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Studies on *in -vitro* Anti Microbial Potential of Rhizospheric Soil Bacteria against Multi Drug Resistant Clinical Isolates

KEYWORDS: Antibiotic, Rhizosphere, Multi-drug-resistant (MDR)

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ABSTRACT *Antimicrobial resistance provides a survival benefit to microbes and makes it harder to eliminate infections from the body. In the present investigation we tried to find a new antimicrobial agent producing bacteria from rhizosphere soil. In this view study has been performed to find out some excellent rhizospheric soil bacteria which are active against multi drug resistant clinical pathogens. In the present study P. aeruginosa , S. aureus, E. coli, K. pneumoniae were isolated from different clinical samples. All the isolates were then subject to antibiotic susceptibility testing by disc diffusion method. Susceptibility testing by disc diffusion method. Out of 86 isolates 62.79 % isolates were found to be multi drug resistant (MDR). The rhizospheric isolates S2 (Bacillus spp) & S5 (P.fluorescens) were found to exert good antimicrobial activity against 8 most resistant clinical isolates. Because of huge emergence of multidrug resistant (MDR) bacteria reported in our study and previously reported studies it is an urgent need to discover new therapeutics that would be effective against MDR strain. Rhizosphere soil gives an excellent option as a source for search of some new alternative medicines.*

INTRODUCTION:-

We are living in the age of microorganisms as they have significant positive and negative impact on human population. A variety of infections are caused by microorganisms (bacteria, virus, fungi, and protozoan's) which are very harmful to both animals and plants. For the treatment of such diseases antibiotics are being used from ancient time.Widespread use of antibiotics is thought to have spurred evolutionarily adaptations that enable bacteria to survive these powerful drugs. Antimicrobial resistance provides a survival benefit to microbes and makes it harder to eliminate infections from the body.Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century.

In recent years, the number of new antibiotics licensed for human use in different parts of the world has been lower than in the recent past. In addition, there has been less innovation in Considerable research is being done in order to find new chemotherapeutic agents isolated from soil (Rondon et al., 2000; Crowe and Olsson, 2001; Courtis et al., 2003). Soil microbial communities are among the most complex, diverse and important assemblages of organisms in the biosphere; and they participate in various biological activities. Accordingly, they are an important source for the search of novel antimicrobial agents and molecules with biotechnological importance.

One of the areas in soil where one can find abundance in microbial populations is the rhizosphere. It is a thin layer of soil adhering to a root system which is rich in microbial diversity. The magnitude of this area depends on the plant and the size of the roots that the plant posses (Rondon et al., 1999; Rondon et al., 2000 and Dakora & Phillips, 2002). These microorganisms produce antimicrobial agents and seem to have unique genetic and biological systems that may have applications outside the host plants, in which they normally reside. In this view the present study has been performed to find out some excellent rhizospheric soil bacteria which are active against multi drug resistant clinical pathogens.

MATERIAL AND METHODS:-

ꕥ Collection of clinical samples:-

Forty five clinical samples (Viz pus, blood, urine) were collected in sterile container and transferred immediately to laboratory for further processing. Samples were collected from Civil Hospital, Private Clinics and Pathology laboratories of Akola city.

ꕥ Isolation and identification of clinical pathogens: -

The samples were inoculated on selective and differential media as Mannitol Salt agar, Eosine Methylene Blue (EMB) agar, Cetrimide agar and MacConkey agar for the isolation of S. aureus, E. coli, K. pneumoniae and P. aeruginosa respectively. The plates were incubated at 37° C for 24 hrs. The identification of these clinical isolates was done on the basis of morphological cultural and biochemical characteristics according to Bergey's manual of Determinative Bacteriology,(1986)

• Determination of antibiotic resistance pattern of clinical isolates: -

The antibiotic susceptibility testing was conducted using disc. Diffusion method (Kirby- Bauer Method) using Muller – Hinton agar (Himedia, India) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI). All the isolates were tested against 13 different antibiotics.

Table 1: Soil Samples from Different Locations from Akola District.

Soil sample no.	Place	Plant Rhizosphere
1	Gudhadi	Lemon
2	Umari Mothi	Cotton
3	Malkapur	Banana
4	Yawalkhed	Chikku
5	Umari	Cotton
6	PDKV,Akola	Googanvilia
7	Ridhora	Toor
8	Kapshi	Soya Bean
9	Shirla	Soya Bean
10	Patur	Soya bean

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- **Isolation of soil bacteria :-**

One gm soil sample was suspended in 9 ml of sterile distilled water, then the suspension was serially diluted and 10^{-7} dilution was used for plating on nutrient agar plates. All the plates were incubated at 37 ° C for 24 hrs. After incubation the well isolated colonies were further purified by streaking on fresh nutrient agar plates. The pure colonies were subculture and maintained in nutrient agar slants.

- **Screening of isolated rhizosphere soil bacteria for antibacterial activity against MDR clinical isolates: -**

Antibacterial activity was screened by agar well diffusion method against selected MDR clinical bacterial isolates (Irobi et al., 1994). Muller Hinton agar plates were swabbed (by a sterile cotton swabs) with 24 hours old broth culture of selected bacterial strain to get a confluent growth. Bores were made by a 6 mm sterile cork borer. Afterwards, cell free supernatant of each isolated bacteria was added. Plates were incubated for 24 hrs at 37°C. After 24h zones of inhibition was observed in plates (Farooq and Bano 2013).

•Identification of prominent rhizospheric soil isolates:-

The isolates from rhizosphere which showed prominent antimicrobial activity against multi drug resistant human pathogens were further identified by morphological, cultural and biochemical characters.

RESULTS AND DISCUSSION:-

In the present study 45 clinical samples comprising 15 bloods, 15 urine and 15 pus were collected from various privates clinics, Civil Hospital and Pathology laboratories. The samples were inoculated on various selective and differential media for the isolation of some human pathogens of health significance which includes S. aureus, E. coli, K. pneumoniae and P. aeruginosa. The distribution of these clinical pathogens isolated from various samples was obtained (Table 2).

It was found that more no. of isolates were isolated from urine samples as 50% clinical pathogens were obtained from urine sample. It is followed by pus sample (27 %) and blood sample (18 .60%).The prevalence of selected clinical pathogen was studied (fig 1). It was found that P. aeruginosa was most prevalent pathogen with isolation percentage 31.40 followed by K. pneumoniae, E. coli, and S. aureus with isolation percentage 27.90, 20.93 and 19.76 respectively.

All the isolates were then subjected to antibiotic susceptibility testing by disc diffusion method (Table. 3). Out of 86 isolates 62.79% isolates found to be multi-drug resistant P. aeruginosa isolates exhibit highest resistance (74.07%) toward different antibiotics. Only 25.92% isolates showed sensitivity toward tested antibiotics. In case of K. pneumoniae 66.66% isolates showed resistance. This was followed by E. coli, which exhibit 61.11 % & S. aureus which exhibit 41.17 % resistance. All these isolates showed resistance toward two or more antibiotics tested. Out of these multi drug resistant (MDR) clinical isolates. 2 isolates of each showing drug resistance to 5 or more antibiotics were selected as the most resistant target. The resistance pattern of these 8 MDR isolates including P. aeruginosa, S. aureus K. pneumoniae, & E. coli, is shown in Table 4.

The 10 soil samples from different rhizosphere were collected a total of 60 pure colonies were isolated from the soil samples. These isolates were tested for antimicrobial activity against selected 8 MDR clinical isolates. Only 5 isolates were found to showed antibacterial activity against selected target pathogens (Fig.3). Out of these 5 isolates only two isolates S2 and S5 showed activity against all drug resistant pathogens.

The isolates S2 & S5 were further proceed for identification by performing series of morphological, cultural and biochemical reaction. On the basis of this the isolate S2 was identified as Bacillus spp and isolate S5 was P.fluroscence (Table 5).

Table 2:- Distribution of clinical pathogens isolated from various sample.

Name of isolate	Clinical sample			Total
	Blood (n=15)	Urine (n=15)	Pus (n=15)	
P. aeruginosa	03	14	10	27
K. pneumoniae	08	11	05	24
E. coli	02	15	01	18
S. aureus	03	03	11	17
Total	16	43	27	86

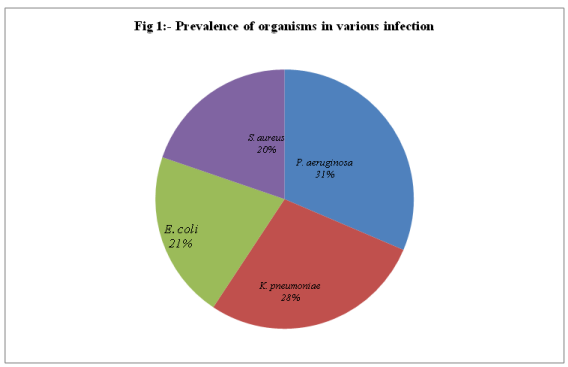

Table 3:- Overall distribution of resistance and sensitivity of clinical pathogens.

Clinical isolate	Resistance (%)	Sensitivity (%)
P. aeruginosa	74.07 %	25.92 %
K. pneumoniae	66.66 %	33.33 %
E. coli	61.11 %	38.88 %
S. aureus	41.17 %	58.82 %

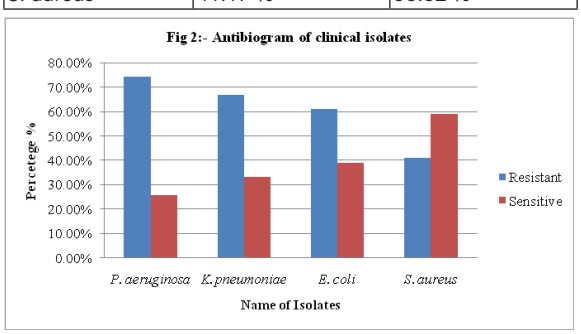

Table 4:- Antimicrobial resistance pattern of the most resistant target clinical pathogens.

Clinical Isolates	Antibiotics												
	GEN	C	Cip	AMC	NA	AMP	E	VA	CTX	TE	M	Pen	IE
PS 3	R	R	R	R	R	R	R	S	S	R	R	R	R
PS 15	R	R	R	S	R	R	R	S	S	S	R	R	S
KP 10	R	R	R	R	R	R	R	R	R	S	R	R	S
KP 14	R	R	S	R	R	R	R	S	S	S	R	R	S
EC 9	S	R	R	S	R	R	R	R	S	R	R	R	R
EC 13	R	S	R	S	S	R	R	S	R	S	R		S
SA 6	R	S	S	S	R	R	R	S	R	R	R	R	S
SA 15	R	R	S	S	S	R	R	R	S	S	R	R	S

(PS-Psudomonas aeruginosa, EC-Escherichia coli, SA-Staphylococcus aureu, KP- Klebsiella Pneumonia, GEN-Gentamycin, C- Chloramphenicol,Cip- Ciprofloxacin,

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AMC- Amoxyclav, NA- Nalidixic Acid ,E- Erythromycin, AMP- Ampicillin, VA- Vancomycin, CTX- Cefotaxmine, TE- Tetracycline, M- Methicillin, Pen- Penicillin, IE- Imipenem).

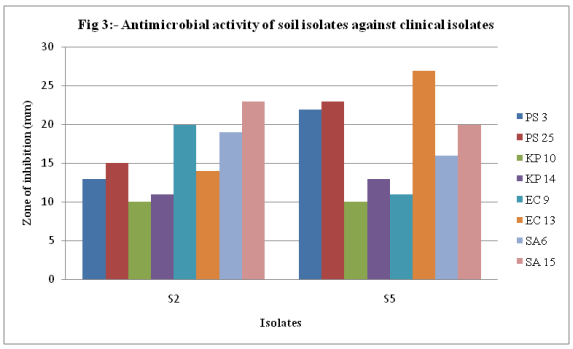

Table 5:- Morphological and Biochemical Characters of Rhizosphere isolate.

Characteristics	Variables		Microbial isolates	
		S2	S5	
Colony and cell morphology	Size		0.5-1.5 mm	1.5-2.0 mm
	Gram's Nature		Gram positive	Gram Negative
	Shape		Coci	Bacilli
	Motility		Motile	Motile
	Elevation		Raised	Convex
	Opacity		Opaque	Opaque
	Texture		Smooth	Rough
	Color		White	Yellowish green
Biochemical characteristics	Idole		Negative	Negative
	Methyl red		Positive	Negative
	Voges Proskeurs		Positive	Negative
	Citrate		Negative	Positive
	Oxidase		Positive	Positive
	Urease		Positive	Positive
	Catalase		Positive	Positive
	Gelatinase		Positive	Positive
Carbohydrate fermentation test	Glucose	Acid	+	+
		Gas	-	-
	Lactose	Acid	+	-
		Gas	-	-
	Sucrose	Acid	+	-
		Gas	-	-
	Mannitol	Acid	+	-
		Gas	+	-
Probable isolates			Bacillus spp	P.fluorescens

DISCUSSION

The infection dynamics of pathogens, it was obvious that antibiotic sensitive pathogens have a limited capacity of virulence as the employed antibiotic controls them. At several levels, the host defense system also helps to control of pathogens when the later are in a smattering number. Most often than not, an infection from a MDR bacterial strain leads to a disease, particularly when an emulating control-agent/antimicrobial is absent, i.e., the employed antibiotic has been won over by it.

Slowly, the use of number of antibiotics for the control of infectious diseases in last decades have led to multiple resistances in one cell, the MDR strain of a species, paradigmatically with any of notorious pathogens. As conjectured from retrospective follow-ups, it is clear that older antibiotics slowly became obsolete, by the resistant mechanism. The clinical concern is that antibiotic resistance was reported in several pathogenic bacteria for which, particular antibiotics were never applied. (Sahu et al., 2013).

In the present study the resistance pattern amongst the clinical isolates was determined which showed high degree of antibiotic resistance (Fig. 2). The literature also agrees with the emergence of antibiotic resistance amongst P.aeruginosa, S. aureus, E.coli & K. punemoniae (Sahu & Pandhy, 2013; Shadi et al., 2010; Marwa et al., 2012 & Sharmeen et al., 2012).

The increase in the frequency of multidrug resistant pathogenic bacteria is created an urgent demand in the pharmaceutical industry for more rational approaches and strategies to the screening of new antibiotics with a broad spectrum of activity, which resist the inactivation processes exploited by microbial enzymes (Saadoun and Gharaibeh, 2003; Motta et al., 2004).

Screening and isolation of promising rhizosphere bacteria with potential antibiotics is still a thrust area of research and it is suggested that the exploration of materials from different areas and habitats have a vital role to play in the search for new microbes and novel metabolites and is urgent to counter the threats posed by the fast emerging phenomenon of antibiotic resistance (Saadoun and Gharaibeh, 2003).

Thus, in the present work, different bacteria were isolated from rhizospheric soil of Akola district and then screened with regard to their potential to generate antibacterial substance. For the purpose of obtaining antimicrobial agents against resistant target bacteria, isolation of different microorganisms from the rhizosphere of different soil localities was carried out.

Accordingly, 60 isolates from rhizosphere soil tested against MDR clinical isolates Only 5 were found to show antimicrobial potential. The isolate S2 and S5 were most prominent isolates latter on identification it was found that isolate S2 was belongs to Bacillus spp & S5 belongs to P. fluroscence. This is in agreement with other studies who reported the bacillus amyloliquefaciens and P. fluroscence isolated from rhizosphere soil exerting good antibacterial activity against bacterial pathogens of health significance (Rekha et al., 2010 & Das et al., 2013).

Interactions that take place in the rhizosphere can be beneficial for the plant and also for the microbial community present. The Exudates released by plants have various effects in the surrounding ecosystem as altering the physical- chemical properties of soil by inhibiting the growth of other plants, enhancing symbiotic relationships, and selecting the type of microbiota that can colonize the area. Also, the microflora present in the rhizosphere can produce antagonistic molecules that will inhibit or kill the pathogens present (Rondon et al., 1999; Rondon et al., 2000; Jaben et al., 2004).

Thus, the present piece of work have an important implication for the discovery of novel antimicrobial compounds from rhizosphere soil bacteria and may allow the development of new methods for screening novel compounds active against multi drug resistance bacteria.

Conclusion:-

The drug resistance was found to be high in the study among the clinical isolates of P. aeruginosa, K. pneumoniae , E coli & S. aureus. Because of huge emergence of multidrug resistant (MDR) bacteria reported in our study and previously reported studies it is an urgent need to discover new therapeutics that would be effective against MDR strain. Rhizosphere soil gives an excellent option as source for search of some new alternative medicine. Rhizosphere soil isolates SP2 & SP5 found to exert prominent antibacterial activity even against MDR isolates further study about these isolates results in development of a potential drug.

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