INNOVARE JOURNAL OF LIFE SCIENCE

Vol 1, Issue 2 , 2013

ISSN:2321-550X

Review Article

DIFFERENT MODELS TO EVALUATE ANTIMICROBIAL AGENTS-A REVIEW

HEMRAJ VASHIST*, DIKSHA SHARMA, AVNEET GUPTA

Department of Pharmacy, L.R.Institute of Pharmacy, Solan (H.P), India. E-mail: Shimla_pharmacy@rediff.com

Received: 8 July 2013, Revised and Accepted: 26 July 2013

ABSTRACT

Objective-To study different antimicrobial models for modern drug development for microbial infections.
Method- Different type of antimicrobial methods has been searched from different books and internet. The review is an attempt to combine all available literature about such models.
Result- During the literature survey it was found that such antimicrobial models complement human investigations to study in detail pathogenic hypothesis and therapeutic strategies. To achieve this several new screening models have been produced. Various methods have been used for this purpose by using several microbes on different culture media.
conclusion-This review work would further help to get the literature of discussed antimicrobial models at one place. These methods would help to provide new antimicrobial agents for the treatment of various microbial diseases of human

Keywords: Antimicrobial, model, Screening.

INTRODUCTION

Although the causes of chronic illnesses are for the most part unknown, the complex signs and symptoms that evolve in many patients with fibromyalgia (FM), Gulf War Syndrome (GWS) and rheumatoid arthritis (RA) may be due in part to systemic chronic infections from bacteria, viruses and fungi. Such infections can follow acute or chronic chemical or other insults (viral, environmental, trauma, etc.) that have the potential to suppress the immune system. These illnesses probably evolve over time as a multi-step process that may require multiple toxic exposures, including infections that can be causative for the illness in some patients, cofactors for the illness (not causative but important) in others or opportunistic in immune-compromised patients.

Most microorganisms like mycoplasmas are not considered important human pathogens when they are found at superficial sites, such as the mouth or intestines, but some species, such as *M. fermentans, M. penetrans, M. pneumoniae, M. genitalium, M. pirum*and *M. hominis*, have the capacity to penetrate the blood circulation and colonize various tissues.

These microorganisms probably do not actually cause FM, GWS or RA on their own, but they appear to be important in causing chronic illness progression or exacerbating the major signs and Symptom in patient with those symptom. There is evidence for microorganism infections in FM, GWS and RA patients. My lab and others are finding mycoplasmal blood infections in about about 70% of FM and about 50% of GWS and RA patients examined. In our studies on GWS, a CFIDS-like illness, we found mycoplasmal infections in the blood of about 50% of more than 200 patients, and these patients were found to have principally one infectious species of mycoplasma, *M. fermentans*. We have found a variety of pathogenic Mycoplasma species, such as *M. fermentans, M. penetrans, M. pneumoniae, M. genitalium* and *M. hominis* in the white blood cells of about 60% of more than 200 civilians with CFIDS and FM. These infections are found in less than 10% of healthy controls. Interestingly, the majority of CFIDS and FM patients had multiple mycoplasmal infections (more than one species), but multiple infections were not found in any of the healthy control subjects. The tests that we use to identify mycoplasmal infections, forensic polymerase chain reaction and nucleoprotein gene tracking, are very sensitive and highly specific. These tests are a dramatic improvement over the relatively insensitive serum antibody and other tests that are currently being used to assay for systemic infections[1].

Infectious diseases, also known as transmissible diseases or communicable diseases comprise clinically evident illness (i.e., characteristic medical signs and/or symptoms of disease) resulting from the infection, presence and growth of pathogenic biological agents in an individual host organism. In certain cases, infectious diseases may be asymptomatic for much or even their entire course in a given host. In the latter case, the disease may only be defined as a disease (which by definition means an illness) in hosts who secondarily become ill after contact with an asymptomatic carrier. An infection is not synonymous with an infectious disease, as some infections do not cause illness in a host[2].

The term infectivity describes the ability of an organism to enter, survive and multiply in the host, while the infectiousness of a disease indicates the comparative ease with which the disease is transmitted to other hosts[3]. Transmission of pathogen can occur in various ways including physical contact, contaminated food, body fluids, objects, airborne inhalation, or through vector organisms[2].

Among the almost infinite varieties of microorganisms, relatively few cause disease in otherwise healthy individuals[4]. Infectious disease results from the interplay between those few pathogens and the defenses of the hosts they infect. The appearance and severity of disease resulting from any pathogen depends upon the ability of that pathogen to damage the host as well as the ability of the host to resist the pathogen. Clinicians therefore classify infectious microorganisms or microbes according to the status of host defenses - either as primary pathogens or as opportunistic pathogens:

Primary pathogens cause disease as a result of their presence or activity within the normal, healthy host, and their intrinsic virulence (the severity of the disease they cause) is, in part, a necessary consequence of their need to reproduce and spread. Many of the most common primary pathogens of humans only infect humans; however many serious diseases are caused by organisms acquired from the environment or which infect non-human hosts.

Organisms which cause an infectious disease in a host with depressed resistance are classified as opportunistic pathogens. Opportunistic disease may be caused by microbes that are ordinarily in contact with the host, such as pathogenic bacteria or fungi in the gastrointestinal or the upper respiratory tract, and they may also result from (otherwise innocuous) microbes acquired from other hosts (as in *Clostridium difficile* colitis) or from the environment as a

result of traumatic introduction (as in surgical wound infections or compound fractures). An opportunistic disease requires impairment of host defenses, which may occur as a result of genetic defects (such as Chronic granulomatous disease), exposure to antimicrobial drugs or immunosuppressive chemicals exposure to ionizing radiation, or as a result of an infectious disease with immunosuppressive activity (such as with measles, malaria or HIV disease). Primary pathogens may also cause more severe disease in a host with depressed resistance than would normally occur in an immunosufficient host[2].

Diagnosis

Diagnosis of infectious disease sometimes involves identifying an infectious agent either directly or indirectly. In practice most minor infectious diseases such as warts, cutaneous abscesses, respiratory system infections and diarrheal diseases are diagnosed by their clinical presentation. Conclusions about the cause of the disease are based upon the likelihood that a patient came in contact with a particular agent, the presence of a microbe in a community, and other epidemiological considerations. Given sufficient effort, all known infectious agents can be specifically identified. The benefits of identification, however, are often greatly outweighed by the cost, as often there is no specific treatment, the cause is obvious, or the outcome of an infection is benign.

Diagnosis of infectious disease is nearly always initiated by medical history and physical examination. More detailed identification techniques involve the culture of infectious agents isolated from a patient. Culture allows identification of infectious organisms by examining their microscopic features, by detecting the presence of substances produced by pathogens, and by directly identifying an organism by its genotype. Other techniques (such as X-rays, CAT scans, PET scans or NMR) are used to produce images of internal abnormalities resulting from the growth of an infectious agent. The images are useful in detection of, for example, a bone abscess or a spongiform encephalopathy produced by a prion. However different biochemical tests have been performed to check and differentiate the bacteria [5] but still various new anti- infectious agents are being tested by different screening methods. The researchers may or may not be getting the procedures for the screening of new antimicrobial agents in one article. Therefore this article is an attempt to provide all possible literature about such procedures.

Evaluation of Antimicrobial agents

Antimicrobial assay is the method to evaluate the antibiotics as well as to compare the antimicrobial potential of a claimed antimicrobial agent against any standard antimicrobial agent.

Different antimicrobial procedures are available the list of such are as below.

Cylinder plate method

The cylinder plate method depends on diffusion of the antibiotic from a cylinder through an inoculated agar gel in a petridish forming a zone of inhibition.

Growth of inoculated microorganism is inhibited entirely in a circular area zone, around cylinder or a cavity containing a solution of antibiotic.

Procedure in general

- Prepare microbial inoculum with the required quantity of test organism.
- Add prepared microbial suspension in the media and mix it and transfer into petridish
- Prepare solution of known concentration of the standard preparation with respect to the assumed concentration of the antibiotics/antimicrobial agent to be examined.
- Apply the solutions to the surface of the solid media in sterile cylinder or in cavity prepared in agar plate.
- Leave the dishes or plates for one to four hours at room temperature.
- Incubate the dishes at 20-30oc for 28 hours.
- Observe the zone around the hole by antibiotic zone reader or visually.

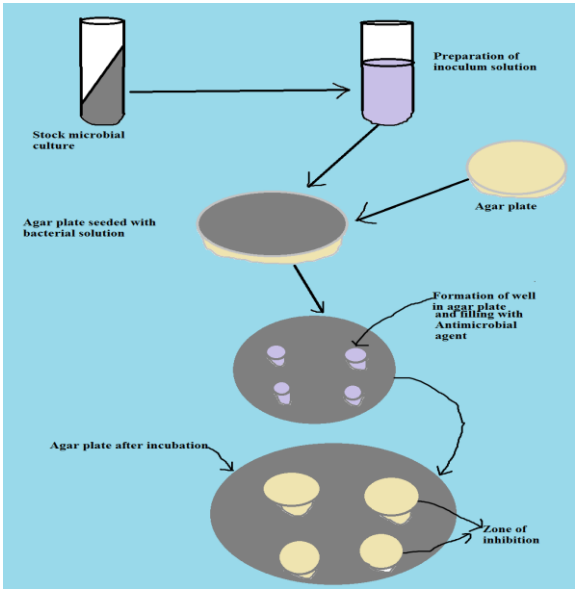

Fig.1: Shows evaluation of antimicrobial by well diffusion method.

Specific procedures

Preparation of Nutrient agar plate (NA) -There are several ways to make NA plates, all depending on what you have to start with. The methods for the same are as below-

Method 1[3-4]

- Measure and record the amount of water that half-fills a custard cup.
- Determine the number of plates (custard cups) you will need to use, and from that the amount of medium you need to make to half-fill all the cups.
- Empty a can of fat free beef or chicken broth into a large measuring cup and dilute, if necessary, with water as per the instructions on the side of the can. Mix. Discard the excess beyond what you need for your cups. Pour this into a sauce pan.
- Add 1 level teaspoon of agar per 2/3 cup of broth. Stir the required amount of agar into the cold broth in the sauce pan.
- Turn on the heat and with constant and continuous stirring and/or swirling bring just to a boil. Keep swirling, and then adding small amounts of heat, and do this until all the granules of agar are dissolved. Agar solutions burn very easily - just like making gravy (in which you have added another polysaccharide as a thickening agent). An alternative way to do it is to place the broth/agar mix into a glass bowl and place it in a microwave oven. But do this cautiously as the mix will suddenly foam up. So be ready to shut off the energy at any moment. Give the dissolving solution a swirl, and microwave a bit longer. Swirl and energize repeatedly until all the granules are dissolved.
- Distribute the hot medium among the cups which are need.
- Plate pieces of double-thick aluminum foil over the cups so that at least a half inch (1 cm) over hangs.
- To the empty, clean pressure cooker, add enough tap water so that at least a half inch of water is in the bottom. Place the device on cooking stove.
- Arrange your cups in the bottom and add anything else you need to sterilize between and on top of the foil covered cups.
- Put the lid on the pressure cooker making sure that all the lugs are in place and the top twisted to the right (clockwise).
- Turn on the heat.
- For awhile the water will boil inside the pot, but steam will not spew from the vent. Only hot air will come out.

- Finally, when steam spews from the vent, place the special weight atop the vent. Some weights have several holes in them labelled with numbers indicating the amount of pressure they will produce in the pot. It should be made sure that the use of the hole that says '15' (which means 15 psi, which is one atmosphere above ambient air pressure).
- Soon the steam pressure inside the pot will reach 15 psi, and at that time will be able to lift the special weight, which will now jiggle and hiss as it allows excess pressure to be vented. As soon as the hissing begins, begin the timer.
- While the hissing and jiggling continues, turn down the heat a little - just so long as hissing and jiggling continues.
- After 20 minutes, turn off the heat.
- Using a fork, for example, slightly lift the weight. If more steam hisses out, put the weight back down, and wait a little longer for the pot to cool.
- Finally, lifting the weight does not result in hissing. Lift off the weight and put it aside in a safe place.
- Twist and remove the top from the pot. When the top is raised, lift it so that the rear edge lifts first so that the steam inside rises away.
- Reach in with mitts and gloves to remove the various items in there. Do not remove foil covers or other wrappings. Remember that the cups are half-filled with nearly boiling liquid, so lift them carefully and set on the counter top where they will cool and solidify.
- For safety, dump a pan of cold water into the pot before moving it. That will cool the hot contents so that splashes and misdirected spills will not burn anyone. Dump the pot into a sink and rinse well with warm water and set aside inverted to drain and dry.
- In an hour or so, the nutrient agar in the cups will have cooled enough to solidify.
- Immediately before use they must be dried.

Method 2

- Use a standard organism.
- Grow the bacteria for 80 to 24 hours in broth and standardize the suspension to one billion organisms per milliliter.
- Extract agar pH 6.8 may be employed. Some bacteria require the use of beef infusion agar pH
- Both type of medium are satisfactory, but the medium as well as pH must be designated.
- The final agar concentration should be 1.5 % percent. A 3% agar diluted to 1.5 percent agar with saline is satisfactory. Such an agar can also be reduced in concentration with protein (serum, blood) or protein saline mixtures. In this manner protein agar mixture containing upto 50 percent protein solution may be conveniently made for special studies.
- Seed the agar medium on the basis of 0.1 ml of the standard suspension of organism for each 30ml. volume of medium. The temperature of the agar should be about 48oc that is the temperature ordinarily used in making blood agar medium.
- Pour approximately 30 ml of seeded agar medium into a standard 100x 15 petri dish bottom. Cover the petri dish with a porcelain lid glazed on the outside.
- After the agar solidified, remove a disc of agar measuring 1.5 cm in diameter. In some instances it is feasible and desirable to prepare three or more cups from a single plate.
- Prepare appropriate dilution of the antiseptic using a graduated 1ml pipette place 0.2 ml of a given dilution in each cup.
- The antiseptic should be added and the plates should be ready for incubation within one hour from the time the bacteria are added to the agar medium.
- Incubate test plates upright at 37oc for 24 hrs. and then measure the width of the zone of inhibition of bacterial growth with hand lens in measuring the zone in an aid in an accurate results.

A simple device used to remove the disc of agar from the medium. This instrument consist of a thin walled stainless steel cylindrical chamber measuring 2.5 cm in length and having diameter o 1.5 cm; the cutting edge is beveled on the inside. A capillary metal tube about 10 cm long is attached to the bottom of the cylindrical chamber. The chamber is sterilized by dipping in alcohol and flaming. Placing the open end of the chamber on the surface of a poured agar plate, the disc is cut easily with slight pressure. A finger is then placed over the tip.

Disk diffusion method [6]

Plate sizes-100mm

Disc size- 6mm.

Storage and use of Discs-Prepared Discs should be stored at at -20oc and the working ones at <8oc. Discs should be warmed slowly to room temperature then the discs should be taken out from the refrigerator 1-2 hours before applying on the culture medium.

Kirby-baur diffusion method[7,8]

Purpose

The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds in order to assist a physician in selecting treatment options for his or her patients. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism.

Formula for Mueller-Hinton agar per liter of purified water.

Beef, Infusion from	300.0g
Casamino acid, technical	17.5g
Starch	1.5
Agar	17.0g

A 0.5 McFarland standard may be prepared in-house as describe below.
1. Add a 0.5-ml aliquot of a 0.048 mol/liter BaCl2 (1.175% wt/vol. BaCl2 • 2H20) to 99.5 ml of 0.18 mol/liter H2SO4 (1% vol/vol) with constant stirring to maintain a suspension.
2. Verify the correct density of the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvette. The absorbance at 625 nm should be 0.08 to 0.13 for the 0.5 McFarland standard.

3. Transfer the barium sulfate suspension in 4- to 6-ml aliquots into screw-cap tubes of the same size as those used in standardizing the bacterial inoculums.

4. Tightly seal the tubes and store in the dark at room temperature.

Preparation of Mueller Hinton plate

1. Allow a MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.

2. If the surface of the agar has visible liquid present, set the plate inverted, ajar on its lid to allow the excess liquid to drain from the agar surface and evaporate. Plates may be placed in a 35°C incubator or in a laminar flow hood at room temperature until dry (usually 10 to 30 minutes).

3. Appropriately label each MH agar plate for each organism to be tested.

Preparation of inoculums

- Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.
- Suspend the organism in 2 ml of sterile saline.
- Vortex the saline tube to create a smooth suspension.

- Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.
- Use this suspension within 15 minutes of preparation.

Inoculum preparation

Organisms to be tested must be in the log phase of growth in order for results to be valid. It is recommended that subcultures of the organisms to be tested be made the previous day.

Never use extremes in inoculum density. Never use undiluted overnight broth cultures or other

unstandardized inoculation for inoculating plates. If the organism is difficult to suspend directly into a smooth suspension, the growth method of preparing the inoculums should be used. However, the recommended organisms listed in this procedure all produce smooth suspensions with little difficulty. See the Clincial Laboratory Standards Institute document (3) for the growth procedure method for preparing the inoculums, if needed.

Inoculation of the MH plate

- Dip a sterile swab into the inoculum tube.
- Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet (Fig. 2).
- Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculums.
- Rim the plate with the swab to pick up any excess liquid.
- Discard the swab into an appropriate container.
- Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface

of the agar plate to dry before proceeding to the next step.

Placement of the antimicrobial disks.

Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multidisc dispenser to dispense multiple disks at one time and individual disk placement with forceps.

- To use a multidisc dispenser, place the inoculated MH agar plate on a flat surface and remove the lid.
- Place the dispenser over the agar plate and firmly press the plunger once to dispense the disks onto the surface of the plate.
- Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure compl.ete contact with the agar surface. This should be done before replacing the petridish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid.
- Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.
- To add disks one at a time to the agar plate using forceps, place the MH plate on the template provided in this procedure. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.
- Using the forceps carefully remove one disk from the cartridge
- Partially remove the lid of the petridish. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air.
- Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in steps f. and g. above.

2. Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C-37oC air incubator for 16 to 18 hours.

Disk placement[8-9]

Disks should not be placed closer than 24 mm (center to center) on the MH agar plate. Ordinarily, no more than 12 disks should be placed on a 150-mm plate or more than 5(usually 6) disks on a 100-mm plate. However, the semiautomatic disk dispensers hold 16 and 8 disks respectively and may not maintain the recommended 24 mm center to center spacing. The template provided in this protocol maintains the recommended 24 mm center to center spacing and allows the placement of up to 8 disks on the plate. You should avoid placing disks close to the edge of the plate as the zones will not be fully round and can be difficult to measure.

The generally 7 discs are used. 3 discs are kept on each edge and one in the center. Each discs should be at 24mm far from each other.

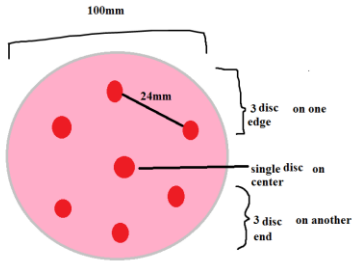

Fig.2: **Shows disc placement on agar plate.**

Each disk must be pressed down with forceps to ensure complete contact with the agar surface or irregular zone shapes may occur. If the surface of the agar is disrupted in any way (a disk penetrating the surface, visible lines present due to excessive pressure of the swab against the plate during inoculation, etc.) the shape of the zone may be affected. When printing the template for use in your microbiology lab, be sure that the diameter of the circle on the template is the same size as the Mueller-Hinton agar plates that you use in lab (100 mm). The reduce or enlarge function on a photocopier can be used to change the size of the template if needed. You may also make your own template by drawing a circle around a MH agar plate on a sheet of paper. Add the placement marks based on the number of disks you plan to use in your lab session, maintaining the recommended spacing as indicated above.

Incubation of the plates

A temperature range of 35°C ± 2°C is required.

Note that temperatures above 35°C may not allow the detection of methicillin-resistant Staphylococcus.
Do not incubate plates in CO2 as this will decrease the pH of the agar and result in errors due to incorrect pH of the media.

Results can be read after 18 hours of incubation unless you are testing Staphylococcus against oxacillin or vancomycin, or Enterococcus against vancomycin. Read the results for the other antimicrobial disks then re incubate the plate for a total of 24 hours before reporting vancomycin or oxacillin.

Measuring zone sizes

- Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper; include the diameter of the disk in the measurement.
- When measuring zone diameters, always round up to the next millimeter.
- All measurements are made with the unaided eye while viewing the back of the petridish. Hold the plate a few inches above a black, nonreflecting surface illuminated with reflected light.

- View the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading.
- Record the zone size on the recording sheet.
- If the placement of the disk or the size of the zone does not allow to read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter.
- Growth up to the edge of the disk can be reported as a zone of 0 mm.
- Organisms such as Proteus mirabilis, which swarm, must be measured differently than non swarming organisms. Ignore the thin veil of swarming and measure the outer margin in an otherwise obvious zone of inhibition.
- Distinct, discrete colonies within an obvious zone of inhibition should not be considered swarming. These colonies are either mutant organisms that are more resistant to the drug being tested, or the culture was not pure and they are a different organism. If it is determined by repeat testing that the phenomenon repeats itself, the organism must be considered resistant to that drug.

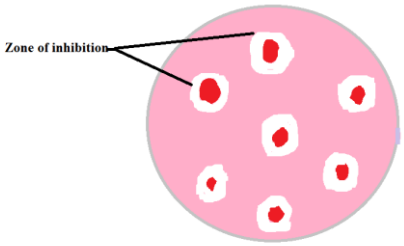

Fig.3: Shows zone of inhibition

Strokes disk diffusion method[9]

In stroke disk diffusion method the plate is divided in three parts. The test organism is inoculated at central one third and control on upper and lower third of the plate. However in the modified strokes disk diffusion method, the test organism is inoculated in upper and lower third and control on the central one third.

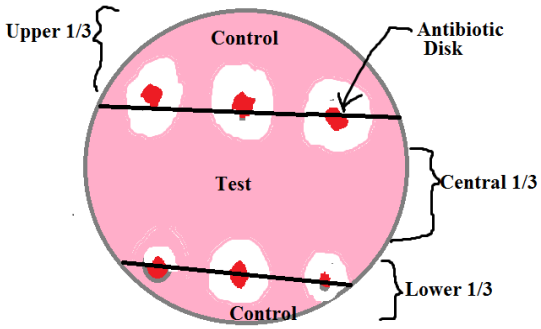

Fig.4: Shows disk placement and zone of inhibition.

Modified strokes disk diffusion method-

In this method the upper and lower one third remains test and central one remain is control.

The uninoculated gap of 2-4 mm should be between control and test area on which antimicrobial disks are applied.

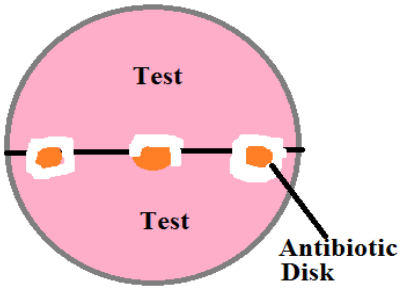

Fig.5: Shows disk placement and zone of inhibition according to modified stroke diffusion method

Principle of antimicrobial disk diffusion- The concentration of the antimicrobial decreases as the distance from the disk increases. The diffusion of antimicrobial also flow in the depth of the agar medium surface

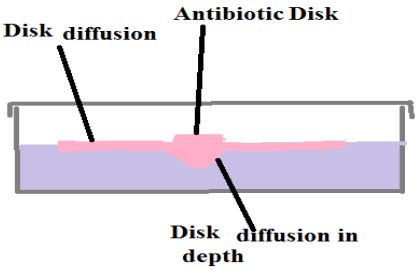

Fig.6: Shows principle of disk diffusion .

Measurement of zone of clearance-The zone of clearance is measured from the edge of antimicrobial disk. Following types of zone of inhibition may be found.

- Sensitive- In this the zone size is equal to longer than or not more than 3mm smaller than the control.
- Intermediate-Here the Zone size of the test strain is at least 2mm but also at least 3mm smaller then that of the control strain.
- Resistant- The zone of the test strain is smaller than 2mm.

Broath Dilution Method[8]-

- Make dilutions of various concentrations like 120, 60,30,7.5 and 3.5 mg/ml of test antimicrobial agent.
- Add these dilution to the test parasites culture.
- After three days of incubation at 25oc,The parasites were counted and the MIC was determined by finding the lowest concentration of drug that inhibited the growth of the parasite.

Agar dilution method[8]-

- Make dilutions of various concentrations of test antimicrobial agent onto blood agar.
- Cultivate the parasites on the surface of the agar.
- Plates are incubated in candle jar at 25oc for 3-5 days
- The growth is cheked by observing the lowest drug concentration that inhibited visible parasite growth.
- Calculate the MIC.

Assay of disinfectants [9, 10]

Evaluation of Disinfectants

Rideal walker test

Rideal walker (RW) test is used to measure the effectiveness of the disinfectant as compare to phenol. If the RW coefficient is 1 or more, then the disinfectant is effective otherwise the it is considered as ineffective.

PROCEDURE

- Prepare the broth by adding 20g meat extract ,20g peptone and 10g sodium chloride in 1000ml water**.**
- Adjust the pH to7.6 and sterilized by autoclaving.
- Filter it and take the filtrate in 5ml capacity broth tube and autoclave it.
- Separately make a subculture of *Salmonella typhi* in nutrient agar by incubating at 28oc for 23 hours.
- Add a loopful of this to the broth tube prepared in step 1-3 and autoclave it for 23hrs at 38oc.
- A loopful of the above is added to second tube and repeate this process for 3 times.
- Prepare 5% pure phenol solution in distilled water.
- Different dilutions are prepared from this with 95,100,105, and 115ml of amount.
- Similarly the solution of the test is performed with 5ml of portion of the test sample in 500ml of distilled water with thorough mixing in sterile measuring cylinder.
- Five ml of this is taken in each 4 tubes.
- All the tubes are kept on water bath with such arrangement that the tube with the strongest concentration should be on left side.
- The fifth test tube is kept on write side filled with 5ml of standard phenol solution.
- When the temperature reaches to 17oc-19oc 0.2ml of broth culture is added to each tube after 30 second.
- A loopful of above is added after 30 seconds to next 5 tubes containing 5ml broth.
- This procedure is repeated until the period of 2.5, 5, 7.5 and 10 minutes is maintained.
- Incubate these tubes for 48hrs at 37oc.
- Growth of the microbes is recognized by turbidity.

Chick martin test [8]- Chick and Martin in1908 recommended the test they recommend the use of human dried faeces for this test Later, Garrod suggested the use of dried yeast as a substitute.

PROCEDURE

- Inocculate the Salmonella typhi in the graded solution of test disinfectant and standard phenol solution .
- Make sure that all solution contain dried yeast.
- Allow the contact period of 30 minutes at 20oc and at the end of this period.
- At the end of this period make subcultures in duplicate in nutrient broth.
- The subculture tubes are incubated for 48 hrs. at 37oc and the presence and absence of growth is noted.
- The concentration of phenol which prevent the growth in both is determine and calculate the mean value. Similar procedure should be followed for test sample.
- Calculate the co-efficient by dividing that value found for the test.

CONCLUSION

This review work would further help to get the literature of discussed antimicrobial models at one place. These methods would help to provide new antimicrobial agents for the treatment of various microbial diseases of human.

REFERENCE

1. Nicolson,G. Role of Microorganism Infections in Chronic illnesses The CFIDS . The role of microorganism infections in chronic illnesses: Support for antibiotic regimens. www.cfids.org/archives/1999/1999-5-article06.asp
2. Ryan KJ; Ray CG (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. ISBN 0-8385-8529-9.
3. Glossary of Notifiable condition. Washington State Department of Health. Retrieved 2010-02-03.
4. 4.This section incorporates public domain materials included in the text: Medical Microbiology Fourth Edition:Chapter 8 (1996) . Baron, Samuel MD. The University of Texas Medical Branch at Galveston.
5. Vashist, H., Sharma, D., Gupta,A. A Review on Commonly Used Biochemical Test for Bacteria. IJBSP 2013; 4(1) :36 - 51
6. Bhattachary,A., Zaman1, M. K., Haldar, P. K. Antibacterial Activity Of Stem Bark And Root of Indian Zanthoxylum Nitidum. Asian Journal of Pharmaceutical and Clinical Research 2009; 2(1):30-34.
7. Jan Hudzicki. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol.American society for microbiology.2012.
8. Mohammadzadeh T., Sadjjadi SM, Habibi P, and Sarkari B. Evaluation of Anti-leishmanial Drugs on *Leishmania promastigotes*. Iran J Parasitol 2012; 7(3): 43–47.
9. Arora, D.R., Arora, B. (2008) Text book of Microbiology, 3rd edition. CBSE publishers and distributors. New Delhi.
10. N.K. Jain. Pharmaceutical Microbiology. Revised and updated second edition. Delhi, Vallabh Prakashan, 2005.