REVISION DE LITERATURA

EVOLUTIONARY MECHANISMS THAT GENERATE BACTERIAL RESISTANCE IN LIVESTOCK PRODUCTION

MECANISMOS EVOLUTIVOS QUE GENERAN RESISTENCIA BACTERIANA EN LOS SISTEMAS DE PRODUCCION GANADEROS

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ABSTRACT

From a strictly Darwinian evolution reference the result of continuous adaptation of an organism to changing a different environments. Any modification of gene structure of an individual assumes the ability to adapt to new environments. The irrational use of antibiotics in livestock production, self-medication and lack of knowledge of the mechanisms of bacterial resistance have led to a marked decline of therapeutic options in health services and problems associated with food security. Multidrug resistance is a challenge to the various treatments leaving little chance to successfully tackle infections. Transfer of resistance genes is performed horizontally across several genetic elements within the best known are, plasmids, transposons, bacteriophage DNA and more recently, gene cassettes and integrons. The transfer of these elements can occur between different bacteria by conjugation, transformation or transduction. This transfer allows the transmission to other generations and also other bacterial species, thus bacteria can acquire resistance to one or more drugs without being in contact with these. The mechanisms used by bacteria to defend against antibiotics are constantly evolving. Resistance to antibiotics is due to genetic and structural processes of microorganisms through which mechanisms have been developed as efflux pumps, altered site of action, enzymatic modification of the antibiotic by beta-lactamases, Carbapenemases and other modifying enzymes, changes in membrane permeability closing mainly porins

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RESUMEN

Desde una referencia estrictamente darwinista, la evolución es el resultado de la adaptación continua de un organismo a ambientes cambiantes o diferentes. Cualquier modificación de la estructura génica de un individuo supone la posibilidad de adaptarse a nuevos ambientes. El uso irracional de antibióticos en las producciones pecuarias, automedicación y la falta de conocimiento de los mecanismos de resistencia de las bacterias han llevado a una disminución acentuada de las opciones terapéuticas en los servicios de salud y problemas asociados a la seguridad alimentaria. La multirresistencia representa un reto a los diversos tratamientos dejando pocas posibilidades para enfrentarse exitosamente a las infecciones. La transferencia de genes de resistencia se realiza horizontalmente a través de varios elementos genéticos dentro de los más conocidos están, los plásmidos, transposones, el ADN de bacteriófagos y, más recientemente, los integrones y cassettes genéticos. La transferencia de estos elementos entre diferentes bacterias puede ocurrir por conjugación, transformación o transducción. Esta transferencia permite la trasmisión a otras generaciones y también a otras especies bacterianas, de esta forma una bacteria puede adquirir resistencia a uno o varios medicamentos sin necesidad de haber estado en contacto con estos. Los mecanismos que utilizan las bacterias para defenderse de los antibióticos están en constante evolución. La resistencia a los antibióticos se debe a procesos genéticos y estructurales de los microorganismos, a través de los cuales se han desarrollado mecanismos como: bombas de expulsión, alteraciones del sitio de acción, modificación enzimática del antibiótico por betalactamasas, carbapenemasas y otras enzimas modificadoras, cambios en la permeabilidad de la membrana principalmente por cierre de porinas.

Palabras clave: bacteria, Resistencia, antibioticos, produccion ganadera.

INTRODUCCIÓN

The evolutionary process of a living being is accelerating the larger is its ability to produce genetic variability, either by mutation or recombination. However, the higher this capacity, also increased the risk of accumulating deleterious mutations. Genetic variability is therefore a highly regulated, so that bacteria tend to maintain a low rate of mutation process. In different bacterial populations analyzed there is always a variable percentage of strains with a mutation rate of the modal frequency than the rest of the population (1).

There is a direct relationship between the proportion of strains mutate and degree of environmental stress. For example, in chronic infectious diseases, in which the antibiotic regimens for prolonged periods, the highest percentages of mutating bacteria, about 50% of the population are observed. This positive selection of bacteria that mutate is due to the enormous potential that have to develop antibiotic resistance (100 times higher than normal bacteria). This capability has been capitalized in research centers as a natural model of accelerated evolution to predict the ease with which certain resistant variants may occur, know what positions are the most susceptible to changes and what the cost to the bacteria (1).

The evolution of antibiotic resistance in bacteria is of great importance especially in the field of health and livestock production. This condition is the result of natural selection causes phenotypic differences. Evolution requires a phenotypic variation, selection and heritability. This usually determines that a mutation provides a unique groove biodiversity originates evolution (2). The resistance phenomenon has intrinsic or acquired genetic substrate that is phenotypically expressed by biochemical mechanisms. Thus resistance can be seen both from the biological perspective and biochemistry (3).

In the early fifties, several antibacterial drugs used to treat bacterial infections, including penicillin, streptomycin and tetracycline. It soon became clear that it was difficult to eliminate certain bacteria with these drugs, pathogens have developed resistance to antibiotics. By exposing the bacteria continuously to antibiotics, humans introduced inadvertently agent natural, strong selection within the microbial world. Bacteria reproduce and mutate quickly. Those bacteria that produced resistance mutations drug effects, survived and prospered quickly dominating bacterial populations. The researchers found that drug-resistant bacteria could transfer their genes to counter medicines even in other bacterial species (4). The widespread use of antibiotics in livestock production systems, the hospital system for self-medication and consumption lead to the selection of multidrug resistant bacteria, that is, a bacterial strain carries the resistance to more than one antibiotic. This phenomenon shows the importance of studying the spread of resistant strains by the emergence of increasingly complex (5).

Natural or intrinsic resistance, permanent mechanism is genetically determined, not be correlated with increasing doses of the antibiotic (3). It belongs to each family, species or bacterial group and its appearance predates the use of antibiotics, as evidenced by the isolation of antimicrobial resistant bacteria, an estimated age of 2000 years found in the depths of the glaciers in the regions Arctic Canada. Besides all bacteria of the same species are resistant to some families of antibiotics (6), for example, all gram-negative bacteria are resistant to vancomycin, and this situation is not variable. The resistance achieved is variable and is acquired by a strain of a bacterial species. Thus, there *pneumococcal* strains that have acquired resistance to penicillin resistant strains of *Escherichia coli* to ampicillin resistant strains of *Staphylococcus aureus* (8). Transmissible resistance is the most important, being mediated by plasmids, transposons or integrons, which can move from one bacterium to another (9).

For the above ideas, the work explores the main evolutionary mechanisms that have allowed the generation of bacterial resistance to antibiotics, which begins by describing genetic and structural processes of microorganisms through which mechanisms have been developed and dynamic patiently traced and identified.

EPIGENETICS

The term epigenetics has evolved to include any process that alters gene activity without changing the DNA sequence, and leads to modifications that can be transmitted to daughter cells (although experiments show that some epigenetic changes can be reversed). Many types have been identified in bacteria epigenetic processes which include methylation, acetylation, phosphorylation. Probably other considerations and epigenetic mechanisms as the time advances arise. Epigenetic processes are natural and essential for many body functions, but if they occur improperly significant adverse effects on health and behavior may occur. Perhaps the best known epigenetic process, partly because it was easier to study with existing technology, is DNA methylation. This is the addition or removal of a methyl group (CH3) that occurs predominantly where cytosine bases are arranged consecutively. DNA methylation was first confirmed in human cancer in 1983, and has since been observed in many other diseases and health conditions (10).

Another significant epigenetic process is chromatin modification. Chromatin is a complex of proteins (histones) and DNA that are tightly packaged to fit into the nucleus. The complex can be modified by substances such as acetyl groups (acetylation process called), enzymes, and some forms of RNA such as microRNA and small interfering RNA. This modification changes the chromatin structure to influence gene expression. In general, the strongly bent chromatin tends to be turned off, or not expressed, while the looser chromatin is functional, or expressed. One effect of such processes is the primer. In genetics, primer describes the condition that one of the two alleles of a typical pair of genes is silenced by an epigenetic process as methylation or acetylation. This becomes a problem if the allele is expressed altered or contains a variant that increases the body's vulnerability to microbes, toxic agents or other drugs (2).

RESISTANCE BACTERIA EVOLUTION

Two mechanisms are involved in antibiotic resistance: mutation and acquisition of resistance genes by horizontal (horizontal gene transfer HGT = gene transfer). Human pathogens are susceptible to antibiotics, but with the use of medications for treatment of infections, have originated acquired antibiotic resistance by HGT. In some instances the antibiotic resistance genes originate by environmental microorganisms systems (11).

Mutation

Microbial evolution depends, therefore, two opposing forces: on the one hand, maintenance of genetic information (the bacteria have a low rate of mutation to stay in the environment for which are optimally adapted) and, secondly, a certain level of genetic variation allows them to conquer new environments. This balance of forces is the key to evolution and survival. Any stress to the bacteria (lack of nutrients, ultraviolet light, antibiotics, etc.) breaks this balance and increases the mutation rate (1). The two main mechanisms that increase the mutation rate are those that affect replication (SOS system) and DNA repair (MMR system or system mismatch repair). The SOS system is induced when a DNA damage and the MMR system is responsible to recognize and repair mismatched bases during replication (8).

Horizontal gene transfer

In a study of horizontal gene transfer in bacteria and archaea genome, show that horizontal gene transfer is important in the evolutionary process of prokaryotes. There is a statistical method for predicting whether genes of a genome are acquired by horizontal gene transfer. This based on an analysis of the G + C content, codons, amino acids and position of the genes. The horizontal gene transfer can be present in one or both lineages and include genes constituting prophage isolated pathogens, transposons, integrase or recombinase, genes in *Helicobacter pylori* and functionally related gene regions (12).

GENETICS OF RESISTANCE

Bacteria are able to acquire resistance depending on their genetic variability. The emergence of resistance in a bacterium occurs through mutations and by the transmission of extrachromosomal genetic material from other bacteria. In the first case, the resistance is transmitted vertically from generation to generation. Then, the gene transfer is done horizontally across movable plasmids or other genetic material and transposons integrons; the latter not only allows the transmission to other generations, but also other bacterial species (transmissible resistance). Thus bacteria can acquire resistance to one or more antibiotics without being in contact with these (13). In the acquisition of antibiotic resistance mutations may occur during treatment with antibiotics, while the resistance acquired by HGT (horizontal gene transfer) requires contact between a microorganism in the biological environment (or in the case of DNA transformation) and bacteria associated with human (11).

Transfer Mechanisms of genetic material between bacteria

The mechanisms of transfer of genetic material between bacteria are conjugation, transduction and transformation. Conjugation, is a gene transfer process that requires cell-cell contact. This mechanism requires a donor bacterium containing a conjugative plasmid and a recipient bacterium which lacks it. In transduction, DNA is transferred from one bacterium to another by a bacteriophage. The transduction can be generalized or specialized and finallyTransformation, that is a process by which free DNA is incorporated into a competent host bacteria is carried out and genetic recombination. When a bacterium

is able to take a DNA molecule and transformed is said to be competent. Only some strains are transformable, suggesting it may be a heritable (14).

Dispersal mechanisms resistance genes

The biological phenomenon of resistance depends on the emergence and maintenance of resistance genes such as chromosomal and extrachromosomal genetic elements. In short it is the change in the genome that determines the appearance of these genes; these changes are classified into macroevolution and microevolution. The first are the result of unique mutations that compromise paired nucleotides, while macroevolutionary affect DNA segments (3). Dispersal of the resistance genes are mediated by plasmids, transposons and integrons carrying the antibiotic resistance genes (14).

Plasmids

Genes coding for bacterial antibiotic resistance may be located in both the chromosome and plasmids. Plasmids are genetic elements that replicate independently of the bacterial chromosome, consist of double-stranded DNA, are circular and vary in size from 103 to 106 base pairs (bp). Plasmids found in Gram positive and Gram negative bacteria, knowing thousands of different plasmids. Only in *E. coli* strains over 300 natural plasmids were isolated. Resistance plasmids (plasmids R) are one of the most widespread groups of clinical isolates plasmids confer resistance to antibiotics and growth inhibitors (9).

Transposable elements

Transposable elements were first identified as spontaneous bacterial operons inserts as nullify the transcription or translation of genes which are inserted. Thereafter various types of transposable elements were characterized (14).

Insertion sequences

The simplest transposon are called insertion sequences (IS). Each type carries a SI prefix followed by a number that identifies it. The IS elements are normal constituents of the bacterial chromosome and plasmids. Transposons compounds are formed by a central region carrying the marker gene, flanked on both sides by IS elements, may be in the

same or reverse orientation. In replicative transposition element is duplicated during the reaction, thus transposing the unit is a copy of the original item. Then, transposition involves an increased number of copies of transposon (12).

Integrons

Integrons potentially a family of mobile genetic elements able to integrate and express genes for antibiotic resistance. To date, several families have integrons described according to the nucleotide sequence of intI gene, at least three of them are related to the expression of resistance genes. Your integrase have between 45% and 58% homology, suggesting an evolutionary divergence for more than 50 years period, which corresponds approximately to the antibiotic era (15).

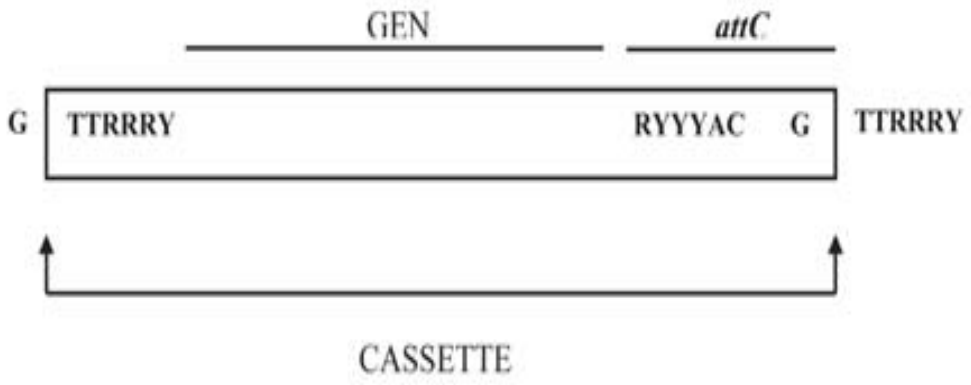

Figure 1. Gene cassette structure; the cassette is made by gene recombination site and attC. The arrows indicate the beginning and end of the cassette, denoting the sites where the site-specific recombination for mobilizing the same is done (14).

Cassettes genes

Cassette genes is a typically structure that include two functional components: the gene and the recombination site or element attC 59 bases (59-be), located at the 3 'end of the gene (Figure 1). The gene cassettes are a diverse group of small moving parts that usually contain only complete open reading frame (ORF), or coding region (gene). Although it is considered that the cassettes are movable members, not encode enzymes or other

products involved in their own mobilization. Cassettes encoding resistance to a wide range of antibacterial compounds, including ßlactámics antibiotics, aminoglycosides, trimethoprim, sulphonamides, phenicols, tetracycline, rifampicin, erythromycin and Quinolones (15).

RESISTANCE MECHANISMS

The resistance mechanisms can be grouped into four categories (Figure 4).

Enzymatic modification of the antibiotic

Bacteria expressing the enzymes can create changes in the structure of antibiotic causing it to lose its functionality. The ß-lactamases are the most prevalent. Proteins are capable of hydrolyzing the beta-lactam antibiotics possessing ring of this family such as hydrolytic modification acetylations, phosphorylations adenilaciones inactivating or aminoglycosides can also occur (16).

ß-lactamase

The beta-lactam antibiotics have in common their molecular structure with a ß-lactam ring, which is largely responsible for its antimicrobial action. The ß-lactamases are enzymes capable of breaking this ring and inactivate these antibiotics. The ß-lactamases are ubiquitous Gram negative bacteria and represent an important form of resistance. The genes encoding these enzymes can be found in the bacterial chromosome or plasmid, which allows easy transfer between different bacteria, which represents a major challenge to control infections (17, 18).

Betalactamase as plasmids

The plasmid betalactamases are fundamentally the class A enzymes which are typically found on plasmids, whereas the chromosomal be C (19). In class are primarily gram positive penicillinase, excluding action cephalosporins. Beta-lactamases in gram-negative bacilli, originally had a smaller chromosomal action spectrum, but were more effective (20).

Chromosomal beta-lactamase

Confer resistance to second-generation cephalosporins, and depending on their size, they are also able to confer resistance to third-generation cephalosporins. Its expression mechanism is closely related to the synthesis of peptidoglycan and recycling, which acts through a promoter that is normally inhibited (21).

Carbapenemases

Carbapenemases are able to hydrolyze penicillin, first-generation cephalosporins and carbapenems weakly; not hydrolyze third generation cephalosporins and aztreonam (22). Due to their low activity against carbapenems, the carbapenemase OXA type are capable of conferring resistance to carbapenems when the bacterium expresses some other mechanism of resistance, such as closing porins and exaggerated expression of efflux pumps (23, 24).

Other modifying enzymes

In addition to beta-lactamases are other enzymes responsible for the emergence of antimicrobial resistance methylases such as, acetyl-transferases, and transferases phosphotransferases nucleotidil- inactivating especially aminoglycosides. This enzyme can also generate resistance to fluoroquinolones, non-synthetic antibiotics aminoglycosides (25). The genes responsible for producing these methylases by bacteria found in other plasmids carrying resistance genes, leading to patterns multiresistant Gram negative bacteria (26).

Efflux pumps

Taking the antibiotic operate the periplasmic space and expelling the outside, thereby avoiding coming to their site of action. This mechanism is used frequently in Gram negative bacteria (27). Efflux pumps are responsible for antimicrobial resistance not only in bacteria but also in other common pathogens such as parasites (*Plasmodium spp*., For example). Found in the outer membrane of the cell and ejected outward of the bacterium

many molecules, including metabolites, detergents, organic solvents and antibiotics. They use ATP hydrolysis or counter-transport mechanism ion as an energy substrate (28, 29).

The main role of this mechanism is to maintain low concentrations of toxic substances within the cell. Output pumps may be specific to a drug (generally plasmid encoded and, therefore, transmitted by) or nonspecific (usually expressed in the bacterial chromosome (30). If expression is increased nonspecific pump may generate cross-resistance to multiple classes of a single mechanism being used drugs (31).

Changes in the permeability of the outer membrane (closing porins)

Bacteria can generate changes in the lipid bilayer, but the permeability of the membrane is altered primarily by changes in porins. porins are proteins that form water-filled channels embedded in the outer membrane that regulate the input elements, including antibiotics. changes in conformation may lead to the outer membrane not allowing passage of these agents to the periplasmic space (29).

These molecules have the ability to retard access of antibiotics into the bacteria. The beta-lactam antibiotics must penetrate through these channels; when a porin is lost by mutations increase the CIM for the antibiotic. Porins may be specific or nonspecific depending on their selectivity for molecules which pass. Changes in permeability of the membrane may include three types of alterations (19), changes in bacterial membranes, changes in input energy dependent antibiotics and increased output antibiotics.

Site of action disturbances

Bacteria can alter the site where the antibiotic is bound to the bacteria to interrupt this vital function. This mechanism is primarily used by Gram-positive bacteria, which cause structural changes in the sites of action of ß-lactam-level penicillin binding proteins antibiotics. There are other strategies to achieve this goal. Action sites can be found in different bacterial components vital cellular activities involving (32).

Another site of action of antibiotics is protein synthesis, this process can be inhibited by targeting the nuclear components of DNA replication and RNA transcription. For example,

quinolones inhibit topoisomerase, an enzyme responsible for the cleavage of DNA replication. Similarly, protein synthesis, conducted on ribosomes, can be inhibited by drugs such as aminoglycosides, tetracycline, clindamycin, macrolides and chloramphenicol. It is usually due to chromosomal abnormalities although recently it has been associated with plasmid-borne genes (33).

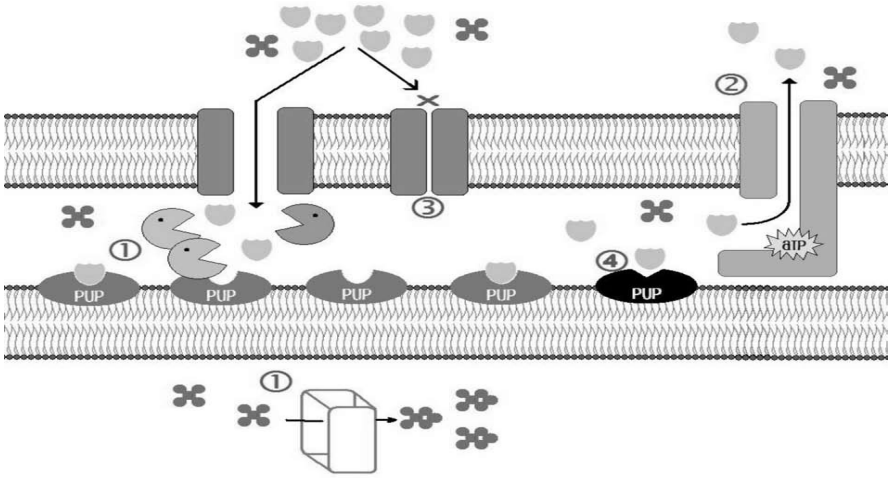

Figure 2. Main mechanisms of resistance to antibiotics. 1. modifying enzymes. 2. Pumps expulsion. 3. Close porin. 4. Penicillin-Binding Proteins (PUP) (30).

The role of the environment in the evolution of resistance in pathogenic bacteria (34), suggest that there are three important points in the evolution of resistance of human pathogens:

1. Bacteria in the biological environment contains a large number of genes that confer antibiotic resistance and transfer HGT. The amount of antibiotics and resistance genes appear as a result of human activities on natural ecosystems.

2. In the habitat there is frequent contact of bacteria associated with human to environmental microbiota.

3. Treatment of patients by itself. The DNA is transferred to pathogenic commensal bacteria, generating resistance to β-lactamases by Gram positive bacteria.

CONCLUSIONES

Resilience to antibiotics bacteria can be present an intrinsic characteristic, or may result from the selective pressure arises from the use of antibiotics. Bacteria develop various mechanisms to survive in the presence of an antibiotic due to mutation resistant alleles of native genes and how easy it is to exchange information, intra- and interspecies, using elements such as plasmids, transposons, integrons or cassettes capable of mobilize resistance genes.

The transfer of genetic information, together with the selective pressure by overuse of antibiotics has led to the emergence of strains resistant to multiple antibiotics, which given the complexity and speed of the phenomenon in clinical therapeutic disadvantage to intervene timely and successfully an uncertain future in terms of production and availability of antibiotics to fight infection, superlative why multidrug resistance has become one of the most serious public health problems, recognized by the World Organization Health.

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