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Review Article -Antibiotics Resistant to Different Microorganisms

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Abstract
Over a years, people mostly who were struggled with primary cause of infection and diseases by microorganisms, and utmost care has been taken that some antibiotics are highly resistant to bac- terial species and emergence of broad-range of antibiotic therapy. Broad-spectrum antibiotics have widely emerged in various ways to kill microorganisms that tend to cause illness and diseases in the human era. New resistance mechanisms are emerging and spreading worldwide. However, some bacteria may become resistant to commonly used antibiotics. Antibiotic-resistant bacteria are bacteria that are inhibited or killed by antibiotics as they can able to survive and even rapidly spread by multiplying in the human system in the presence of antibiotics. The major mechanisms interrupted in bacterial resistance are limitation of drug uptake, modification of a drug target, in- activation of a drug, and active efflux of a drug. Those bacteria are resistant to many antibiotics and they are termed Multi-resistant organisms. For example, benzylpenicillin has very little effect on most organisms in the human digestive system. *Staphylococcus aureus* and *Neisseria gonor- rhoeae* are resistant to benzylpenicillin, Methicillin-resistant Staphylococcus aureus, Vancomycin- resistant Enterococcus, Multi-drug-resistant Mycobacterium tuberculosis, Carbapenem-resistant *Enterobacteriaceae*. Different methods were used for detecting the antibiotic resistance to different microorganisms mainly Gram-negative bacilli, and Gram-positive bacteria. The common ways that antibiotic-resistant bacteria can be transmission in hospitals from person to person is through contact with contaminated hands of hospital staff, door handles, hospital beds, and equipment. Important ways can be followed to prevent antibiotic resistance by minimizing unnecessary over-prescribing of antibiotics by medical practitioners and maintaining proper hygiene such as hand washing by use of regular infection control.

Keywords: Antibiotic resistance organisms; Enterobacteriaceae, Antibiotics group; Testing antibiotic resistance to different organisms

Introduction:

Over a decade, antibiotic resistance arose to high risk worldwide and new resistance mechanisms are emerging and spreading globally, threatening our ability to treat various infectious diseases. These may also be due to the medical practitioners who have prescribed the overdose resistance antibiotics likely to treat the infections, but in some cases, Unlike most of the diseases that have emerged and sustainable that killed people can be treated with antibiotics that become harder and less effective like Pneumonia, Tuberculosis, Gonorrhea, and Salmonellosis. Common bacterial infections including urinary tract infections, sepsis, sexually transmitted infections, and some forms of diarrhea are at high rates of resistance against less effective antibiotics. For example, the rate of resistance to ciprofloxacin used to treat urinary tract infections may vary from 8.4% to 92.9% for Escherichia coli, 4.1% to 79.4% for Klebsiella pneumonia as reported to the Global Antimicrobial Resistance and Use Surveillance System. (GLASS) (2). As the carbapenem re- sistance rates are more in Klebsiella pneumonia among half of the patients in hospitals. Colistin is the last resort treatment for life-threatening infections caused by carbapenem-resistant Enterobac- teriaceae (E.coli, Klebsiella). Staphylococcus aureus is part of our skin flora and is also the common cause of infections both in community and healthcare facilities. Methicillin-resistant Staphylococcus aureus (MRSA) infections are 64% more likely to die than people with drug-sen- sitive infections. This indicator is used to monitor the two specific drug-resistant pathogens such as Methicillin-resistant Staphylococcus aureus(MRSA), and E.coli resistant to third-generation cephalosporins (3GC). As N.Gonorrhea is highly resistant to sulphonamides, penicillin, tetra-cyclines, macrolides, and fluoroquinolones. Ceftriaxone is the only antibiotic therapy for gonorrhea. Rifampicin-resistant Tuberculosis (RR-TB) and Multidrug-resistant Tuberculosis (MDR-TB) are the two

pathogens resistant to anti-TB drugs. MDR-TB sustains treatments that are

longer, less effective, and far more expensive than those for non-resistant TB. Antiviral drug re- sistance is an increasing concern in immune compromised patients where ongoing viral replication and prolonged exposure to drugs lead to the selection of resistant strains. People receiving an- tiretroviral drugs can acquire HIVDR and people who get infected with HIV are also drug- resistant. The emergence of drug-resistant parasites poses one of the greatest threats to malaria control and results in increased malaria morbidity and mortality. Artemisinin-based combination therapies are recommended first-line treatments for uncomplicated P. falciparum malaria. The prevalence of drug-resistant fungal infections is increasing and difficult to treat the disease. Many fungal infections have existing treatability issues such as Drug-resistant Candida Auris, which is already with increasing resistance to fluconazole, amphotericin B, and voriconazole. (1,2)

Types of Antibiotic resistance

1. Natural resistance: This is otherwise named Structural resistance as it is caused by the structural characteristics of bacteria and is not associated with the use of antibiotics. E.g., In Gram-negative bacteria, vancomycin does not pass through the outer membrane so Gram-negative bacteria are naturally resistant to vancomycin.
2. Acquired resistance: This may occur in the chromosomal characteristics of bacteria. This kind of resistance occurs due to mainly structures of chromosomes or extra chromosomes (Plasmids, transposons). Chromosomal resistance arises from mutations developing in the spontaneous bacterial chromosome, such mutations may occur by physical and chemical factors. Streptomycin, erythromycin, aminoglycosides, lincomycin. Extrachromosomal re- sistance by extrachromosomal genetic elements can be transferred in various ways like plasmids, transposons, and integrons. Resistance genes and plasmids carry the genetic ma- terial from a bacterium in three ways those are transduction, transformation, conjugation, and transposition mechanism.
3. Cross-resistance: Some microorganisms are resistant to a certain drug, that acts with the same or similar mechanism and are also resistant to other drugs, such as resistance between erythromycin, neomycin-kanamycin, or resistance between cephalosporin and penicillin.
4. Multi-drug resistance and pan-resistance: Multi-drug-resistant organisms are usually bac- teria that have become resistant to the antibiotics used to treat them, It can occur in two
5. mechanisms, these bacteria may accumulate multiple genes, each coding for resistance to a single drug. It may also express by the enhancing expression of genes that code for mul- tidrug efflux pumps. If the bacterial strains are resistant to three or more classes of antimicrobials, it is considered multi-drug resistant. For example, Acinetobacter species can be defined as the bacterial isolate resistant to at least three classes of antimicrobial agents such as All penicillin, cephalosporins, fluoroquinolones, and aminoglycosides). If the strains are resistant to all but one or two antibiotic groups, they are considered exten- sively drug-resistant. Acinetobacter species are resistant to three classes of antimicrobials and also be resistant to carbapenems, they are termed extensively drug-resistant. If the strains are resistant to all available antibiotics, they are considered pan-drug-resistant. Aci- netobacter spp is resistant to polymyxins and tigecycline, they are termed pan-drug resistant or pan-resistant. (3)

Mechanisms of Resistance to Antibiotics

1. Alterations in the ribosomal target: The antibiotic penicillin is resistant and involves alter- ation in the ribosomal structure of Staphylococcus aureus, Streptococcus pneumonia, Neisseria meningitides, and Enterococcus faecium strains. Beta-lactam, quinolones, gly- copeptides, and macrolides are involved in the mechanisms. (3)
2. Enzymatic inactivation of antibiotics: Enzyme synthesizing by Gram-positive and Gram- negative bacteria can degrade antibiotics. E.g., Beta-lactamases, aminoglycosides, and modifying enzymes. (3)
3. Reduction in the cell membrane permeability: This mainly occurs in Gram-negative bacilli in part of the alteration in the inner and outer membrane permeability so that the drug uptake by cell wall refluxed from the active pump systems and as a result, it may cause porin mutations. Eg., Pseudomonas aeruginosa strain-specific porin mutations - OprD to carbapenem resistance and also reduction in cell permeability which is more resistance to quinolones and aminoglycosides. (3)

4. Flush out of the drug (Active pump system): Tetracycline drug plays an important role in developing resistance by flushed out with an energy-dependent active pumping system and cannot sustain inside the cell. Such resistance is in control of the plasmid and chromosome and is effective in resisting quinolones, chloramphenicol, and beta-lactams. (3)
5. Alternative metabolic pathway: New metabolic pathway may occur changes in bacteria by synthesizing folate on its own by gaining the property of getting folate from the environ- ment. This pathway resistance among the sulfonamide and trimethoprim. (3)

Mechanisms of resistance to the antibiotic group

1. Beta-lactam group antibiotic resistance: Beta-lactam antibiotics include penicillins, cephalo- sporins, monobactams, and carbapenems. The resistance mechanism is responsible for the synthesis of beta-lactamase enzymes. Gram-positive and Gram-negative bacteria like Klebsiella pneumonia and Escherichia coli are included in this group class A beta-lactamases group. Stenotrophomonas maltophilia, Bacteroides fragilis, Aeromonas, and Legionella hy- drolyze carbapenems as well as penicillin and cephalosporins are in class B beta-lactamases. Cephalosporins are usually found in Gram-negative bacteria. Produced in high levels in the presence of beta-lactam antibiotics inducible and they named as inducible beta lactamases (class C beta-lactamases) found on P.aeruginosa, Enterobacter cloacae, Citrobacter freundii, Serratia marcescens. Oxacillin degrades enzymes (oxacillin) produced by Gram-positive cocci of S.aureus that are induced by Class D beta-lactamases. Anaerobic bacteria like Bacil- lus fragilis also produce beta-lactamase namely cephalosporins. Gram-positive bacteria like Staphylococcus aureus possess peptidoglycan synthesis in the cell membrane and are respon- sible for penicillin-binding proteins, transpeptidase, and PBPs carboxypeptidase enzymes. Methicillin-resistant staphylococcus aureus is responsible for methicillin resistance in strains. S.pneumoniae is responsible for penicillin and cephalosporin resistance. Gram-negative bac- teria like P.aeruginosa possess porin protein channels which may develop resistance to carbapenems, beta-lactams, tetracyclines, chloramphenicol, and quinolones. (3)
2. Aminoglycoside group antibiotics resistance: Aerobic Gram-negative bacteria play an im- portant role in the aminoglycoside antibiotic resistance mechanism by enzymatic inactivation in which enzymes change the structural molecule of the aminoglycoside group. Acetyl trans- ferases, nucleotidyltransferases, and phosphotransferases are enzymes involved in the high resistance to gentamycin in enterococci., Decreased cell permeability by the passage of drug to the cytoplasm by anaerobic bacteria, Increased efflux, Modifications of the 30S ribosome's subunits that encountered with aminoglycosides, and finally point mutations and posttran- scriptional modification entered into aminoglycoside resistance. (4)
3. Tetracycline resistance: Three general mechanisms have been involved. efflux, ribosomal protection, and enzymatic inactivation of drugs. Binding to bacterial ribosomes and interact- ing with a highly 16S (rRNA) target in the 30S ribosomal subunit & interfere with aminoacyl- transfer RNA during elongation of protein synthesis. (7) Tetracycline Specific Efflux pumps extrude tetracycline antibiotics from the inside of cells at the expense of a proton and have been assigned to seven different groups. (7) In Gram-negative bacterial cell- like E.coli, tetra- cycline undergoes passive diffusion through the outer membrane which produces porins OmpF and OmpC. (5,6,7)While in Gram-positive bacteria mobile genetic elements carry re- sistance genes, mutations within the ribosomal binding site, and mutations causing induced expression of intrinsic pathways. (8) Enzymatic inactivation of tetracyclines encoded by a Bacteroides plasmid expressed in E.coli and this activity was subsequently characterized as a flavin-dependent monooxygenase, capable of covalently inactivating all tetracyclines. (9)
4. Macrolide, Lincosamide, and Streptogramin (MLS) resistance: In Gram-negative bacteria, impermeability occurs which have hydrophobic outer membranes and are naturally resistant to these MLS antibiotics. (3) whereas Gram-positive cocci possess two mechanisms of re- sistance, N6- dimethylation of adenine in 23S rRNA undergoes by erythromycin-ribosomal methylase is encoded by erm genes. (12) Structural changes to 23S rRNA prevent macrolide binding and allow bacterial protein synthesis to continue and bacteria carrying the gene en- coding macrolide efflux gene (mefE gene). (10) inactivation of streptogramin-B (STG-B) and lincosamide by the products of the such (encoding streptogramin B hydrolase) and linA' (en- coding 3-lincomycin 4-clindamycin O-nucleotidyltransferase) genes, respectively; and active efflux of Mac and STG-B antibiotics determined by the more and more genes in Staphylo- coccus epidermidis and Staphylococcus xylosus, respectively, both of which appear to act as an ATP-dependent efflux pump. (11)

5. Chloramphenicol resistance: Chloramphenicol is a broad-spectrum antibiotic naturally pro- duced by streptomyces after a decade. The most common mechanism of resistance is enzymatic inactivation by acetylation mainly by acetyltransferases and chloramphenicol phosphotransferases. (14) Due to target site mutation/modification, decreased outer mem- brane permeability, and the presence of efflux pumps act as multidrug extrusion transporters.

(15) Chloramphenicol-dependent inhibition of bacterial protein biosynthesis is mainly due to the prevention of peptide chain elongation. It is based on a reversible binding of 70S rRNA. It can be inactivated by acetylation and also other ways such as dehalogenation, glucuronida- tion, and reduction of the nitro group. (16)

6. Fluoroquinolones resistance: Resistance to fluoroquinolones arises as a result of alteration in the target enzymes (DNA gyrase and topoisomerase IV), change in drug entry, and efflux. The mechanisms of resistance to quinolones areas ., mutations that alter the drug targets, mu- tations that reduce drug accumulation, and plasmids that protect cells from the lethal effect of quinolones. It can target the cell wall and cell membrane of Gram-positive bacteria and in Gram-negative bacteria, it can regulate membrane permeability by altering outer membrane porin proteins for passive diffusion. Decrease in drug accumulation by mutations that impair target enzyme function and plasmid-mediated quinolones are involved in protein-protein in- teractions and purified Qnr protein bind to and protects both DNA gyrase and topoisomerase IV from inhibition. (17)

7. Rifampicin resistance: In Gram-positive bacteria such as Mycobacteria species undergo re- sistance to Rifampicin by a mutation that alters DNA-RNA synthesis by binding with the beta- subunit of DNA-dependent RNA-polymerase responsible for DNA transcription. (18)

In Mycobacterium tuberculosis, both gene mutation and an efflux pump mechanism play a major role in the resistance mechanism, and in transcription -level analysis among non-mutated isolates shows three efflux pumps are involved in exporting drug from the cell. (19)

8. Sulfonamide and trimethoprim resistance: By targeting the sulfonamide resistance is medi- ated by the enzyme dihydropteroate synthase in prokaryotes plays a crucial role in the folic acid pathway, whereas mammalian cells are not dependent on the endogenous synthesis of folic acid it lacks enzyme DHPS. In many pathogenic bacteria, sulfonamide resistance is me- diated by the horizontal transfer of foreign folP. (20) In Gram-negative bacteria are plasmid- borne and are caused by gene encoding alternative drug-resistance variants of the DHPS en- zymes. Escherichia coli, Shigella, and Campylobacter are resistant to sulfonamides and trimethoprim. (21) For trimethoprim, dihydrofolate reductases enzyme-mediated by transpos- ons and plasmids are mainly due to the horizontal spread of resistance genes. (22)

9. Glycopeptide antibiotic resistance: Glycopeptide groups mainly include vancomycin, teicoplanin, Telavancin, corbomycin) and vancomycin bind to the c-terminal of acyl-D-alanyl D-Alanine dipeptide at the end of the peptidoglycan layer and inhibit bacterial cell wall syn- thesis in Gram-positive bacteria by glycosylases and peptidases enzymes involved in transglycosylation and transpeptidation in peptidoglycan synthesis pathways, whereas these antibiotics cannot enter the outer membrane of Gram-Negative bacteria and are naturally re- sistant to vancomycin and vancomycin-resistant enterococci is a deadly recommended therapy for a major problem with methicillin-resistant staphylococcus aureus infections. (23) Inducible glycopeptides resistance (VanA type) is mediated by plasmids and genes carried by transposons. (24)

Methodology for detecting antibiotic susceptibility testing:

Over a decade, Invitro methods have been widely accepted and performed in detecting the antibi- otic susceptibility tests were as Disk diffusion, Agar dilution - Broth macro dilution, Broth microdilution, E test, and a concentration gradient test. When compared to the manual method, Automated instrumental systems also can be performed to detect antibiotic resistance in a quick and accurate method. However, also can be done by molecular testing in detecting antibiotic re- sistance assays were DNA microarray, PCR, DNA chips, and loop-mediated isothermal amplification (LAMP)

Disk diffusion test: The purpose of the disk diffusion test by the Kirby-Bauer susceptibility method is simple, it does not require any special equipment and is also well-standardized to per- form and the viability can be determined using the various antimicrobial compounds and to measure the sensitivity or resistance of pathogenic aerobic and facultatively anaerobic bacteria on the agar plate. Approximately 1-2x10 8 CFU/ml of bacterial inoculum are applied on the Mueller- Hinton agar with 150 mm in diameter has been used for the growth of organisms with the presence of 12 prepared various antimicrobial impregnated filter paper disks with a size of 6mm. The plates are incubated at 35 degrees Celsius for a 16-24 hrs period The presence or absence

of the growth of microorganisms can be determined by the measurement of a zone of inhibition around the anti- microbial disk and the diameter can be measured through a measuring scale. The determined results are qualitative (ie., susceptible, intermediate, or resistant) and zones of size can be com- pared with the commercially-available zone reader systems published by the Clinical and Laboratory Standards Institute. (CLSI) (25)

Broth macro dilution test: In earlier days, the Broth dilution test has named macro broth dilution or tube-dilution method and it can be performed by using test tubes containing a liquid medium dispensed by two-fold dilutions of antibiotics ie., 1.2.4.8) and standardized bacterial inoculum of 1-5x 10 5 CFU/ml are inoculated in an antibiotic tube and incubated at 35 c for overnight temper- ature in an incubator and visible bacterial growth with turbidity can be examined and lowest concentration prevents the growth that shows the minimal inhibitory concentration (MIC). And compare to the disk method, it is a quantitative result in measuring the concentration of a drug. The possibility of errors when performing the test, antibiotic preparation, the large number of re- agents, and space required for the test. (25)

Broth microdilution test: In this method, use small, disposable, plastic microdilution trays it con- tains 96 wells each containing volume of 0.1 ml that make 12 antibiotics to be tested in a range of 8 two-fold dilutions in a single tray. Microdilution panels are prepared using dispensing aliquots in the volume of diluted antibiotics in broth into the individual wells. Inoculation of panels with the standard 5x 10 5 CFU/ml using a disposable instrument that transfers 0.01 to 0.05 ml of stand- ardized bacterial suspension into each well of the microdilution tray followed by incubation. MIC can be determined using an automated examining device for bacterial growth. The most valuable advantages of this method are convenience, less space, and economy reagents, and drawbacks that come across in this method are the inflexibility of drug selections in standard commercial panels. (25)

Concentration gradient method: This method uses the principle of an antimicrobial concentra- tion gradient in agar in determining susceptibility. It underlies thin plastic strips which are impregnated with a dried antibiotic concentration gradient and shown on the upper part of the antibiotic strip. Around 5-6 strips can be placed radially manner on the surface of a 150mm Mueller-Hinton agar plate that has been inoculated with a standardized organism suspension as that for disk diffusion test followed by overnight incubation. The test is examined by viewing the intersection of the lower part of the ellipse-shaped growth inhibition area with the test strip. It has flexibility by being able to test the drugs in laboratory use. It is likely chosen to need 1-2 drugs for fastidious organisms. It can be determined the systematic higher biases toward MICs of certain organisms. (25)

Agar dilution method: For anaerobic bacteria, the Agar-dilution method is the common reference standardized method and can be performed in a petri dish likely to detect the MICs of multiple tested strains in a single plate. Two-fold dilution of standard antibiotic agents and incorporated into molten agar plates in different concentrations. Medium gets inoculated with standardized sus- pensions adjusted to 0.5 Mcfarland standard containing a concentration of 5x 108 CFU/ml. Around 107 CFU/ml and 2 ul of bacterial diluted with multi-points into agar plates contain approximately 104 CFU/spot on one agar plate as control seeded without an antibacterial agent. After incubation period under the anaerobic condition at 35 c for 16-18 hrs for non-fastidious and 18-24 hrs for fastidious organisms. Determination of MICs is based on the lowest concentration of drug tested that entrapped the bacterial growth. It is recommended by CLSI for fastidious bacteria and anaer- obic bacteria like campylobacter species and helicobacter, and also for antifungal detection tests. (25)

Automated instrumental systems: As it is a part of the automated method, it is well-standardized in reading the endpoints and produces susceptibility test results in a shorter period than the manual method. It is likely the presence of optical detection systems which is very sensitive to detecting bacterial growth. As of now, FDA has recommended and approved four automated systems. Among four systems, three of them are generated rapid and accurate, precise test results while the fourth is an overnight system.

The Microscan walkaway(From Seimens healthcare diagnostics) is a large self-contained incuba- tor that can incubate and analyze 40-96 microdilution trays. These trays are hydrated and inoculated manually and then placed in an incubator slot in the instrument and examined periodi- cally with a photometer to determine bacterial growth. Separate Gram-positive and Gram-negative panels can be read using turbidimetric endpoints in 4.5-18 hrs.

The BD phoenix automated system has an incubator reader with a capacity of 99 panels with 84 wells for antibiotic dilutions and is operated manually. It takes 20 mins for every panel to process in both turbidometric

and colorimetric growth and can detect both Gram-positive and Gram-neg- ative bacteria and results can be detected within 6-16 hrs.

The Vitek2 (Biomerieux) system is an automated and very compact plastic reagent card that con- tains a microliter of antibiotics and test media in 64 wells. This method uses turbidometric growth in an incubation period. It can be configured to accumulate 30-240 tests and allows Gram-positive and Gram-negative aerobic bacteria.

The Sensititre ARIS 2X (Trek diagnostic) is an automated, overnight incubator method and read- ing with a capacity of 64 panels which is highly standard 96 well microdilution plates inoculated with an auto incubator. Growth is determined by fluorescence in 18-24 hrs incubation and the panel encounters to detect both Gram-positive and Gram-Negative bacteria and also non-ferment- ative Gram-Negative bacilli. (25)

Isothermal microcalorimetry

Isothermal microcalorimetry is a heat-inducing calorimetric detection method where the heat gets generated or consumed by various processes in microorganisms and production can be measured in parallel with growth curves and with growth phases of organisms such as lag, log, and stationary phases. The antibiotic dose increases gradually only when the rate of heat production is due to the resistance mechanisms and is energy-dependent and decays suddenly decreases once it losses its activity and energy and occurrence of the ultimate destruction of bacteria. This method is to deter- mine the Gram-Positive and Gram-Negative bacteria such as Staphylococcus species, Escherichia. coli, and Mycobacterium species. (26)

Polymerase Chain reaction-based methods

This method is a molecular-based genotypic assay to detect the microbial pathogen that is highly specific on DNA sequence amplification-based and also to detect encoded resistance genes in Gram-Negative bacteria and Gram-positive bacteria. Loop-mediated isothermal amplification also genotypic assay to detect the antibiotic resistance and susceptibility using PCR enzyme at a con- stant temperature of 60-65 C, however, in real-time PCR using hydrolysis probes, double-stranded DNA-binding fluorescent dyes can enumerate the number of nucleic acid copies in a bacterial sample for growth and also measuring phenotypic resistance. (26) Examples: Methicillin-re- sistance encoding mecA gene in Staphylococci, Rifampicin resistance in Mycobacterium tuberculosis. Nucleic acid-based technology has two systems such as hybridization and amplifica- tion.

MALDI-TOF-MS (Matrix-assisted laser desorption/ionization-time of flight mass spectrometry)

This is a device that analyzed large biomolecules and strains that can be differentiated by ribosomal proteins and can directly identify colonies of Gram-Negative bacteria and Gram-Positive bacteria without possessing the metabolic activity. Cocrystallisation is the first step where the crystal is created by agitating the microbial sample and the matrix has formed and placed on the target plate to dry at normal room temperature and continue to do processes up to 384 samples per plate. It is then further kept in a machine and transported in a vacuum chamber. Through an electromagnetic field from the laser, the beams produces from the laser desorb the proteins of the bacteria, and ions get accelerated in a pulsed fashion into a linear flight tube. Time-of-flight detects the molecules of ionized particles using the mass spectrometry detector at the vacuum flight tube end.

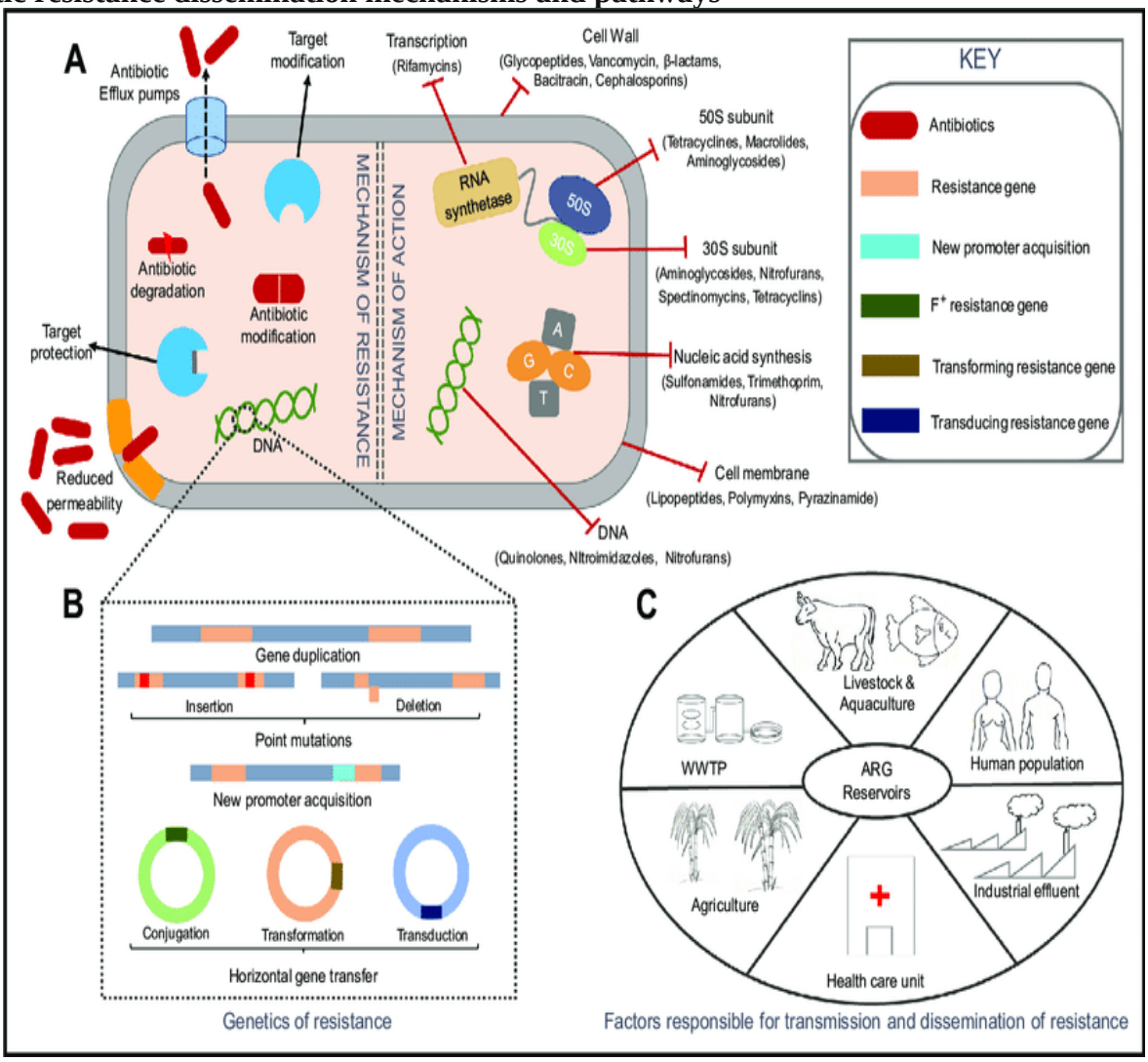
Microfluidic devices and microdroplets

In nanotechnology and bioengineering molecular assays, microfluidic methods use lab-on-chip techniques and they can be designated for cell lysis, and nucleic acid amplification to detect anti- biotic susceptibility and toxicity testing antibiotics. It requires microchannels measures of 10-100 micrometer, and a very small volume of reagent and nanoliter analyte can flow through micro- channels on one chip and detect by a detector using a microcalorimeter. As the size of the chip is too small and so this method can be performed by portable devices a self-loading microfluidic instrument that determines the MIC. Chambers can be molded by a polydimethylsiloxane layer filled with dried antibiotics and reversibly sealed with a second layer containing channels connect- ing the chambers. To detect the MIC, the pH indicator is used for bacterial growth indication that can degrade the activity of indicators such as glucose and phenol red and produces organic acids and can be observed by colorimetry. Reflective interferometric spectroscopy is the most effective method in the mean of evaluating the swelling and shrinking activity by which pH-responsive chitosan hydrogel gets immobilized through a microfluidic channel and undergoes swells and shrinks in response to pH changes and optical thickness determines the activity of bacterial growth and growth curves can be generated within two hours,

however, in the microfluidic agarose chan- nel system determines the MIC of single bacterial growth time and undergoes different antibiotic culture conditions and CLSI has tested the three bacteria with different antibiotics within 3-4 hours.

The Microdroplet method is a fluorescent-based method in which nanodroplets or microdroplets of microorganisms can be emitted through fluorescence signal detected and measured the growth of bacteria and compare with reference databases and the entire process takes place in a small transport reaction vessel where the microorganisms get embedded and the inverted droplets can be manipulated and carried out in the dispersed liquid phase and then into the continuous phase, hence these two phases are immiscible liquids to measure the activity and viability of the bacteria. (26)

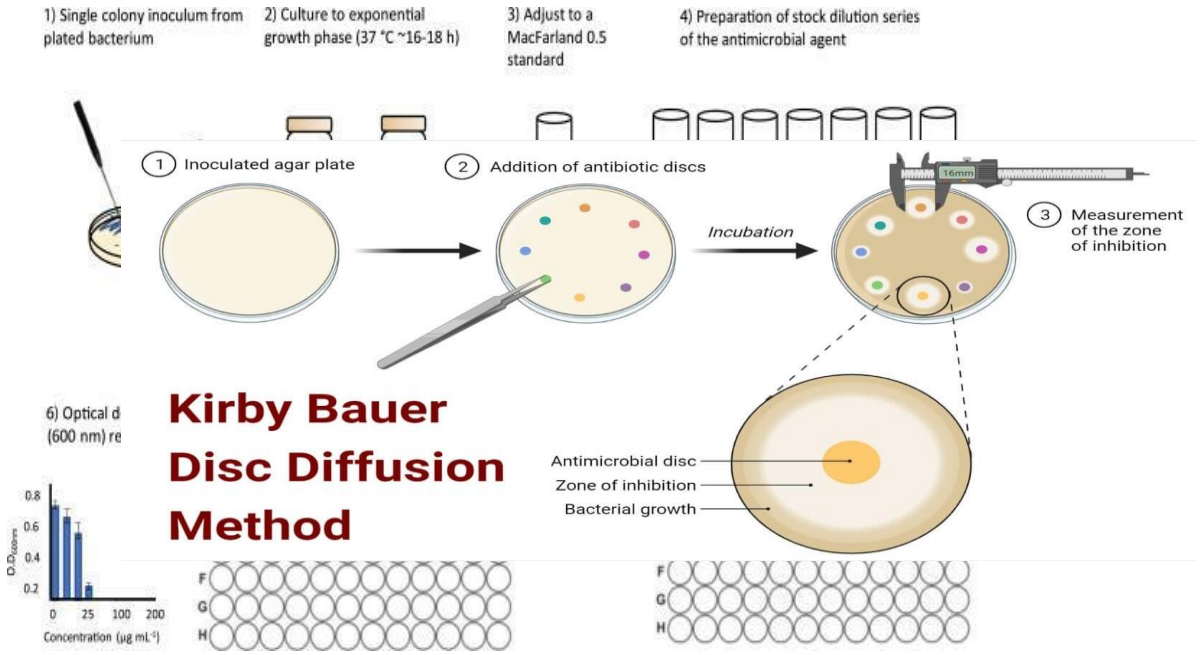
Antibiotic resistance dissemination mechanisms and pathways



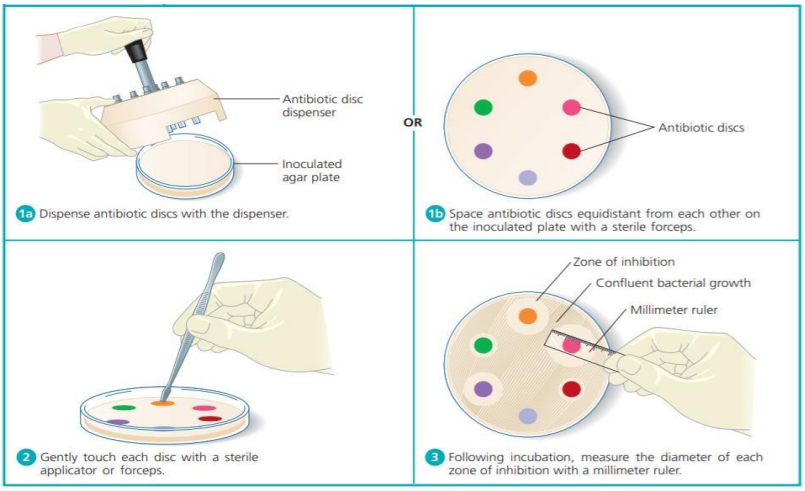
(A) Schematic overview of antibiotic modes of action, and subsequent mechanisms of antibiotic resistance,
(B) mechanistic insight into the genetic basis of antibiotic resistance and modes of dissemination,
(C) Schematic overview of genetic factors governing the dissemination of acquired antibiotic re- sistance gene. (13)

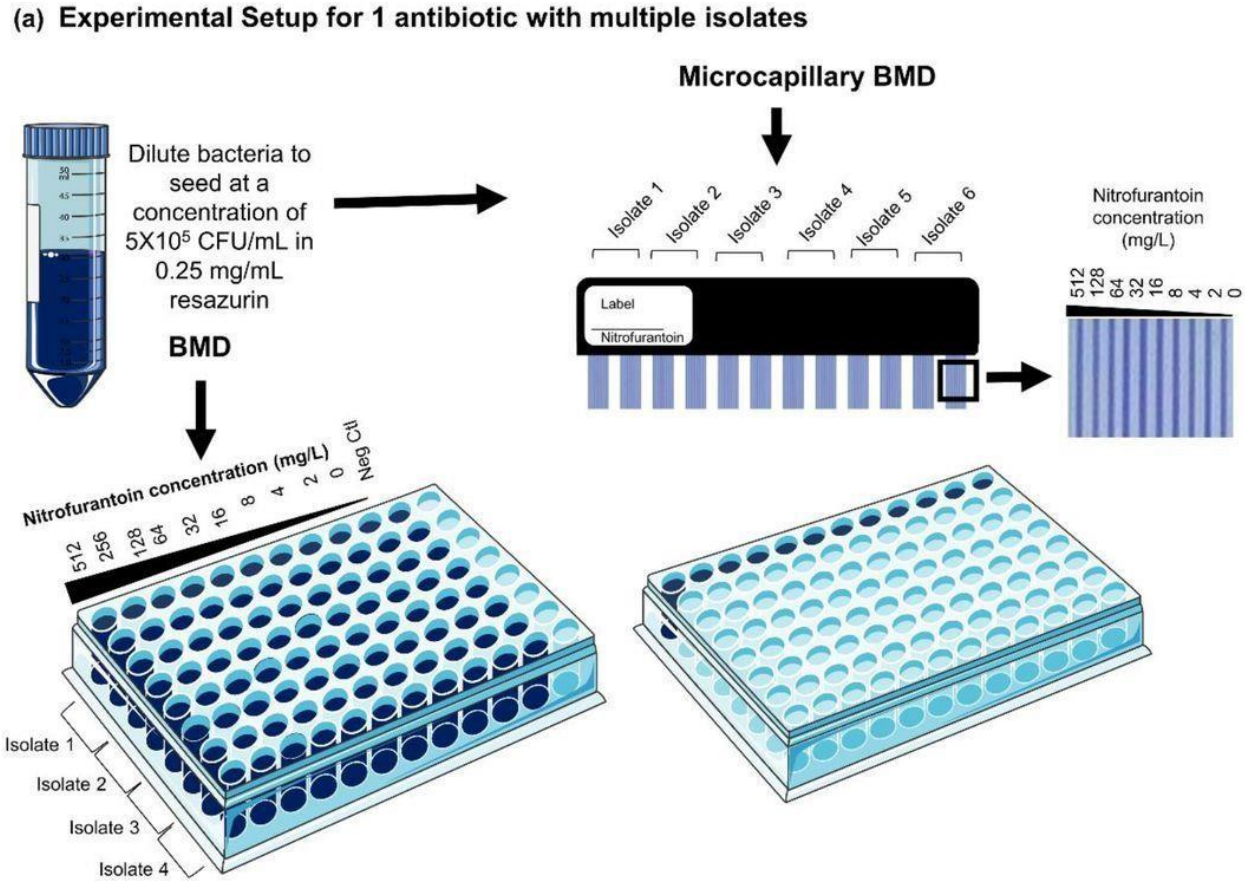
Kirby Bauer Method (Old Method)

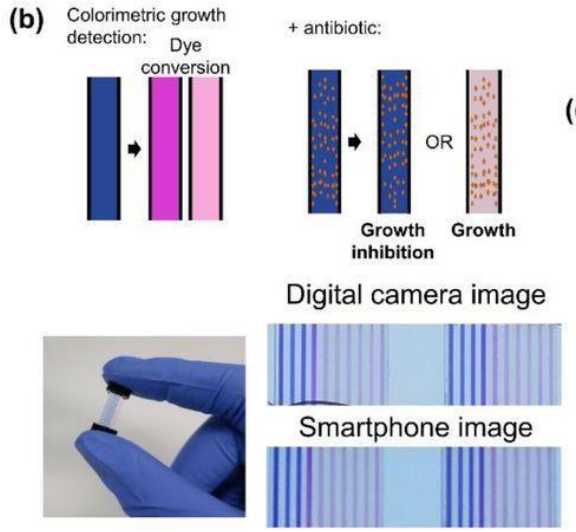
Modified Kirby Bauer Disc diffusion Method

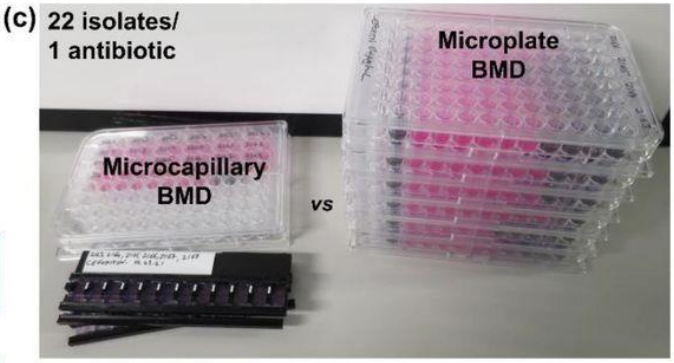


Broth Macro Dilution Method



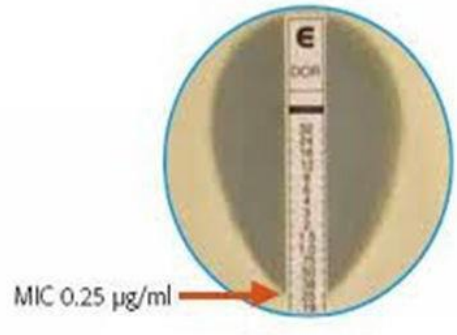

(a) Experimental Setup for 1 antibiotic with multiple isolates
Microcapillary BMD
Dilute bacteria to seed at a concentration of 5X10^5 CFU/mL in 0.25 mg/mL resazurin
BMD
Isolate 1
Isolate 2
Isolate 3
Isolate 4
Isolate 5
Isolate 6
Label
Nitrofurantoin
Nitrofurantoin concentration (mg/L)
512
128
64
32
16
8
4
2
0
Nitrofurantoin concentration (mg/L)
512
256
128
64
32
16
8
4
2
0
Neg Ctl
Isolate 1
Isolate 2
Isolate 3
Isolate 4

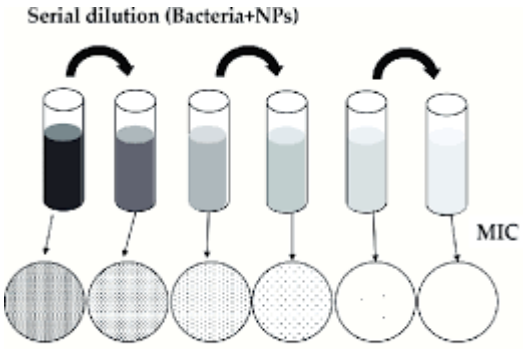

(b)
Colorimetric growth detection:
Dye conversion
+ antibiotic:
OR
Growth inhibition
Growth
Digital camera image
Smartphone image


(c)
22 isolates/ 1 antibiotic
Microplate BMD
Microcapillary BMD
vs

E- test/ Gradient Diffusion Method

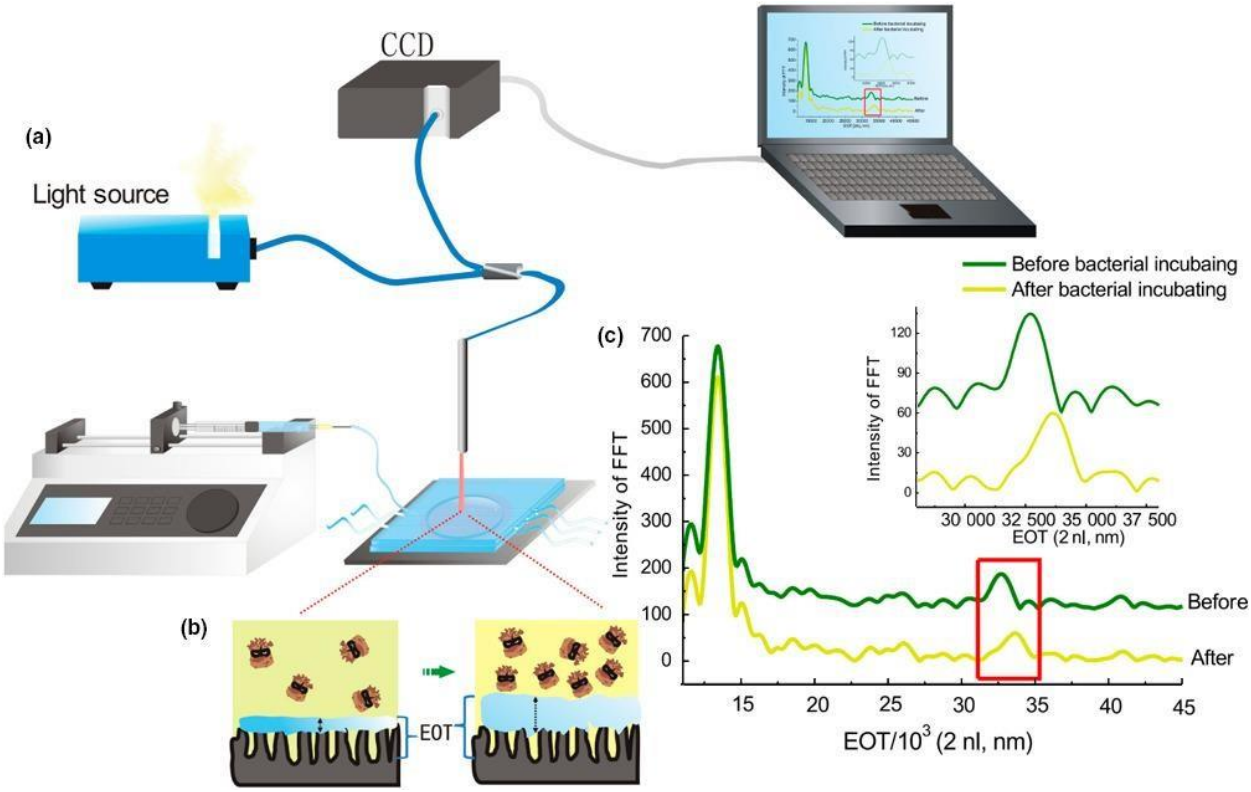
- "MIC on a stick"
- Plastic strips impregnated with antimicrobial on one side
- MIC scale on the other side
- Read MIC where zone of inhibition intersects E strip scale





Agar di

Microfluidics and Microdroplets Method



a) Instrumental setup of the microfluidic sensor – camera, Light source from a tungsten lamp, syringe pump, pH response chitosan hydrogel incorporated in a microfluidic chan- nel, PC as a data collector
b) Monitoring of bacterial metabolic activity and cell growth

Recent Developments

As per research and their recent developments correlate to antibiotic resistance in molecular techniques such as molecular beacons and DNA chips are very well coincided with Mycobacte- rium tuberculosis. DNA chip technology has used the rpo B gene for sequencing and identified two point mutations in all nine isolates that have suitable for the detection, However, molecular beacons designed five fluorogenic probes in five separated PCRs, which are monitored in real- time. (27)

Prevention and control measures of antibiotic resistance

To overcome such untreatable diseases caused by antibiotic resistance and most of the preventive control measures can come across globally by protecting ourselves by regular washing our hands properly, recognizing the occurrence of infection in early-stage, Administered proper medicines by a medical practitioner, Not using leftover antibiotics administered by a medical practitioner, Safely food preparation, vaccinated and immunized to prevent illness. (28)

According to the Centers for Disease Control and Prevention, the US government has taken nec- essary action by responding to treat ongoing antibiotic-resistant threats, Strengthening the detection of antibiotic resistance, Improving antibiotic use, and reporting how and when to be used, Recent development of antibiotic resistance diagnostics, Exaggerate research on new antibiotics exposure to patients, Improving newer prevention measures to overcome antibiotic-resistant infec- tions. (29)

By having a strong tie-up with CDC data, collaborations with CMS & AHRQ recommend a unique way to make in reducing antibiotic-resistant infections, expanding the HAI/AR programs. CDC has created AR Laboratory Network by Nationwide testing to fill data gaps, Forecasting inform prevention and response, Forecasting changes in resistance for hard-to-treat microbes, Sentinel surveillance, with robust testing and standardized alert values, for new and unusual resistance threats, and Pilot strategies to collect public health data.

Whole-genome sequencing provides a DNA fingerprint that enables to detection of genes that make bacteria resistant to antibiotics and is also built up to conduct the program and enhance investigations, and patient care.

(30)

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