**ARTÍCULO DE REVISIÓN**/*REVIEW ARTICLE*

# Antibiotic Resistance: Origins, evolution and healthcare-associated infections

# Resistencia a antibióticos: Origen, evolución e infecciones asociadas a la atención en salud

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**Abstract**

*The increased incidences of Healthcare-associated Infections (HAI) caused by multidrug-resistant bacteria, have led to an enlarged number of morbidity and mortality cases. Besides, other factors that are affected are patients, families and institutions providing health services. Therefore, the permanent study of the subject is necessary to identify possible strategies that contribute to the reduction of the issue. A critical review of the literature based on the origin of antibiotics, the evolution of their respective resistance, and the impact on public health from a historical and current perspective was developed. The search of the literature was carried out in the bibliographic databases: Pubmed, Web of Science, Scopus, SciELO, The Cochrane Library and Lilacs. The reviewed literature showed, from the historical viewpoint, the discovery of antibiotics to the last-generation antibiotics. The rapid coevolution of genes for antibiotics resistance and its subsequent spread to hundreds of species of microorganisms by Horizontal Transfer gene (HTG) was also reviewed. It is also discussed how the expansion in antimicrobial resistance (AMR) generates a series of factors that increase health-care associated infections care (HAI) and their impact on public health. The development of antibiotics from the discovery to recent changes in the behavior and response of the microorganisms with the generation of AMR shortly after, is one of the most fantastic examples of the evolution that exists in nature.*

**Key words**: Microbial Drug Resistance, Bacterial Genes, Infection, Horizontal Gene Transfer, History.

*Fecha de recepción:* 29 de octubre de 2017
*Fecha de aceptación:* 8 de febrero de 2018

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Salud Uninorte
Vol. 34, N° 2, 2018
ISSN 0120-5552
eISSN 2011-7531

**Resumen**

*El aumento en la incidencia de infecciones asociadas a la atención en salud causada por microorganismos multiresistentes a antibióticos, han incrementado la morbilidad, mortalidad y otros factores que afectan a paciente, familias e instituciones prestadoras de servicios de salud; por lo que se ha hecho necesario el estudio permanente del tema, para identificar posibles estrategias que contribuyan a disminuir la situación. Se realizó una revisión de la literatura sobre el origen de los antibióticos, la evolución de su respectiva resistencia, el impacto en la salud pública; desde una perspectiva histórica y actual. La búsqueda de la literatura se realizó en las bases de datos bibliográficas: Pubmed, Web of Science, Scopus, SciELO, The Cochrane Library y Lilacs. El análisis de la literatura mostró desde el punto de vista histórico, el descubrimiento de los antibióticos hasta los últimos antibióticos de última generación, y la rápida coevolución de los genes de resistencia a los antibióticos y su posterior diseminación a cientos de especies de microorganismos mediante la Transferencia Horizontal de Genes (THG). También es discutido como el incremento de la resistencia a los antibióticos (RAM) genera una serie de factores que potencian las infecciones asocia de las a los cuidados de la salud (IACS) y su impacto en la salud pública. La historia desde el descubrimiento, los cambios en el comportamiento de uso de los antibióticos y la respuesta de los microorganismos con la generación de la RAM poco tiempo después, es uno de los ejemplos más fantásticos de coevolución que existe en la naturaleza.*

**Keywords:** Resistencia microbiana a los medicamentos, genes bacterianos, infección, Transferencia horizontal de genes, Historia.

## INTRODUCTION

Since the beginning of the antibiotics era, the resistance to these substances has described, during several decades; antimicrobial resistance (AMR) has been an increasing menace for the effective treatment of a wide range of infections caused by bacteria, parasites, virus and fungi. AMR produces a reduced efficacy of antibacterials, antiparasitics, antivirals and antifungals; turning difficult the treatment of patients who have got this kind of microorganisms (1). The origin, evolution and resistance mechanisms have appeared during the last 60 years; at the beginning, the problem was solved with the synthesis of new substances which were capable to control bacteria with AMR, then other medicaments appeared such as aminoglycosides, macrolides, glycopeptides, among others (2). During the first world congress about antibiotics resistance, the World Health Organization (WHO)) exposed that the so called "killer bacteria" are a worldwide menace, with a great ability to mutate; even avoiding broad-spectrum antibiotics, in the same way the data of 114 countries previously analyzed and it was also exposed that AMR is currently present worldwide and at every social level (3). The alarming increase of AMR is, without a doubt, one of the biggest problems of current public health, since these compounds are one of the main tools to control and treat bacterial infections, in human medicine as well as in veterinary.

Recent studies estimated the economical effects of ARM, for example; the annual cost for health system in USA is estimated from 21 to 34 billion dollars, accompanied by more than 8 million days in hospital (4); in Europe, it is estimated a cost around €1.600-6.000 per year in patients with resistance to third-generation cephalosporines (5); a study made in 12 European countries (Belgium, France, Germany, Italy, Netherlands,

Poland, Slovakia, Slovenia, Spain, Sweden and the United Kingdom) evaluated the costs of medical attention and the resistance to multiple medicines finding that this amounted to € 727.4 135 (£ 589 856) (6); the costs in Singapore are around USD$ 8638.58 in multiresistant infections (7); in Spain, the average economical cost per admission of patients who have got strains resistant to multiple medicines is higher than the ones with non-resistant strains with €15.265 compared to €4.933 for the others (8); in South Africa, the average cost of a successful tuberculosis treatment in patients resistant to rifampicin was USD $8359 (9). Therefore, the objective of this study was to make a critical review of the literature about the evolution of AMR, from different perspectives: Historical, molecular, mechanisms and its impact in public health.

## METHODOLOGY

A thorough review about the origin and impact of antibiotics and their endurance regarding the infections associated with health assistance worldwide was made with a historical and current perspective. This review is the result of the execution of the research project entitled: "Molecular typing of resistance genes in gram-negative bacilli associated to infections in a health service provider institution in Boyacá". For the identification of the studies, the bibliographic databases consulted were; Pubmed; Web of Science; Scopus; SciELO; The Cochrane Library and Lilacs. It was set a deadline for publications from 2010 to 2017. They were considered original researches or review articles, available in English or Spanish. Key words validated in Descriptors in Health Sciences were also used, which included microbial resistance to drugs, bacterial genes, infection, horizontal gene transfer and history.

## RESULTS

### Evolution of AMR

Since the emergence of humankind, the use of natural resources ha sbeen sought for its benefit, as an adaptive strategy to different environments.Several natural products were used by observation or intuition, in order to improve their health and welfare, mainly, facing the presence of pathogenic agents.Once these resources were considered exhausted and measured by advances in science, they resorted to chemical synthesis (10).

AMR has been described since the beginning of the 30s. After the use of penicillin in World War I, the first resistant bacteria emerged; in 1945, Fleming postulated the potential risks associated to the use of antibiotics; he showed that the use of a large and prolonged scale can select resistant bacteria, observing in his laboratory that bacteria sensitive to penicillin multiply in the presence of increasing concentrations of the antibiotic (11). During the 40s, the first report of penicillin resistance by strains of *Escherichia coli* (*E. coli*) and *Staphylococcus sp* was reported (12). In 1947, resistance to streptomycin among patients with tuberculosis was detected, where 80% of them relapsed within three months due to the formation of resistant bacilli (13). In the years 1952 and 1957, resistance to tetracycline and chloramphenicol was reported and in the decade of the 60s, β-lactamases producing strains, such as TEM and SHV of wide spectrum (detected in gram-negative bacilli) were discovered (14) (15).

Extended-spectrum β-lactamases (ESBLs) are phenotypically resistant to penicillins and cephalosporins; they were registered for the first time in *E. coli* in 1964 (16). Later, in

the 80s, antibiotics such as aminoglycosides (including vancomycin) were detected from resistant strains of *Enterococcus*; a short time later, it was found resistance to ampicillin in different species and the list kept growing (17). At the end of the 70s, bacteria resistant to ampicillin and cephalosporins were reported (12). In 1980, it was estimated that between 3-5% of *Streptococcus pneumoniae* (*S. pneumoniae*) was resistant to penicillin; but in 1998, 34% of these bacteria increased their resistance to this antibiotic. In the same decade, resistance to vancomycin and erythromycin was observed (13). Subsequently, in 1999 the multiresistance of gram-negative bacteria was described. For the period from 2002 to 2009, an increase in strains of *E. coli* resistant to broad-spectrum cephalosporins was observed, which is present in most of European countries. In 2008, a new enzyme called "New Delhi Metallobetalactamase" was observed, which confers resistance to all beta-lactam antibiotics, except aztreonam; showing a global alert against AMR to several non-beta-lactam antibiotics (18), leaving a few therapeutic options for the treatment of patients infected with these bacteria. After this report, the presence of this type of resistance was identified in 2010 in Canada, in 2011 in the United States and Guatemala; in 2012 in Uruguay, Paraguay and Colombia (19).

AMR is one of the most spectacular and documented natural event in microbial evolution, from the origin and application of antibiotics, passing through a few years in order to make that the different phenotypes arise; twelve years after the origin and application of penicillin, the first resistance mechanism was detected (20). This is how it is observed that, in a few years, bacteria can increase the speed of AMR generation. For example, in ten years after having resistant strains to penicillin and methicillin, resistance to chloramphenicol emerged, and in four years to streptomycin. Therefore, AMR is a growing public health problem, seen barely some years after the discovery of penicillin (21). That is why, a question arises from the emergence of AMR: What is or what are the mechanisms involved in this fast production of AMR?

**Origin of AMR**

Throughout the five decades that followed the synthesis and the indiscriminate use of antibiotics in people, animals and agriculture; a selective process unprecedented in the history of evolution has been observed, due to the fact that it has been an unregulated practice that lacks control and supervision (22); but this selection, considered by many researchers as artificial, has many components to consider in this review; in addition to the selective effect, the high rate of mutations in bacteria, the formation of bacterial communities and the horizontal transfer of genes, are important factors in the generation of AMR (23). There are several examples in the literature of spontaneous mutations in bacteria; some researchers consider that the evolution of resistance, through the acquisition of spontaneous mutations is particularly relevant for certain drugs, such as quinolones and rifamycins, for which the high-level resistance can result from a point mutation (22, 24). For example, in *Salmonella typhimurium* (*S. typhimuriun*), with a point mutation in the *henC* gene, the resistance of the bacteria to protamine increases, but with a cost in the reduction in bacterial growth (25); similar results were described in *Salmonella enterica*, where mutations in the tRNA-isoleucine gene confer resistance to mupirocin but with a reduction in growth (26). Other authors consider that AMR can evolve through the accumulation of multiple

sequential mutations and not by single point mutations (27); this mechanism would be responsible of the high levels of AMR that currently present many of the microorganism species, pathogenic species(28). These results, have shown that microorganisms that have a strong selective pressure (high concentrations of antibiotics), have RAM in a short period; similar to the one presented currently, especially in treatments against infections in humans (29).

It is presumed that there exist around 20 thousand resistant genes, predicted through the analysis of DNA sequences of different bacterial genomes however, they are functionally expressed in just some of them (28); many of them are originated by unique or consecutive punctual mutations, or also by gen duplications. But, what is the reason for these genes to be distributed in other strains or bacterial species presented in different environments? The answer to this question can be found in studies of comparative genomics. The identification of the sequences of bacterial genes in eukaryotic genomes, as the presence and genomics of pathogenicity islands presented in *E.coli*, found in other animal pathogenic, in human genome and some plant species; they confirm the theory of horizontal gene transfer (30). Horizontal gene transfer (HGT) has been considered as the mechanism responsible of the dissemination of antimicrobial resistance genes through different bacterial species (31). Actually, the genes that present resistance to certain antibiotics in non-related phylogenetically bacteria, demonstrate to have identical nucleotide sequences, including Gram positive and negative bacteria; it emerged at the beginning of the decade of 1990; it was a way to explain the phylogenetic incongruence using different gene trees. This process can also occur among the domains in all the possible directions, from bacteria to archaea, bringing new data about the rise of the genomic era, which has permitted the comparison of genes among different species (32). The interchange of genetic material in HGT among genomes is carried in different ways, acquiring a great relevance in the prokaryotic evolution due to the resistance to antibiotics that contribute to the inclusion of new mechanisms by bacteria (33, 34).

HGT is a phenomenon which takes place in and within the three domains of life (Fig. 1). The acquisition of genes by bacteria has got accelerated by the increase of adaptive and selective pressure needs, specifically the use of antibiotics in infections control in medicine, veterinary, agriculture and animal nutrition (35); the mark of the transference corresponds to the existence of a gene or genic sequence in the phylogenetic tree of the organism and to the observation of the same genic disposition in the donor and receptor bacterial population (31, 36).

With the recent increase of the studies in metagenomics, in which resistance to antibiotics has been identified in different ecosystems (37), for example; in human micro biome which generated complete genome sequences of several hundreds of human microbes, it has confirmed this HGT theory. Liu *et al*, detected a total of 13.514 genes coming from HGT identified in 308 human microbes in different parts of the body (including intestine, mouth, skin, etc.), with an average of 43,9 HGT per microbe THG (30). Besides this finding, researchers discuss the possibility of THG among the micro biome and the cells of our body; and how this event can be related to human health due to the fact that the total number of microbial cells hosted by the human body is 10 times greater than the number of human

cells in the body (100 times the number of genes in the human genome); The theory of HGT between the microbiome and the cells of our body is more than supported, but this behavior is not exclusive of the human microbiome (38).

The acquisition of genes by bacteria is accelerated, increasing the need of adaptation and selective pressure, specifically, by the use of antibiotics to control infections in human medicine, veterinary medicine and agriculture. Therefore, being in permanent contact with diverse environments, farm or domestic animals, plants, insects, among others HGT could be present even more frequently than it is commonly thought (31, 39)

Among the most probable mechanisms of HGT are conjugation, transformation and transduction; in which mobiñe genetic molecules take part such as plasmids, bacteriophages, transposons, integrons and gene cassettes that have genes with functions for their own transfer and / or bacterial resistance (40). In chat 1 examples can be found of mobile genetic elements that transfer resistance genes.

One of the most common and known mechanism is the conjugation by means of plasmid transfer, taking resistance genes; in Gram-negative bacteria, resistance genes are found as a part of small mobile genetic elements or "cassettes", integrated in greater elements (integrons) (41). Integrons are structures of interest because they are found in the bacterial chromosome structure presented in the cassettes of genes related to resistance; it has been observed that more than a cassette can be inserted in the same integron to generate molds that contribute to the spreading of the multiple resistance (42). Resistance genes spreading is higher when these are part of mobile genetic cassettes, which permit them to be transferred by several mechanisms (43). There exists enough scientific evidence of the high rate of HGT among gram-negative and positive bacteria, generated mainly by conjugation.

Cassettes can codify several compounds that generate resistance for a huge range of antibiotics including ß-lactam, aminoglycosides, trimethroprim, amphenicol, sulfonamide, tetracyclines, rifampicin, erytromycin and quinolones (44).Therefore, integrons and cassettes that bring multiple ARM are, currently, the most studied genic elements by researchers in order to explain the origin of ARM and its impact in public health.

## AMR in health-care associated infections

During more than 60 years, antibiotics have been considered as the panacea to cure infections, with enormous benefits for human health. The development of the resistance to this important class of medicaments, and the consequent loss of its efficacy as an antimicrobial therapy, represents a serious health menace. Despite the efforts of hospitals to improve the caring process and health of the patient, infections still occur with a higher frequency; it has been complex to determine the world range exactly; it is estimated that every year billions of patients get affected (45). Health-care associated infections (HAI) are defined as any infectious process, general or localized, that occurs due to the stay or attendance to a health center and appears during or after the discharge more than 48 hours after the entry. They include blood infections, affected area by a surgery, skin and soft tissue, pneumonia, and urinary tract infections which are the most common (46). Therefore, HAI, besides entailing an adverse

effect for the patient, are also an indicator of the caring quality. The rise and reemergence of HAI, caused by ARM microorganisms, has as a consequence the increase of morbidity and mortality in hospitals around the world (47, 48). They are associated to economic effects in institutions, in health systems and therefore in economical ranges for the countries (49-51).

In the American continent, a prevalence of HAI is present which varies from 4.5% in the United States, to 14% in Brazil (52). Other studies in several countries of America reveal a wide variation in the incidence of resistance in common bacterial pathogens, as an example, the resistance to third generation cephalosporines observed in *E. coli*, varying from 0% in the case of Brazil to 50% in Peru; in comparison with the world reports where they are found in 26.8% (53).

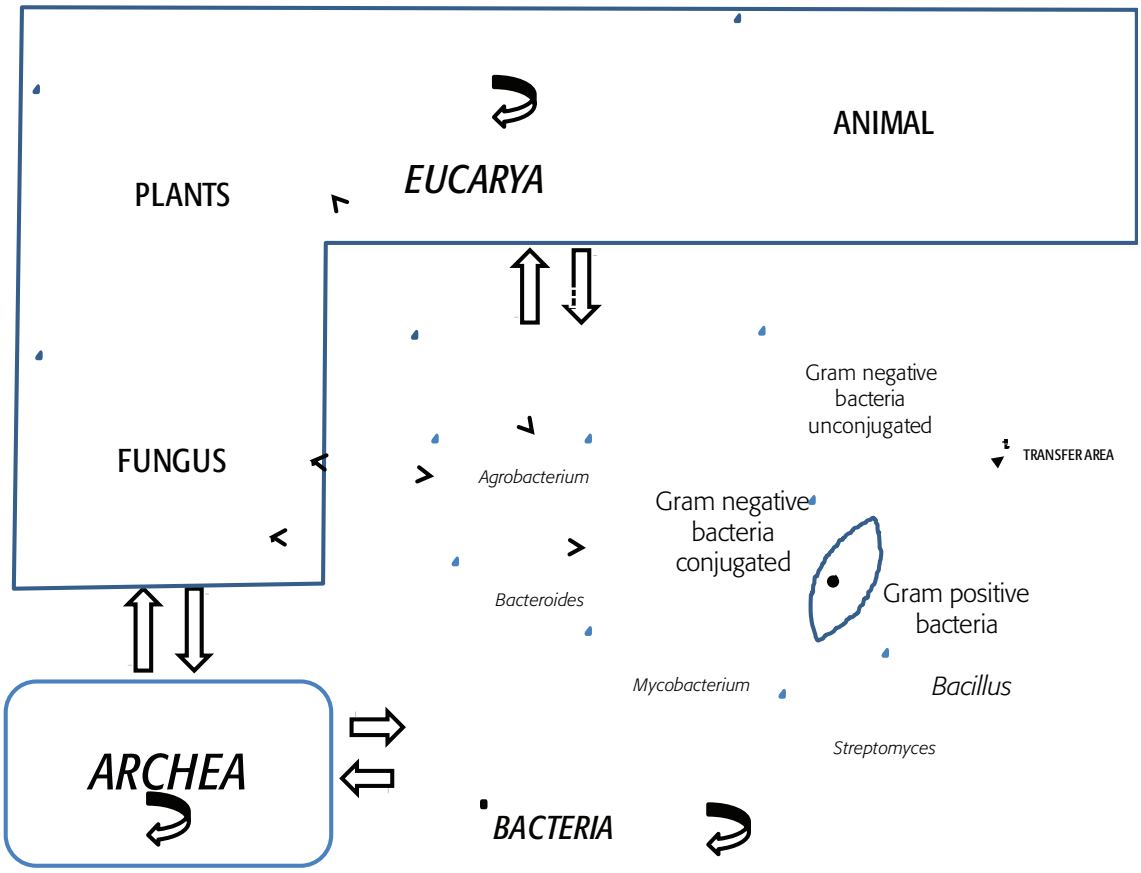
The increase of the resistance has become one of the most important aspects in the world and this is why, the antimicrobial resistance was declared as a public health problem by the World Health Organization in 1999; it is related to the excessive and indiscriminate use of antibiotics in the community and hospitals, as a decisive factor in the origin of the rise of resistant pathogens nowadays (3).Therefore, it is necessary to promote strategies of control of AMR through the exact identification of the microorganism and its resistance phenotype, besides the opportune information of these results to the service of infectious disease treatment in the hospital, in order to avoid the proliferation of multiresistant strains that produce new HAI (46).

## CONCLUSIONS

The history of the discovery and usage of antibiotics and their corresponding ARM generation a short time later, is one of the most fantastic examples of coevolution that exist in nature. One of the decisive factors in this case is the indiscriminate use of antibiotics at different levels: In human, animal and environmental medicine. The last mentioned, can emerge when the antibiotics that were not consumed are thrown, taking the risk of generating resistant bacteria in the environment.

On the other hand, the rise of resistance genes can have several origins: (a) Punctual mutations, (b) Consecutive mutations of high frequency and, (c) genic duplications. Besides, microorganisms are held to strong selective pressures, due to the indiscriminate use of antibiotics which cause multiresistant strains. In the same way, these ARM microorganisms are able to transfer their resistant genic pool to other strains or sensitive species to antibiotics by different HGT mechanisms as transduction, transformation and conjugation. HGT can be presented in different auspicious environments as health attention centers where different types of infections are treated and their different origins increasing the HGT potential. The increase of HAI is originated for multiple factors, most of them, avoidable. Unfortunately, in many HGT cases, infections are originated by multiresistant microorganisms, even to last generation antibiotics, increasing the morbidity and mortality around the world.

In a consistent way with the alarms of the WHO, the studies related to ARM must be increased as well the respective restrictions of antibiotics usage, mainly in developing countries as the ones of South-America.




Las HSPs tienen 4 regiones funcionales conservadas. En Azul claro dominio N-terminal (llamado dominio J), En azul la región flexible rica en glicina/fenilalanina, en verde la región M de unión al sustrato y en rojo la región N-terminal. Fuente: Realizada por los autores de la revisión.

**Figure 1**. The horizontal transfer of genes between the domains of life

**Chart 1**. Examples of mobile genic elements that transfer resistance genes

| Donor bacterium | Receptive bacterium | Vector (genetic element) / genes | Resistance phenotype |
|---|---|---|---|
| Klebsiella pneumoniae, Eschericha coli, Enteroacter cloacae | Eschericha coli | Plasmids R6K, RP4, R1 y pUA21 / BLEEs tipo SHV-2 y SHV-5 (54, 55). | Cephalosporins |
| Escherichia coli MKD13 | Klebsiella pneumoniae | Plasmids pNU147 / blaTEN-1 (56). | ß-lactam, gentamicin, kanamycin, tetraciclyne and chloranphenicol |
| Ancestral | Pseudomonas aeruginosa | Integron In0 - plasmid pVS1 / sul 1 (57). | Sulfonamide |
| Ancestral | Acinetobacter baumannii biotype 9 | intI1 - intI2 (Tn7, Tn21)/ sul1 (50). | ß-lactam, sulfonamides, trimethoprim, tetraciclyne, chloranphenicol, and aminoglycosides |

**Conflict of interests:** The authors have declared that there are no conflict of interests.

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