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Review Article

# ANTIBIOTIC RESISTANCE AND ITS DETECTION: ROLE OF SPECIFIC PROTEINS OF MULTIDRUG RESISTANCE (MDR) STRAINS.

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**ABSTRACT: Antibiotic resistance is emerging as a serious health problem at global level and it is acquiring alarming proportions in developing and developed countries recently. Multidrug resistant bacteria gain resistant genes by different mechanisms (Horizontal gene transfer) in developing resistance against a broad spectrum of antibiotics. Several mode of action to contain resistant pathogen has already been elucidated in numerous publications. Currently available methods of resistant pathogen diagnosis are not cost effective and require technical expertise which is largely unavailable to common masses. Appropriate method of identification and detection may lead to suitable course of treatment which in turn saves valuable resources and discomfort caused to patients. Present review explores the potential of selected proteins specifically present in MDR strain of Gram- negative bacteria to be exploited in diagnostic tools.**

**Key Words: Multidrug resistance (MDR), Horizontal gene transfer, Efflux, Diagnostics.**

## Introduction

Antibiotics have been critical in the fight against many diseases and infections. The gradual increase in resistance rates of several important pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus* (VRE), multidrug-resistant (MDR) *Pseudomonas aeruginosa*, imipenem-resistant *Acinetobacter baumannii*, and third-generation cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumonia*, poses a serious threat to public health (Meyer *et al*., 2010). The frequency of antibiotic resistance in many bacterial pathogens is increasing around the world and resulting in failures of antibiotic therapy that cause hundreds of thousands of deaths annually (Palmer and Kishony, 2013).

Multidrug efflux transporters are normal constituents of bacterial cells. These transporters are major contributors to intrinsic resistance of bacteria to many anti-microbial agents (Giamarellou *et al*., 2009). Intrinsic mechanism are those specified by naturally occurring gene found on host chromosome such as β-lactamase of gram negative bacteria and many MDR efflux system. There are five families of efflux pump proteins that are associated with multi drug resistance: The ATP binding cassette (ABC) superfamily, The major facilitator superfamily (MFS), The multidrug and toxic compound extrusion (MATE) family, The small multidrug resistance (SMR) family and resistance nodulation division (RND) family (Paulsen *et al*., 2003). Efflux pumps consist of a single or multiple components. Efflux systems in Gram-positive bacteria always comprise a single polypeptide located in the cytoplasmic membrane. The cell envelope of Gram-negative bacteria is a major barrier for antibiotics and consists of the plasma membrane, the periplasm and the outer membrane. The outer membrane is the major barrier for antibiotics antibiotics penetrate through porins or by passive diffusion through the outer membrane phospholipids (inner leaflet) -lipid A (outer leaflet) bilayer. The lipopolysaccharide (LPS) forms another barrier for many antibiotics but polycationic compounds such as gentamicin and colistin are being transported through the outer membrane via interaction with LPS in a process called self-promoted uptake. Antibiotic molecules then enter the cell from the periplasm either via partition into and passive diffusion through the plasma membrane or are actively transported via transporters into the cytoplasm. Efflux pumps of the resistance nodulation cell division (RND) superfamily are major players in antibiotic resistance of Gram-negative bacteria (Table 1). These tripartite systems span the entire cell envelope and are composed of an RND transporter, a membrane fusion protein (AcrA), transporter (efflux) protein (AcrB) and an outer membrane protein channel (TolC) (Koronakis *et al*., 2004).

**Table 1**: Examples of RND efflux systems involved in the antibiotic resistance of different pathogens. *(AG -amino glycosides, BL- β-lactamase, CM- chloramphenicol, EM-erythromycin, FQ- fluoroquinolones, ML- macrolides, NB- novobiocin, RF- rifampin, TC- tetracycline, VM-Vancomycin).

| **Species** | **Pump** | **Regulator** | **Antibiotic resistance to** |
|---|---|---|---|
| *S.* Typhimurium | AcrAB | AcrR | BL, CM, FQ, NB, EM, RF, TC |
| *E. aerogenes* | AcrAB-TolC | AcrR | CM, FQ, NB, TC |
| *E. coli* | AcrAB-TolC | AcrR, MarA, | BL, NB, EM (ML), CM, TC, FQ |
| *P. aeruginosa* | MexJK-OprM | MexL | AG, CI, EM, TC |
| *K. pneumonia* | AcrAB | AcrR | FQ |

In Enterobacteriaceae, TolC can function as the protein channel for different RND family efflux pumps. And it can also interact with MFS (major facilitator superfamily) transporters (EmrAB of E.*coli*). The antibiotic resistance determinant most frequently found was *lsa* gene (*Enterobacter*, *Pantoea* and *Salmonella* isolates). Erythromycin resistance genes (*ereA, ereB*) were detected among *Enterobacter*, *Klebsiella* or *Salmonella*. The drug substrate of bacteria that over express an efflux pump range of antibiotics involved such as Chloramphenicol, Quinoloes including nalidixic acid, ciprofloxacin, norfloxacin, and tetracycline such as SDS and Triton X-100 (Baucheron *et al*., 2004).

**Epidemiology**

In 2007, morbidity because of MRSA in European Union membrane states, Iceland and Norway was found to be 12% and mortality was found to be 37%. Where as in Vancomycin-resistance Enterococcus faecium morbidity was found 19% where as mortality found to be 52%. Antibiotic

resistance in Gram-negative bacteria (*E*.coli, Klebsiella pneumonia, Pseudomonas aeruginosa) recorded morbidity 27%,27%,37% and mortality 52%,52%,7% respectively (WHO, 2012).

According to a 2013 report by CDC, USA multi drug resistance bacteria related morbidity and mortality are quite high as more than 2 million people becoming infected and more than 23000 people die on annual bases (Hota and Ahmad, 2009).

Likewise Antibiotic resistance in India in**:** Neonates1 million India children die in the first four week of life each year. Out of these death rates 190,000 are caused by sepsis, a bacterial infection that overtake bloodstream and 30% of sepsis death are attributed to antibiotic resistance (Fig. 1).

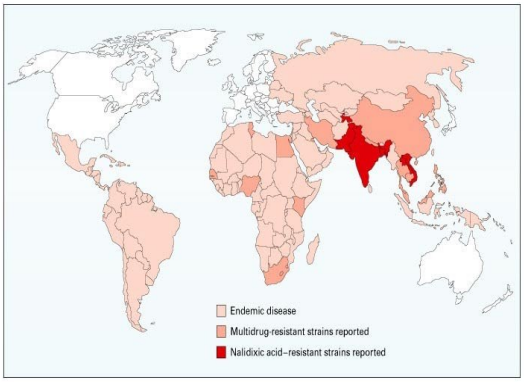


Fig1: Multidrug resistance endemic areas worldwide (Sefton *et al*., 2002).

**Molecular mechanism of antibiotic resistance**

Several mechanisms have evolved in bacteria which confer them with antibiotic resistance. These mechanisms chemically modify the antibiotic. Some bacteria are naturally resistant to certain types of antibiotics. Genetic mutation yield different types of resistance. Some mutations enable bacteria to produce enzymes that inactivate antibiotics. While other mutations eliminate the cell target that antibiotic attack. Bacteria can acquire antibiotic resistance genes from other bacteria in several ways (Yoneyama *et al*., 2006) By undergoing a simple mating process called conjugation in which bacteria transfer genetic material, including genes encoding resistance to antibiotics (found on plasmids and transposons) from one bacterium to another bacterium. Any bacteria that acquire resistance genes by spontaneous mutation or genetic exchange with other bacteria have the ability to resist one or more antibiotics. As bacteria can collect multiple resistance traits over time, they can become resistant to many different families of antibiotics.

Antibiotic resistance can occur via three general mechanisms: a) prevention of interaction of the drug with target, b) efflux of the antibiotic from the cell, c) direct destruction or modification of the compound (Fig.2).The most common mode is enzymatic inactivation of the antibiotic. Existing enzyme is modified to process the antibiotic and modifies it so that it no longer affects the microorganism. An alternative strategy utilized by bacteria is the alteration of the antibiotic target site. Bacteria may become resistant to many antibiotics like amino glycosides (netilmicin, tobramycin, gentamicin, streptomycin, neomycin, amikacin, etc.) by enzymatically adding new chemical groups to these antibiotics, thus inactivating the drug (Rachakonda *et al*., 2004) (Table 2).

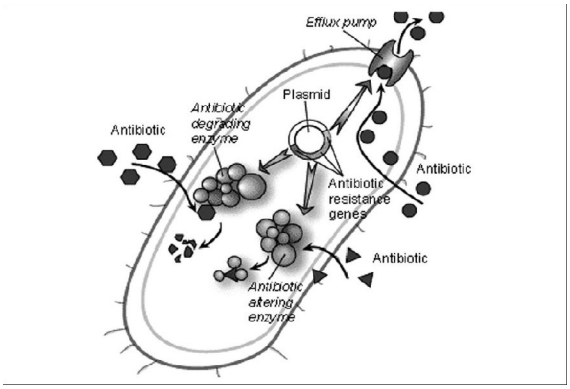


Fig.2. Molecular mechanism of antibiotic resistance (Sosa *et al*., 2010).

**Table 2.** Examples of antibiotics and reasons of their inefficacy.

| | |
|---|---|
| B-lactamase, erythromycin, lincomycin | Reduce binding of antibiotic to target |
| B-lactamase, erythromycin | Hydrolysis |
| Aminoglycosides, Chloramphenicol | Inactivation of antibiotics by enzymatic modifications |
| Sulfonamides,trimethoprim | Overproduction of antibiotic target |
| Bleomycin | Binding of specific immunity protein to antibiotic |
| Tetracycline | Active efflux from the cell |
| Chloramphenicol | Reduce uptake into cell |

**Resistant pathogen**

***Salmonella* and *E. coli***

Infection with *Escherichia coli* and *Salmonella* can result from the consumption of contaminated food and water. When both bacteria are spread, serious health conditions arise. Many people are hospitalized each year after getting infected, and some dying as a result. Some strains of *E.coli* become resistant to multiple types of fluoroquinolone antibiotics (Johnson *et al.*, 1999).

***Klebsiella pneumoniae***

*Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and mortality whose incidence is rapidly increasing in a variety of clinical settings around the world. Carbapenemase antibiotics are generally not effective against KPC-producing organisms (Arnold and Ryan, 2011).

***Clostridium difficile***

*Clostridium difficile* is a nosocomial pathogen that causes diarrheal disease. Clindamycin-resistant *C.difficile* was reported as the causative agent of large outbreaks of diarrheal disease in hospitals in New York and was responsible for death of several patients. It is transmitted via the oral-fecal route and forms spores that are resistance to heat and acid resistance hence able to survive in gut (Gerding *et al*., 1995).

**Method of Antibiotic resistance: Treatment and Its Management**

**Role of Anti microbial peptide**

Antimicrobial peptides (AMPs) are small molecular weight proteins with broad spectrum antimicrobial activity against bacteria, viruses, and fungi (Richard *et al.*, 2005). Antimicrobial peptides are an essential part of innate immunity that evolved in most living organisms over 2.6 billion years to combat microbial challenge. Antimicrobial peptides are wide spread in the form of life, from the multicelluar oraganism to bacterial cells. Anti microbial peptides are effective against multidrug resistance bacteria. These peptides are produced in large quantities at sites of infection and inflammation and can have broad-spectrum antibacterial, antifungal,

antiviral, antiprotozoan and antisepsis properties (Aumelas *et al*., 1996) (Fig. 3).

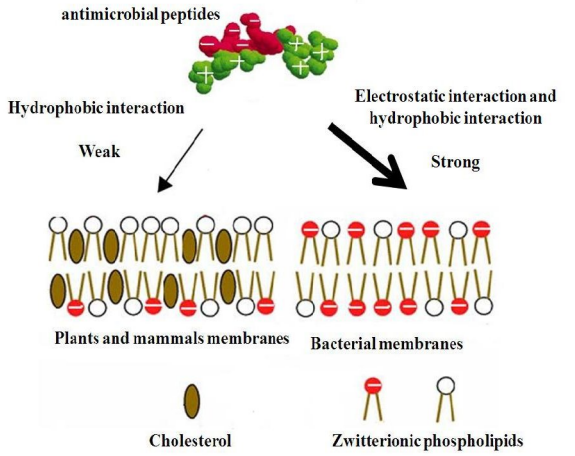


Fig. 3. Molecular basic of cell selectivity of antimicrobial peptide (Hancock *et al.*, 2006).

Positively charged antimicrobial peptide interacts with negatively charged bacterial membrane via electrostatic interaction leads to cellular association. They interact directly with host cells to modulate the inflammatory process. Larger antimicrobial protein contains sites that target specific microbial macromolecule. In most bacterial species, after exposing microorganisms to antibiotic peptides, several systems are affected such as energy and nitrogen metabolism regulation, glucan biosynthesis, amino acid, protein, and nucleotide synthesis, and, moreover, various proteins show a stress response. Beside their direct antimicrobial function, antimicrobial peptides have multiple roles as mediators of inflammation with effects on epithelial and inflammatory cells, such as proliferation, immune induction, wound healing, cytokine release, chemotaxis, protease–antiprotease balance, and redox homeostasis. (Yount and Yeaman, 2004).

**Potential strategies of antimicrobial peptides for their general therapeutic application:**

1) As single anti infective agent
2) As Immunostimulatory agent that enhance natural innate immunity
3) As an endotoxin neutralizing agents to prevent the potentially fatal complications associated with bacterial virulence factors that cause septic shock.
4) In combination with conventional antibiotics or antiviral to promote any additive or synergistic effects.

**Role of Bacteriophage**

Antibiotic-resistance genes are often carried by conjugative plasmids, which spread within and between bacterial species. It has long been recognized that some viruses of bacteria (Bacteriophage) have evolved to infect and kill plasmid-harbouring cells. Generally, phages as obligatory parasites of a bacterial cell show several life cycles: lytic, lysogenic, pseudolysogenic and chronic infections (Weinbauer *et al*., 2004). The main interest in phage application as antimicrobials has focused on lytic tailed phages representing three families of *Caudovirales* order: (i) *Myoviridae* with the biggest capsid head (~150nm) and contractile tail; (ii) *Siphoviridae* with a relatively small capsid head (~50-60 nm) and a long flexible, noncontractile tail; and (iii) *Podoviridae* with a small capsid head (~50-60 nm) and a short tail (Matsuzaki *et al*., 2005).

Phages have been proposed as natural antimicrobial agents to fight bacterial infections in humans, in animals or in crops of agricultural importance. The

intensive research on phage biology has led to increasing potential phage application in different aspects of human activity. Possible applications of bacterial viruses and phage-encoded may concern: (i) phage therapy proteins; (ii) phage typing; (iii) bacterial detection; (iv) disinfection of medical tools and devices; (v) food decontamination; and (vi) drug delivery vehicles (Table 3).The utilization of phage or phage proteins has both advantages and limitations. Phages are extremely specific, which requires detailed diagnostics of etiological factors causing infection. On the other hand, the narrow specificity protects the endogenous microbial flora. Therapeutic phages need to be precisely characterized in terms of biology and genetic features, because the use of not well defined lytic phages may lead to the expression of undesirable virulence factors as an adaptive response to phage infection and must be carefully studied prior to any clinical trials (Table 4).

Table 3. The Characteristics of selected Endolysins of Gram Negative and Gram Positive Bacteria (Kawa *et al*., 2012).

| **Bacteria** | **Phage** | **Host** | **Phage Protein** | **Name(displayed activity)** | **Reference** |
|---|---|---|---|---|---|
| Gram-Negative | T5 phage | *E. coli* | Globular | LysT5 (peptidase) | Mikoulinskaia *et al*., 2009. |
| | P2 phage | *E. coli* | Globular | P2gp09(lytic transglycosylase) | Walmagh *et al*., 2012. |
| | SPN1S phage | *S.* Typhimurium | Modular | SPN1S(endolysin (lysozyme) | Lim *et al*., 2012. |
| | PVP-SE1 phage | *S. enterica* | Modular | PVP-SE1gp146 (lysozyme) | Walmagh, *et al*., 2012. |
| | K11 phage | *K. pneumoniae* | Globular | K11gp3.5 (amidase) | Walmagh and Boczkowska, 2012. |
| Gram - Positive | φP68 phage | *S. aureus* | modular | Lys16 (endopeptidase, amidase) | Takac and Witte, 2005. |
| | EFAP-1 phage | *E. faecalis* | modular | EFAL-1 (amidase) | Son *et al*., 2010. |
| | Cp-1 phage | *S. pneumoniae* | modular | Cpl-1 (muramidase) | Loeffler *et al*., 2003. |
| | Mu1/6. | *S. aureofaciens* | modular | mu1/6Lyt (amidase) | Farkasovska *et al*., 2003. |
| | C1 phage | *S. pyogenes* | modular | PlyC (amidase) | Nelson *et al*., 2001. |

Table 4: Features of Antibiotics, Lytic Bacteriophages and Non- Lytic Phage Proteins as antimicrobials. (Kawa *et al*., 2012).

| **Selected feature** | **Antibiotic** | **Phage** | **Non-lytic phage protein** |
|---|---|---|---|
| **Resistance development** | Vertical - mutation and selection, Horizontal - acquisition of resistance genes from another organism via transformation, transduction and conjugation | Vertical - mutation and selection, Temperate phage acquisition, Low level of induced resistance. | Vertical - mutation and selection, Low level of induced resistance. |
| **Multidrug therapy** | drugs in combined therapy, Prevention of resistance development, Eradication of multidrug resistant Strains. | Cocktail of phages (3-5) or phage antibiotic Combination, Prevention of resistance development, Extended activity spectrum | Combined therapy of protein-protein, phage-protein, antibiotic-protein, antibiotic-phage-protein, Prevention of resistance development, Extended activity spectrum, Synergistic effect possible. |
| **Biofilm eradication** | Difficult effective drug concentration in biofilm structure limited. | Relatively effective phage penetration into the biofilm structure possible by means of EPS degradation (phage enzymes). | Effective biofilm degradation possible by EPS degrading phage enzymes. |
| **Serious side effects on the host** | Allergy, dysbiosis, secondary infections, Endotoxin (LPS) and other toxins release possible. | Endotoxin (LPS) and other toxins release during cell lysis possible. | Endotoxin (LPS) and other toxins release during cell lysis possible. |
| **Efficient bacterial killing** | Bacteriostatic or bactericidal, concentration- or time-dependent killing, PAE effect possible (post antibiotic effect), MIC (minimum inhibitory concentration), Effective on growing cells. | Bacteriolytic, Phage titer-dependent killing, Virulence efficacy: MOI, burst size, growth rate, Effective on growing cells. | Bacteriostatic or bacteriolytic, Concentration-dependent killing, MIC (minimum inhibitory concentration), Effective on growing and nongrowing cells. |

**Strategies to Minimize Antibiotic Resistance**

Strategies to minimize antibiotic resistance can be reduced by using antibiotics based on guidelines of antimicrobial stewardship programs ASPs (Drew *et al*., 2009) and various data such as pharmacokinetic, pharmacodynamic and properties of antibiotics, for diagnostic testing, antimicrobial susceptibility testing, clinical response, and effects on the microbiota, and development of new antibiotics. The controlled use of antibiotics in food animals is another effort to reduce antibiotic resistance. Antimicrobial susceptibility testing (AST) can also be an important aid for a rapid and reliable prediction of antimicrobial success in the treatment of bacterial infections (Pulido *et al*., 2013).

All the major resistance control strategies recommend education for patients, children through schools and day care centers. The public, and relevant healthcare professionals like primary-care physicians, pharmacists, and medical students, regarding unique features of bacterial infections and antibiotics, prudent antibiotic prescribing as a positive construct, and personal hygiene like hand washing (Pittet *et al*., 2000). The problem of antibiotic resistance can be minimized only by efforts of all members of society for ensuring the continued efficiency of antibiotics. It is important to try to understand that appropriate antibiotic use can really take place in a variety of different points within antibiotic therapy (Butler and Cooper, 2012).

**Development of Novel Antibiotics:**

Antimicrobial drugs are a unique class of drugs that does not directly target human biochemical processes but instead affect the growth of invading pathogens and commensal microbiota. Bacteria can easily adapt environmental changes and decrease their susceptibility to antibiotics by several mechanisms, including mutation and horizontal gene transfer within and in between species (Thomas *et al*., 2005). New weapons are always indispensable for combating bacterial infections (Spellberg *et al*., 2004). During the past 30 years, only two new systemic classes of antibiotics oxazolidinones in 2000 (Table5) and cyclic lipopeptides in 2003 and topical classes pleuromutilins in 2007 were introduced in the market neither of these new systemic classes of antibiotics were effective act against Gram-negative bacteria in which MDR is an acute problem and the treatment options were limited. Gram-positive bacteria, Gram-negative bacteria have an additional outer membrane comprised of lipopolysaccharide (LPS) which offers an additional barrier to block the invasion of antibiotics (Butler *et al*., 2013).

Table 5. Antibiotic discovery.

| Year | Antibiotic Discovered | Reference |
|---|---|---|
| 1928 | Penicillin | Levin, *et al*., 2004. |
| 1943 | Aminoglycosides, streptomycin | Overbye, *et al*., 2005. |
| 1944 | Tetracycline's, chlortetracycline | Chu, *et al*., 1996. |
| 1946 | Chloramphenicol | Chu and Plattner., 1996. |

| | | |
|---|---|---|
| 1948 | Erythromycin | Hutchinson, *et al*. 1997 |
| 1953 | Vancomycin | Hutchinson, *et al*., 1997. |
| 1963 | Streptogramin B | Bronson, *et al*., 2001. |
| 1997 | Bedaquiline | Levin and Bonten, 2004. |
| 1986 | Daptomycin | Raghunath *et al*., 2010. |
| 2000 | Streptogramins | Bronson, *et al*., 2001. |

Recent report shows that compounds with molecular weight less than 600 Da can penetrate the outer membrane of Gram-negative bacteria, whereas compounds with MW of more than 1,000 Da like Vancomycin of 1,449 Da and daptomycin of 1,620 Da can pass through the Gram-positive bacteria cell wall (O'Shea and Moser, 2008). A limited number of antibiotics targeting Gram-negative bacteria, e.g. polymyxin, colistin and azithromycin with high molecular weight can penetrate the outer membrane of Gram-negative bacteria by using active transport mechanisms that facilitate transport through the outer membrane of bacteria (Livermore *et al*., 2005). A new business models and political actions are required. Such political actions include innovative financial support, such as offering subsidies, reducing financial and transactional costs of research and development (R&D) process, and introduction on outcome-based rewards. To discover new classes of antibiotics, novel strategies for rational design and screening-based approaches are required. Strategies are also presented for the treatment of microbial diseases, such as host defense peptides, bacteriophages, vaccines, immunoglobulin's, and probiotics (Lloyd *et al*., 2012).

**Awareness regarding drug usage:**

To optimize antimicrobial prescribing, the prescribers should have appropriate knowledge of general medicine, pharmacokinetic (PK) and pharmacodynamic (PD) properties of drugs, knowledge of epidemiology, microbial virulence, immunological and genetic host factors (Pulcini *et al*., 2007). Most of the antimicrobial agents are used in primary care education on antibiotic prescribing in primary care is important (Harnden *et al*., 2007). Teaching postgraduate students about the prescribers of antibiotics in the community requires internship, foundation training or close collaboration between local healthcare providers and academicians. The teacher, who offers the guidelines for antibiotic treatment, must be trained on the available educational strategies, and the current information on antimicrobial stewardship (Pulcini and Gyssens, 2013). Antibiotic management requires effective teamwork between all healthcare professionals. But, for an effective education of patients and the public, the role of professionals is very important. The professionals must give the public clear information about the duration of symptoms, self-care, benefits and limitations of antibiotics, and antibiotic resistance (Huttner *et al.,* 2010).

**Hygiene and Disinfection:**

MDR pathogens often cause hospital acquired infections, which require more expensive antibiotics and hospitalization. In the United States (U.S.), 1.7 million

hospital-acquired infections are recorded each year, which result hundred thousand deaths (Klevens *et al*., 2007) The main source of Multidrug resistance pathogens is endogenous flora of patients, healthcare workers are also considered an important source(Caron *et al*., 2010).

Appropriate hospital disinfection and personal hygiene of healthcare workers are required to prevent hospital-acquired infections. The Centers of Disease Control and Prevention (CDC) and the SHEA (Society of health care epidemiology of America) offered guidelines for preventing nosocomial transmission of multi drug resistant bacteria in hospitals (Muto *et al*., 2003). Transmission of pathogens through the hands of healthcare workers is particularly the most common cause for spreading (Sax *et al*., 2009).Contamination of the hands of healthcare workers result either directly from contact with patients or indirectly from touching contaminated environmental surfaces (Weber *et al*., 2010). Several studies have demonstrated that an increase in hand washing compliance significantly decreases nosocomial infections by MRSA in intensive care units (Peacock *et al*., 1980). The World Health Organization (WHO) and the CDC presented hand hygiene guidelines in healthcare.

**Techniques**

Antimicrobial susceptibility testing can also be an important aid for a rapid and reliable prediction of antimicrobial success in the treatment of bacterial infections. The most conventional methods are those that detect phenotypic resistance by measuring bacterial growth in the presence of the antibiotic (Pulido *et al*., 2013). These standard phenotypic resistance tests are highly sensitive to detection of resistance, they require rather large numbers of viable cells, limited organism spectrum, analytical variability, long time to obtain results, and high cost (Van Belkum *et al*., 2013). In recent years, novel approaches for the rapid detection of resistance in bacterial pathogens have been developed and developing. The PCR-based techniques, mass spectrometry, microarrays, microfluidics, flow cytometry, isothermal microcalorimetry (IMC), cell lysis-based approaches and whole-genome sequencing, and their ability to detect resistance in various bacterial species has been demonstrated. Rapid and accurate organism-identification also benefits the patient and the effectiveness of ASP (Antimicrobial Stewardship Programs to optimize antimicrobial therapy. For rapid identification of *Staphylococcus* species, *Enterococcus faecalis*, *Enterococcus faecium*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Clostridium difficile*, and *Candida* species from clinical samples, molecular diagnostic methods have been developed (Pulido *et al*., 2013). Remarkable advances in next-generation sequencing technology have enabled to elaborate molecular characterizations of the microbial ecosystem, and recent reports using this technology have shown that the microbiota seriously affects human health and physiological development, including nutritional processing, prevention from pathogen invasion, host development, maturation and homeostasis of immune system (Muegge *et al*., 2011)

Antibiotic treatment to remove pathogens is likely to cause both short-term and long-term impacts on the commensal microbiota and this disturbance in the microbiota can trigger both transient and persistent changes in host immunity and physiology (Almeida Da Silva *et al*., 2011) Therefore, while prescribing antibiotics to patients, physicians should be aware of the effects that antibiotics will have on the particular patient's microbiota. Like cases of ciprofloxacin (Dethlefsen *et al*., 2008) and Vancomycin (Robinson *et al*., 2010) antibiotic treatment typically causes a dramatic and immediate decrease in the phylogenetic diversity of the previously stable microbiota, Studies using animal models have demonstrated that different antibiotic therapies cause distinct effects on the microbiota. For instance, treatment with a combination of amoxicillin, metronidazole, and bismuth could be rapidly recovered to pretreatment levels after treatment withdrawal (Antonopoulos *et al*., 2009).

Interestingly, bacteria that are affected by antibiotic treatment are not limited to those which are directly susceptible to antibiotic. Despite the fact that antimicrobial activity of Vancomycin being restricted to Gram-positive bacteria, some Gram-negative bacteria were significantly reduced and this phenomenon is partly caused by the dysregulation of host immune homeostasis as a consequence of changes in the microbiota. Recent many reports have shown antibiotic-associated changes in host immunity and the reduced expression of REG3γ by broad-spectrum combination antibiotic treatment (Vancomycin, neomycin, and metronidazole) reduced expression of REG3β by streptomycin therapy, depletion of T helper 17 cells by Vancomycin therapy, reduced production of RELMβ by ampicillin therapy , reduced expression of TLR2 and TLR4 in peritoneal macrophages by the treatment of streptomycin and cefotaxime , and disrupted mucus layer by metronidazole therapy.

**Conclusion**

The indiscriminate use of antibiotic drugs has resulted in bacterial resistance to a large number of common antibiotics**.** Multidrug resistance is a worldwide problem because of enhanced intercontinental mobility and randomly affects members of all socioeconomic classes. Bacteria can adopt complex strategies to avoid the lethal effects of antibiotics. The dual nature of antibiotic acting both as signaling as well as growth inhibitor molecule suggests that the antibiotic resistance genes have to co-evolve to safeguard the producer organism from antibiotic threat. With growing number of evidences that multidrug resistance efflux pumps can have a role in bacterial pathogenicity, more research is required urgently to explore the link between MDR pumps and increased virulence. Multidrug resistance efflux pumps inhibitors would be invaluable tool to help clear bacterial infection. Knowledge of these mechanisms of resistance can help in designing new tools for disease diagnosis and its management. Healthcare institutions need robust infection control and antibiotic stewardship programs to prevent transmission of resistant bacteria. By adopting these strategies, the life of current antibiotics can be enhanced against

bacterial infection and development of future antibiotic therapies can be secured.

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