Research Article

Optimization, Production and Characterization of Multidrug Resistant Cultures Isolated from Hospital Premises

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ABSTRACT

The present study is carried out by isolation of Multidrug resistant culture from hospital premises. The multidrug resistant cultures showed growth in the presence of multiple antibiotics. In this work the 4 soil samples were used and out of 4 total 5 cultures were isolated but only 2 cultures were characterized through Bergey's manual. The cultures were *S.equisimilis and N.sicca* and antibiotics were Oofloxacin, Erythromycin, Floxip, Trimexazole, Ampilox, Cephalaxin and Almox. The Oofloxacin showed best resistant activity against 2 bacterial cultures.Further optimization parameters involved suitable carbon sources, suitable nitrogen sources and suitable pH.The best carbon source obtained in Sucrose for *S.equisimilis* and for *N.sicca* Lactose was best carbon source. The best nitrogen source obtained inNa_2HPO_4 for *S.equisimilis* and for *N.sicca*. The best growth obtained for *S.equisimilis* and for *N.sicca* at pH 7.

Keywords: Multidrug resistant, Antibiotics, Bergey's manual.

INTRODUCTION

Present time the antibiotic resistance has become a major problem in the clinical and public health prospects.The increasing levels of multi-drug resistance in human pathogenic bacteria are compromising our ability to treat infectious disease. Since antibiotic resistance determinants are readily exchanged between bacteria through lateral gene transfer, there is an increasing interest in investigating reservoirs of antibiotic resistance accessible to pathogens.There is growing public health concern over the contribution of agricultural antibiotic use to the global rise of drug resistant bacteria.Microorganisms cultured from soil have provided most of the antibiotics and many other medicinal agents that have dramatically improved human health in later half of the 20th century[1,2].One of the richest sources of new antibiotics may be the uncultured microorganisms of soil. Antibiotic resistance is a well-recognized threat to public health. Search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research. Natural products having novel structures have been observed to possess useful biological activities. Soil is a natural reservoir for microorganisms and their antimicrobial products[3].The emergence and spread of antimicrobial resistance are complex problems driven by numerous interconnected factors.Studies have been shown that introduction by these routes have changed the antibiotic susceptibility of the microbes in those environments .One of these routes is the sewage, the antibiotics that we take in are not all processed by our bodies. Some of them are expelled as waste and wind up in our wastewater treatment plants of bacteria isolated from sludge remaining after wastewater treatment at one plant, 46.4% were resistance to multiple antibiotics. Sewage from hospitals and pharmaceutical plants has been shown to contribute to antibiotic resistance in treatment plant[4,5].The volume of antibiotics used in hospitals and private households and released into effluent and municipal sewage indicates a selection pressure on bacteria.Waste effluent from hospitals contains high numbers of resistance bacteria and antibiotics residues at concentration able to inhibit the growth of susceptible bacteria.Although sewage treatment process residue the numbers of bacteria in waste water, the effluent will still generally contains large numbers of both resistant and susceptible bacteria shows the decrease in VRE from 16% in untreated waste water to 12.5% at the outlet[6].

The present study is carried out to isolate and characterize the multidrug resistance (MDR) pathogens from hospital premises area of Lucknowto check the activity of culture in the presence of various antibiotics.

MATERIALS AND METHODS

At first soil samples were collected from hospital area for the experimental purpose and then various tests were performed.

Collection of soil sample
The soil samples were collected form Hospitals ofLucknow. These samples were isolated for bacteriological analysis by serial dilution and then agar plate culture techniques.

Serial dilution
This method is based on the principle that when soil sample or water sample along with bacterial colonies taken, the results obtained in the form of reduced number of bacterial colonies. The microbes are having importance in the industries for enzyme and antibiotic production.
Dilution=volume of the sample/total volume of the sample and the diluents.Labelled the dilution blanks as 10^{-1},10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}.Prepare the initial dilution by adding 0.5gm of soil sample into 5 ml distilled water.Mix the control and then from the first dilution, transfer 0.5ml of suspension to the next test tube and mixed properly and perform this work till 10^{-5}. Prepare Nutrient agar plates.Spread 50 µl sample from each test tube.Incubate at 37˚C for overnight.Observed result in the form of bacterial colonies.

Pure culture
A pure culture is a culture containing a single colony of an organism. A pure culture is usually derived from mixed culture by transferring a small sample in to a new sterile growth medium in such a manner as to disperse the individual cell across the surface medium or by thinning the individual cells so that when multiply each will form a discrete colony.100 ml nutrient agar was prepared and poured in a sterile Petri plate.Type of colonies present in every sample named. All the obtained in mixed were streaked with the help of sterile inoculation loop. Quadrant and zigzag streaking was done.All the plates were incubated at 37˚C for overnight.

Charaterization of bacterial culture
Characterization was done with the help of Bergey's manual using biochemical tests.It included Gram staining of bacteria, Endospore staining, Catalase test[7] etc.

Antibiotics sensitivity test
Antibiotics are the chemical which inhibit the growth of bacterial cultures. The Mode of action of antibiotics can be of 2 types, eitherthey will inhibit the protein synthesis i.e block the translation or they will destroy the cell wall.AST is used to check the sensitivity of antibiotics against pathogens[8].

Growth kinetics
Growth is the orderly increases in all major constituents of an organism, involving several strivers, nucleic acid, protein and all other cell components from nutrient obtained from outside the cell. Growth kinetics process was to determine the time period at which the culture showed optimum activity. Growth kinetics was done with the help of day by day OD method to know the log phase at 600 nm.

Optimization Parameters
Optimization involved suitable conditions for the growth of culture using suitable carbon, nitrogen sources as well as pH.

Carbon sources
The effect of carbon source such as glucose,dextrose, sucrose, beef extract at a concentration of 1% was examined by replacing in the production media.

Nitrogen sources
Various nitrogen sources like- Peptone, urea, NH_4Cl, Na_2HPO_4at a concentration of 1% was examined by replacing in the production media.

pH
The pH of media (N.B.) were used 5, 7, 9, &11.

MBC (Minimum bactericidal concentration)
The bactericidal culture showed the growth in the form of bacteriostatic and if cultures were not showing growth then the concentration of antibiotic is bacteriocidal for culture.

RESULTS
Bacterial culture were isolated from hospital premises of Lucknow and out of 5 cultures 2 cultures wereused for their antibiotic sensitivity test and that isolated culture was maintain for optimization of media, pH, suitable carbon source and nitrogen source. The MBC test also performed.

Serial dilution Method
The serial dilution method was performed in order to get pure and reduce number of bacterial colonies and there were total 5 isolates were found and out of 5 isolates 2 cultures were used for further work.

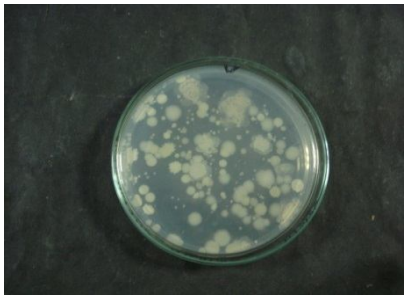


Fig. 1: Bacterial colonies in a mixed culture form

Colony morphology

Table 1: Colony morphology of Isolates

Characteristics	C1	C2	C3	C4	C5
Shape	Fusiform	Regular	Irregular	Regular	Irregular
Elevation	Elevated	Flat	Flat	Flat	Flat
Color	White	Off white	White	Off white	White
Texture	Rough	Smooth	Rough	Smooth	Rough
Margin	Entire	Lobate	Entire	Lobate	Entire
Opacity	Opaque	Transparent	Opaque	Transparent	Opaque

Sub culturing

The procedure to get single isolated colonies from one medium to another.These isolated cultures had named as C1,C2,C3,C4 and C5.

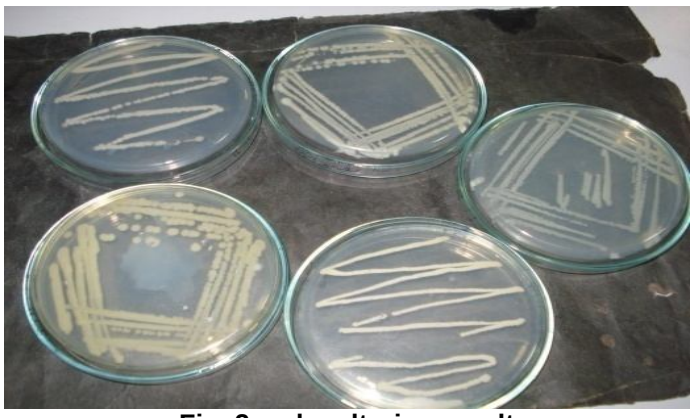


Fig. 2: sub culturing result

Table 2: Biochemical analysis of isolated MDR bacterial cultures

biochemical test	Isolates				
	C1	C2	C3	C4	C5
Gram's staining	positive	positive	negative	negative	negative
cellular morphology	chain/ purple	chain/ purple	thread/ pink	thread/ pink	thread/ pink
Endospore test	negative	negative	negative	negative	negative
catalase activity	negative	negative	negative	negative	negative
acid fast staining	negative	negative	negative	negative	negative
Group confirmed	Group VII *streptococcus*	Group VII *streptococcus*	Group XI *Neisseria Villonela*	Group XI *Neisseria Villonela*	Group X *Klebsiella Shigella*

Table 3: Confirmatory test

biochemical test	Isolates				
	C1	C2	C3	C4	C5
Glucose test			positive	positive	positive
grwoth in 6.5NaCl	negative	negative			
Acid from glycerol	positive	positive			
Nitrate test			negative	negative	negative
Starin confirmed	*S. equisinilise*	*S. equisinilise*	*N. sicca*	*N. sicca*	*N. sicca*

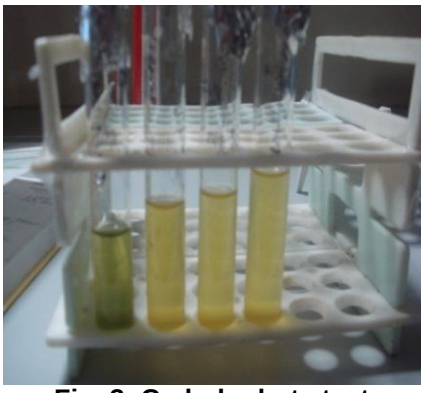


Fig. 3: Carbohydrate test
Fig. 3: showed that carbohydrate is positive the color changes green to yellow

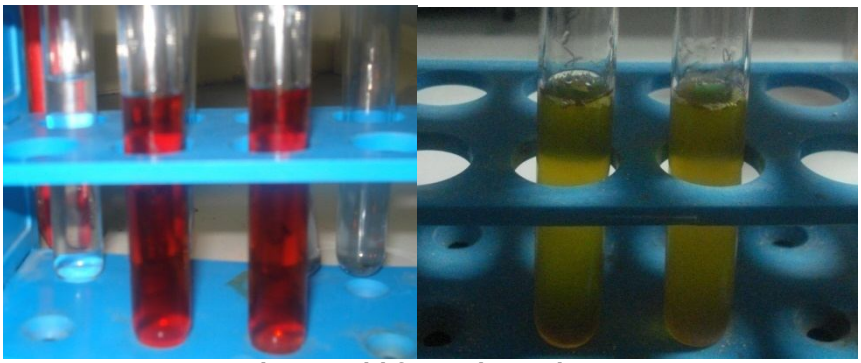


Fig. 4: Acid from glycerol test
Fig. 4: showed positive test for *Streptococcus equisimilis* color changes red to yellow

Antibiotic sensitivity test

AST is used to check the sensitivity of the antibiotics against pathogens. If the antibiotic is sensitive to that pathogen, they make a clear zone surrounding the disc. If the culture were showing growth in the presence of antibiotics means culture were resistant for that antibiotic. Antibiotics are used with different concentration/ml- 100µg, 50µg and 10µg.

Fig. 5: Ofloxacin

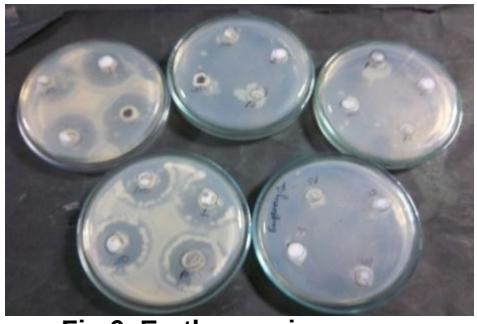


Fig 6: Erythromycin

Fig. 5: showed the growth in the presence of antibiotics it showed that the cultureswere MDR culture

Fig. 6: does not show maximum growth in the presence of antibiotic so these cultures were not MDR culture

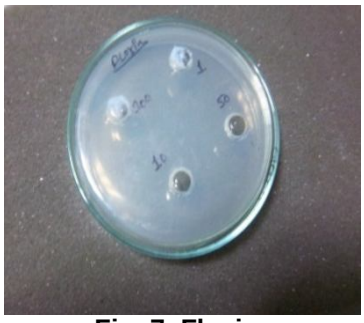


Fig. 7: Floxip

Fig. 8: Trimexazole

Both fig 7 and fig 8 are not showing the growth in the presence of antibiotics it showed that the cultures were MDR culture.

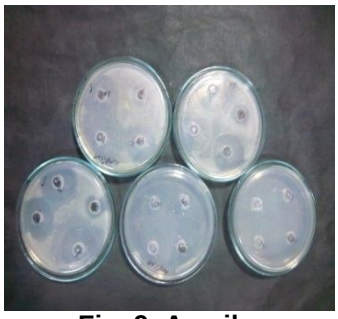


Fig. 9: Ampilox

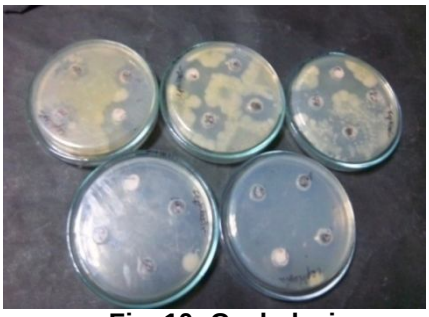


Fig. 10: Cephalaxin

Fig 9 and fig 10 not showed the growth of inhibition means antibiotic is sensitive

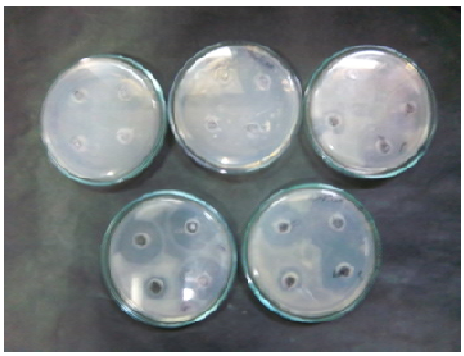


Fig. 11: Almox

Table 4: MDR test for culture

Antibiotic	C1	C2	C3	C4	C5
Oofloxacin	R	R	R	R	R
Erythromycin	S	S	S	R	S
Floxip	R	S	R	S	S
Trimexazole	R	R	R	S	R
Ampilox	S	R	S	R	R
Cephalaxin	S	R	S	R	R
Almox	S	S	R	R	S

R= Resistance, S= Sensitive

Production media

For study about growth kinetics prepared production media for those two isolated bacterial culture *Streptococcus equisimilis* and *Neisseria sicca.*

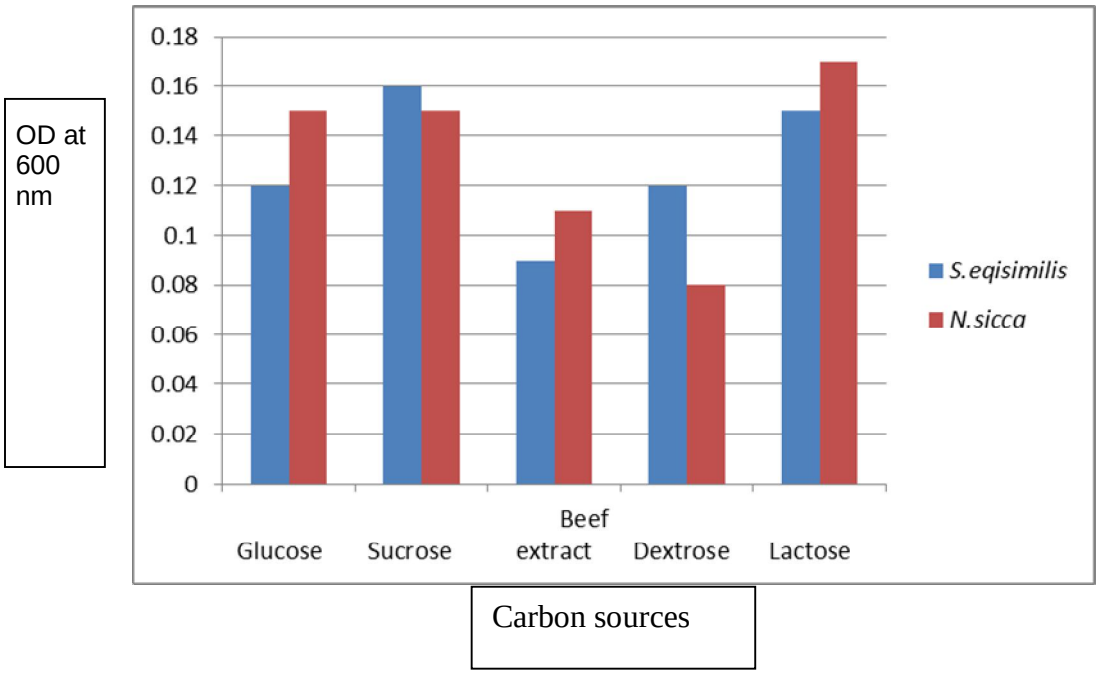
Optimization of culture condition

For *Streptococcus equisimilisand N.sicca.*

Table 5: Suitable carbon sources

Carbon source	OD at 620 nm (*S.equisimilis)*	OD at 620 nm (*N.sicca*)
Glucose	0.12	0.15
Sucrose	0.16	0.15
Beef extract	0.09	0.11
Dextrose	0.12	.08
Lactose	0.15	0.17

Table 5: showed that best carbon source obtained in Sucrose for *S.equisimilis* and for *N.sicca* Lactosewas best carbon source.

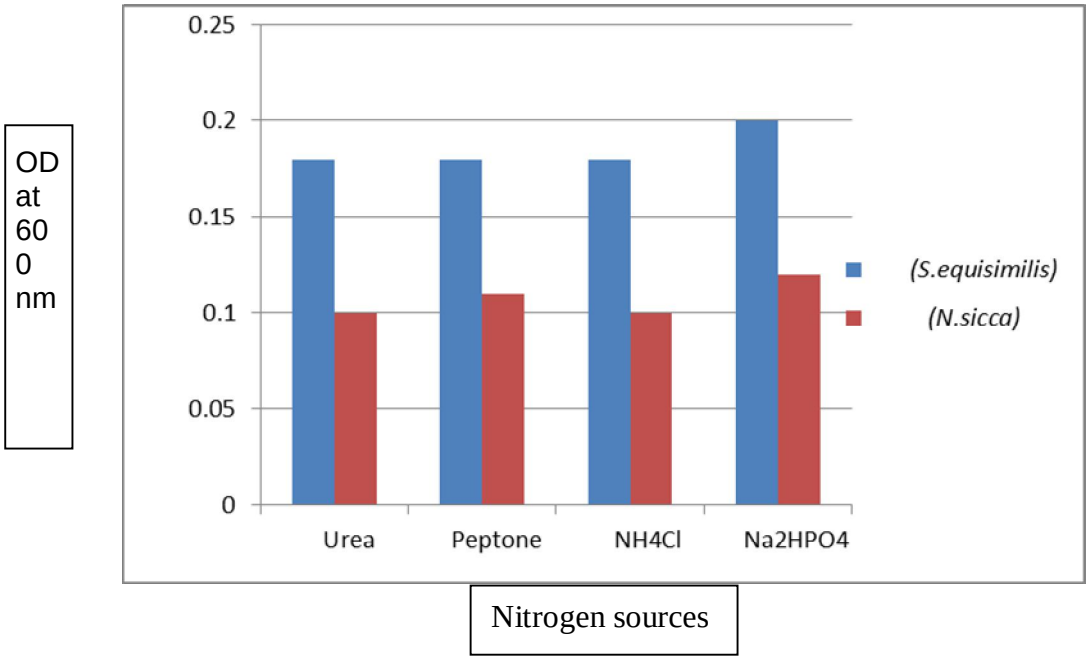


Graph 1: Optimization of carbon sources

Table 6: Suitable nitrogen source

Nitrogen source	OD at 620 nm (*S.equisimilis)*	OD at 620 nm (*N.sicca*)
Urea	0.18	0.10
Peptone	0.18	0.11
NH_4Cl	0.18	0.1
Na_2HPO_4	0.20	0.12

Table 6: showed that best nitrogen source obtained inNa_2HPO_4 for*S.equisimilis* and for *N.sicca*

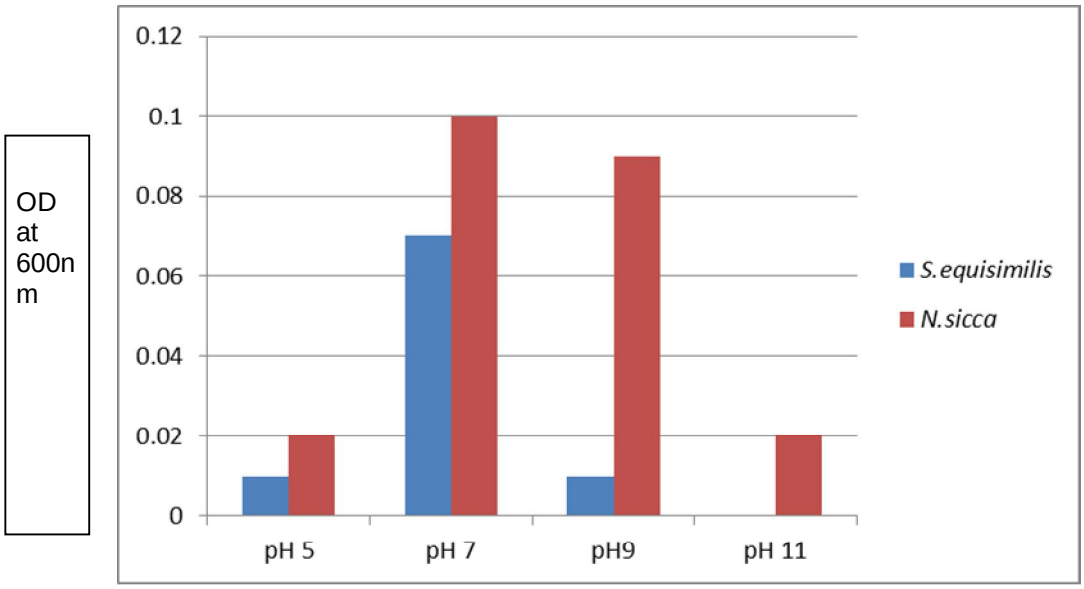


Effect of nitrogen source in growth curve

Table 7: Suitable pH

pH	OD at 620 nm (*S.equisimilis*)	OD at 620 nm (*N.sicca*)
5	0.01	0.02
7	0.07	0.10
9	0.01	0.09
11	0.00	0.02

Table 7: showed that best growth obtained for *S.equisimilis* and for *N.sicca*at pH 7



Effect of pH in growth curve

MBC: (Minimum bactericidal concentration)
After providing suitable carbon, nitrogen source and pH of isolated bacterial culture AST has performed.

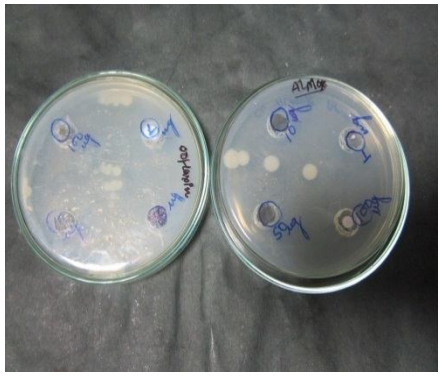


Fig. 12: MBC plates of *Neisseria sicca*
Fig. 12: showed the minimum growth in the presence of antibiotic Ofloxacin and Almox, that showed cultures are less MDR

Fig. 13: MBC plates of *Streptococcus equisimilis*
Fig. 13: showed the maximum growth for *streptococcus equisimils* in the presence of antibiotic Oofloxacin, Trimexazole, Ampilox and Almox, that showed cultures are MDR

DISCUSSION

An antibiotic can be isolated through various microbes, but out of these microbes, some microbes are capable of producing resistant against various types of antibiotics, these are called "**Multi drug resistant culture".** The present study is carried out by optimization, production and characterization of MDR cultures isolated from soil samples. The soil samples were collected from Devine hospital, Gomti Nagar, Lucknow. The isolation was done by serial dilution and further the cultures were characterized through Bergey's manual. The antibiotic sensitivity tests were performed by Agar well diffusion method and the antibiotics were used Oofloxacin, Erythromycin, Cephalaxin, Trimexazole, Almox, Ampilox and Floxip[9]. And the ranges of antibiotics are 1mg, 100µg, 50µg and 10µg/ml. further growth kinetic study was done to know the log phase, in this phase the culture will produce max resistant. Optimization will provide suitable condition for this work suitable Nitrogen source, carbon source media and pH. The Oofloxacin and Trimexazole were the most resistant antibiotic because they do not show most inhibition. The Almox, Erythromycin and Floxip were the most sensitive antibiotic because they showed most zone of inhibition. The parameters which we used for optimizing the growth of microorganism, the Lactose was gave best carbon source for *Streptococcus.*The parameters which we used for optimizing the growth of microorganism, the Na2HPO4 was gave best nitrogen source for both *Streptococcus* as well as *Nesseria.*The suitable pH for both were7.

CONCLUSION

At the end of all the experiment it was identified that bacterial culture isolated from

wastage area, cultures were multi drug resistance, isolates C1 and C2 as gram positive and C3, C4 and C5 as gram negative.C4 culture is identified as *Nisseriasicca*and C2 culture is identified as *Streptococcus equisimilis*.

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