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Analysis of antibiotic resistance of organisms from different industrial soil sources

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ABSTRACT

Bacterial resistance to antibiotics poses a serious challenge to the use of antibiotics. Rational use of antibiotics is most desirable but it cannot provide a permanent solution to the problem. The present study concentrates on analysis of antibiotic resistance of microorganisms from two different soil samples collected from different industrial regions. The bacterial population in the samples was isolated and analyzed using serial dilution procedure followed by pour plate method. The antibiotic resistances of microorganisms were studied with 7 broad and narrow spectrum antibiotic discs and the zone of inhibition in mm was measured. Based on the zone of inhibition the antibiotic resistance was conferred, which in turn can provide the data for assessing the range of resistance of microorganisms in the region.

KEYWORDS

Antibiotics;
Antibiotic resistance;
Industrial soil;
Isolation;
Zone of inhibition.

INTRODUCTION

The intense use and misuse of antibiotics form the major cause of development of resistance in pathogenic and commensal bacteria throughout the world. Though the volume and way of use of antibiotics contribute to the selection of resistant strains, yet other social, ecological and genetic factors also directly affect the use and frequency of resistance. The resistant bacteria once emerged subsequently continue to proliferate and maintain the resistant strains even in the absence of antibiotics[11].

It is reported that human therapy accounts for approximately half of the total consumption of antibiotics in the European Union and the USA[6]. Until last decade, resistance was found chiefly in hospitals where intensive use of antibiotics was prominent; recently with the increase in number of treated patients resistance has become widespread among the commensal bacteria[6,8,9].

According to Centers Disease Control and Prevention report[1], new multi-drug resistant bacteria continue to emerge, In response to which hospitals have used a variety of infection-control measures, some of which are costly and difficult to implement[7]. National Nosocomial Infections Surveillance (NNIS) System Report[5] says that despite the effort taken to reduce transmission of antibiotic resistant bacteria (ARB) the nosocomial infections occur with alarming frequency and continues to increase[2].

Certain class of bacteria such as multi-resistant Gram-negative bacteria are troublesome and are reported to cause two thirds of death due to bacterial infections in the US[3,10]. The consequences of antibiotic

resistance affects patients' lives but also reaches far beyond the individual patient affecting health care systems and societies across the world.

In the present study bacteria was isolated from different industrial soil samples using serial dilution procedure and pour plate method. The antibiotic resistance of the isolated bacteria was assayed using antibiotic disc sensitivity assay and the resistance in the measure of zone of inhibition is reported.

EXPERIMENTAL

Luria-Bertani agar and LB broth were used as bacterial medium. 7 different antibiotic discs were used for the sensitivity assay.

Sample collection

Soil samples from two different regions near pharmaceutical industries in the locality were collected from the periphery using sterile collectables in sterilized plastic bags.

Isolation of bacteria from soil sample

The soil sample was diluted in sterile distilled water and then was serially diluted to obtain dilutions upto10^{-7}. Of these dilutions 10^{-5}, 10^{-6}& 10^{-7} were selected and used for the isolation procedure. Luria-Bertani agar plates were prepared and 1ml of the serially diluted samples was poured and incubated at 37°C for 24hrs (pour plate method)[4].

Antibiotic sensitivity assay

A Single colony, each from all three dilution (10^{-5}, 10^{-6} and 10^{-7}) culture plates were picked and inoculated into 100ml LB broth incubated overnight at 37°C. Each overnight culture was plated onto LB agar plates by spreading 100µl of the culture using sterile L-rod evenly. 7 different antibiotic discs were placed equidistant on the plates and the plates were incubated overnight at 37°C. All procedures were carried out in triplicates.

RESULTS AND DISCUSSION

Isolation of bacteria

Growth with appropriate cell density was observed in the 10^{-6} dilution of sample 1 and 10^{-5} dilution of sample 2 of the industrial samples and in all three dilutions plated (10^{-5}, 10^{-6}, 10^{-7}) of the hospital sample. These bacterial colonies were further isolated by repeated plating, until a single colony of bacterial isolate was obtained which was utilized for antibiotic sensitivity assay.

Antibiotic sensitivity assay

7 different antibiotic discs were used for the assay. TABLE 1 shows the zone of inhibition in mm for the various antibiotic discs for industrial samples. From which it is evident that the sample 1 at 10^{-6}dilution showed strong resistance to Streptomycin, and sample 2 at 10^{-5}dilution was found to be strongly resistant to Piperacillin and Clindamycin. TABLE 2 represents the results of antibiotic sensitivity assay showing zone of inhibition for various antibiotics of hospital sample. The results indicate that the sample at all three dilutions are strongly resistant to ampicillin and 10-7 dilution showed stong resistance to clindamycin. TABLE 3 represents the

TABLE 1 : Results of antibiotic sensitivity assay showing zone of inhibition for various antibiotics of industrial sample

Antibiotic disc	Sample 1 (10^{-6})	Sample 2 (10^{-5})
	Zone of inhibition (mm) (diameter)	Zone of inhibition (mm) (diameter)
Ampicillin	25	28
Clindamycin	30	Nil
Erythromycin	31	36
Gentamycin	28	35
Piperacillin	23	Nil
Streptomycin	Nil	10
Tetracyclin	37	35

TABLE 2 : Results of antibiotic sensitivity assay showing zone of inhibition for various antibiotics of hospital sample

Antibiotic disc	Sample (10^{-5})	Sample (10^{-6})	Sample (10^{-7})
	Zone of inhibition in mm (diameter)	Zone of inhibition in mm (diameter)	Zone of inhibition in mm (diameter)
Ampicillin	Nil	Nil	Nil
Clindamycin	10	10	Nil
Erythromycin	14	16	15
Gentamycin	20	18	20
Piperacillin	14	14	12
Streptomycin	20	20	22
Tetracyclin	20	20	20

standard assay values for the antibiotics used. Comparing the standard values to that of experimental few other susceptible results were observed in the case of ampicillin, streptomycin for both industrial and hospital samples.

TABLE 3 : Standard values for antibiotic sensitivity assay

Antibiotic	Zone of inhibition (mm)		
	Resistant	Intermediate	Susceptible
Ampicillin	28		29
clindamycin	14	15-20	21
Erythromycin	13	14-22	23
Gentamycin	12	13-14	15
Piperacillin	17	18-20	21
streptomycin	14	15-20	21
tetracyclin	14	15-18	19

CONCLUSION

From the results of antibiotic assay it could be concluded that the industrial soil sample contains bacteria showing influencing resistance to streptomycin, piperacillin and clindamycin. The organisms from hospital soil were resistant to Ampicillin; and incase of other antibiotics most are in its closest for becoming resistant. This indicates that these broad spectrum antibiotics are used maximally in the region of sample collection which poses a challenge to the rational use of these antibiotics in the community.

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