ABSTRACT Antimicrobial agents of various types have important bearing on the outcomes of microbial infections. These agents may be bacteriostatic or –cidal, exert their impact via various means, originate from a living organism or a laboratory, and appropriately be used in or on living tissue or not. Though the primary focus of this chapter is on resistance to the antimicrobial agents used to treat uropathogenic Escherichia coli (UPEC)-caused urinary tract infections (UTIs), some attention will be given to UPEC’s resistance to silver-containing antiseptics, which may be incorporated into catheters to prevent foreign body-associated UTIs.

INTRODUCTION

Without doubt, antimicrobial agents, in particular antibiotics, have saved countless lives and revolutionized medicine in many respects, leaving few to question their importance to modern society. However, early optimism that antibiotic usage would conquer bacterial infections was soon eclipsed by reports of emerging resistance. Certainly, this has been the case with uropathogens, such as uropathogenic Escherichia coli (UPEC), where multidrug-resistant strains are emerging and causing outbreaks worldwide (1). Here, we will review some of the common mechanisms of antibiotic resistance and the prevalence and dissemination of antibiotic-resistance determinants among UPEC.

OVERVIEW TO THE ANTIMICROBIAL AGENTS USED TO CONTROL UPEC-CAUSED UTIS

Antimicrobial agents may originate from a living organism or a laboratory, exerting their ‘inhibitory’ or ‘killing’ effects via various mechanisms after appropriate delivery into or on living hosts or abiotic surfaces. Though antimicrobial agents encompass a wide range of chemical and physical agents, the primary foci of this chapter are antibiotics and resistance to the antibiotics used to treat UPEC-caused urinary tract infections (UTIs). In addition, some attention is given to UPEC’s resistance to silver-containing antiseptics, which may be incorporated into catheters to prevent foreign-body associated UTIs. Antibiotics, as defined by Waksman (2), are chemical substances produced by microorganisms that are antagonistic to the growth of other microorganisms. An example here would be aminoglycosides. By contrast, synthetic antibacterial agents, like sulfonamides and quinolones, do not have a biologic origin, and semisynthetic drugs are produced by modification of natural antibiotics. Examples of semisynthetic agents are carbapenems, which are produced by chemical modification of naturally produced β-lactam antibiotics. However, more recently, usage of these terms has blurred with some regarding natural,
synthetic, and semisynthetic antibacterial agents as antibiotics (3).

Antibiotics are designed to exhibit selective toxicity, meaning that their toxic activity is directed towards vulnerable targets in the pathogen that do not exist in the host. Fortunately, several differences in the cell machinery and functions of prokaryotes and mammalian cells exist that can be exploited to selectively inhibit the growth or reproduction of the pathogen without harming its host. Typical targets of antibiotics include bacterial cell wall synthesis, membrane structure, protein synthesis, DNA or RNA synthesis, and certain metabolic pathways (4–6).

Origins of Antibiotic Resistance

Though there is evidence that antibiotic-resistant bacteria existed prior to clinical use of antibiotics, clinical use of these agents has certainly been accompanied by an acceleration in the emergence of antibiotic-resistant pathogens, forcing changes in the ‘drugs of choice’ used to treat UTIs and other infections. Today, just a little less than three generations after the discovery of penicillin, a return to the ‘pre-antibiotic era’ due to the emergence of multidrug-resistant (MDR) pathogens is a disturbing possibility. For instance, some strains of Mycobacterium tuberculosis, Acinetobacter baumannii, Staphylococcus aureus, and Clostridium difficile have undergone multiple mutations, resulting in high levels of resistance to the antibiotic classes specifically recommended for their treatment, thus, leading to enhanced morbidity and mortality due to infections with these organisms.

The origins of antibiotic resistance are murky at best with the recent emergence of certain resistances being interpreted by some to mean that resistance is a modern phenomenon. This view is supported by the finding that bacteria collected between 1914 and 1950 (Murray collection) were completely sensitive to antibiotics (7), although they did contain a range of conjugative plasmids capable of carrying resistance genes. However, identification of a bacterial penicillinase before the clinical use of penicillin (3) raised an interesting question: did resistance elements originate before or as a result of our use of antibiotics? Recent metagenomic studies provide insight into this issue. Though the cold-seep sediments of the deep-sea Edison seamount are estimated to be 10,000 years old, metagenomic analysis revealed that sediment bacteria contained genes encoding antibiotic-resistance factors like TEM-type β-lactamases (8). Similarly, a study of a metagenomic library from 30,000-year-old Beringian permafrost sediments identified a highly diverse collection of genes encoding resistance to β-lactam, tetracycline, and glycopeptide antibiotics (9). In addition, resistant microorganisms have been identified from historic culture collections prepared before the advent of modern day antimicrobial usage. For example, E. coli that were resistant to sulfadiazone, spectinomycin, and tetracycline were identified in isolates collected prior to 1950, providing added evidence that resistance existed in nature prior to the clinical use of antibiotics and even into ancient times.

Further, antimicrobial resistance may be broadly distributed across environmental locales regardless of the existence of antimicrobial pressure. The soil microorganisms, actinomycetes, were found to be resistant to an average of seven or eight antibiotics with some strains resistant to as many as 20 antibiotics (10). Novel resistance mechanisms, including those not traditionally found in pathogens, were identified in a collection of these organisms. The identification of antibiotic-producing microorganisms in soil has led to the idea that a primary ecological role of antibiotics is to inhibit the growth of competitors. Following this line of reasoning, antibiotic-resistance determinants may have arisen to protect microorganisms from antibiotic activity. This ‘fight-for-survival’ view is supported by the discovery in aminoglycoside-producing organisms of aminoglycoside-modifying enzymes that display marked homology to the modifying enzymes found in aminoglycoside-resistant bacteria (11). Interestingly, recent work indicates that some bacteria may utilize low concentrations of antibiotics for intercellular signaling rather than warfare, with antibacterial resistance genes in turn providing a means to modulate, silence, or otherwise disrupt interbacterial communication (12). According to this view, resistance genes may serve purposes other than providing an edge in dealing with competing microbes.

Some antimicrobial resistance genes may have originated from bacterial “genetic juggling”. Resistance to fluoroquinolones (FQs) is a case in point. Mutations in gyrases, an efflux pump, and aminoglycoside N-acetyltransferases all resulted in emergence of FQ-resistant bacteria. A comprehensive survey of the Keio E. coli mutant library to determine the extent of the intrinsic resistome identified a total of 140 different mutants showing enhanced susceptibility to at least one of seven different antibiotics (13). Later work identified 283 mutants within the Keio library that harbored intrinsic resistance to at least one of 22 antibiotics (14). These observations demonstrated that in the E. coli K12 genome, and likely in UPEC, genes are present that confer a resistance phenotype. These ‘so-called’ proto- or quasi-resistances are referred to as intrinsic resistance. In
addition, susceptible UPEC become resistant through horizontal gene transfer (HGT) by acquisition of mobile genetic elements such as transposons, integrons, plasmids, and phages, all of which can harbor resistance genes.

In general, bacterial sampling from the mobilome (the pool of mobile genes) likely plays a crucial role in microbial evolution, providing bacteria a means to compensate for their lack of sexual reproduction, the major mechanism of genetic innovation in higher organisms. Acquisition of “ready-made” genes on plasmids and other mobile genetic elements enables bacteria to respond quickly to change, such as the introduction of disinfectants and antibiotics into their environment. This would not be the case if bacterial fitness were solely reliant on de novo evolution. For example, resistance (R) plasmids, occurring in some extraintestinal pathogenic E. coli (ExPEC), the E. coli pathotype that includes UPEC, have been shown to encode resistance to up to eight or more antimicrobial agents, including antibiotics, synthetic agents, disinfectants, and heavy-metal compounds. The acquisition of resistance transposons, which are mobile genetic elements consisting of insertion sequences (IS elements) flanking a DNA sequence that can include a resistance gene(s) that can move both intra- and inter-molecularly, is another means by which bacteria can quickly respond to environmental perturbations. IS elements are usually very similar in sequence and occur in inverted orientation on either end of the transposon. Transposons contribute to the dissemination of antibiotic-resistance elements by their insertion in conjugative plasmids or by acting as conjugative transposons. One well-known example of a transposon is Tn10, which encodes tetracycline resistance and is found among certain ExPEC strains of avian origin. However, despite their possible importance to UPEC resistance, transposons have been given only scant attention for their role in the dissemination of antibiotic resistance among uropathogens.

More attention has been given to the role of integrons in the rapid dissemination of antibiotic resistance in UPEC. Integrons, which were first identified and characterized by Stokes and Hall in 1989, are ‘assembly platforms’ into which exogenous genes are incorporated by site-specific recombination. All characterized integrons contain three key elements necessary for the capture of exogenous antimicrobial-resistance genes: a tyrosine-recombinase family integrase gene (intI); a primary recombinant site (attI); and an outward-oriented promoter. Integron-encoded integrases can recombine an antimicrobial resistance “gene cassette” in a RecA-independent manner. Gene cassettes are free circular-DNA structures that are not expressed on their own due to the lack of promoters. Integration occurs downstream of the resident promoter at the attI site, allowing the expression of the exogenously acquired resistance gene in the cassette. In general, a gene cassette contains only a single gene and an imperfect inverted repeat at the 3’ end of the gene called the attC site, which is recognized by the site-specific integrase. Gene cassettes can be arranged in tandem, and more than 60 distinct cassettes have been identified. In UPEC, cassette-associated genes have been shown to confer resistance to trimethoprim, sulfonamides, streptomycin/spectinomycin, beta-lactams, chloramphenicol, lincomycin, gentamicin, and aminoglycosides.

A strong association in UPEC has been established between the presence of integrons and resistance to both multiple and single antimicrobial agents. Based on sequence analysis of encoded integrases, at least five classes of integrons have been identified with class I and II found most frequently in UPEC strains. The reported prevalence of integrons in UPEC strains range from 16.6% to 64%. Class I integrons, whose prevalence range from 26.9% to 64% among UPEC isolates, have a functional integrase gene allowing integration of new cassette genes into the bacterial genome. Dihydrofolate reductase (dfr) genes that confer resistance to trimethoprim are commonly inserted into the UPEC genome in this manner. To date, about 20 variant sequences of the dfr gene have been described with dfrA1, dfrA5, dfrA7, dfrA12, and dfrA17 often associated with gene cassettes found in UPEC’s class I integrons. In Europe, dfrA1 is the most common variant found in UPEC that contain class I integrons, while in Korea and Australia, dfrA17 and dfrA12 are more common. These regional differences may be the result of clonal expansion of isolates containing different dfr alleles and limited contact between continents.

In UPEC, sulfamethoxazole-resistance genes are also associated with class I integrons but can also occur independent of the integron. Currently there are three types (sul1, sul2, and sul3) of sulfamethoxazole-resistance genes that have been characterized, with sul1 frequently observed as conserved or semi-conserved regions within class I integrons carried by UPEC isolates. Interestingly, combined use of trimethoprim and sulfamethoxazole has been the standard treatment for acute uncomplicated UTI. The fact that resistance to these drugs is encoded by class I integrons in UPEC underscores the importance of these mobile elements in the dissemination of antimicrobial resistance among UPEC isolates.
Class II integrons occur less often in UPEC than class I integrons, occurring in 4% to 20% of UPEC strains (28, 33). However, in UPEC from children in Iran, class II integrons (10.41%) were more prevalent than class I integrons (6.25%) (21). Interestingly, class II integrons cannot acquire new gene cassettes, because their integrase is inactive due to the presence of a premature in-frame stop codon (34). They do, however, contain a static array of resistance genes: dfrA1 (trimethoprim), sat (streptomycin), and aadA1 (spectinomycin/streptomycin) (30).

Exceptionally, a functional class II integrase was found in a UPEC strain (35) in which the in-frame stop codon of the integrase had been replaced by a glutamine codon. This finding suggests that such class II integrons could acquire new antibiotic-resistance genes through integration of gene cassettes. Subsequently, a polymerase chain reaction (PCR) protocol was developed whereby the functional class II integrase could be distinguished from the non-functional variant. However, use of PCR to distinguish the two has not proved reliable. To confirm these PCR results, it is now recommended that the amplicon be sequenced.

Bacterial plasmids are extra-chromosomal, double-stranded, circular DNA molecules that replicate independently of the bacterial chromosome (36). In general, plasmids do not encode genes essential for survival but rather carry accessory genes and genes enabling bacteria to adapt to particular situations, such as environments in which an antibiotic has been introduced. Some plasmids encode genes enabling bacteria to resist bactericidal or bacteriostatic antimicrobial agents, including antibiotics and heavy-metal compounds. These are called R (resistance) plasmids or R factors. R plasmids may harbor these resistance genes within transposons and integrons, and these elements plus other resistance genes may be clustered together in R plasmids forming multidrug-resistance (MDR)-encoding islands (16, 37, 38). R plasmids are widely distributed among UPEC (39–42), and UPEC’s acquisition of MDR-encoding R plasmids have been major factors in the emergence of resistant UPEC clones in recent years (1, 43–47).

In avian pathogenic E. coli (APEC), a subpathotype of ExPEC that bears certain similarities to UPEC in disease-causing abilities and other traits, R plasmids frequently co-transfer with so-called virulence plasmids (16, 37, 48–51). These virulence plasmids can harbor genes involved in host cell adherence, invasion, iron acquisition, and other pathogenic processes. Sequence analysis of these plasmids revealed that the virulence genes that they harbor are often clustered into pathogenicity islands (PAIs) (48, 49). For instance, pAPEC-O2-ColV (Fig. 1), a 180-kilo-base pair (kb) virulence plasmid isolated from APEC O2, harbors a 93-kb PAI encoding several virulence traits, including the serum-survival protein Iss, the autotransporter toxin Tsh, and several iron acquisition and transport systems. During conjugation, this virulence plasmid co-transfers with pAPEC-O2-R (Fig. 2), a 101-kb IncF R plasmid, which possesses an MDR-encoding island (37). This 33-kb pair island contains 15 genes responsible for resistance to at least eight antimicrobial agents, including silver and other heavy-metal compounds, quaternary ammonium disinfectants, tetracyclines, sulfonamides, trimethoprim, aminoglycosides, and beta-lactam antimicrobial agents. Also, pAPEC-O2-R harbors a Tn21-like region and a class I integron. In this case, the class I integron encodes resistance to chloramphenicol (catB3), aminoglycoside (aad5), and trimethoprim (folA).

Interestingly, acquisition of these co-transferring R and virulence plasmids by an avirulent recipient E. coli strain enhanced its abilities to grow in human urine and cause UTI in the murine model of human disease (52). Thus, the occurrence of similar plasmids among UPEC and related strains could have important implications for management of UTIs. These plasmids were also transferable to a human UPEC isolate (37), and E. coli contaminants of retail poultry meat appear to harbor similar plasmids (53). Still other APEC-associated plasmids are hybrids of resistance and virulence plasmids, harboring both an MDR-encoding island and a PAI (38). For instance, plasmid pAPEC-O103-ColBM (Fig. 3) harbors a 35-kb MDR-encoding island encoding ten resistance elements and a 55-kb conserved portion of the PAI found among APEC-virulence plasmids (38) (Fig. 3). Localized at the 5′ end of the MDR-encoding region is a portion of Tn1721, including tetracycline-resistance genes (tetAB) and transposase gene (tnpA) with a downstream region flanked by exact 25-base pair (bp) inverted repeats. This 22-kb region contains sul2, encoding sulfonamide resistance; strAB, encoding streptomycin resistance; and a class I integron. In the gene-cassette region of this class I integron is dfrA1, encoding trimethoprim resistance and the core class I integron genes intI1, aadA1, qacEAl, and sul1. Other antimicrobial resistance genes found in the MDR-encoding island were mphB, encoding macrolide resistance, and merEDACPTR genes, encoding mercury resistance. Among the antimicrobials to which pAPEC-O103-ColBM confers resistance were several agents that are frequently used for treatment of community-acquired UTI (32).
In addition to the potential impact of such a plasmid on successful treatment of UTIs, it also could impact uropathogenesis, as its PAI encodes three iron-uptake systems (aerobactin, SitABCD, and salmochelin), the putative hemolysin HlyF, and the serum resistance-determinant Iss. Not surprisingly, this plasmid contributes to the pathogenesis of ExPEC in several in vitro and multiple animal models of human and avian disease (38). Thus, plasmids harboring MDR-encoding islands and/or PAIs are common among APEC, found in the E. coli contaminating retail poultry, can be transferred to human ExPEC isolates, and can promote resistance to multiple antimicrobial agents as well as uropathogenesis in models of human UTI. Collectively, these results demonstrate the 1) potential of plasmids to enhance both the resistance and virulence of UPEC, 2) the presence of plasmid-containing E. coli strains in food destined for human consumption, and 3) the potential of contaminated food to be a reservoir of mobile resistance and virulence genes capable of contributing to the pathogenesis of human UTIs and the outcomes of these infections.
Manges and Johnson (54) summarized additional evidence of a possible link between human-associated ExPEC and APEC-contaminated poultry. This evidence includes overlapping virulence genes and antimicrobial-resistance profiles in APEC, UPEC, and other human ExPEC strains. If confirmed, these findings would lend further support to the hypothesis that contaminated poultry is a source of drug-resistant ExPEC and large R and virulence plasmids important in human disease. Collectively, such observations suggest that continued vigilance and a proactive approach to meat safety are warranted.

Most, if not all, classes of antibiotics used in the clinical treatment of UTIs can be countered by plasmid-borne antibiotic resistance harbored in one or more UPEC isolates. Notable among these are such commonly used agents as trimethoprim, sulfonamides, streptomycin, beta-lactams, chloramphenicol, lincomamide, gentamicin, and aminoglycosides. Most R plasmids described among UPEC are small, ranging in size from 2–10 kb (55), and often mobilizable, meaning that they are transferable only when using the transfer apparatus encoded by a co-resident conjugative plasmid. By contrast, conjugative plasmids are relatively large, ranging in size from 30 kb to more than 200 kb in size, and harboring a transfer region of about 20–30 kb in size that encodes the machinery for cell-to-cell transfer (56). Suhartono showed that 22.2% (8/36) of UPEC isolates harbored large plasmids (55), and Rijavec et al. demonstrated that at least 17% (19/110) of UPEC strains carried conjugative plasmids encoding antibiotic resistance (57). Plasmids with the same replication machinery cannot propagate in the same bacterial cell. Based on this
property, plasmids have been categorized into incompatibility (Inc) groups or replicon types (58). To date, 26 Inc groups have been identified among the Enterobacteriaceae. Although classification of plasmids into Inc groups is desirable, early methods of Inc testing were laborious and time-consuming. Recently, a polymerase chain reaction (PCR)-based typing method developed by Carattoli et al. (59) allowed for the rapid identification of 18 common types of plasmid replicons in the Enterobacteriaceae. Johnson et al. (60) streamlined this method and applied it to the replicon typing of commensal and pathogenic E. coli isolates. IncFIB and IncB/O plasmid replicons were most often identified among UPEC (60). However, IncFIB and IncP1-α replicons are associated with the greatest numbers of resistance genes. Specifically, IncFIB plasmids can provide resistance to amoxicillin, ceftriaxone, gentamicin, kanamycin, streptomycin, and ceftiofur, while IncP1-α replicons are associated with resistance to gentamicin, kanamycin, nalidixic acid, streptomycin, and tetracycline (61). Of note, many ExPEC-associated plasmid-replicon types, including IncA/C, IncP1-α, IncFIB, and IncI1, carry class I integrons (61).

Mechanisms of UPEC Resistance

Though bacterial antibiotic resistance existed prior to the clinical usage of antibiotics, the mechanisms of resistance have continued to evolve over time, at least in part due to selective pressure associated with antimicrobial use. Mechanisms of antimicrobial resistance can be categorized into five broad areas based on how a pathogen copes with the stress incurred by an antimicrobial agent (4) (Table 1). Here, some of the more common antimicrobial resistance mechanisms of UPEC will be examined. However, this list can be expected to expand as new drug-resistance mechanisms and mobility units evolve and disseminate.

Antimicrobial agents are designed to exhibit selective toxicity, meaning that their toxic activity is directed towards vulnerable targets in the pathogen that do not
exist in the host. Typical targets of antimicrobial agents include bacterial cell wall synthesis, protein synthesis, DNA or RNA synthesis, certain metabolic pathways, and bacterial membrane structures (4–6).

Bacteria use a range of resistance strategies in order to protect themselves from the host’s defenses as well as the activity of antimicrobial agents. Andersson and Hughes (5) suggest that most resistance mechanisms incur a fitness cost for the bacterium, which is manifested as a decreased bacterial growth rate. Logic would suggest that in the presence of a selective agent, such as an antimicrobial agent, only the fittest cells (i.e., resistant cells) are capable of survival but that they do so at a high fitness cost. This line of thought also leads to the expectation that removal of the selective pressure will result in the susceptible strains outcompeting the resistant ones. However, data to date suggest that this is not usually the case (5).

Van Hoek et al. (62) noted that some of the more common mechanisms of drug resistance in bacteria include changes in cell wall permeability that restrict access of the drug to its target site; active efflux of the drug out of the cell; enzymatic modification of the drug; acquisition of alternative metabolic pathways to those against which the drug is designed; modification of antimicrobial targets; and overproduction of a target enzyme. Resistances via these processes are acquired in a number of ways, including the mutation of antibiotic targets. For example, resistance to macrolide antibiotics, which bind the 23S ribosomal RNA (rRNA) within the 50S ribosomal subunit of the bacterial ribosome, can be achieved by nucleotide-base substitutions in the 23S rRNA. Single-nucleotide polymorphisms (SNPs) can also result in resistance to quinolone, sulfonamide, and trimethoprim drugs. Mutations in the rpsL gene (encoding a 30S ribosomal protein subunit) have been associated with streptomycin resistance, and frame-shift mutations in the chromosomal gene ddl (encoding a cytoplasm enzyme) resulted in glycopeptide resistance in Enterococcus.

Acquisition of mobile genetic elements that carry genes or cassettes encoding antimicrobial resistance is another common means by which bacteria become resistant to antimicrobial agents (5). In addition, in members of the Enterobacteriaceae, inherent resistance can be associated with efflux pumps, chromosomal mutations, and enzymatic inactivation of drugs (63).

Specific resistance mechanisms

In this section, we focus on the mechanisms of resistance to some of the more commonly used antimicrobial agents that are recommended for treatment of UTIs under the current guidelines from the Infectious Diseases Society of America. These guidelines were recently updated for the treatment of patients with catheter-associated infection (64) and for acute uncomplicated cystitis and pyelonephritis in women (65). Among the drugs considered below are the quinolones, which have superseded trimethoprim-sulfamethoxazole (TMP-SMX) as the first choice of therapy for UTIs in some regions where the prevalence of resistance to TMP-SMX is high (66). Mechanisms of resistance to other classes of drugs used to treat UTIs are also examined, including the β-lactams, fluoroquinolones, tetracyclines, aminoglycosides, nitrofurantoin, and fosfomycin (66, 67). Aside from these drugs, the resistance of UPEC to silver compounds is discussed, as these antimicrobial agents are sometimes used to coat urinary catheters to prevent nosocomial UTIs.

Trimethoprim-sulfamethoxazole (TMP-SMX)

Sulfonamides were first implemented as treatments for human infections in the mid-1930s (68). Currently,
long-acting sulfonamides, such as SMX and sulfadiazine, are the most commonly used members of this drug class (69). TMP was first used in treating humans in the 1960s (68).

**Mode of action of TMP-SMX.** TMP and sulfonamides are antimetabolite antimicrobial agents. Each drug blocks distinct steps in folic acid synthesis (70), and their combined use is synergistic (71) and effective against a wide spectrum of bacteria (68). TMP-SMX are selectively toxic for bacteria because, unlike mammalian cells, bacteria cannot use pre-formed folic acid and so must synthesize their own (69). Sulfonamide drugs competitively inhibit dihydropteroate synthase (DHPS), the enzyme that is involved in the catalysis of the condensation reaction of p-aminobenzoic acid (PABA) and dihydro-6-hydroxymethylpterin-pyrophosphate (DHIPP) to form dihydropteroic acid, an important intermediate in the formation of dihydrofoleric acid (69). Sulfonamides are PABA analogs and, as such, are competitive inhibitors of the synthase in this reaction.

TMP works by inhibiting the enzyme dihydrofolate reductase (DHFR), which catalyzes the formation of tetrahydrofolate from dihydrofolate (70). It is thought that TMP has fewer side effects than sulfonamides (70). Typically, SMX and TMP are used in combination as broad-spectrum antimicrobial agents, effective against E. coli and other members of the Enterobacteriaceae (68). It was initially thought that the use of these combination drugs would prevent emergence of drug-resistant strains; however, the worldwide prevalence of resistance to these drugs does not support this claim (70).

**Resistance to TMP-SMX.** Resistance to TMP-SMX or TMP alone is widespread and has been linked to UTI treatment failures (70). TMP and SMX resistances are often encoded by genes linked to mobile genetic units such as transposons, integrons, and plasmids (68). TMP resistance is mediated by several mechanisms including 1) changes in the bacterial permeability barrier or efflux of the drug and 2) altered regulation or sensitivity of the target enzyme (70).

Chromosomal encoded TMP resistance may be due to insertion of the transposon Tn7 in the bacterial chromosome. A second, low-level resistance may be linked to a mutation that results in the failure of bacteria to methylate deoxyuridylic acid, resulting in dependence on an exogenous source of thiamine. A third mechanism of TMP resistance may be related to a mutation requiring an increase in the binding affinity of the drug to exert its toxic effect. Most often, TMP resistance has been associated with overproduction of the enzyme DHFR to combat the effect of TMP, alteration of the enzyme, or a combination of these two mechanisms. The number and type of transferable dhfrI genes linked with TMP resistance continues to increase with at least 20 such genes currently recognized (72).

Chromosomal sulfonamide resistance is associated with the ability of the SMX to act as a competitive inhibitor of DHPS. Sulfonamides, which are analogs of the DHPS substrate PABA, competitively inhibit DHPS activity, thereby blocking the formation of folate in the bacterial cell. Chromosomal mutations in the dhps gene may alter the susceptibility of DHPS to inhibition by sulfonamides. However, resistance to sulfonamides may also be transferable and linked to mobile genetic units. Transferable resistance is mediated by two drug-resistant DHPS enzymes, encoded by sulI and sulII (68). These genes are largely plasmid-borne. sulI is usually linked with other resistance genes on Tn21 transposons, while sulII is usually linked to small IncQ and pBP1 plasmids. Indeed, almost all sulfonamide resistance appears to be due to sulI and sulII genes with integrons typically carrying sulI and small multi-copy plasmids carrying sulII (68).

**Prevalence of TMP-SMX resistance.** Although TMP-SMX is the primary choice of antimicrobial agent to treat uncomplicated UTI in adults and children (66), resistance is increasingly common resulting in substitution of FQs for TMP-SMX in certain countries, where the effectiveness of TMP-SMX is limited. Prevalence rates of resistance to TMP-SMX among UPEC range from 51% to 58% in pediatric and adult cases in Brazil (73, 74), 27.3% in Greece (75), 18% in uncomplicated cases of UTIs in Canada (76), 64% in Nicaragua (77), and 34% to 43% in Turkey (78, 79). Data for the U.S. shows a prevalence of 24.2% for 2010 with this number representing an overall 6.3% increase for the period of 2000–2010 (80).

**Fluoroquinolones**

Quinolones are a family of chemosynthetic broad-spectrum bactericidal agents, which include the FQs. Nalidixic acid is the predecessor of all the quinolones, including the FQs, which include ciprofloxacin, norfloxacin, levofloxacin, and many others (81). FQs are commonly used as a second drug choice for the treatment of UTIs and are recommended where the prevalence of resistance to TMP-SMX among uropathogens is high (66).
Mode of action of FQs. The FQs are the only antimicrobial agents that act by direct inhibition of DNA synthesis (6). They target type II topoisomerases: DNA gyrase (topoisomerase II) and DNA topoisomerase IV, both of which are essential in bacterial DNA replication (82). DNA gyrase is a tetramer composed of two types of subunits, GyrA and GyrB. Topoisomerase IV has a similar structure with two types of subunits, ParC and ParE. DNA gyrase introduces negative superhelical twists into DNA, which are important for initiation of DNA replication. Topoisomerase IV is involved in decatenation or separation of the daughter chromosomes in the latter part of DNA replication so that segregation of the daughter cells can occur (83). Both enzymes are critical to bacterial growth, as the topology of chromosomal DNA affects DNA replication, transcription, recombination, and repair (82, 83). FQs inhibit DNA gyrase or topoisomerase IV by stabilizing the drug-DNA-enzyme complexes. As a result, replication-fork complexes accumulate leading to inhibition of DNA synthesis, strand breaks, and ultimately, cell death (83).

Antimicrobial resistance associated with FQs. FQ resistance in the Enterobacteriaceae is most often the result of an accumulation of mutations in DNA gyrase (GyrA) primarily and topoisomerase IV (ParC) secondarily (83). Mutations in GyrA that result in FQ resistance mainly occur in the quinolone resistance-determining region (QRDR) (83). Mutations that alter these two enzymes or that limit FQ’s access to them are responsible for classically recognized chromosomally encoded FQ resistance (84). Restriction of FQ’s access to these enzymes can result from decreased bacterial permeability or enhanced expression of energy-dependent efflux pumps (62).

More recently identified mechanisms of FQ resistance are plasmid-encoded and involve FQ-resistant proteins (Qnr proteins) such as the efflux pumps QepA and OqxAB (83) and the aminoglycoside acetyltransferase AAC(6’)-Ib-cr (where ‘cr’ stands for ciprofloxacin resistance) (85, 86), an enzyme able to inactivate both aminoglycosides and FQs (84).

Qnr was the first plasmid-linked FQ determinant identified. More recently, it has been referred to as QnrA1 (87). All known qnr genes reside on plasmids ranging in size from 7–320 kb, in association with sul1-containing class 1 integrons (83, 85, 88). These R plasmids often encode resistance to multiple drugs including β-lactams, aminoglycosides, chloramphenicol, tetracycline, sulfonamides, trimethoprim, and rifampin. Qnr proteins contribute to low-level FQ resistance via alterations in target enzymes, enhanced expression of drug efflux pumps, or changes in outer membrane porins (83). Qnr-containing isolates appear to ‘supplement’ FQ resistance and facilitate selection of strains bearing chromosomal mutations conferring FQ resistance when under FQ-selective pressure (83).

Another plasmid-encoded FQ-resistance mechanism is the aminoglycoside acetyltransferase AAC(6’)-Ib-cr. This variant of the AAC(6’)-Ib aminoglycoside acetyltransferase acetylates kanamycin, tobramycin, and amikacin, but also confers a low-level resistance to FQs (83). This enzyme is encoded by a widely distributed cassette localized within sul1-type class I integrons on plasmids (86, 88). Still other plasmid-encoded FQ resistance mechanisms involve efflux pumps associated with some human E. coli isolates. The first, known as the quinolone efflux pump (Qep), is encoded on large mobilizable plasmids (83). Another known as OqxAB, which confers resistance to multiple agents including FQs (83), was found in one study to be associated with IS26 on a 43- to 115-kb IncF transferable plasmid (89).

Though most frequently associated with bacterial isolates from animals, farm environments, and farm workers, OqxAB was also encoded, though infrequently, among a group of 261 E. coli isolates recovered from human blood between 1998 and 2006 (90).

Prevalence of FQ resistance. The overall prevalence of FQ resistance among E. coli implicated in UTIs has increased significantly, with FQ-resistance rates varying worldwide. In Brazil, FQ-resistance rates of 17.1% to 21.6% have been reported (91), while Karaca et al. (92) noted the emergence of resistance to the FQs ofloxacin and ciprofloxacin had increased from 4.1% and 5.2% in 1995 and 1996 to 25.3% and 27.6% in 2002, respectively. A similar study in Italy reported resistance rates of approximately 17% (93). Arslan et al. (94) noted ciprofloxacin resistance varying from 17% to 38% in uncomplicated versus complicated UTIs in Turkey, while resistance to nalidixic acid, norfloxacin, and ciprofloxacin in Senegal were 23.9%, 16.4%, and 15.5%, respectively (95). In Norway, a FQ-resistance rate of 1.2% was observed among 7,302 UPEC cases (96), and a 10-year study in the U.S. reported an overall increase (14.1%) in ciprofloxacin resistance from 3% in 2000 to 17.1% in 2010 (80). Recently, Khawcharoenporn and colleagues (97) examined levofloxacin resistance among E. coli from catheter-associated UTIs and found that resistance was significantly higher in UPEC from nosocomial UTIs, compared to community-associated UTIs.
The names of these ESBLs have varied origins. The term SHV is derived from ‘sulfhydryl variable’ as it relates, or was thought to relate, to inhibition of SHV activity by p-chloromercuribenzoate (105). The name of the TEM-type ESBLs was derived from the name of the patient from which the first known TEM-1-containing E. coli was isolated (103). CTX reflects the strong hydrolytic activity of CTX ESBLs against cefotaxime (103), and OXA-type ESBLs are named for their oxacillin-hydrolyzing abilities (103). Many of these ESBLs have broad activity and are able to hydrolyze many of the new extended-spectrum third-generation cephalosporins. Genes encoding the ESBLs are typically plasmid-linked within integrons (98). Most ESBL-producing organisms are resistant to penicillins, first- and second-generation cephalosporins, third-generation oximino-cephalosporins, and monobactams. However, most ESBL-producing bacteria are still susceptible to fourth-generation cephalosporins (98).

There are currently more than 200 TEM- and 160 SHV-derived ESBLs recognized. An accurate list of the TEM-, SHV- and OXA-derived β-lactamases is housed at http://www.lahey.org/Studies/ and is updated as new β-lactamases are identified. These enzymes have been found in a range of Enterobacteriaceae including E. coli.

Excellent reviews by Poole (98) and Paterson and Bonomo (103) provide detailed overviews of β-lactam resistance and ESBLs.

β-lactam resistance associated with efflux pumps is also recognized. There are five main families of efflux mechanisms for bacterial strains; these include the major facilitator superfamily (MFS), ATP-binding cassette (ABC), resistance-nodulation division (RND), multidrug and toxic compound extrusion (MATE), and small multidrug resistance (SMR) families. The RND family has been reported to accommodate β-lactams including the third- and fourth-generation cephalosporins and carbapenems (98).

Prevalence of β-lactam resistance. Resistance to β-lactams among UPEC is prevalent worldwide. A study in Switzerland by Meier et al. (106) reported a prevalence rate of 69.6% to amoxicillin/clavulanic acid. In Japan, Shigemura et al. (107) reported decreasing susceptibility to a range of β-lactam drugs in complicated versus uncomplicated UTIs ranging from 0% to as high as 21.5% and 47.2%, respectively. In India, Taneja et al. (108) reported resistance rates in UPEC against β-lactams ranging from 70% to 93.9% with the lowest resistance observed to imipenem. In the U.S., Sanchez et al. (80) reported resistance prevalence increasing from 38.2% in 2000 to 43.4% in 2010.
Of special concern with regards to the β-lactams is the emergence of the new metallo-β-lactamase 1 (denoted New Deli NDM-1). This metallo-β-lactamase was first identified in Klebsiella pneumoniae and E. coli from an Indian patient in Sweden in 2008 (109). Strains possessing NDM-1 can hydrolyze all penicillins, cephalosporins, and carbapenems and are typically broadly resistant to other drug classes in addition to the β-lactams (110). Data suggest that these NDM-type E. coli strains may also be linked with UTIs and have been recovered from UTI patients in Thailand (111) as well as from the urine, blood, and feces of patients in New Zealand (112), Lebanon (113), and Denmark (114). The emergence of such a strain type in human illness including UTIs could have devastating consequences. Active monitoring for the emergence of NDM-1 strain types in UPEC warrants careful attention and proactive design of methods for its control should be undertaken.

Alternative antimicrobials for the control of β-lactam-resistant strains include fosfomycin (115) and tigecycline (116).

Nitrofurantoin
Nitrofurantoin has been used as an option for treating UTIs for more than 50 years. Its use has declined in favor of other antibiotics such as TMP-SMX and the FQs (76). However, the emergence of uropathogens that are resistant to many front-line drugs, has led some researchers to re-examine the empirical therapeutic value of nitrofurantoin (117). Upon administration, the drug becomes concentrated in urine, where it can be effective against bladder infections (cystitis) (76). However, nitrofurantoin has a lower cure rate than other drugs, but is nonetheless considered appropriate for use during pregnancy, in pediatric medicine, and as prophylaxis against recurrent UTI.

Mode of action of nitrofurantoin. Nitrofurantoin is a nitroheterocyclic compound. The active site of the drug is where the nitro group is coupled to the heterocyclic furan. The drug is activated within bacterial cells by nitrofuran reductases that rapidly reduce nitrofurantoin to various active intermediates that interfere with protein and DNA synthesis, energy metabolism, and cell wall and carbohydrate synthesis (118). Intracellularly produced intermediate products attack the chromosomal DNA of the cell causing a range of mutations, and it is believed that hydroxylamine (one of the intermediate products produced during the reduction of the nitrofurantoin) activity results in bacterial DNA and protein damage (118, 119).

Antimicrobial resistance associated with nitrofurantoin. Antimicrobial resistance to nitrofurantoin can be chromosomal or plasmid-mediated and is associated with inhibition of nitrofuran reductase (120, 121). McCalla et al. (122) showed that E. coli resistance to nitrofurantoin compounds is associated with loss of enzyme activity. However, the full mechanism of nitrofurantoin resistance is still not fully understood. McOsker and Fitzpatrick (123) showed that nitrofurantoin itself inhibits the synthesis of bacterial nitrofuran reductase by interacting with bacterial ribosomes. Sandegren et al. (119) also noted that resistance may be linked to the nitroreductase activity, which is of two types – type I that is insensitive to oxygen and type II that is inhibited by oxygen.

Prevalence of resistance to nitrofuran compounds. A study from Crete found that the overall prevalence of nitrofuran resistance over a five-year period ranged from a low of 3.7% to a high of 13% (124). In Brazil, the prevalence of nitrofuran resistance among UPEC was also low (2.9%) (125), while the rates of resistance in Portugal, Spain, Finland, and Sweden were 5.8%, 4.2%, 0.5%, and 0%, respectively (126). In the U.S., Sanchez et al. (80) reported that the prevalence of nitrofurantoin resistance in UPEC has ranged from 0.8% in 2000 to 1.6% in 2010. These data contrast significantly with Sire et al. (95) who reported a resistance rate of 10.1% among UPEC isolates in Senegal. Given the low resistance rates observed, recommendations from the Infectious Diseases Society of America list nitrofurantoin as an option for treating acute uncomplicated cystitis (65).

Fosfomycin
Fosfomycin is a phosphoric acid antibacterial agent, which is indicated in the treatment of uncomplicated cystitis (76). Fosfomycin is a broad-spectrum drug used in the treatment of uncomplicated UTIs, bacteriuria during pregnancy, and pediatric UTIs, and as a prophylactic agent before urological procedures (127).

Mode of action of fosfomycin. Fosfomycin was first described in 1969 (128). It has activity against both Gram-positive and -negative organisms, and most organisms seem to be sensitive to the agent or show low resistance rates. Activity of fosfomycin is related to its ability to inhibit cell wall biosynthesis by inactivating the enzyme UDP-N-acetylmuramyl-L-alanine-N-acetylmuramyl-L-alanine amidophosphoribosyltransferase (MurA), which is involved in peptidoglycan biosynthesis (129).

Mechanisms of fosfomycin resistance. Bacterial resistance to fosfomycin is primarily chromosomal, however,
plasmid-mediated resistance to fosfomycin has also been observed in rare instances (127). Chromosomally mediated fosfomycin resistance is associated with mutations that interfere with the L-α-glycophosphate and hexose phosphate uptake transport systems (127, 129). Plasmid-mediated resistance has been linked to carriage of fosA gene, which encodes a protein (FoS) involved in the conjugation of glutathione to fosfomycin, thus rendering it useless (130).

Prevalence of fosfomycin resistance. The occurrence of fosfomycin resistance among UPEC is low, if present at all. Liu et al. (131) reported a rate of 4.5% resistance among E. coli in Taiwan, while earlier work by Schito (132) reported that the worldwide prevalence was 1% to 3%. Sire et al. (95) reported a prevalence of 0.7% resistance in Senegal. An extensive countrywide study in Europe reported an overall prevalence of <2% (126), and a rate of 6% was noted in Pakistan by Noor et al. (133). These findings suggest that this ‘old world’ drug may find ‘new world’ use, as resistance to the more commonly used antimicrobial agents for UTI treatment continues to emerge.

Tetracyclines

The tetracyclines were first discovered in the early 1940s. They are broad-spectrum antibiotics produced by members of the Streptomyces (134). Tetracyclines in general have been used to treat UTIs and other infections caused by organisms such as Mycoplasma and Chlamydia.

Mode of action of tetracyclines. The results of several studies suggest that tetracyclines function by binding the 30S subunit of microbial ribosomes. In doing so, they inhibit protein synthesis by blocking attachment of charged aminoacyl-transfer RNA (tRNA) to the A site of the ribosome, resulting in inhibition of bacterial growth (135, 136). However, there is evidence that their mode of action may be more complex than once appreciated (137).

Tetracycline has a broad spectrum of activity against a range of Gram-positive and -negative organisms, mycoplasmas, rickettsias, and protozoan parasites; however, Pseudomonas aeruginosa and Proteus spp. appear to have intrinsic resistance to tetracycline (134). Alternative tetracyclines, such as tigecycline, a derivative of minocycline, have good activity against bacteria that are resistant to tetracyclines and ESBL-producing E. coli. Tigecycline is, however, considered to be an alternative when aminoglycosides and carbapenems are unavailable (138).

Resistance associated with tetracyclines. Antimicrobial resistance to tetracycline occurs by ribosomal protection, energy-dependent efflux of the drug, or enzymatic drug inactivation (62). Ribosomal protection relies on alteration of drug target sites. Here, bacterial cells produce ribosomal protection proteins that bind to the ribosome and alter its conformation, thus altering the active site where tetracycline would normally act (134). Though resistance to tetracyclines is primarily due to active efflux or ribosomal protection, oxygen-dependent, TetX-catalyzed destruction of tetracycline has been described in certain organisms (139). To our knowledge, enzymatic inactivation of tetracycline is not a mechanism of tetracycline resistance that has been found among E. coli. There are at least 46 different tetracycline-resistance determinants recognized (134) with novel determinants identified as unique if they share less than 79% amino acid-sequence identity with all of the known genes (62).

As the list of tetracycline-resistance genes has grown over the years, the nomenclature describing these determinants has had to evolve. Where once the letters of the English alphabet provided enough coverage, modifications of the naming schemes had to be designed to accommodate new and hybrid tetracycline-resistance genes (140–143). Determinants include several tet (tetracycline resistance), a few otr (oxytetracycline resistance), and a few others (62). The designations tet and otr do not reflect inherent differences in these determinants, but instead refer to the source organism from which these determinants were originally identified (136). Typically, resistance to tetracyclines is due to acquisition of new genes on plasmids or transposons although, in a few cases, resistance may be the result of mutations (136).

Prevalence of resistance to tetracyclines. Antimicrobial resistance to tetracyclines appears to be widespread among UPEC. Thus, tetracyclines may not be the best first choice for treatment of a UTI. For instance, the prevalence of tetracycline resistance in Nigerian UPEC ranges from 88% to 100% (144, 145). Although the prevalence is less in Brazil, it is still high, ranging from 30% to 73% (73, 125), and while it is still lower in the U.S., the rate of tetracycline resistance has increased from 22.6% in 2000 to 24.9% in 2010 (a 2.3% increase over a 10-year period) (80). The high and increasing prevalence of tetracycline resistance likely reflects the multiple mechanisms by which a strain can become resistant and the linkage of these mechanisms to mobile genetic elements.
Aminoglycosides
The aminoglycosides are broad-spectrum antibiotics with concentration-dependent killing action that have been in clinical use for over 60 years (62, 146). Aminoglycosides include several natural and semisynthetic antimicrobial compounds, which contain one of several aminated sugars joined to a dibasic cyclitol via glycosidic linkages (147). The first aminoglycoside discovered was streptomycin, which was produced by Streptomyces griseus. Not long after, additional aminoglycosides from Streptomyces spp. were described including neomycin and kanamycin (62). Gentamicin was recovered from the actinomycete, Micromonospora purpurea (62) in the 1960s, and in the 1970s the first semisynthetic aminoglycosides were developed. These included netilmicin, dibekacin, and amikacin (62, 147). Aminoglycosides derived from Streptomyces spp. can be identified by their ‘-mycin’ suffix, while Micromonospora-derived aminoglycosides have the ‘-micin’ suffix (62).

These drugs are indicated in the case of serious bacterial infection or a complicated UTI (148). The drug is usually given by injection and is excreted in the urine unchanged. Often, aminoglycosides are given in combination with other drugs such as β-lactams and are valued for their ability to act in synergy with other agents (62). However, their use is complicated by their toxicity to the host and the emergence of aminoglycoside resistance among certain bacterial pathogens (62, 147).

Mechanism of action of aminoglycosides. Aminoglycosides work by inhibiting prokaryotic protein synthesis by binding to 16S rRNA and also by disrupting the integrity of the bacterial cell membrane (149). At high concentrations, they may also affect protein synthesis in mammalian cells, resulting in their toxic effects (147).

Resistance associated with aminoglycosides. Resistance to most aminoglycosides, other than streptomycin, which is greatly impacted by alteration of ribosomal binding sites, is owed to one of two overall strategies: 1) decreased accumulation of the drug in the bacterium or 2) bacterial production of drug-modifying enzymes, (147). The specific mechanisms by which aminoglycoside resistance occurs include, 1) deactivation of the drug by N-acetylation, adenylation, or O-phosphorylation; 2) decreased outer membrane permeability to the drug; 3) decreased inner-membrane transport, active efflux, and drug trapping; 4) alteration of the 30S ribosomal subunit target by mutation; and 5) methylation of the aminoglycoside-binding site (149).

Active efflux, membrane impermeabilization, and ribosomal alteration may be intrinsic properties of the resistant bacterium or the result of chromosomal mutations (62). However, the most common aminoglycoside-resistance mechanism is enzymatic inactivation of the drug. Over 50 such modifying enzymes have been identified (150) and include acetyltransferases (AAC), adenylation transferases (ANT), phosphotransferases (APH), and bifunctional enzymes (62). The genes encoding these modifying enzymes are generally located on mobile genetic elements, including plasmids, transposons, and integrons (149). This location has no doubt facilitated the emergence of aminoglycoside resistance. Indeed, the first plasmid-linked aminoglycoside-resistance gene, armA (the aminoglycoside resistance methyltransferase) was found in Klebsiella in 2003 (151), but by 2005, Galimand et al. (152) had found it in clinical isolates of Citrobacter freundii, Enterobacter cloacae, E. coli, Klebsiella pneumoniae, Salmonella enterica serotype Enteritidis, and Shigella flexneri from across Europe. Several other such genes have since been described (153, 154).

Efflux pump-associated resistance. Aminoglycosides are substrates for a number of efflux pumps, including chromosomally encoded members of the RND family of efflux pumps (155).

16S rRNA methylation. Another mechanism of resistance to aminoglycosides is 16S rRNA methylation. Bacteria producing rRNA methylases can alter the nucleotides normally bound aminoglycosides, preventing the drugs from disrupting ribosomal function.

Prevalence of aminoglycoside resistance. Because of the relative toxicity of these drugs, their use is often limited to the treatment of complicated infections. In a study in Iran, Mohammad-Jafari et al. (156) reported the prevalence of gentamicin resistance among E. coli recovered from UTIs to be 23%, while a study in Tunisia reported <14% of isolates were resistant to amikacin and <5% resistant to gentamicin (157). In contrast, Gad et al. (158) examined the aminoglycoside resistance of Gram-negative bacteria from patients with UTIs, ear, skin, and GI infections in Egypt. Overall, over 80% of these isolates were resistant to streptomycin, but only ~18% were resistant to amikacin. Intermediate degrees of resistance were found to neomycin, kanamycin, gentamicin, and tobramycin. Of the 50 E. coli isolates they examined, almost 40% were from UTIs. Among these UTI-associated strains, 78% to 82% were resistant to
streptomycin, 48% to 54% to neomycin, 44% to 46% to kanamycin, 36% to 40% to gentamicin, 30% to tobramycin, and 4% to 16% to amikacin. Such widely varying results demonstrate that resistance rates can differ greatly depending on regional use.

**Silver-containing antimicrobial agents**

Heavy metals such as silver, copper, cadmium, lead, and mercury are considered toxic to bacteria and, as such, have often been used in their salt forms as antimicrobial agents in hospital settings and other environments (159). Of particular interest here is the use of silver compounds since they are used to coat urinary catheters in order to prevent nosocomial UTIs. These agents also find frequent use in burn wards, where they may be applied to wounds in creams and dressings. Silver salts can also be applied to the eyes of newborns to prevent neonatal eye infection and are commonly used in dental amalgams, water-filtration systems, impregnated plastics (cutting boards), and fabrics (160). The application of silver as a coating agent on urinary-tract catheters and heart valves has become a common practice in an effort to prevent the growth of bacterial biofilms (159, 160). The introduction of silver-coated catheters in the U.S. occurred more than a decade ago (161).

Johnson et al. (162) reported that catheter-associated UTIs account for 40% of hospital-acquired infections. A number of systematic reviews suggest that use of silver-coated catheters results in fewer UTIs (161, 163, 164). Indeed, Rupp and colleagues (164) reported a 57% decrease in the prevalence of catheter-associated UTIs when silver-coated catheters were used. However, Hooton et al. (64) concluded that there are insufficient data on which to make a recommendation for use of coated catheters for short- or long-term indwelling use.

Considering the potential of silver coatings to prevent catheter-associated UTIs, the prevalence of silver resistance among UPEC and other uropathogens warrants monitoring. Silver (160) reported on the presence of silver-resistant strains in environments where silver use as an antiseptic was high (e.g., burn wards). A silver-resistance cluster consisting of nine genes has been identified that encodes a periplasmic protein, two efflux pumps, and a chemiosmotic RND-exchange system (160). Evidence for the presence of the silver-resistance genes in IncH plasmids has been described by Gupta and colleagues (159). Plasmids of this replicon type were found among UPEC isolates in Denmark (165) and elsewhere, but in UPEC and human fecal commensal *E. coli* they are observed infrequently (60).

A recent report that may have negative implications for the future use of silver compounds in the prevention of UTIs is found in the description of the sequence of plasmid pAPEC-O2-R (37). This IncF plasmid was recovered from an APEC strain isolated from a bird with colibacillosis. pAPEC-O2-R, which contains a functional silver-resistance gene cluster, was easily transferred by conjugation into other *E. coli* strains, including a human UPEC isolate. In addition to silver resistance, this plasmid encodes resistance to quaternary ammonium compounds, tetracycline, sulfonamides, aminoglycosides, trimethoprim, and beta-lactam antimicrobial agents. IncF plasmids occur at greater prevalence than IncH plasmids in a variety of *E. coli*, including in human UPEC and fecal strains (60). Results like these suggest that the promiscuous nature of ExPEC R plasmids may have a significant bearing on the future of UTI control. Another R plasmid (the IncHI2 type plasmid pAPEC-O1-R (16)), containing a functional silver resistance operon, was found in APEC O1, an APEC strain with many similarities to human UPEC isolates (166).

Such findings, coupled with recent reports linking plasmid-containing, APEC-contaminated retail poultry to the occurrence of human disease (53), serve as important reminders that many microbes containing a variety of resistance determinants are circulating in the environment. Many times, all that may be needed for these strains to emerge is the use of some key selective agent. Thus, silver compounds to prevent UTIs must always be used thoughtfully, recognizing that their use may select for bacteria containing plasmids encoding resistance to silver and many other drugs, and perhaps even virulence genes. For this reason, continued and close monitoring for the development of silver resistance among UPEC is justifiably warranted.

**CONCLUSIONS**

Though the authors would be remiss if we failed to encourage prudent use of antimicrobial agents when treating UTIs and other infections, we must recognize that any use, including prudent use, may select for the emergence of resistant strains of bacteria. This line of reasoning is especially sobering in terms of organisms containing R plasmids like pAPEC-O1-R, pAPEC-O2-R, or pAPEC-O103-ColBM, as use of even a single antimicrobial agent (including antibiotics, silver, mercury, or copper-containing antiseptics and disinfectants, or even benzalkonium chloride antiseptics and disinfectants) can select for MDR pathogens with enhanced abilities to resist therapy and disinfection. This scenario is even
more sobering when considering pathogenic strains harboring hybrid R-virulence plasmids - in which case use of even a single antimicrobial agent could conceivably select for pathogens with both enhanced drug resistance and increased virulence potential.

As noted by many researchers and experienced first-hand all too often by physicians, veterinarians, and their patients, the emergence of antimicrobial resistance among uropathogens and other pathogens is a growing problem, one that has surpassed the pace of drug discovery and development \(^{(105, 167)}\). Continuing the successful fight against bacterial pathogens will likely require a multipronged approach involving enhanced awareness, increased vigilance, intensified control measures to limit the dissemination of resistance, strict implementation of infection control procedures, and investment in drug discovery \(^{(105)}\). For the latter to be successful, new drugs or drug combinations might incorporate an anti-plasmid approach, where small molecules target plasmid-replication control as a means to eliminate MDR-encoding plasmids and the transmission of drug-resistance genes. Such adjuncts to routine therapies could ‘rejuvenate’ many antimicrobial agents whose effectiveness has waned due to plasmid-mediated multidrug resistance \(^{(36)}\). Kunin \(^{(168)}\) also reminds us that it is better to avoid a UTI than to treat one and finds that there is no substitute for thoughtful care. Avoiding unnecessary or prolonged use of urinary catheters, avoidance of invasive procedures that put patients at greater risk of infection, and the development of improved catheter and drainage bags are all commonsense approaches that can decrease the occurrence of UTIs in hospitals. Maki and Tambyah \(^{(169)}\) also advocate for ‘engineering out’ the risk of infection with improved urinary catheters and suggest that our greatest hope for reducing catheter-associated UTIs is vaccine development. Gilbert and Mc Bain \(^{(170)}\) find hope in good hygienic practice in appropriate settings and the wise application of biocides. Thus, there are multiple approaches that deserve study with preventive strategies and judicious application of antimicrobial agents, all playing important roles in the future control of UPEC-caused UTIs.

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Antimicrobial Resistance in Uropathogenic Escherichia coli


