

Antibiotic Resistance During Therapy: Mechanisms and Means of Control

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Abstract: Antibiotic resistance is a serious public health problem. The most effective way to control this phenomenon is to make rational use of antibiotics. However, antibiotic resistance is a complex process in which clinical, pharmacodynamic, pharmacokinetic and microbiological factors all play a part.

Since antibiotic therapy is usually performed empirically, clinicians should follow guidelines that take all these factors into account together with the concepts of evidence based medicine. These guidelines may be elaborated using information technology tools that help to collect, analyze and weigh up all the information available on a certain pathogen.

Therefore, the administration of antibiotics should be controlled with the help of multi-disciplinary working groups and in accordance with objective data collected following a thorough analysis of all the available information.

Keywords: Resistance, therapy, mechanism, control.

ARTICLE

The increase in antibiotic resistance is a phenomenon related to globalization and makes it difficult to treat infections in many patients [1]. It is essential to adopt measures to control this phenomenon; however, since it is a multi-factorial problem, its control is complex and requires the adoption of many complementary measures in various fields.

From the microbiological point of view, resistance to antimicrobial drugs in bacteria can result from mutations in housekeeping, structural or regulatory genes. Alternatively, resistance can result from the horizontal acquisition of foreign genetic information. The two phenomena are not mutually exclusive and can be associated in the emergence and more efficient spread of resistance [2]. These processes are favoured by incorrect administration of antibiotic treatment.

Clinical outcome is dependent upon antibiotic-mediated bacterial eradication in a number of infections. An increase in antimicrobial resistance reduces the probability of achieving eradication; whereas failure to eradicate bacteria may promote the emergence and dissemination of antimicrobial-resistant clones [3].

Rational use of antimicrobial drugs is the main measure that may be adopted to control this problem and great efforts are being made in this respect [4, 5]. However, advances in microbiology, molecular biology, information technology, pharmacokinetics, pharmacodynamics and evidence based medicine may provide new tools with which to try and alleviate this problem, bearing in mind that the situation can only be improved in the future if a multidisciplinary analysis of each therapeutic decision is carried out.

Traditionally, the minimum inhibitory concentration (MIC) has been used as the microbiological marker to determine the antibiotic susceptibility of microorganisms, thereby enabling the correct antibiotic therapy to be chosen. However, in order to achieve bacteriologic and clinical success, sufficient concentrations of the antimicrobial at the site of infection must be maintained for an adequate period of time [6]. Therefore, it is essential to take into account pharmacokinetic and pharmacodynamic criteria, since the activity of cefepime and ceftazidime against *Pseudomonas aeruginosa*, for example, is seen to be greatly influenced by the drug doses used [7].

Duration of the treatment is also an essential factor. Short therapies are known to be beneficial to the patient since the secondary effects of the antibiotics are minimised and treatment is more likely to be completed. However, the optimum duration of each antibiotic treatment to restrict the selection of drug-resistant bacteria during therapy is not known [8]. Antibacterial dose administration may be optimised by means of continuous or prolonged infusion; the use of smaller doses administered more frequently for the time-dependent beta-lactam agents; or higher, less frequent dose administration of the concentration-dependent aminoglycosides and fluoroquinolones [9].

Each antibiotic has also been seen to have a different capacity to select resistant mutants. Thus, cefotaxime, imipenem, and piperacillin-tazobactam are seen to be the drugs that most frequently generate resistance during treatment of infections due to *Pseudomonas aeruginosa* [10]. Macrolides have also been reported to select strains resistant to both macrolides and beta-lactams more efficiently than aminopenicillins [11].

Another phenomenon to take into consideration when deciding on the antibiotic to use is cross-resistance among the different antibiotics. Thus, selective pressures generated by the indiscriminate use of beta-lactam antibiotics have resulted in increased bacterial resistance across all beta-lactam classes. However, each antibiotic has a different capacity to carry out this process, since it is known that cefepime, piperacillin-tazobactam, and ampicillin-sulbactam have not demonstrated such strong selective pressures [12].

It is also known that in order to slow the development of resistance to antimicrobial agents, it is optimal to use drugs with more than one mechanism of action or target and prescribe those with demonstrated ability to minimise or reverse resistance problems. Hence, it is best to limit empirical use of beta-lactam plus fluoroquinolone combination therapy, since these two classes activate some common resistance responses and using them together can facilitate multidrug resistance in important pathogens, particularly *Pseudomonas aeruginosa* and *Acinetobacter* species [13].

The MIC is a microbiological parameter that provides only partial information on the interaction between the antibiotic and the microorganism. Therefore, minimal bactericidal concentration (MBC) values, indicating the antibiotic's bactericidal capacity and the study of the killing rate and post-antibiotic effect to determine the time-related antimicrobial effects must be taken into consideration since it has been seen that there can be profound differences in the microbiological efficacy of anti-biotics with identical MICs [14].

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Recently, the concept of mutant prevention concentration (MPC) has been developed to determine the concentrations above which resistance is unlikely to occur [9], since it denotes the antibiotic concentration that minimizes the selection of first-step resistant mutants present in large, $>$ or $= 10^{10}$ CFU/mL, heterogeneous bacterial populations. When this parameter is analyzed for ciprofloxacin against *E. coli*, *E. cloacae* and *K. pneumoniae*, and for levofloxacin against *E. coli*, *C. freundii* and *K. pneumoniae*, MPC results were below the susceptible breakpoints and within clinically achievable and sustainable drug concentrations for >24 hours of the dosing interval. Incorporation of MPC strategies represents a realistic approach for preventing the further selection of resistant organisms [15].

Furthermore, the concept of pharmacokinetic and pharmacodynamic (PK/PD) modelling has been introduced to help interpret determinations of susceptibility breakpoints, as well as the new parameter called area under the inhibitory concentration-time curve (AUC). Values greater than 250 have been reported to be associated with a rapid bactericidal activity, and values higher than 100 with the prevention of selection and induction of resistance, but this parameter needs to be validated for the different infections and microorganisms [16].

Whether or not resistant mutants will be present before the start of antibiotic treatment of an initially susceptible population of bacteria depends on the size of the infecting population, the rate of mutation to resistance, and the amount of time that the population has been maintained. It has been shown that the problem of acquired resistance to treatment with single antibiotics can be thwarted by combination therapy with pairs of antibiotics of different classes with synergistic activities [17].

Recently, the phenomenon of hypermutable *P. aeruginosa* strains has been described, characterized by increased (up to 1,000-fold) spontaneous mutation rates due to alterations of the DNA mismatch repair (MMR) system frequently found in the lungs of patients with cystic fibrosis. Multiple-antimicrobial resistance was documented in the hypermutable strains. Hypermutation is found to be a key factor for the development of multiple-antimicrobial resistance, especially for the treatment of chronic infections [18]. In *E. coli*, differences in the prevalence of these strains have been found depending on the geographical region studied and the clinical sample from which the microorganism is isolated. They were found to be more prevalent in southern Europe and in blood isolates [19].

It is also known that since cefotaxime selects hypermutators, the risk of secondary acquisition of antibiotic resistance is increased; as expected, the cefotaxime-resistant mutants had a mutation frequency 10 times higher in response to ciprofloxacin [20].

Another phenomenon to take into account is phenotypic tolerance. When growing bacteria are exposed to bactericidal concentrations of antibiotics, the sensitivity of the bacteria to the antibiotic commonly decreases with time, and substantial fractions of the bacteria survive, due to the emergence of antibiotic-mediated enrichment subpopulations, physiologically tolerant but genetically susceptible to these antibiotics. It has been shown that tolerant subpopulations generated by exposure to one concentration of an antibiotic are also tolerant to higher concentrations of the same antibiotic and can be tolerant to antibiotics of the other types [21].

The phenomenon of biofilm-associated resistance to antimicrobial agents is also important. The increased antimicrobial resistance of microorganisms in biofilms may be due to many factors, such as the fact that the extracellular matrix might physically restrict the diffusion of antimicrobial agents, or that the nutrient and oxygen depletion within the biofilm cause some bacteria to enter a nongrowing (i.e., stationary) state. Some

organisms in biofilms have been shown to express biofilm-specific antimicrobial resistance genes [22].

It has been seen that resistance and bacterial virulence are present in the same genetic determinants [23]. In order to characterise this important phenomenon, new microbiological tools are being developed, such as an oligonucleotide microarray that detects 189 *Escherichia coli* virulence genes or markers and 30 antimicrobial resistance genes [24].

Another little known phenomenon is the selection of low-level resistant populations due to the exposure of microorganisms to low antibiotic doses. This process favours secondary selections for more specific and effective mechanisms of resistance, since exposure to low antibiotic doses generates substantial stress in bacterial populations, which eventually influences the rate of genetic variation and the diversity of adaptive responses [25].

In addition to the influence of incorrect antibiotic treatment on the selection of resistant mutants during such treatment, the incorrect, indiscriminate use of antibiotics may produce other phenomena, of which little is known at present that may contribute to the increase in antibiotic resistance in many bacteria. Non-pathogenic bacteria that inhabit the ground are known to be a reservoir of resistance determinants and exposure of these to antibiotics may lead to the emergence of new resistance mechanisms in the microbial community [26, 27]. In a patient, it is necessary to take into consideration undesired effects of the antibiotics on the commensal flora of the mucosa, since treatments with fluoroquinolones have been reported to select quinolone-resistant viridans group streptococci in the oropharynx of neutropenic patients [28].

Analysis of each of the above parameters for each isolate prior to administration of antibiotic treatment is not technically feasible, since, in many cases the rapid empirical administration of antibiotics is essential [29]. However, it is important to monitor and administer antibiotics, both in hospital and in the community, in accordance with certain guidelines. Moreover, a great effort should be made to give all the professionals who use these compounds the necessary training, and multidisciplinary working groups should be fomented [30].

When designing these guidelines, the concepts of evidence based medicine should be applied. Due to the great number of factors involved and the difficulty in determining the importance of each one, it would probably be advisable to develop computer programmes to collect, analyze and weigh up all the data available on a specific microorganism, similar to the database developed by Stanford University to determine the resistance of the human immunodeficiency virus to anti-retroviral drugs. Patterns of antibiotic susceptibility of the microorganisms found in multicentre studies [31], drug characteristics, experimental microbiological studies and data provided by clinical studies should be included. A global analysis of all these data could provide reliable information on the therapies recommended to treat a certain pathogen. The clinician should use this information bearing in mind other factors such as the patient's characteristics, local antibiotic susceptibility data and the antibiotic policy of each centre. In order to integrate these data with the overall information obtained, it would be advisable to develop software applications that facilitate the decision making process.

Only by monitoring and using antibiotics correctly at all levels - human, animal and food - will the phenomenon of anti-biotic resistance be brought under control. It is, therefore, essential to administer antibiotic treatment in accordance with evidence based medicine and to do this multidisciplinary groups should work together analyzing all the available data and making use of the new methodologies in order to develop useful recommendations for the clinician which contribute to the patient's cure and protect the community from drug resistant microorganisms.

ABBREVIATIONS

MIC = Minimal inhibitory concentration

MPC = Mutant prevention concentration

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