Horizontal gene transfer-emerging multidrug resistance in hospital bacteria[1]

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ABSTRACT

The frequency and spectrum of antibiotic resistant infections have increased worldwide during the past few decades. This increase has been attributed to a combination of microbial characteristics, the selective pressure of antimicrobial use, and social and technical changes that enhance the transmission of resistant organisms. The resistance is acquired by mutational change or by the acquisition of resistance-encoding genetic material which is transfered from another bacteria. The spread of antibiotic resistance genes may be causally related to the overuse of antibiotics in human health care and in animal feeds, increased use of invasive devices and procedures, a greater number of susceptible hosts, and lapses in infection control practices leading to increased transmission of resistant organisms. The resistance gene sequences are integrated by recombination into several classes of naturally occurring gene expression cassettes and disseminated within the microbial population by horizontal gene transfer mechanisms: transformation, conjugation or transduction. In the hospital, widespread use of antimicrobials in the intensive care units (ICU) and for immunocompromised patients has resulted in the selection of multidrug-resistant organisms. Methicilin-resistant *Staphylococci*, vancomycin resistant *Enterococci* and extended-spectrum beta-lactamase (ESBL) producing Gram negative bacilli are identified as major problem in nosocomial infections. Recent surveillance studies have demonstrated trend towards more seriously ill patients suffering from multidrug-resistant nosocomial infections. Emergence of multiresistant bacteria and spread of resistance genes should enforce the aplication of strict prevention strategies, including changes in antibiotic treatment regimens, hygiene measures, infection prevention and control of horizontal nosocomial transmission of organisms.

INTRODUCTION

Multidrug-resistant bacteria in both the hospital and community environment are important concern to the clinician and the pharmaceutical industry, as it is the major cause of failure in the treatment of infectious diseases[1]. Acquired antimicrobial resistance results in escalating healthcare costs, increased morbidity and mortality and the evolution of new pathogens[1,2]. During the last few decades the frequency and spectrum of antibiotic resistant infections have increased steadily within the United States[3], Europe[4-7] and the developing world[8]. This increase has been attributed to a combination of microbial characteristics, the selective pressure of antimicrobial use, and social and technical changes that enhance the transmission of resistant

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organisms factors, such as increased use and misuse of antimicrobial agents, increased use of invasive devices and procedures, a greater number of susceptible hosts, and lapses in infection control practices leading to increased transmission of resistant organisms[1].

Microorganisms have a remarkable array of mechanisms with which to overcome the effects of antimicrobial agents. These include the production of structure-altering or inactivating enzymes (eg, beta-lantanas- or amino glycoside-modifying enzymes), alteration of penicillin-binding proteins or other cell-wall target sites, altered DNA gyrase targets, permeability mutations, active efflux and ribosomal modification[9-12]. Selective pressure resulting from antimicrobial administration can lead to the growth of previously susceptible strains that have acquired resistance or to the overgrowth of strains that are intrinsically resistant. In general, resistance is acquired by mutational change or by the acquisition of resistance-encoding genetic material. The escape of resistance genes to mobile DNA fragments (plasmids) is enabling the process of transfer of antimicrobial resistance not only between bacteria of the same population, but between bacterial genera. These evolutionary old genetic recombination mechanisms for gene transfer in bacteria have been adapted for new antibiotic environment that has been created due to liberal use of antibiotics in human medicine, agriculture, fisheries and animal husbandry[13,14]. In clinical practice, widespread use of antimicrobials in the intensive care units (ICU) and for immunocompromised patients has resulted in the selection of multidrug-resistant organisms[15-17]. Treatment of nosocomial infection caused by multidrug resistant microorganism is directly (increased infection control cost) and indirectly (prolonged hospital stay, increased laboratory cost) increasing health care cost[18]. Increased incidence of multidrug resistant bacteria and rising evidence of resistance transfer from one organism to another may lead to combined growth of nosocomial pathogens, for which there are no antibiotic solutions[1].

HORIZONTAL GENE TRANSFER

Acquired resistance to antibiotic occurs either by mutations (point mutations, deletions, inversions etc. in bacterial genome) or by horizontal transfer of resistance genes located on various types of mobile DNA elements[19].

In bacterial populations (plasmid bearing or not), the generation of antibiotic resistance depends on the rate of emergence of resistant mutants, ie, on the bacterial mutation rate. A correlation between high mutation rate and antibiotic resistance has been reported in the case of *Pseudomonas aeruginasa* isolated from the lungs of cytic fibrosis patients[20]. Some antibiotic treatments can be selected for mutator bacteria present at low frequencies among all wild-type populations[21]. These strains are of particular interest. They exhibit much higher mutation rate than the normal strains. Although the mutation rate of cell is controlled by multiple pathways any of which can lead to a mutator phenotype when defective, of particular significance is the fact that all mutators identified among natural isolates are defective in methyl-directed mismatch repair (MMR)[22]. This repair pathway, encoded by *mutH, mutS, mutL* and *mutU* genes is necessary for editing replication and recombination[23]. MMR proteins recognize mismatched bases in the DNA and abort recombination process between diverged DNAs. Thus MMR is a potent inhibitor between non-identical sequences. MMR system is also involved in the control of DNA replication fidelity.

Inactivation of MMR genes (mutator phenotype) greatly increases the frequency of mutations as well as recombination between diverged bacterial species thus enabling horizontal gene transfer between diverged bacteria[24]. Therefore inactivation of MMR gene should increase the probability of any gene transfer between diverged bacteria (eg, antibiotic resistance gene).

High incidence of mutator phenotypes among human pathogens was reported. Numerous mutator *E coli*, *Salmonella enterica* and *P aeruginosa* isolates from patients and other natural environments have defective MMR systems[20,21]. Defect in the *mut S* gene, the mutator gene most often found in nature enhances bacterial mutation rate up to 1000-fold and relaxes the barriers that normally restrict homeologous recombination. These mutators thus afford the opportunity for horizontal exchange of DNA between disparate strains[25].

The unique role of MMR defects in promoting homeologous recombination (horizontal gene transfer) between and among species provides the selective advantage for successful pathogens. That is, mutator with relaxed speciation barriers could acquire sequence elements (eg, resistance islands) from either similar or disparate genomes after which this rare recombinant might rise to prominence by selective enrichment in particular environments. Actually, by selecting for a resistance

allele, the antibiotic selective pressure was also selected for a mutator phenotype as the mechanism that generated the resistance[17]. It has been demonstrated that the fraction of mutator cells is increased in bacterial population under strong or prolongated selection in the course of antibiotic treatments[26]. Mutation frequencies to resistance can vary dramatically depending on the mechanism of resistance and whether or not organism exhibits a mutator phenotype. Resistance usually has a biological cost for the microorganism, but compensatory mutations accumulate rapidly that abolish this fitness cost and this explains why many types of resistances may never disappear in bacterial population.

Three mechanisms of gene transfer in bacteria have been identified: transformation, involving the uptake and incorporation of naked DNA; conjugation, a cell contact-dependent DNA transfer mechanism found to occur in most bacterial genera; and transduction, whereby host DNA is encapsidated into a bacteriophage which acts as the vector for its injection into a recipient cell[27]. Genes encoding for the resistance to antibiotics are often carried by large self-transmissible plasmids, or by smaller plasmids that can be mobilized by self-transmissible plasmids. They are frequently part of trans-posons or conjugative transposons. Self-replicating plasmids, prophages, transposons, integrons and resistance islands all represent DNA elements that frequently carry resistance genes into sensitive organisms. These elements add DNA to the microbe and utilize site specific recombinases- integrases for their integration into the genome[19].

The impact of lateral gene transfer on bacterial evolution is understood by the realization that foreign DNA can represent up to one-fifth of a given bacterial genome[27]. Perhaps the most striking embodiment of its effect on microbial adaptation has been rapid and widespread emergence of similar antibiotic resistance profiles among phylogenetically diverse Gram-negative clinical and environmental isolates over last 50 years. The localization of antibiotic-resistance determinants to mobile entities such as plasmids and transposons readily explained this phenomenon, but closer examination revealed that in many cases a new type of genetic element, termed an integron, harbored the resistance determinants.

In Gram-negative pathogens, multiple antibiotic resistance is common and many of the known resistance genes are contained in mobile gene cassettes. Cassettes can be integrated into or deleted from their receptor elements, the integrons or infrequently may be integrated at other locations via site-specific recombination catalysed by and integron-encoded recombinase. As a consequence, arrays of several different antibiotic resistance genes can be created. Over 40 gene cassettes and three distinct classes of integrons have been identified to date[28].

Integrons were first identified as the primary mechanism for antibiotic resistance gene capture and dissemination among Gram-negative bacteria. More recently, their role in genome evolution has been extended with the discovery of larger integron structures, the super-integrons, as genuine components of the genomes of many species. Integron-driven gene capture is likely to be an important factor in the more general process of horizontal gene transfer in the evolution of bacterial genomes. It appears that multiresistant integrons have evolved from super-integrons specific capture of resistance cassettes from many different kinds of bacteria[29].

Such resistance determinants most probably were acquired by pathogenic bacteria from a pool of resistance genes in other microbial genera, including antibiotic-producing organisms[2].

Incorporation and uptake of naked DNA (transformation) are dependent on bacterial competence which is variable between species. Some species are already competent, and the others (eg, *E coli*) can be induced to take up DNA by a number of chemical or physical processes. Despite its sensitivity to nucleases, DNA is relatively common in almost all environments and may be excreted by living bacteria or be liberated during autolysis. Environmental DNA adsorbed to sand or clay particles may retain its transforming ability for weeks or even months[27].

In the process of transduction, bacterial genes are incorporated by bacteriophage particles and transferred to another bacterium. Bacteriophages are very common in the environment and are relatively stable, being protected by the protein coat. Phages are also more compact and thus more diffusible than naked DNA[27]. Some bacteriophages may encode for virulence factors that are expressed, like bacteriophages coding for shiga toxin are involved in the pathogenicity of *E coli*[30].

The vast majority of reports of bacterial gene transfer in the environment concern conjugation. Many plasmids and conjugative transposons are of very wide host range. Such transfer systems may have wide evolutionary consequences and have been implicated in the

horizontal transfer of antibiotic resistance and xenobiotic degradation genes. Knowledge of conjugal transfer in the human and animal intestinal tracts is important for understanding epidemics caused by drug-resistant bacteria, and the evolution and origin of multiple drug-resistant transfer factors. Studies have demonstrated that the transfer of antibiotic resistance genes can take place in the intestine between a variety of different gram-positive or Gram-negative bacilli[31].

EPIDEMIOLOGY OF NOSOCOMIAL INFECTIONS AND MULTIDRUG RESISTANCE

Numerous surveillance studies have documented trends in nosocomial infections worldwide that might affect resistance patterns. The percentage of the lower respiratory tract and bloodstream nosocomial infections in the USA (from 1975 to 1996) increased, while the percentage of the urinary tract or in surgical wounds infections decreased, which indicates trend toward more severely ill patients[3].

Concerning multidrug resistant bacteria causing nosocomial infections, three types of bacteria are identified as major problem: methicilin-resistant *Stahylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and extended-spectrum beta-lactamase (ESBL) producing Gram-negative bacilli[4,5]. The type and incidence of each pathogen and resistance genes are differing within continents, countries and even regions within the country, but as it is influenced by many factors, the ethiology of variability is still controversial.

Many programs and studies concerning following the spread of resistance genes and bacteria are going on worldwide, like the European Network for Antimicrobial Resistance and Epidemiology (ENARE) and SENTRY antimicrobial surveillance program[5,6]. Recent European study confirmed the proportion of MRSA in the various European countries ranging from <1 % in Scandinavia to alarmingly high >30 % in Spain, France, and Italy (12.8 % mean)[6]. Rates of resistance to the non-glycopeptide antibiotics were lowest for rifampin and highest for ciprofloxacin[5]. Most frequently MRSA is isolated in the intensive care units (ICU) patients and in surgical departments (60 %), with wounds being the most common isolation source. Another emerging problem is threatening appearance of intermediate-level resistant *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA)[32].

ESBL producing Gram-negative bacilli are of special clinical concern, as they may transfer resistance gene to other, more pathogenic bacterial genera. ESBL are plasmid-mediated beta lactamases of predominantly Bush class A, so far described only in Gram-negative bacilli[33]. ESBL are capable of efficiently hydrolyzing penicillins, narrow spectrum cephalosporins, many extended-spectrum cephalosporins, the oxyimino group containing cephalosporins (cefotaxime, ceftazidime), and monobactams (aztreonam), but are generally inhibited by beta-lactamase inhibitors[34,35]. In the United States in 1990 to 1993 a survey of ICU of 400 hospitals recorded an increase from 3.6 % to 14.4 % in ESBL producing strains of *Klebsiella ssp*[36]. By 1994 the Center for Disease Control and Prevention National Nosocomial Infections Surveillance System (NNIS) reported that 8 % of *Klebsiella spp* had ESBL with producers predominately from a few large centers[35,37]. In Europe as of 1995, ESBL occur in 20 %-25 % of *Klebsiella ssp* from patients in ICU, although they have been found in up to 30 %-40 % in France. Rates vary greatly worldwide and within geographic areas and are rapidly changing over time[38].

Most ESBL producing organisms are in the family *Enterobacteriaeae* and have been described in almost all members strains and have been isolated from abscesses, blood, catheter tips, lung, peritoneal fluid, sputum, and throat culture[34,38,39].

The incidence of *Enterococcal* nosocomial infection has increased since 1980s[40]. Enterococcal antimicrobial resistance, particularly VRE, is a serious concern. Multiple vancomycin resistance phenotypes have been identified, including van A, van B, and van C. The van A gene, which is often plasmid borne, confers high-level resistance to vancomycin and can be transferred to other microorganisms. Recent findings provide strong evidence that the horizontal transfer of resistance genes contribute largely to the emergence of multidrug-resistant *Enterobacteriaceae*[41]. VRE are most commonly isolated from highly compromised patients, such as those in the ICU. Enterococcal resistance includes beta-lactamase-mediated resistance, ampicillin resistance based on altered penicillin-binding proteins (PBP), and high-level aminoglycoside resistance[42].

However, since the first report of penicillinase producing *E faecalis* in 1983, ampicillin-resistant strains have been increasing, furthermore, these strains often associated with high-level resistance to aminoglycosides[43]. In the USA, colonisation with VRE is endemic

in many hospitals and increasingly causes infection, but colonisation is absent in healthy people. In Europe, colonisation seems to be endemic in healthy people and farm animals due to use of avoparcin, a vancomycin-like glycopeptide, widely used in the agricultural industry, explaining the reservoir in animals[40,43]. Avoparcin has not been used in the USA, which is consistent with the absence of colonisation in healthy people. Considering the spread of antibiotic-resistant bacteria and resistance genes, the emergence of VRE has emphasized the non-existence of boundaries between hospitals, between people and animals, between countries, and probably between continents[1].

PREVENTION AND TREATMENT STRATEGY

Despite the fact that powerful new antibiotics are introduced, morbidity and mortality resulting from infectious disease have increased in recent years, after decades of decline[1]. Inadequate use of antibiotics contributes to increased bacterial resistance both by selecting for the more resistant members of a population and by eliminating the patient's resident flora, which might otherwise compete with the pathogen. The spread of antibiotic resistance is not only related to the overuse of antibiotics in human health care but also in animal feeds, agriculture, fisheries[44], *etc*. A call for prudence and control has often been made during the past 25 years but has largely been ignored. Prevention programs in the last decade have brought some hope that rational use of antibiotics can restore bacterial susceptibility to some extent. For instance, widespread use of certain antibiotics, particularly third-generation cephalosporins, has been shown to foster development of generalized ß-lactam resistance in previously susceptible bacterial populations. Reduction in the use of these agents (as well as imipenem and vancomycin) and concomitant increases in the use of extended-spectrum penicillins and combination therapy with aminoglycosides have been shown to restore bacterial susceptibility[45-48]. Some prevention programs considering reduction in certain antibiotics use in the community-acquired infections, like reduction of macrolide use in Finland by 40 % has restored susceptibility of group A *Streptococci* from 16.5 % to 8,6 % in 4 years[49]. After abandon of avoparcin use as feed additive in animal husbandry in Germany, VRE prevalence in the gut flora of healthy persons has declined from 12 % to 3 % from 1994 to 1997[50]. On the other hand, resistance to streptomycin in *Enterobacteriaceae* was not restored after 20 years after abandon of its clinical use[51]. Measures to prevent horizontal transfer of resistance genes should be also employed both in community and in health care institutions by increasing hygiene measures and applying infection prevention programs[52,53].

Treatment modalities may also influence the development of antimicrobial resistance. One of the most important risk factors is repeated exposure to suboptimal antibiotic concentrations. The presence of sublethal concentrations of a drug exerts selective pressure on a population of pathogens without eradicating it. Under these circumstances, mutant strains that possess a degree of drug resistance are favored and tend to dominate the population. From such populations with low-level resistance, more highly resistant organisms are more readily selected. It thus follows that one tactic to prevent the emergence of resistance is to minimize the time that suboptimal drug levels are present by thoughtful attention to dosing[54].

Based on their pharmacodynamic properties, antimicrobial agents can be divided into two major groups. The first group, which includes the fluoroquinolones and the aminoglycosides, consists of agents that exhibit concentration-dependent killing of pathogens. For this group, the higher the drug concentration, the faster the eradication of pathogens. In the second group, which includes the β-lactam antibiotics, peak concentrations are relatively unimportant. Instead, the length of time that concentrations are maintained above the pathogen's minimal inhibitory concentration (MIC) is critical to bacterial eradication. Members of this group are referred to as concentration-independent or time-dependent drugs. An understanding of the pharmacodynamic characteristics of an antibiotic can provide insights into the best regimen for a given drug. For concentration-dependent antibiotics, a high once-daily dose is the best way to eradicate pathogens. This approach has been successful for aminoglycosides but cannot be applied to fluoroquinolones because CNS toxicity may result from high doses. Possible ways to optimize fluoroquinolone regimens include the administration of larger doses than conventionally used or the addition of a second agent to the regimen[55]. For concentration-independent drugs, the goal is to maximize the time that drug levels at the site of infection exceed the pathogen's MIC. One way of achieving this is through continuous infusions, which provides a prolonged time >MIC compared with bolus dosing[55]. The enhanced duration of

the antibiotic effect may be particularly important in immunosuppressed patients or in the treatment of pathogens with high MICs. Continuous infusions also reduce the amount of drug required for treatment and appear to have advantages over standard intermittent bolus dosing, particularly with respect to time >MIC. In addition, continuous infusions allow a reduction in total daily dose compared with conventional regimens[56]. No adverse effects were observed in healthy volunteers or critically ill patients receiving continuous infusions of ceftazidime[56,57].

Clinicians need to consider pharmacodynamic properties when choosing an antibiotic therapy. Choosing a drug with the appropriate spectrum of activity will always remain important in treating bacterial infections. However, choosing the right dose and dosing interval may also be critical to achieving optimal clinical responses and preventing the emergence of resistant pathogens.

Special precautions should be considered in the presence of hypermutable strains (mutators) in the bacterial population causing nosocomial infection. Mutator phenotypes among pathogenic bacteria may be found in significant proportion[20,21,58]. Treatment strategy should employ previous diagnostic assay for the presence of mutators, because the generation of resistance depends on the rate of emergence of resistant mutants[59]. Antibiotics whose mode of action involves inhibition of only one enzyme should be avoided (because mutators can easily overcome such mechanism). Recent experiment has indicated that initial treatment failure, which had induced high rate of mutator alleles, might lead to failure to subsequent treatments with other antibiotics[26].

Emergence of multiresistant bacteria and spread of resistance genes should enforce the application of strict prevention strategies, including changes in antibiotic treatment regimens, hygiene measures, infection prevention and control of horizontal nosocomial transmission of organisms.

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