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Studies on Antimicrobial Activity of *Bacillus Species* isolated from soil samples against Multi-Drug resistant Enteric Bacteria

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Abstract- *Bacillus* species is one of the largest sources of bioactive natural products exhibiting a wide range of antibiotic activities. Antibiotics have been used against various infectious bacteria and fungi for over many years. The increase in the resistance amongst enteric bacteria limits the existing options of antibiotics. In the present study, resistance was highest among the *E. coli* (73.33%) followed by *K. pneumoniae* (59.09%), *S. typhi* (57.14%) and *P. vulgaris* (33.33%). 17 *Bacillus spp.* isolated from soil samples includes *Bacillus subtilis, Bacillus amyloliquifacience* and *Bacillus licheniformis*. It was found that isolate BS_7, BS_3 and BS_1 showed prominent antimicrobial activity against tested MDR enteric bacteria.

Keywords- Antimicrobial activity, *Bacillus* spp., MDR,

I. INTRODUCTION

Bacillus species are aerobic spore forming rods that stain gram positive or gram variable. Except for few species the large majority has no pathogenic potential and has never been associated with disease in man or animals [1]. Member of the genus have significant microbiological uses. Numerous enzymes antibiotic and other metabolites have medical, agricultural, pharmaceutical and other industrial applications. *Bacillus* species are rod shaped endospore forming aerobic or facultatively anaerobic gram positive bacteria that are widely spread in nature [2]. *Bacillus* species are one of the largest sources of bioactive natural products, exhibiting a wide range of antibiotic activities [3]. Members of the bacillus species are generally found in soil and most of these bacteria have the ability to disintegrate protein (proteolytic activity) [4].

In antimicrobial activities of the polypeptide antibiotics which constitute the *Bacillus* bacteria have been gaining importance as a result of studies. The *Bacillus species* that produce antibiotics are *B. subtilis, B. polymyxa, B. brevis*. The *Bacillus* spp have a wide range of antimicrobial activities since they are use as antifungal agents, antiviral agent, antiameobocytic agent and antimycoplasma agents. Many *Bacillus* species are of remarkable importance because they construct antibiotics [1]. The potential of *Bacillus* species to synthesize a wide variety of metabolites with antimicrobial activity has been widely used in medicine and pharmaceutical industry; one of its abilities is to control various diseases in animals, humans and plants when applied as a biological control agent [5,6]. These organisms are usually found in decaying organic matter, dust, vegetables, water, and some species are part of the normal flora.

Enteric bacteria are Bactria in the family *Enterobacteriacae*. These bacteria reside normally in the guts of many animals including humans and some are pathogenic causing disease in certain animal species. Many cases of the food poisoning are caused by infection with enteric bacteria as are some more serious condition, such as plague [7].

Enterobacteriaceae are Gram-negative, rod shaped 1-3 µm large bacteria. They are facultative anaerob and their natural host is the human and animal intestine, where they belong to the commensal microbial flora e.g.: *Escherichia coli, Klebsiella* spp., *Proteus* spp., *Morganella* spp., *Providentia* spp., *Enterobacter* spp., *Serratia* spp. These bacteria can be pathogens of urinary tract, respiratory tract, bloodstream and wounds. Obligate human intestinal *Enterobacteriaceae* pathogens include *Salmonella* spp., *Shigella* spp. and *Yersinia* spp.etc. Infections caused by *Enterobacteriaceae* are treated with antibiotics.

Members of the *Enterobacteriaceae* family are medically important as infectious agents, exhibits antibiotic resistance and are present in large member in the animal gut. In addition antibiotsic producing microorganisms are found naturally in soil, suggesting intrinsic chromosomal antibiotic resistant originated in the soil in response to inhibitory environments. The increasing frequency of multi-resistant pathogenic bacteria is created an urgent demand in the modern world for more rational approaches and strategies to the screening of new antibiotics with a broad spectrum of activity that can resist the inactivation processes exploited by microbial enzymes [8]. The origin of resistant genes could be due a natural process whereby the resistant genes are maintained in nature because of the presence of antibiotics producing bacteria in soil. Emergence of multidrug resistance among bacterial

pathogens of hospital environment, domestic and industrial environment and in biofilms is reported globally. *Enterobacteriaceae* species are important human pathogens while increasing number of antibiotic resistant strains are detected worldwide. The drug resistance was found to be high in the various studies among the clinical isolates of *Enterobacteriacae* family. Because of huge emergence of multidrug resistant (MDR) bacteria it is an urgent need to discover new therapeutics that would be effective against MDR strain [9]. In accordance to this attempt has been made in the present study to isolate some potential *Bacillus* spp. which can inhibit drug resistant pathogens.

II. MATERIAL AND METHODS

1. Collection of samples-

Soil samples were collected in sterile plastic bags from different areas of Akola district for the isolation of *Bacillus* spp. and carried to the lab for further investigation. While for the isolation of enteric bacteria the clinical samples i.e. blood, urine & pus were collected from various pathology, laboratory and hospitals of Akola city.

2. Isolation and identification of isolates-

Bacillus species were isolated by serial dilution method and isolates were subcultured and maintained in nutrient agar slants. Whereas the Clinical samples were inoculated on various selective and differential media viz. MacConkey agar, EMB agar for isolation of enteric bacteria. All the isolates were then subjected to various morphological, cultural and biochemical characteristics and identified according to Bergey's Mannual of determinative bacteriology.

3. Antimicrobial Activity of *Bacillus species* against enteric bacteria.

All the enteric bacteria were checked for antibiotic susceptibility by Kirby Bauer Method and those showed MDR were used to check antimicrobial activity of *Bacillus* spp.. The Agar well diffusion method was used to detect the antimicrobial activities of *Bacillus species* against enteric bacteria [10].

III. RESULTS AND DISCUSSION

In the present study 50 clinical samples comprising 25 blood & 25 urine were collected from various private hospitals, civil hospital and pathology laboratories. From 50 clinical samples total 81 isolates were obtained. The distribution of these clinical pathogens from the blood and urine samples is shown in Table 1. The samples were then inoculated on various differential and selective media for the isolation of selected pathogens from *Enterobacteracae* family. The isolates were then identified by standard conventional methods.

The prevalence of the isolates from *Enterobacteriacae* family was observed and shown in figure 1. The results showed that *E. coli* (73.33%) was the most prevalent pathogen followed by *K. pneumoniae* (59.09%), *P. vulgaris* (33.33%) and *S. typhi* (37.41%) from *Enterobacteriacae* family.

All the isolates were then subjected to antibiotic susceptibility test by Kirby Bauer method. The overall distribution of resistance and sensitivity of all 81 isolates to different antibiotics were recorded and collectively depicted in Table 2.

Results showed that out of 81 isolates about 59.25% found to be multidrug resistant (MDR). Resistance was highest among the *E. coli* (73.33%) followed by *K. pneumoniae* (59.09%), *S. typhi* (57.14%) and *P. vulgaris* (33.33%)(Figure.2). Out of these MDR isolates of *Enterobacteracae* few isolates from each species were selected for further studies. The selection was based on drug resistance of isolates to 5 or more antibiotics. The details of resistance of these isolates to tested antibiotics is given in Table 3.

The soil samples which were collected from various places were used to isolate the *Bacillus spp*. A total of 17 pure colonies were isolated on the basis of primary morphology resembling of *Bacillus*. These were further identified according to standard conventional methods. These were probably identified to the *Bacillus subtilis, B. cerus, Bacillus licheniformls* and *B. amyloliquifacence.*

All these isolates were then subjected to antimicrobial activity against selected MDR clinical pathogens. The cell free supernatant checked against these selected clinical pathogens showed good inhibitory activity by showing zone of inhibitions (Table 4).

Table 1: Distribution of clinical pathogens isolated from various samples.

Name of isolate	Clinical samples		Total
	Blood (n=25)	Urine (n=25)	
E. coli	09	21	30
K. pneumoniae	04	18	22
S. typhi	10	04	14
P. vulgaris	03	12	15
		Total	81

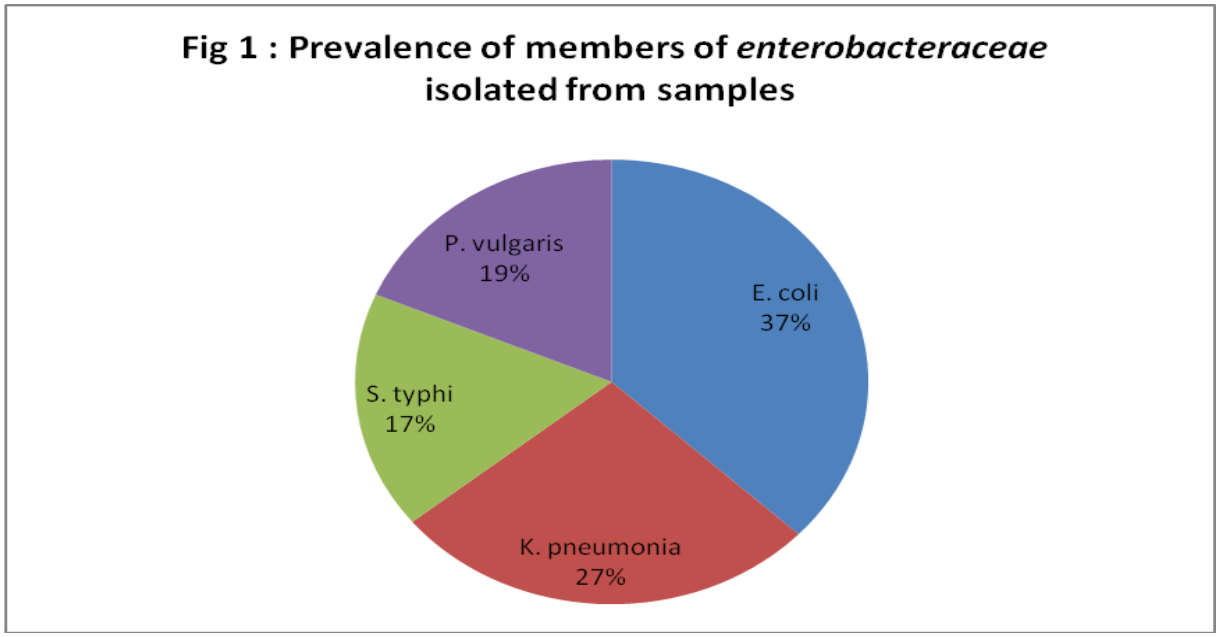

Table 2: Overall distribution of Resistance and Sensitivity among the members of *Enterobacteriacae*.

Clinical Isolates	Resistant (%)	Sensitive (%)
E. coli	73.33%	26.66%
K. pneumoniae	59.09%	40.90%
S. typhi	57.14%	42.85%
P. vulgaris	33.33%	66.66%

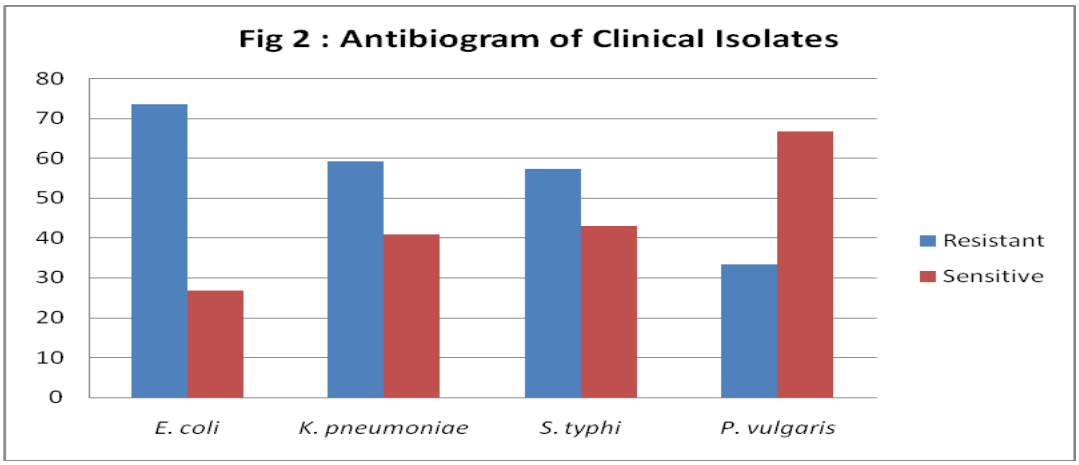

Table 3 : Antibiotic resistance pattern of selected MDR isolates.

Isolates	Te_{30}	Cip_5	E_{15}	C_{30}	A_{30}	Na_{30}	Nx_{10}	Nf_{300}	Amp_{10}	Gen_{10}	I_{10}	Ce_{30}
EC_5	I	R	R	S	R	R	S	S	R	R	I	R
EC_{20}	R	R	S	I	R	S	S	R	S	I	R	R
KP_8	I	R	S	R	S	S	I	R	S	R	I	S
KP_{15}	S	R	R	S	I	S	R	R	S	I	R	S
ST_{10}	R	S	S	R	S	R	R	I	S	S	S	R
ST_{11}	R	R	S	R	S	S	I	R	R	R	S	I
PV_7	R	S	R	I	R	S	R	R	S	R	R	S
PV_{13}	S	R	S	R	R	S	I	S	R	S	R	R

Table 4 : Antimicrobial activity of cell free supernatant of *Bacillus spp*. against MDR enteric bacteria.

Isolate	Name of isolate	EC_5	EC_{20}	KP_8	KP_{15}	ST_{10}	ST_{11}	PV_7	PV_{13}
BS_1	*B.licheniformis*	21	11	19	20	19	09	18	22
BS_2	*B.amyloliquifacience*	11	12	09	12	10	-	15	12
BS_3	*B.amyloliquifacience*	23	-	14	12	08	11	15	24

BS_4	*B.subtilis*	16	08	09	15	11	11	09	17
BS_5	*B.licheniformis*	17	13	06	-	15	18	11	-
BS_6	*B.amyloliquifacience*	15	09	11	-	14	13	22	20
BS_7	*B.subtilis*	20	13	23	27	08	12	18	23
BS_8	*B.amyloliquifacience*	08	12	09	24	13	14	19	26
BS_9	*B.subtilis*	21	11	09	14	11	11	10	-
BS_{10}	*B.subtilis*	17	13	14	13	10	21	21	20
BS_{11}	*B.licheniformis*	08	09	12	17	19	08	21	17
BS_{12}	*B.amyloliquifacience*	09	12	11	13	09	06	14	09
BS_{13}	*B.licheniformis*	14	19	-	13	16	12	12	20
BS_{14}	*B.licheniformis*	11	07	12	20	11	13	-	-
BS_{15}	*B.amyloliquifacience*	-	07	-	20	11	13	-	12
BS_{16}	*B.subtilis*	10	09	17	-	12	09	09	-
BS_{17}	*B.licheniformis*	-	14	09	11	14	13	15	15

IV. DISCUSSION

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 [9]. Resistance to antibiotics has resulted in morbidity and mortality from treatment failures and increased health care costs. The increases in antibiotics resistant have been attributed to inappropriate use, inadequacies on the part of the manufacturers and leads to the steady decline of effective antibiotics annually worldwide. Unfortunately, legislative is still not enough to curb the menace. The situation has become a serious challenge to drug manufacturers, public health practitioners worldwide. Therefore, this study is an attempt to identify bacillus species with potential of antibiotic production that could be use to control the problem of drug resistance.

In the present study, the resistance pattern among the clinical isolates from *Enterobacteracae* showed high degree of antibiotic resistance. The literature also agrees with the emergence of antibiotic resistance among selected *Enterobacteracae* family members viz. *E. coli, K. pneumoniae, S. typhi* and *P. vulgaris* [11,12,1314,9,15].

Bacillus species are known to inhibit soil, because the organisms are documented to withstand both high and low temperature condition. This special attribute exhibited by *Bacillus species* makes the organisms most successful among other bacterial species known. The peculiarity of *Bacillus species* in an environment with elevated nutrient is an indication that the organisms have better competitive ability compare to other bacteria species present in the environment under study.

Thus in the present study *Bacillus spp.* isolated from soil samples were examined against selected MDR pathogens. It was found that most of the *Bacillus* isolates exert good antibacterial role against tested *Enterobacteracae* family members. As most of these clinical isolates were not found sensitive to many standard antibiotics. Amongst all *Bacillus spp.* BS_7 (*Bacillus subtilis*), BS_3 (*Bacillus amyloliquifacience*) and BS_1 (*Bacillus licheniformis*) showed prominent activity against all test bacteria. This is in agreement with other studies who also reported the *Bacillus spp*. exerting good antibacterial activity against pathogenic bacteria of health significance [16,17,3].

Since, some of the *Bacillus* isolates showed interesting antimicrobial properties it would be useful to further exploit these isolates for future studies in order to isolate,characterize and identify antimicrobial compounds against the pathogens.

V. CONCLUSION

The drug resistance was found to be high among the selected pathogens of *Enterobacteracae*. The *Bacillus spp*. isolated in the present study showed good antimicrobial property to combat these drug resistant pathogens. The further detail studies regarding isolation and purification of metabolites from these isolates in order to determine their structure and composition may be useful in formulation of new drug in near future.

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