Chapter

Antimicrobial Resistance in *Escherichia coli*

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Abstract

In the last decades, antimicrobial resistance has become a global threat to public health systems worldwide. Among those bacteria that pose the greatest threat to human health because of its growing resistance to antibiotics are the members of the *Enterobacteriaceae* family, particularly *Escherichia coli* and *Klebsiella* spp. Among the different antibiotic-resistant mechanisms developed by bacteria, the ones found in *Enterobacteriaceae* are more diverse than those in other families and include resistance to different antibiotic groups, advantages that partially explain why these microorganisms are among the most common causes of antibiotic-resistant bacterial infections in humans. Due to the continuously increasing number of infections caused by multidrug-resistant *E. coli* due to its ease of transmission via the fecal-oral route among humans and from environmental sources, the understanding of the epidemiology of these strains and their mechanisms of resistance are key components in the fight against these infections.

Keywords: antimicrobial resistance, multidrug resistance, antibiotics, *Escherichia coli*, *Enterobacteriaceae*

1. Introduction

Escherichia coli is one of the most studied bacteria in the world and is arguably the best understood of all model microorganisms [1]. In the context of human and animal ecology, this microorganism participates as both a commensal of the gut, being one the first bacterial species to colonize it right after birth [2], and one of the most important human and animal pathogens, being able to cause intestinal and extra-intestinal infections. In humans, *E. coli* is the most frequent cause of urinary tract infections and has been identified as the causative agent of disease in practically every anatomical site of the human body, causing appendicitis, pneumonia, bloodstream, gastrointestinal infections, skin abscesses, intra-amniotic and puerperal infection in pregnant women, meningitis and endocarditis. Furthermore, *E. coli* can cause both community-acquired infections and health care-related infections, and is able to cause disease in all age groups.

Since the introduction of penicillin in the 1940s, which started the era of antibiotics, these agents have been recognized as one of the greatest advances in modern medicine and a turning point in human history. In 1900, infectious disease was a leading cause of death; in 2000, infectious diseases were responsible for only a small percentage of deaths in developed nations [3]. Unfortunately for humans, bacteria have evolved different mechanisms that have rendered them resistant to

antibiotics, to the point that since not long ago antimicrobial resistance has become a global threat to public health systems worldwide.

The ability of bacteria to develop resistance against antibiotics began soon after their introduction, as penicillin resistance by *S. aureus* was identified just a few years after its introduction in hospitalized patients [4]. In the case of *E. coli*, resistance against antibiotics has been steadily increasing since the first reported cases and, due to its impact in human health, is now included, along with the rest of the *Enterobacteriaceae* family, in the World Health Organization's (WHO) list of the 12 families of bacteria that pose the greatest threat to human health [5].

The contribution of *E. coli* to the antimicrobial resistance phenomenon should be analyzed under two different, but complementary, contexts that at some point meet in one common issue: a broad impact on human health. These two perspectives include the increasing number of infections worldwide caused by multidrug-resistant *E. coli* strains *per se* and the ability of this bacterium to transmit its genetic-resistant traits to other bacteria. *E. coli* has evolved these two attributes that have made this microorganism such a key player in the antibiotic resistance pandemic due to its ease of transmission among humans and from animals to humans via the fecal-oral route. Secondly, the microorganism's ability to colonize the gut of humans and animals allow it to be in close interaction with an abounding number of different bacteria, interaction that grant *E. coli* the duality to behave as a donor of genetic material to other bacteria and the ability to acquire resistance genes from other microorganisms.

This chapter describes the human actions that have contributed to the development of *E. coli* resistance to antibiotics, including the major impact of hygiene on the transmission and maintenance of its multidrug-resistant strains, and the known mechanisms developed by this organism to resist the actions of commonly used antibiotics.

2. Onset and spread of *E. coli* resistance to antibiotics

The emergence of antibacterial resistance in *E. coli* and other bacteria is multifactorial, but has paralleled the incorporation of these agents into the therapeutic arsenal in human and veterinary medicine. Data show that *E. coli* present the highest rates of resistance against those antibiotics that have been in use the longest time [6], as is evidenced by the high resistance rate worldwide against sulfonamides [7], whose use in humans started in the 1930s and its first *E. coli*-resistant clones were identified as early as 1950 [6]. Additionally, it is of no coincidence that those regions of the world with the highest consumption of antibiotics are low- to mid-income countries (**Table 1**), whose antibiotic-resistant rates are higher than those found in high-income nations.

Antibiotic resistance (AR) is largely believed to be the sole result of human activity and antibiotic chemotherapy; however, genomic studies of human bacterial commensals and environmental bacteria have revealed the presence of considerable numbers of resistance determinants within their genomes [9] that were not acquired from horizontal transmission and predated the clinical introduction of antibiotics. This type of AR is known as intrinsic resistance and provides a selective benefit for the producing strains by inhibiting or eliminating other bacteria competing for resources. Intrinsic resistance differentiates from the newly developed extrinsic antibiotic resistance in that in the former there is no contribution of human activities and the latter is mainly driven by antibiotic selection pressure [10]. In the current era of increasing AR and lack of new antibacterial agents, the study of intrinsic resistance becomes highly attractive as a new mechanism to counteract

Country	Daily doses per 1000 inhabitants/day (% of total) [8]
Mongolia	64.4
Iran	38.8
Turkey	38.2
Sudan	35.3
Serbia	31.6
Montenegro	29.3
Romania	28.5

Table 1.
Countries with the highest antibiotic consumption in the world.

bacterial resistance, as inhibition of elements that comprise the intrinsic resistome renders bacteria hyper-susceptible to antibiotics [11]. In the case of Gram-negative bacteria, like *E. coli*, two major contributors to the bacterium intrinsic resistance are its outer membrane, which is impermeable to many molecules, and its expression of numerous efflux pumps, that effectively reduce the intracellular concentration of certain antibiotics [12].

The acquired, or extrinsic, and continuously increasing resistance of *E. coli* to antibiotics is already considered a major public health problem around the world. In 2018, more than half of the *Escherichia coli* isolates reported to the European Centre for Disease Prevention and Control were resistant to at least one antimicrobial group under surveillance, and combined resistance to several antimicrobial groups was frequent [13]; in the United States in 2017, the national prevalence of extended spectrum β-lactamases (ESBL)-producing *E. coli* strains isolated from urinary tract infections (UTI) was 15.7%, whereas levofloxacin and trimethoprim-sulfamethoxazole-resistant rates were ≥24% among all isolates [14]. In developing countries the situation worsens, as reported by national surveillance data from Mexico, China and Turkey, where *E. coli*-resistant strains has been shown to have a prevalence >40% to cephalosporins, quinolones and trimethoprim/sulfamethoxazole (TSX), drugs widely used around the world to empirically treat bacterial infections (**Table 2**).

During 1945, just a few years after the introduction into clinical practice of penicillin, Alexander Fleming warned the world about antibiotic overuse, warning that became reality a few years later when the first *S. aureus* strain was reported to be resistant to penicillin. Several human activities have been identified as key drivers of the current AR crisis, but it has been demonstrated that the overuse of antibiotics clearly influences the evolution of resistance [18]. The reported actions that have led to the overuse of antibiotics are multifactorial and include different players in different industries such as the health, the livestock and the pharmaceutical industries. Examples of these actions comprise inappropriate prescription of antibiotics by healthcare providers, extensive use of antibiotics in livestock and fish farming, patients not following antibiotic treatment regimes, poor hygiene, bacterial mutations and lack of new antibiotics developed [19].

2.1 Overuse/inappropriate prescribing

One of the most significant factors that have contributed to the current antibacterial resistance crisis is the rapid evolution of bacteria under selective antibiotic pressure, since a continuous interaction between any given antibiotic and bacteria is an important aspect for the increase in multidrug-resistant strains [20].

Country	Resistance rates (%)		
	Cephalosporins	Quinolones	TSX
Mexico [15]	54.4	59.0	62.1
China [16]	52.4	69.8	ND
Turkey [17]	40.7	47.2	58.0

ND: not done.

Table 2.
Escherichia coli *antibiotic resistance rates to different antibiotics.*

Unfortunately, overuse and inappropriate prescription of these drugs are two large contributors to such issue. In any given antibiotic treatment against a bacterial infection, susceptible bacteria will be killed; if properly targeted, the pathogenic microorganism will be eradicated; however, along infecting bacteria, those members of the individual's microbiota, sensitive to the antibiotic in use, will also be wiped out. In case resistant microorganisms exist, either belonging to the normal microbiota or the pathogenic microorganisms being targeted, these survivors will replicate and will become the prevailing strain within the respective anatomical site.

The discovery and use of antibiotics have revolutionized the field of medicine and saved millions of lives each year; unfortunately, seen as the "miracle drug," healthcare providers and patients around the world have abused their use. Despite the marked increase of infections caused by multidrug-resistant bacteria around the world, the global response to this crisis has been inadequate, as people not only continue to misuse antibiotics but have continuously increased their abuse. Using a global database of antibiotic sales, Klein et al. [21] found that the antibiotic consumption rate around the world increased dramatically from 11.3 daily doses/1000 inhabitants per day to 15.7, an increase of 39%, between 2000 and 2015. In this same study, it was reported that the mean antibiotic consumption rate was primarily driven by the consumption in low- and mid-income countries, as no coincidence present the highest prevalence of multi drug-resistant bacteria-related infections. To make matters worse, the consumption of last-resort antibiotics such as carbapenems and colistin is also on the rise [21], situation that is consistent with the appearance of *E. coli*-resistant strains to these agents. To date, resistance of this organism to carbapenems is rare, with its prevalence depending on the area of the world under study, but not exceeding 3% [22]. However, in the future, an increase of resistance to this agent might be seen in *E. coli*, as the enzymes responsible for its hydrolysis, and thus inactivation, carbapenemases, are encoded mainly on plasmids, and are highly transmissible [23].

A key contributor to the increasing selective pressure of antibiotics is their overprescription. Recent data indicates that over 70% of prescribed antibiotics by primary care providers in the United States are inappropriate, the majority of which are for acute respiratory tract infections [24]; unfortunately, this rate of antibiotic misuse is probably a situation found in most countries. Coincidently, ciprofloxacin, one of the two most likely antibiotic to be prescribed inappropriately [24] is one to which *E. coli* present the highest rates of resistance around the world [15–17].

In addition to the contribution of the abuse of antibiotics to the selection of resistance, Zhang et al. [25] found epidemiological evidence that antibiotic resistance and *E. coli* diarrheagenic virulence phenotypes might be partially linked. They found that subjects with diarrhea had more frequent use of antibiotics before their onset of symptoms, linkage that might be explained as antibiotics might disrupt the intestinal microbiota, allowing overgrowth of resistant pathogens [25].

2.2 Use of antibiotics in livestock

Antibiotics are used in livestock to treat clinical disease, to prevent and control common disease events, and to enhance animal growth [26]. Unfortunately, this use of antibiotics has favored spread and persistence of resistant bacteria in humans by means of two different mechanisms: (a) human ingestion of antibiotics by means of the antibiotic-contaminated meat that enters the body and induces selective pressure on the host's microbiota and (b) resistant bacteria found in the gut of food animals are transmitted to humans via contaminated meat.

When livestock are treated or are provided with antibiotics, these agents exercise the same selective pressure on their microbiota as when humans ingest these drugs; thus, overuse of antibiotics on food animals has led to a high colonization rate of intestinal bacteria, including members of the *Enterobacteriaceae* family, such as *E. coli* and *Klebsiella* spp., that become resistant to different antimicrobials. Different studies around the world have shown that ready-to-eat animal products are contaminated with *E. coli* strains resistant to different kinds of antibiotics, mainly to β-lactams by means of the bacterial production of extended spectrum β-lactamases (ESBL) [27, 28]. These studies show that animal meat contaminated with *E. coli*-resistant strains is far more prevalent in developing than in developed nations, probably due to different hygiene habits; German studies have reported a prevalence of ESBL-contaminated meat of 24.1% [27], whereas in Mexico this prevalence has been reported to be above 60.0% [28]. *E. coli* strains isolated in meat have also shown resistance to other antibiotics, including to last-resort ones such as carbapenems [29] and colistin [30]. If this contaminated meat is ingested undercooked by humans, gut colonization is likely, establishing a reservoir for future antibiotic-resistant infections, as Ruppé et al. have shown that people with high gut colonization rates of ESBL-producing *E. coli* strains present higher risk to develop urinary tract infections with these clones than patients with no ESBL gut colonization [31].

Figure 1 shows a resumed representation of the main reservoirs, including livestock, of antibiotic-resistant *E. coli* and their interaction with humans.

2.3 Hygiene/fecal colonization

Higher consumption of antibiotics in unprivileged areas of the world plays a key role in the emergence and maintenance of antimicrobial resistance due to selective pressure by these agents on resident microbiota. However, studies have shown that inhabitants of these areas can be highly colonized with antibiotic-resistant *E. coli* strains despite not being in contact with antibiotics for 3–6 months [28], indicating that additional factors play important roles in the increased prevalence of AR worldwide. Global evidence suggests that elements in people's environment such as poor waste, non-potable drinking water, housing overcrowding and lack of hygiene facilitate the development and transmission of resistant bacteria [32].

The ability of *E. coli* to colonize different environments, including the gut of humans and animals, has provided this organism with the evolutionary advantage to acquire antibiotic resistance traits from other bacteria within its environment, as well as to be easily transmitted via the fecal-oral route. The gut microbiota of humans can harbor more than 1000 different antibiotic-resistant genes [33] and transmission of these traits among gut commensals is a constant phenomenon. Major examples of the transference of resistance genes between environmental bacteria, including gut commensals, and human pathogens, are the bla*CTX-M* genes, which is the most prevalent ESBL gene in *E. coli* and *Klebsiella* spp., and

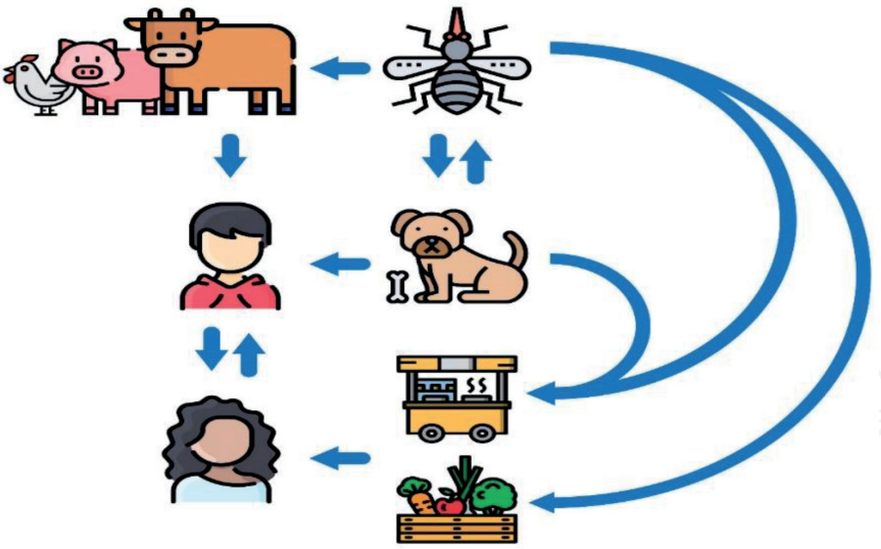

Figure 1.
Reservoirs of antibiotic-resistant E. coli *and their interaction with humans. Arrows show the* E. coli *flux from the different reservoirs.*

the OXA-48-type carbapenem-hydrolyzing β-lactamase genes, which are increasingly reported in *Enterobacteriaceae* around the globe. The potential origin of the bla*CTX-M* genes was identified in the chromosomal DNA of various environmental *Kluyvera* species [34], whereas that of OXA-48 was found to originate from the waterborne, environmental *Shewanella* species [35].

As many antibiotic resistance genes are associated with elements such as plasmids or transposons, and while the transfer of these elements may also occur through transformation or transduction, conjugation is often considered as the most likely responsible mechanism for the transmission of these traits [36]. The aforementioned ESBL and carbapenemase genes are primary examples of resistant genes with high impact on human health that have spread between bacteria via plasmid conjugation. Studies in China [37] have demonstrated that transmission via conjugation of ESBL genes in *E. coli* do occur even in the food chain, situation that partially explain the high fecal prevalence of ESBL-producing *E. coli* around the world.

The gut of humans and animals is a major reservoir of antibiotic-resistant *E. coli* and shedding of these strains through the feces of colonized individuals, livestock and domestic animals allows them to reach humans via contaminated water and food (see **Figure 1**). Human fecal colonization by antibiotic-resistant *E. coli* strains present the highest rates in deprived areas of the world, situation that begins since birth. Whereas in high-income nations the prevalence of *E. coli* strains resistant to antibiotics colonizing the gastrointestinal system of neonates is low [38], in low-income countries the prevalence of *E. coli* strains resistant to antibiotics such as tetracycline, ampicillin and trimethoprim/sulfamethoxazole exceeds 50% [39]. Fecal colonization of humans by resistant *E. coli* is on the rise around the world since the mid-2000s and the situation has worsened as fecal colonization by strains resistant to last-resource antibiotics, such as colistin, has been recently reported in different countries [40, 41]. As the prevalence of fecal colonization by these *E. coli* strains increase, so will the number of human infections caused by them, as it has been previously shown that fecal colonization with resistant microorganisms increases the risk factor of developing urinary tract infections by a factor of 13.0 [31].

Antibiotic consumption has contributed to the selection of resistance and is largely accepted as one of the major drivers of AR development; however, the high

prevalence of antibiotic resistance around the world, especially in low- and mid-income countries, can be more likely attributed to the dissemination and maintenance of resistant clones via poor sanitation and lack of hygiene habits [32]. Ingestion of contaminated food and water, close contact with colonized animals and household members and abundance of flies are factors that contribute to the transmission of kl *E. coli* strains. As these conditions are considerably less frequent in developed areas of the world, this situation partially explains the reduced prevalence of these strains in these nations. However, due to the current globalization, resistant strains can easily be transmitted from one country to another. In a large cohort study of Dutch travelers to regions of the world with high prevalence of ESBL-producing bacteria, 34.3% subjects who were ESBL negative before travel had acquired these clones during their time abroad, with the highest number of acquisitions being among those who traveled to southern Asia, and remained colonized at 12 months after return [42]. Additionally, this same study showed that the estimated probability of onward transmission within households was 12%. Similar results were reported in a study in Spain, in which up to 66% of the isolates from patients with ESBL-producing *E. coli* infections were indistinguishable from those isolated from fecal samples from their household members [43]. These results indicate that acquisition of *E. coli*-resistant clones during travel is high and that transmission between household members can maintain such clones in the community for long periods of time.

As anthropogenic activities largely shape the resistome of different environments, transmission of resistant genes between bacteria in a community can be influenced by its contamination with human and animal feces and its impact is largely driven not by the presence of resistant bacteria but rather from the presence of human-related mobile resistance genes [44]. If poor sanitation, manifested by fecal contamination, of a given community is the key to transmit and maintain resistant clones, the reduction of antibiotic consumption will not be sufficient to control antimicrobial resistance. Thus, strategies to control the AR pandemic should also include improving sanitation conditions in all parts of the world.

3. Antibiotic resistance mechanisms

Few microorganisms have shown the ability to develop resistance to as many classes of antibiotics as the *Enterobacteriaceae.* Of the large list of bacterial genus that belong to this family, *E. coli* is only surpassed by *Klebsiella* in the number of human infections associated to multidrug-resistant bacteria [15–17, 27] and the past two decades have witnessed major increases in the emergence and spread of *E. coli* resistance strains to major classes of antibiotics such as β-lactams, quinolones, aminoglycosides, sulfonamides and fosfomycin. Unfortunately, this resistance has spread to last resource antibiotic classes such as the polymyxins and carbapenems. The following sections will briefly described the resistance mechanisms developed by *E. coli* against one of the major antibiotic groups currently used in the treatment against this organism: the β-lactams.

3.1 Resistance to β-lactams

Antibiotics belonging to the β-lactams class share a common feature: a three-carbon and one-nitrogen ring (beta-lactam ring), which is the molecular constituent responsible for the bacteriolytic mechanism of action of these agents against bacteria. β-Lactams act by inhibiting the bacterial synthesis of peptidoglycan, a vital constituent of the microorganism cell wall. The targets for the actions of beta-lactam antibiotics are known as penicillin-binding proteins (PBPs).

Bacteria have evolved different mechanisms of resistance against β-lactams: (a) Inactivation of these agents by the production of beta-lactamases; (b) decreased penetration of the antibiotic to the target site; (c) alteration of target site PBPs; and (d) efflux from the periplasmic space through specific pumping mechanism. However, in the case of *E. coli*, resistance to these antibiotics is mediated by the production of a group of enzymes referred as the "β-lactamases." These enzymes are ancient compounds, currently exceeding 2800 unique proteins, which emerged from environmental sources [45].

To date, β-lactamases are usually classified based on functional or structural criteria. Currently, the most widely used classification for these enzymes is the Ambler structural classification, which is based on sequence similarity, and separates these proteins into four classes: the classes A, C, and D of serine-β-lactamases and the class B of metallo-β-lactamases [46].

Gram-negative bacteria have evolved the production of different β-lactamases; in the case of *E. coli*, the most important ones from the medical point of view are the extended spectrum β-lactamases (ESBL), AmpC β-lactamases (AmpC) and the carbapenemases. Each of these groups of enzymes presents different spectrum of hydrolytic activity, thus presenting resistance to different types of β-lactams, as shown in **Table 3**.

3.1.1 Extended spectrum β-lactamases (ESBL)

Among the β-lactamases, ESBL are worthy of the attention of the scientific and medical community over the last decades because of their increasing prevalence as cause of antibiotic-resistant infections around the world. These enzymes can be produced by any member of the *Enterobacteriaceae*, but *Klebsiella* spp. and *E coli* are the predominant ESBL-producing genus.

ESBL belong mostly to class A of the Ambler classification, are generally plasmid encoded and confer resistance to those bacteria that produce them to penicillins, first-, second-, and third-generation cephalosporins and monobactams (e.g., aztreonam), but cannot hydrolyze cephamycins (cefoxitin) or carbapenems (imipenem, meropenem), and are inhibited by β-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam [47].

β-Lactamase	**Spectrum of activity**	**Inhibition by β-lactamase inhibitors**	**Activity against broad-spectrum cephalosporins**
ESBL	Penicillins First to third generation cephalosporins Monobactams	Yes	No
AmpC	Penicillins First to third generation cephalosporins Monobactams	No	Yes
Carbapenemases New Delhi metallo-β-lactamase	All β-lactams except aztreonam	No	Yes
Carbapenem-hydrolyzing oxacillinase-48	All β-lactams except broad spectrum cephalosporins	No	Weak

Table 3.
Spectrum of activity of the major types of β-lactamases produced by Escherichia coli.

When ESBL were first identified, most ESLB-related infections were caused by strains producing the TEM and SHV types. However, since then, ESBL CTX-M has emerged as the predominant type, both in humans and animals, in commensal organisms and in pathogenic strains and in community and healthcare-associated infections. Since the first isolation of SHV- and TEM-producing strains, more than 100 different variants of each type have been described and all have arisen from the original strains; contrary to the SHV and TEM types, CTX-M groups seem to have originated from the chromosomally encoded ESBL genes from different *Kluyvera* species [48].

HSV, TEM and CTX-M show different hydrolytic activities against different β-lactams. When first identified, SHV β-lactamases proved its activity against penicillins and first generation cephalosporins; as of today, the three sub-groups used to classify this group of enzymes present different antibiotic resistance phenotypes: (a) subgroup 2b hydrolyze penicillins and early cephalosporins (cephaloridine and cephalothin) and are strongly inhibited by clavulanic acid and tazobactam; (b) subgroup 2br are broad-spectrum β-lactamases that acquired resistance to clavulanic acid; and (c) subgroup 2be comprises ESBL that can also hydrolyze one or more oxyimino β-lactams (cefotaxime, ceftazidime, and aztreonam) [49]. In the case of TEM β-lactamases, the bacteria carrying these genes are able to hydrolyze penicillin and first generation cephalosporins such as cephaloridine; furthermore, TEM-1 is able to hydrolyze ampicillin at a greater rate than carbenicillin, oxacillin, or cephalothin, and has negligible activity against extended-spectrum cephalosporins [50]. Finally, CTX-M enzymes have the property of having potent hydrolytic activity against cefotaxime, with CTX-M-producing microorganisms showing cefotaxime MICs in the resistant range (>64 μg/ml), while ceftazidime MICs are usually in the apparently susceptible range (2 to 8 μg/ml); however, some CTX-M-type ESBLs may actually hydrolyze ceftazidime and confer resistance to this cephalosporin; aztreonam MICs are variable. CTX-M-type β-lactamases hydrolyze cefepime with high efficiency [50].

The exponential global increase in the number of infections caused by ESBL-producing strains has coincided with the appearance of the *CTX-M* genes. When originally reported, these strains were predominantly found in three geographic areas: South America, the Far East, and Eastern Europe. However, due to the extremely transferable plasmids which harbor bla*CTX-M* genes [49], with a frequency of transmission from 10^{-7} to 10^{-2} per donor cell [48], these strains are now increasingly reported as cause of human infections in every continent, to the point that it could be speculated that CTX-M-type ESBLs are now the most frequent ESBL type worldwide [50]. An additional factor that has been suggested as a key contributor to the dissemination of these clones is the frequent co-existence of bla*CTX-M* with genes conferring resistance to other classes of antibiotics like fluoroquinolones and aminoglycosides, situation that might lead to high rates of co-selection [51].

To date, over 150 CTX-M types have been identified and described (https://www.lahey.org/studies/other.asp). These ESBL types have been grouped into five clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) [52], with each cluster presenting different variants, and more variants constantly being described, as shown by the recent discovery of two novel ones, named $bla_{\text{CTX-M-14.2}}$ and $bla_{\text{CTX-M-15.2}}$ [53]. Out of the different CTX-M variants, different reports in different continents indicate that $bla_{\text{CTX-M-15}}$, belonging to cluster CTX-M-1, is now the most prevalent ESBL in *E. coli* around the world [54]. The increasing predominance of the $bla_{\text{CTX-M-15}}$ allele might be due to the powerful ability of this enzyme to hydrolyze different β-lactams, which probably offers the producing bacteria a selective advantage, especially when multiple antibiotics are concomitantly or consecutively prescribed [53].

One of the key players in the global dissemination of CTX-M-15-producing *E. coli* strains is clone ST131. A study performed in *E. coli* ST131 strains isolated between 2002 and 2004, before de ESBL pandemic, showed that only 2% of those strains carried the *CTX-M-15* gene [55]; almost two decades later, ST131 is one of the main clones isolated in the worldwide spread of ESBL-producing *E. coli* [56], particularly subclone H30Rx [57]. How this *E. coli* clone went from being a non-factor in the global ESBL transmission to a key player is probably multifactorial. Although ST131 strains are not considered hypervirulent, most of them show the presence of fluoroquinolone-resistant genes, they have the ability to be persistent gut colonizers even in the absence of antibiotic exposure, a condition that precedes some infections such as those in the urinary tract, and can be easily transmitted between people of all ages [58]. All of these factors have allowed this clone to be successful human pathogen, even before the spread of the ESBL genes; however, the acquisition by ST131 strains of the CTX-M-15 plasmid has made this *E. coli* lineage an even more successful pathogen and has probably exasperated the spread of such clone [59] and the rapid global spread of CTX-M-15-producing *E. coli*.

3.1.2 AmpC β-lactamases (AmpC)

Although the production of class A extended spectrum β-lactamases is the most common mechanism of resistance in *E. coli* against β-lactam agents, class C β-lactamases, or AmpC, can also confer those strains that produce them the ability to inactivate some of these compounds. Similar to ESBL, AmpC-producing organisms hydrolyze amino- and ureidopenicillins, oxyimino-β-lactams such as ceftazidime, ceftiofur, and aztreonam, but contrary to the former enzymes, AmpC also inactivates broad and extended-spectrum cephalosporins such as cephamycins (cefoxitin) and are not inhibited by β-lactamase inhibitors such as clavulanic acid. Neither ESBL nor AmpC confer bacteria resistance to carbapenems.

Originally, AmpC were described as chromosomally encoded enzymes and were detected in a few bacterial species such as *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens, Acinetobacter* spp., *Aeromonas* spp. and *Pseudomonas aeruginosa* [60]. As the use of β-lactamase inhibitors increased among the population, dissemination of AmpC genes among bacterial species began by means of horizontal travel through plasmids, phenomenon that led to the appearance of AmpC-resistant traits in bacteria that previously lacked such genes or expressed them at low levels, such as *E. coli*, *Klebsiella* spp. and *Shigella* spp. [60].

In *E. coli*, the subject of this chapter, resistance by AmpC can be plasmid encoded or due to the overexpression of the chromosomal AmpC genes. Contrary to the AmpC enzymes of other members of the *Enterobacteriaceae*, such as *Enterobacter* spp. and *Citrobacter freundii*, that of *E. coli* exhibits a non-inducible phenotype that is constitutive and its production depends on either the strength of the *ampC* promoter [61], the presence of >1 copy of the *ampC* gene, the incorporation of a stronger promoter sequence as part of an insertion element or by the acquisition of a strong promoter of other bacterial species [62]. As stated before, this organism can carry *ampC* genes either chromosomally or in plasmids; however, the latter is being recognized as the major threat since plasmid-encoded AmpC are easily transferable between bacterial species, can cause nosocomial outbreaks, is associated with multidrug resistance and, in combination with porin loss, may lead to resistance to carbapenems [63].

Bacterial resistance to β-lactams is a major public health problem around the world. Although ESBL production clearly exceeds AmpC production as the major cause of β-lactam resistance, the later enzymes are now being recognized as a growing problem in different members of the *Enterobacteriaceae*, including *E. coli*, as

evidenced by the increasing number of these strains being reported across the globe. Sources of AmpC-producing *E. coli* strains include livestock [64], the environment [65], as colonizers of the human gut [66] and as cause of human infections. The prevalence of these strains isolated as causative agents of human infections varies, ranging from 2.0% reported in a Portuguese hospital [67] to 16.7% from three university hospitals in Iran [68] to 29.0% from five referral hospitals in Sudan [69].

When comparing the epidemiology of today's AmpC-producing *E. coli* to that of ESBL-producing bacteria of two decades ago, they present several common features: high gut colonization in both animals and humans, reduced prevalence as cause of human infections, environmental contamination by these multidrug-resistant strains, higher isolation of both types of β-lactamase-producing strains in developing countries and their ability to be transmitted via plasmids among different bacterial species. As these two types of β-lactamase-producing strains behave similarly, it would be of no surprise to witness in the near future a booming increase of reports of infections caused by AmpC-producing strains, as witness two decades ago with ESBL. To make matter worse, infectious disease specialists are starting to see an increase of cases of *E. coli* strains that co-express ESBL and AmpC genes, complicating antimicrobial treatment even further. Different reports in India [70, 71] have shown that co-expression of bla*ESBL* and bla*AmpC* genes by *E. coli* strains isolated from different human infections is not uncommon, thus continuous monitoring of these resistance patterns is a necessity that will help prevent the further spread of these multidrug-resistant microorganisms.

3.1.3 Carbapenemases

Since ESBL- and AmpC-producing *E. coli* are increasingly being reported as cause of severe infections, carbapenems represent in many cases the last option for effective treatment against these infections. Nevertheless, with an increasing consumption of these agents, carbapenem-resistant strains, particularly *Klebsiella* spp. and in a lesser degree *E. coli*, have become a public health concern, particularly in the hospital setting. Carbapenems bind to penicillin-binding proteins and induce spheroplast formation and cell lysis without filament formation. The carbapenems include four agents: imipenem, meropenem, ertapenem and doripenem.

As in the case of ESBL- and AmpC-producing *Enterobacteriaceae*, reports from different countries show that resistance to carbapenems has been constantly increasing in the last few years, becoming a public health problem. In Europe, 11 countries have reported an increase in the number of infections caused by carbapenemase-producing *Enterobacteriaceae* in the period from 2015 to 2018 [72] and in China, Tian et al. [73] have reported an increase in the prevalence of carbapenemase-producing *E. coli* from 0% in 2011 to 1.9% in 2017.

The reported carbapenemases in *E. coli* primarily include *Klebsiella pneumoniae* carbapenemases (KPC), metallo-β-lactamases (MBL), including the VIM, IMP, GIM and NDM type, and oxacillin-hydrolyzing metallo-β-lactamases (OXA) [74]; however, different reports around the world have shown that the predominant types in *E. coli* are of the New Delhi metallo-β-lactamase (NDM-1) and carbapenem-hydrolyzing oxacillinase-48 (OXA-48) types [73, 75, 76].

3.1.3.1 New Delhi metallo-β-lactamase

The New Delhi metallo-β-lactamase (NDM-1) and closely related enzymes are a group of zinc-requiring metallo-β-lactamases capable of hydrolyzing a broad range of β-lactams including all penicillins, cephalosporins and carbapenems, just sparing monobactams, and are among the most recently identified carbapenemases. The

gene encoding these enzymes, bla_{NDM}, has been identified on bacterial chromosomes and plasmids [77]; however, in the case of *E. coli*, bla_{NDM} is mainly plasmid encoded with only few strains carrying it chromosomally [78].

NDM-1 was first identified in 2008 in India, a country that has been pointed out as the primary reservoir of NDM strains [77], followed by the Balkan states [79] and the Middle East [80]. From these three spots, bla_{NDM-1}-carrying bacterial strains have spread around the world, mainly due to the ability of the carrying microorganisms to horizontally transfer the carbapenemase resistance trait via plasmids. An additional factor that has contributed to the worldwide dissemination of NDM-1-producing strains is the frequent co-existence of the bla_{NDM-1} gene on plasmids carrying additional antibiotic resistance genes, situation that has allowed the plasmid-carrying strains to thrive under environments of antibiotic selective pressure.

Since the first report of NDM-1, over 20 NDM variants have been reported; however, in *E. coli*, NDM-1, followed by NDM-5, are the predominant variants in human infections in different parts of the world [81, 82]. Surprisingly, in a study by Shen et al. [83] published in 2018, the highest prevalence in the human gut and livestock was of the NMD-5 variant, suggesting a possible shift from NDM-1 to NDM-5 in the community in China. An additional, and important finding of this study, was the identification, albeit small, of NDM-5 *E. coli* strains that co-express colistin resistance genes, *mcr*-1, in the gut of healthy individuals, situation that if not properly controlled might contribute to the future dissemination of *E. coli* strains that are resistant to last resource antibiotics.

3.1.3.2 Carbapenem-hydrolyzing oxacillinase-48 (OXA-48)

As with any other β-lactamase, OXA-48 hydrolyzes β-lactam antibiotics, including carbapenemases, but paradoxically spares broad-spectrum cephalosporins. OXA-48 genes were originally traced to the aquatic bacterium *Shewanella oneidensis*, but further studies now trace its origin to *Shewanella xiamenensis* [84]. Since the first description in Europe of OXA-48-carrying *Enterobacteriaceae*, several variants have been reported, including OXA-162, OXA-163, OXA-181, OXA-204, OXA-232, OXA244 and OXA-245.

Mainly found in *Klebsiella* species, reports on the detection of bla_{oxa}-carrying *E. coli* have increased in the last 3 years in different parts of the world, being reported in studies in Myanmar [85], the United States [86] and Thailand [87]. In all three studies, the isolated strains were co-expressing bla_{OXA-48} or its variants and bla_{NDM5}. Oxa-48-carrying *E. coli* strains have also been isolated in Europe, between January and October 2019, 134 cases of *E. coli* strains carrying the OXA-48 variant OXA-244 were isolated from clinical samples in Germany; this same variant was further identified in 119 *E. coli* strains isolated from other European countries [88]. The source and route of transmission of these strains is currently unclear.

As carbapenems are considering in many clinical instances as a last resource antibiotic, worldwide monitoring on the prevalence of *E. coli* carrying resistant traits against these agents should be continuously performed in order to prevent the spread of these strains, situation that can jeopardize even further the current antibiotic resistance crisis.

4. Conclusions

The ability of *Escherichia coli* to colonize the gut of humans and animals, thus facilitating its transmission via the fecal-oral route, and its ability to transmit and

uptake antibiotic resistance genes via plasmids to and from other bacteria have made this organism a key target in the fight against antimicrobial resistance. As discussed in this chapter, *E. coli* has evolved different mechanisms to fight off the action of antibiotics, and in many cases a single strain can carry resistance genes to distinct classes of these agents, thus complicating treatment.

The emergence of antibiotic resistance has been shown to be multifactorial, but all elements coincide in a major topic: antibiotic over abuse, both in human and veterinary medicine. The establishment of antibiotic stewardship programs is a major necessity in all nations as a way to reduced antibiotic resistance. However, as the spread and maintenance of *E. coli*-resistant traits among humans and between animals and humans is driven by additional, and probably more difficult to tackle, social issues such as lack of hygiene, lack of drinking water and house overcrowding, these factors must be taken care of in order to truly impact antibiotic resistance.

Conflict of interest

The author declares no conflict of interest.

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