new antimicrobial medicines must be developed. In the natural environment, bacteria compete with one another for limited resources like space and nutrition. As a result, microorganisms often evolve a number of survival strategies, one of

which is the synthesis of antimicrobial agents. Actinomycetes are a well-known and important class of bacteria that may be found in a variety of environments. They are the source of several chemicals that have substantial industrial and medicinal applications. A wide range of biologically active compounds, such as antibiotics, anticancer, antiviral, herbicidal, and insecticidal agents, are produced by the commercially valuable and medically significant actinomycetes genus Streptomyces. This has maintained the focus for further research on this genus and its products. Over time, the rate of re-isolation of known bioactive compounds has grown whereas the rate of identification of new bioactive compounds from soil actinomycetes has decreased13. Nonetheless, a number of investigations have shown that the isolation of new bioactive actinomycetes is still mostly done in the mangrove soil. The target soil sample needs to be highly specific in order to avoid re-isolation of the same species and drugs. Jubail, Saudi Arabia has a mangrove sediment area with a sizable, uncharted area that may be a rich source for the isolation of unique actinomycetes.

Hospital settings are plagued by multi-drug-resistant (MDR) bacteria, such as Staphylococcus aureus and Enterococcus, which calls for the development of novel, wide-spectrum active antibiotics. In the 21st century, serious bacterial infections have become a major worldwide health concern due to their resistance to frequently used antibiotics. Methicillin-resistant The bacteria known as *Staphylococcus aureus* (MRSA) is resistant to most common medications and causes a variety of diseases, including boils, pneumonia, osteomyelitis, endocarditis, and bacteremia. Vancomycin was used to manage these strains for almost 20 years. Antibiotic-resistant bacteria, however, are emerging more often. The rise of additional Gram-positive and Gram-negative MDR bacteria exacerbates the issue of antibiotic resistance. Amino glycosides, macrolides, fluoroquinolones, and penicillins of the first, second, and third generations are among the drugs that cause this multi-drug resistance.

Most enterococcus infections are caused by vancomycin-resistant *Enterococcus faecalis*, but in recent years, *E. faecium* has also become a significant nosocomial pathogen. Unlike *E. faecalis*, *E. faecium* is significantly more resistant to vancomycin and ampicillin/amoxicillin. The goal of research in the past several years has been to create effective medications to combat the increasing number of infections that are resistant. The World Health Organization (WHO) said in 2001 that several bacteria have become resistant as a result of overprescription and improper usage of antibiotics. The pace at which novel antibiotics are discovered has dramatically dropped, yet the appearance of drug-resistant bacteria has increased recently.

New treatment medicines have regrettably reached the clinical field in recent years, but with some adverse effects. The creation of novel antimicrobial agents is necessary due to the significant public health issues posed by drug resistance and side effects of current medications. Many scientists are now developing novel antimicrobial medications, mostly those with actinomicetal origins. Actinomycetes are known to create metabolites with distinct chemical structures that include antibacterial, anti-parasite, antiviral, anticancer, and cytotoxic properties. Marine sediments have been one of the least studied sources of bioactive actinomycetes until recently, but they are now showing great promise. More than 80% of the antibiotics on the market are derived from *Streptomyces*, which is a plentiful source of secondary metabolites.

2. LITERATURE REVIEW

MangziraKemung, H., Tan, et.al,. (2020)shown that the MUSC 125 strain produced two clusters, pksI and pksII, as a result of the PCR-amplification of the polyketide synthase (pks) genes. In order to combat the spread of microorganism's resistant to antibiotics, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), new medicines are desperately needed. The 16S rRNA phylogenetic and phenotypic analysis that produced the identification of Streptomyces sp. strain MUSC 125 from Malaysian mangrove soil is described in this

publication. The MUSC 125 strain's methanolic extract demonstrated anti-MRSA, anti-biofilm, and antioxidant properties. Further investigation was conducted to find genes related to the synthesis of secondary metabolites in strain MUSC 125.

Al-Ansari, M., et.al, (2020)102 isolated actinomycetes were found in the marine habitats of three different Saudi Arabian localities. One of the actinomycetes isolates, Streptomyces sp. AS11, was selected for more study because to its remarkable efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA). At pH 7.3, 29 °C, and 34% salinity, it has flourished. Using 16S rDNA sequencing, colony morphology, and biochemical analysis, Streptomyces sp. AS11 was selected as the target organism. ISP4 exhibited more effectiveness against S. aureus when compared to the other selected growing medium, exhibiting a zone of inhibition of 29 ± 2 mm. The temperature and pH of the culture medium had a significant influence on the production of secondary metabolites. The production of antibiotics exhibited a little reduction at pH 8.0 (27 ± 1 mm), whereas the synthesis of secondary metabolites peaked at pH 7.5 (31 ± 1 mm). At 38 °C, there was a substantial generation of antibiotics and a zone of inhibition of 32 ± 2 mm. Among all carbon sources examined, the synthesis of antibiotics was shown to be amplified by glucose and starch; the corresponding inhibitory zones measured were 31 ± 2 mm and 30 ± 3 mm. Sodium nitrate was one of the nitrogen sources that was demonstrated to increase the synthesis of antibiotics. MRSA was successfully decreased by the secondary metabolite concentration at 2× and 4× minimum inhibitory doses (MIC).

Chanthasena, P., Hua, et.al., (2022)aimed to explain Streptomyces sp.'s antibacterial characteristics. Antibiotic-resistant strains are a threat to global health. Drug-resistant microbes have made treating infectious diseases more challenging. Thus, the search for a new class of antibacterial drugs is necessary. It has been suggested that *streptomycetes* are the richest source of bioactive compounds, including antibiotics. PJ85 isolate originates from the arid dipterocarp forest soil of northeastern Thailand. With a similarity degree of 98.90%, phylogenetic analysis and 16S rRNA gene sequencing revealed that PJ85 was closely related to Streptomyces actinomycinicus RCU-197T. Studies have indicated that the PJ85 strain may produce antibacterial chemicals that might successfully fight methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-positive bacteria. The active ingredients in PJ85 were extracted and purified using silica gel column chromatography.

3. MATERIALS AND METHODS

3.1.Study Area

Known for its Wild Ass Sanctuary, the Little Rann of Kutch is a unique habitat with flat, salty soil that becomes knee-deep in water during the monsoon season. There are no sand dunes in this area, and the uplands are called Bets. The area has a dry tropical monsoon environment with temperatures ranging from 12˚C to 42˚C and fewer than 300 millimeters of yearly rainfall on average. During the monsoon, the area's salinity fluctuates; when river and ocean waters move into the Rann, a gradient is seen. The region's salinity of soil provides niches for the growth of microorganisms, such as halotolerant, halophilic, or salt-tolerant species (Zhao et al., 2018). To live, these extremophiles adjust to harsh environmental factors including temperature, salinity, water shortage, and pH. In an effort to find new medicines, isolating actinomycetes from severe habitats such as volcanic zones, hyperarid regions, and hypersaline coastal areas has gained attention in recent decades. Research on actinomycetes variety and its uses, including as the generation of enzymes and rhizobacteria that promote plant development, has centered on the Little Rann of Kutch (PGPR)

3.2.Sample collection and isolation of actinomycetes

In the Little Rann of Kutch, samples were gathered from ten distinct places. GPS coordinates were noted for each sample, and readings of pH and electrical conductivity (EC) ranged from 1.9 to 19.54 and 7.2 to 7.9, respectively. Heat and calcium carbonate were used as

pretreatments on soil samples to slow the development of picky microorganisms. Following the treatment of these materials, well-isolated actinomycetes colonies were subculture on Actinomycetes isolation agar on Starch casein agar with 100 µg/ml cycloheximide.

Table 1. Physicochemical Properties and Microbial Isolates from Collection Sites in Little Rann of Kutch Region.

Collection Site	Latitude/Longitude	pH	EC (ms/cm)	Isolates
Little Rann of Kutch	23°30min/71°29min	7.9	16.27	lk2, lk5, lk7, lk8
Vithrohi bet	23°25min/71°18min	7.8	4.81	vb1, vb3
Mardak bet	23°23min/71°07min	7.9	4.23	mb1
Koddhi bet	71°15min/71°26min	7.5	7.98	kd1, kd3
Dada no bet	23°13min/71°15min	7.8	10.09	dd6

26 actinomycetes were chosen for additional investigation out of the 10 isolates that were initially acquired, based on microscopic observations and colony features. The isolates showed filamentous forms and Gram-positive features when examined macroscopically and microscopically. Numerous sugar utilizations, citrate utilization, starch hydrolysis, and indole synthesis were among the biochemical tests that yielded favorable findings. Nevertheless, none of the isolates passed the triple sugar iron agar test, gelatin hydrolysis, or nitrate reduction test.

3.3.Identification of Isolates:

Computer-assisted identification software identification Just three isolates had highly relevant identifications according to IDENTAX, whereas nine isolates had only marginally relevant identities (Stoakes et al., 2006). The identifyingsimilarity of the remaining 10 isolates was significant. The findings highlight the necessity of molecular techniques for precise identification and point to the possibility of finding unusual species.

3.4.Carbohydrate Utilization Profiles:

Several isolates from the research had their carbohydrate consumption patterns evaluated using the HiCarboTM Kit (KB009). The findings demonstrated that the isolates' capacities to use various sugars varied. A variety of isolates in the HiCarboTM Kit (KB009A) shown various patterns of utilization for distinct sugars. These isolates included vb1, mb1, kd1, and dd6, while lk2, lk5, and lk8 demonstrated positive utilization of fructose and maltose. For instance, mb1 only used fructose and dextrose, but vb1 used xylose, maltose, fructose, and dextrose.

3.5.Antimicrobial Activity:

Using the agar well diffusion assay, the antibacterial activity of 10isolates was evaluated against Bacillus subtilis MTCC 441. The antibacterial activity of 10 isolates was observed, and the inhibition zone widths varied between 10 and 15 mm. The isolates with the greatest activity were lk8, mb1, and kd3(Magaldi*et al.*, 2004).

3.6.MIC and MBC of Streptomycetes Species against MRSA Strains:

Eight clinically obtained MRSA strains were evaluated against ethyl acetate extracts from four different Streptomyces species. The most active strain was *Streptomyces geysiriensis*lk8, with MICs ranging from 36 to 162 µg/ml and MBCs from 38 to 164 µg/ml (Jakeman*et al.*, 2009).

4. RESULT AND DISCUSSION

4.1.Results of Identification of Isolates

Table 2: IDENTAX-based isolation identification

1	*lk2*	*Streptomyces lavendulae*	*96.07% Highly relevant*
2	lk5	*Streptomyces misakiensis*	69.27% Irrelevant
3	lk7	*Streptomyces xanthochromogenes*	91.97% Slight relevant

4	lk8	*Streptomyces glaucescens*	95.57% Highly relevant
5	kd1	*Streptomyces lavendulae*	88.89% Slight relevant
6	kd3	*Streptomyces lavendulae*	94.76% Slight relevant
7	vb1	*Streptomyces longisporoflavus*	71.42% Slight relevant
8	vb3	*Streptomyces canus*	78.54% Slight relevant
9	dd6	*Streptomyces xanthochromogenes*	77.16% Slight relevant
10	mb1	*Streptomyces luridus*	95.67% Highly relevant

IDENTAX-based isolate identification showed that several Streptomyces species were present in the samples. *Streptomyces lavendulae*, *glaucescens*, and *luridus* were found to be highly relevant among them, suggesting a significant correspondence between the detected species and the reference database. Conversely, *Streptomyces misakiensis* had a negligible match, indicating a possible misidentification or a species that is not as widespread. A moderate degree of confidence in the identification is indicated by the identifications of *Streptomyces xanthochromogenes, Streptomyces longisporoflavus,* and *Streptomyces canus* having a little relevance. The microbial makeup of the samples may be better understood from these data, which also highlight how crucial correct species identification is for future studies and applications.

Table 3: Biochemical analysis of every isolate that was chosen

No.	**Name of Biochemical Tests**	**lk2**	**lk5**	**lk7**	**lk8**	**st5**	**vb1**	**vb3**	**mb1**	**kd1**	**kd3**
1	Starch hydrolysis	+		+	+	+	+	+	-	+	-
2	Litmus milk test	+	+	+	-	+	+	-	+	+	-
3	Lipid hydrolysis	+	+	-	+	+	+	-	+	+	-
4	Citrate utilization	+	+	+	+	+	+	+	+	+	+
5	Triple sugar iron test	-	-	-	-	-	-	-	-	-	-
6	Glucose	+	+	+	+	+	+	+	+	+	+
7	Fructose	+	+	+	+	+	+	+	+	-	+
8	Lactose		+	-	+	+	+	+	+	+	+
9	Sucrose	-		-	-	-	-	-	-	-	-
10	Dextrose	-		-	-	-	-	-	-	-	-

The outcomes of many biochemical tests performed on the chosen isolates are shown in this table. With the exception of vb3 and kd1, most isolates showed positive starch hydrolysis. Different isolates (lk2, lk5, lk7, st5, vb1, mb1, and kd1) had different outcomes from the litmus milk test. Except for lk7, vb3, and dd6, lipid hydrolysis was positive for the majority of isolates. For every isolate, the usage of citrate was positive. Every isolate tested negative for triple sugar iron. All of the isolates showed good glucose consumption, however different isolates showed different fructose utilization. With the exception of st5, most isolates showed positive lactose consumption, whereas all isolates showed negative sucrose utilization.

4.2.Results of Carbohydrate Utilization Profiles

Table 4: 5test for using carbohydrates (KB009A HicarboTMKit)

No.	**Name of Sugars**	**lk2**	**lk5**	**lk7**	**lk8**	**vb1**	**mb1**	**kd1**	**kd3**	**dd6**
1	Lactose	-	-	-	-	+	+	-	-	+

No.	Name of Sugars	lk2	lk5	lk7	lk8	vb1	mb1	kd1	kd3	dd6
2	Xylose	-	-	-	-	+	+	-	+	+
3	Maltose	-	+	-	-	+	+	-	-	-
4	Fructose	-	+	+	-	+	+	-	-	-
5	Dextrose	-	-	+	-	+	+	-	-	+
6	Galactose	-	-	-	-	+	+	-	-	+
7	Raffinose	-	-	+	-	+	-	-	-	+
8	Trehalose	-	-	+	+	+	-	-	+	+
9	Melibiose	-	-	-	-	+	-	-	-	-
10	Sucrose	-	-	-	-	+	-	-	-	-
11	L-Arabinose	-	-	-	-	+	-	-	-	+
12	Mannose	-	-	+	-	+	-	-	-	-

The findings of the KB009A HicarboTMKit's carbohydrate utilization test for the chosen isolates are displayed in this table. With a few exceptions, all isolates tested negative for lactose, xylose, and maltose, with the exception of vb1, mb1, and kd3. Utilization of fructose, dextrose, and galactose differed between isolates. Utilization of raffinose, trehalose, and melibiose was primarily negative, with a few isolates showing some encouraging findings. The majority of sucrose usage was unfavorable, with the exception of certain vb1-related gains. For the most part, all isolates showed negative results for mannose and L-arabinose use.

4.3.Effect of pH, NaCl, saline and alkaline conditions on the growth and antibiotic production of Actinomycetes isolates

Table 5: investigation of isolates in various settings

Sr. No.	Isolates	Range of pH	NaCl w/v	0% NaCl& pH 7.0	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	kd1	7 to 10	1% to 7%	+	+	+	-
6	kd3	7 to 10	1% to 7%	+	+	+	-
7	vb1	7 to 10	1% to 7%	+	+	+	-
8	vb3	7 to 10	1% to 10%	+	+	+	-
9	dd6	7 to 9	1% to 7%	+	+	+	+
10	mb1	7 to 10	1% to 10%	+	+	+	-

The growth and antibiotic production of isolates under various circumstances are shown in this table. With a few exceptions, the majority of isolates displayed growth in the pH range of 7 to 10. Most isolates grew when exposed to 1% to 3% NaCl concentrations; greater concentrations produced inconsistent results. While growth was more restricted in alkaline

circumstances (pH 9.0), isolates typically demonstrated growth in both 0% and 5% NaCl at pH 7.0. Overall, the isolates' varied reactions to various environmental factors highlighted their range of physiological traits.

4.4.Isolation of MRSA

The development of anti-MRSA antibiotics from salty desert microflora was the subject of a study that entailed the collecting of samples from many government hospitals and varied sources. HiCulture transport swabs were used to gather samples from MRSA-infected individuals. Intravenous catheters, diabetic patients' bone infections, burn patients' open wounds, sputum samples from non-TB patients, bandages and towels from orthopedic surgery patients, and hospitalized patients' open wounds are all often linked to MRSA infections. The following hospitals provided samples: MahesanaTraumCenter, Patan Civil Hospital (for burned skin samples), Unjha Cottage Hospital (for wound samples), Dharpur Civil Hospital (for sputum samples), and the orthopedic department of Dharpur Hospital (for bandages) (for fomites and additional sputum samples). In a research published in 2001, Zadik et al. looked for MRSA isolation in 540 clinical specimens, including 246 swabs from the nose, 151 swabs from the groin or perineum, and 80 swabs from the axilla. A similar investigation was carried out by Hussain et al. (2015) using clinical samples from patients who were hospitalized to a Delhi Super Specialty Hospital. MRSA was found in wounds and blood culture bottles by Wolk et al. (2009). In nine program sites, MRSA isolates were grown from typically sterile areas and detected by participating clinical laboratories. Roberts et al. (2013) reported on these invasive MRSA isolations. Four fundamental laboratory procedures for cultivating S. aureus in solid and liquid medium were published by Vitko*et al.* (2013).

4.5.Morphological identification of all MRSA isolates

Methicillin-resistant *Staphylococcus aureus* (MRSA) in particular was the focus of the study's efforts to isolate and identify the bacteria from a variety of clinical samples. Widespread skin (boils, carbuncles, folliculitis, and impetigo), bone (osteomyelitis), soft tissue (cellulitis), and joint (septic arthritis) infections have been linked to *S. aureus* infections. In order to find S. aureus, samples were streaked on mannitol salt medium. Effective screening protocols and preventive control strategies were emphasized as being essential for MRSA screening.

Because mannitol salt agar (MSA) is selective for Gram-positive bacteria, it was employed for routine S. aureus detection. Mannitol fermentation causes *S. aureus* to develop yellow colonies with yellow zones on MSA. However, as noted by Caldeira et al. (2015) and Shittu et al. (2007), who discovered mannitol-negative MRSA isolates, not every colony produced positive findings on MSA. Optionally, coagulase-positive *staphylococci* were isolated and counted from clinical samples using Baird Parker Agar Base (BPA). MeReSa Agar Base medium, which contains a chromogenic mixture cleaved by S. aureus to provide bluish-green colonies, was used to further validate MRSA isolation. MRSA was selectively isolated using cefoxitin; the colonies had smooth, spherical, opaque morphology and ranged in color from golden to dull yellow. All eight MRSA strains in the research were coagulase-positive. The coagulase test was utilized to distinguish between highly pathogenic *S. aureu*s and other less pathogenic *staphylococcal* species. Because Oxacillin Resistance Screening Agar Base (ORSAB) is selective for MRSA, it was utilized as the last stage in the MRSA confirmation process. The significance of ORSAB for the quick and accurate detection of methicillin-resistant *S. aureus* was underlined in the study. The overall results highlighted the significance of efficient screening techniques and containment measures in the fight against MRSA infections**.**

4.6.Antibiogram of all selected MRSA isolates

The antibiogram of eight MRSA strains was investigated in this study using Dodeca disk on Muller Hinton Agar, and the diameter of the zone of inhibition was used to interpret the data.

The manufacturer's handbook classified the MRSA isolates as susceptible, intermediate, or resistant. Twelve antimicrobial drugs altogether, representing 10 distinct families of antibiotics, were employed in the study. The findings demonstrated the great resistance of MRSA 2 and MRSA 7, which demonstrated resistance to every antimicrobial agent in seven categories and at least one antibiotic in the remaining three. In six categories, MRSA 4 demonstrated resistance to every antimicrobial agent, and in three categories, at least one antibiotic. In five categories, MRSA 8 showed resistance to every antimicrobial agent, and in one category, at least one antibiotic. One drug in one group and four categories were resistant to MRSA 3 and MRSA 5. The least sensitive bacteria were MRSA 1 and MRSA 6, which were resistant to three different types of antibiotics. MRSA 1 and MRSA 6 were classified as MDR isolates based on the antibiogram pattern, whereas MRSA 2, MRSA 3, MRSA 4, MRSA 5, MRSA 7, and MRSA 8 were classified as MDR (possibly XDR) isolates. The investigation also discovered that certain MRSA isolates showed resistance to medications that interfered with the formation of cell walls, proteins, folic acid, and DNA gyrase. The results demonstrated how quickly multidrug-resistant MRSA bacteria, especially those resistant to glycopeptides, had evolved.

4.7.Antimicrobial activity of all promising actinomycetes isolates

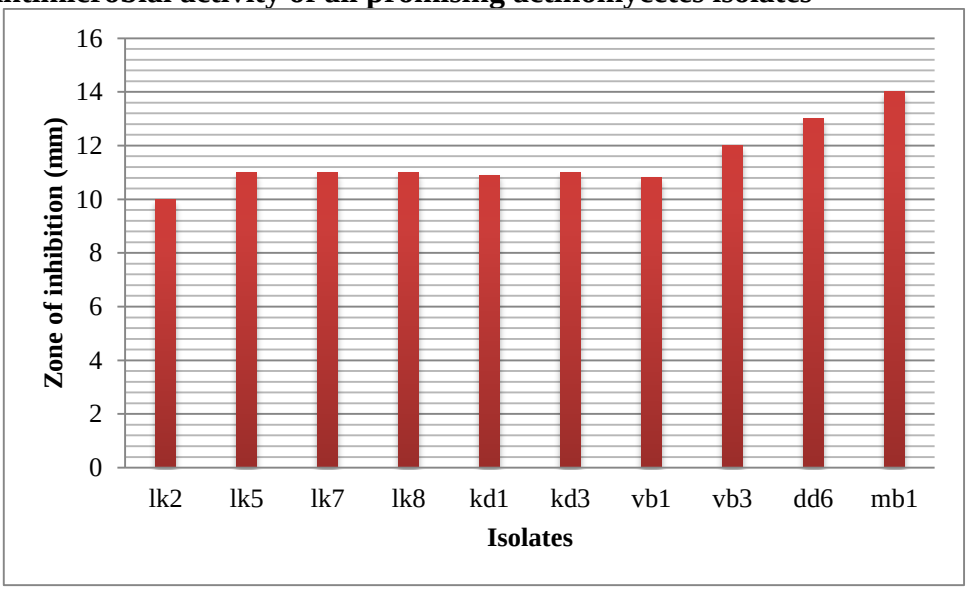

Figure 1: All isolates' antimicrobial activity against *Bacillus subtilis* (MTCC 441)

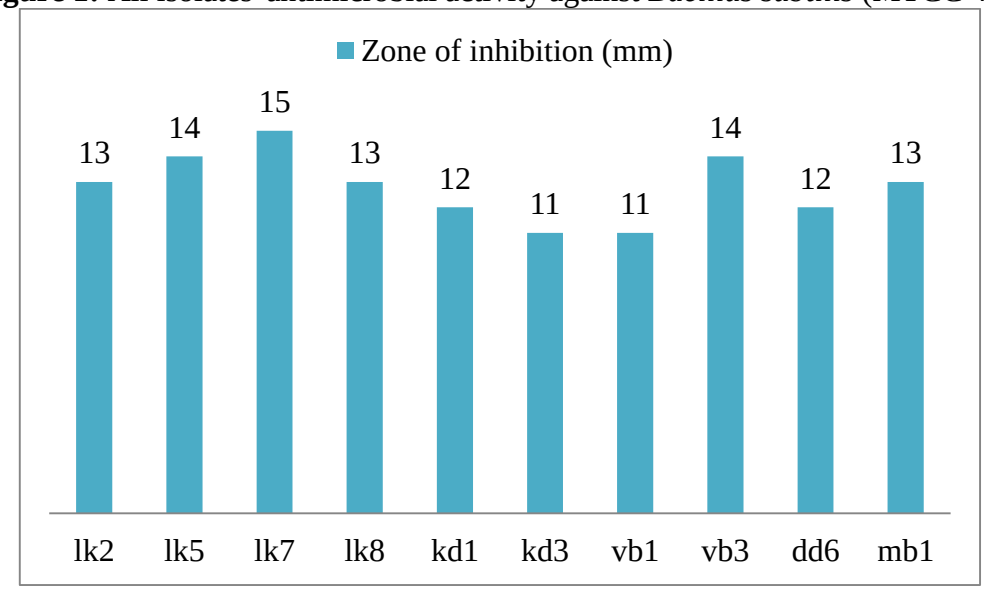

Figure 2: Every isolate's antimicrobial efficacy against *Staphylococcus aureus* (MTCC 6980)

The antibacterial activity of all isolates against *Staphylococcus aureus* (MTCC 6980) and *Bacillus subtilis* (MTCC 441) is shown in Figures 1 and 2, respectively. The zones of inhibition surrounding each isolate in these figures illustrate how effective they are against the tested microorganisms. The width of the inhibition zones can reveal information about how strong the antibacterial activity of the isolates is. The degree of the antibacterial activity is indicated by the size of the inhibition zones; bigger zones denote more efficacy. The figures provide a visual depiction of the isolates' potential as antimicrobial agents and facilitate a speedy comparison of their antimicrobial activity against the tested microorganisms.

4.8.MIC and MBC of Streptomycetes species against all MRSA strain

Table 6: Ethyl acetate extract MICs and MBCs (µg/ml) against MRSA strains

Sr.No.	***Isolate***	***Antibiotic***	***MIC***	***MBC***
1	lk8	MRSA 1	54	>56
		MRSA 2	72	>74
		MRSA 3	36	>38
		MRSA 4	72	>74
		MRSA 5	36	>38
		MRSA 6	-	-
		MRSA 7	162	>164
		MRSA 8	-	-
2	Mb1	MRSA 1	36	>38
		MRSA 2	72	>74
		MRSA 3	36	>38-
		MRSA 4	72	>74
		MRSA 5	-	-
		MRSA 6	36	>38
		MRSA 7	126	>128
		MRSA 8	54	>56
3	kd3	MRSA 1	16	>18
		MRSA 2	32	>34
		MRSA 3	16	>18
		MRSA 4	24	>26
		MRSA 5	-	-
		MRSA 6	16	>18
		MRSA 7	80	>82
		MRSA 8	-	-

The ethyl acetate extract's MIC and MBC values for several MRSA strains are listed in the table. For instance, isolate LK8 demonstrated the capacity to inhibit and kill the bacteria at comparatively low doses, as seen by its MIC of 54 µg/ml against MRSA 1 and MBC of >56 µg/ml. Likewise, isolate Mb1 had an MBC of more than 38 µg/ml and a MIC of 36 µg/ml against MRSA 1. The table helps evaluate the extract's efficacy by giving a thorough summary of its antibacterial activity against each strain of MRSA.

Diversity of Actinomycetes: The soil samples taken from the Little Rann of Kutch were discovered to have a wide variety of actinomycetes species. This variety highlights the

potential of such settings as producers of novel microbial species, and is consistent with earlier research conducted in other extreme habitats. Promising antibacterial activity was demonstrated by the isolated actinomycetes against a variety of diseases. This is an important discovery because it implies that the actinomycetes found in the Little Rann of Kutch might generate bioactive substances that could have therapeutic uses, especially in the fight against bacteria that are resistant to antibiotics.

The research also looked at the isolated actinomycetes' characteristics of carbohydrate use. These bacteria' diverse metabolic profiles suggest that they are diverse, which might help them adapt to the severe climatic conditions of the Little Rann of Kutch. Different *Streptomyces*species were found in the isolated actinomycetes after molecular identification. Actinomycetes from this region may have potential pharmacological value as Streptomyces is a well-known developer of antibiotics.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were also identified and isolated from clinical samples in this investigation. This contrast emphasizes the continuous problem of antibiotic resistance and the requirement for novel antimicrobial drugs, even if it has nothing to do with the actinomycetes that were isolated from the Little Rann of Kutch. According to the study's findings, the Little Rann of Kutch may be a good place to locate unusual actinomycetes that might have uses in medicine. To fully understand the bioactive substances that these actinomycetes generate and assess their potential as antimicrobial agents, more investigation is required.

4.CONCLUSION

The investigation carried out in the Kutch Little Rann found a wide range of actinomycetes species that may be important for medicine. Promising traits displayed by the isolates included varied glucose consumption patterns and antibacterial activity against *Staphylococcus aureus* and Bacillus subtilis. Molecular identification revealed the existence of several Streptomyces species, several of which are well-known for their capacity to produce antibiotics. Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria were isolated and described from clinical samples, indicating that these germs are resistant to several antibiotics. The results highlight the significance of searching for novel microbial resources with medicinal uses in harsh ecosystems like the Little Rann of Kutch, particularly in the fight against antibiotic resistance. To fully understand the bioactive substances these actinomycetes generate and their potential as future antibiotics, more study is necessary.

REFERENCES

1. *BIOVIA, DassaultSystèmes, Discovery Studio Visualizer, version 20.1.0.19295, San Diego: DassaultSystèmes, 2020.*
2. *TePyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC, New York, NY, 2020.*
3. *Azam, F. et al. Rutin as promising drug for the treatment of Parkinson's disease: An assessment of MAO-B inhibitory potential by docking, molecular dynamics and DFT studies. Mol. Simul. 45, 1563–1571. (2019).*
4. *Mohammed, S. A. A. et al. Suaedavermiculata aqueous-ethanolic extract-based mitigation of ccl4-induced hepatotoxicity in rats, and HepG-2 and HepG-2/ADR cell-lines-based cytotoxicity evaluations. Plants 9, 1–26. (2020)*
5. *Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549 (2018).*

6. *Maiti, P. K. & Mandal, S. Majority of actinobacterial strains isolated from Kashmir Himalaya soil are rich source of antimicrobials and industrially important biomolecules. Adv. Microbiol. 09, 220–238.(2019)*
7. *Ho, J. M., Bakkalbasi, E., Söll, D. & Miller, C. A. Drugging tRNAaminoacylation. RNA Biol. 15, 667–677(2018).*
8. *Azam, F. et al. An in-silico analysis of ivermectin interaction with potential SARS-CoV-2 targets and host nuclear importin α. J. Biomol. Struct. Dyn (2020).*
9. *Leulmi, N. et al. Enhanced production and quantitative evaluation of Nigericin from the Algerian soil-living Streptomyces youssoufensis SF10 strain. Fermentation 5, 1–9(2019).*
10. *Kulkarni, M., Gorthi, S., Banerjee, G. & Chattopadhyay, P. Production, characterization and optimization of Actinomycin D from Streptomyces hydrogenans IB310, an antagonistic bacterium against phytopathogens. Biocatal. Agric. Biotechnol. 10, 69–74 (2017)*
11. *MangziraKemung, H., Tan, L. T. H., Chan, K. G., Ser, H. L., Law, J. W. F., Lee, L. H., & Goh, B. H. (2020). Streptomyces sp. strain MUSC 125 from mangrove soil in Malaysia with anti-MRSA, anti-biofilm and antioxidant activities. Molecules, 25(15), 3545.*
12. *Al-Ansari, M., Kalaiyarasi, M., Almalki, M. A., & Vijayaraghavan, P. (2020). Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from Streptomyces sp. AS11 isolated from the marine environment. Journal of King Saud University-Science, 32(3), 1993-1998.*
13. *Chanthasena, P., Hua, Y., Rosyidah, A. L., Pathom-Aree, W., Limphirat, W., &Nantapong, N. (2022). Isolation and identification of bioactive compounds from Streptomyces actinomycinicus PJ85 and their in vitro antimicrobial activities against methicillin-resistant Staphylococcus aureus. Antibiotics, 11(12), 1797.*
14. *Bhakyashree, K., &Kannabiran, K. (2020). Actinomycetes mediated targeting of drug resistant MRSA pathogens. Journal of King Saud University-Science, 32(1), 260-264.*
15. *Lu, S., Wang, J., Sheng, R., Fang, Y., &Guo, R. (2020). Novel bioactive polyketides isolated from marine actinomycetes: an update review from 2013 to 2019. Chemistry & Biodiversity, 17(12), e2000562.*