Quinolone resistance in *Escherichia coli*

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Abstract – *Escherichia coli* is an important pathogen of animals and humans that causes great financial cost in food production by causing disease in food animals. The quinolones are a class of synthetic antimicrobial agents with excellent activity against *Escherichia coli* and other Gram-negative bacteria used in human and veterinary medicine. Different quinolones are used to treat various conditions in animals in different parts of the world. All members of this class of drug have the same mode of action: inhibition of topoisomerase enzymes, DNA Gyrase and Topoisomerase IV. *Escherichia coli* can become resistant to quinolones by altering the target enzymes, reducing permeability of the cell to inhibit their entry, or by actively pumping the drug out of the cell. All these resistance mechanisms can play a role in high-level fluoroquinolone resistance, however target site mutations appear to be most important. As all quinolones act in the same way resistance to one member of the class will also confer decreased susceptibility to all members of the family. Quinolone resistant *Escherichia coli* in animals have increased in numbers after quinolone introduction in a number of different case studies. The resistance mechanisms in these isolates are the same as those in resistant strains found in humans. Care needs to be taken to ensure that quinolones are used sparingly and appropriately as highly resistant strains of *Escherichia coli* can be selected and may pass into the food chain. As these drugs are of major therapeutic importance in human medicine, this is a public health concern. More information as to the numbers of quinolone resistant *Escherichia coli* and the relationship between resistance and quinolone use is needed to allow us to make better informed decisions about when and when not to use quinolones in the treatment of animals.

fluoroquinolone / *E. coli* / ciprofloxacin / poultry / resistance

Résumé – Résistance aux quinolones chez *Escherichia coli*. *Escherichia coli*, agent pathogène important chez les animaux et les humains, est responsable d’un coût financier très important dans la production alimentaire dû aux maladies qu’il provoque chez les animaux d’élevage entrant dans la chaîne alimentaire. Les quinolones sont une classe d’agents antimicrobiens synthétiques, utilisés en médecine humaine et vétérinaire, ayant une activité excellente contre *E. coli* et d’autres bactéries à Gram-négatif. Différentes quinolones sont utilisées dans le traitement de diverses affections chez les animaux dans différentes parties du monde. Tous les membres de cette classe d’antibiotiques

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1. INTRODUCTION

*Escherichia coli* are a common cause of infection in humans and animals responsible for diseases including infections of the intestine and septicemia. The quinolones are a synthetic class of antimicrobial agents which have been used widely in human and veterinary medicine since their introduction in the late 1980s and early 1990s [19]. Quinolones have very potent activity against Gram-negative bacteria including *E. coli* and as a result have become common in human and veterinary medicine [3, 36]. *E. coli* cause several infections in animals, notably those of the respiratory and intestinal tracts including colibacillosis and septicemia in poultry [15] and diarrheal diseases such as scours, septicemia and pneumonia in cattle and pigs [21]. All these diseases contribute significantly to mortality and morbidity in animals and are extremely costly to food production.

Due to the broad spectrum of activity for various animal infections, different quinolones and fluoroquinolones have been approved for use in the treatment of animal disease around the world (Tabs. I and II). The compounds in use vary between different countries as do the regulations for their use. Exact figures as to the amount of quinolones currently used in veterinary medicine were difficult to obtain.

Due to their excellent in-vitro activity it was initially hoped that resistance to the quinolones would be rare, however since their introduction into human and veterinary medicine there has been a rise in the numbers of resistant strains of Gram-negative bacteria, including *E. coli* [12]. Usually, resistant bacteria arise spontaneously and resistance is not due to transfer of genes encoding resistance or the selection of a few clones of fluoroquinolone resistant *E. coli* which are disseminated within an
Table I. Quinolones currently licensed for use in animals worldwide (adapted from [3]).

<table>
<thead>
<tr>
<th>Name</th>
<th>Cattle</th>
<th>Swine</th>
<th>Chickens</th>
<th>Turkeys</th>
<th>Fish</th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarafloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orbifloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flumequine</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxolinic acid</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difloxacin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II. Quinolones licensed for use in different regions of the world (adapted from [3]).

<table>
<thead>
<tr>
<th>Region</th>
<th>Livestock</th>
<th>Poultry</th>
<th>Pet animals</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>enrofloxacin, flumequine, marbofloxacin, danofloxacin</td>
<td>enrofloxacin, difloxacin, flumequine, oxolinic acid</td>
<td>enrofloxacin, difloxacin, sarafloxacin</td>
<td>sarafloxacin (oxolinic acid)</td>
</tr>
<tr>
<td>USA</td>
<td>enrofloxacin</td>
<td>enrofloxacin, sarafloxacin</td>
<td>enrofloxacin, difloxacin, orbifloxacin, marbofloxacin</td>
<td>none</td>
</tr>
<tr>
<td>Japan</td>
<td>enrofloxacin, danofloxacin, orbifloxacin, difloxacin, oxolinic acid</td>
<td>enrofloxacin, danofloxacin, ofloxacin, vebufloxacin, oxolinic acid</td>
<td>enrofloxacin, orbifloxacin</td>
<td>oxolinic acid</td>
</tr>
<tr>
<td>Asia</td>
<td>enrofloxacin, danofloxacin, ciprofloxacin</td>
<td>enrofloxacin, ciprofloxacin, danofloxacin, ofloxacin, oxolinic acid</td>
<td>enrofloxacin</td>
<td>oxolinic acid</td>
</tr>
<tr>
<td>Latin America</td>
<td>enrofloxacin, ciprofloxacin, danofloxacin, norfloxacin, flumequine</td>
<td>enrofloxacin, ciprofloxacin, danofloxacin, norfloxacin, flumequine, oxolinic acid</td>
<td>enrofloxacin</td>
<td>oxolinic acid</td>
</tr>
<tr>
<td>Canada</td>
<td>none</td>
<td>enrofloxacin</td>
<td>enrofloxacin</td>
<td>none</td>
</tr>
<tr>
<td>Australia</td>
<td>none</td>
<td>none</td>
<td>enrofloxacin</td>
<td>none</td>
</tr>
</tbody>
</table>
environment. Rather, the appearance of resistance in genetically diverse backgrounds reflects the intrinsic ability of *E. coli* to develop quinolone resistance if under sufficient selective pressure [35]. All quinolones (and fluoroquinolones) act in a similar manner, by interacting with a DNA-topoisomerase complex. As a result the resistance mechanisms employed by *E. coli* give rise to decreased susceptibility to all quinolones, human or veterinary. This leads to the possibility of a veterinary quinolone selecting resistant strains in food animals, which have the potential to be resistant to quinolones used in human medicine. This could lead to a situation in which humans have acquired a quinolone resistant *E. coli* via the food chain, which either causes an infection or becomes part of the human commensal flora. In the former an infection may fail therapy with a fluoroquinolone. In the latter the commensal could cause an opportunistic infection in a susceptible host (e.g. a neutropenic individual), which may fail fluoroquinolone therapy. In addition a human commensal bacterium could become more resistant if exposed to a fluoroquinolone during treatment for an infection (e.g. a respiratory tract infection). This could lead to the emergence of highly resistant organisms.

2. MECHANISMS OF RESISTANCE TO QUINOLONES

Since their introduction, resistance to the quinolones has emerged and spread at a surprising rate. There are two main mechanisms of resistance to the quinolones; reduced accumulation mediated by reduced cellular permeability and/or enhanced efflux [29]. The second mechanism of resistance is alteration of the intracellular target proteins (DNA gyrase, topoisomerase II or topoisomerase IV [18]). Recently, plasmid mediated resistance has been reported [25] but the importance of this mechanism of resistance remains unclear. However the prevalence of plasmids mediating quinolone resistance appears to be extremely low (G. Jacoby, personal communication).

The major mechanism of quinolone resistance is alteration of the target enzymes of fluoroquinolones, DNA gyrase and topoisomerase IV [18]. Most attention has focused on DNA gyrase which is composed of two GyrA and two GyrB subunits [14]. DNA gyrase is a topoisomerase enzyme responsible for introducing negative supercoils into DNA, a necessary step in DNA replication [38]. This process involves the topoisomerase complex breaking the double stranded piece of DNA, passing another one through it and then resealing the broken strand to complete the reaction [38]. The quinolones interact with the enzyme-DNA complex and prevent the resealing of the DNA by the topoisomerase [18]. This inhibits the synthesis of DNA and the release of double stranded DNA is a lethal event for the cell, although the molecular mechanism of this lethality is still not fully understood. The topoisomerase IV enzyme (made up of two ParC and two ParE subunits) has extensive homology [34] to DNA gyrase (especially at the N-terminus) and acts on DNA in a similar way. The quinolones can also interact with the topoisomerase IV DNA complex and inhibit DNA synthesis [18]. From genetic studies with *E. coli*, the primary target for the quinolones has been shown to be DNA gyrase, specifically the GyrA subunit. In other species, notably Gram-positive organisms *Staphylococcus aureus* and *Streptococcus pneumoniae*, topoisomerase IV is the primary target [18]. Both GyrA and ParC have regions at their N-termini associated with quinolone resistance known as the quinolone resistance determining regions (QRDRs) [17, 41]. Amino acid substitutions within these areas can give rise to resistance to the quinolones (Tab. III). These important residues are located near the active sites of the enzymes which have a tyrosine residue at their core. The most favoured residues at which alteration leads to resistance in GyrA
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are at serine 83 and aspartate 87 [35]. It is thought that these mutations alter the sites at which the quinolones interact and thereby reduce the affinity of the drugs for the enzyme. Once one or two substitutions within the QRDR of GyrA have accumulated further substitutions in the QRDR of ParC [35] (most often at serine 79 or aspartate 83) can lead to incremental increases in resistance to quinolones (Tab. III). Mutations in gyrB and parE have also been described [8, 42] but are much rarer than mutations in gyrA or parC. Despite initial optimism that quinolone resistance would be rare and that high-level resistance would not occur, quinolone resistant bacteria have emerged and have risen in numbers in recent years in both clinical and veterinary isolates of E. coli.

The E. coli cell wall consists of an inner and an outer membrane, separated by a periplasmic space. The outer membrane contains porin proteins which allow essential solutes and other molecules into the cell [31]. The two major porin proteins of E. coli are OmpF and OmpC (Omp = outer membrane protein) [16]. Down regulation of these porins can lead to reduced accumulation of quinolones (and other agents) within the cell [27, 28]. OmpF is a member of the mar regulon (mar = multiple antibiotic resistance), whose expression can be regulated by MarA, a transcriptional regulator which can control more than 60 genes at different loci around the chromosome [4]. One of these genes is micF, an antisense RNA to the ompF mRNA. When micF is induced it binds to the ompF mRNA [9] and prevents translation of ompF and thereby inhibits production of the OmpF protein [9]. MarA also induces expression of the acrAB efflux pump [33]. Efflux pumps reduce the concentration of their substrates from within a cell by actively exporting them from the cytoplasm to the external media [30, 37] (Fig. 1). E. coli has a large number of putative efflux transporters, perhaps the most important is the acrAB system. AcrAB [22] is a member of the RND (resistance nodulation division) family of pumps which consist of an inner membrane pump (AcrB) linked to an outer membrane efflux pump (TolC [11]) held together by a membrane fusion protein (AcrA) which helps hold AcrB and TolC together allowing the complex to span the periplasm. The AcrAB-TolC efflux system has a wide substrate range including quinolones, tetracycline, chloramphenicol, ampicillin, rifampicin, puromycin, organic solvents, pine oils, dyes, disinfectants and detergents [8]. AcrAB is normally produced at a low level but can be de-repressed in response to stress [24] by a number of global regulatory systems (marA, soxS, rob and maybe others) as well as by mutations in the repressor of acrAB, acrR [20, 39]. De-repression of acrAB does not lead to high-level quinolone resistance on its own but rather a low level multiple drug resistance (the minimum inhibitory

Table III. Effect of resistance mechanisms on MIC of Ciprofloxacin.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MIC (µg·mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>0.015⁻¹</td>
</tr>
<tr>
<td>GyrA substitution at Ser83</td>
<td>2–4⁻¹</td>
</tr>
<tr>
<td>GyrA substitution at Ser83 + Asp87</td>
<td>4–8⁻¹</td>
</tr>
<tr>
<td>ParC substitution + 1 GyrA substitution</td>
<td>4–8⁻¹</td>
</tr>
<tr>
<td>ParC substitution + 2 GyrA substitutions</td>
<td>8–32⁻¹</td>
</tr>
<tr>
<td>Down regulation of OmpF</td>
<td>0.25–0.5⁻¹</td>
</tr>
<tr>
<td>Over expression of AcrAB-TolC</td>
<td>0.12⁻¹</td>
</tr>
<tr>
<td>Over expression of MarA</td>
<td>0.12⁻¹</td>
</tr>
</tbody>
</table>

⁻¹ Reference [38],⁻² reference [10],⁻³ reference [26].
concentrations (MIC) of most antibiotics are elevated between 2 and 4 fold [8]). The importance of *acrAB* in the overall fluoroquinolone resistance phenotype is not fully understood, however *acrAB* over-expressing strains appear to be common amongst highly resistant human and veterinary isolates of *E. coli* [39]. Recent studies of ciprofloxacin resistant isolates from humans and animals found 90% and 31% over-expressed AcrA or *acrB*, respectively [26, 39]. Recent work has suggested that the presence of a functional *acr* locus is required for mutations in topoisomerase genes to be effective [32]. There are also other efflux pumps in *E. coli* [23], including a number of homologues of *acrAB* (*YhiV, acrSEF* and *acrD*) which may well also be involved in quinolone resistance. The incidence of efflux in clinical and veterinary isolates of *E. coli* suggests that it may be an important factor in antibiotic resistance [14, 39].

High-level quinolone resistance occurs after a combination of chromosomal mutations in *gyrA, parC* and genes associated with efflux pumps and outer membrane permeability (Tab. III) [10, 26].

### 3. EPIDEMIOLOGY OF QUINOLONE-RESISTANT *E. coli*

There is a scarcity of data relating to the numbers of quinolone resistant *E. coli* from animals; those reports that do exist agree that the incidence of quinolone resistance tends to be higher than anticipated and that it has risen after the introduction of quinolones. In 1996, Bazile-Pham-Khac et al. [5] reported fluoroquinolone resistant isolates from poultry in Saudi Arabia. Flumequine (the first fluoroquinolone) and oxolinic acid have been used in veterinary

![Figure 1. Regulation of OmpF and AcrB. Antibiotics can enter the cell via OmpF and are exported by AcrAB-TolC. Down regulation of OmpF by over-expression of *micF* and over-production of AcrAB-TolC leads to reduced permeability of the cell to antibiotics and increased efflux. Both these events can be controlled by MarA and SoxS.](image-url)
Quinolone resistance in *E. coli*

Subsequently after its introduction the numbers of quinolone resistant avian *E. coli* began to increase. Fifteen quinolone resistant isolates (MIC of nalidixic acid 32–2056 μg·mL⁻¹) that displayed decreased susceptibility to eight different quinolones (including the fluoroquinolones; ciprofloxacin; MIC 0.12–16 μg·mL⁻¹, sparfloxacin; MIC 0.12–16 μg·mL⁻¹, norfloxacin; MIC 0.5–128 μg·mL⁻¹, and pefloxacin; MIC 0.5–128 μg·mL⁻¹) were investigated. All the strains showed reduced resistance (MICs fell to wild-type values) when complemented with wild-type, quinolone susceptible *gyrA* (wild-type *gyrA* is dominant to mutant alleles so introduction of the wild-type gene on a plasmid leads to restoration of the wild-type phenotype) suggesting that *gyrA* mutations were important in the resistance phenotype. None of the strains displayed reduced accumulation of ciprofloxacin, ofloxacin, pefloxacin or sparfloxacin when the uptake of these drugs was measured. Interestingly 75% of the strains were also cross-resistant to chloramphenicol and trimethoprim.

In 1992–1993, Blanco et al. [6] isolated 468 *E. coli* strains from chickens (311 from septicaemic birds, 157 from healthy birds) in Spain and determined the numbers resistant to a range of antimicrobials. They reported 13% of strains resistant to ciprofloxacin (5 μg disks used) and norfloxacin (10 μg disks used), and 44% resistant to nalidixic acid (30 μg disks used) based on disk diffusion assays. The authors concluded that the high numbers of fluoroquinolone resistant *E. coli* observed in this study were due to the injudicious over-use of antimicrobial agents in Spain. Similar resistance rates were reported in *E. coli* from chickens with colibacillosis in Morocco by Amara et al. [1].

Most recently White et al. [40] reported on the mechanisms of resistance in 29 nalidixic acid resistant *E. coli* isolated from chickens with colibacillosis during 1996 to 1999 in the USA. One of the aims of the study was to assess the incidence of resistance to the fluoroquinolones, sarafloxacin and enrofloxacin. The Food and drug administration (FDA) licensed these agents in 1995 for treatment of poultry with colibacillosis to reduce the financial impact of this condition. The study found that sarafloxacin resistance among pathogenic avian *E. coli* (MIC > 0.25 μg·mL⁻¹) increased from 15% in 1996 to 40% in 1999 (Fig. 2), also dual resistance to both enrofloxacin (MIC > 2 μg·mL⁻¹) and sarafloxacin rose from 9% in 1997 to 30% in 1999. The FDA has recently recommended that both sarafloxacin and enrofloxacin be withdrawn from the treatment of drinking water on poultry farms. The resistant *E. coli* also displayed cross-resistance to other agents (the MICs were determined using the broth microdilution method and interpreted using resistance breakpoints from the National committee of clinical and laboratory...
standards (NCCLS): sulfamethoxazole (98%), tetracycline (86%), streptomycin (83%), gentamicin (62%) and ampicillin (55%). Many of the strains (66%) were resistant to five or more antibiotics although none displayed organic solvent tolerance (a marker of mutations at the mar locus). All of the strains had a substitution of leucine for serine at amino acid position 83 of GyrA, seven also had changes at position 87 but none had alterations in ParC. The strains were shown to be genetically diverse by random amplified DNA polymorphism (RAPD) analysis, ruling out the possibility of the selection and transfer of clonal isolates which harboured resistance. Another study from the USA of cephalosporin-resistant strains from cattle with scours in 1996, found a resistance rate of about 13% to the quinolones (nalidixic acid, 10 μg disks and ciprofloxacin, 5 μg disks), again these isolates were multiply antibiotic resistant and were not clonally related. These animals had not been treated with a quinolone so the resistance rate of 13% was surprisingly high [7].

In 1996, Everett et al. [10] reported on the mechanisms of resistance in eight ciprofloxacin resistant isolates from an outbreak of diarrhoea in calves and chickens in the United Kingdom. The MIC of ciprofloxacin ranged from 2 to 8 μg·mL⁻¹ in these strains. All of the strains had a mutation in the gyrA gene, leading to an amino acid substitution at serine 83 of GyrA, those with an MIC of 4 or 8 also had substitutions at aspartate 87 of GyrA or within the QRDR of parC. Three of the strains also had a strong efflux phenotype for ciprofloxacin, these strains have subsequently been shown to over-express the acrAB efflux system [39]. The same study also investigated a set of strains from humans and found similar topoisomerase mutations and over-expression of efflux pumps.

In contrast to the above reports, the experience of the Danish is more reassuring, in 1998 Danish food producers withdrew the use of antibiotics as growth promoters in poultry, cattle and pigs [2]. In line with this, total antibiotic use including quinolones has also dropped dramatically since that date, 400 kg of quinolones were used in 1998 and only 150 kg in 1999 (most of this decrease is due to the withdrawal of enrofloxacin from use in feed and water), a fall of 65% [2]. In the same period resistance to nalidixic acid (MIC > 16 μg·mL⁻¹) in broilers fell from 26% in 1998 to 13% in 1999 (these animals were healthy, a greater decrease in resistance has been observed in isolates from diagnostic submission – Frank Aarestrup, personal communication); the level of ciprofloxacin resistance (MIC > 2 μg·mL⁻¹) remained steady in the same period (a rise from 0.5% in 1998 to 1.6% in 1999, this is not statistically significant).

4. CONCLUDING REMARKS

The use of antibiotics in the treatment and prevention of animal disease is clearly a necessity, however much care must be given to the choice of antimicrobial agents to be used and when to, or just as importantly when not to, use certain agents. The fluoroquinolones are a highly active class of agents which are effective in treating different infectious animal diseases, making them an attractive treatment option. However, resistance to fluoroquinolones is mediated mainly by mutations in genes encoding the topoisomerase enzymes that are the target for this class of compounds. All quinolones act in the same manner and development of resistance against one leads to decreased susceptibility against all members of the class. It has been shown that, as for most antibiotics, resistance to fluoroquinolones emerges and the number of resistant strains increases after their introduction. The mechanisms of resistance in veterinary quinolone resistant isolates are the same as those seen in laboratory mutants and in isolates from humans. It is for this reason that care must be taken in deciding when to use this class of drugs in food
animals that are of major importance in human medicine. The passage of *E. coli* from food to humans has been well documented (e.g. outbreaks of serotype O157:H7 [13]) and the over use of quinolones in veterinary practice may lead to quinolone resistant strains being transferred to humans via food with potentially worrying clinical implications. Although the degree of risk to human health created by the selection of a fluoroquinolone resistant population of *E. coli* in food animals is unsure, there is a clear theoretical risk. It seems sensible that the fluoroquinolones are limited to situations where few other agents would be effective and that their use is subject to regulation. The example of the Danish food production industry clearly indicates that a reduction in the use of antibiotics as a whole can be achieved without collapse of the industry and that reduction of use may lead to a reduction in levels of resistance to antibiotics, including the quinolones.

More research is needed into the incidence of fluoroquinolone resistant *E. coli* and the relationship between the numbers of fluoroquinolone resistant strains and fluoroquinolone use. It is important that there is an understanding of how resistant strains are selected and to characterise the mechanisms of resistance involved. For example if it appears that efflux is an important first step in resistance, then there would be a case for administering compounds that inhibit efflux pumps with the fluoroquinolone being used in order to repress the development of resistant strains. More information about the selection and spread of fluoroquinolone resistance in *E. coli* will allow veterinarians to make more informed decisions about the use of these drugs and will shed more light on the potential risks of their overuse.

**ACKNOWLEDGEMENTS**

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multiple-
lactam compounds lack the ompF porin, Antimi-
in fluoroquinolone-resis-
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