INTRODUCTION

It is difficult for modern physicians to appreciate the impact that the sudden availability of antibiotics had on the practice of medicine in the 1930s and 1940s (Spellberg, 2009).

The medical community failed to understand that microbes have been waging war among themselves with antibiotics, and creating resistance mechanisms to defeat antibiotics, for more than two billion years. We will never ‘defeat’ microbes with antibiotics (Spellberg et al., 2008).

The global spread of carbapenem-resistant Enterobacteriaceae (CRE) has become a major challenge in clinical and public health settings. Infections with CRE organisms that are multidrug-resistant (that is, non-susceptible to at least one antimicrobial in at least three antimicrobial classes), extensively drug-resistant (that is, non-susceptible to at least one antimicrobial from all but one or two antimicrobial classes), or pan-drug-resistant (that is, nonsusceptible to all antimicrobial agents) are difficult to treat (Magiorakos et al., 2012).

A prospective intervention of having an infectious disease specialist interact regularly with the medical ICU team was conducted to assess guideline compliance and antibiotic and health-care costs; it achieved significantly reduced use of extended spectrum penicillins, carbapenems, vancomycin, and metronidazole (Rimaw et al., 2013).
Specifically, the intervention group had a significantly lower rate of treatments not corresponding to guidelines, with fewer MV days, shorter stays, and lower in-hospital mortality (Rimawi et al., 2013).

Optimal antibiotic use is crucial in the critical care setting, especially in an era of rising antibiotic resistance and lack of new antimicrobial development (Leuthner et al., 2013). Study results indicate that 30% to 60% of antibiotics prescribed in ICUs are unnecessary, inappropriate, or suboptimal (Kollef et al., 2001). Overprescribing and misprescribing antibiotics are undoubtedly contributing to the growing challenges posed by antibiotic resistant bacteria, and epidemiological studies have clearly demonstrated direct relationships between antibiotic consumption and the emergence and dissemination of resistant strains in hospitals and ICUs (Kollef et al., 2001).

More than 140 antibiotics have been developed for use in humans over the past 80 years (Spellberg et al., 2009). Thus, we face considerable scientific barriers to discovering the next generation of antibiotics because the low-hanging fruit has been plucked.

Using the same screening methodologies and the same chemical libraries tends to identify the same lead scaffolds over and over again (Spellberg et al., 2011).

Most published observational data suggest that the time to appropriate antibiotic administration is a major outcome determinant for ICU patients with severe bacterial infections. However, owing to methodological concerns, the harmful effects of inadequate therapy are not accepted by all (Amaral et al., 2014).
In ICU, signs and symptoms of infection due to non-infectious causes are common, so rushing to prescribe antibiotics may mean that many uninfected patients receive unnecessary treatment (Yahav et al., 2013).

Optimizing in-ICU antimicrobial therapy is difficult. No single measure alone can succeed, emphasizing the need to devise a structured antibiotic stewardship program (Yahav et al., 2013).

Unfortunately, the exact set of key interventions essential to this multifaceted and multidisciplinary ‘care bundle’ remains unknown, as do the factors contributing to its success (Leuthner et al., 2013) (Cotta MO et al., 2014).

Several parenteral antimicrobial therapies are currently under investigation for the treatment of multidrug-resistant Gram-negative infections, including CRE. Ceftazidime-avibactam (a new beta-lactamase inhibitor) is active against extended-spectrum beta-lactamase-producing organisms, some resistant Pseudomonas aeruginosa strains, and CRE of the KPC type, but not against metallo-beta-lactamases such as New Delhi metallo-beta-lactamase and Verona integron-encoded metallo-beta-lactamase. It is currently undergoing phase 3 studies for complicated UTI and intraabdominal infections (Boucher et al., 2013).
AIM OF THE STUDY

To identify causes of spread of antibiotics resistance in ICU, and trying to find strategies to follow to decrease this resistance and decrease the rapid emergence and dissemination of antimicrobial-resistant microorganisms in ICUs.
Antibacterial Resistance

General concepts:

Resistance is a measure of decreased ability of an antimicrobial agent to kill or inhibit the growth of a microbial organism. In practice, this is determined by testing a patient isolate against an antimicrobial in an in vitro assay system (Fraimow and Tsigrelis, 2011).

For bacteria, the common in vitro testing system are automated liquid media microdilution system, disc diffusion, and the epsilometer test (E-test). For quantitative systems like broth microdilution or E-test, the measure of drug activity is the minimum inhibitory concentration (MIC) (Fraimow and Tsigrelis, 2011).

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents (Andrew, 2001). A lower MIC is an indication of better antimicrobial agent. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism (Turnidge et al., 2003).

From testing large number of isolates, breakpoints that define the threshold of susceptibility for each organism-drug combination are established by groups such as the US Clinical and Laboratory Standards institutes (CLSI) and the Eureapen Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoints are included in the US Food and Drug Administration (FDA)-approved product labeling for new
antibacterial agents (*Fraimow and Tsigrelis, 2011*).
A strain reported as susceptible in vitro has an MIC value at or below the defined susceptibility breakpoints, which is believed to correlate with high likelihood of therapeutic success (Fraimow and Tsigrelis, 2011).

For strains reported as intermediate or indeterminate, therapeutic effect is uncertain; for strains reported as resistant, use of that agent is associated with high likelihood of therapeutic failure (Woodford and Sundsfjord, 2005).

Some resistance traits are not reliably detected by standard method, and require additional microbiologic or molecular confirmatory testing which may lead to delay and increased cost for correctly identifying resistant organism (Woodford and Sundsfjord, 2005).

Intrinsic resistance is an inherent failure of a species resulting in the lack of activity of a drug or drug class. Intrinsic resistance may be due to such factors as lack of the appropriate antimicrobial target, inability of the drug to access target, or presence of species-wide antimicrobial inactivating enzymes (Fraimow and Tsigrelis, 2011).

An example is the intrinsic resistance of gram-negative organism to the glycopeptides vancomycin and teicoplanin, which cannot penetrate the outer membrane to reach their target. Circumstantial resistance reflects the disparity between in vitro and in vivo activity. Antibiotics that are active in vitro may not be clinically effective, due to lack of drug penetration to protected sites such as cerebrospinal fluid, or the inactivity of drug at low pH or in an anaerobic environment (Fraimow and Tsigrelis, 2011).

Foucsing in acquired resistance: a change in phenotypic characteristics of an organism resulting in decreased effectiveness of a previously active drug. Acquired resistance is a natural consequence of
genetically adaptable microorganisms responding to selective pressure of antimicrobial agent (*Fraimow and Tsigrelis, 2011*).

The phenotype of acquired resistance has a genotypic correlate, although the genetics of some resistance traits remain poorly characterized. Some important acquired traits can be directly selected for in vitro and in vivo via one or several points mutations in antimicrobial target genes. There are many important resistance phenotypes, such as methicillin resistance in Staphylococci, that cannot be selected for in vitro or in vivo, and only occur through susceptible organisms acquiring exogenous genetic material (*Fraimow and Tsigrelis, 2011*).

**Molecular genetics of antibiotic resistance:**

Analysis of bacteria collected before widespread introduction of antibiotics reveals, excluding intrinsic resistance, almost complete sensitivity. Organism with intrinsic resistance are often of low virulence but do become a problem in vulnerable patients managed in selection pressure environments (*pseudomonas, Acinetobacter*). Acquisition is based on the mechanism of genetic mutation and inter-cell transfer. Mutation is often disadvantageous to the bacteria but will, infrequently, affect antibiotic resistance. However, the transfer of resistance between bacteria is of greater importance, the mechanism of which are not mutually exclusive (*Vareley et al., 2009*).

**1-Naked DNA (transformation)**

Naked DNA is released from killed bacteria and as such is common in the ICU patient on antibiotic. DNA in this form is unprotected and is quickly degraded. Bacteria have a varying ability to take up this DNA. Transformation is the process where this DNA is incorporated into the genome of another bacterium (*Varely et al., 2009*).
2- Bacteriophages (transduction)

These are viruses that infect bacteria. A protein coat protects the DNA within and the virus relies on the bacteria's cellular machinery to propagate it. The DNA within the virus may be exchanged or transferred to the host, and through this mechanism can transfer genetic information encoding resistance. This is known as transduction. Bacteria vary in their susceptibility to infection with bacteriophages; Coryne- bacterium diptheriae and Vibrio cholerae are examples of bacteria that commonly receive genetic information through this route (Varley et al., 2009).

3. Plasmids (conjugation)

These are self-replicating circles of DNA that exist within the bacteria but are separate from the chromosome Figure (1). They lack the protective coat of the bacteriophage and are unable to move independently of the bacteria. Despite these limitations, they are the most important routes of transmission of genetic information within the intensive care unit (Varley et al., 2009).

![Diagram of Bacterial DNA and Plasmids]

**Figure (1):** Illustration of a bacterium with plasmid enclosed showing chromosominal DNA and plasmids (Varley et al., 2009).

4. Transposons

These are small segments of DNA that can encode resistance genes. They also encode for their mobility, thus allowing them to move from plasmid to plasmid, or within the main genotype. As such they can transmit resistance between cells (Varley et al., 2009).
Evolution and spread of antimicrobial resistant organism:

In a patient exposed to antimicrobial agent, resistant organism can emerge by selection for and expansion of subpopulations of spontaneously generated, less susceptible mutants of antimicrobial target (Martinez et al., 2007).

More commonly, colonization or infection with drug-resistant organism results from super infection rather than by evolution of resistance in the original target organism.

New drug-resistant"invaders" are selected from organisms already part of the patient's endogenous flora, living on mucosal surface or in the gastrointestinal tract, or are newly acquired from the health care environment (Plesiat, 2010).

Emergence of resistant organisms and superinfection are both concerns in patients failing to respond to antimicrobial therapy, but there are multiple other reasons for therapeutic failure: inadequate source control, host immune status, and pharmacological issues of drug bioavailability and optimal dosing are only a few of these. Bacteria employs several basic strategies for evading the effects of antibiotics, including enzymatic modification and inactivation of antimicrobial agents, restriction of drug access to the cellular targets, and modification or even complete elimination of the target (Plesiat, 2010).

The most important classes of inactivating enzymes are the many b-lactamases in gram-positive and gram-negative bacteria and the aminoglycosides-modifying enzymes (AME) (Jacoby and munoz-price, 2005).

Restriction of drug target access can occur from alteration in membrane permeability to decrease drug entry, or by "trapping" of an
antimicrobial agent before access in the target (Jacoby and Munoz-price, 2005).

Target modification occur through mutations in target genes, such as the gyrase and topoisomerase targets of fluoroquinolones, by enzymatic modification of target genes, by introduction of new, non-susceptible targets such as the MecA protein in Staphylococci areus{ the MecA gene is a gene found in bacterial cells. The MecA gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin, erythromycin, tetracycline and the other penicillin-like antibiotics} (Ubukata et al., 1989), or through novel synthetic pathways like the Enterococcal vanAand vanB clusters that eliminate the bacteria's need for the original antimicrobial target (Jacoby and Munoz-price, 2005).

Levels of resistance are magnified by combining different mechanisms. For example, permeability changes and efflux pumps that decrease intracellular b-lactam concentrations enhance effectiveness of b-lactamases present in the gram-negative periplasmic space (Jacoby and Munoz-Price, 2005).

Organisms expressing acquired resistance traits can clonally disseminate, transmitting their resistance traits to their multiple descendants. The extra" work" required for maintaining resistance traits may result in decreased fitness of the organism, thus resistance may ultimately disappear in the absence of selective pressure. However, other resistance traits are relatively stable and persist even in the absence of antibiotic exposure (Andersson and Hughes, 2010).

Resistance genes or gene clusters can also be transmitted horizontally between organisms, as well as between species. Resistance genes are typically carried on transposons which are mobile genetic
Chapter (1)  Antibacterial resistance

elements that can move in and out of the bacterial chromosomes and into
plasmids, facilitating horizontal gene transfer. Unrelated resistances genes are often clustered together, enabling transfer of multiple resistances as a single package. Transfer of resistance occurs among gram-negative flora in the human gastrointestinal tract, and exchange of vanA resistance clusters in vivo from Enterococci to satphylococcus aureus has led to emergence of highly vancomycin-resistant Staphylococcus aureus (VRSA) (Rice, 2010).

**Multidrug resistant organisms:**

Multidrug-resistant (MDR) organism have acquired resistance to multiple unrelated classes of antimicrobials (Livermore, 2009).

Multidrug resistance can be selected by sequential exposure to different antibiotics, or by acquisition of multiple resistance traits clustered on mobile genetic elements. Some selectable resistance, such as permeability changes or up regulated broad range efflux pumps, can contribute to expression of resistance to multiple antimicrobials classes (Poole, 2006 a).

MDR pathogen of greatest concern in the hospital environment include methicillin-resistant Staphylococcus areus (MRSA), vancomycin-resistant enterococci (VRE), and drug-resistant Streptococcus pneumoniae (DRSP), and the MDR gram-negative bacteria (MDR-GNB) including Pseudomonas, Acinetobacter, Klebsiella pneumoniae, Enterobacter, and the other species (Boucher et al., 2009).