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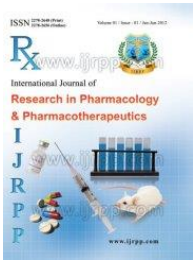

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# An analytic comparative study about the antimicrobial activity of different generic and branded *Amikacin* on *Escherichia* coli - an *In-vitro* study.

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## ABSTRACT

### Background

A Generic drug is a pharmaceutical product usually intended to be interchangeable with an innovator product that is manufactured without any license from the innovator company and marketed after the expiry date of the patent or other exclusive rights. The generic drug can be equivalent to branded formulations if they have the same pharmaceutical form, therapeutic indications and similar bioequivalence [20%]. There is an increased report of adverse events against the active drug ingredient of the generic drugs. Many of these factors lead to the increased confusion in the medical field on prescribing drugs. The antimicrobial resistance is a global problem, probably due to the indiscriminate and irrational use of antibiotics, prescriptions for incorrect medicines or incorrect determinations of dose, route and/or duration [16]. Antibiotics are type of antimicrobial drugs used in the treatment and prevention of bacterial infections. There is increased incidence of resistance against many antibiotics which contributes to the serious emerging and re emerging infections. In this study the antibacterial activities of different generic and branded amikacin were compared against *escherichia coli -In vitro*.

### Methods

In this experiment one generic amikacin (mikastar) and five branded amikacin were taken and coded. The urine samples of the patient with UTI were collected in the hospital under aseptic precautions and the *E.coli* colonies sensitive to amikacin were isolated by using antibiotic sensitivity test by means of Kirby Bauer disk diffusion method using amikacin disc and the *E.coli* sensitive to it were isolated based on the zone of inhibition to the amikacin.

To know the antimicrobial activities of the drug, the minimum inhibitory concentration (MIC) of the antibiotics were calculated by using serial macro broth dilution method.

### Results

Based on the above experiments done, the generic and the branded antibiotics were equally efficacious against the *Escherichia coli*.

**Conclusions**

All the samples of the antibiotics taken are pharmaceutical equivalents and the products can be used in antimicrobial therapy [16].

**Keywords:** Branded drug, Generic drug, Minimal inhibitory concentration, Bioequivalence, Amikacin, *E.coli*, Macrobroth dilution, Antibiotic sensitivity test, Disc diffusion method.

## INTRODUCTION

As the debate of replacing branded drugs with generic drugs heats up, it also brings the issue of substandard drugs in highlight. Physicians and patients have prejudices against substitution of generic drug and there are concerns regarding quality and effectiveness of these drugs [15].

A Generic drug is actually a pharmaceutical product usually intended to be interchangeable with an innovator product, that is manufactured without a license from the innovator company and marketed after the expiry date of the patent or other exclusive rights [as per the definition of WHO]. The generic drug manufacturer must prove the drug is bioequivalent as the branded one.

Generic drugs are medicines which are identical and are bioequivalent to brand name drugs in dosage, formulation, safety, strength, route of administration, quality, performance characteristics and intended use [12].

They are chemically equivalent to their branded counterparts in terms of active ingredients but may differ in peripheral features such as pill colour or shape, inert binders and fillers and the specific manufacturing process. The 1984 Hatch-Waxman Act first authorized the FDA to approve generic drugs demonstrated to be "bioequivalent," which is defined as absence of a significant difference in the availability of the active ingredient [7]. All generic manufacturing, packaging and testing sites must pass the same quality standards as those of brand name drugs and the generic products must meet the exact specifications as any brand name product.

The primary drivers of elevated drug costs are brand-name drugs, which are sold at high prices during the period of patent protection and market exclusivity after approval by the Food and Drug Administration (FDA). To control spending, many payers and providers have encouraged substitution of inexpensive bioequivalent generic versions of these drugs which can legally be marketed by multiple manufacturers after the brand-name manufacturer's market exclusivity period end [7].

There has been a dramatic increase in clinical use of generic medicines since 1980, but there are not systematic evaluations of their therapeutic efficacy compared with innovator products [6].

The physicians and patients might also have prejudices against generic drug substitution or concerns regarding quality and generic effectiveness. A meaningful proportion of physicians expressed negative perceptions about generic medications, representing a potential barrier to generic use [13].

Some physicians and patients have expressed concern that bioequivalent generic and brand-name drugs may not be equivalent in their effects on various clinical parameters [6]. There is a fear that the generic drugs are ineffective, they can result in adverse clinical outcomes such as treatment failure. There is an increasing reports of adverse events against the active drug ingredient of the generic drugs.The average difference in C(max) and AUC between generic and innovator products was 4.35% and 3.56%, respectively over 12 years [8].

In general 93% of generic and 87% branded drug users believed that their drugs were effective ($P$ = 0.238) in controlling their ailments. No significant difference (9% generic, 10% branded drug users, $P$ = 1.000) was observed in reported adverse effects between generic and branded drug users. Of these82% generic drug users and 77% branded drug users were adherent to their generic and branded drugs respectively ($P$ = 0.289). As expected, a significantly lower cost of generic drugs was observed compared to its branded counterpart [14]. Savings from newer generic medicines—those that have entered the market since 2002—continue to increase exponentially and account for more than one-third of the total savings. The IMS analysis found that the savings from generics introduced in the past 10 years has now reached approximately $481 billion and accounts for more than 40% of the overall generic savings. In 2011 alone, the U.S.

health care system saved nearly $130 billion from these recently generalised drugs, or more than two-thirds of the savings for the entire year [9].

Generic substitution of brand prescriptions is an accepted practice in many parts of the world and this is often done for economic reason [8, 9]. *India is one among the largest manufacturers* of generic drugs for export to US and Europe. In India, however, generic substitution is not a universally accepted practice. This results from various factors including no availability of generic formulations, distrust of generic medicines by practitioners often due to perceived inferior quality and counterfeiting of drugs [10]. Patients and providers perceptions regarding the efficacy, safety, or value of generic drugs may be responsible for these inconsistencies [11]. The Medical Council of India (MCI) has guidelines for physicians to prescribe drugs with generic names. Ministry of Chemicals and Fertilizers, Government of India since 2008, opened dedicated outlets called "Jan Aushadhi Stores" where generic medicines are sold at low prices [12], the "Jan Aushadhi yojana" is actually setting up of generic stores all over India to provide medicines at cheaper rate and to increase affordability to the poor people.

We, therefore, undertook this study to evaluate the experiences and attitude of patients toward generic drugs.Antibiotics, as well as antimicrobial drug used in the treatment and prevention of bacterial infections, They may either kill or inhibit the growth of bacteria. The aminoglycosides group consist of two or more amino sugars joined in glycosidic linkage to a hexose nucleus which usually is in central position. This hexose is either streptimide or 2 deoxystreptamine [1].

The amikacin is identified in 1970s.The patency was expired and it came into use in 1976. This drug is kept under the list of essential medicine by the World Health Organization (WHO).

**A**mikacin is a bacteriostatic drug which inhibits the growth of the bacteria. They affect the protein synthesis of the bacteria by binding to the 30s ribosomes. They cause misreading of the mRNA template, incorporate the inappropriate aminoacids into the growing polypeptide chain and they also causes premature termination of protein and blocks further translation.The initiation of protein synthesis were also sometime blocked[1]. The advantage of the amikacin is that pharmacologically they have good synergistic activity and they have post antibiotic effect which means that the antimicrobial activities of the amikacin last in the body even when the antibiotics level in the body decreases below the minimum inhibitory concentration (MIC). The amikacin action is based on the concentration dependant killing where the increase in concentration of drug, increases the antimicrobial activities of it. The amikacin is also economically advantageous as they are easily available and cheaper in the market.

The amikacin is also acquiring cross resistance with other antibiotics of the amino glycosides. The bacteria acquire resistance to amino glycosides by producing amino glycoside metabolising enzyme which makes the antibiotics ineffective. The gene acquiring aminoglycoside modifying enzyme is acquired by conjugation and transfer of resistance plasmids. The bacteria they acquire mutation which alters the amino acid in the drug binding area of the ribosome as a result the drugs cannot bind and hence there is no antimicrobial activity but comparatively the amikacin have least resistance among the aminoglycoside family.

The amikacin have poor oral bioavailability hence should not be administered orally. It can be given intravenously, intramuscularly, rectally and also in inhalational rote. It can also be given in intrathecal or intraventricular route for central nervous system infections. The amikacin has a half life of 2-3hours and also have a post antibiotic effect which last even after the MIC level of the antibiotic reduces in the body. The amikacin is widely distributed throughout the body and can be used for CNS infection also as it crosses the blood brain barrier when infected and the amikacin is protein bound and is metabolised in liver and excreted via kidney.

The amikacin have a broad spectrum of activity than any other aminoglycosides against the gram negative bacteria usually aerobic and they act poorly against the anerobes such as Escherichia. Coli, serratia, proteus, pseudomonas aeroginosa, klebsiella, acinetobacter, enterobacter,providencia and some other gram negative species[1].The amikacin is the widely prescribed antibiotic for the urinary tract infections, hospital acquired pneumonia, meningitis, pneumonia bronchiectasis, non tuberculous mycobacterial infections, intra abdominal infections, bone and joint infections, respiratory tract infections, skin and suture site infections etc.. The amikacin can also be used for streptomycin resistant strains, atypical mycobacteria and it is one of the second

lineanti tuberculosis drugs and it is started as an empiric therapy in fever. The amikacin is contraindicated in pregnancy and in renal failure patients. The adverse reaction of amikacin are ototoxicity, nephrotoxicity and neurotoxicity and other mild side effect are hypertension, headache, rashes, eosinophilia, arthralgia, allergic reaction, etc.. The general dosing of amikacin is estimated as 15mg/kg/day usually given in 2-3 divided doses.

The Escherichia coli is the bacteria living only in the human or animal intestine. They are excreted normally in faeces and they remain viable in the environment only for few days, at that time they remain infective for the human beings. The infection is acquired through feco-oral route.

The *E.coli* is a gram negative bacilli measuring 1-3x 0.4-0.7microns arranged singly or in pairs. It is motile by peritrichate flagella, some were non motile. The *Escherichia coli*is the major cause for the naturally acquired urinary tract infections, diarrhea and pyogenic infections.

The E.coli consist of three types of antigen which are somatic antigen O, flagellar antigen H, capsular antigen K based on which the serotyping will be done. The virulence of the E.coli is based on the somatic antigen O and the toxin produced by it. There are two kinds of exotoxin produced by the E.coli they are hemolysin and enterotoxin. The enterotoxins, produced by the E.coli, is the important cause of diarrhea. The distinct types of enterotoxins are heat labile toxin (LT), heat stable toxin (ST) and verotoxin (VT).

The E.coli is the most common cause of urinary tract infections and these infections are usually precipitated by urine flow obstruction. They present initially as asymptomatic bacteriuria which undetected and untreated leads to significant bacteriuria and can even produces pyelonephritis.

The E.coli also comes under the major bacteria which are suspected for diarrhea. Five different types of diarrheagenic E.coli strains have been identified they are

1. Enteropathogenic-E.coli,
2. Enteroinvasive-E.coli,
3. Enterotoxigenic-E.coli,
4. Enterohemorrhagic-E.coli,
5. Enteroaggregative-E.coli. Of these, the Enteropathogenic-E.coli is the common cause for diarrhea in children and infants, the Entero-toxigenic-E.coli is the most common cause for travellers diarrhea and the Enterohemorrhagic-E.coli can give rise to severe diarrheal disease to fatal hemmorhagic colitis and is the common cause for Hemolytic uremic syndrome particular in elderly individuals.

The major pyogenic infection caused by the E.coli are the intra-abdominal infections such as peritonitis and abscesses usually due to the spillage of the bowel contents into the abdominal cavity. The blood stream infections by it can lead to fatal conditions like septic shock and systemic inflammatory response syndrome.

The complications of E.coli infections are Hemolytic uremic syndrome, Hemmorhagic diarrhea, severe dehydration and death finally. The drugs commonly employed for *E.coli* were aminoglycosides ,beta lactamase antibiotics .

## METHODS

### Isolation of e.coli

The urine samples were taken to isolate the Escherichia coli from UTI patients. The amikacin is an aminoglycoside antibiotic which is more effective against gram negative infections and one of the commonly preferred drug for urinary tract infections. Only those *E.coli* sensitive to amikacin alone is taken by means of antibiotic susceptibility test by Kirby Bauer disk diffusion method under standard CLSI guidelines. Briefly a McFarland 0.5 standardised suspension of bacteria is swabbed over the surface of Mueller Hinton agar plate and the paper disk containing single concentration of each antimicrobial agent are placed over the inoculated surface and after overnight incubation the diameter of zone of inhibition were calculated[2]. In this method zone of inhibition to Escherichia coli isolate for Amikacin was 18mm and is taken into account.

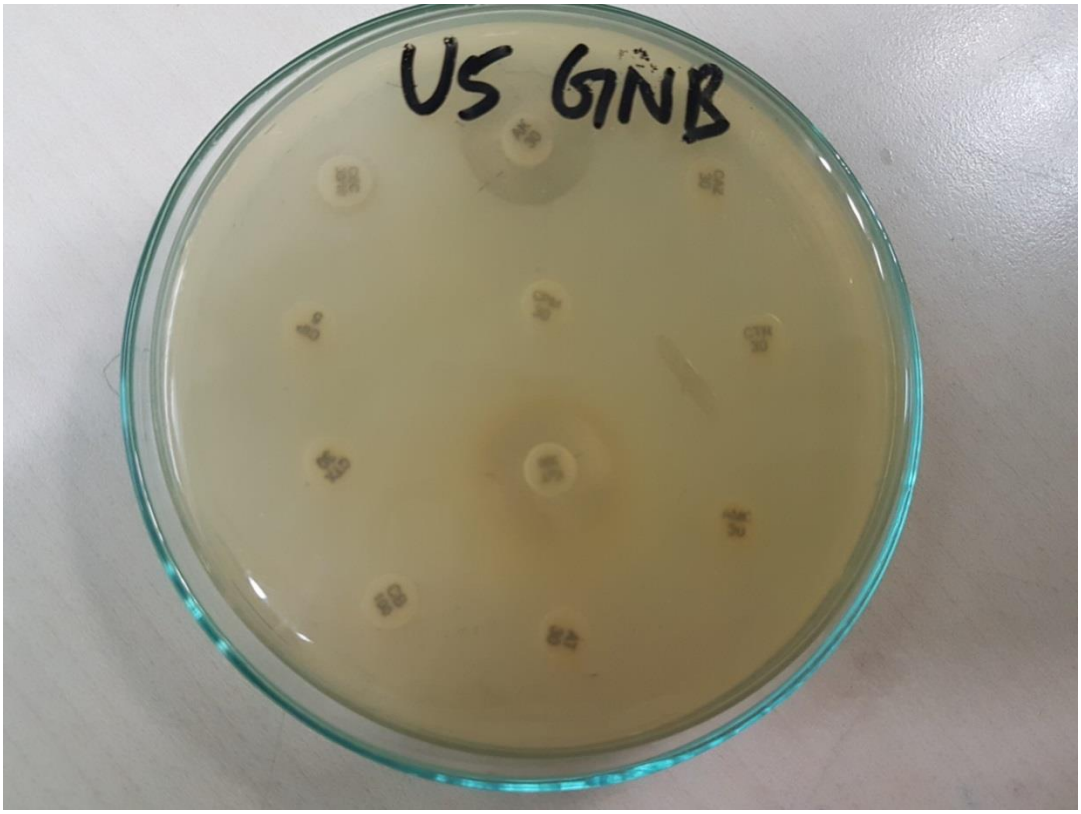



**Fig1; Antibiotic sensitivity test in Kirby bauer disk diffusion method.**

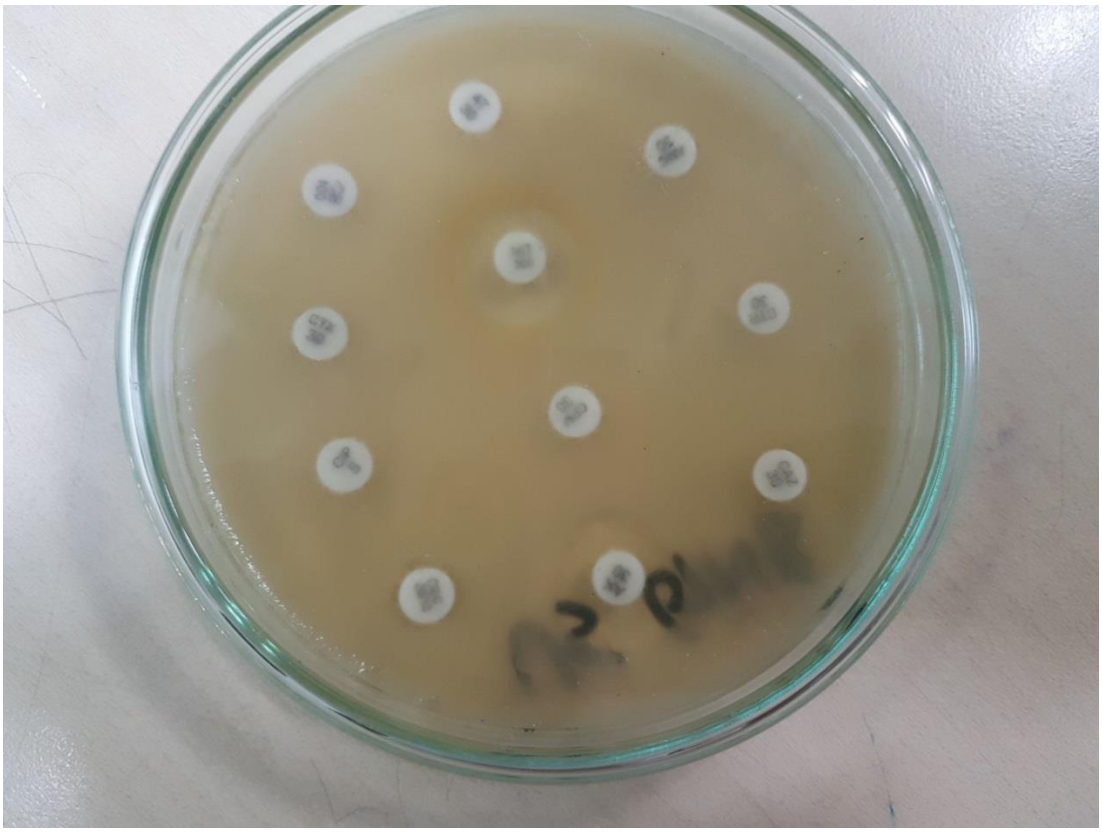
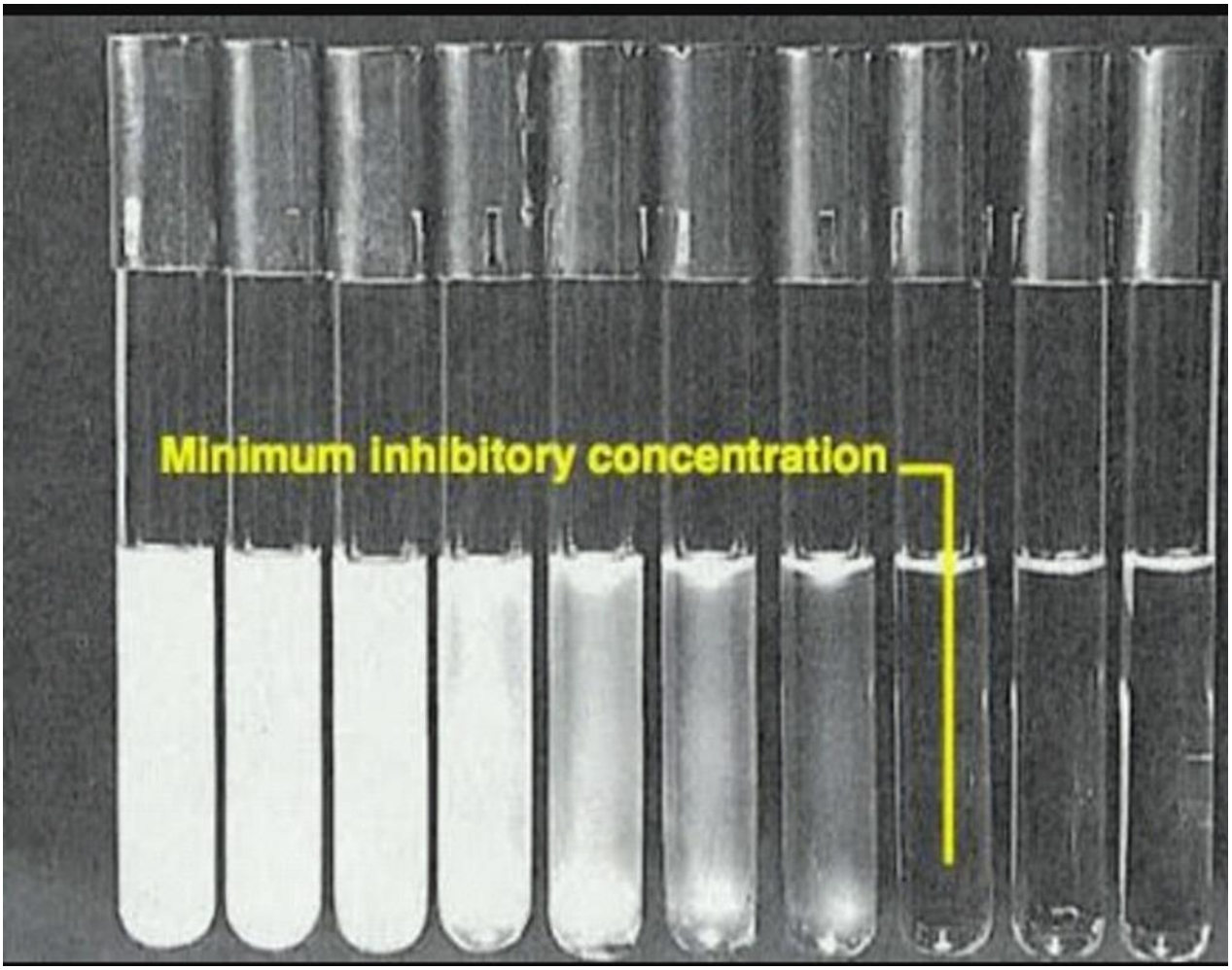


**Fig 2; Antibiotic sensitivity test in Kirby bauer disk diffusion method**

## Macrobroth dilution

The antimicrobial activities of the antibiotics were calculated by means of Minimal Inhibitory Concentration of the drug using serial macrobroth dilution method.

The dilution method was the first susceptibility procedure that was developed where serial dilutions of antibiotics were compared either in agar or in liquid media. Typically two fold dilution scheme is used(eg 100,50,25,12.5,6.25,3.1,1.5,0.75microgram/ml) and each inoculated is diluted with a standardised concentration of test organism and after a specific incubation period the lowest concentration of antibiotic that inhibits the organism is determined[4].




One generic [A] and 4 branded formulations of the branded drugs[B,C,D,E] were taken for microbiological assay. Coding were done.

Escherichia coli isolated from urine sample was tested for the antimicrobial activity of Amikacin, generic drug of mankind (mikastar), coded as A and branded drugs of sun pharmacy, Macleod, Workhardt, Cipla were coded as B,C,D and E respectively for experiment purposes.

For each antibiotic(A,B,C,D,E), their serial dilutions were prepared in different concentration by means of serial dilutions as 0.5,1,2,4,8,16,32 mg/ml and constant amount of E.coli, isolated will be added and each tube will be checked out for turbidity which indicates growth.

Based on CLSI guidelines the antibiotic amikacin disk of concentration 30mcg was considered sensitive when the zone of inhibition is greater than 17mm and were said to be resistance when its zone of inhibition is less than 14mm and of intermediate sensitive when the zone is of range from 15-16mm.

The minimal inhibitory concentration to E.coli for amikacin to all branded and generic drug was done in MH broth by macro broth dilution method according to CLSI guidelines [64microgram-0.5microgram]. The MIC of amikacin for E.coli less than 16 microgram is considered as sensitive and upto 32 microgram is considered as intermediate and those above 64 microgram were resistant

## RESULTS

The minimum inhibitory concentration of the drug by broth dilution method for all four branded [B,C,D,E] and generic [A] Amikacin after 24 hours of incubation showed no growth on naked eye examination at different concentration of drugs from 64ug/ml upto 0.5ug/ml.

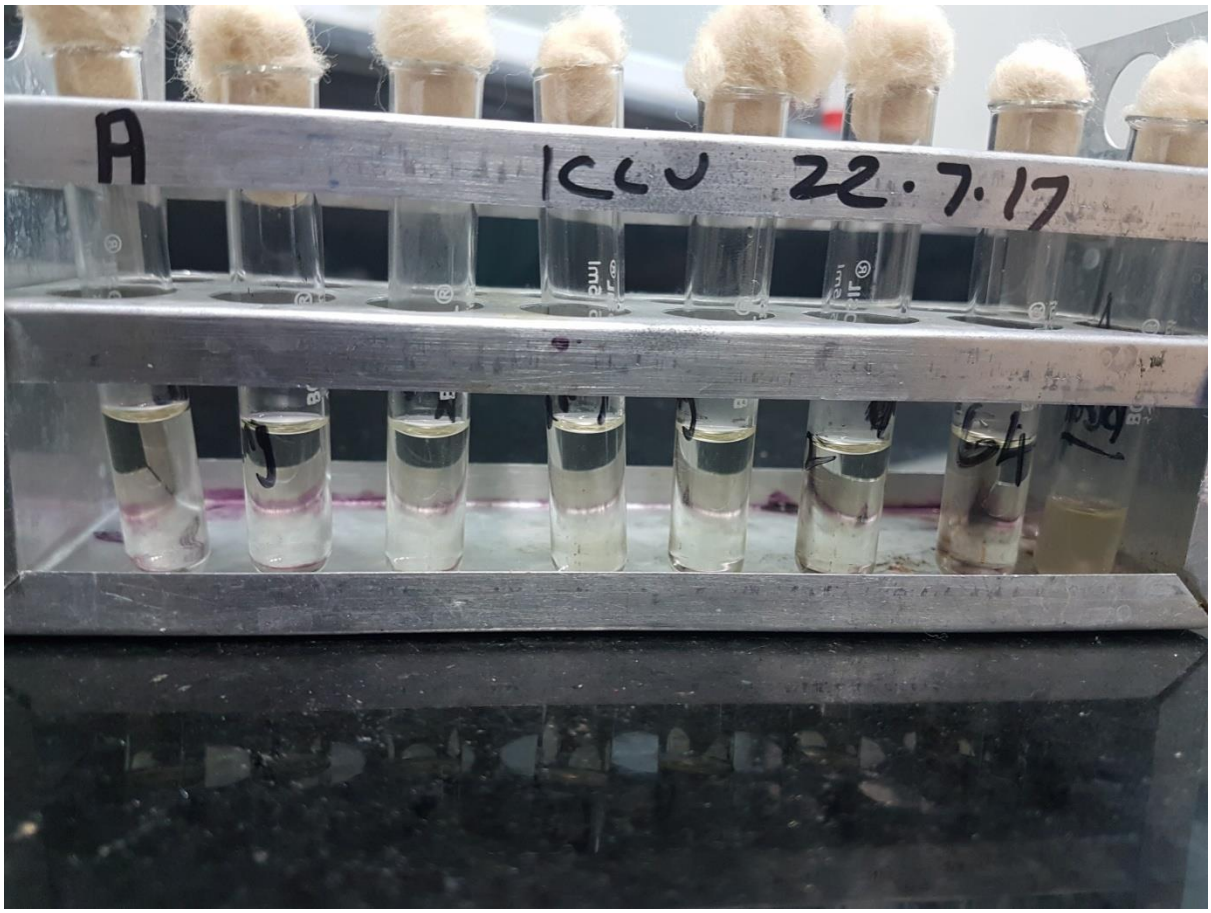



**Fig 3; Macrobroth dilution of drug A (generic drug)**

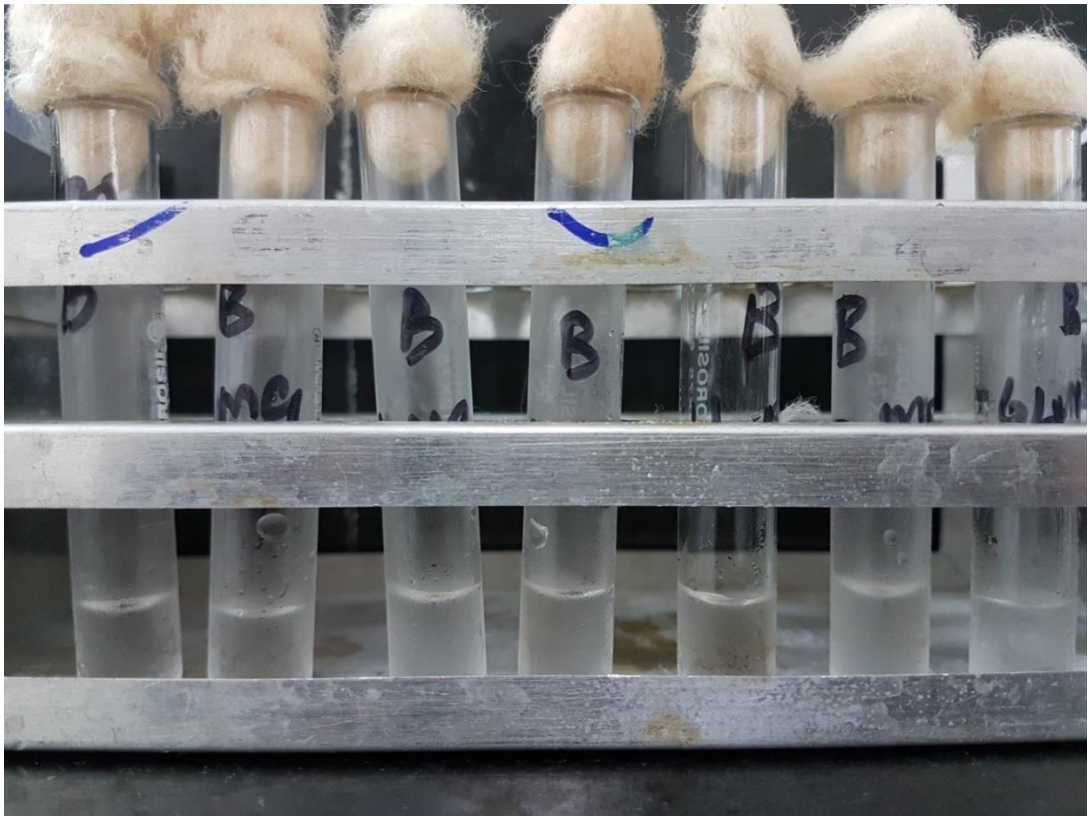



**Fig 4; Macrobroth dilution of drug B**

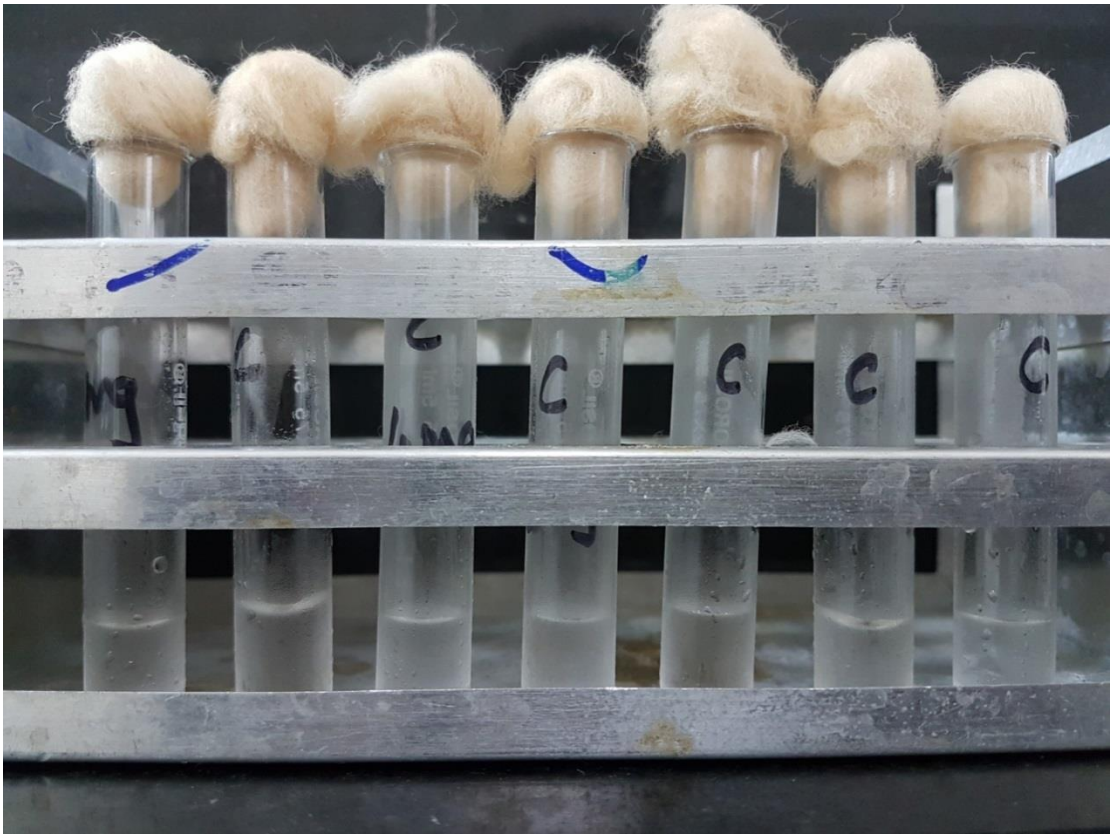



**Fig5: Macrobroth dilution of drug C**

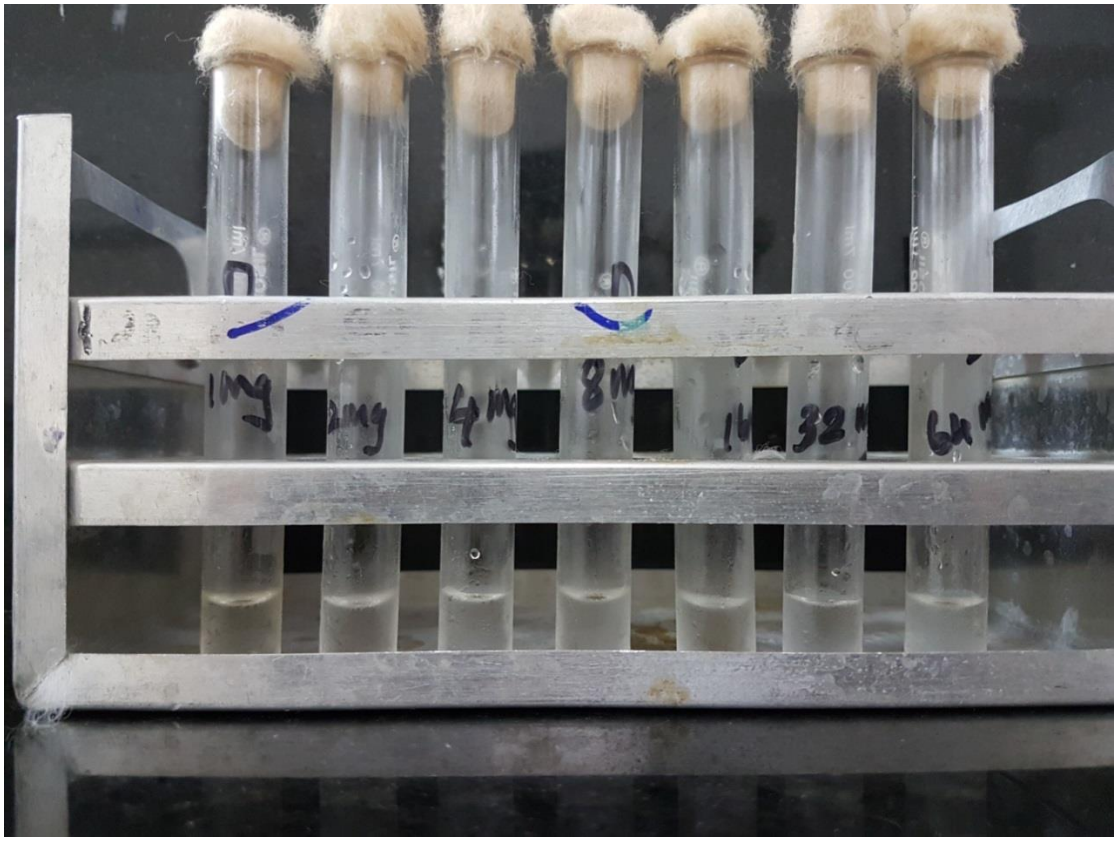


**Fig 6; Macrobroth dilution of drug D**

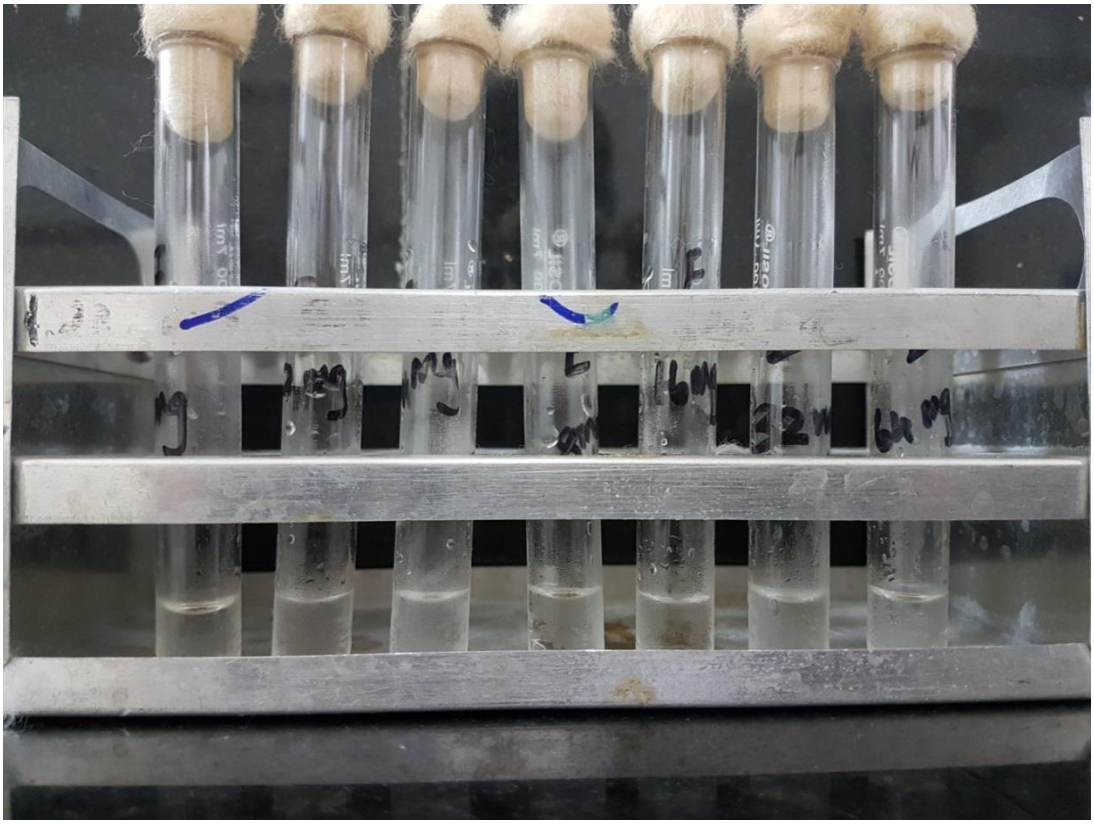


**Fig 7; Macrobroth dilution of drug E**

## DISCUSSION

The in-vitro efficacy of the antibiotics were measured by means of minimal inhibitory concentration of the amikacin against *Escherichia coli*.

MIC is defined as lowest concentration of the antimicrobial agent that prevents the visible growth of the microorganism after 18-24hrs of incubation. The significance of MIC is that it helps us to know about the efficacy of these drugs. The MIC of amikacin up to 16mg is considered as significant for its antibacterial activity .The MIC level were identified by serial macrobroth dilution where serial dilution of antibiotics are made and were inoculated with the microorganism and the growth of the organism can be measured on the basis of its turbidity and then it is confirmed by inoculating that on MH agar where no growth were seen.

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## DECLARATIONS

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*Conflict of interest: nil*
*Ethical approval:* Approval letter from IECHS of DSMCH – attached.

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