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Isolate, Characterization and Optimization of Soil Sample and Mobile Sample and Also Test the Antimicrobial Activity of that Sample

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Abstract: *In this study, soil bacteria were isolated and characterized including its antimicrobial Activity. For this, soil samples were collected from Environment. Soil samples were diluted and cultured in nutrient agar plates to obtain the isolated bacterial colonies. Antimicrobial activity producers were screened by stab overlay, agar well diffusion, cross streak plate, pour plate & spread plate methods. The strain isolates with significant antimicrobial activity producing potential, which inhibited the growth of sensitive strains in all applied assays have been identified as S. aureus. Maximum antimicrobial activity of the isolated strain was observed at pH 7, 24 hrs& incubation at ± 37 ˚C. Under optimized growth conditions, inhibitory zone was 18-14. 5 mm.T hese antimicrobial activity lost antibacterial activity after treating with Cefuroxime & Methanolic Extract. Antimicrobial activity obtained from producer strain was active agaist Staphylococcus aureus, E. coli. A total of 91 mobile phones belonging to staff members in Taif University screened for bacterial isolates using bacteriological methods. Bacteriological analysis revealed that about (85.1 %) of mobile phone samples were contaminated with bacteria. Some bacterial species were isolated from mobile phone samples. They identified as Staphylococcus and Bacillus spp. Genetic diversity of these bacteria was investigated by Random Amplified Polymorphic DNA (RAPD) analysis. The fingerprinting patterns revealed two main clusters of strains with a similarity level of approximately 55.8%.*

Keywords: Staphylococcus Aureus,,E.Coil, Antimicrobial Activity, Zone of inhibitions, Growth Curve of Bacteria. Gram staining, sterilization

I. INTRODUCTION

1.1 Microbiology & History of Microbiology

The word microbiology describes exactly what the discipline is: the study of small living things. Micro = small, Bio = living, and logy = to study. Microbiology (or specifically, bacteriology) is still a very young science and not yet completely understood. Only about three hundred years have passed since the discovery of the first bacteria. Many estimates suggest that we have studied only about 1% of all the microbes in any given environment1. In the scope of the world, it is obvious to see that the discipline of microbiology is still in its infancy. Microbiology defined as the study of organisms too small to be seen with the naked eye. These organisms include viruses, bacteria, algae, fungi, and protozoa. Microbiologists are concerned with characteristics and functions such as morphology, cytology, physiology, ecology, taxonomy, genetics, and molecular biology. Microbiology research has been, and continues to be, central to meeting many of the current global aspirations and challenges, such as maintaining food, water and energy security for a healthy population on a habitable earth.

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1.2 Environmental Microbiology

The function and diversity of microbes in their natural environments.

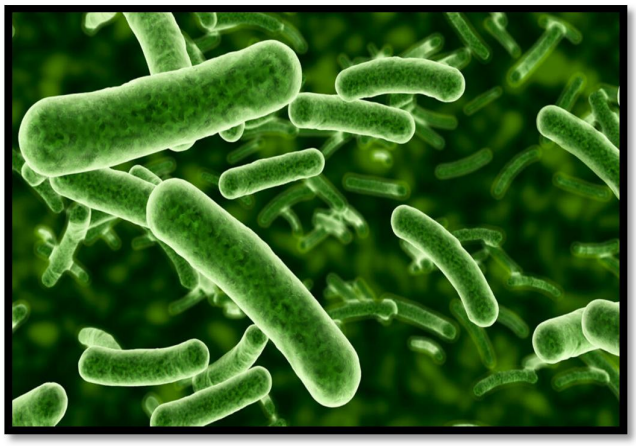

Fig 1. Bacteria

1.3 Sterilization

Sterilization, which is any process, physical or chemical, that destroys all forms of life, is used especially to destroy microorganisms, spores, and viruses. Precisely defined, sterilization is the complete destruction of all microorganisms by a suitable chemical agent or by heat, either wet steam under pressure at 120 °C (250 °F) or more for at least 15 minutes, or dry heat at 160 to 180 °C (320 to 360 °F) for three hours An autoclave is a device that works on the principle of moist heat sterilisation, wherein saturated steam is generated under pressure in order to kill microorganisms such as bacteria, viruses, and even heat-resistant endospores from various types of instruments. Autoclave is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials.A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents.

1.4 Antimicrobial Activity of Ceufuroxime Axetil

Antimicrobial activity of cefuroxime axetil (CXM-AX) was compared with those of other cephem antibiotics against clinically isolated strains obtained mainly from outpatients of our center in a period from January to September of 1990 and 1993. Minimum inhibitory concentrations were determined and the following results were obtained.The results suggested that, compared with reports of studies conducted with clinical isolates in early 1980's, MIC80 of CXM were equal to or lower against Staphylococcus Aurues , Escherichia coli, MIC90 of comparator drugs reflected those of new resistant organisms recently appeared, such as benzylpenicillin (PCG)-insensitive S. pneumoniae (PISP), cephem-resistant E. coli and Klebsiella spp., new quinolone-resistant H. influenzae and N. gonorrhoeae. Methicillin-resistant Staphylococcus aureus (MRSA) was detected also from specimens of community acquired infections. From the nature of MRSA detected in those situations MRSA appeared to present a continuing problem.MIC90 against strains obtained from patients with community acquired infections was a good index of increases of multidrugresistant organisms in the past. Therefore, the determination of MIC90 is important in examining changes with time of sensitivities or resistances of clinically isolated strains to antimicrobial drugs. Antimicrobial activities of CXM against recent clinical isolates showed the existence of problems as mentioned above. However, MIC of CXM as well as those of comparator drugs indicated that antimicrobial activities of CXM against Staphylococcus spp., Streptococcus spp., H. influenzae appeared to be relatively strong, and it is concluded that cefuroxime axetil still is one of the clinically useful oral antimicrobial drugs in the 1990's. Staphylococcus aureus is a major human pathogen associated with invasive disease such as deep abscess formation, endocarditis, osteomyelitis, and sepsis (Lowy, 1998). Because of the great genetic variability of S. aureus and the ability to develop changes in sensitivity to antimicrobials, most clinical isolates of S. aureus are resistant to a number of antibiotics (Sibanda et al., 2010).

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1.5 Zone of Inhibitions

The Zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The zone of inhibition can be used to measure the susceptibility of the bacteria towards the antibiotic.

1.6 Gram Staining

A Gram stain is a test that checks for bacteria at the site of a suspected infection such as the throat, lungs, genitals, or in skin wounds. When the stain combines with bacteria in a sample, the bacteria will either stay purple or turn pink or red. If the bacteria stays purple, they are Gram-positive.

II. MATERIALS AND METHOD

2.1Materials

Table 1: Composition of Nutrient agar

Sr No	Ingredients	Quantity(100ml)
1	Yeast Extract	0.2 g
2	Peptone	0.5 g
3	Sodium chloride	0.5 g
4	Agar	1.5 g
5	Distilled Water	100 ml

Table 2: Composition of Nutrient Broth

Sr. No	Ingredients	Quantity
1	Yeast extract	0.2 g
2	Sodium chloride	05 g
3	Peptone	0.5 g
4	Distilled water	100 ml

Table 3: Composition of Mannitol Salt Agar

Sr. No	Ingredients	Quantity
1	Proteose Peptone	1 gm
2	D-Mannitol	1 gm
3	Meat Extract	0.1 gm
4	Sodium chloride	7.5 gm
5	Phenol red	0.0025 gm
6	Agar	2 gm
7	Distilled water	100 ml
8	pH	7.4±0.2

Table 4: Composition of Herbal Preparation

Sr. No	Ingredients	Quantity
1	Plant fresh Leaves	200 gm
2	Methanol	100 ml
3	Extract	50 ml
4	Dimethyl Sulfoxide	10 mg/ml
5	Distilled Water	Q.s

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2.2 Method of Preparation

A. Cup-plate Method

In these methods, the agar is melted, cooled at 45°C, inoculated with the test microorganisms and poured into a sterile Petri plate. In the cup-plate method, when the inoculated agar has solidified, holes about 9 mm in diameter are cut in the medium with a sterile cork borer. The antimicrobial agent is directly placed in the holes (Fig) In the filter paper and cylinder plate method, the antimicrobial agent is applied to the surface of the solidified, innoculated agar by using a filter paper disc and cylinder respectively. The zone of inhibition is observed after incubation at 30 to 35°C for 2 to 3 days. The diameter of the zone of inhibition gives an indication of the relative activities of different antimicrobial substances against the test microorganisms.

Fig 2. Cup Plate Method

B. Tube dilution and agar plate method

The chemical agent is incorporated into nutrient broth or agar medium and innoculated with the test microorganisms. These tubes are incubated at 30 to 35°C for 2 to 3 days and then the results in the form of turbidity or colonies are observed. The results are recorded and the activity of the given disinfectant is compared as shown in the Fig.

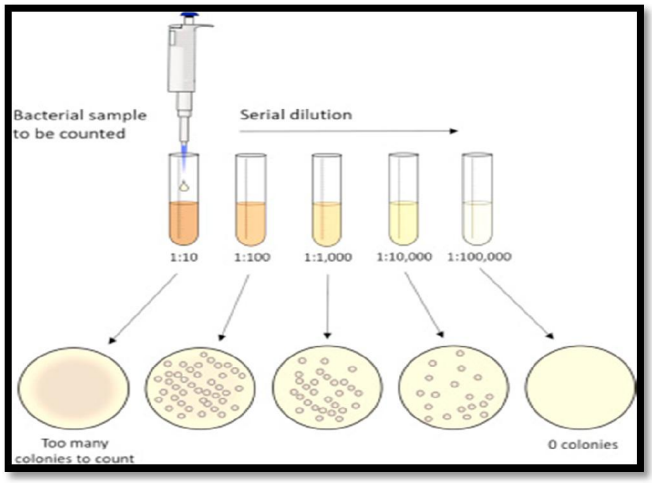

Fig 3: Tube dilution and agar plate method

C. Phenol Coefficient Method

In the phenol coefficient method a test chemical is rated for its microbicidal property with reference to phenol under identical conditions. In this test similar quantities is microorganisms are added to rising dilutions of phenol and of the disinfectant to be tested. in the U.K. the organism used is Salmonella typhi, and the U.S.A. Salmonella_typhi, Staphylococcus aureus and Pseudomonas aeruginosa are used. The phenol coefficient test includes: 1 Rideal - Walker test (RW test) 2. Chick - Martin test (CM test) 3. United States Food and Drug Administration test (FDA test) 4. The US Association of Official Agricultural Chemists test (AOAC test)

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$$\text{R.W Coefficient} = \frac{\text{Dilution of disinfectant kiling in 7.5 but not in 5 min}}{\text{Dilution of Phenol kiling in 7.5 but not in 5 min}}$$

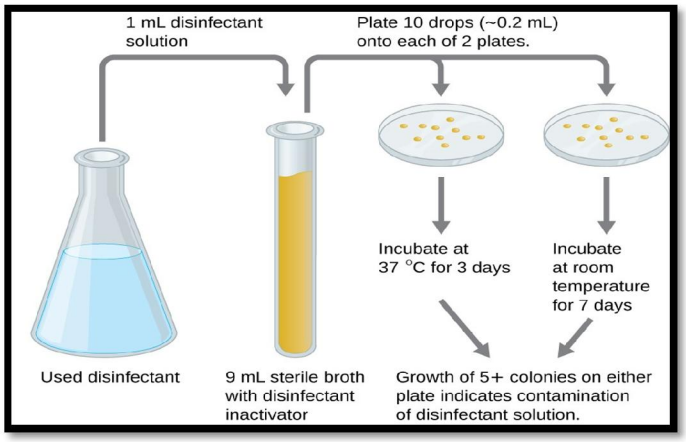

Fig 4. Phenol coefficient method

D. Kelsey-Sykes Method

In this method several test bacteria such as staphylococcus aureus Proteus vulgaris, Escherichia coli and Pseudomonas aeruginosa are Used. This test can be carried out in clear or dirty conditions. In both cases the final concentration of bacterial cells should be about 10%/ml. Clean conditions are simulated by Using broth as the suspending fluid and dirty conditions by the use of a yeast suspension or activated horse serum as the suspending fluid. The dilutions of the disinfectant are made hard water.

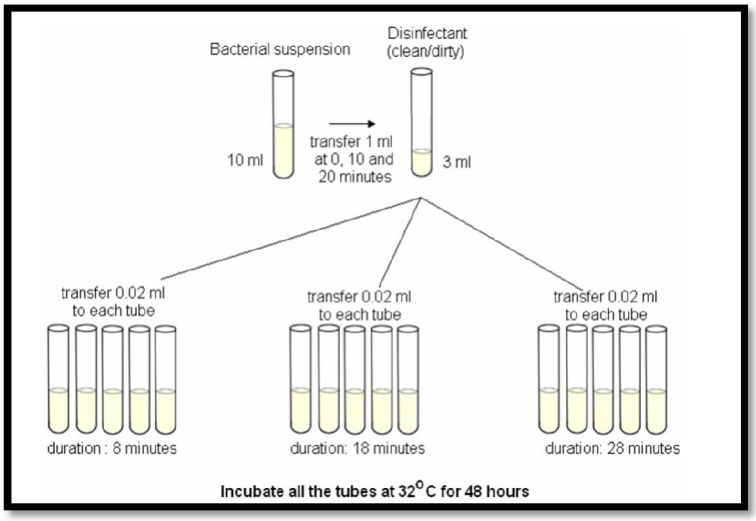

Fig 5: Kelsey-Sykes method

E. Ditch-Plate Method

The nutrient agar is melted, cooled suitably, poured into petri dish. The solidified media is cut with a sterile blade to make a ditch. The drug is poured very carefully into the ditch. Various microorganisms are streaked on the sides ofthe ditch.This method is used to find out the potency of drug against various microorganisms by the means of inhibition of growth on streaked area.

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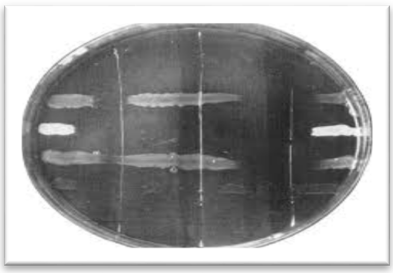

Fig 6: Ditch-Plate Method

2.3 Detection and Isolation Technique S.Aureus:-

Table 5: Detection of S.aureus

Characters	**Styphylococcus aureus**
Gram Staining	positive (Purple)
Capsule	Non Capsulated
Size	1 micrometer (um)
Motility	Non motile
Aerobic & Non Aerobic	Aerobic Facultative
Media	Casein, Soyabin
Sugar Fermentation Test	Lactose,Fructose,Maltose,Mnitose
I- Indole Test	+
M-Methyl Red Test	+
Vi- Voges-Proskauer Test	+
C- Citrate Test	-
Track	Skin Noise

2.4 Isolation Technique of S.Aureus :

100 ml of fluid Casein soyabean + 1 gm or 1ml of test sample

↓

Mix and incubate at 30 to 35°C for 18 to 24 hours

↓

Growth is present, streak the culture on the surface of the following media

↓

Mannitol-salt agar

↓

Incubate at 30 to 35°C for 18 to 72 hours

↓

Yellow or white colonies with yellow zones

↓

Absence of Staphylococcus aureus to carry out coagulase test

↓

Incubate in water broth at 37°C

↓

If no coagulation is observed that indicates absence of Staphylococcus aureus.

Fig 6: Procedure for detection of Staphylococcus aureus

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2.5 Isolation Technique

A. Spread Plate Technique

This technique is used to readily quantify the amount of bacteria present in a solution. In this technique, the sample is diluted and then a little amount of it is added to the agar plate. Then the sample is spread over the agar surface evenly with the help of a spreader. After the colonies grow, the number of colonies is counted and the original number of bacteria in the sample is counted. The end point of our analysis is the number of colony forming units per milliliters.

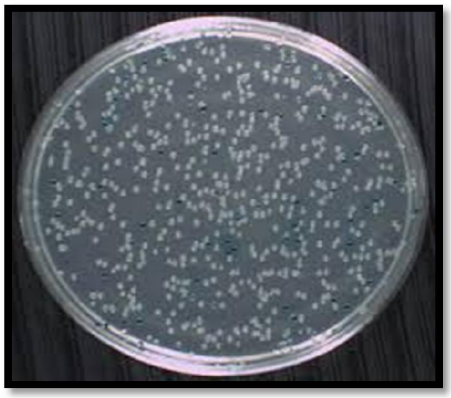

Fig 7: Spread plate technique

B. Pour Plate Method

Culture and liquid agar mediumare mixed together. After mixing the medium, the medium containing the culture poured into sterilized petridishes (petriplates), allowed solidifying and then incubated. After incubation colonies appear on the surface.

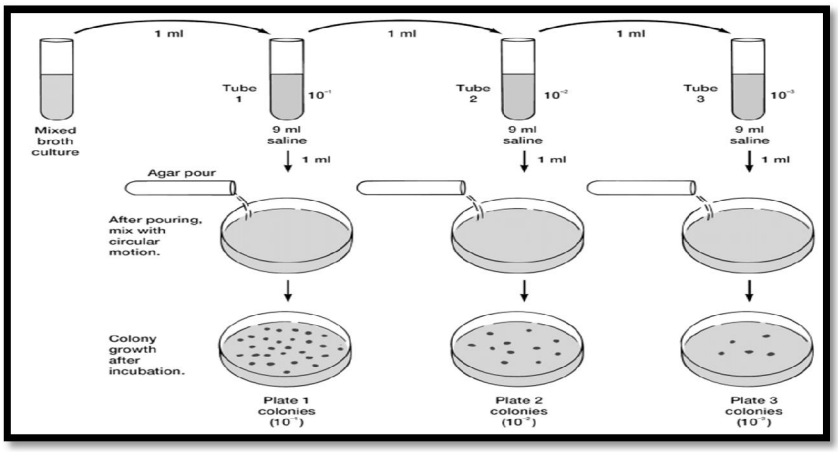

Fig 8: Pour plate method

C. Streak Plate Technique

Streak plate technique is used for the isolation into a pure culture of the organisms (mostly bacteria), from a mixed population. The inoculum is streaked over the agar surface in such a way that it "thins out" the bacteria. Some individual bacterial cells are separated and well-spaced from each other. As the original sample is diluted by streaking it over successive quadrants, the number of organisms decreases. Usually, by the third or fourth quadrant, only a few organisms are transferred which will give discrete colony forming units (CFUs).

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Organism	Zone of inhibition(mm)diameter of herbal product	Zone of inhibition(mm)diameter of cefuroxime
E.Coli	13.5mm	17.5mm
S.aureus	14.5mm	18mm

Fig 9: Streak Plate Technique

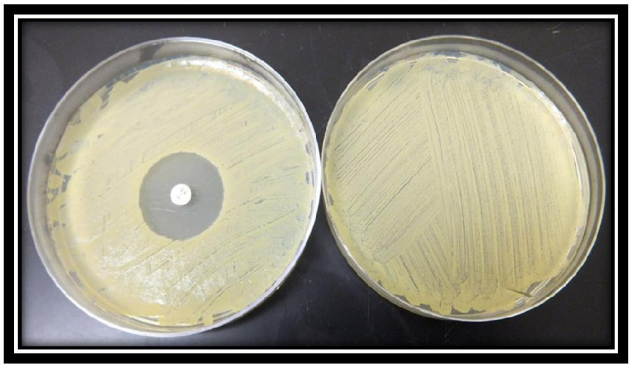
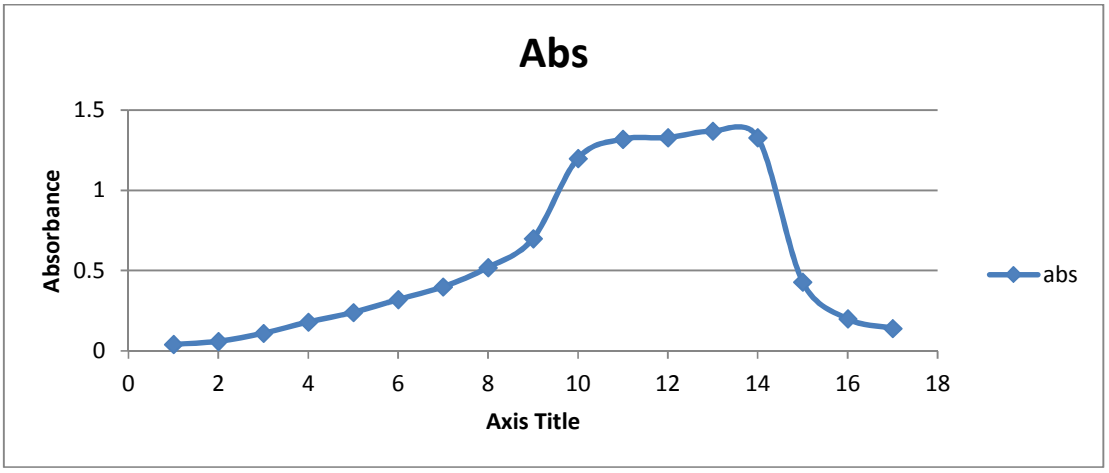


Fig. 10: Antimicrobial test for Herbal preparation and Cefuroxime tablet

2.6 Growth Curve of E.Coli

The standard laboratory strain E. coli MG1655 K-12 has a doubling time of about 30 min at 37°C. Once nutrients in the medium are exhausted, bacterial culture enters a stationary phase, which is characterized by equilibrium between the numbers of dividing and dying cells and represents a plateau in the growth curve.



Graph 1: Growth curve of E.Coli

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III. RESULT AND DISCUSSION

Media sterilization:-

Fig 11: Media Preparation

Growth of Microorganism for soil & mobile sample:

Fig 12: Growth of Microorganism for soil & mobile sample

Gram Staining Test

Result of test sample (Mobile and Soil) is gram positive (purple colour under microscope) rod (bacillus family) and cocci (coccous family) shape.

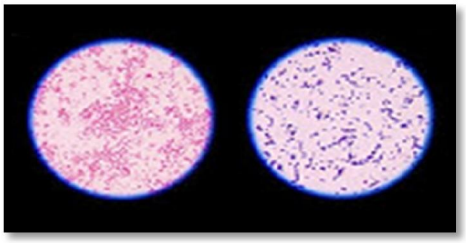

Fig 13: Gram Staining moble sample & soil sample

Zone of Inhibition Cefuroxime Tablet & Herbal Product

Fig 14. Zone of Inhibition Cefuroxime Tablet & Herbal Product

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Biochemical Test of S. Aureus

Sr. No	Biochemical test	Observation
A	I-Indole Test	Positive (+)
B	M-Methyl Red Test	Positive (+)
C	Vi- Voges-Proskauer Test	Positive (+)
D	C- Citrate Test	Negative (-)

IV. CONCULUSION

Findings from this research work are encouraging and could proceed further to applied aspects. Antibacterial activity against human pathogens S. aureus, E. coli.They are Grampositive. Each isolate preferred different carbon and nitrogen sources for their enhanced antibacterial activity. 3).Efficacy of the culture filtrates of these isolates was tested by filter sterilization, autoclaving. Filter-sterilized culture filtrates showed higher antibacterial activity than other treatments. A comparison of the antibacterial activity of culture filtrates and antibiotic Cefuroxime produced an inhibition zone agent 18 mm and Herbal product 14.5 mm respectively. This is the first report on the antibacterial activity of all the 3 bacterial strains against all the human pathogens, mentioned earlier.It is also found that the antibiotic factor is considerably reduced the antibacterial activity of the culture filtrates. With the above significant results, these 2 bacteria are considered to be promising candidates for the isolation of new antibacterial agents.

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