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PRODUCTION AND CHARACTERIZATION OF MULTIPLE DRUG RESISTANT CULTURES ISOLATED FROM HOSPITAL PREMISES

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ABSTRACT

A current phenomenon of great concern among the medical communities in developing countries is raising multi-drug resistant organisms, and the challenges of curing the infections in human and animals. Five MDR cultures of bacteria were isolated from two different locations of Bikaner, Govt. P.B.M. Hospital & Kothari Hospital. Based upon Gram staining two isolates (S1C-V & S2SC-V) were identified as Gram negative which are Cocci and out off three Gram positive isolate (S2SC-II, S2SC-III, S2SC-IV) two were roads and one was cocci. Based upon the biochemical analysis S1,C-V bacterial culture was identified as *Veillonella*, for multidrug resistance (MDR) test eight different antibiotics in different concentration were used. It was observed that S1C-V (*Veillonella*) bacterial culture showed resistance to Seven different antibiotics at different concentration from 10 µg/ml to 100 µg/ml. Bacterial culture S2SC-II (Bacillus) showed resistance to Six different antibiotics at 100 µg/ml concentration. Bacterial culture S2SC-III (*Sprorlactobacillus*) showed resistance to five different antibiotics at 100 µg/ml concentration. Bacterial culture S2SC-IV (*Streptococcus*) showed resistance to four different antibiotics at 100 µg/ml concentration. Bacterial culture S2SC-V (*Neisseria*) showed resistance to seven different antibiotics at 10 µg/ml to100µg/ml concentration.

KEYWORDS : Antibacterial Properties, Secondary Metabolites, Multi Drug Resistant Pathogens

Antibiotic resistant bacteria have been a source of an ever-increasing therapeutic problem. Continued mismanagement and resulting selective pressure have contributed towards the emergence of multiple drug resistant bacteria which has been regarded as an inevitable genetic response to antimicrobial therapy. Drug resistant infectious microbes have become an important public health concern warranting organizations in public and private sectors worldwide to work together (Blanch et al., 2003 and NIAID, 2011). Mostly the antibiotic resistant bacterial cultures are found in clinical and veterinary land areas whereas the existence of antibiotic resistance (AR) bacteria in water has also observed and supported to be present in higher concentrations and diversity in hospital areas as compared to domestic areas. Antibiotics are either synthesized industrially or also produced by microorganisms; these antibiotics have microstatic or microcidal activity. Mainly these antibiotics producing microbes interrupt the microbial metabolism by various mechanisms (Jalal et al., 2010). Several researches were performed that dealt with advanced antimicrobial resistance in bacteria isolated from food, animal and environment (Jensen et al., 2001). The use of antibiotics is increasing continuously in different fields like veterinary medicine, agriculture, etc. but the awareness of knowledge regarding the quantity of antibiotics present in the environment after their use is very less (Hirsch et al., 1998). Multidrug- tuberculosis (MDR-TB) an increasing global problem with most cases arising non-compliance during treatment of susceptible TB the extent and burden of MDR-TB varies significantly from country to country and region to region. Contribution of hospital wastewater to the spread of antibiotic resistance in comparison to non-health institution has explained a potential post-antibiotic era threatening present and future medical advances. The current worldwide increase in resistant bacteria and simultaneously the downward trend in the development of new antibiotics had serious implication. Isolation of multi-drug resistant *Paenibacillus* sp. from fertile soil has explained an imminent menace of spreading resistance. The multi-drug resistant bacterium isolation from soil exhibited a resistance to various classes of antibiotics namely Glycopeptides, Beta-lactams, Aminoglycosides Macrolides & lincosomides (Pallavi et al., 2010). Spontaneous mutation and DNA transfer are the two main reason of resistance found in bacterial isolates. Spontaneous mutation takes place through (1) Tautomerision, (2) Depurination, (3) Deamination and (4) Slipping where as DNA transfer

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occurs through (1) Transduction, (2) Conjugation and (3) Transformation. The main objective of this work is to isolate multidrug resistant (MDR) pathogens from hospital premises in Bikaner (UP) and characterise them and also observe their growth kinetics in different production media.(Ahmad and Beg, 2001; Bhatta and Kapadnis, 2010).

MATERIALS AND METHODS

The soil samples were collected from two different locations of Bikaner; S1 (Govt. P.B.M. Hospital, Bikaner), S-2 (Kothari Hospital, Bikaner). Samples were serially diluted and bacteria were isolated on Nutrient Agar, Eight bacterial colonies were selected and designated as: S1C-I, S1C-II, S1C-V, S2SC-I, S2SC-II, S2SC-III, S2SC-IV and S2SC-V respectively. Bacterial colonies were sub cultured several times up to pure culture, maintained for further biochemical analysis, and preserved at low temperature. After isolation, identification of the bacterial isolates was carried out according to Bergey's Manual of Determinative Bacteriology. All the bacterial isolates were analyzed for their sensitivity against commonly used antibiotics by agar well diffusion method. Different antibiotics having different mechanisms of action and antibiotics taken were Tetracycline, Amoxycilline, Ampicilline, Ofloxacin, Cefixime, Ciprofloxacin, Doxyciline, Chloremphenicol and Erythromycine of concentration 10µg/ml, 20 µg/ml, 30 µg/ml, 50µg/ml, 100µg/ml. Wells were prepared on nutrient agar plate previously spread with 50 µl of isolated bacterial broth culture. These wells were loaded with 50µl of antibiotic solution and then plates were incubated at 37°C for overnight, then the zone of inhibition were examined. The obtained multidrug resistant isolates were further characterized by microscopic and Biochemical studies. The purified cultures were characterized by staining (Gram staining and Endospore staining) and biochemical activity (IMVIC) as par Bergey's manual given in Aneja (2003).Carbohydrate test and starch hydrolysis test were performed as a confirmatory test for Bacillus species. For carbohydrate test NB media were prepared with 10% glucose as a source of carbon which is than inoculated with cultures and incubated at 37°C for 24 to 48 hours colour change from blue to yellow gives the positive result.

Production media used here were the specific type of media which were used for larger scale production of specific bacterial culture. Specific types of production media were used for *Bacillus*, Neisseria and Straptococcus species for which their growth kinetics were studied by taking OD (optical density) at different time interval.

RESULTS

Five MDR cultures of bacteria were isolated from two different locations of Bikaner which were selected for further characterization. Based upon Gram staining two isolates (S1C-V & S2SC-V) were identified as Gram negative which are cocci and out off three Gram positive isolate (S2SC-II, S2SC-III, S2SC-IV) two were rods and one was cocci. Three isolated bacterial cultures were aerobic (S1C-V, S2SC-III, S2SC-V) and two are anaerobic (S2SC-II, S2SC-IV). Colony morphology of the cultures isolated from sample-1are given in table 1 and Colony morphology of the cultures isolated from sample-2 are given in table 2.

Based upon the biochemical analysis (Table, 3), S1C-V bacterial culture was identified as *Veillonella*, S2SC-II as *Bacillus*, S2SC-III as *Sporolactobacillus*, S2SC-IV as *Streptococcus* and S2SC-V as *Neisseria*.

Table 1: Colony Morphology of Sample-1 for S1C-V

Characteristics	C-V
Shape	Regular
Pigmentation	White
Texture	Smooth
Margin	Entire
Opacity	Opaque
Elevation	Flat

Table 2 : Colony Morphology of Sample-2 for S2 (SC-II, SC-III, SC-IV, SC-V)

Characteristics	SC-II	SC-III	SC-IV	SC-V
Shape	Regular	Regular	Regular	Regular
Pigmentation	Bluish	Bluish	Bluish	White
Texture	Smooth	Smooth	Smooth	Smooth
Margin	Entire	Entire	Entire	Entire
Opacity	Opaque	Opaque	Opaque	Transluce nt
Elevation	Elevated	Elevated	Elevated	Elevated

Table 3: Biochemical Analysis of Isolated MDR Bacterial Cultures

Tests	S1,C-V	S2,SC-V	S2,SC-II	S2,SC-III	S2,SC-IV
Gram stain	-ve (cocci)	-ve (cocci)	+ve(rod)	+ve(rod)	+ve (cocci)
Endospore stain	-ve	-ve	+ve	+ve	-ve
Catalase test	+ve	+ve	-ve	+ve	-ve
Carbohydrate test	.....	.....	+ve	-ve	-ve
Amylase test	.....	.....	+ve	-ve	-ve
Oxidase test	-ve	+ve	.....	.....	.....

Table 4: MDR Test Using Tetracycline as Antibiotic at Different Concentrations

Cultures	10 µg	20 µg	50 µg	100 µg
S1,C-I	R	R	S	S
S1,C-II	S	R	S	S
S1,C-V	R	R	R	R
S2,SC-I	S	R	S	S
S2,SC-II	R	R	S	S
S2,SC-III	R	R	S	S
S2,SC-IV	R	R	S	S
S2,SC-V	R	R	R	R

R= Resistance, S= Sensitive

For the multi- drug resistance (MDR) test eight different antibiotics in different concentration were used. It was observed that for tetracycline only bacterial cultures S1C-V (*Veillonella*) and S2SC-V (*Neisseria*) showed resistance at 100µg/ml concentration (Table, 4). For Cefixime all isolated bacterial cultures showed resistance at 100µg/ml except S2SC-I (Table, 5). For Ofloxacine S1C-V, S2SC-II and S2SC-III showed resistance but S1C-V and S2SC-V showed partial resistance (Table, 6). For Amoxyciline all culture showed resistance at 100µg/ml concentration except S2SC-II (Table, 7). For Doxyciline, bacterial culture S1C-V (*Veillonella*) showed resistance at100µg/ml and bacterial culture S2SC-V (*Neisseria*) shows partial resistance (Table,8). For Chloremphenicol, bacterial culture S1C-V, S2SC-II, S2SC-III, S2SC-V showed resistance while S1C-I showed partial resistance (Table, 9). For Erythromycine, bacterial culture S1C-V, S2SC-II, S2SC-III, S2SC-IV and S2SC-V showed resistance at 100µg/ml concentration (Table,10). For Ciprofloxacine, only two bacterial cultures S2SC-II and S2SC-IV showed resistance at 100µg/ml concentration (Table,11).

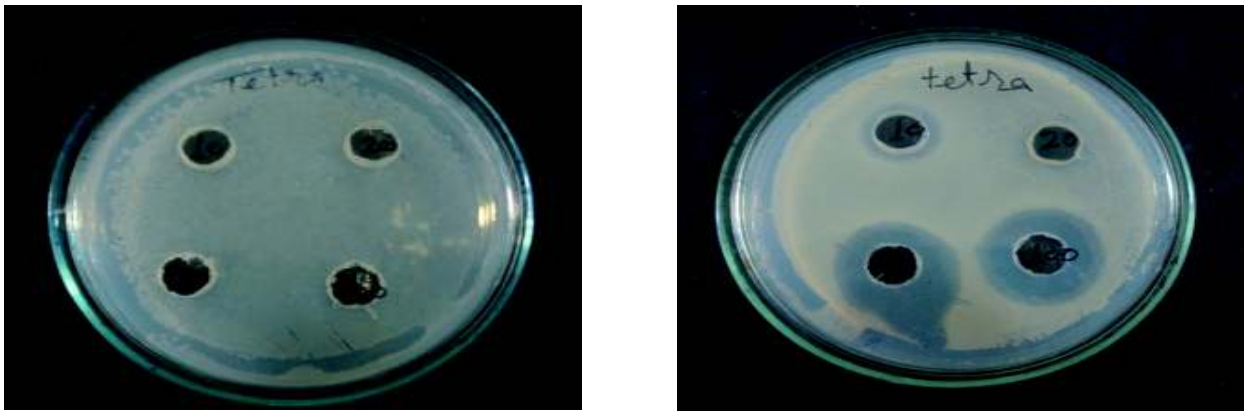


Figure 1: Antibiotic Sensitivity Test By Tetracycline

Figure, 1 showed that One Culture (S2, SC-V) show growth in presence of Tetracycline while other zone of Inhibition (S2,SC-I).

Table 5: MDR Test Using Cefixime as Antibiotic at Different Concentration

Cultures	10 µg	20 µg	50 µg	100 µg
S1,C - I	R	R	R	R
S1,C - II	R	R	R	R
S1,C - V	R	R	R	R
S2,SC - I	S	S	S	S
S2,SC - II	R	R	R	R
S2,SC - III	R	R	R	R
S2,SC - IV	R	R	R	R
S2,SC - V	R	R	R	R

R= Resistance, S= Sensitive

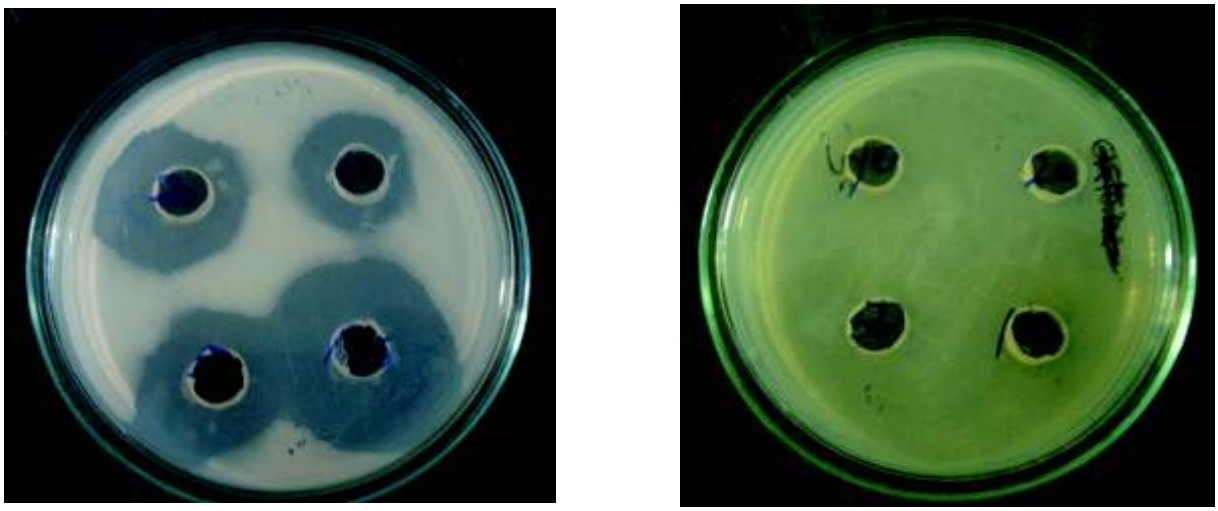


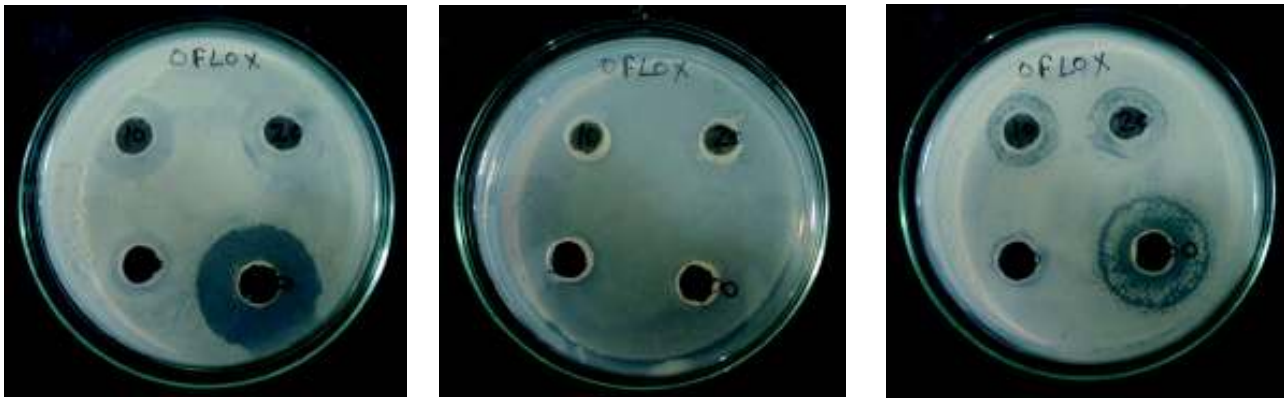
Figure 2 : Antibiotic Sensitivity Test By Cefixime

Figure, 2 showed that One Culture (S2, SC-III) show growth in presence of Antibiotic Cefixine, while other zone show Resistance (SI,SC-I).

Table 6 : MDR Test Using Ofloxacine as Antibiotic at Different Concentration

Cultures	10 µg	20 µg	50 µg	100 µg
S1,C-I	R	R	R	S
S1,C-II	Partial R	Partial R	Partial R	Partial R
S1,C-V	R	R	R	R
S2,SC-I	R	R	R	S
S2,SC-II	R	R	R	R
S2,SC-III	R	R	R	R
S2,SC-IV	S	S	Partial R	S
S2,SC-V	R	R	R	Partial R

R= Resistance, S=Sensitivity



(a) **(b)** **(c)**

Figure 3: Antibiotic Sensitivity Test by Ofloxacine

Figure, 3 showed that One Culture (a) (S2, SC-I) show sensitivity at 100 µg concentration of Antibiotic Ofloxacine, while other Culture (b) (SI,C-V) zone show Resistance of all concentration and Culture (C) (SI,C-II) show partial Resistance to all concentration.

Table 7: MDR Test Using Amoxyciline as Antibiotic at Different Concentration

Cultures	10 µg	20 µg	50 µg	100 µg
S1,C-I	R	R	R	R
S1,C-II	R	R	R	R
S1,C-V	R	R	R	R
S2,SC-I	S	S	S	S
S2,SC-II	R	R	R	R
S2,SC-III	R	R	R	R
S2,SC-IV	R	R	R	R
S2,SC-V	R	R	R	R

R= Resistance, S=Sensitive

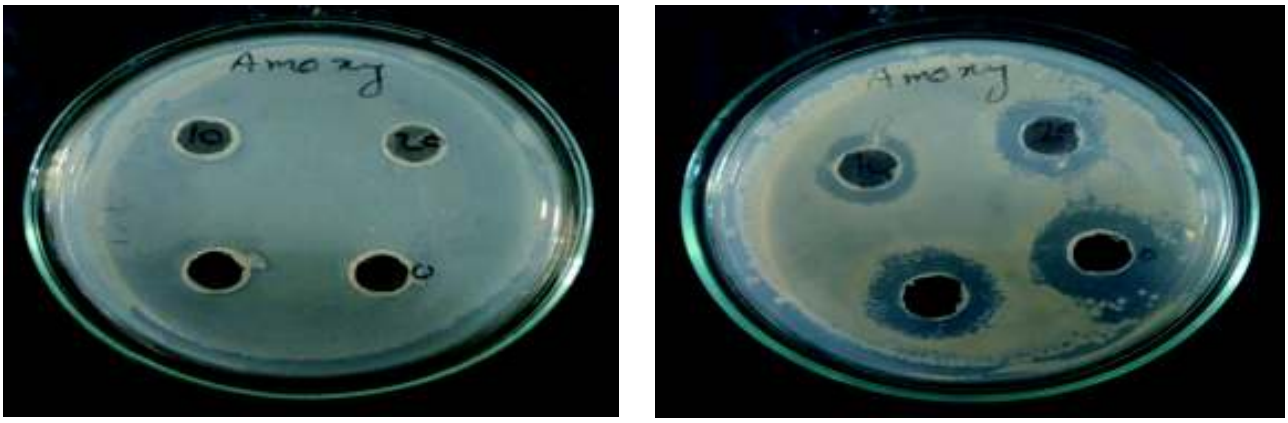


Figure 4: Antibiotic Sensitivity by Amoxyciline

Figure 4 showed that One Culture (a) (S1, C-I) show resistance in presence of Antibiotic Amoxyciline, while other Culture (b) (S2,SC-I)show sensitivity.

Table 8: MDR Test Using Doxyciline as Antibiotic at Different Concentration

Cultures	10 µg	20 µg	50 µg	100 µg
S1,C-I	S	S	S	S
S1,C-II	S	S	S	S
S1,C-V	R	R	R	R
S2,SC-I	S	S	S	S
S2,SC-II	S	S	S	S
S2,SC-III	S	S	S	S
S2,SC-IV	S	S	S	S
S2,SC-V	R	R	Partial R	Partial R

R= Resistance, S= Sensitivity

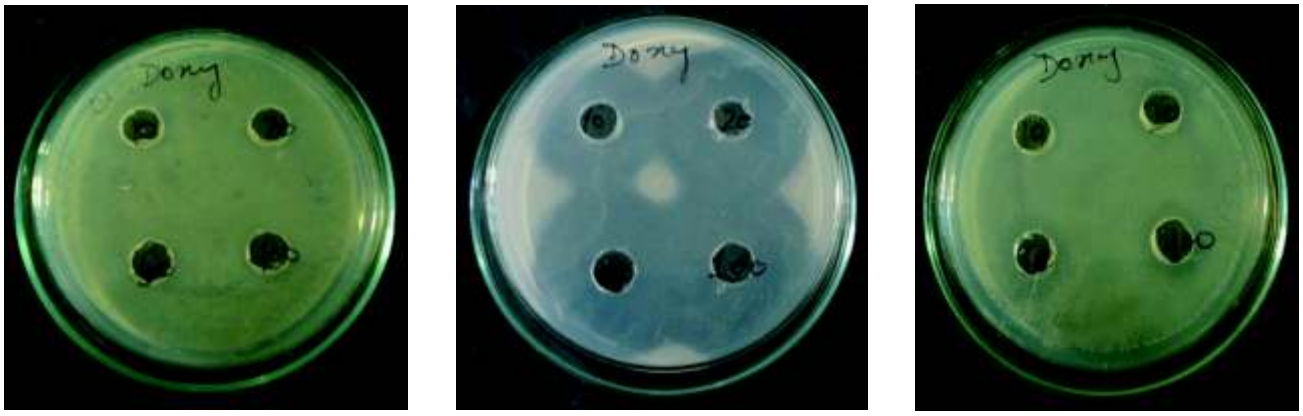


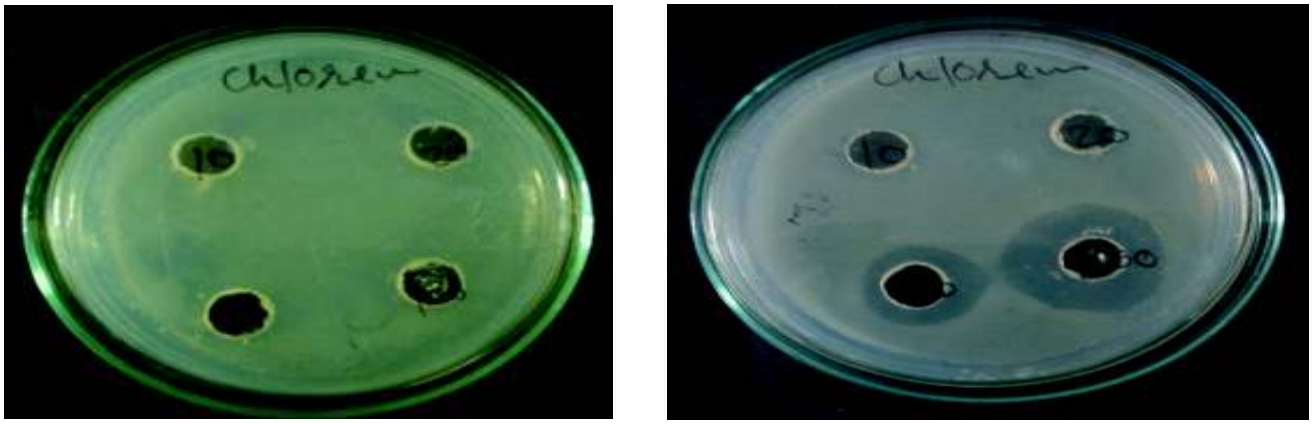
Figure 5 : Antibiotic Sensitivity by Doxyciline

Figure 5 showed that One Culture (a) (S1, C-V) show resistance in presence of Antibiotic Doxyciline, Culture (b) (S2, SC-V) show partial resistance at 50 µg & 100 µg concentration of Antibiotic and Culture (c) (S2, SC-I) show sensitivity of all concentration of Antibiotic.

Table 9 :MDR test using Chloremphenicol as antibiotic at different concentration

Cultures	10 µg	20 µg	50 µg	100 µg
S1,C -I	R	R	R	Partial R
S1,C - II	R	R	S	S
S1,C - V	R	R	R	R
S2,SC -I	R	R	Partial R	S
S2,SC - II	R	R	R	R
S2,SC - III	R	R	R	R
S2,SC - IV	R	R	S	S
S2,SC - V	R	R	R	R

R= Resistance, S=Sensitive



(a) (b)

Figure 6 :Antibiotic sensitivity by Chloremphenicol

Figure 6 showed that Culture (a) (S2, SC-III) show resistance in presence of Antibiotic Chloremphenicol, while other Culture (b) (S2,SC-IV)show resistance to only 10 µg & 20 µg concentration while at 50 µg & 100 µg concentration it show sensitivity toward antibiotics.

Table 10: MDR Test Using Amoxyciline as Antibiotic at Different Concentration

Culture s	10 µg	20 µg	50 µg	100 µg
S1,C -I	R	R	R	R
S1,C - II	R	R	R	R
S1,C - V	R	R	R	R
S2,SC -I	S	S	S	S
S2,SC - II	R	R	R	R
S2,SC - III	R	R	R	R
S2,SC - IV	R	R	R	R
S2,SC - V	R	R	R	R

Three bacterial isolate *Neisseria, Streptococcus*, and *Bacillus* were produced in production media and their activity was checked by antibiotic sensitivity test at 1mg/ml concentration only ofloxacine and ciprofloxacine showed sensitivity rest all the antibiotics showed resistance.

Table 11: Antibiotic Sensitivity Test for Cultures Produced in Production Media

Antibiotics	*Neisseria*	*Streptococcus*	*Bacillus*
Tetracycline	R	R	R
Cefixime	R	R	R
Ofloxacine	S	S	R
Amoxyciline	R	R	R
Doxyciline	R	R	R
Chloremphenicol	R	R	R
Erythromycine	R	R	R
Ciprofloxacine	S	S	R

Significance of Results

In this paper result is showing that today due to pollution and use of excess amount of antibiotics Microorganism is now going to adaptive against these antibiotics.

REFERANCE

Ahmad I. and Beg A. J.,2001. Antimicrobial and phytochemical studies on 45 indian medicinal plants against multidrug resistance human pathogens. J. Ethnapharma.

Aneja K. R. ,2003.Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International (P) Limited,New Delhi, **4**:607.

Bhatta D. R and Kapadnis B. P.,2010. Production Optimization and Characterization of Bioactive Compound Against Salmonella From *Bacillus subtilis*. Scientific World, **8**(8):19-27.

Blanch A. R., Caplin J. L., Iversen A., Kühn I., Manero A., Taylor H. D. and Vilanova X. ,2003. Comparison of Enterococcal populations related to urban and hospital wastewater in various climatic and geographic European regions. J. Appl. Microbiol., **94**:994-1002.

Hirsch P., Ludwig W., Hethke C., Sittig M., Hoffmann B., Gallikowski C.A., 1998. Hymenobacter roseosalivarius gen. Nov. From continental Antarctic soils and sandstone : bacteria of the Cytophaga / Flavobacterium / Bacteroides line of phylogenetic descent. Syst. Appl. Microbiol., **21**: 374-383.

Jalal K.C.A., Nur Fatin U.T., Mardiana M.A., Akbar John B., Kamaruzzaman Y.B., 2010. Antibiotic resistance microbes in tropical mangrove sediments in east coast peninsular, Malaysia. African Journal of Microbiology Research, **8**:640-645.

Jensen LB., Baloda S., Boye M. and Aarestrup F.M.,2001. Antimicrobial resistance among *Pseudomonas* spp. and the Bacillus cereus group isolated from Danish agricultural soil. Environ. Int., **26**: 581-587.

NIAID,2011.http://www.niaid.nih.gov/TOPICSIANTIMICROBIALRESISTANCE/RESEARCH/Pages/translating.aspx.

Pallavi B. Pednekar, Roopesh J., Narsinh L., Thakur and Girish B,M.,2010. Isolation of Multi-Drug Resistance *Paenibacillus* sp. from fertile soil: An Imminent Menace of Spreading Resistance. .Journal of life Sciences.