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Antimicrobial Resistance in *E. coli* Isolated from the Biological Samples in Zacatecas Patients

De la Cruz López María Margarita¹, López Luna María Argelia¹, Nava Maldonado Flavio Manuel¹, Noemí Gaytán Pacheco¹, Infante Espinoza Margarita María² and Flores de la Torre Juan Armando^{1*}

¹Academic Unit of Chemistry Sciences, Autonomous University of Zacatecas, México

²Academic Unit of Odontology, Autonomous University of Zacatecas, México

*Corresponding author: Dr. Flores de la Torre Juan Armando, Academic Unit of Chemistry Sciences, Autonomous University of Zacatecas, México, Tel: 4492055221; E-mail: armando.flores@uaz.edu.mx

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Abstract

The problem of antimicrobial resistance has led Health Services take to stop population from self-medicating. The use of antibiotics to trigger a possible mutation of the most common bacteria of which the human being has contact, so it is important to evaluate the consequences. The present study was to evaluate the antibacterial sensitivity necessary to determine the antibiotic to be prescribed; were analyzed cultures of 48 patients who already referred symptoms were used between hospital inpatients and outpatients, of different sex and age ranges groups. The biological samples were vaginal exudates and urine cultures. The results reports indicate that resistance predominates over sensitivity, a fact that must draw attention to taking decisive measures in this regard.

Keywords: Antimicrobial resistance; *E. coli*; Zacatecas patients

Introduction

Antimicrobial chemotherapy can be defined as treatment with antimicrobial chemicals by general or systemic means (as opposed to local or topical antiseptics). Antimicrobial chemotherapy, antibiotics or anti-infective are substances of selective toxicity, that is, they must be much more toxic to the microorganism (or parasite) that causes the infection than to the host. Scientific antimicrobial chemotherapy began in 1909 with Paul Ehrlich, using the treatment of syphilis with Salvarsán. Ehrlich spoke of a magical projectile capable of killing the infectious agent without causing harm to the patient.

Antibiotics were originally considered to be natural antimicrobials and chemotherapeutic ones were those of synthetic origin. Today this distinction is not used, and both terms are considered synonymous. Hence, the term

antimicrobial has been generalized to encompass both. When an infection is treated with bacteriostatic antibiotics, we rely on the host's defenses, to finally destroy the infecting agent (whose growth is only stopped by the antibiotic) and completely eliminate the infections.

When chemotherapy is used to treat infections in patients with diminished defense mechanisms (immunosuppressed), always use bactericidal antibiotics, because not only can rely on the action of the antibiotic [1].

Desirable properties of antimicrobials

The ideal antimicrobial used for chemotherapeutic purposes is characterized by a number of properties.

- Selective toxicity is an essential property of a chemotherapeutic agent, which must inhibit or destroy the pathogens without harming the host.
- The ideal chemotherapeutic agent has a bactericidal rather than bacteriostatic action. 3) The ideal chemotherapeutic agent is one for which susceptible microorganisms do not become genetically or phenotypically resistant.
- That the agent is effective against a broad spectrum of microorganisms that are found most frequently in clinical practice.
- The ideal agent should not be allergenic nor cause adverse side effects due to the continuous administration of large doses.
- The agent must remain active in the presence of plasma, body fluids or exudates.
- The agent must be water-soluble and stable, and bactericidal levels in the organism must be reached quickly and maintained for prolonged periods [2,3].

Antibiotics

The term "antibiotic" refers to a substance produced by a microorganism or a similar substance made completely or partially by chemical synthesis, which at low concentrations inhibits the development of other microorganisms. The microorganisms that produce the different antibiotics have a

wide distribution in nature, where they play an important role in the regulation of the microbiological populations of soil, water, and manure. The chemical, physical and pharmacokinetic properties of antibiotics are very varied, as are their antimicrobial spectrum and their mechanisms of action. Of the several hundred naturally-produced antibiotics that have been purified, only a few have proved sufficiently non-toxic to be used in medical practice. Those that currently have the greatest use have been obtained from a relatively small group of microorganisms belonging to the genera: *Penicillium*, *Streptomyces*, *Cephalosporium*, *Micromonospora* and *Bacillus* [4].

Mechanism of action

Antibiotics are classified based on their chemical structure and mechanism of action as follows, observing some examples in **Table 1**:

- Antimicrobials that act in the synthesis of the cell wall.
- Inhibitors of protein synthesis
- Inhibitors of nucleic acid synthesis
- Folate inhibitors
- Antimicrobials that act on the outer and cytoplasmic membranes [5,6]

Table 1 Classification of antibiotics by their mechanism of action [4].

Target	Group of antibiotics
Bacterial wall	Betalactamics, vancomycin
Ribosomes	Aminoglycosides, macrolides
Via folic acid	Sulfonamides, trimetropin
DNA replication	Quinolones

Antimicrobial resistance

It is called the clinical resistance of a bacterium to an antibiotic, to the inability of that antibiotic to cure an infection for that bacterium.

Natural resistance: There are groups and species of bacteria that are always resistant to one type of antibiotic: for example, all anaerobic bacteria are resistant to aminoglycosides. *Pseudomonas aeruginosa* is resistant to penicillin.

Acquired resistance: A microorganism that was originally sensitive to an antimicrobial becomes resistant to it (the antibiotic that previously served to treat these infections is no longer valid), nowadays very frequent with the abuse and its massive use of antibiotics. Microorganisms can develop resistance by very different mechanisms: a) because they learn to produce inactivating enzymes that destroy the antibiotic (destruction of beta-lactamases, by beta-lactam antibiotics), b) because they alter the wall in such a way that they prevent the penetration of the antibiotic [7].

Transmissible resistances: When the resistance is the consequence of a mutation, when a microorganism becomes

resistant to the antibiotic its descendants inherit this characteristic, and over time this resistance diffuses widely among all the bacteria of the same species, another the microorganism transmits this resistance without the need for these to be their descendants, using the mechanism of transfer of genetic material.

Multiresistant: The bacteria are simultaneously resistant to many antibiotics: 1) against a group of analogous antibiotics (cross resistance), usually due to the appearance of a mechanism of inactivation of this group of antibiotics. Eg: emergence of resistance of *P. aeruginosa* to penicillin and cephalosporin by production of highly active beta-lactamases. Or the appearance of *Staphylococcus aureus* resistant to all beta-lactams by a mutation that causes a change in its target cell wall. 2) Multiresistance can also arise due to a generalized loss of permeability of the bacteria to antibiotics. Eg: *S. aureus* simultaneously resistant to beta-lactams, quinolones and other antibiotics. This happens because the wall of bacteria does not allow the passage of antibiotics inside them and then they cannot act on their targets [8].

Causes of resistance to antimicrobial agents

For antimicrobial drug therapy to be effective, the drug must reach the site of contamination or infection in adequate concentration.

The bacterial resistance to an antibiotic can be attributed to three general mechanisms: 1) the drug does not reach its target; 2) the drug is not active or 3) the target is altered [8-10].

The outer membrane of gram-negative bacteria is a permeable barrier that prevents the penetration of large polar molecules. Small polar molecules, like many antibiotics, penetrate the cell through a group of protein channels called porins.

The absence, mutation or loss of a porin decreases the speed with which the drug penetrates the cell or even prevents its entry, thus reducing the concentration of the drug in its target. When the target is intracellular, and the drug requires active transport through the cell membrane, any mutation or phenotypic change that interrupts this transport mechanism confers resistance. For example, gentamicin, which targets ribosomes, is actively transported through the cell membrane using the energy provided by the electrochemical gradient of the membrane. This gradient is generated by respiratory enzymes that combine the transport of electrons with oxidative phosphorylation.

Any mutation in an enzyme of this route or anaerobic circumstances (oxygen is the terminal electron acceptor of this route and its absence decreases the energy potential of the membrane) reduces the speed with which gentamicin penetrates the cell, causing resistance. In addition, the bacteria have an exit pump that can transport the drugs to the outside of the cell.

The second general mechanism of drug resistance is pharmacological inactivation. Bacterial resistance to

aminoglycosides and β -lactam antibiotics is almost always due to the production of an enzyme that modifies the aminoglycosides or a β -lactamase, respectively. A variation of this mechanism is the failure of the bacterial cell to activate a prodrug [11].

The third general mechanism of pharmacological resistance is the presence of an altered target. The reason is perhaps the mutation of the natural target (E.g. resistance to fluoroquinolone), the modification of the target (e.g., ribosomal protection resistance to macrolides and tetracyclines) or the acquisition of a resistant variety of an objective that is naturally sensitive (e.g., *Staphylococcus* resistance to methicillin through the production of a low affinity penicillin binding protein).

Likewise, pharmacological resistance can be acquired by mutation and selection, with which the character is transferred vertically to the daughter cells. For mutation and selection to generate resistance, the mutation must not be fatal, nor must it modify virulence to an important degree [6].

Drug resistance is usually acquired by horizontal transfer of the factors that define it from a donor cell, often from another bacterial species, by transduction, transformation or conjugation. The horizontal transfer of resistance offers several additional advantages to mutation-selection.

a) Mutation-selection: The mutation and selection of the antibiotic from a mutant and resistant strain constitutes the molecular basis of resistance to streptomycin (ribosomal mutation), to quinolones (mutation of the gyrase gene or topoisomerase IV), rifampicin (mutation of the polymerase gene) of RNA), and linezolid (mutation of ribosomal RNA). Mutations can appear in the gene encoding: 1) the protein target, modifying its structure so that it no longer binds to the drug; 2) a protein that participates in the transport of the drug; 3) a protein that is important for the activation or inactivation of the drug, as is the case of broad-spectrum beta-lactamases, or 4) in a regulatory or promoter gene that modifies the expression of a target, a transport protein or a inactivating enzyme. Mutations are not produced solely by contact with the drug [7].

b) Horizontal gene transfer: The horizontal transfer of resistance genes is facilitated by a group of mobile genetic elements. However, in this process also other mobile elements, transposable elements, integrases and genetic cassettes participate. The transposable elements are of three general types: insertion sequences, transposons and transposable phages; two of these, insertion sequences and transposons, are important for resistance. The insertion sequences are shorter segments of DNA encoding enzymatic functions for the specific recombination of certain sites with inverted repeat sequences at either end. They can be copied themselves and inserted into the chromosome or a plasmid. Insertion sequences do not encode resistance, but function as sites of integration for other resistance encoding elements, for example, plasmids or transposons.

c) Transduction: It is the acquisition of bacterial DNA from a phage (a virus that spreads in bacteria) that has incorporated

DNA from a previous bacterial host into its outer protein coat. When the DNA comprises a drug resistance gene, the newly infected bacterial cell acquires resistance. Transduction is of special importance in the transfer of resistance to antibiotics among *Staphylococcus aureus* strains.

d) Transformation: The transformation is uptake and incorporation into the host genome, through homologous recombination of free DNA, released into the environment by other bacterial cells. This phenomenon constitutes the molecular basis of resistance to penicillin in pneumococci and *Neisseria* [12-15]. Penicillin-resistant pneumococcus produces altered penicillin-binding protein (PBP) proteins that have a very low affinity for this antibiotic. The nucleotide sequence analysis of the genes encoding these modified PBPs indicates that they are mosaics, where foreign DNA blocks have been imported from a similar species of streptococcus [6].

e) Conjugation: The conjugation is the transfer of genes by direct contact between two cells through a pilus or sexual bridge. This complex and fascinating mechanism for the dissemination of antimicrobial resistance is very important since it is possible to transfer several resistance genes in a single event. The transferable genetic material costs two groups of genes encoded by plasmids that are in the same plasmid or in different plasmids. One group encodes the real resistance and the other encodes the genes necessary for the bacterial conjugation process [12]

Materials and Methods

For the processing, samples were taken from patients with previous indications, (vaginal exudate and urine culture). Then the samples were identified in the log, (with registration number, type of sample, shift and date).

The urine samples were cultivated in agar muller Hilton, blood, Mac Conkey, MS and Biggy, by the technique of stria, incubated at 37 degrees centigrados for 24 hours, proceeded to realize the colony count (CFU/mL), staining was performed to perform the morphological identification (Gram positive or negative, cocci or bacilli) perform biochemical tests (LIA, TSI, MIO, CITRATE OF SIMMONS, UREA), catalase test and finally antibiogram [13]

The samples of vaginal exudate were measured pH, with a swab made a direct gram, placed in Stuart transport medium, then in thioglycollate, from there cultivate on Tayer Martin agar, blood, Mac Conkey, salt and mannitol and Biggy, by the technique of stria, to later perform antibiogram

After the colonies were identified, Gram was performed, biochemical tests were performed on LIA, TSI, MIO, Simmons Citrate, Ures oxidase, and antibiograms with gram (-) and Gram (+) multidiscs [14-16].

Results and Discussion

From a total of 168 antimicrobial resistance tests for *Escherichia coli*, it was found that 52% of the bacteria are resistant to some type of antibiotic, 44% are sensitive and 4%

are classified as intermediate sensitivity, in the same way it was observed that the Amikacin has total sensitivity, to a lesser degree, Netilmicin, followed in descending form of chloramphenicol and nitrofurantoin. The resistance is observed marked although there was some sensitivity for some patients, the antibiotic Ampicillin is one of those that

have reached more resistivity, followed by Cefalotin, and Trimethoprim-Sulfamethoxazole, followed by Ceftriaxone, Cefotaxime and Pefloxacin, so it can be mentioned that some bacteria reviewed show both sensitivity and resistance, as can be seen in **Tables 2 and 3** and **Figure 1**.

Table 2 Statistics on the results of sensitivity and drug resistance of the microorganism *Escherichia coli*.

Gram (-) antibiotic	Patients													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CF	R	R	R	0	I	R	R	R	R	R	R	0	R	R
CL	R	R	R	0	0	0	R	0	0	R	0	0	0	0
CRO	R	R	R	0	0	R	R	R	R	R	R	0	R	0
AM	R	R	R	0	0	R	R	R	R	R	R	R	R	R
AK	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SXT	R	R	R	R	0	R	R	R	R	R	R	0	0	R
CTX	R	R	R	0	0	R	R	R	R	R	R	0	R	0
GE	R	R	R	0	0	0	R	R	0	R	0	0	R	0
NET	0	I	0	0	0	0	0	0	0	R	I	0	R	0
FEP	R	R	R	0	0	R	R	R	R	R	R	0	R	0
NF	I	I	0	0	0	R	0	R	0	R	0	0	0	0
LEV	R	R	0	0	0	0	R	R	R	R	R	0	R	0
TE	R	R	-	-	-	-	-	-	-	R	-	-	-	-
CXM	R	R	-	-	-	-	-	-	-	R	-	-	-	-
SAM	-	0	-	-	-	-	-	-	-	R	-	-	-	-
DC	-	R	-	-	-	-	-	-	-	-	-	-	-	-
IPM	-	-	-	-	-	-	-	-	-	0	-	-	-	-
MEM	-	-	-	-	-	-	-	-	-	0	-	-	-	-

*Note: Sensidisks only used with those microorganisms in the majority of the others are resistant. TE, CXM, SAM, DC, IPM, MEM.

Table 3 Summation of results of *E. coli* bacteria, 168 tests.

Antimicrobial agent	<i>Escherichia coli</i>		
	Sensitive	Resistant	Intermediate
Cefalotina (CF)	2	11	1
Chloranphenicol (CL)	9	5	-
Ceftriaxone (CRO)	4	10	-
Ampicilin (AM)	2	12	-
Amikacin (AK)	14	0	-
Trimethoprim-Sulfamethoxazole (SXT)	3	11	-
Cefotaxime (CTX)	4	10	-
Gentamicin (GE)	7	7	-
Netilmicin (NET)	10	2	2

Pefloxacin (FEP)	4	10	-
Nitrofurantoin (NF)	9	2	3
Levofloxacin (LEV)	6	8	-
Tetracycline (TE)	-	3	-
Cerufloxime (CXM)	-	3	-
Ampicilin-Salbutam (SAM)	-	1	1
Imipenem (IPM)	1	-	-
Meropenem (MEM)	1	-	-

Of the antibiotics for Gram-negative microorganisms, Amikacin (AK) was the one that predominated with sensitivity, followed by Netilmicin (NET), Chloramphenicol (CL), Gentamicin (GE), and Levofloxacin (LEV), and with resistivity on the part of Ampicillin (AM) was found in all bacteria, and

resistance was found in most of the bacteria found: Trimethoprim-Sulfamethoxazole (SXT), and Cefotaxime (CTX).

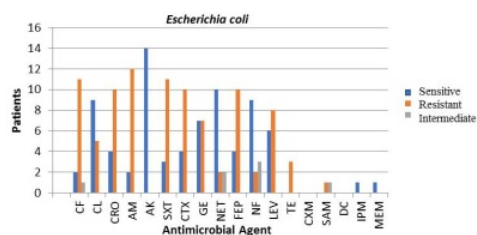


Figure 1 Results of 168 antimicrobial susceptibility tests for *Escherichia coli*.

Conclusion

It was observed that the inadequate use of drugs by patients is generating a resistance to antimicrobials by microorganisms, which has led to possible bacterial mutations, so that day by day is observed greater lack of effectiveness of drugs employees as part of the treatments established by doctors.

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