Chapter 6

Antibiotic Resistance among *Escherichia coli*: Isolates and Novel Approaches to the Control of *E. coli* Infections

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Abstract

Bacteria are the microorganisms that most frequently cause infectious diseases in humans. The synthesis of silver nanoparticles (AgNPs) has attracted interest due to the new and different physical and chemical characteristics with applications in new fields. AgNPs, alone or supported on ceramic, are used as antimicrobial fillers in textiles and polymers for food-packaging and biomedical applications, for antimicrobial paints, and potentially for drug delivery. The evaluation of mesoporous nanostructures or nanocomposites as FDU-12/lignin/silver was effective in inhibiting *Staphylococcus aureus, E. coli, Enterococcus faecalis*, and *Candida albicans*. The best results were achieved against the inhibition of *E. coli* and with the structures FDU-12/silver. In plates with FDU-12/lignin/silver, FDU-12, FDU-12/lignin, and the positive control, it was enumerated at 0, 6, 14, and 27 colonies, respectively. While the development of resistance to a new antibiotic is expected, the time course and degree of resistance are uncertain and depend on various factors. The application of AgNPs as nanocomposites can alter the expression of bacterial proteins and could be used for inactivation. This review explores such aspects and a number of factors arising like the use of nanostructures against *E. coli*, from the knowledge acquired.

Keywords: *Escherichia coli*, nanostructures, nanocomposites, FDU-12/lignin/silver, *E. coli* resistance

1. Introduction

Bacterial survival and persistence in an inappropriate substrate can be defined as the ability of bacteria to tolerate exposure to lethal concentrations of bactericidal antibiotics. This view

was first noticed in 1944 regarding that treatment of cultures of *Staphylococcus aureus* with high concentrations of penicillin did not kill all the strains, and that a fraction of the order of 10^{-6} of these strains survived. Bacteria can cause frequently infections in humans, and the resistance to antibiotics is a primary cause of disease and sometimes death in the intensive-care units of hospitals worldwide [1] and the cause of numerous clinical problems [2]. The development and increase of resistance among pathogens causing nosocomial and community-acquired infections are known to be associated with the widespread utilization of antibiotics [3–5].

Antimicrobial is a general term for drugs, chemicals, and/or other substances that either kill or slow the microbial metabolism. Various antimicrobial agents that are in use today are antibiotic, antiviral, antifungal, and antiparisitic drugs. An antibiotic is a type of antimicrobial agent produced by a fungi or a bacterium that has a direct influence on other microbes, specifically bacteria. Many of antibiotic resistance genes are found on transposons, integrons, and/or plasmids that can be transferred to another bacteria belonging to the same or different species [6] and resistance elements can be transferred to the human commensal or pathogenic microbiome [7].

The genes conferring resistance to antibiotics have been widely distributed in the environment since before the introduction of antibiotic chemotherapies, but human activities are probably the major driven force of the resistant bacteria found in air and water, principally with *S. aureus* and *E. coli* isolates. The antibiotic presence in the environment can exert selective pressure on the organisms living closely, but the mechanisms of cross resistance to antibiotics are unknown [6].

Studies with animals [8] showed that the proximity with humans has the tendency to generate more antibiotic-resistant enteric bacteria as saw with African baboons. With birds, a study [9] showed that 8% of *E. coli* isolated from arctic region presented resistance. This can enhance the pressure of antibiotic resistance by an anthropogenic activity. In pristine environments, the determinants of an antibiotic resistance existed naturally and were probably subjected to horizontal gene transfer [10]. This predisposition for the genetic exchange of resistance elements has facilitated the antibiotic resistance in pathogenic bacteria.

Antibiotics kill or inhibit bacteria that are susceptible to that antibiotic. Bacteria that are intrinsically resistant or that can acquire resistance will survive and replace the drug-susceptible bacteria. The production of an antibiotic is associated with the presence of genes encoding one or more self-protection processes. Antibiotic biosynthesis gene clusters that encode resistance proteins specific to the compound made (modification of the compound or target) or multifunctional (efflux systems) are important systems. The resistance genes that are contiguous with the biosynthesis genes could be involved in regulation of the biosynthetic pathway.

There are consistent results [11] that showed that antibiotic resistance genes are found in natural sources. Another form of resistance in isolates not producing antibiotics is the mutation of the target gene product, which reduces or prevents inhibition by antibiotic binding. Spontaneous mutations causing resistance often lead to different bacterial phenotypes.

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The presence of antibiotic resistance strains in environment may be understood as a response to the selective pressures. Thus, any antibiotic use will provide a selective pressure that perpetuates resistant bacteria. The introduction of antibiotics in the treatment of diseases that were prior incurable, enabled the effective treatment of these, promoting the increase in longevity. This kind of medicine is widely used in the treatment of people in the community and health services and is also used to treat animals in agricultural environments. Thus, the increasing levels of resistance are compromising the effectiveness of them. Therefore, it is essential that we assess the use of antibiotics carefully, regardless of setting, and use them only when necessary, to avoid promoting the development of resistance among bacteria [12].

Various infections are caused by important pathogens, such as *Staphylococcus aureus*, and up to 50% are resistant to stronger drugs, such as methicillin [13], and with *E. coli* [12, 14–16] the resistant trait is growing.

The food supply can be a source of antimicrobial-resistant and virulent *E. coli* strains that could be lethal to humans or, in a major scale, can cause intestinal as well as extra intestinal infections. *Escherichia coli* (**Figure 1**) is a ubiquitous and versatile microorganism, moving from commensal to opportunistic and specialized virulent bacteria, with potential to cause different diseases. To cause infection, harmless commensal *E. coli* can acquire a set of combination of mobile genetic elements to become a highly adapted pathogen capable of causing different diseases in different hosts, ranging from gastroenteritis to extra intestinal infections of the urinary tract, bloodstream, and central nervous system. Seven diarrheagenic and two extra intestinal *E. coli* pathovars can cause disease in various hosts.

Interestingly, one outbreak happens, caused by one not expected *E. coli* pathotype [18] in 2011. An uncommon high number of haemolytic uremic syndrome (HUS) cases were reported in

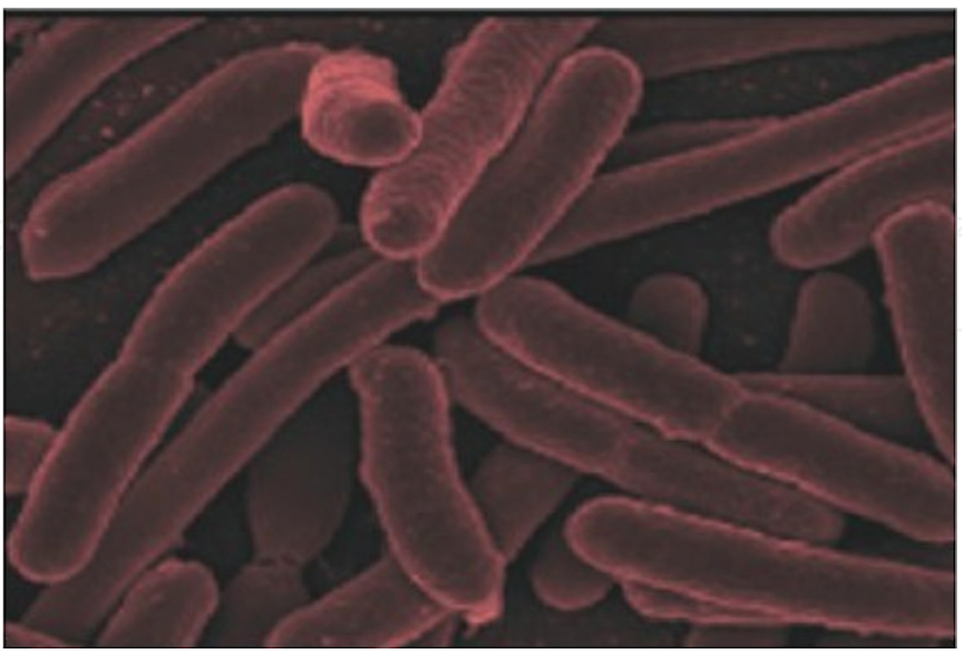

Figure 1. *E. coli* in scanning electronic microscopy [17].

Europe, most precisely in Germany. The implicated agent was an enterohemorrhagic *E. coli* (EHEC), which presented virulence traits of both, a verotoxigenic *E. coli* (VTEC) and enteroaggregative *E. coli* (EAEC).

A relevant study involving enterotoxigenic *E. coli* STb toxin was conducted in 2014 [19], regarding the STb toxigenicity. STb is a heat-resistant toxin responsible for diarrhea in farm animals and in humans as well. The toxic effect of STb in host cells is due to allowing the passage of electrolytes and water through the paracellular space. The authors [19] demonstrated that STb could promote the delocalization of transmembrane proteins such as claudin-1.

This event among others can prove how the versatility of this bacterium can reach a worldwide proportion, with a high concern for public health. These types of outbreaks are not solely a consequence of health conditions in developing countries. With the increase in international travel and trade globalization, diarrheagenic *E. coli* has become a worldwide public health threat [20].

On the other hand, antibiotic resistance rates in *E. coli* are rapidly rising, especially concerning to the use of fluoroquinolones and third- and fourth-generation cephalosporin. These strains are acquired predominantly in the community [12]. In these conditions, drug-resistant *E. coli* are readily acquired via the consumption of contaminated food and beverage. Some authors [14] studied 287 *E. coli* samples isolated from meats regarding their virulence factors. They observed that drug-resistant isolates had similar characteristics to those collected from the same types of meat.

This review shows the immune view of *E. coli*, and focus on the presence of these bacteria highlighting the acquisition of resistance and discussing various aspects of *E. coli* pathotypes. Because the antibiotic treatment is our primary method of threaten diseases, studies on this field are important to a better understanding of bacterial evasion, circumventing, and subverting mechanisms to acquire resistance characteristics.

2. Immune view

A good immune system is essential for the survival of any organism because of the protection against infectious beings. It is the principal infections evolution blocker that may cause elevated decease rate. This is a well-established fact for almost all known infective illness; the number of subjects in contact with the infectious agents is greater than those who really evolve diseases.

Contaminations occurred by no cell invasive bacteria are the most common. In these cases, the immune system of shield is mostly associated to the harborer's innate barriers, natural protection mechanisms, and antibody production. The importance of innate barriers (**Table 1**) in the combat against no cell invasive bacterial infections is well-known [21]. The integrity of skin and mucosa prevent adherence and penetration of bacteria; mucociliary movement eliminates bacteria from the respiratory tract; the stomach's acidic pH destroys some bacteria penetrating by the upper digestive tract; and in the saliva, eyes secretion, and prostatic secretion, lysozyme and other substances have antimicrobial activity.

I. Natural barriers against infection	II. Innate immunity	III. Acquired immunity
1. Integrity of skin and mucosa	1. Extracellular molecules (C reactive protein, complement)	1. Antibodies
2. Mucociliar movement	2. Natural killer cells, neutrophils, macrophages	2. Cytokines produced by T cells
3. pH variations	3. Chemokines, cytokines	–
4. Antimicrobial substances	–	–

Note: Ref. [21] with modifications.

Table 1. Barriers against infectious microorganisms.

On the other hand, the main characteristic of intracellular bacteria is the ability to survive within the macrophages. In this context, some important pathogens are *L. monocytogenes*, *Mycobacterium tuberculosis* and, *M. leprae*. Invasion of the macrophage is also a parasite's getaway strategy. Though paradoxical, the last mechanism is benign to the harborer, while the lack of cell penetration by bacteria can induce a strong inflammatory effect and excessive injury to the host.

Adaptive immunity, principally by means of antibodies plays an important function versus bacteria outside of the cell. Antibodies may exert its inhibition in three steps: (i) opsonization, (ii) activation of the complementary system, and (iii) furthering the neutralization of bacteria or their metabolites.

Extracellular bacteria are prone to undoing when phagocytozed. So subverting this system, they developed substances such as evasive mechanism with an antiphagocytic system.

Antibodies directed against these substances not only avoid their action, also facilitate phagocytosis, while neutrophils and macrophages have receivers for the fc part of immunoglobulin (opsonization).

The antibodies also coassist in the destruction of complement by bacteria, and activate this system by the classical pathway. Through neutralization mechanism, IgA antibodies, in particular, can bind to bacteria and therefore prevent the latter from settling on the intestinal mucosa and the respiratory tract. Antibodies bind frequently to toxins produced from bacteria, such as tetanus (*Clostridium tetani*) and diphtheria toxin (*Corynebacterium diphtheriae*), and neutralize the action of these metabolites. The fine balance between health and disease is found in this scenario, in which deficiency is as much as excess may result in tissue damage.

3. *E. coli* antibiotic resistance

A mature human gut harbors a vast number of bacterial resident microbiota, accounting for more than 10^{14} individual bacteria. Notably, the composition of the microbiota is individual host specific and the type of species living in the gastrointestinal tract varies with the host age, diet, habits, health, and idiosyncratic status [22]. The intestinal mucosa is a first contact between the immune system and the external environment and plays a central role in a

microbe and host cross talk [23]. The indigenous intestinal microbiota provides important protective, metabolic and trophic functions, principally offering resistance to colonization by exogenous microorganisms, and preventing invasion by incoming pathogens.

The intestinal epithelium can resist against microbial invasion, but through evolution mechanisms, potential pathogenic enteric microorganisms developed strategies to circumvent and subvert this strong barrier. As an initial step in the infection process, some pathogens target specific epithelial cell structures, as glycoprotein and glycolipid [24], which act as receptors for attachment, permitting the microorganisms to exploit the underlying signal transduction pathway.

Other strategies utilized by invasive pathogens such as *Salmonella enterica* serovar, *S. thyphimurium, Shigella* spp., and invasive *E. coli* orchestrated their entry into intestinal epithelial cells. This strategy uses the expression of a bacterial type III protein secretion system, to deliver various effectors proteins into the host cell [25]. This effectors protein subverts normal host cell processes by triggering a marked rearrangement of the host cytoskeleton. This procedure facilitates the pathogen to cross the epithelial barrier and induces an inflammatory host response [25].

The latter strategy can be done by direct cytotoxic injury, intracellular migration, disruption of the epithelial tight junctions, or indirectly by inducing neutrophil infiltration. Pathogenic *E. coli* have been shown to increase chloride ion secretion from intestinal epithelia by upregulating the expression of the receptor for the neuropeptide galanin-1 [26].

Enteric pathogens have the propriety to perturb the intestinal epithelial barrier and impact paracellular permeability, most often with an alteration in the arrangement of tight junctional component proteins by mechanisms that are unique for different pathogens. With respect to enteropathogenic *E. coli*, they disrupt the epithelial barrier by the phosphorylation of myosin light chains [27].

E. coli isolated from human and animal gut, and as well as from environmental sources presenting antibiotic resistance is a public health problem, especially in developing countries [28]. Work conducted in 2005 [29] showed that extended spectrum lactamase producing *E. coli* (ESBL) strains have spread as a hospital infection. The large plasmid genes coding this resistance also carry genes for resistance to other antibiotics [30]. The frequency of resistance to fluoroquinolone antibiotics (ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin, and nalidixic acid) in *E. coli* has increased worldwide [31].

In one review about *E. coli* producing fimbrial or afimbrial adhesins [32], authors showed that some *E. coli* strains (ETEC STb positive) associated with diarrhea, presented an afimbrial adhesin, named AIDA-I (adhesion involved in diffuse adherence). This adhesin originally found in human *E. coli* isolates showed that the establishment of a persistent and chronic infection could also help the microorganism to resist antimicrobial agents and prevent effective treatment of diseased animals.

In 2010, some authors [33] detected high resistance rates among *E. coli* (up to 30%) to ampicillin, tetracycline, streptomycin, ciprofloxacin, enrofloxacin, and cotrimoxazol. These authors [33] also found lower resistance to gentamicin (6.5%) and chloramphenicol (3.2%).

Also in 2010, one study [34] reported that morbidity and mortality attributable to third -generation-cephalosporin-resistant *E. coli* are significant. They also believe that if prevailing

resistance trends continue, high societal and economic costs can be expected and that better management of infections caused by resistant *E. coli* is becoming essential.

Work with neonates in a single center concluded that the use of minor antibiotic therapy with reducing preemptive treatment resulted in a moderate reduction of the antibiotic use and did not increase mortality [35].

Another study [36] was conducted to determine the antimicrobial susceptibility patterns among common pathogens in the intensive care unit of a university hospital in Iran between 2006 and 2009. Authors worked with 606 isolates from respiratory, urine, blood, and wound specimens of 456 patients. *E. coli* was present in 8.3% of isolates, and presented high antimicrobial resistance.

Scientists worked with 1163 clinical isolates in Taiwan [37]. The frequencies of Gram-positive and Gram-negative bacteria isolates were 30.4 and 56.2%, respectively. *Staphylococcus aureus* was the most common isolate among the Gram-positive organisms, while *Pseudomonas aeruginosa, E. coli*, and *Klebsiella pneumoniae* were the leading Gram-negative isolates.

The antimicrobial resistance in one intensive care unit in Canada was investigated. In 2008, it was found high antibiotic rates to *E. coli*: cefazolin, 20.1%; cefepime, 0.7%; ceftriaxone, 3.7%; gentamicin, 3.0%; fluoroquinolones, 21.1%; piperacillin-tazobactam, 1.9%; and trimethoprim-sulfamethoxazole, 24.8%. *E. coli* was the most prevalent Gram-negative bacterium [38].

According to a work conducted in 1975 [39], a hospital acquired urinary tract infection account for approximately 45% of nosocomial infection and 2–4% of the cases may develop septicemia. In this context, it was observed that 40% of the Gram-negative septicemia acquired in hospital originates in the urinary tract. This observation can enhance the *E. coli* importance for acquiring resistance. In 2009 was observed that, Gram-negative bacteria were the most frequent isolates, with *E. coli* being the most common followed by *Pseudomonas aeruginosa* and *Klebsiella* spp. *Candida albicans* accounted for almost 11% of the organisms, followed by *Acinetobacter baumanni* (**Table 2**) [39].

Bacteria and fungi	**Total**	**Resistance to all**	**Sensibility**
	N (%)	N (%)	N (%)
Escherichia coli	27 (49.1)	22 (81.5)	5 (18.5)
Pseudomonas aeruginosa	7 (12.7)	5 (71.4)	2 (28.6)
Klebsiella spp.	7 (12.7)	4 (57.1)	3 (42.9)
Candida albicans	6 (10.9)	–	–
Acinetobacter baumanni	3 (5.5)	3 (100)	0
Others	5 (9.1)	2 (40)	3 (60)
Total	55 (100)	36 (73.5)	13 (26.5)

Note: Ref. [39] with modifications.

Table 2. Profile resistance of main microorganisms isolated from hospitals.

4. Mechanisms of antibiotic resistance in Gram-negative bacteria

Bacterial antimicrobial resistance in both the medical and agricultural fields has become a serious problem worldwide. Resistant bacteria isolated from agriculture, farm or hospital can transfer the resistance genes to human pathogens [40]. The selection pressure applied by the antibiotics that are used in clinical and agricultural settings has promoted the evolution and spread of genes that confer resistance, regardless of their origins. Several factors can be implicated with resistance, sensibility, and antibiotic resistance dissemination such as: (i) impermeable barriers [6]; in this case, some bacteria are intrinsically resistant to certain antibiotics because they have an impermeable membrane or lack the target of the antibiotic; (ii) multidrug resistance efflux pumps; these pumps protect the bacterial cell against toxic molecules. It is an active transport mechanism for outside the cell. Some transporters, such as those of the resistance-nodulation cell division family, can pump antibiotics directly outside the cell, whereas others, such as those of the major facilitator superfamily, secrete them into the bacterial periplasm; (iii) resistance mutations; these mutations can cause a modification in the target protein, for example, by disabling the antibiotic-binding without changing the protein functionality. Specific examples include mutations in the gyrase, which cause resistance to fluoroquinolones, in RNA polymerase subunit B, which cause resistance to rifampicin, and in the 30S ribosomal subunit protein S12 (encoded by *rpsL*), which cause resistance to streptomycin; and (iv) antibiotic inactivation; inactivation can occur by covalent modification of the antibiotic, such as that catalyzed by acetyltransferases acting on aminoglycoside antibiotics, or by degradation of the antibiotic, such as the hydrolytic degradation of the β-lactam ring on antibiotics by the β-lactamases. The emergence of drug resistance among diarrheagenic *E. coli* is important, and in infant, is a cause of morbidity and mortality principally in developing countries. Analyzing stools of infants in India was verified that about 90% of *E. coli* strains presented resistance to the most antibiotics tested [41]. All isolates were resistant to ampicillin, imipenem, cotrimoxazole, and sensitive to amikacin, and presented 29 different antibiotic profiles. Most of the isolated *E. coli* harbored plasmids (64%) and up to 76% could transfer their plasmids. The transconjugant strains were carrying plasmids and presented resistance to ampicillin, imipenem, and cotrimoxazole. The authors found an increase in the prevalence of drug resistance among *E. coli* isolates, and conjugation transfer of plasmids contributed to a rapid spread of an antibiotic resistance.

Cyclomodulins are a growing functional family of toxins, which hijack eukaryotic cell cycle. Four cyclomodulin types are actually known in *E. coli*: cytotoxic necrotizing factors (CNFs), cycle inhibiting factor (Cif), cytolethal distending toxins (CDTs), and the pks-encoded toxin.

One interesting work [42] isolated ceftriaxone-resistant *E. coli* from 1.5% of participants in Maryland and Michigan, United States. One *E. coli* isolate collected from an apparently healthy person, presented resistance to eight antibiotics, and the resistance genes were contained on an incompatibility plasmid. These plasmid types are common among *Enterobacteriaceae* and can carry multiple resistance genes, generating multidrug resistance [43]. In Krueger's work [42], the source of the extensively resistant *E. coli* is not known, but the isolated strain may have been acquired from food.

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5. Diarrheagenic and extra intestinal *E. coli* pathotypes

Several distinct pathogenic categories (i.e., pathotypes or virotypes) of diarrheagenic *E. coli* strains are recognized. Each pathotype is defined by a characteristic set of virulence-associated determinants that act in a concert to determine the clinical, pathological, and epidemiological features of the disease they cause [44].

By definition, the virulence determinants of each *E. coli* pathotype are distinct. However, they can generally be categorized as either colonization factors (adhesins), which enable the bacteria to bind closely to the intestinal mucosa and resist removal by peristalsis, or secreted toxins, which interfere with the normal physiological processes of host cells. The key virulence determinants of the primary pathotypes of diarrheagenic *E. coli* are summarized in **Table 3**.

Pathotype	Common genotype	Most common presentation	Intestinal pathology	Susceptibile groups
EPEC	eae +, bfp +, EAF +	Non-specific gastroenteritis, noninflammatory diarrhea	Intimate adhesion, attaching–effacing lesions throughout the intestine, loss of brush border enterocyte	Children under 2 years of age in developing countries
ETEC	LT, ST, (STa, STb toxins)	Watery, cholera-like diarrhea, noninflammatory diarrhea	No notable change, adhesion to small intestinal mucosa	Children in developing countries; travelers
EHEC	eae +, stx +	Bloody diarrhea 'Hemorrhagic colitis';	attaching–effacing lesions confined to the large intestine; necrosis in severe cases; HUS, hemorrhagic colitis	Children and the elderly in industrialized countries
EIEC	Inv	Bacillary dysentery	Inflammation and disruption of the mucosa, mostly of the large intestine; necrosis and blood loss	All ages; more common in less-developed countries
EAEC	AA +, aaa −/aaa +	Persistent diarrhea Inflammation;	cytotoxic changes in enterocytes	Children in less-developed countries; travelers to those countries
A-EPEC	eae +, bfp (−/+), EAF −	Nonspecific gastroenteritis	Some lesions throughout the intestine; toxin production as EAST1	Children and adults; reservoir for human infection

Note: Ref. [44] with modifications.

Table 3. Key virulence determinants of diarrheagenic *E. coli*.

It can be seen in **Table 3**, that the number of virulence traits varies from each pathotype and have implications on intestinal pathology. Besides Enteropathogenic *E. coli* (EPEC) causing intimate adhesion, attaching-effacing lesions throughout the intestine and loss of brush enterocytes, Enterotoxigenic *E. coli* (ETEC) do not present notable change to intestinal mucosa. Enterohaemorragic *E. coli* (EHEC) provoke a similar intestinal pathology, with necrosis. Enteroinvasive *E. coli* (EIEC) cause inflammation and necrosis, but Enteroaggregative *E. coli* (EAEC) present enterocytes changes. Atypical *E. coli* (A-EPEC) and Diffusely adherent *E. coli* (DAEC) can cause lesions in the intestine.

Based on genetic variation within *E. coli*, it was found [45] that pathogenic strains have accelerated the rates of mutation and recombination and virulence is the driving force for more frequent recombination. These characteristics can impulse the bacterial population to acquire more resistance. Some studies [45–46] proposed a model where commensal *E. coli* maintains low frequencies of homologous recombination and acquisition of novel genes that result in virulence by horizontal genetic exchange. The pathogenic condition results in exposure to immune system barriers and antibiotic selection. These population presents higher mutation and recombination rates. Epidemic strains are exposed to stronger selection by pressures imposed by immune defenses and antibiotic use, resulting in highest levels of mutation, recombination, and infection.

In one study, conducted in Ontario, Canada, the authors [15] showed that the most common bacteria identified on urine culture over a 5 year period were *Escherichia coli* (71.6%), *Enterococcus* spp. (5.7%), and *Klebsiella* spp. (5.0%) and that these bacteria were frequently resistant to ampicillin (54.4%) and trimethoprim-sulfamethoxazole (TMP-SMX) (40.4%) [15].

Another study showed that resistance was more commonly seen in typical EPEC than in atypical pathotypes. The most prevalent resistances observed were to ampicillin, tetracycline, streptomycin, and the sulfonamides [16].

EPEC, an established etiological agent of human infantile diarrhea, is a pathogen that subverts intestinal epithelial cell function to produce distinctive "attaching and effacing" (A/E) lesions. These types of pathogens are typically found on the surface of the host epithelial cell. They can cause severe lesions on intestinal microvilli. Other pathogens can display similar characteristics, which includes *Hafnia alvei, Citrobacter rodentium*, and enterohemorrhagic *E. coli*.

The interactions between EPEC and host cells have been divided into three stages. Initial adherence to cultured epithelial cells is mediated by the formation of type IV fimbriae known as bundle forming pili (BFP) [47]. Initial adherence helps bring the bacteria in intimate contact with the host cell. BFPs mediate bacterial interactions in a human intestinal organ culture model [48].

The genetic answer for the formation of A/E lesions can be explained by the presence of the *locus* of enterocyte effacement (LEE) [49]. This cluster includes the genes of following bacterial proteins: *E. coli* attaching and effacing that encodes the protein intimin (*eae*); *E. coli* secretion (*escs*); *E. coli*-secreted protein (*esps*); secretion of *E. coli* proteins (*sep*), and translocated intimin receptor (Tir).

The second stage of EPEC pathogenesis involves the secretion of bacterial proteins, some into the host cell, including EspA, EspB, and EspD at the temperature of the body [50], and particularly the gastrointestinal tract, the expression of these proteins is maximal, which

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implies that they may be involved in virulence. The translocation of these proteins is essential for activating a number of signal transduction pathways.

The third stage of EPEC interaction with the eukaryotic cells is characterized by the intimate attachment with the host cell. A 94-kDa outer membrane protein and intimin, encoded by the *eae* gene [51], binds to a 90-kDa tyrosine phosphorylated protein in the host membrane. This receptor is of bacterial origin and has been designated as the translocated intimin receptor (Tir). Tir is translocated from the bacterial cell into the host membrane, where it becomes phosphorylated on one or more tyrosine residues and functions as a receptor for its binding partner, intimin. The resultant tight association is accompanied by the formation of actin pedestals. The most remarkable change in the cellular structure of the eukaryotic cell is the formation of typical actin pedestals. Within 3 hours of infection by EPEC, host-cell actin, a-actinin, talin, erzin, and villin accumulate directly under the bacteria. EPEC presents a strong and intimate adhesion to the intestinal mucosa leading to dissolution of the brush border by inducing vesiculation of the microvilli. This is the attaching and effacement step, and in the jejunum and ileum results in a loss of brush border disaccharidase enzymes and a large area of absorptive surface.

Typical kinds of EPEC are EPECs that have lost the EAF plasmid. ETEC strains are a major cause of secretory diarrhea in both humans and animals. They produce heat-labile and/or heat-stable (STa and STb) toxins that also cause diarrhea. EHEC strains are implicated in foodborne diseases principally due to ingestion of uncooked minced meat and raw milk. These strains produce shiga-like toxin 1 (stx1), shiga-like toxin 2 (stx2), and variants thereof. These toxins can destroy colonic enterocytes and produce hemorrhagic colitis. EIEC can attach to enterocytes and penetrate by endocytosis and replicate therein. DAEC strains are diffusely adhering *E. coli* that are also implicated with episodes of diarrhea. EAEC damage and blunt colonic villi by hemorrhagic necrosis, although the precise pathogenic mechanisms are unclear. EAEC are a major cause of chronic diarrhea in children. ExPEC are the cause of a diverse spectrum of invasive human and animal infections, often leading to septicemia and sometimes to death.

Extraintestinal *E. coli* (ExPEC) strains have amazing behavior and possess virulence mechanisms to invade, colonize, and induce disease in sites outside of the gastrointestinal tract. Human diseases caused by the ExPEC include urinary tract infections, neonatal meningitis, sepsis, pneumonia, surgical site infections, as well as infections in other extraintestinal locations. ExPEC strains have been isolated from food products, in particular from raw meats, and poultry, indicating that these organisms potentially represent a new class of foodborne pathogens [52–53].

Extraintestinal *E. coli* infections are associated with specialized strains presenting antimicrobial resistance. The food supply may disseminate ExPEC and antimicrobial-resistant *E. coli*. Retail foods may be an important vehicle for community-wide dissemination of antimicrobial-resistant *E. coli* and ExPEC, which may represent a newly recognized group of medically significant foodborne pathogens.

E. coli contamination exhibited a prevalence gradient from miscellaneous foods (9%), through beef or pork (69%), to poultry (92%) [54]. Among *E. coli*-positive samples, similar prevalence gradients were detected for antimicrobial resistance (27, 85, and 94 of samples, respectively)

and ExPEC contamination (4, 19, and 46%, respectively). Indirect evidence suggested on-farm selection of resistance.

Uropathogenic strains can invade bladder cells and at this local, form reservoirs, which is possibly the storage local of the bacterium. *E. coli* causing infant meningitis is resistant to host immune responses and has the ability to cross the blood-brain barrier and cause disease. ExPEC from human and avian hosts encounter similar challenges in establishing infection in extraintestinal locations.

Extended spectrum beta-lactamases (ESBLs) are the bacterial enzymes that make them resistant to advanced generation cephalosporins and might lead to the failure on therapy.

The importance of this resistance in one children population in India was studied. CTX-M-15 enzyme is increasingly being reported from this part of the world together with TEM-1 [55]. TEM-1 is the most commonly encountered beta-lactamase in Gram-negative bacteria. Up to 90% of ampicillin, resistance in *E. coli* is due to the production of TEM-1 and *E. coli* is the most common cause of neonatal sepsis. The authors found that 97 ESBL-producers were identified among 266 *E. coli* strains isolated from 238 neonates. The isolates were screened for blaCTX-M, blaTEM, armA, rmtA, and rmtB, the last three genes being responsible for aminoglycoside resistance. The authors [55] concluded that male neonates colonized or infected by ESBL-producing *E. coli* have longer stay in NICU compared to their female counterparts. This happened because of male neonates getting colonized and/or infected earlier than their female counterparts do. Plasmid-mediated-conjugal transfer was found to be the mechanism of transfer of blaCTX-M-15 resistance marker in the described setting [55].

Antimicrobial drug resistance is a large and growing problem among organisms that cause diarrheal disease. Although most diarrheal diseases are self-resolving and should not be treated with antimicrobial agents, invasive or protracted infections require chemotherapy and are typically managed empirically [56].

The more recently defined enteroaggregative *E. coli* are typically multidrug-resistant and are one of the most common causes of childhood diarrhea, particularly persistent infections [56]. Antimicrobial drug-resistant diarrheagenic *E. coli* pathotypes, including enteroaggregative *E. coli*, are also emerging as important diarrheal pathogens in AIDS patients [57].

According some data [18, 20] about *E. coli* outbreaks, new pathotypes can emerge and cause disease and death in different populations in both, developed [20] and in developing [53] countries. Other authors [58] observed that both EHEC O157 and non-O157 STEC infections can occur at the same time. These authors presented some interesting reasons to this, such as: (i) they are common and may be increasing in frequency; (ii) could be associated with high morbidity and mortality; (iii) utilizing ideal laboratory conditions these pathogens should be detected by both, culture procedures and using protocols to detect Shiga toxin; and (iv) these strains cannot be readily detected with certainty by selective targeting of patients age, time of year or presence of blood in the stool. These observations can be understood in a globalized world. Humans are embedded into the microbial world.

In healthy populations, saprophytic microorganisms constitute a rich source of genetic material which pathogens can readily acquire resistance. The study conducted by NIS in Nigeria showed

that resistance of commensal *E. coli* to almost all agents studied increased rapidly over time [59]. Additionally, urban residents in Nigeria, Ghana, and Zimbabwe were more likely to carry multidrug-resistant *E. coli* than were rural or provincial residents [60]. This finding has important consequences in light of the rapid rate of urbanization in these countries and other parts of the continent. Travel networks have become more efficient and are more extensively used.

Most antibiotic-producing strains carry genes encoding resistance to the antibiotics that they produce, and they are located in the same gene cluster as the antibiotic biosynthesis pathway genes. The sources by which antibiotic resistance genes can be found are presented in **Table 4**.

Resistance genes exist naturally in the environment owing to a range of selective pressures in nature. Humans have applied additional selective pressure for antibiotic resistance genes because of the large quantities produced, consumed, and applied in daily activities. Physical and biological forces also cause widespread dissemination of resistance throughout many natural environments.

In lifetime, humans are exposed to antibiotic resistance bacteria. The potential routes for human exposition with wild animals and its microbiota include [6]: (i) translocation of wildlife into suburban areas, habit destruction, pollution ,and changes to water storage, irrigation or climate changes; (ii) human contact with nature such as hunting and camping; (iii) consumption of exotic foods, bushmeat and game farms; (iv) acquisition of exotic pets and transport of live animals from long distances; (v) incorporation of animal's habitats in human life as zoos; and (vi) trapping of fur-bearing animals.

Some microorganisms and some environments harbor antibiotic resistance genes irrespective of the human use of antibiotics. The prevalence and diversity of resistance genes in the environment inspire hypotheses about the native roles of so-called resistance genes in natural microbial communities.

Selection for antibiotic resistance	**Environment**	**Utilization**
Nature	Medicine	Agriculture
Protection against endogenous antibiotics	Industrial antibiotic production	Utilization of antibiotics onto fields
Protection against naturally occurring antibiotics and heavy metals	Antibiotic consumption	Antibiotic consumption
Alternative cellular functions of the resistance protein	–	–
Spread of antibiotic resistance genes	–	–
Physical forces	Biological forces	–
Air currents	Human activities	–
Water	Animal presence	–

Note: Ref. [6] modified.

Table 4. Sources and movement of antibiotic resistance genes in the environment.

6. Antibiotic resistance requires a coordinated response

Antibiotic use in animals has led to the emergence of resistant bacteria, and sometimes these resistant bacteria can be transferred from animals to humans by direct contact or by handling and/or consuming contaminated food.

High levels of resistance were observed for tetracycline as well as intermediate resistance against tetracycline, amikacin, and gentamicin. Gentamicin was the most effective out of these antibiotics [61]. Some authors [62] have showed high rates of tetracycline resistance in strains of enteric *E. coli*. Preventing resistant infections provides the greatest opportunity to limit resistance. Strategies to prevent and control resistant bacteria vary by the pathogen and the setting in which the infection is acquired. Infections were diagnosed in 188 patients from a single healthcare institution [63]. The medical costs for antibiotic resistant infections were estimated between $13.35 and $18.75 million dollars. In United States, antibiotic resistance is also an economic burden on the healthcare system, in other words, resistant infections cost more to treat.

Unfortunately, infections caused by antibiotic resistant bacteria are an everyday occurrence in healthcare settings. In United States, an effort of the National Antimicrobial Resistance Monitoring System (NARMS) contributes to minimize the impact of resistance. NARMS consists in a lab-based system for surveillance. This system is presented in all 50 states and detects resistance in pathogens that are commonly transmitted from animals to humans or through food, such as *Salmonella, Campylobacter*, and *E. coli*. Outbreaks caused by resistant bacteria can occur in community settings where people are concentrated, such as athletic teams, childcare centers, and prisons, or in healthcare settings, including hospitals, long-term care facilities, and ambulatory care facilities. Because the impact of resistance is extensive, activities may be done. The action plan could focus on: (i) reducing inappropriate antimicrobial use; (ii) reducing the spread of antimicrobial resistant microorganisms in institutions, communities, and agriculture; (iii) encouraging the development of new antiinfective products, vaccines, and adjunct therapies; and (iv) supporting basic research on antimicrobial resistance.

Another interesting goal to the process used to inhibit pathogens is linked to ancient knowledge. Many plant products are known to be able to inhibit the growth of several pathogens [64]. These compounds are used by plants in defense mechanisms such as predation by herbivores, insects, and microbial infections.

On the other hand, some studies [65] have shown that microorganisms living in intimate interaction with the host plant without causing any apparent disease symptoms produce most of these compounds. These microorganisms are defined as endophytes [65].

Some studies [65] recently showed that the phytochemicals produced by endophytes have revolutionized the use of these microorganisms as a source of bioactive compounds in recent years [65]. Among the great diversity of the different biomes, many plants stand out for its medicinal properties.

Streptomyces tubercidicus can produce tubercidin. Scientists working with a strain isolated from the Brazilian tropical savannah tree (*Solanum lycocarpum* St. Hill), named this strain (RND-C) [66]. In this study, different fractions with strong antimicrobial activity against *E. coli* and

S. aureus were obtained. The fractions showed a diverse chemical structure and molecular weight, suggesting the presence of new bioactive compounds.

In another study [67], *Paenibacillus polymyxa* was isolated as an endophyte from *Prunus* spp. in the same environment (Brazilian tropical savannah) as *S. tubercidicus*. This study reported the isolation of potent bioactivity of small molecules (<403 Da), against *E. coli* and. *S. aureus*. The previous author with collaborators [68] conducted studies highlighting conditions for production and characterization of these bioactive substances isolated from *P. polymyxa* RNC-D. Recently, a new group [69, 70] showed as well that endophytes isolated from *Miconia albicans* had that potential to inhibit *E. coli* and other pathogens.

According to these data, the bioprospection of endophytes consists in a promising and unexplored reserve for phytochemical agents. Thus, there is a great opportunity to find new antimicrobial substances [64, 65].

7. Nanotechnology in health sciences

Nanotechnology is the technology that deals with materials and products at the nanoscale. It is able to provide more effective solutions to some of the biotechnology issues, such as the development of drugs, due to the reduction of the proportion between contact surfaces and volume of materials, optimizing their action and consequently reduces the consumption of substances and products.

Mesoporous nanostructures, as FDU-12 silica, have high specific surface area, mesoporous large volume, diameter, and adjustable pore surface properties that can be directed to the desired needs. They also have a great importance in catalysis processes, adsorption separation of large molecules, sensors, photonics, optical, drug release or drug, acoustic, nanoreactors, nanotechnology with advanced integrated systems, among others [71].

Lignin, besides being the second vegetal macromolecule found naturally in abundance, can functionalized mesoporous nanostructures, as it has in its structure phenolic and carboxylic groups. These groups are still capable of reducing metal to form nanoparticles and they also have the advantage coat of the silver nanoparticles.

8. Nanoparticles linked to silver

Metallic nanoparticles have different functions, like the following: (i) the marking of a particular stretch of DNA; (ii) the increase in resistance of metals and in the case of nanoparticles linked to silver; (iii) the antimicrobial action (both against Gram-negative bacteria, which have a thin layer of peptidoglycan and against Gram-positive, whose layer is thicker); and (iv) fungicide, which makes these particles a special nanostructured material to be incorporated into the control of such pathogens [72, 73].

However, there are no general consensuses about the mechanisms that can explain the action of silver nanoparticles in the inhibition of microbial growth. Some researchers claim that silver

reacts with the thiol group of some vital enzymes to microorganisms and inactive them. Others claim dimerization of the pyrimidines of DNA, thus preventing the replication and thus their growth [74]. Another hypothesis is that the silver nanoparticle causes a change in the cell membrane, causing the output of reducing sugars of the membrane and thus causing cell death [75].

A study conducted by Xu et al. [76] concluded that reactive oxygen species (ROS) played a very important role in the mechanism of AgNPs antibacterial activity, because in anaerobic conditions the efficiency was significantly lower.

Recently [77], α-Ag_2WO_4 microcrystals were synthetized and tested for antimicrobial activity against *E. coli*. The successful of the inactivation was directly related to the presence of specific defects in crystal surfaces. This interaction crystal-bacteria leads to a production of OH* and O_2H* radicals that interact with several components of bacteria such as peptidoglycan, DNA, cell wall, proteins, and other bacterial structures (**Figure 2**) [77].

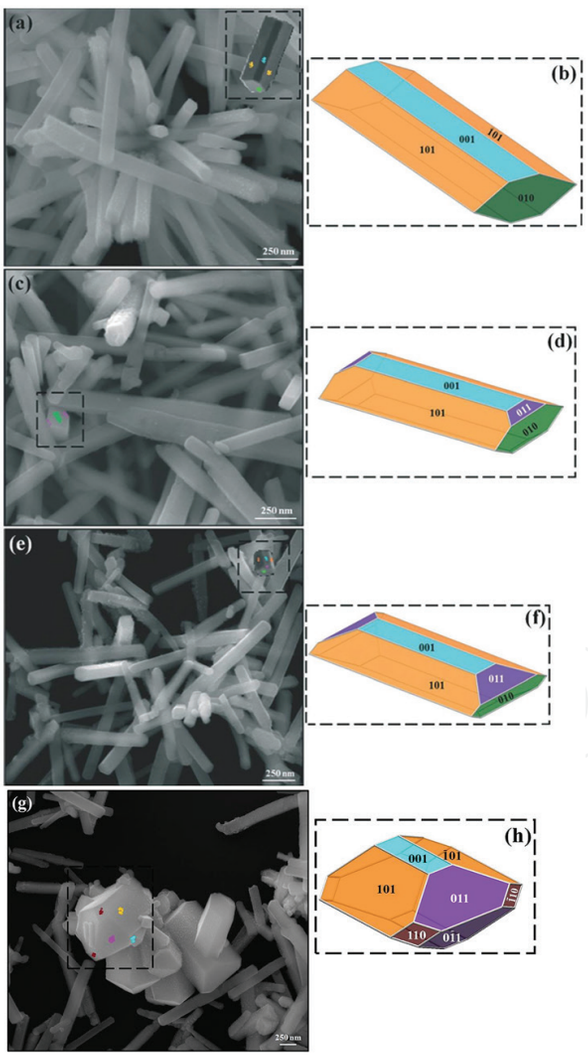

Figure 2. α-Ag_2WO_4 microcrystals in FE-SEM images (a, c, e, and g) and, respectively crystal shape (b, d, f, and h). The crystals were synthetized by MH method. The points highlighted in different colors corresponds each to its respective crystallographic planes [17].

http://dx.doi.org/10.5772/67400

It is known that silver is a toxic metal, for both humans at high concentrations, as for most microorganisms, it is the preferred substance for inhibition thereof, when compared to gold nanoparticles, zinc, and magnesium titanium [78].

The nanocomposites efficiency, containing silver, for the silver ion is much higher than the single metal species, as it has been proved in experiments [78]. It is not completely understood yet, but it is believed that connecting silver to other nanoparticles, such as silica and lignin, can inhibit the growth of microorganisms and these nanoparticles contribute to the destabilizing effect of the cell membrane.

9. Bioactivity of propolis nanoparticles against *E. coli*

Propolis is a natural resinous substance collected from the leaf buds of different tree species by honeybees and known for its biological properties (antibacterial, antifungal, and antioxidant) [79].

Some authors [80] evaluated the antimicrobial activity of propolis nanoparticles in comparison with ethanol-propolis extract against *E. coli*. Ethanol-propolis extract was obtained from green propolis resin, in absolute ethanol under agitation during 15 days. To obtain the propolis nanoparticles, ethanol-propolis extract at 13.75% (w/v) was mixed with polyvinyl-alcohol solution at 0.1% (w/v). The size of the nanoparticles was determined by dynamic light scattering (DLS), atomic force microscopy (AFM), and it was about 70 nm in average [80].

Antimicrobial activity of propolis nanoparticles and ethanol-propolis extract was tested against *E. coli*. Qualitative minimum inhibitory concentrations (MIC) of both solutions were evaluated by agar-well diffusion method, as shown in **Figure 3**. The result was 3.44% (w/v) for ethanol-propolis extract and 1.15% (w/v) for propolis nanoparticles.

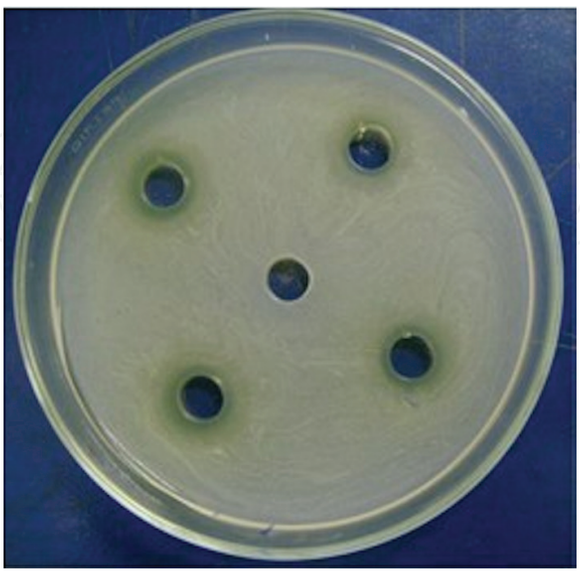

Figure 3. Determination of qualitative MIC of propolis nanoparticles against *E. coli* by agar-well diffusion method in plate dish [80].

The shown antimicrobial activity of propolis nanoparticles is of potential interest for direct applications or in film formulations, for example. Therefore, results obtained in this study, set the bases for future studies, using films as support for propolis nanoparticles, and for application in many products.

10. Conclusions

In the preantibiotic era [81], it was showed that from 30 lyophilized strains before 1950, four were multidrug resistant. The study of bacterial resistance can contribute to the discovery of the potential sources and novel alleles of antibiotic resistance genes. Considering that antibiotic treatment is our primary, and in many cases only, method of treating infectious diseases. We conclude that studies of environmental reservoirs of resistance are crucial to our future ability to fight infection. It is important to establish measures and politics to control the use of antibiotics, but an immediate modification of resistant profile in bacteria is not expected. Patients may follow procedures and use the antibiotics according prescription. The usual techniques of hand wash and use of barriers to prevent bacterial spread is important.

In the experiments, the FDU-12/silver nanoparticles showed the greatest inhibition in the growth of *E. coli*, as was observed fewer colonies or even their absence. Based on these results, we can infer that the best nanoparticle, among tested to inhibit the growth of *E. coli* is described above. The second nanocomposites with proven efficiency in inhibiting the growth of *E. coli* were nanoparticles containing only FDU-12, with an intermediate efficiency. The last nanoparticle studied, FDU-12/lignin/silver, showed the lowest efficiency in inhibiting the growth of *E. coli*, allowing a greater number of colonies to grow in the culture medium.

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