IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 9, Issue 4 Series I (July. – August. 2023), PP 23-41
www.iosrjournals.org

Gram Positive Bacteria Also Resistance In Ampicillin, Vancomycin And Suceptable In Gentamycin, Nor Floxacin.

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Abstract
Anti- microbial Resistance and drug pattern analysis is a very useful methods to treat the anti biotic resistance microorganisms . Antibiotic resistance microorganism have proper cellular modified structure and properties to servive against drugs. Some bacterial modified structure Such as ABC transpoteror antibiotic efflux, antibiotic sequestration by special proteins (timA, BlmA), antibiotic target modification, target bypass and target protection. Some microbs has plasmid through the horizontal gene transfer . Antibiotic resistance bacteria in pond water(bacillus sp. , Salmonella spp.) isolated and it grow in media. Basically they resistance to third generation of cephalosporin.4 samples is detected from the pond water. Bacteria identify by bio chemical analysis and then it check antibiotic sensitivity assay. Antibiotic resistance depends of their concentration and high Consumption Of broad spectrum antibiotic takes the resistance to the specific organisms. To overcoming this problem use to narrow spectrum antibiotic which easily kill this organisms. What is antibiotic dose ? Antibiotic dose represent that the concentration of antibiotic which kill the specific organisms. Upper and lower concentrations of antibiotic concentration may increase value of resistance. Isolated the antibiotic resistance from the environment and detect the resistance drug against them and modified the drug pattern or drug action . Clinically check the drug against antibiotic resistance strains of bacteria. Antibiotic resistance database helps us to know this types of bacteria. Example- E.coli, klebsiellapneumoniae resistance in ciprofloxacin and also carbapenemase. N.gonorrehea resistance from penicillin, tetracycline, sulfonamides etc. Snasocomial infection like staphylococcus aureus skin infection also resistance from methicilin or multi drug resistance.

Keywords:- *Antibiotic, policies, future and resistance.*

Date of Submission: 01-08-2023 Date of Acceptance: 10-08-2023

I. Introduction

Anti- microbial resistance is a property of bacteria which able to grow in media with antibiotic.Bacterial plasmid helps this property gaining.some horizontal gene transfer may convert from simple bacteria to antibiotic resistance bacteria. Antibiotic resistance are three types that –Resistance , Intermediate and suceptable. Resistance means that- in such concentration of antibiotic bacteria grow in media with antibiotic. Intermediate means that- in such concentration of antibiotic slow growth rate of bacteria. Suceptable means that in such concentration of antibiotic fully inhibit the growth of bacteria. It is a number which we found in antibiotic sensitivity test assay in disk diffusion methods. We give the antibiotic disk in media and found a zone of inhibition around the disk , not growth any bacteria and calculate the length of inhibition and check it with reference s. The example is that –650 mg Amoxycillinis resistance for e.coli bacterial growth but 25mg Amoxycillininhibit the e.coli growth . So it means antibiotic concentration varies the resistance or inhibit of growth. In such high concentration of antibiotic not inhibit to bacterial growth because activation of bacterial resistance plasmid gene to survive them. Another example is –Some cancer cells and some gram negetive bacteria have abctranporter to efflux the antibiotic or modified the target side of cell by methylation, acetylationsand b lactamase enzymes.Antibiotic terget bypass by dnagyrase subunit B and target protection by removal DrrC . Anti microbial resistance occurred when bacteria, virus , fungi and other parasites no longer response to medicine making infection harder to treat. Increasing the risk of diseases spread , severe death and death. Some gram negetive bacteria are multi drug resistance , also called super bugs. Some nasocomialinfection or hospital born infections increasing now a days . There is not proper hyzine maintain or sarounding patients infected organs or immuno compromise patients or already treated a surgery or other infection , they are infected this secondary infection and also cause death. Example - staphylococcus aureus or streptococcus payogens skin infection s, E.coli urinary track infection, in COVID situation black fungus infection , multi drug resistance mycobacterium tuberculosis infection. In tuberculosis or HIV infection we use multiple drug therapy not single of antibiotic working. Ciprofloxacin resistance bacteria are – E.coli , klebsiellapneumoniae (also carbapenemase resistance). Staphylococcus aureus is methicilin resistance. Ecoli also resistance third

generation drug cephalosporin .N.gonorrhooeae resistance from penicillin, tetracycline, sulfonamides, fluoroquinolones, cephalosporin. Some virus or parasites, fungi also multi drug resistance.According to WHO reports of 2019, Antibiotic resistance bacteria is responsible for the deaths of 700,000 people while it's estimate that's by 2050 the figure will be risen 20 million . As a result it has become a major problem, posing a serious danger to our lives and our economy. In now days people use monoclonal antibody or other vaccine for bacterial treatment and make aproject work is called "bionic spleen". Orthodox synthetic or non synthetic chemicals used against antimicrobial resistance.

II. Review of literature (Historical prospect)

In twentieth century, the term as a chemical compound generated from microorganisms that inhibit or destroy other microbs . Most of antibiotic synthesized by actinobacteria sp. Nearly 80% . Genus streptomyces produce many of antibiotic like sulfa drugs , quinolons. At frist discovered discovered penicillin from penecilliumnotetum by Alexander Fleming .penecillin inhibit the trans peptidase reaction to from peptydoglycan layer of gram positive bacteria but not show in gram negetive bacteria because it has LPS layer in outer membrane. Penecillinhvethiazoliten chain and B lactam ring and B lactam show antibiotic effect. Now a days some of bacteria has B lactamase enzymes to breakdown the theB lactam ring . So it is penicillin resistance bacteria.Penecillinis two types that- Synthetic or semi – synthetic and biological penicillin. In 1943 the biggest streptomycin produce By streptomycesgriseus. Streptomycin inhibit protein synthesis by binding to the 30s subunit of the prokaryotic ribosome and effective gram negetive bacteria and tubercle bacillus.United States and other countries discovered many other antibiotic like that- amainoglycosides, macrolides, tetracycline, dopamycine,glycopeptides. It 's all are natural and direct produce by microorganisms. In the evolution the resistance mechanisms are devolopedin the bacteria. In streptomycin is self resistance . Antibiotic have been used to cure bacterial infections for more than 70 years . This time infection not caused by multidrug resistance bacteria. But in 20th century infection caused by many types of multidrug resistance bacteria. In pre – antibiotic period the out break "Plauge" is caused by Yersinia Pestis which is transmitted by animal fleas. It is also called black death and 100 of millions people were killed in thisout break. In 1676 discovered "animacules' by Antony Van leeuweenhoek and discovered antibiotic against them. In 1871 Josef Lister discovered penicilliumglaucum has inhibitory effect of bacterial growth. In early era , discover mycophenolic acid from P. Glaucum which has inhibitory growth of BaccillusAnthracis. In 1909 Paul enrich discovered "salverson' which is inhibit syphilis.Neosalverson is less harmarful to inhibit the syphilis than salverson. After that discovered sulfonamides by garhardDomagk in 1930.After neuclic acid synthesis by salvage pathway and sulfonamides also suppressed by penicillin. In 1928 Alexander Fleming discovered penicillin by penecilliumnotetum. staphylococcus aureus inhibit by penicillin G which is semi – synthetic penicillin. After Many of B lactam family antibiotic discovered by the X ray crystallography of penicillin. In golden era, 1939 discovered Tyrothricin from soil bacteria Baccillusbrevis which inhibit gram positive bacteria. In 1940 , discovered streptomycin from soil bacteria streptomycin sp.which is also antifungal agent.1952,1948 discovered nitrofuran and tetracycline. In present days , 5 to 20 pharmaceutical company discovered many other antibiotic or advance effective.

Aim of the project

- To reduce the incidence of infection.
- To optimise the use of antimicrobial medicines.
- To ensure sustainable investment in countering antimicrobial resistance.
- Fight infections caused by antibiotic resistance bacteria.
- Discovered new generation of drugs against antibiotic resistance bacteria.

Materials

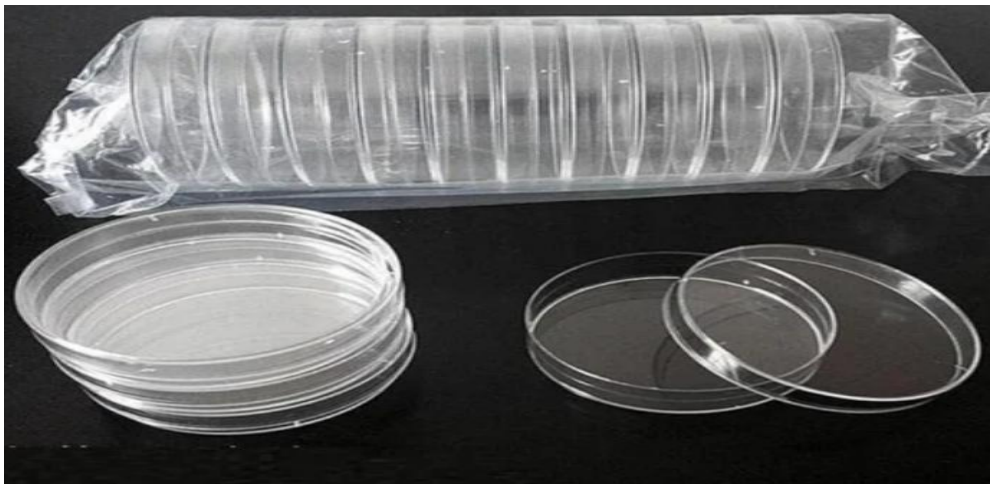
1.	Netrient Agar media
2.	Netrient broth
3.	Petriplate
4.	Spreder, innoculation loop
5.	Testube, spirit lamp, slide
6.	Autoclave, hot air oven
7.	Conical flask, cotton
8	Paper, laminar air flow
9.	Refrigerator, Incubator
10.	Chemicals and other neutrients

11.	Spectophotometer, antibiotic disk
12.	Water., Antibiotic.
13.	Measuring cylinder, Beakers

- Nutrient agar media:- It is essential for bacterial growth. Media composition is that - Nacl -0.5%, Peptone0.5%,beefextract 0.3%, and agar 2%.Nacl maintain osmotic pressure, Peptone - partially diggested N2 source, beef extract – carbon source, agar – solidifying agent.

- Nutrient broth:- It is also important for bacterial growth. It's composition is same as netrient agar media but agar is not present. It mainly use to fresh the bacterial culture. It is used to culture any microorganisms.

- Petriplate:- It is made only glass or thick plastic. It is use for spreading, streaking and poring for bacterial growth and it is round in shape with lidand transparent. We poring it netrient agar media.



Spreder and Innoculation loop:-spreder use to spread the sample in Petri plate for bacterial growth. It is made in glass or Metals.

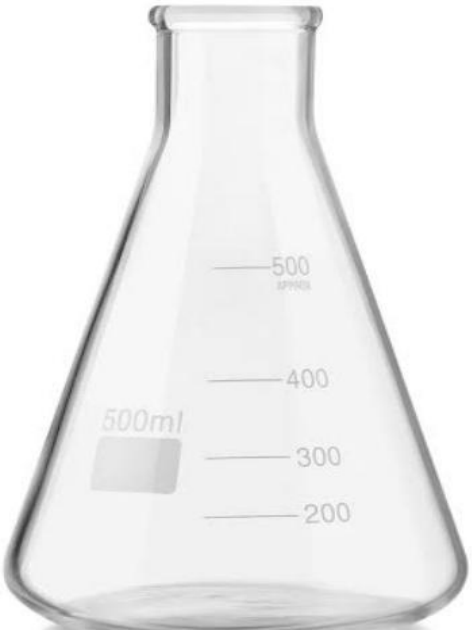
Innoculation loop used to inoculate the bacterial culture in broth or plate. It is made up in nicromewire or other metals. It is incinerate or sterile by the candle and alcohol before using.

Slide, test-tube and spirit lamp:-. Slide made up in glass . It used in gram staining or other bio chemical test. Test tube made up in glass . It is used in prepare cell culture, biochemical test. Sprit lamp used to sterile the loop and no contamination in culture.

Autoclave and hot air oven:-Autoclave made in metals and it have a vessels for pressurerealese or rising pressure , and 10 nobs for tightly close the autoclave and pressure rising and a temparature meter for measure temparature and pressure meter for measure pressure. It works like pressure cooker 121C°, 15lb/ich^2 and 30 min for sterile media, instruments and sterile the culture plate which isalready culture. But not volatile compounds sterile because destroy the important components of it's high temperature. Basically it sterile by steam because it's penetrations power high and energy high. It also called moist heat sterilization.

Hot air oven is called dry heat sterilization. It made up by metals and it have two or three raker for keep the instruments dry. It's working temperature 180°c , 10mins and only sterile instruments not media because it's nutrient components are destroying.

Conical flask :- It made up only thick glass for heat resistance and not breakdown and it is used for media preparetionand measuring water and other substances .



Cotton :- It is used for rapping of conical flask or test tube because not any other organisms enter to this . It also used for washing slides or plates.

Laminar Air flow:- It is also made up metals and it have air filter, UV light regulators and a light . It's air pore size 0.3 um only air is passing not other microorganisms except virus .UV light sterilized the whole laminar and microorganism kill before using it. UV light is physical control of microorganisms it damage the dna level of bacteria (purin – pyrimidin dimer) and also cause point mutations of bacteria. Air filter is HEPA filter used and used light for working like poring media, making pure culture and any other bacterial working. It is also maintain aseptic condition.

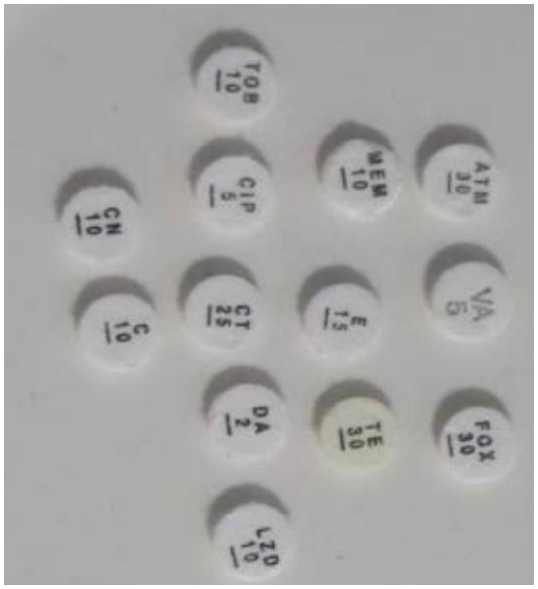
Refrigerator:- Refrigerator used to stored the bacterial culture or mediain 4° c , it also called chilling temperature.It is the dormant phase of bacteria not growth but still alive, growth rate or enzymeticactivity rate slow. In refrigerator, we stored culture or media in few week but not year. We preserve the culture for year in liquid N2 in-196^°c. It is called cryo preservation.

Incubator :- It is made up in metals and it have 3 raker and a shaker . In raker we keep cultur for bacterial growth aseptically. Culture broth keep in shaker for shaking and O2 availability for growth. It's optimum temperature 37°c because this temperature is favourable for enzymetic activity of bacteria and highly growth rate.

Spectophotometer:- It is physical method for measuring cell density by the light absorption. Cells are absorbed the light and rest of lights are transmitted. We get on the screen a value is called O.D value and we measure how many cells are presence this.

Antibiotic disk or antibiotic:- Antibiotic is very useful substances which kill the bacteria . It is very expensive and use only microbs infections. It is found directly in medical store and make from some useful microorganisms. Antibiotic disk mainly use in laboratory scale in experiment to see how much resistance of bacteria . It is found in redimate.

- Measuring cylinder and Beakers :- In measuring cylinder we measure water and other chemicals. It is made in glass or plastic with scaling.

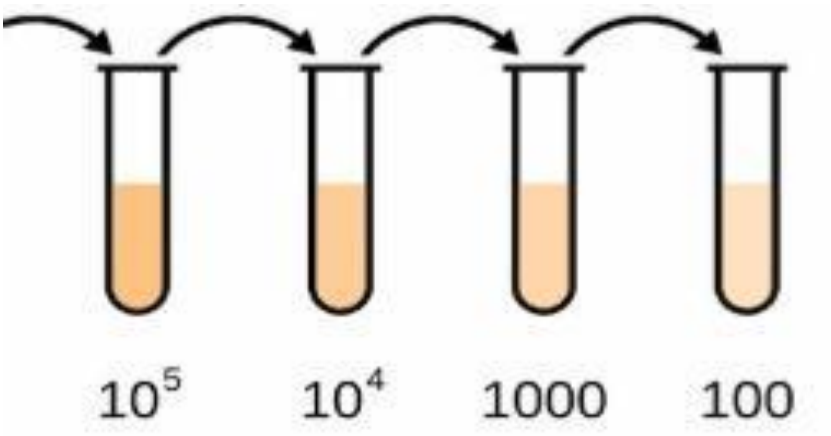


Beakers made up only glass with scaling . It is use in measuring water , keep the testtubesin it for autoclaving.

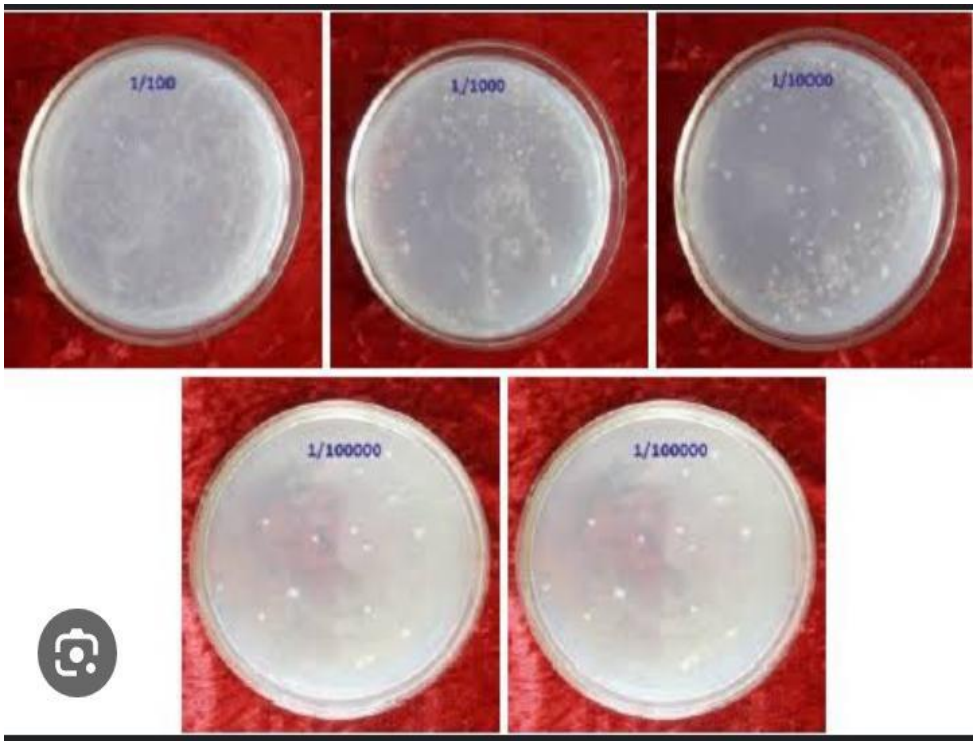
Methods

1.	Serial dilution
2.	Spreading
3.	Patch culture
4.	5 th quadrant streaking
5.	Simple streaking
6.	Gram staining
7.	Catalase, oxidase
8.	Amaylase
9.	Gelatin Hydrolysing test
10.	SIM Indole test
11.	Making MC farland solution
12.	MIC /MBC

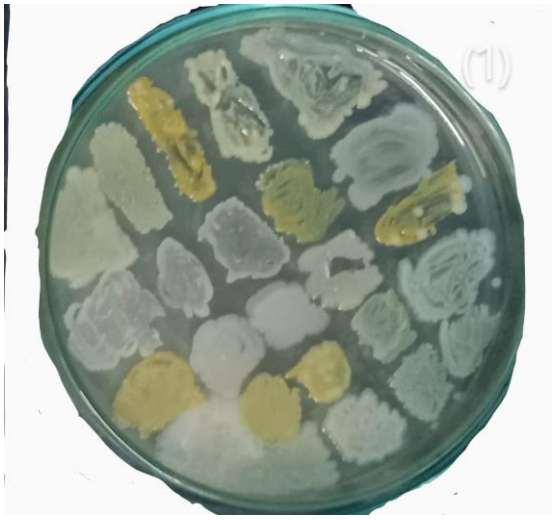
- Serial dilution:- It is a physical method to dilutes the samples. 5 testtubes are given and frist test tube has 9 ml distile water and 1ml sample. So 2^{nd} , 3^{rd}, 4^{th} and 5^{th}testubeare diluted from 1^{st}testtube and from 5^{th} test tube 1ml of sample discarded. So all test tubes volumes is same. Then fom each tubes 0.1 ul sample is spreding in each plates .



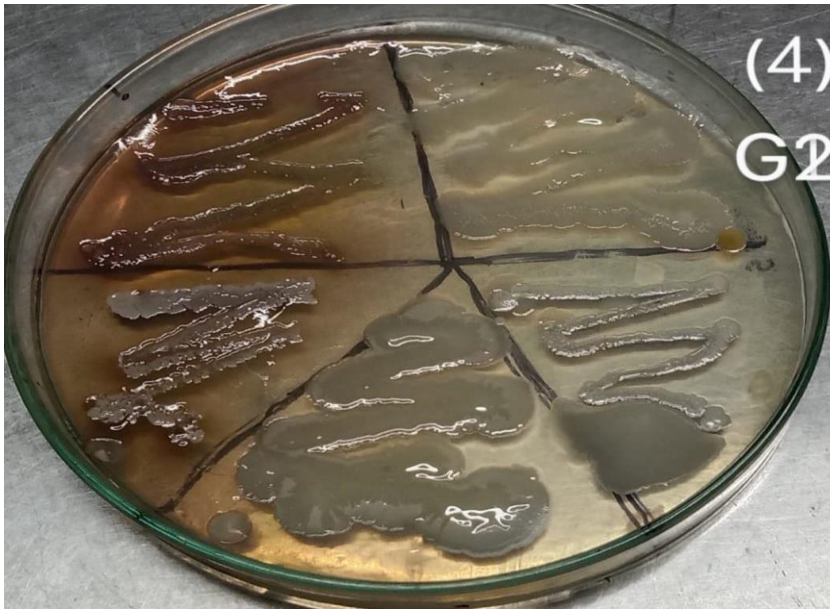
- Spreding:- After the serial dilution from each tubes 0.1ul sample is spreding in each plate and incubation. After incubation colonies we found uniform coloniesand see colony morphology and calculate cfu count. Cfu= number of colony/total volume of sample ×dilution factors.



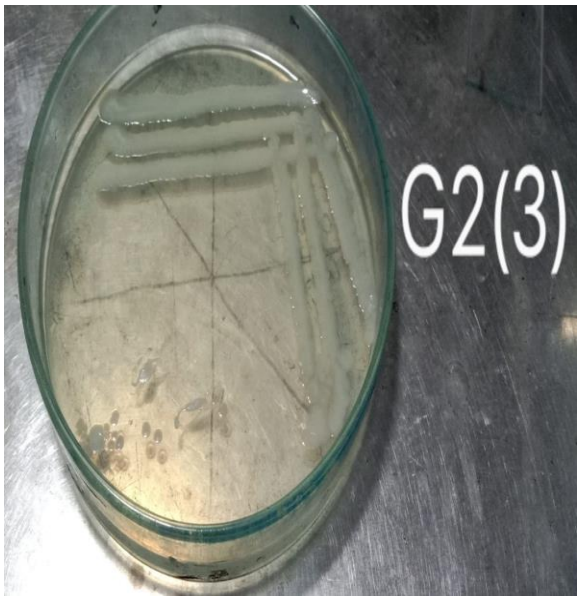
- Patch culture:-patch culture is a technique where maximum number of colony innoculatefrom the spread plate . In this we see clear morphology Of bacteria and it helps to further making pure culture.I use 2plates or isolated 50 colonies.



- 5th quadrant streaking:- It is another method for making pure culture . After patch culture the different colony morphology Streaking the platesand from similar colony morphology one colony picked and streaking. I used1 plates for 5th quadrant streaking for pure culture.

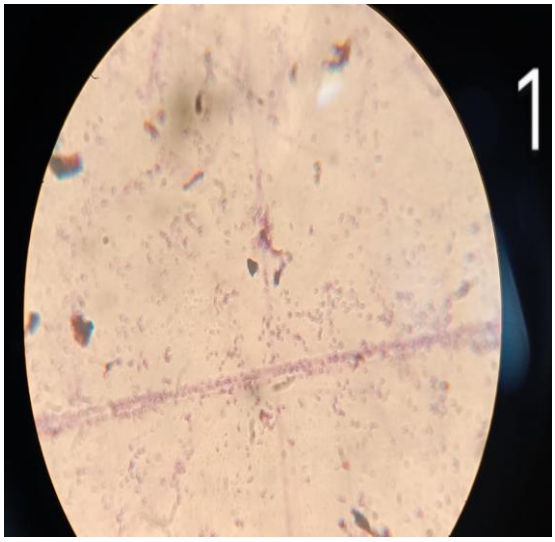


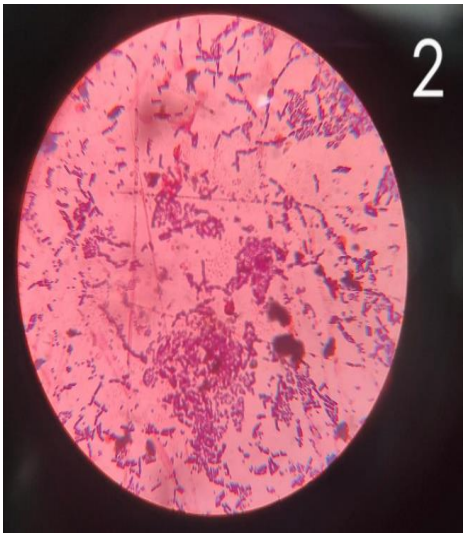
- Simple streaking:-. After 5th quadrant streaking this we isolated specific organisms in each plate is called pure culture and get isolated colonies. 4 organisms isolated each plate 1 is same morphology .so I get 4microorganisms in pure culture.

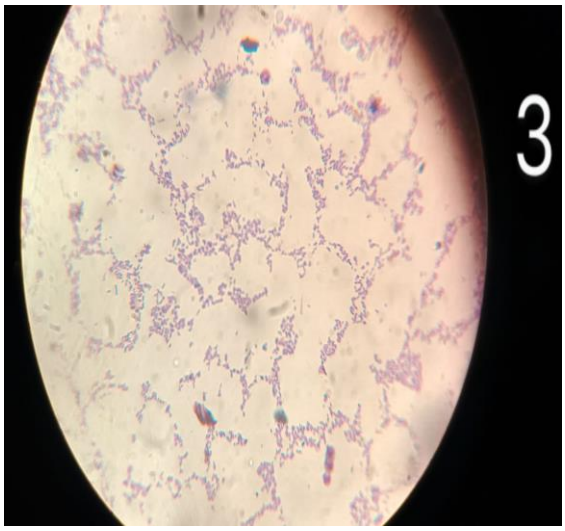


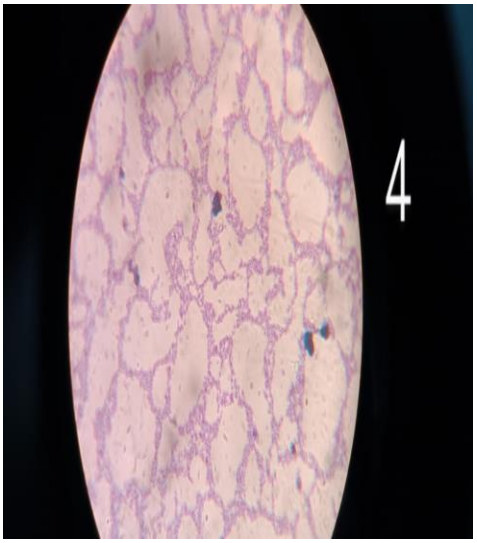
- Gram staining:- After isolated pure culture , identify the bacteria in gram staining. My staining results is that- all are gram positive short rod bacteria. In gram staining we add crystal violet at 1st,

After 1min it wash and add iodine. After 1min it also wash and wash by alcohol in 1min . At last it dry and add saffranin as a counter stain. In gram (+)bacteria has thick peptydoglycan layer and pore size small. So crystal violet and iodine make complex in the pore and it don't wash out by alcohol and colour stabilize and cells see in purple colour. But in gram (-) bacteria has thin peptydoglycan layer and it pore size large. So crystal violet and iodine make complex in the pore and wash by alcohol and colour not stabilize. So cells see pink in colour.

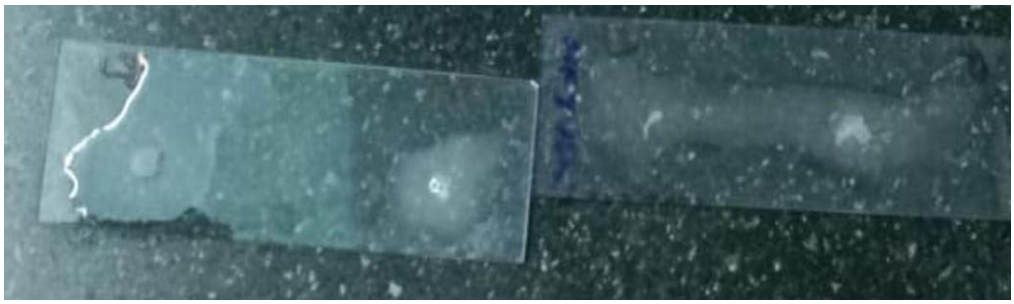




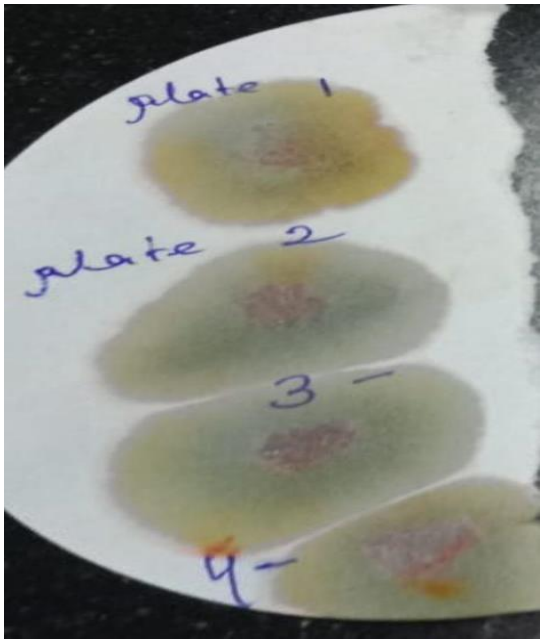




Catalase:- It is another method for bacterial identification. In catalase test 3% h2O2 solution with one loop full culture and see O2 bubble .If bacteria has catalase enzymes then it produce O2 in bubble form with reduce H2O2. I use 2 slides for 4 sample. My result is catalase positive.



- Oxidase:- It is another method for bacterial identification. In filter paper one drop of oxidase reagent and loop full of culture and see blue in colour is positive and purple or pink negetive in test. I use one filter paper and my result is negetive because there is no e- transport in cytochrome.



- Gelatin Hydrolysing test:-. It is another way of identify the bacterial culture. Bacteria produce gelatinase enzymes for breakdown gelatin. The media composition is that –gelatin–6 gm/50ml, agar 1 gm , peptone-0.25, beef extract -0.15 gm . After Innoculation ,it keep incubation and next day it keep 4°c in 1hrs. If it semi solid then it positive and solid it negetive results. My result is positive.
- Amaylase hydrolysing test:- It is another method for bacterial identification. Bacteria produce amaylase for starch degredation. Amaylase hydrolysing test media composition –Starch 1gm/ 100ml, peptone-0.5 mg,
- yeast extract- 0.3gm Agar- 1.5gm. After Innoculationand it keep incubation and next day it flooded with iodinsolution. If it see clear zone it is positive and not zone it is negetive. My result is negetive.

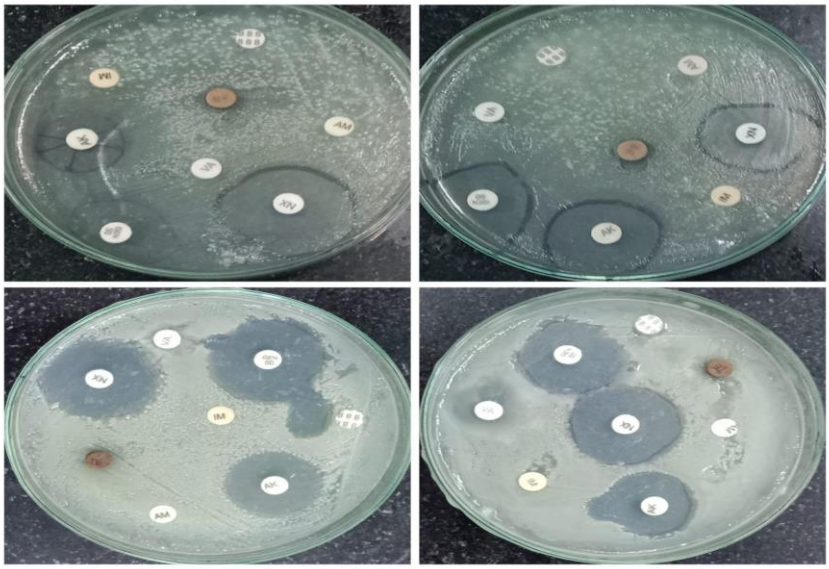
- SIM Indole Test:-. It is used to check bacterial cellsmortile or non mortile . It's media composition are that –Trypton- 1 gm , beefextract – 0.3 gm , sodium thiosulfate – 0.015gm, ferric ammonium sulphate – 0.01gm and agar –0.175 gm. Bacterial culture deep straightly in the media and see observation . If bacteria grow into deep point then it non mortile and it grow in sarroundings then it mortile . It also produce h2s gas in black colour . My result is non mortile and grow in deep point.





MC farland solution:- MC farland is a method where approximation the bacterial cell culture concentration 1.5×10^8 and it's OD value 0.5 . It's solution making by 1%bacl2 and 1%h2SO4 . Bacl2 and H2SO4 reacts each other and make BaSO4 and HCl. It's turbidity compare with the bacterial culture in broth and approximation 1.5×10^8 cells concentration .

MIC:- After MC farlandsolution making , we compare the bacterial culture broth with this solution and then from this broth 0.1 ml culture spreading in the Nutrient agar plate with antibiotic disk and incubation. Next day we found zone of inhibition and measure the length. And this concentration of antibiotic is called minimal inhibitory concentration. The length described the bacterial resistance, susceptibility and inhibitory of antibiotic.



III. Discussion

From the methods, at 1st we isolated bacteria , then make in it pure culture and then check the antibiotic resistance capasity. I isolated the bateria in my areal pond which is only use for fishing and bathing and other working. The pond latitude is 22.434, 88.394 in my area .

At fristI serial diluted the sample and then spredingit . After incubation I found uniform colonies and then maximum number of colonies are collected and innoculate the 2plated is called patch culture. Then study morphology and count cfu. After that same morphology of cells are re innoculate in 5th quadrant streakingand

then isolated pure culture by quadrant streaking. Then we identify the bacteria with gram staining and other biochemical test like – catalase , oxidase, gelatin hydrolysing test, and amaylase and check motility to use SIM Indole test. My result is gram positive short rod bacteria and catalase positive, oxidase negetive, amaylasenegetive and gelatinpositive and cells are non mortile. So it is Baccillus spp. After I make MC farland solution for standardization the turbidity and compare broth culture turbidity. Then it spread in the plate with antibiotic disk and Measure the zone of inhibition length and identify the resistance, suceptable orintermidiate range of bacteria.

Colony morphology:-

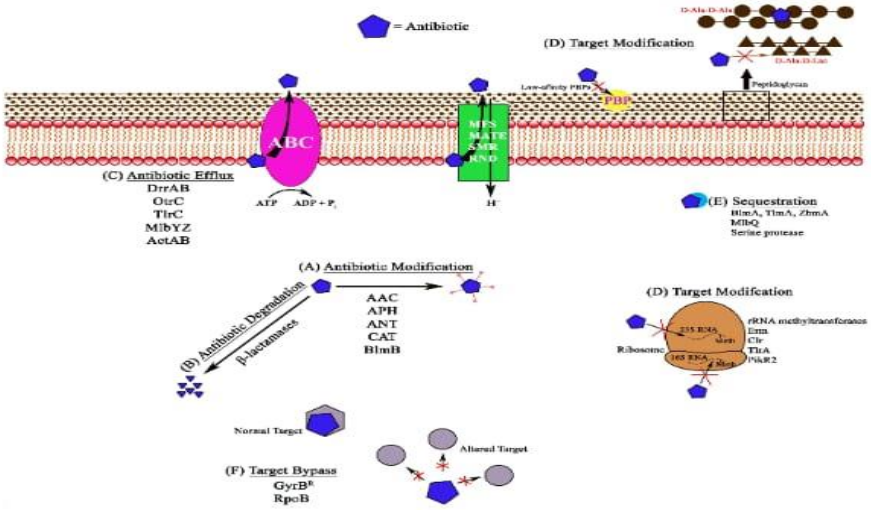
Bacterial No:-	Shape	Colour	Elevations	Margin
1.	Circular	Yellowish	Flat	Entire
2.	Circular	Deep white , translucent	Raised	Entire
3.	Circular	Light yellowish	Flat	Entire
4.	Circular	Light greenish	flat	Entire
5.	Circular	Pinkish	Raised	Lobate

Biochemical test result:-

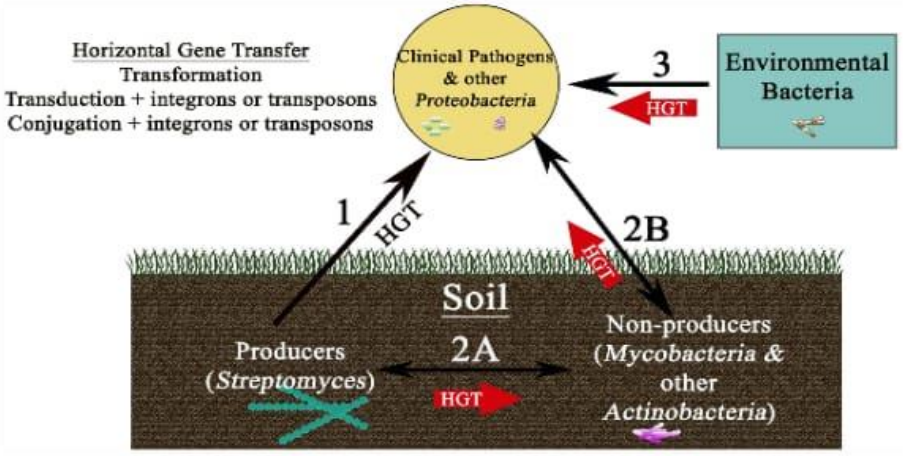
Plate No	Gram staining	Colony morphology	Catalase	Oxidase	Gelatin hydrolysing test	Amaylase hydrolysing test
1.	Gram (+)	Short Rod	+	-	+	-
2.	Gram(+)	Medium rod	+	-	+	-
3.	Gram (+)	Short rod	+	-	+	-
4.	Gram(+)	Short rod	+	-	+	-

How bacteria Resistance itself against Antibiotic??

1. Antibiotic Modification or degredation
   It is a commonly used strategy for rendering an antibiotic in effective, especially in the case of amainoglycosides antibiotic. Some amaino glycosides antibiotic a lage number of modification such as acetylations, methylation or Phosphorylation. Example – streptomycin , penicillin B lactam .
2. Antibiotic efflux.
   It is another method for self resistance . Example – it frist found in streptomycespeucetius , which produce two closely related to anti cancer antibiotic which is preventing the further round of replication. This antibiotic efflux in in ABC family transforter which encoded by the DrrAB genes . It is two subunit that DrrA And DrrB is the integral protein. It is also called multidrug resistance.
3. Antibiotic sequestration.
   It involves the function of drug binding proteins, which prevents the antibiotic from reaching its target . It producers of the bleomycin family of antibiotic . It is the metal free or metal bound antibiotic proteins like TlmA , BlmA. One or more genes related to ABC transpoter s family.
4. Target Modification/ Bypass of antibiotic.
   It is another method as a self resistance mechanisms against several antibiotic such as B lactam, glycopeptides and streptogramins. Target site modified by methylation, acetylations or Phosphorylation. Example –23s r RNA methayltransferaseoccure mono methylation in MLS type 1. Antibiotic also bypass the original target sites by producing low affinity targets.



5. Horizontal gene transfer
It is also another method for bacterial resistance. The gene transfer by environment , conjugation, transformation and transduction. In horizontal gene transfer involves in the plasmid , integrons . Directly transfer by the soil. Examples – streptomyces .



6. inactivation:- In penicillin , B lactam ring is degredated by the penicillin resistance bacteria and open the ring for prevent antibiotic binding target side. Example -staphylococcus aureus. It also breakdown B lactam family of antibiotic including carbapenems.

7. Over production of target sides:-Some bacteria can also over produce target sides of the antibiotic. Example – E.coli, Haemophilis influenza. The over expression is sometime found in combination with mutations to bind antibiotic in target.

Result (zone of Inhibition) mm

Bacterial ID	AM (10mcg)	VM (30mcg)	Bacitracin	Norfloxacin (10mcg)	Imp (10mcg,)	AK(10mcg)	GN(50mcg)
N.P.1	0	0	0	29.5	0	23	34.5
N.P.2	0	0	0	23	0	22.5	22.5
N.P.3	0	0	0	28	0	26	25
N.P.4	0	0	0	29	0	19	20

Determination of range of Bacteria:-

Antibiotic Name	N.P.1	N.P.2	N P.3	N.P.4
Amikacin	S	S	S	S
Ampicillin	R	R	R	R
Vancomycin	R	R	R	R
Norfloxacin	S	S	S	S
Gentamycin	S	S	S	S
Bacitracin	R	R	R	R
Imipenem	R	R	R	R

Keywords:- R- resistance, S- suceptable, , I- intermidiate.

IV. Conclusion

The antibiotic amikacinhas the zone of inhibition 23 ,22.5, 26, 19 in 10 mcg concentration. So it is suceptable for all 4 bacteria. Ampicillin has the zone of inhibition 0 in 10 mcg . So it is resistance for all 4 bacteria. Vancomycinhas the zone of inhibition 0 in 30 mcg concentration. So it is resistance for all4 bacteria. Norfloxacin has the zone of inhibition 29.5, 23,28,29 in 10 mcg. So it is suceptable for all 4 bacteria. Gentamycin has the zone of inhibition 34.5, 22.5, 25,20 in 50 mcg. So it is suceptable for all 4 bacteria. Bacitracin has the zone of inhibition 0 in 10 mcg. So it is resistance for all 4 Bacteria. Imipenem has the zone of inhibition 0 in 10 mcg . So it is resistant for all 4 bacteria. So my all 4 bacteria (Baccillusspp) is resistance in ampicillin, Bacitracin,vancomycin and imipenem and suceptablein norfloxacinand gentamycin and amikacingram (+) rod shape bacteria. So causing any diseases of these bacteria we inhibit of norfloxacin, gentamycin and amikacin antibiotics.

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