ISOLATION AND SCREENING OF ANTIBIOTIC PRODUCING BACTERIA AGAINST MULTI-DRUG RESISTANT (MDR) CLINICAL ISOLATES

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Abstract
Antibiotics are antimicrobial metabolites which are used for disease management. But recently, antibiotic resistance against pathogenic bacteria is a critical issue to treat the bacterial infections. Thus there is a need for the invention of novel antibiotic which is lethal to the clinical pathogens. The present study is aimed to screen antibiotic producing bacteria from the soil environment. The crude extracts of bacterial culture were used to evaluate their antibacterial spectrum against multi-drug resistant (MDR) clinical isolates. The bacterial sensitivity was analyzed by the determination of the size of the inhibition zone. This finding would have an increased importance in medicine and in health care industry and further research on the above aspects may be undertaken.

Keywords: Antibiotic producing bacteria, Multi-drug resistant, Screening.

Introduction
Antibiotics are compounds produced by microorganisms that are able to kill or slow the growth of other microorganisms. These compounds were used therapeutically and sometimes prophylactically in the control of infectious diseases. Antibiotics have enormous economic values in health because these can be used to cure many diseases caused by bacteria, virus, fungi and parasites. Typically, antibiotics have selective toxicity. It means those antibiotics are dangerous for parasites only but not for the host. According to Browdy one of the most significant technologies that have evolved in response to disease control problems is the use of probiotics. Several mechanisms of probiotic action have been described, the most common relating to their abilities to strengthen the intestinal barrier, to
modulate the host immune system, and to produce antimicrobial substances. Indeed, the production of antimicrobial substances is often regarded a priori as an important trait in the context of bacterial fitness but also in terms of probiotic efficacy. Although several hundreds of compounds with antibiotic activity have been isolated from microorganisms over the years, but only a few of them are clinically useful.

However, improper and massive uses of antibiotics are leading to microbial resistance. Microbes are able to produce enzymes that can destroy antibiotics. Different antibiotics like penicillin, erythromycin and gentamycin which used to be one of the important cures are now less effective because bacteria have become more resistant. Antibiotics are the most important bioactive compounds for the treatment of infectious diseases. But now, because of the emergencies of multi-drug resistant pathogens, there are basic challenges for effective treatment of infectious diseases. Thus, due to the burden for high frequency of multidrug resistant pathogens in the world, there has been increasing interest for searching effective antibiotics from soil bacteria in diversified ecological niches. According to WHO, much needs to be done to reduce the overuse and inappropriate use of antimicrobials.

The emphasis in disease management should be on prevention, which is likely to be more cost effective than cure. Hence the need of the hour is a search for novel antibacterial compounds with therapeutic potential for which the pathogens may not have resistance. Hence, the present investigation was aimed isolate antagonistic bacteria present in soil environment and to assess their inhibitory action against selected multi-drug resistant (MDR) clinical isolates.

Materials and Methods

Collection of soil sample and isolation of bacteria

Soil samples were randomly collected from salt pan, Kanyakumari, Tamil Nadu. Samples were taken from 2-3 cm depth and collected in sterile polythene bag. The samples stored at 4°C. The soil samples were serially diluted up to 10^9 dilutions, in distilled water and 1ml sample from 10^-8 to 10^-9 were pouring plated in Nutrient Agar (NA) (Hi-Media, Mumbai, India) plates. The plates were kept for incubation at 37°C for 24 hr in an inverted position. The bacterial isolates were purified by pure culture techniques and refrigerated in agar slants for further studies.

Test microorganisms

To evaluate the antibacterial potential the following test microorganisms, Gram- positive bacteria including Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Enterococcus feacalis, Micrococcus proteus, Gram-negative
bacteria including *Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella enterica, Proteus vulgaris, Salmonella typhi* were procured from local hospitals and maintained on NA slant at 4°C.

**Inoculums preparation**

Test bacterial inoculums were prepared by growing cells in Nutrient Broth (NB) (Hi-Media, Mumbai, India) for 24 hr at 37°C in a rotary shaker (150 rpm). These cell suspensions were diluted with sterile NB to provide cell counts of about $10^8$ CFU/ml and used for antagonism study.

**Primary screening of active bacterial isolates**

Scratching method was used to screen the antimicrobial producing active isolates. *S. aureus* (representative of Gram-positive bacteria) and *P. aeruginosa* (representative of Gram-negative bacteria) were used as test organisms. The test bacterial suspension was spread on solid Mueller Hinton Agar (MHA) (Hi-Media, Mumbai, India) plate using sterile swab aseptically and allowed to dry. Then pure isolated cultures were scratched on it and incubated for 24-48 hr at 37°C.

The isolates showed positive potential antimicrobials, were screened and used for further test.

**Identification of active isolates**

The potent isolates selected were characterized by morphological and biochemical methods. The results of microscopic examination were compared with Bergey's manual of systematic bacteriology and the organism was identified upto genus level.

Various biochemical tests were performed for the identification of the potent isolates are as follows; Fermentation of sugars, Hydrolysis of starch, Indole production, Methyl red, Vogues-Prauskauer, Citrate utilization, Nitrate reduction test, Catalase test, Oxidase test.

**Extraction of antimicrobial metabolites**

The antimicrobial metabolites were obtained from the crude culture filtrate by solvent extraction method. The active strains were broth cultured in a rotary shaker at 200 rpm for 5 days at 37°C. Then the broth culture was extracted using equal volume of ethyl acetate (1:1) using a magnetic stirrer for 1 hr. The ethyl acetate containing active metabolite was separated in a separatory funnel and the ethyl acetate phase was removed and concentrated by evaporation in water bath at 80-90°C. The concentrate (crude extract) was used for the determination of antibacterial study against test organisms.
Antibacterial assay

To determine the potentiality of the selected isolates, the antibacterial assay was done by agar cup method\textsuperscript{13}. The wells (6 mm diameter) were cut using a sterile cork MHA plates. 24 hr young fresh culture of test microorganisms were swabbed with sterilized cotton swab on the surface of plates. 60 μl crude extract was loaded into each well and left for 30 min until the metabolite was diffused. Then the plates were incubated for 24 hr at 37°C. After incubation, the zone of inhibitions was evaluated in millimeter and the individual test was performed for three times. The antagonistic activity of the antibiotic was evaluated by measuring the resulting diameters of zone of inhibition in millimeters.

Results and Discussion

Primary screening of active bacteria

Three soil samples were randomly collected from salt pan to isolate antibiotic producing bacteria. A total of 5 strains were primarily screened for antibacterial activity against two representative pathogens (\textit{S. aureus} as representative of Gram-positive bacteria and \textit{P. aeruginosa} as representative of Gram-negative bacteria) and out of that 2 strains (AB2 and AB5) were found to be most active because it exhibited broad spectral activity.

Identification of active isolates

After the primary screening, the isolate AB2 and AB5 were found to be the best antimicrobial metabolite producing strain and were further analyzed for the rest of the studies. Result of Table 1 revealed that the isolate AB2 is a gram-negative, non-endospore forming, facultative anaerobic, rod shaped bacterium with Catalase, Simmon’s Citrate, Voges-Proskauer, Nitrate reduction positive reactions and Oxidase, Methyl red, Indole negative reactions. Similarly, bacterial strain AB5 is a gram-negative, non-endospore forming, facultative anaerobic, rod shaped bacterium with Catalase, Indole, Methyl red, Nitrate reduction positive reactions and Oxidase, Simmon’s Citrate, Voges-Proskauer negative reactions. Based on the physiochemical, morphological characteristics the isolates AB2 and AB5 were identified as \textit{Enterobacter} sp. and \textit{Escherichia coli}, respectively according to the Bergey’s Manual of Systematic Bacteriology.

Table 1 Morphological, physiological and biochemical characteristics of the isolated strain AB2 and AB5.

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<tr>
<th>Characters</th>
<th>Results</th>
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<tr>
<td>Morphology</td>
<td>AB2: Rod shaped, Gram -ve, AB5: Rod shaped, Gram -ve,</td>
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**Determinant of antibacterial activity of crude extract**

The excessive side effects of chemical antibiotics and their resistance against the infectious microorganisms have drawn the attention of researchers to discover natural antimicrobial products using bacteria, fungi, actinomycetes, plants. Here crude extracts of bacteria were used to demonstrate the antibacterial activity of *Enterobacter* sp. (AB2) and *Escherichia coli* (AB5) against multi-drug resistant clinical isolates. There was a high degree of variation in the level of antibacterial activity against the test microbes which includes Gram-positive and Gram-negative pathogenic bacteria. The antibiotic produced by strain AB2 and AB5 exhibited better antibacterial activity against Gram-negative bacteria than the Gram-positive one. Bacterial strain AB2 showed maximum zone of inhibition against Gram-negative bacteria *P. aeruginosa* of 22 mm and AB5 produced highest antibacterial activity against Gram-negative bacteria *K. pneumoniae* of 21 mm (Figure 1). In case of Gram-positive bacteria, AB2 and AB5 were found to inhibit with maximum inhibition zone of 15 and 12 mm against *S. aureus* respectively (Figure 2).

The above phenomena can be explained by the structure of the cell wall of bacteria. The Gram-positive bacteria is containing a thick peptidoglycan layer about 20-80 nm in their cell wall whereas Gram-negative bacteria is having a thin peptidoglycan layer of 2-3 nm with thick lipid outer membrane. The crude extract might not be penetrated easily in the

<table>
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<th>Characteristics</th>
<th>AB2 (Gram-positive)</th>
<th>AB5 (Gram-negative)</th>
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<tbody>
<tr>
<td>Motility</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Catalase</td>
<td>+ve</td>
<td>+ve</td>
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<tr>
<td>Oxidase</td>
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<td>Methy red</td>
<td>-ve</td>
<td>+ve</td>
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<td>Voges- Proskauer</td>
<td>+ve</td>
<td>-ve</td>
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<td>Indole production</td>
<td>-ve</td>
<td>+ve</td>
</tr>
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<td>Citrate utilization</td>
<td>+ve</td>
<td>-ve</td>
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<td>Nitrate reduction</td>
<td>+ve</td>
<td>+ve</td>
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<tr>
<td>Hydrolysis of starch</td>
<td>-ve</td>
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<td>Fermentation with glucose, lactose, sucrose, mannitol</td>
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cell wall of Gram-positive bacteria compared to Gram-negative bacteria because the polysaccharide layer of Gram-positive bacteria preventing the entry of molecules like antibiotic to reach its site of action, which is the cell membrane and not showed significant antibacterial potential. Thus Gram-positive bacteria are poorly sensitive to antibiotics.

**Figure 1: Antibacterial activity of antibiotic producing bacteria against Gram-positive bacteria**

**Figure 2: Antibacterial activity of antibiotic producing bacteria against Gram-negative bacteria**

**Conclusion**

In view of the above statement, it can be concluded in a positive note that the bacteria isolated from salt pan, have a broad spectrum of antibacterial activity. This antibiotic producing bacterial strain may useful in control in diseases caused by bacterial and fungal pathogenic species. These findings will assist in formulating a potential antibiotic for arresting the growth and metabolite activities of various pathogenic microorganisms. These significant results make the...
strain suitable for further investigation on nature of antibacterial compounds and its various industrial and biological applications.

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References

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