ANTIMICROBIAL RESISTANCE (AMR) AND ITS DETECTION METHODS

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Antimicrobial resistance:

Antimicrobial resistance (AMR) is the non-responsiveness of microorganisms to antimicrobial agents in standard doses making it difficult to treat infectious diseases. Emergence of antibiotic resistance in bacterial pathogens is recognized as a major public health threat affecting humans worldwide and World Health Organisation has named antibiotic resistance as one of the three most important public health threats of the 21stcentury. AMR can affect anyone, of any age, in any country and is a threat to food security and sustainable development. Globally, AMR is responsible for 7,00,000 deaths annually and is predicted that AMR might cause 10 million deaths per year by 2050. Economy-wiseAMR might cause USD 100 trillion loss and 3.5% reduction in GDP by 2050.

A recent analysis of the effect of AMR in 204 countries and territories in 2019 had estimated that 4.95 million deaths were associated, and 1.27 million deaths were attributable to bacterial AMR. The main bacteria involved with the deaths were *Escherichia coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Antimicrobials are vital for the treatment of bacterial infections in humans, terrestrial animals and aquatic animals. Antibiotics exert their antibacterial action by several mechanisms such as inhibiting the bacterial cell wall synthesis (penicillins, cephalosporins, monobactams, carbapenems), disrupting the bacterial cell membrane (polymyxin, colistin, daptomycin), inhibiting protein synthesis (phenicols, macrolides, tetracyclines) or inhibiting nucleic acid synthesis or replication (sulfonamides, trimethoprim, quinolones, fluroquinolones).

More and more species of bacteria are becoming resistant to existing antibiotics. Multidrug resistant (MDR)bacteriumis a strain that hasacquired resistance to at least one agent in three or more antimicrobial classes. Extremely drug resistant (XDR)bacteria aresusceptible to only one or two antimicrobial classes and Pan drug resistant (PDR) bacteriaare non-susceptibile to all categories of antimicrobials. The MDR, XDR and PDR superbugs are emerging due to multiple mutations which have the brutal power to enhance morbidity and mortality. There is lack of interest by the pharmaceutical companies to develop new antibiotics as the bacteria were becoming rapidly resistant to newly introduced antibiotics, thus making the business less profitable. The mean duration of resistance was reported to be 6.9 years.

Antibiotic resistance in bacterium is classified as 'intrinsic resistance' and 'acquired resistance'. Intrinsic resistance refers to the natural existence of genes in the bacteria that make the bacteria resistant to that particular antibiotic. For example, resistance to penicillin is expressed by most Gram-negative bacteria. *Aeromonas* spp., commonly found in freshwater aquaculture environments, have been reported to have intrinsic resistance to ampicillin. In clinical settings, the resistance is usually 'acquired', wherein the bacterial population that was originally susceptible to an antibiotic acquires resistance. Acquired antibiotic resistance results, either from mutations in the chromosomal gene (vertical transmission) or due to acquisition of resistance genes from other bacteria in the environment (horizontal gene transfer, HGT). Horizontal gene transfer occurs through uptake of free DNA by a competent bacterial cell (transformation) or by mobilization of bacterial DNA from one bacterial cell to another by a bacteriophage (transduction) or by mobilization of DNA from a donor bacterium to a recipient bacterium through conjugative machinery (conjugation). HGT is the most relevant mode of resistance emergence and spread in bacterial populations.

Bacteria develop resistance to antibiotics by adopting different strategies such as modifying the antimicrobial molecule, preventing the antibiotic from reaching

the target site, changing the antibiotic target sites and bypassing the antibiotic target sites.

a) *Altering the antibiotic:*Bacteria produce enzymes such as acetyltransferase, phosphotransferase, adenyltransferase, that introduce chemical changes in the antibiotic molecule and modify them leading to loss of their antimicrobial property. This type of resistance was reported towards chloramphenicol, aminoglycosides and lincosamides

b) *Destroying the antibiotic:*Bacteria produce enzymes such as â-lactamases, Extended Spectrum â-Lactamases (ESBLs), that completely destroy the antibiotic making it incapable of executing its antibacterial function. This type of resistance was reported againstpenicillins, cephalosporins and monobactams,

c) *Target protection:* Bacteria alter the ribosomal conformation. This type of resistance was reported towardstetracycline, fluoroquinolones andfusidic acid.

d) *Target modification:*Bacteria modify the target site and decrease the affinity of the antibiotic for the target site. This type of resistance was reported against fluoroquinolone, rifampin and erythromycin

e) *Decrease permeability to prevent antibiotic entry:*Bacteria develop mechanisms to prevent the antibiotic from reaching its intracellular or periplasmic target. This mechanism limits the influx of substances from the external environment. This type of resistance was reported towards â-lactams, tetracyclines and some fluoroquinolones

f) *Efflux Pumps to flush out antibiotics from the bacterial cell:* Efflux pumps are transport proteins involved in the extrusion of toxic substrates. Efflux pumps have been characterized in both Gram-negative and Gram-positive bacteria. This type of resistance was reported against fluoroquinolones, â-lactams, carbapenems and polymyxins.

Detection of antimicrobial resistance

AMR is detected phenotypically by employing the disk diffusion assay (qualitative) or by determining the minimum inhibitory concentration (quantitative). Genotypic methods are used to detect the antibiotic resistance genes either by conventional PCR (qualitative) or real time PCR (quantitative).

Disk diffusion assay is performed as per Kirby-Bauer method by placing six discs of selected antibiotics on Mueller-Hinton agar plates seeded with the target bacterium (1.5 x 10^8cfu/ml). The inhibition zone size is measured and the results are interpreted as per internationally recognized guidelines such as Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial susceptibility testing (EUCAT).

The minimum inhibitory concentration (MIC) method determines the lowest concentration of an antibiotic that inhibits the growth of the target bacterial isolate.MIC test can be performed either by liquid broth dilution methods (tube dilution, microbroth dilution) or solid agar dilution methods. The preferred method is the microbroth dilution method in 96 well plates using cation adjusted Mueller-Hinton broth and using the target bacterium at an inoculum level of 5 x 10^5cfu/ml.

E-test (epsilometer test) is also a quantitative method that uses an inert strip with a pre-defined gradient of specific antibiotic that is placed on a Mueller-Hinton agar plate seeded with the target bacteria. The MIC is derived from the symmetrical inhibition ellipse that is seen after the incubation period. It is necessary to regularly employ quality control strains to evaluate the performance of the antibiotic susceptibility test method in the laboratory as several factors such as media composition, inoculum size, quality of antibiotic discs, incubation temperature etc., severely affect the test results.The commonly used quality control strains were *E.coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923 and *Streptococcus pneumoniae* ATCC49619.

Interpretative criteria can be of two types viz., clinical breakpoints or epidemiological cut-off values. Clinical breakpoints categorize a bacterial isolate as

either sensitive (S), intermediate (I) or resistant (R) and indicate the most probable outcome of specific therapy of a specified infected host. 'Sensitive' indicates that using that particular antibiotic would be helpful in treating the infection whereas 'resistant' suggests that the clinical outcome of using that specific antibiotic would not have a positive therapeutic outcome.

On the other hand, epidemiological cut-off values categorize bacterial isolates as wild type (fully susceptible) or non-wild type (reduced susceptibility) when compared to other members of its species. Advanced tools such as antibiotic resistance gene detection chips,microarray expression analysis, transcriptomics, metatranscriptomics, functional metagenomics, next-generation sequencing, single moleculereal-time sequencing methods werealso used for diagnostics and AMR deciphering.

The 'One Health' strategy i.e. involving the human, animal, food and the environment was adopted by the 'Quadripartite' namely Food and Agriculture Organization (FAO), World Health Organization (WHO), World Organisation of Animal Health (WOAH) and United Nation Environment Programme (UNEP) for addressing the menace of AMR. The Global Action Plan on AMR in 2015 and India's National Action Plan on AMR in 2017 were prepared with the 'One Health' centric approach. On similar line Kerala (2018), Madhya Pradesh (2019), Delhi (2020) and Andhra Pradesh (2022) have prepared the State Action Plans to combat AMR. This indicate the significance and thrust being given at the global, national and state level to mitigate AMR and save human and animal life.