Chapter 5

Antimicrobial Mechanisms of *Escherichia coli*

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Additional information is available at the end of the chapter

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Abstract

Increasing antimicrobial resistance in strains of *Escherichia coli* is having a major impact on the healthcare industry worldwide. The appearance of extended-spectrum β-lactamase (ESBL) and carbapenem-resistant Enterobacteriaceae (CRE) strains has caused clinicians to worry that these strains might become as deadly as methicillin-resistant *Staphylococcus aureus* (MRSA) strains. It is vital that physicians have resources available to help keep them updated on these bacteria and the potential impact on healthcare. This chapter reviews the major strains of *E. coli* (intestinal and urinary), along with a review of the virulence factors, main diseases caused, and pertinent pathogenesis. The chapter then discusses antimicrobial therapy, what drugs are effective against these *E. coli* strains, and the development of resistance to these specific drug classes. Lastly, the molecular aspects of antimicrobial resistance mechanisms in this organism are discussed. This information will be especially helpful for physicians in providing them with a concise review of *E. coli* and an understanding of what is involved in antimicrobial resistance. Hopefully this information can be used to improve the outcomes for patients with *E. coli* infections.

Keywords: *Escherichia coli*, antimicrobial resistance, ESBL, CRE

1. Introduction

Increasing resistance to antimicrobial drugs is of major concern worldwide. For many developing countries, the possibility of having cheap antibiotics available may now be threatened. There are antimicrobial resistance issues with most pathogenic bacteria and in virtually all of the opportunistic bacterial-caused infections. This translates into increased healthcare costs. These costs include extended length of hospital stay and increased costs for medical supplies including more expensive antimicrobial drugs. In addition, antimicrobial drug-resistant infections lead to higher rates of mortality, especially in patients who had recent prior exposure to antibiotics [1].

Antimicrobial drugs are classified into groups according to the type of antimicrobial activity. These groups include drugs that inhibit bacterial cell wall synthesis, depolarize the cell membrane, inhibit bacterial protein synthesis, inhibit bacterial nucleic acid synthesis, and inhibit bacterial metabolic pathways. The improper use of these drugs has helped to create resistant bacterial strains. Factors that contribute to resistance include the increased use of all antimicrobial drugs and improper antimicrobial prescribing. Many of the less expensive drugs that have fewer side effects have been used too commonly. Improper prescribing may be choosing broad spectrum or ineffective drugs [2–4].

Many strains of *Escherichia coli* are not harmful. In fact, these commensal strains in the gut are necessary for the synthesis of vitamin K_2, which is an important clotting factor [5]. However, there are pathogenic strains, and these strains may become a larger threat if they possess or acquire certain antimicrobial mechanisms. The main ones of concern are the extended-spectrum β-lactamase (ESBL)-producing strains and the carbapenem-resistant Enterobacteriaceae (CRE) strains. The ESBL strains are resistant to most β-lactam drugs, and the CRE strains are resistant to most carbapenem drugs. Greatly increased healthcare costs are associated with the ESBL and CRE strains. Various studies have shown a hospital stay of up to twice as long and increased costs of 1.5–2.5 times as much [6–8]. One study in the United States estimated the increased costs to be $16,450 per patient [6].

All of the pathogenic strains of *E. coli* are armed with the same types of potential virulence factors. These factors include a capsule (in some strains), flagella, the lipopolysaccharide (LPS) cell wall, fimbriae, outer membrane proteins (OMPs), a hemolysin, cytolysins, and siderophores. The specific types of some of these virulence factors plus the possession of other toxins and effectors may vary with each individual pathogenic strain [9].

2. Pathogenic strains of *Escherichia coli*

Commensal strains of *E. coli* are the predominant facultative organism in the human gut. Even though greatly outnumbered by the anaerobic organisms, the *E. coli* are vital to human health, playing roles in biofilm communities and subsequent digestion of oligosaccharides and polysaccharides, among other things [10, 11]. Unfortunately, there are also several pathogenic strains of *E. coli*. The classification names of these strains may vary some depending on the source, but for the purposes of this chapter, we will use the following names. There are six strains of potentially pathogenic intestinal-based diarrhea causing *E. coli*: diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (ETEC). In addition, there is one extraintestinal pathogenic strain, uropathogenic *E. coli* (UPEC), which causes urinary tract infections. There is some evidence that a second extraintestinal strain exists, the meningitis-associated *E. coli* (MNEC) strain. Findings on MNEC indicate that the infection starts as a blood infection and then gains access to the central nervous system. A majority of MNEC possess the K1 capsular antigen, and there is a high rate of mortality from the meningitis [12]. This chapter will focus on the six intestinal and the UPEC strains.

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2.1. Diffusely adherent *Escherichia coli* (DAEC)

These strains are sometimes referred to as enteroadherent *E. coli* (EAEC) (not to be confused with the enteroaggregative strains, which are sometimes referred to as EAggEC). While not known to cause severe diarrheal disease, the DAEC, which are probably a group of related strains, are thought to be responsible for some types of persistent diarrhea in infants. Whether they possess true virulence factors is still under debate, but these bacteria are able to bind to enterocytes (probably via adhesins such as Afa/Dr) and elicit a response in which the microvilli extend and wrap around the bacteria. Diarrhea in association with DAEC has also shown an ability to induce the production of inflammatory cytokines, such as IL-8 [13–15].

2.2. Enteroaggregative *Escherichia coli* (EAEC)

The EAEC strains were so named because of their tendency to adhere to enterocytes in dense clusters. The bacteria attach to the microvilli and also to other EAEC bacteria. The EAEC are also a heterogeneous group of strains with a similar pathogenesis, resulting in non-bloody diarrhea. Infection is established by adhering to the microvilli via fimbriae (the aggregative adherence fimbriae—AAF), inducing increased mucus production and biofilm formation, inducing an inflammatory response and production of toxins. The main toxins of the EAEC strains are the EAEC heat-stable enterotoxin (EAST1), which enters the enterocytes and activates guanine cyclase, resulting in increased levels of cGMP in the cell and loss of fluid into the intestinal lumen; the plasmid-encoded toxin (Pet), which disrupts the enterocyte cytoskeleton, resulting in cell detachment; and a *Shigella*-like enterotoxin (ShET1), a heat-stable toxin which may also result in fluid secretion [16–18].

2.3. Enterohemorrhagic *Escherichia coli* (EHEC)

The most publicized strain of pathogenic *E. coli* is the EHEC strain. EHEC serotype O157:H7 is well known as the causative agent of outbreaks of food-associated severe diarrhea. Infection with O157:H7 results in sever abdominal cramps and bloody diarrhea and may lead to hemolytic-uremic syndrome (HUS) which can be life-threatening. The most common foods associated with transmission of these bacteria are undercooked meat (especially ground beef), raw milk, and raw vegetables. The EHEC strains do not directly invade the enterocytes, but produce toxins that do enter and severely damage these cells. The responsible cytotoxins are verotoxins I and II (designated as Shiga toxins, Stx-1 and Stx-2). The Shiga toxins are capable of inactivating ribosomes, blocking protein synthesis, and emerging through the basolateral membrane into the subepithelial region. Stx-2 is seen most often in the EHEC strains that cause HUS [19–21].

2.4. Enteroinvasive *Escherichia coli* (EIEC)

In infections with EIEC, the bacteria invade by directly entering M cells. The bacteria pass through these cells and then are able to invade the enterocytes via the basolateral membrane, causing severe damage to the intestinal mucosa. The bacteria are also able

to spread laterally through the cell side walls to adjacent cells (via actin). This damage results in dysentery (watery diarrhea with pus, mucus, and blood). The EIEC bacteria do not produce toxins, but participate in direct damage and induce production of IL-1 and IL-8. The pathogenic mechanisms and disease symptoms associated with EIEC are so similar to *Shigella* spp. that differential diagnosis can be difficult. Diagnosis is usually based on physiological and biochemical characteristics that can be detected in the clinical laboratory [19, 22, 23].

2.5. Enteropathogenic *Escherichia coli* (EPEC)

The EPEC strains do not directly invade enterocytes. Instead, these bacteria adhere to the microvilli and inject effector proteins into the cell via a type III secretion system (T3SS). One of these effectors is the translocated intimin receptor (Tir), which initiates recruitment of the host cell actin to form a pedestal under the bacteria. The recruitment of actin and formation of the pedestals result in destruction of the rest of the microvilli and also inhibit the transport of Na^+ and Cl^- in the cell, which results in the subsequent exodus of water into the intestinal lumen. Another effector, the *E. coli* secreted proteins (Esps), interacts with the host cell cytoskeleton and results in disruption of the cell tight junctions [24–26].

2.6. Enterotoxigenic *Escherichia coli* (ETEC)

The ETEC strains are a common cause of acute travelers' diarrhea. These strains usually colonize the proximal small intestine, adhering to the microvilli via various colonization factors including fimbrial, nonfimbrial, helical, and fibrillar types. The EPEC strains secrete two types of toxin: a heat-labile toxin (LT) and heat-stable toxins (STs). The LT is an AB toxin, and the B subunits bind to the monosialoganglioside GM1, which induces the cell to take in the toxin. The LT toxin activates adenylyl cyclase, which increases the cAMP in the cell, resulting in hypersecretion of water and electrolytes into the intestinal lumen. The STs bind to guanylyl cyclase receptors on the microvilli, which stimulate guanylate cyclase and activate the cystic fibrosis transmembrane receptor (CFTR). This results in an increase in cGMP in the cell and impaired absorption of Na^+, which causes hypersecretion of water into the intestinal lumen [19, 24, 27].

2.7. Uropathogenic *Escherichia coli* (UPEC)

The UPEC strains are responsible for most uncomplicated urinary tract infections (UTIs). These strains possess a capsule and bind to uroepithelial cells via fimbriae. The interaction of the bacteria with the host cell induces the internalization of the bacteria where the bacteria multiply rapidly and form biofilm-like intercellular bacterial communities (IBCs). The bacteria are shed intermittently from the uroendothelial cells into the lumen of the bladder. UPEC strains produce several types of toxins including hemolysin A (HlyA) which has pore-forming capability and two cytotoxins, cytotoxic necrotizing factor (CNF-1) and secreted auto-transporter toxin (Sat) [19, 28, 29].

3. Antimicrobial therapy

As mentioned above, antimicrobial drugs are often classified in groups based on their mechanism of antimicrobial action. **Table 1** displays those groups along with examples of the antimicrobial drugs included in each group. The β-lactam drugs, which were among the first antimicrobials to be discovered, target the bacterial cell wall (via peptidoglycan synthesis) and are most useful against gram-positive bacteria (having little effect on gram-negative bacteria because of the lipopolysaccharide cell envelope that protects the thin peptidoglycan cell wall in these bacteria). Over the years, because the β-lactam drugs were readily available and inexpensive and caused few side effects, physicians commonly treated their patients initially with these drugs.

Inhibit cell wall synthesis	β-Lactams Carbapenems Cephalosporins Monobactams Penicillins Glycopeptides
Depolarize cell membrane	Lipopeptides
Inhibit protein synthesis	Bind to 30S ribosomal subunit Aminoglycosides Tetracyclines Bind to 50S ribosomal subunit Chloramphenicol Lincosamides Macrolides Oxazolidinones Streptogramins
Inhibit nucleic acid synthesis	Quinolones Fluoroquinolones
Inhibit metabolic pathways	Sulfonamides Trimethoprim

Table 1. Antimicrobial groups based on mechanism of action.

When bacterial resistance to the β-lactam drugs became an issue (very early on), scientists developed synthetic versions of penicillin such as ampicillin, amoxicillin, and methicillin. In addition, scientists discovered the natural cephalosporin β-lactam drugs. The initial cephalosporins (first generation) were most useful against gram-positive cocci, with some activity against a few gram-negative bacilli. Further development of these drugs has produced second generation (less effective against gram-positive cocci, more effective against gram-negative bacilli); third generation (generally with a broad spectrum of activity against gram-negative organisms); fourth generation (extended-spectrum activity against gram-positive cocci and gram-negative bacilli); and recently, fifth generation (hopefully effective against various

multidrug-resistant organisms), with more still in development. Other β-lactam drugs developed during this time frame were the carbapenems (broad-spectrum activity) and monopenems (aztreonam—activity against gram-negative aerobic bacteria) [30–32].

Differences in structure, metabolism, virulence factors, etc., between gram-negative and gram-positive bacteria predict which antimicrobial drug groups may be effective. Fewer of the drug groups have good activity against gram-negative bacteria. Those groups include some of the β-lactam drugs (especially second-, third-, and fourth-generation cephalosporins): aminoglycosides, fluoroquinolones, trimethoprim/sulfamethoxazole (TMP/SXT), and nitrofurans (for UTIs) [33]. Intestinal infections with *E. coli* are most commonly self-limiting and require supportive therapy (antiemetics, antidiarrhetics, rehydration) only. Severe or recurring infections (e.g., traveler's diarrhea) may be treated with fluoroquinolone drugs. Acute dysentery caused by EIEC strains may be treated with fluoroquinolones or appropriate cephalosporins. For infections caused by EHEC, antimicrobial therapy is contraindicated as it greatly increases the risk for development of HUS. UTIs caused by UPEC strains are usually treated with antimicrobial drugs and uncomplicated UTIs with nitrofurantoin or TMP/SXT; complicated UTIs may also be treated with fluoroquinolones [34–37]. Infections with ESBL or CRE strains severely limit treatment options. For ESBL strains, carbapenems may still be an option or newer β-lactam/β-lactamase inhibitor drug combinations; for CRE strains, gentamicin, amikacin, colistin, tigecycline, and fosfomycin may be options. Unfortunately, some ESBL and CRE strains may be resistant to even some of these drugs [38–40].

At the beginning of antimicrobial drug resistance, physicians did not realize how the various drugs affected the bacteria. In addition, in an effort to begin antimicrobial therapy as quickly as possible, physicians often ordered a broad-spectrum drug before knowing the causative agent of the infection. These issues (among others) have led to a large amount of resistance to β-lactam drugs, especially among the gram-negative bacteria. Bacteria that produced β-lactamases (enzymes that inactivate β-lactam drugs) were identified as early as the 1940s (around the same time as penicillin was discovered), and the number of different β-lactamases produced has increased over the years to around 1000 [2–4, 41].

The issue of resistance is not just with the β-lactam drugs. Over the 70 plus years that antimicrobial drugs have been in existence, resistance mechanisms have been seen for most of these drugs. It does not seem to take the bacteria very long from initial use of a drug to development of resistance to that drug. Important resistant milestones include resistance to aminoglycosides and tetracycline in the 1960s, vancomycin in the 1980s, fluoroquinolones in the 1990s, and linezolid in the 2000s [42]. In addition, some of the bacteria have become resistant to multiple antimicrobial agents from many of the drug classes. These multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are currently a major cause of morbidity and mortality [43].

Similar to the threat of MRSA, members of the gram-negative Enterobacteriaceae, in particular *E. coli* and *Klebsiella pneumoniae*, include strains that are ESBLs and CREs. These organisms can be resistant to most commonly used antimicrobials, which makes the infections they cause extremely difficult to treat, leading to increased morbidity and mortality (and healthcare costs) [44, 45].

4. Antimicrobial resistance mechanisms

There are four general antimicrobial resistance mechanisms that bacteria use. These are limiting uptake of the drug, modifying the target of the drug, inactivating the drug, and active efflux of the drug. These mechanisms may be located on the bacterial chromosome and occur naturally in all members of a species (intrinsic) or come from other bacteria, usually via a plasmid (acquired). Intrinsic resistance genes may be expressed constitutively (usually at a low level) or be induced by the presence of antimicrobial drugs. Gram-negative bacteria widely use all four of these mechanisms and are very capable of horizontal transfer of resistance elements. **Table 2** shows which resistance mechanisms and genes are associated with resistance to various antimicrobial drugs [43, 46, 47].

Antimicrobial agents	Mechanisms of resistance	Genetic basis
β-lactams	β-lactamases—inactivate drugs	*ampC*
Penicillins	Active efflux	*bla* genes—plasmid
Cephalosporins		(TEM, SHV, CTX-M, NDM)
Monobactams		*acrAB(tolC), acrAD(tolC)*
Carbapenems		
Aminoglycosides	Aminoglycoside modifying enzymes	*aac*, *ant*, *aph*—plasmid
Amikacin	Modify target—16S rRNA	*amrA, rmtB*
Gentamicin	Active efflux	*mdtEF(tolC)*
Tobramycin		
Tetracyclines	Limiting uptake	*ompF*
Tetracycline	Active efflux	*acrAB(tolC)*
		tetA, tetB—plasmid
Chloramphenicol	Limiting uptake	*ompF*
	Active efflux	*acrAB(tolC)*
Fluoroquinolones	Limiting uptake	*ompF*
Ciprofloxacin	Modified target—gyrase	*gyrA*
Norfloxacin	Modified target—topoisomerase IV	*parC*
	Active efflux	*acrAB(tolC), acrEF(tolC), mdtABC(tolC)*
Metabolic pathway inhibitors	Target enzyme modification	TMP—*dhfr*
Trimethoprim/Sulfamethoxazole		SXT—*dhps*

Table 2. Common antimicrobial resistance genes and mechanisms in *Escherichia coli*.

4.1. Limiting drug uptake

Gram-negative bacteria have an advantage in combating drugs because of the structure and functions of the LPS cell wall, which provides a natural barrier to certain molecules. The LPS is generally hydrophobic which limits access to small hydrophilic drugs, such as the β-lactams. These hydrophilic drugs gain access by traveling through the OMPs. The main

OMPs in *E. coli* are OmpF and OmpC. In addition to the β-lactams, other drugs that may use the porin channels are chloramphenicol, fluoroquinolones, and tetracycline. Hydrophobic drugs such as the aminoglycosides and the macrolides gain access by permeating through the LPS layer. There are two main mechanisms that are used to limit access to drugs via porins: a decrease in the number of porins or a change in charge, within the porin channel, which reduces its function or binding properties. In *E. coli* porin production may be reduced dramatically or even stopped, or a different porin may be produced instead [48, 49].

4.2. Modifying drug target

Gram-negative bacteria make use of the modifying of drug targets against several of the antimicrobial groups including β-lactams, aminoglycosides, fluoroquinolones, and the combination drug TMP/SXT. Even though not as widely used as in gram-positive bacteria, the gram-negative bacteria are able to produce penicillin-binding proteins (PBPs) that are resistant to some β-lactam drugs. PBPs are actually peptidases that are involved in the making of the peptidoglycan cell wall. Penicillin drugs that are able to bind to PBPs inhibit the assembly process. There are several different native PBPs produced by *E. coli*, some of which have reduced binding affinity for some of the β-lactam drugs. No acquired modified form of PBP has been shown to be significant in β-lactam resistance in *E. coli* [50, 51].

The aminoglycoside drugs inhibit protein synthesis by binding to the bacterial 30S ribosomal subunit at the A-site of the 16S rRNA. Bacteria are able to modify the ribosomal subunit via acquisition of plasmids carrying 16S rRNA methyltransferases. The methyltransferases are able to modify the structure of the 16S rRNA, which decreases the ability of the drug to bind to it. Several of these methyltransferases have been identified and characterized. The genes involved include *armA* (for aminoglycoside resistance methylase) and several *rmt* (for ribosomal methyltransferase) genes, with *rmtB* being the most common. The bacteria quite often possess several of these genes simultaneously. These genes most often confer clinically significant resistance to amikacin, gentamicin, and tobramycin, among other aminoglycosides [52–56].

The fluoroquinolone drugs interfere with nucleic acid synthesis during DNA replication by inhibiting either DNA gyrase or topoisomerase IV. Resistance to these drugs occurs commonly from mutations in either the chromosomally encoded GyrA subunit of gyrase (*gyrA* gene) or the ParC subunit of topoisomerase IV (*parC* gene). These mutations decrease the binding ability of the drugs, most commonly ciprofloxacin and norfloxacin. There is also some evidence that low-level resistance may be acquired via plasmids carrying quinolone resistance (*qnr*) genes [56–58].

The combination drug TMP/SXT is currently a common choice for treatment of UTIs. Both of these drugs target enzymes in the bacterial folate biosynthesis pathway via competitive inhibition. Trimethoprim is an analog of the natural substrate of the dihydrofolate reductase (DHFR) enzyme, and SXT is an analog of *p*-amino-benzoic acid, the natural substrate of the dihydropteroate synthase (DHPS) enzyme. This competitive binding blocks the binding of the natural substrate and stops the pathway at that point. Since TMP and SXT affect two different enzymes on the same pathway, the combination drug makes an effective treatment. Chromosomal mutations (often single point mutations) in the *dhfr* or *dhps* genes are commonly the cause of resistance to these drugs [59, 60].

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4.3. Inactivating the drug

Drug inactivation is accomplished in one of two ways: by actual degradation of the drug or by transfer of a chemical group to the drug. Gram-negative bacteria use drug inactivation against β-lactams and aminoglycosides. The β-lactam drugs are universally inactivated by β-lactamase enzymes, which degrade the drugs, and *E. coli* produces several of these. The aminoglycoside drugs are inactivated fairly universally by enzymes that transfer one of three small chemical groups to the drug. These enzymes include the acetyltransferases (AACs, *aac* genes), nucleotidyltransferases (ANTs, *ant* genes), and the phosphotransferases (APHs, *aph* genes) [43, 49, 61].

4.3.1. β-lactamases

The β-lactam drugs all share a specific core structure, which consists of a four-sided β-lactam ring. The β-lactamases (also originally called penicillinases and cephalosporinases) are capable of inactivating β-lactam drugs via hydrolyzation of a specific site in the β-lactam ring structure causing the ring to open. The drugs are then not able to bind to their target proteins, the PBPs. Within the large number of β-lactamases which have been identified, there are enzymes which can inactivate any of the current β-lactam drugs. The production of β-lactamases is the most common resistance mechanism used by gram-negative bacteria against β-lactam drugs [46, 62].

The β-lactamase enzymes can be classified based on their primary structure or functional characteristics. Structurally they are placed into four main categories (A, B, C, or D). There are three functional groupings: the cephalosporinases, the serine β-lactamases, and the metallo-β-lactamases. These enzymes are also commonly referred to by their enzyme family, for example, the TEM (named after the first patient) family, the sulphydryl variable (SHV) family, and the CTX (preferentially hydrolyze cefotaxime) family [56, 63].

The first β-lactamase to be characterized was from *E. coli* and is chromosomally encoded by the *ampC* gene (so named for ampicillin resistance). This gene is constitutively expressed at a low level, but mutations may result in overexpression of the gene. The AmpC β-lactamases are most effective against the penicillins and some first-generation cephalosporins. There are also many plasmid-borne β-lactamases, which carry a variety of *bla* genes (β-lactamase genes). Because these β-lactamases confer resistance to later generation cephalosporins, they were designated as ESBLs and include the TEM, SHV, and CTX-M enzyme families. The most commonly seen of these in *E. coli* are the CTX-Ms. The ESBLs may also be resistant to multiple drug classes but are generally sensitive to β-lactamase inhibitors. The β-lactamase inhibitors are structurally similar to β-lactamases and have weak antimicrobial ability alone but work synergistically in combination with a β-lactam drug [56, 64–67].

Recently, there has been emergence of β-lactamases that are active against the carbapenems (carbapenemases), found primarily in the *Enterobacteriaceae*. Bacterial strains that carry these are known as CRE strains. The carbapenemases are all metallo-β-lactamases (MBLs), and the most widely distributed are the IMP-1 (for imipenem resistance) and VIM-1 (Verona integron-encoded MBL) types. A new MBL has recently been identified, mainly in strains of *E. coli*. It has been designated as New Delhi MBL (NDM-1). The CRE strains are usually resistance to

all the β-lactam drugs and are not inactivated by the standard β-lactam/β-lactamase inhibitor combination drugs. There is a newer β-lactamase inhibitor, avibactam, which has been approved for use with ceftazidime against gram-negative bacteria. In addition, avibactam is being tested for use with aztreonam against CREs [62, 66–68].

4.4. Drug efflux

Bacteria possess methods for disposal of toxic substances to the outside of the cell. The most commonly used mechanism is the efflux pump. Most bacteria have chromosomally encoded efflux pump genes. Some of these pumps are expressed constitutively, and expression of others is induced by various environmental stimuli. Many of these pumps are capable of transporting a variety of substances and are also described as multidrug (MDR) efflux pumps. There are five efflux pump family groups: the ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, and the resistance-nodulation-cell division (RND) family. The RND pumps are generally found only in gram-negative bacteria as these pumps are multicomponent pumps that function in association with an OMP [69–72].

There is only one ABC efflux pump in *E. coli* that is known to contribute to antimicrobial resistance. That is the MacAB transporter that confers resistance to some macrolides [73]. There is also only one MATE efflux pump found in *E. coli*, the NorE pump which is able to transport fluoroquinolones. It is still in question if the NorE pump has a clinically significant impact on antimicrobial resistance [74, 75]. There are five known MFS efflux pumps found in *E. coli*. These are capable of transporting macrolides (MefB and MdfA pumps), fluoroquinolones (QepA2, EmrAB-TolC, and MdfA pumps), tetracycline (EmrAB-TolC and MdfA pumps), trimethoprim (Fsr pump), and chloramphenicol (MdfA pump). In addition, there are several MFS pumps that may be acquired by *E. coli* (e.g., via plasmids) that are specific for tetracyclines, with *tetA* and *tetB* being the most common [76, 77]. There are no clinically significant SMR efflux pumps found in *E. coli* [78].

The RND efflux pumps are the most clinically significant pumps found in gram-negative bacteria. These pumps consist of three components (tripartite): an inner membrane transporter, an outer membrane porin, and a periplasmic accessory protein that functions to connect the other two components. In *E. coli*, the OMP that is associated with all of the antimicrobial efflux pumps is TolC. There are five known RND pumps in *E. coli*: AcrAB-TolC, AcrAD-TolC, AcrEF-TolC, MdtABC-TolC, and MdtEF-TolC. AcrAD-TolC has been shown to efflux aminoglycosides and β-lactams. AcrEF-TolC has been shown to efflux quinolones and tigecycline. MdtABE-TolC has been shown to efflux quinolones. MdtEF-TolC has been shown to efflux erythromycin. The level of expression of these four pumps is relatively low, and if operating alone, the amount of antimicrobials effluxed would probably not be significant. Because *E. coli* has five efflux systems plus multiple other types of antimicrobial resistance mechanisms in play, these pumps undoubtedly help out. The other RND efflux pump in *E. coli*, AcrAB-TolC, is the most clinically significant and accounts for major antimicrobial efflux. This pump has been shown to efflux β-lactams, fluoroquinolones, tetracyclines, chloramphenicol, and lincosamides [72, 79, 80].

5. Conclusion

For many strains of pathogenic *E. coli*, the most common course of therapy is supportive and does not require the use of antimicrobial drugs, or in the case of EHEC, antimicrobial therapy is not recommended. For severe intestinal infections and UTIs, antimicrobial therapy may be necessary. Unfortunately with the issue of ever increasing antimicrobial resistance, the antimicrobial options are becoming fewer. With the emergence of ESBL and CRE *E. coli* strains, the options have gotten extremely limited, and antimicrobial development has not been able to keep up with the demand. Hopefully the newer carbapenem/β-lactamase inhibitor combination drugs and other drugs being developed under the tetracycline and aminoglycoside drug classes will prove to be equal to the task or at least keep the bacteria under control until better options become available.

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