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***Research article***

# STUDIES ON ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES AGAINST MDR WOUND BACTERIAL ISOLATES

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**ABSTRACT:** A total of five different actinomycete isolates were recovered from mine soil samples collected from Salem, Tamilnadu. These were then assessed for their antibacterial activity against five multidrug resistance bacterial wound isolates. All five isolates of actinomycete exhibited antagonistic activity. The zone of inhibition ranged between 11-25 mm. Among the 5 isolates of actinomycetes A5 isolate has highest antibacterial activity against *S.aureus* and *E.coli*. Out of five bacterial isolates *Pseudomonas aeruginosa* was highly suppressed by actinomycetes followed by *E.coli*. The maximum antibacterial activity was observed on 14th day incubation. The result of primary screening reveals that most of the active actinomycetes isolates were active against gram positive bacteria (*S.aureus*) than gram negative bacteria. The antibiotic profile of these isolates underlined their potential as a source of novel antibiotics.

**Key words:** Mine soil, screening of Actinomycete, MDR wound isolates, antibacterial activity.

## INTRODUCTION

Wound infections have been a problem in the field of surgery for a long time. Most bacteria live on our skin and other parts of the body with little potential for causing disease because of first line defence within the body. Surgical operation, trauma, burns, diseases, nutrition and other factors affect these defences. The skin barrier is disrupted by every skin incision, and microbial contamination is inevitable despite the best skin preparation (Howard *et al.,* 1980). The control of wound infections has not completely eradicated the problem because of development of resistance.

Wound can be infected by a variety of microorganisms ranging from bacteria (Bowler *et al*., 2001). The common gram positive organisms are the β –hemolytic *Streptococcus – Strept. pyogenes* and *S. aureus.* The gram negative aerobic rods are *Ps. aeruginosa*. The facultative anaerobes include *Enterobacter*species, *E. coli*, *Klebsiella* species and *Proteus* species (Gus Gunzalez *et al.*,2006; Mordi and Momoh, 2009). The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by Methicillin resistant *S. aureus* (MRSA) and Vancomycin resistant *Enterobacter* (VRE). Most bacteria have multiple routes of resistance to any drug and, once resistant, can rapidly produce vast numbers of resistant progeny (Livermore, 2003).

People infected with drug-resistant organisms are more likely to have longer and more expensive hospital stays, and may be more likely to die as a result of the infection. When the drug of choice for treating their infection doesn't work, they require treatment with second- or third-choice drugs that may be less effective, more toxic, and more expensive. This means that patients with an antimicrobial-resistant infection may suffer more and pay more for treatment.

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The past decade has seen an increase in the frequency of resistance to modern antibiotics, including the "third generation" cephalosporins due to overuse or misuse of antibiotics (Chaudhary and Aggarwal, 2004). Bacterial resistance has greatly hampered effective treatment of patients in clinical settings. The rise of antibiotic resistant microorganisms is one of the severe problems in health care systems of the world and infectious diseases are the second most serious cause of death worldwide. Therefore, new drugs have to be found, in order to combat such diseases and it is essential to find new compounds that have antimicrobial properties. Concerning the above facts, it is worthwhile to screen microbes which have the above properties to synthesize new drugs (Nascimento *et al*., 2000).

Microbial production of antibiotics is one of the rapidly expanding branch of industrial microbiology. Biotic potentials of actinomycetes are very wide as are capable of synthesizing many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides, enzymes etc. Of these compounds, antibiotics predominate in therapeutic and commercial importance. Rising numbers of antibiotic unresponsive infectious disease agents confront patients worldwide (Richa *et al*., 2011), and consensus has emerged that it is essential that novel antibiotic classes should be developed as part of the strategy to control the emerging drug-resistant pathogens. The present study was carried out to evaluate the effect of antimicrobial compound of actinomycetes against wound bacterial pathogens.

## MATERIALS AND METHODS

### Collection of soil samples

A total of 5 soil samples were collected based on texture, from different places of mine region in Salem, Tamilnadu. The samples were carefully taken with spatula by removing 2-3 inches top soil and kept in sterile polypropylene bags. The collected samples were taken to the laboratory for isolation of actinomycetes.

### Isolation of actinomycetes

Serial dilution agar plating method is used for the isolation and enumeration of actinomycetes (Aneja, 2003). Prepared Starch casein agar medium is used for the isolation of actinomycetes. The pH of media used was set to 7.2. Cyclohexamide (0.050 mg/ml) were added into the medium as antifungal agent (nalidixic acid 20 mg/l). One ml aliquots of various dilutions of soil samples were added over cooled and solidified agar medium The plates were incubated at 28ºC for at least one week. The ISP2 medium also utilized for the isolation of Actinomycetes.

The identification of actinomycetes was done on the basis of morphology of spore chain, pigment production, color of aerial mycelium, color of substrate mycelium, consistency, gram's staining and growth on actinomycetes media (Hucker and Conn (1923).

### Isolation of bacterial isolates from wound infected patients

### Collection of samples

Pus samples were collected aseptically with the aid of sterile swab sticks from 10 patients with different wounds infection at Namakkal Dist surrounding hospitals.

### Bacterial isolation and identification

Culture plates of eosin methylene blue agar, macconkey agar and mannitol salt agar, nutrient agar and cetrimide agar were used. The swab sticks used for the collection of the samples were streaked directly on the labelled agar plates and incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 h. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Biochemical tests were performed to identify microbes. Characterization and identification of the isolates was done using the methods of Cheesbrough (2004).

### Antibacterial stability test

The standard Kirby-Bauer disc diffusion method was used to determine the antimicrobial profiles of the *Klesiella spp, E.coli, Streptococcus mutans, Staphyolococcus aureus* and *Pseudomonas aeruginosa* isolates. Following antibiotics such as Ampicillin, Tetracycline, Kanamycin, Erythromycin, Methycilin, Vancomycin and Ciprofloxacin were used.

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The nutrient broth was prepared and sterilized at 121°C and inoculated the isolates then incubated at 37°C for 24 hrs. After incubation period the broth culture were swapped into surface of the Muller-Hinton agar plates and antibiotic disc were placed, then Plates were incubated at 37°C for 18 to 20 h. The zone of inhibition and resistance was measured, recorded and interpreted according to the recommendation of the disc manufacture.

**Fermentation**

The Actinomycetes was inoculated into 250 ml Erlenmeyer flasks containing 75 ml of liquid starch nitrate medium (seven flasks). The flasks were incubated on a rotary shaker (200 rpm) at 30 °C for 6 days. A twenty liter total volume was filtered through Whatman No.1 filter paper and followed by centrifugation at 5000 rpm for 20 minutes (Atta, 2009). The clear supernatant used as an antibacterial substance.

**Antibacterial activity of actinomycetes against clinical isolates (Shekar et al.,2011)**

Agar well method was used to determine the antimicrobial activity of isolates. Different types of wound bacterial test isolates were inoculated in nutrient broth and incubated at 37ºC for overnight. After overnight incubation, the bacterial culture was swapped in solidified Muller Hinton agar plate and 6mm diameter wells were punched in the plate. Then add five of actinomycetes culture filtrate (100µl) was dispensed in separate wells. Ampicillin was used as a standard. The plates were incubated at 37ºC for 24 hour. After incubation, the diameter of zone of inhibition around the wells were measured and recorded.

## RESULTS

In screening for actinomycetes with antibacterial activity, five isolates were screened. The potent actinomycetes were characterized by morphological and biochemical methods. The observed structure was compared with Bergeys manual of systematic Bacteriology (Kariminik *et al*., 2010). The photographic plate 1 shows the isolated colonies of actinomycetes on Starch Casein Agar. The selected isolates were found to be Gram positive, Sucrose, glucose, Mannitol and Lactose producers these revealed that the isolates have characteristic features of actinomycetes (Table.1). The five of multidrug resistance pathogenic bacteria have isolated from different wound infected patients. All the bacterial isolates were subjected antagonistic activity against actinomycetes (Table.2 & Plate 1). The actinomycetes showed antibacterial activities against five of the multidrug resistance test bacteria with zones of inhibition ranging between 11-25 mm. Among the 5 isolates of actinomycetes A2 isolate has highest antibacterial activity against *S.aureus* and *E.coli*. Out of five bacterial isolates, *Pseudomonas aeruginosa* was highly suppressed by actinomycetes followed by *E.coli*. The maximum antibacterial activity was observed on 14$^{th}$ day incubation.

**Table- 1: Physiological and biochemical character of Actinomycetes isolates**

| S.No | Medium/ Test | Character of Actinomycetes |
|---|---|---|
| 1. | ISP 2 | Creamish white-coloured colonies with a clear zone around them |
| 2. | Starch casein agar | white series colour of aerial mycelium |
| 3. | Grams stain | Gram positive , Rod shape |
| 4. | Indole | Negative |
| 5. | Methyl red | Positive |
| 6. | Citrate | Negative |
| 7. | Catalase | Positive |
| 8. | Oxidase | Positive |
| 9. | Urease | Negative |
| 10. | D- Glucose | Positive |
| 11. | Sucrose | Positive |
| 12. | Lactose | Positive |
| 13. | D- Mannitol | Positive |

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**Table-2: Antibacterial activity of Actinomycetes against wound isolates**

| S.No | Test Organisms | Antagonistic activity of Actinomycetes (mm) | | | | | Ampicilin |
|---|---|---|---|---|---|---|---|
| | | A1 | A2 | A3 | A4 | A5 | |
| 1. | *K. pneumoniae* | 21 | 15 | 14 | 16 | 14 | 20 |
| 2. | *S.aureus* | 14 | 22 | - | 13 | 19 | 15 |
| 4. | *E.coli* | 13 | 20 | 20 | 16 | 13 | 14 |
| 5. | *P.aeruginosa* | 25 | 15 | 13 | 28 | 22 | 23 |
| 6. | *S.mutans* | 10 | 11 | 11 | 18 | 14 | 12 |

**Plate 1**

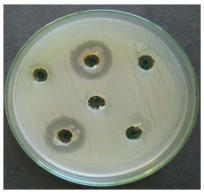
**Actinomycetes on starch casein agar**

**ISP-2 Medium**

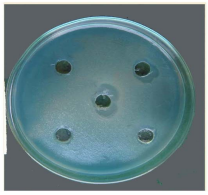
**Plate 2**

**Antibacterial activity against wound isolates**

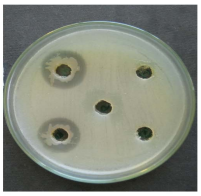
***Klesiella***



***P.aerugionosa***

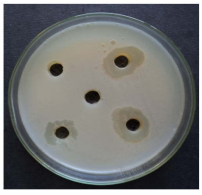


***S.mutans***



***S.aureus***

***E.coli***



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## DISCUSSION

The antibacterial resistance is presently an urgent focus of research and new antibiotics are necessary to combat multidrug resistant pathogens. The emergence and dissemination of antibacterial resistance is well documented as a serious problem worldwide (Gold *et al*., 1996). The emergence of bacterial resistance threatens to return us to the era before the development of antibiotics. This situation recommends the need for the investigation of new, safe and effective antimicrobials for replacement of invalidated antimicrobials (Smith *et al*., 1999). Actinomycetes have been recognized as source of several secondary metabolites like antibiotics and lytic enzymes among which isolates have been shown to have characteristics which make them useful as antagonistic agents against pathogens (Kariminik and Baniasadi, 2010).

The result of primary screening reveals that most of the active actinomycetes isolates were active against gram positive bacteria (*S.aureus*) than gram negative bacteria. The reason for different sensitivity between gram positive and gram negative bacteria could be ascribed to the morphological differences between these microorganisms, gram negative bacteria having an outer polysaccharide membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, The gram positive should more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. The present study result was well correlated with previous studies of Pandey *et al*., (2004), Cwala *et al.,* (2011). They reported that actinomycetes species usually show good activity against Gram-positive bacteria, but usually lacking activity against Gram-negative bacteria. In 2010, Maha *et al*., also reported antagonistic activity of actinomycetes against multidrug drug resistance of gram positive isolates. Several authors have screened soil samples collected from different parts all over the world for AAPMs as Brazil in study of Huddleston et al. (1997); Morocco (Yedir *et al.*, 2001); India (Haque *et al*., 1992; Peela *et al*., 2005; Thakur *et al*.,2007, Yadav *et al*.; 2009).

The crude extract of actinomycetes showed antibacterial activity against various species of human pathogens including both Gram positive and Gram-negative bacteria. These findings indicated that crude extract of A1 and A2 might be the alternative antimicrobial substance as a tool for controlling human diseases causing resistant pathogens.

In this study, we were able to determine the optimal growth conditions to cultivate actinomycetes producer isolates and developed methods to isolate and accumulate preparations of the new antibiotic. The continuing research of the producer had highly efficient against the clinical strains of multidrug resistance bacteria.

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