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President & CEO

October 19, 1999

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fisher's Lane, Room 1061
Rockville, Maryland 20852

Re: Docket No. 98-D-0969 Antimicrobial Resistance in Food-Producing
Animals Public Workshops

The Animal Health Institute (AHI) provides these additional comments to further clarify and expand on suggestions offered during an October 4, 1999 general public meeting held to solicit input on two public workshops on antimicrobial resistance.

AHI is a national trade association representing manufacturers of animal health products – pharmaceuticals, vaccines and feed additives used in modern food production and the medicines that keep pets healthy. AHI supports CVM in its continuing efforts to make evidence-based decisions concerning animal health products, including anti-infective products, and to provide consistent guidance to industry regarding antimicrobial resistance.

GENERAL COMMENTS

Scope – The Animal Health Institute believes that a workshop should begin with a clear, concise statement of purpose. We strongly encourage CVM to begin each workshop with a clear statement of the scope, purpose and objectives of the workshop; what they envision to be the end product of the workshop; and what the next steps and timeline will be after the conclusion of the workshop. This approach should also be applicable to the breakout sessions as well.

Format – Since the workshop will be attended by many individuals with diverse areas of expertise, varying notions of the intent and application of CVM's framework document, and varying appreciation of the complexity of the issues involved, it should begin with the necessary background information to ensure that all participants are at a minimal level of understanding in order to effectively contribute in later sessions. AHI recommends a workshop format that 1) provides the participants with a plenary session summary of the critical issues that impact and influence the topics under discussion; 2) includes breakout sessions to facilitate frank discussions of key topics in conjunction with appropriate experts; and 3) concludes with a closing general session to summarize the key issues, recommendations, and areas for further input regarding the various topics discussed in breakout sessions. Additionally, we encourage CVM to include an informal information exchange medium, such as a poster session, to allow participants access to

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information relevant to the topic(s) under discussion, but which will not be formally presented during the workshop (e.g., for the December workshop MRL's TSN system, the Lipsitch resistance modeling poster, Thomas Oscar salmonellosis risk assessment model, etc).

Workshop on Risk Assessment and the Establishment of Resistance Thresholds

Scope – The discussion should be limited to risk assessment and the scientific feasibility of establishing thresholds. It would also be appropriate to consider how these areas fit into the overall scheme of new drug discovery and development programs and post-approval activities.

Issues – AHI believes a general discussion of the application of and differences between risk assessment, risk management and risk communication is an important introductory session to be addressed in the plenary. Another key topic for the plenary session is in vitro antibiotic breakpoints (for resistance and for susceptibility), methods for determining breakpoints, and how breakpoints are used by the medical community. Finally, we would encourage presentation of the risk assessment model being contemplated for use in the proposed CVM study.

AHI further recommends the following topics for discussion by experts and workshop participants during breakouts.

- An analysis of the components of the CVM risk assessment model, particularly the various populations of concern, and how probability estimates have been applied to the populations at risk, including a discussion of how ongoing changes, modifications to assumptions or contingencies could be evaluated and incorporated into the model. Important discussion points would include CVM proposals to increase certainty of assumptions at each stage in the proposed model.
- A discussion of microbiological specimen sampling methods, in vitro susceptibility testing methods, qualitative and quantitative test results, and breakpoints as reliable indicators for predicting possible impact on human health.
- An examination of how to define both monitoring and resistance thresholds and how data would be applied to each, how each would be interpreted and what actions would be triggered by results which exceed the predefined limits.
- A discussion of possible mitigation steps if a threshold is reached.
- A review, including both policy and legal issues, of the application of the standard “reasonable certainty of no harm.” We believe this is a critical discussion since this standard is the foundation for setting thresholds. We believe there are valid questions as to whether this standard, as applied to the approval process, is properly applicable to actions the FDA may take in attempting to control antimicrobial resistance.

AHI has previously provided CVM with specific comments on the Framework Document which outline relevant subtopic areas for discussion within the breakout groups. CVM is

encouraged to review these key issues and concerns in order to compile a list of discussion topics that must be addressed.

Experts – The following list provides the names of various experts that we believe would enhance the workshop discussions. We have included their areas or expertise along with relevant publications.

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AHI and its member companies appreciate the opportunity to provide input and recommendations for consideration by the Agency as to the scope, format, issues to be discussed and experts for the December 9-10, 1999 Workshop on Risk Assessment and the Establishment of Resistance Thresholds. In the near future we plan to provide input on the February 2000 Workshop on Pre-Approval Studies in Antimicrobial Resistance.

Sincerely,



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1. Taxonomy of Campylobacter and brief review of their clinical significance.

The genera *Campylobacter*, *Arcobacter* and the generically misclassified species *Bacteroides ureolyticus* constitute the family *Campylobacteriaceae* (Vandamme and Goossens, 1992). Within the genus *Campylobacter*, the group of the thermophilic - or more accurately "thermotolerant", campylobacters (*C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*) forms taxonomically a distinct cluster. *C. fetus* and *C. hyointestinalis* are also close relatives, while the remaining species form a loose assemblage of organisms (*C. concisus*, *C. curvus*, *C. gracilis*, *C. mucosalis*, *C. rectus*, *C. showae*, *C. sputorum*).

1.1. "Thermotolerant" campylobacters.

C. jejuni comprises two subspecies: *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*. In general, distinction is not made between both subspecies although several studies have suggested that *C. jejuni* subsp. *doylei* is a rare pathogen in humans. This subspecies has indeed been rarely isolated from ulcerated gastric tissue, diarrhea and blood cultures, notably infants (Goossens et al, 1992; Steele and Owen, 1988). Since this *C. jejuni* subsp. *doylei* is only occasionally isolated from clinical specimens I will not further consider it in my presentation. Indeed, *C. jejuni* subsp. *jejuni*, further referred to as *C. jejuni*, is by far the most important pathogen among the genus *Campylobacter*. *C. jejuni* is differentiated from *C. coli*, both phenotypically and genotypically similar taxa, by the hippurate hydrolysis test in which *C. coli* is negative. However, some *C. jejuni* are hippurate negative.

C. jejuni causes abortion in sheep and bovine, and occasionally in other animals. It may also cause diarrhea in animals and may be responsible for hepatitis in some bird species. However, *C. jejuni* are found as normal intestinal flora of poultry and other bird species, sheep, cattle, goats, dogs, cats, rabbits and monkey. In humans, *C. jejuni* is one of the most important causes of bacterial diarrhea worldwide. Rarely, septicemia, abortion and some other extraintestinal infections occur. *C. jejuni* may be a predisposing factor to the development of Guillain-Barré and Miller-Fisher syndromes.

C. coli may cause diarrhea in pigs and monkeys and abortion in rodents. Like *C. jejuni*, it has been associated with hepatitis in some bird species. In humans, it causes diarrhea, and, occasionally, extraintestinal infections including abortion. *C. hyoilei*, which have been associated with porcine proliferative enteritis, are in fact, *C. coli*.

C. lari have been isolated from intestinal contents of seagulls and other animals, river water and shellfish. In humans, they are a very rare cause of infection, particularly diarrhea. Several *C. lari* variants have been described, which have been isolated mainly from shellfish in the Netherlands (Endtz et al, 1997). The exact relationships between genuine *C. lari* and these variants is unknown at the present time. Their pathogenic role in diarrheal disease is also unknown.

C. upsaliensis have been isolated from fecal samples of dogs and cats. This *Campylobacter* species is a cause of diarrheal disease in humans, and, occasionally of abortion and breast abscess (Goossens et al, 1992).

C. helveticus have been isolated from diarrheic and asymptomatic cats and, rarely, dogs. They have not been isolated from humans at the present time.

1.2. *C. fetus*.

Two subspecies have been differentiated associated with distinct diseases in animals; however, from a taxonomical point of view this distinction is difficult.

C. fetus subsp. *fetus* will grow in the intestinal tract of man and animals and is transmitted orally. It causes abortion in sheep and sporadically in cattle. It is an important cause of infection in patients with immunodeficiency. While some cases may be traced to contact with infected animals, the majority of patients have no identifiable exposure. Therefore, it is not clear that *C. fetus* subsp. *fetus* infection, as compared to infection caused by "thermotolerant" campylobacters, is a zoonotic disease.

C. fetus subsp. *venerealis* will not multiply in the intestinal tract of man and animals. It is a cause of abortion in cattle; it is also pathogenic in some other animal species. *C. fetus* subsp. *venerealis* is not pathogenic in humans and publications on *C. fetus* probably refer to *C. fetus* subsp. *fetus*. Therefore, subsequently I will also refer to *C. fetus* as the representative for *C. fetus* subsp. *fetus*.

1.3. *C. hyointestinalis*.

This *Campylobacter* species has been differentiated in two subspecies: *C. hyointestinalis* subsp. *hyointestinalis* and *C. hyointestinalis* subsp. *lawsonii*. The latter has been isolated only from the stomach of pigs. The former has been isolated from the intestines and stomach of several animal species, including pigs; it has been associated with porcine proliferative enteritis. Although its pathogenicity is unknown in humans, it may be a rare cause of diarrhea. When *C. hyointestinalis* is isolated from humans, this probably refers to *C. hyointestinalis*. Therefore, I will also refer to *C. hyointestinalis* as the representative for *C. hyointestinalis* subsp. *hyointestinalis*.

1.4. *C. concisus*.

The pathogenicity of this organism in humans is unknown. It has been found in the gingival crevices of man with gingivitis and periodontitis; it has also been isolated from persons with normal and diarrheic stools, and from the blood.

1.5. *C. curvus*.

This organism has been very rarely isolated from the blood, peritoneal fluid and from normal and diarrheic stools in humans.

1.6. *C. gracilis*.

This *Campylobacter* species may be responsible for deep tissue infection, pneumonia and empyema. It is frequently resistant to various antibiotics.

1.7. *C. mucosalis*.

This species, together with *C. fetus* and *C. hyointestinalis*, is primarily important in veterinary medicine, causing enteric disease in pigs (although its role in proliferative enteritis in pigs has been questioned). Human infections with *C. mucosalis* have been reported but were found to be misidentified *C. concisus* strains.

1.8. *C. rectus*.

This organism has been associated with periodontal disease in humans.

1.9. *C. showae*.

Their pathogenicity is unknown, although they have been isolated from human dental plaque.

1.10. *C. sputorum*.

C. sputorum comprises three biovars: *C. sputorum* biovar *sputorum*, *C. sputorum* biovar *faecalis* and *C. sputorum* biovar *paraureolyticus*. These strains have been occasionally isolated from clinical specimens in humans.

2. Campylobacter infections where antibiotic use may be indicated.

2.1. Community-acquired infectious diarrhea.

Infectious diarrhea is one of the most frequent causes of acute diarrhea in humans. This diarrhea can be either watery or invasive. Diarrhea may also occur in special circumstances, such as in travelers and in the immunocompromised patient.

2.1.1. Acute infectious diarrhea.

- In general, watery diarrhea is caused by strains of bacteria that produce toxins that, in turn, produce fluid secretion without causing any damage to the epithelial surface. However, the pathogenesis for certain pathogens is not well understood. Pathogens are acting in the small intestine; blood or leucocytes are seldomly present in stools. The most frequent pathogens are: *E. coli* (enterotoxigenic, enteropathogenic), rotavirus, *Salmonella*, *Campylobacter*, calicivirus, *Cryptosporidium*, *Cyclospora*, *Giardia*, *Vibrio cholerae*. Since most patients have a mild self-limited course, the major goal of treatment is the replacement of fluid and electrolytes. Antimicrobial therapy is rarely indicated.

- Invasive diarrhea is characterized by the visible presence of blood and leucocytes. This is usually caused by direct invasion of the gastrointestinal mucosa by the pathogen or via the production of cytotoxins, at the large intestine. The most frequent pathogens are: *Shigella*, *Campylobacter*, *Salmonella*, *E. coli* (enteroinvasive and enterohemorrhagic), *E. histolytica*. Although other "thermotolerant" campylobacters may escape detection, *C. jejuni* appears to be the most important species responsible for bacterial diarrhea. The rate of campylobacter enteritis could be estimated at 40 per 100,000 population, but in a survey of patients seeking treatment for acute diarrhea in the United Kingdom, investigators found an incidence of 1.1% (1,000 cases per 100,000 population per year, or 500,000 cases per year) (Tauxe et al., 1992). The incidence in developing countries is probably much higher, and travelers to these areas are at risk for developing campylobacter infection. The intestinal symptoms produced by *C. jejuni* infections are clinically indistinguishable from those caused by other bacterial enteric pathogens, such as *Salmonella* and *Shigella*. The signs and laboratory findings in *C. jejuni* enteritis are similar to those in inflammatory diarrhea caused by other bacteria. Fever (> 37.5°C) is present in two-thirds of infected persons. Fecal leucocytes are found in more than 75% of cases and gross or occult fecal blood in more than 50%. Duration of symptoms is usually self-limiting, and symptoms resolve within 1 week even without specific antimicrobial therapy. However, symptoms may persist for 1-3 weeks in up to 20% of ill patients.

Campylobacter infections are typically acquired following ingestion of improperly handled or undercooked food, primarily poultry products.

Many patients with campylobacter enteritis do not require antimicrobial treatment, even if these patients seek medical attention. Antimicrobial treatment reduces the duration of

campylobacter excretion in stools, and, provided that the treatment is not begun on the second

or third day of illness, it may shorten the course of uncomplicated enteritis. Patients who may benefit from antimicrobial treatment include those with prolonged (more than 1 week) or worsening symptoms, high fever or bloody stools. Probably, pregnant women may also benefit from antimicrobial treatment considering the deleterious effects *C. jejuni* may have on the fetus.

Macrolides are the treatment of choice for most cases of campylobacter enteritis. The newer macrolides may be better tolerated than erythromycin, but sufficient evidence is lacking with roxithromycin, clarithromycin and azithromycin. In double-blind, placebo-controlled trials of treatment in patients with campylobacter enteritis, erythromycin promptly eradicated campylobacters from the feces but did not alter the natural course of enteritis when administered 4 days or longer after the onset of symptoms. Studies in which therapy was started earlier in the course of illness gave conflicting results with regard to clinical resolution, although *C. jejuni* was rapidly eliminated from stools.

Fluoroquinolones have emerged as drugs of choice for treatment of invasive diarrhea for several reasons: an antibacterial spectrum including most major pathogens associated with this type of diarrhea, rapid bactericidal effect, good absorption, high tissue and intracellular concentrations, high and prolonged drug concentrations achieved in feces and bile, few side effects, preservation of anaerobic flora. However, although the initial small trials with ciprofloxacin and norfloxacin for treatment of campylobacter enteritis were promising (reduction of symptoms, bacterial eradication, no selection of resistance), subsequent studies have shown that differences with the placebo group were slight and of doubtful clinical importance, except for the severely ill. Most studies have shown that treatment with fluoroquinolones for acute campylobacter diarrhea will only be effective if instituted early (? 2 days of symptoms) in the course of more severe enteritis. Wiström et al (1992) found a difference in mean time to cure in the norfloxacin group in comparison with the placebo group of only 1 day. They also observed higher eradication rates in the norfloxacin group than in the placebo, whilst the opposite was true for *Salmonella* species enteritis.

Thus, fluoroquinolones have very limited clinical benefit for the treatment of campylobacter diarrhea; they should not (no longer) be considered treatment of choice for acute campylobacter enteritis. Shigellosis is the enteric infection that seems to respond best to fluoroquinolone treatment. Thus, since invasive diarrhea may be caused by *Shigella* species as well as *Campylobacter* species, and since clinical symptoms do not allow differentiation between these pathogens, increased resistance of *Campylobacter* species to fluoroquinolones may result in (only marginal) prolonged disease and bacteriological eradication failure. However several alternatives are available for treatment of campylobacter enteritis; including macrolides, clindamicin, amoxicillin - clavulanic acid, tetracycline, furazolidone.

2.1.2. Traveler's diarrhea.

Traveler's diarrhea occurs in travelers, usually those visiting a less-developed area of the world. It is estimated that 15 to 20 million persons from industrialized countries travel to developing countries. Median diarrhea rates of 21-100%, 21-100% and 36-62% were found in prospective studies in Latin America, in Asia and in Africa, respectively. Watery loose stools are the most common complaint. The most frequent pathogens are: *E. coli* (enterotoxigenic), *Campylobacter*, *Shigella*, *Salmonella*, *Vibrio species*, rotavirus, *Giardia*, *Entamoeba histolytica*. No pathogens are identified in 22%-83% of ill patients.

As opposed to invasive diarrhea in developed countries, where stool cultures are readily available and indicated in more severe forms of enteritis, in travelers, immediate antibiotic treatment has been advised to travelers suffering from acute diarrhea. If there is indeed no delay in the initiation of fluoroquinolone therapy, clinical benefit can be expected if the traveler's diarrhea is caused by *Campylobacter* species. Compared with placebo, quinolones reduce the mean duration of diarrhea by 24 to 48 h, they reduce the intensity of illness during treatment (usually measured as mean number of loose stools or by different severity scoring systems). Treatment should however be started early in the course of diarrheal illness, ideally within 24 h of onset. Overall approximately 80% of travelers receiving antibiotics are cured after 48 h of treatment compared with

approximately 40% of placebo-treated subjects (Wiström and Norrby, 1995). There are no significant differences in clinical efficacy between quinolones and other antibiotics for treatment of traveler's diarrhea.

2.1.3. Diarrhea in the compromised host.

Prospective studies of diarrhea in patients with the human immunodeficiency virus (HIV) in the industrialised countries have found that microsporidia are the most common causes of chronic diarrhea followed closely by *C. parvum*. Prevalence rates of enteric cytomegalovirus infection vary greatly. Bacteria including *Campylobacter*, may also cause chronic or recurrent bloody diarrhea associated with fever and abdominal pain in immunocompromised subjects. Although the relative importance of campylobacter infection in patients with AIDS is unknown, one study showed *C. jejuni* to cause more frequently diarrhea in HIV-infected patients (Sorvillo et al., 1991). In this study, the incidence of *C. jejuni* infection among patients with AIDS (mean annual incidence, 519 cases per 100,000 persons) was higher than that in the healthy population (mean annual incidence, 13.3 cases per 100,000 persons). It has also been shown that *C. jejuni* infection is more severe, prolonged and relapsing in immunosuppressed patients, including AIDS patients, with sometimes bacteremia.

It has been speculated that the incidence of campylobacter infection in HIV-infected patients is underestimated, because many ("thermotolerant") campylobacters grow only at 37°C, are more fastidious and slow-growing, and are susceptible to antibiotics present in selective media. Snijders et al (1997) reported on the enhanced sensitivity and usefulness of the membrane filter technique, performed on nonselective agar, for detecting *Campylobacter* species other than *C. jejuni* in HIV-infected patients. They performed a cross-sectional study of 201 HIV-infected patients and detected *Campylobacter* species in 12, including 11 "atypical" campylobacters; these "atypical" *Campylobacter* species were associated with diarrhea. Their study showed also the increasing need for more-sophisticated methods of differentiating and identifying *Campylobacter* species. They also found, as we had suggested previously (Goossens et al., 1991), an association between *C. upsaliensis* and dogs. This suggests that "atypical" campylobacters may be more prevalent than *C. jejuni* and *C. coli*, and that the animal source of infection may also be different from non-HIV-infected subjects.

2.1.4. Treatment failures with fluoroquinolones.

Fluoroquinolone resistance among campylobacters is emerging (Endtz et al., 1991; Piddock, 1995; Sjögren et al., 1997). Goodman et al (1990) reported relapse in 2 of 10 *Campylobacter*-infected patients treated with ciprofloxacin twice daily for 5 days: one patient showed persistent symptoms in addition to persistent positive cultures and one patient showed only microbiological failure. Subsequently, they characterized the mechanism of quinolone resistance, and showed that the pretreatment isolates were susceptible to ciprofloxacin (MICs, 0.125 - 0.5 mg/l), whereas the posttreatment isolates were resistant (MICs 32 mg/l) (Segreti et al., 1992). Obviously, mutation in *C. jejuni* had occurred in vivo and was associated with clinically significant resistance. Development of resistance during therapy with lomefloxacin was also observed in a clinical trial by Ellis-Pegler et al (1995).

It has also been shown that emergence of resistance in *Campylobacter* species may occur as rapidly as within 1 day of treatment (Adler-Mosca et al., 1991; Wretling et al, 1992).

The main advantages of fluoroquinolones in treatment of traveler's diarrhea are safety, low risk of selecting resistant organisms, and good in-vitro activity against most bacterial enteropathogens. However, treatment failures were reported during a treatment trial of traveler's diarrhea with ciprofloxacin given as a single dose (one case) and as a 3-day course (one case) (Petrucci et al., 1992). Both traveler's with *C. jejuni* enteritis relapsed clinically and microbiologically (MICs were 0.2 and >16 mg/l before and after treatment, respectively). It is noteworthy that duration of

treatment with fluoroquinolones in traveler's diarrhea is in general shorter (from single dose to maximum 5 days) than in acute invasive diarrhea not related to traveling. This short-term treatment with fluoroquinolones in traveler's diarrhea may be largely responsible for selecting resistant campylobacters.

Sequential development of multidrug resistance in *C. jejuni* isolates recovered from stool specimens from 3 HIV-infected patients was also reported (Tee et al, 1995). The results indicated that each strain had progressively acquired resistance to the antibiotics used during treatment, including erythromycin and fluoroquinolones. Moreover, the emergence of resistance appeared to correlate with clinical relapse. *C. jejuni* has been considered a treatable cause of AIDS-related diarrhea, but in these cases infection failed to respond to antibiotic therapy. All three patients died; two with severe diarrhea, although other enteropathogens may have contributed to the diarrhea (cytomegalovirus in patient 1; *E. histolytica* in patient 2) and the third one from disseminated fungal infection. Although the isolates remained susceptible to gentamicin in vitro, long-term oral gentamicin treatment did not benefit patient 1.

Although published data on resistance to fluoroquinolones are most likely referring to the selection of resistant mutants following the use of fluoroquinolones in patients, we can expect also increased clinical and microbiological failures due to infection with *Campylobacter* species that are resistant a priori to fluoroquinolones. Indeed, very high resistance rates to fluoroquinolones among *C. jejuni* have been published recently in various developed as well as developing countries, both in humans and in animals. Kuschner et al (1995) published clinical and microbiological failures of ciprofloxacin (500 mg daily for 3 days) for the treatment of *Campylobacter* enteritis in travelers to Thailand, where resistance of *Campylobacter* species to fluoroquinolones is more than 50%! These investigators found the number (%) of patients recovered by 48 hours 2/7 (57%) in the ciprofloxacin-susceptible group vs 4/7 (29%) in the ciprofloxacin-resistant group; the mean duration of illness was 48.3 in the ciprofloxacin-susceptible group vs 59.6 hours in the ciprofloxacin-resistant group. Although these numbers are small, there is clearly a tendency. In the same study, eradication of *Campylobacter* species from stools after 7 days of treatment did not fail in the ciprofloxacin-susceptible group vs 3/5 (60%) in the ciprofloxacin-resistant group. Other antibiotics have been evaluated for the treatment of *Campylobacter* enteritis in travelers, such as azithromycin. This drug has good activity against common bacterial pathogens. Although there are no strong data showing that there is cross-resistance between fluoroquinolones and macrolides, increase use of macrolides, such as azithromycin in travelers, may increase antibiotic selective pressure.

Interestingly Bahtiar and Shanson (1991) published paired isolates where the post-therapy ciprofloxacin resistant isolate had also become resistant to both erythromycin and tetracycline. Endtz et al (1993) found no difference between macrolide activity against quinolone-susceptible and quinolone-resistant *C. jejuni* strains. However, Reina et al (1995) in Spain found that among 308 quinolone-resistant strains 19 (6.1%) displayed simultaneous resistance to erythromycin, while only 6 of 382 (1.5%) quinolone-susceptible strains were also resistant to erythromycin ($P=0.0026$). Very recently, Hoge et al (1998) published trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Resistance to ciprofloxacin among *Campylobacter* species had increased to 84% in 1994; azithromycin resistance was found in 7-15% of *Campylobacter* isolates, and all isolates that were resistant to azithromycin were also resistant to ciprofloxacin. Despite the fact that the resistance mechanisms for these two antimicrobials are different, it may be that the large and abusive use of quinolones, both in humans and animals, facilitates the selection of campylobacters resistant to erythromycin. Increased use of quinolones in humans can be expected with the arrival of trovafloxacin and grepafloxacin. The value of frequent use of fluoroquinolones for prophylactic purposes (Du Pont et al, 1993) has also to be questioned.

2.2. Extraintestinal infections.

Extraintestinal manifestations of *C. jejuni* are the result of local invasion. Bacteremia may occur early in some cases of *C. jejuni* enteritis, but, since most strains of *C. jejuni* are susceptible to killing by normal human serum, this bacteremia is transient and clinically insignificant. Unlike other enteric infections, such as salmonellosis, infection with "thermotolerant" campylobacters is not often associated with a systemic illness. However, clinical outcome is different in immunosuppressed patients, including HIV-infected subjects. Tee and Mijch (1998) retrospectively evaluated and compared clinical and bacteriologic features and clinical outcomes of *C. jejuni* bacteremia in 9 HIV-infected and 12 non-HIV-infected patients. In HIV-infected patients, *C. jejuni* bacteremia was more severe, prolonged, and debilitating: the median duration of diarrheal disease was 1.5 days for non-infected subjects compared to 5 days for HIV-infected patients and the median duration of fever was 1.5 and 2 days respectively. Extraintestinal infection occurred in eight of nine HIV-infected patients, but in only two of twelve non-HIV-infected subjects. Death was attributable to *C. jejuni* infection in three of nine HIV-infected patients but in none of the non-HIV-infected patients. All three patients had a CD4 cell count of $< 20/\text{mm}^3$. Travel history was a risk factor in non-HIV-infected patients but none of the HIV-infected patients had traveled outside Australia. Therefore, these patients may have acquired their infection locally, probably through ingested food or contact with domestic pets. Two *C. jejuni* isolates among the HIV-infected group and one isolate among the non-HIV-infected group were resistant to ciprofloxacin; none of the strains were resistant to erythromycin. Therefore, antibiotic treatment failure was not directly responsible for death in the three HIV-infected patients who probably died of *C. jejuni* septicemia.

This and other studies have shown that excess of *C. jejuni* infections affects only patients in the later stage of HIV disease, with low CD4 cell counts. HIV-infected patients with relatively high CD4 counts are not usually prone to either *C. jejuni* infections or relapses.

C. fetus subsp. *fetus* are more likely to be isolated from the blood and other extraintestinal sites, even in the non-compromised host. This is probably due to the intrinsic resistance to killing by normal human serum. The organisms have a vascular tropism, and infection may result in endocarditis, mycotic aneurysm, septic thrombophlebitis or relapsing fever, most notably in the immunocompromised hosts. Fluoroquinolones demonstrate excellent in vitro activity against *C. fetus*. However, Meier et al (1998) recently described acquisition of resistance to these agents in two patients treated with ciprofloxacin. In one patient, resistance occurred after oral treatment with 750 mg twice a day for 6 weeks and clinical resolution could only be achieved with erythromycin (2 g/day orally). Resistance to fluoroquinolones was associated with a single nucleotide change at residue 87.

3. Conclusion.

Resistance of campylobacters to fluoroquinolones is increasing. This resistance has been responsible for bacteriological and clinical failures. These failures have been well documented in patients developing resistance to fluoroquinolones during fluoroquinolone treatment. We may expect similar failures to occur in patients infected with campylobacters a priori resistant to these compounds, derived from animals.

Alternatives for treatment of fluoroquinolone-resistant strains are available (macrolides, doxycycline, aminoglycosides, ampicillin, chloramphenicol). However, with increased resistance to fluoroquinolones, selective pressure on these alternatives will increase. Therefore, resistance of campylobacters to fluoroquinolones should be reduced by further limiting its use for treatment in acute invasive diarrhea and in travelers with acute diarrhea, as well as in animals.

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Review of the Clinical Use of Quinolones in Human Medicine: Western Hemisphere

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Since the development of fluoroquinolones and their release in the United States in the mid 1980s, there has been extensive clinical use of these agents in both inpatients and outpatients (1). Until recently there were five fluoroquinolones available in the United States, norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin, and enoxacin, and ciprofloxacin and ofloxacin have received the widest use. In the past two years, four new agents have been released in the United States, levofloxacin, sparfloxacin, grepafloxacin, and trovafloxacin and are anticipated to contribute to increased usage of the class as a whole. This brief review will focus on the major areas of indicated uses in the United States and will include commentary on the circumstances in which acquired fluoroquinolone resistance has occurred in association with human use. Data from formal monitoring of clinical usage for specific indications are not available in the United States.

Urinary tract infections. All approved fluoroquinolones except sparfloxacin and grepafloxacin are indicated for the treatment of urinary tract infections. Efficacy is high in uncomplicated cystitis in young women, but other agents such as trimethoprim-sulfamethoxazole or nitrofurantoin are preferred as more cost-effective first-choice therapy (2). When fluoroquinolones are chosen, a 3-day regimen has been shown to be sufficient. Single-dose treatment may also be effective, but infections due to *Staphylococcus saprophyticus* respond less well to single-dose therapy. In uncomplicated pyelonephritis, studies indicate high cure rates for 7- to 10-day courses of ofloxacin or norfloxacin (3). Complicated urinary tract infections occurring in patients with structural and functional abnormalities of the the urinary tract are more often caused by more difficult to treat pathogens including *Pseudomonas aeruginosa*. Cure rates of 75-80% for *P. aeruginosa* infections have been reported, but recurrent infections are common in this group of patients. Failures have been associated with acquired fluoroquinolone resistance in 10-20%. Surprisingly, quinolone-resistant *E. coli* infections have become a particular problem in Spain, and resistance has been associated with prior use of fluoroquinolones, urinary tract abnormalities, and presence of a catheter (4).

Prostatitis is an indication for ofloxacin, ciprofloxacin, and trovafloxacin. Chronic infections generally require 4- to 6-week courses of therapy with eradication rates of 67-91% in open studies (5). *E. coli* infections have been best eradicated with poorer response rates with infections caused by *P. aeruginosa* and enterococci.

Sexually transmitted diseases. Many fluoroquinolones (ofloxacin, enoxacin, ciprofloxacin, grepafloxacin, trovafloxacin) have approval for use in treatment of gonococcal urethritis and cervicitis and single-dose therapy is usually highly effective (6; 7). Gonococci with reduced susceptibility to fluoroquinolones, however, have been identified in certain parts of the United States and have been associated with therapeutic

failure (8). Ofloxacin, grepafloxacin, and trovafloxacin are approved for treatment of chlamydial infections but must be given for 7 days to be effective. For pelvic inflammatory disease, which may result for mixed infections that include gonococci, chlamydia, enteric bacteria, and anaerobes, trovafloxacin is the only quinolone approved for use alone, but ciprofloxacin in combination with an agent active against anaerobes is also approved and effective (9).

Gastrointestinal and abdominal infections. For treatment of bacterial gastroenteritis, ciprofloxacin is the only fluoroquinolone with approval in the United States, although other agents have been shown to be effective. The duration of diarrhea in both campylobacter and salmonella gastroenteritis may be shortened by norfloxacin (10), but persistence of *Campylobacter jejuni* in the stool after ciprofloxacin treatment has been associated with acquisition of resistance (11) and treatment failure. Fecal carriage of salmonella has also been prolonged after treatment with ciprofloxacin (12), but bacterial resistance was not reported. Several quinolones are effective in reducing symptoms in shigellosis, and a single 1-gram dose of ciprofloxacin is effective except in infection caused by *Shigella dysenteriae* type 1 (13). For travelers to areas of risk for bacterial gastroenteritis, presumptive therapy at the onset of diarrhea with ciprofloxacin given as a single dose (750 mg) or for 3 days with or without loperamide is recommended rather than use of quinolones in prophylaxis (14). For enteric fever ciprofloxacin and ofloxacin have been clinically effective with resolution of fever within 5 days (15), and because of resistance to other antimicrobial agents these quinolones are considered the agents of choice for typhoid fever.

Use of quinolones for treatment of other abdominal infections has included small numbers of patients with biliary tract infection with good response rates to ciprofloxacin (16), and in a more recent trial a combination of ciprofloxacin and metronidazole was shown to be comparable to imipenem for treatment of complicated intraabdominal infections largely related to disease of the colon, appendix, or small bowel (17). Trovafloxacin is also approved for this use, but data are not yet published. Treatment of peritonitis associated with chronic peritoneal dialysis with systemically administered quinolones has heretofore been limited by the susceptibility of the usual staphylococcal pathogens (18). Norfloxacin has been shown to be effective as prophylaxis of spontaneous bacterial prophylaxis in patients at high risk due to cirrhosis (19), but this prolonged usage in low doses was associated with the emergence of quinolone resistance (20).

Respiratory tract infections. Many fluoroquinolones have approval for treatment of respiratory tract infections [ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, grepafloxacin, trovafloxacin, and (bronchitis only) lomefloxacin]. For acute bacterial exacerbations of chronic bronchitis and community-acquired pneumonia there has been concern about the potency of ofloxacin and ciprofloxacin against the most commonly identified bacterial pathogen, *Streptococcus pneumoniae*. Eradication of *S. pneumoniae* has in some studies been less than eradication of the *Haemophilus influenzae*, a more susceptible pathogen (21). Sparfloxacin, levofloxacin, grepafloxacin, and trovafloxacin have increased potency against *S. pneumoniae*, and comparative studies have documented the ability of each these agents to eradicate pneumococcal respiratory infections, some associated with bacteremia (22-24). Quinolone resistance has not been identified as a problem in *H. influenzae* or *S. pneumoniae* as yet, but a small number of resistant pneumococcal isolates have been reported from the UK (25). The spectrum of activity of these four newest quinolones like that of ciprofloxacin and ofloxacin also

covers atypical pneumonia pathogens such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* spp., and thus are being recommended and marketed for routine and empiric treatment of community-acquired pneumonia in the setting of rising penicillin and cephalosporin resistance in *S. pneumoniae* (26). There is, however, concern that extensive use of newer fluoroquinolones for community-acquired respiratory tract infections may promote increasing pneumococcal resistance to these agents, and a concern that their future application for pediatric uses may pose a particular risk because the reservoir of pneumococci resides in this younger population. The documented overuse of antibiotics for treatment of respiratory tract infections, particularly those of likely viral origin, may further exacerbate the potential for resistance (27).

Pneumonia acquired in the hospital usually in association with endotracheal intubation more commonly involves *S. aureus* and gram-negative bacilli. For this indication, ciprofloxacin in high dose has been shown to be comparable to imipenem (28). Responses, however, were less in the subgroups with infections due to *S. aureus* and *P. aeruginosa*, and these pathogens persisting in sputum often acquired quinolone resistance. Ciprofloxacin has also been widely used for treatment of respiratory exacerbations in patients with cystic fibrosis in whom *P. aeruginosa* is the most common respiratory pathogen (29). Clinical responses were comparable to other conventional regimens in patients with mild to moderate exacerbations, but repetitive uses was associated with rising resistance in *P. aeruginosa* isolated from sputum.

For mycobacterial infections, ciprofloxacin and ofloxacin have been used as second-line agents, particularly when needed for multidrug resistant strains. In comparative trials of multidrug regimens for pulmonary tuberculosis caused by susceptible strains of *M. tuberculosis*, rifampin appeared to be superior to ciprofloxacin, and ofloxacin appeared comparable to ethambutol (30; 31). Ciprofloxacin has been used in multidrug regimens that are active in AIDS patients with disseminated *M. avium-intracellulare* infection, but these regimens were inferior to clarithromycin-containing regimens (32).

Bone and joint infections. Ciprofloxacin is approved for treatment of bone and joint infections in the United States. In small comparative trials, ciprofloxacin and ofloxacin have produced similar results to broad-spectrum cephalosporins or combinations of gentamicin and penicillins (33-35). Most studies have been dominated by patients with mixed infections that include enteric gram-negative bacilli with overall provisional cure rates of about 75%, but patients with *S. aureus* and *P. aeruginosa* infections have responded similarly to the group as a whole. Infections associated with joint prostheses are difficult to cure without joint removal, but combinations of ofloxacin and rifampin given for 6-9 months have apparently cured as many as a half to two-thirds of patients with staphylococcal infections without joint removal (36). Failures have been associated with acquisition of resistance.

Skin and skin-structure infections. Ciprofloxacin, ofloxacin, levofloxacin, and trovafloxacin are all approved for this indication. Ciprofloxacin has been shown to be comparable to cefotaxime for cellulitis, wound infections, and infected skin ulcers, when mixed infections dominated gram-negative bacilli were the principal pathogens (37). Selection of quinolone resistance has been particularly problematic in this setting, and has been reported in a high proportion of staphylococci from diabetic patients treated with ciprofloxacin (38). For infections of the diabetic foot, additional coverage for anaerobic and gram-positive bacteria may be needed. Levofloxacin appears to have adequate

coverage of gram-positive cocci for skin and soft-tissue infections (39), and trovafloxacin with its broad activity including gram-positive cocci and anaerobes is only fluoroquinolone approved as single agent therapy of diabetic foot infections; published data are not yet available, however.

Systemic infections. In patients with fever and neutropenia, ciprofloxacin or ofloxacin alone may not be adequate for those patients who are most severely ill (40), but ofloxacin has been used safely in the outpatient management of carefully selected low-risk patients (41). Choice of a quinolone would not be appropriate for therapy if a quinolone had been used for prophylaxis because of problems with resistant *E. coli* bacteremias which have been seen at some centers in which patients routinely received quinolone prophylaxis during episodes of neutropenia (42-44). Ciprofloxacin in combination with rifampin has been used for treatment of patients with *S. aureus* right-sided endocarditis (45; 46). Quinolones or third-generation cephalosporins are now commonly used in many U.S. hospitals in place of aminoglycosides for gram-negative coverage in systemically ill patients in intensive care units. In some hospitals, there has been an increase in resistance to quinolones since their introduction, with the most commonly affected organisms being *S. aureus*, usually methicillin-resistant strains (MRSA), and *P. aeruginosa* (47).

Uses in prophylaxis or eradication of colonization. In a number of studies use of quinolones as prophylaxis in patients with neutropenia has been effective in reducing the occurrence of gram-negative bacteremia, but in bone marrow transplant recipients their use has also been associated with an increased incidence of viridans streptococcal bacteremia (40). In some cancer centers prophylactic use of quinolones has been associated with an increasing incidence of quinolone-resistant *E. coli* bacteremias (42-44). The organisms involved appear to be largely distinct strain types and to have multiple resistance mutations, suggesting sequential selection of endogenous flora. Eradication of nasopharyngeal colonization with *Neisseria meningitidis* has been accomplished by single doses of ciprofloxacin and ofloxacin (48), but attempts at eradication of nasal and skin carriage of MRSA have been largely unsuccessful and associated with selection of resistance (49). Secretion of fluoroquinolones in sweat may contribute to selection of resistance among staphylococci colonizing the skin (50). Only trovafloxacin has been approved for prophylaxis in colorectal and pelvic surgery in the United States. Data on this use are not yet published.

Epidemiologic features of quinolone resistance associated with human quinolone use. In many studies the single strongest risk factor for acquisition of quinolone resistance has been use of quinolones for either therapy or prophylaxis in individual patients, and in some studies there has been a dose-response relationship with increasing amounts of drug exposure correlating with increasing risk (42; 51; 52). Opportunity for spread of resistant organisms may also contribute in some cases, such as with infections caused by gonococci with reduced quinolone susceptibility that were shown to be less likely to be seen on gram staining of urethral specimens than fully susceptible isolates and thus may be more likely to go undetected and possibly untreated (8). Also for nosocomial pathogens such as *S. aureus*, which may be multidrug resistant, two additional factors may amplify quinolone resistance. First, these strains may be spread from patient to patient in the hospital environment, as supported by the observation that over time after the introduction of use ciprofloxacin in one hospital an increasing proportion of patients with ciprofloxacin-resistant MRSA had not received a ciprofloxacin and thus would not have selected a resistant strain from their own endogenous flora (53; 54). Second, once

ciprofloxacin resistance is acquired by an already multiply resistant strain of staphylococci, this strain may be easily amplified or selected by patient exposure to any of several antibiotics (51). In vitro studies and studies simulating drug concentration-time profiles in serum suggest that exposure of susceptible bacteria to peak drug concentrations in excess of 10-fold the MIC reduces the likelihood of selection of resistant subpopulations (55; 56), a finding that is likely related to the observation that single spontaneously occurring mutations usually cause an increment of resistance of 10-fold or less for current fluoroquinolones (57). Thus, such mutants in a population may still be killed by drug concentrations in excess of this amount. This principle would imply that use of high doses of fluoroquinolones for brief periods will carry a lower risk of resistance than lower doses used over prolonged periods. Inverse correlations have been made between the ratio of AUC (which is related to dose) of ciprofloxacin and MIC of the infecting pathogen and development of resistance in patients undergoing for treatment of lower respiratory tract infections (58).

Summary. The newer quinolones have been widely and in many cases effectively used in human medicine. Associated with their use (and perhaps overuse) has been the emergence of resistance in some organisms, *P. aeruginosa* and MRSA in particular. Resistance acquired during therapy has also occurred with campylobacters causing gastroenteritis. Resistance due to multiple mutations developing in initially highly susceptible pathogens such as *E. coli* and gonococci was initially surprising and may have resulted from intense selection pressures, reservoirs for persistence of organisms with intermediate levels of resistance, and person-to-person spread. As expanded indications for treatment of community-acquired respiratory tract infections are developed with the newest quinolones with enhanced potency against pneumococci, (including penicillin-resistant strains), the challenge will be to guide the appropriate targeting of their usage to minimize the risks of development of resistance in pneumococci.

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Mechanisms of Quinolone Resistance

Attachment C

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Bacterial mechanisms of resistance to quinolones can be divided into two general categories, alterations in drug target enzymes and alterations in drug permeation that affect drug access to these target enzymes. As yet, no specific quinolone-modifying enzymes have been identified as causing resistance, although certain fungi are capable of degrading quinolones through metabolic pathways (1).

Modifications of drug target enzymes

DNA gyrase. DNA gyrase, one of two targets of quinolones, is an essential bacterial enzyme responsible for introducing negative superhelical twists into bacterial DNA (2; 3). It is also capable of removing both negative and positive DNA superhelical twists. Negative supercoiling of DNA catalyzed by DNA gyrase is necessary for initiation of DNA replication, and positive supercoils that accumulate ahead of the DNA replication fork would impede fork propagation if not removed by DNA gyrase. The enzyme is composed of two A (GyrA) and two B (GyrB) subunits encoded by the *gyrA* and *gyrB* genes, respectively. Shortly after the discovery of *Escherichia coli* DNA gyrase, resistance to nalidixic acid was shown to be caused by mutations in the *gyrA* gene (4). Subsequently many studies in a wide range of gram-negative bacteria have identified amino acid changes in the GyrA and GyrB subunits that cause quinolone resistance or reductions in activity (5). These alterations result from single nucleotide changes in *gyrA* or *gyrB* that occur as spontaneous mutations and are selected by exposure to quinolones. There are some data indicating that quinolone exposure is mutagenic for bacteria and may thereby increase the frequency with which resistance mutations occur (6).

Resistance mutations have been clustered in the amino terminus [usually between amino acids 67 and 106 based on numbering in *E. coli*, the "quinolone-resistance-determining region (QRDR)] of GyrA near the active site tyrosine at position 122 (7). The two most common single sites of change are at positions 83 and 87. Quinolones bind specifically to the complex of DNA gyrase with DNA rather than DNA gyrase alone, and alterations at position 83 have been associated with reduced drug binding to this complex (8). The recently reported x-ray crystallographic structure of a fragment of GyrA localizes the QRDR to a positively charged surface along which DNA is thought to bind (9). Thus, a common model envisions that amino acid changes in the QRDR of GyrA alter the structure of the site of quinolone binding near the interface of the enzyme and DNA and that resistance is then caused by reduced drug affinity for the modified enzyme-DNA complex. Direct structural information on the site of quinolone binding within the complex is as yet lacking, however.

Alterations in the GyrB subunit also cause reductions in quinolone susceptibility but usually to a lesser degree than the most common GyrA mutations (10). These mutations have been clustered in the mid-portion of the GyrB amino acid sequence. There has been no reported crystallographic structure of GyrB that includes this region, but the homologous region of the crystal structure of yeast topoisomerase II enzyme is distant

from the region homologous to the QRDR of GyrA (11), suggesting that this QRDR region of GyrB may not be directly involved in a putative quinolone binding site and that the molecular mechanism of resistance caused by alterations in GyrB may differ from that caused by alterations in GyrA.

Topoisomerase IV. Topoisomerase IV is also a quinolone target within bacterial cells. This enzyme, like DNA gyrase, is essential for DNA replication, but its role appears primarily to be the decatenation or unlinking of daughter chromosomes at the completion of a cycle of DNA replication to allow their segregation into daughter cells (12). Topoisomerase IV has a structure similar to that of DNA gyrase and is composed of two ParC (or GrlA in *Staphylococcus aureus*) and two ParE (GrlB in *S. aureus*) subunits (13; 14). ParC is homologous to GyrA, and ParE is homologous to GyrB. Particularly highly conserved is the QRDR homologous region of ParC.

Resistance mutations in ParC similar to those in GyrA have been clustered in the equivalent QRDR region, with the most common mutations occurring at positions 80 and 84 (*E. coli* numbering) (13; 15), although additional mutations outside this region near the active site have been described (16). Resistance mutations in ParE have also been identified in regions homologous to those causing resistance in GyrB (17). There have as yet been no studies of drug binding to topoisomerase IV-DNA complexes and no crystallographic structure of topoisomerase IV reported, but the similarities in overall subunit structure and amino acid sequence between topoisomerase IV and DNA gyrase suggest that the models of these two enzymes will be similar.

Relative roles of the two target enzymes in resistance and stepwise incremental resistance. In *E. coli*, genetic studies have clearly demonstrated that *gyrA* (and *gyrB*) mutations alone can cause quinolone resistance or reduced susceptibility. In contrast, *parC* (and *parE*) mutations alone have no effect on drug susceptibility (18). Double mutants with both *gyrA* and *parC* mutations, however, have higher levels of resistance than the same *gyrA* mutant alone. For *S. aureus*, this pattern is reversed. Mutations in *grlA* or *grlB* alone can cause quinolone resistance, and *gyrA* mutations only affect susceptibility when they occur together with *grlA* or *grlB* mutations (16; 19). These patterns can be best understood in terms of the relative sensitivities of the two target enzymes to a given quinolone. In the case of *E. coli*, purified DNA gyrase is more sensitive to most quinolones than is purified topoisomerase IV, and the reverse is true for the two enzymes purified from *S. aureus* (20). Thus, mutations in the most sensitive target enzyme contribute to first-step resistance. Mutations in the less sensitive enzyme alone have no effect on susceptibility because quinolone interaction with the more sensitive enzyme causes cell death regardless of the drug affinity status of the less sensitive enzyme. The primary target of a particular quinolone in a particular species then is determined by which enzyme is more sensitive to that quinolone. Patterns have emerged indicating that for current quinolones for most species of gram-negative bacteria DNA gyrase is the primary drug target and for many species of gram-positive bacteria topoisomerase IV is the primary target (21). Exceptions do occur, however, in that the primary target of sparfloxacin in *Streptococcus pneumoniae* is DNA gyrase, indicating that relative targets are determined by drug structure (22).

Differences in quinolone sensitivity between DNA gyrase and topoisomerase IV have implications for risks of resistance development. With the occurrence of a resistance mutation in the more sensitive target enzyme, the level of susceptibility of this first-step mutant is determined either by the degree of alteration in primary target enzyme

sensitivity determined by the particular mutation or the intrinsic level of resistance of the secondary target, whichever is less. Thus, the closer the levels of quinolone sensitivity of the two enzymes are to each other, the lower the increase in resistance that can occur with a first-step mutation in the primary target enzyme. This principle implies that the drug concentration above which two mutations will be required to select resistance will decrease as the level of concordance of sensitivity of the two enzymes increases. Furthermore, the extrapolation of this principle implies that drugs with potent and equal activity against both enzymes will have exceptionally low levels of resistance related to altered enzyme targets, since mutations in the genes of both enzyme targets must occur concurrently for initial resistance by target modification to occur. With many current quinolones, however, there appear to be sufficient differences in sensitivity of the two target enzymes in many species for stepwise resistance to be selected. In which case highly resistant isolates can be selected sequentially with increasing or repeated quinolone exposure, resulting in accumulating mutations in *gyrA* and *parC*; in the most resistant isolates mutations have numbered two or more in each gene (23).

Alterations in quinolone permeation

Alterations in the outer membrane and efflux systems. In gram-positive bacteria, quinolones must traverse the cytoplasmic membrane, and in gram-negative bacteria they must traverse both the cytoplasmic membrane and the outer membrane to reach their topoisomerase targets. For many quinolones their size and zwitterionic charge configuration enhance their ability to diffuse across porin channels in the outer membrane (24). Reductions in porins have been associated with quinolone resistance, but more detailed studies have suggested that seldom is the level of reduction in diffusion by porin change alone sufficient to account for resistance and reductions in steady-state drug accumulation in growing bacteria (25). Increasingly recognized has been the common occurrence of endogenous efflux systems in many species of bacteria (26). These efflux systems are composed of a protein pump present in the cytoplasmic membrane either alone in gram-positive bacteria or linked to other proteins that span the periplasm and outer membrane in gram-negative bacteria. The pumps that have been shown to affect quinolone susceptibility belong to the major facilitator class of multidrug (MDR) pumps in gram-positive bacteria and the RND class in gram-negative bacteria (27). These MDR pumps are energized by proton motive force across the membrane and have broad substrate profiles. Intrinsic resistance in *Pseudomonas aeruginosa* and resistance in Mar mutants of *E. coli* associated with reductions in porin channels have been shown to dependent on intact MDR pumps such as the MexAB-OprM system in *P. aeruginosa* and the AcrAB-TolC system in *E. coli* (28; 29). In gram-positive bacteria, mutations causing hyperexpression of the NorA pump of *S. aureus* cause low-level resistance to some quinolones (30).

Quinolones appear to differ in the extent to which they are substrates for certain efflux pumps. In the case of NorA of *S. aureus* in particular, quinolones with greater hydrophobicity and other properties are less affected by hyperexpression of NorA (31). Similar correlations have been made for the several efflux systems identified in *P. aeruginosa* (32). The normal physiologic functions of these MDR pumps are not yet certain, but they are thought generally to function to remove toxins from the cell (33). Because quinolones are synthetic antimicrobials, they presumably played no direct role in the evolution of MDR pumps in Nature but are accidental substrates.

Contribution of efflux pumps to quinolone resistance. It is unclear the extent to which NorA-hyperexpressing mutants of *S. aureus* or Mar mutants of *E. coli* contribute to

quinolone resistance in clinical isolates (34). But Mar mutants exhibit pleiotropic resistance (including tetracycline and chloramphenicol) and can be readily selected with tetracycline in the laboratory (35). In many cases in vitro, initial selections for quinolone resistance result in mutations in topoisomerase genes, but with *P. aeruginosa* pleiotropic mutants likely due to altered permeation are readily selected (36). In gram-positive bacteria, regulation of expression of some MDR pumps has been demonstrated. Mutants with increased expression of NorA induced by exposure to the quinolone norfloxacin have been described, implying regulation of expression (37). The factors that normally regulate the expression of these pumps, however, is unclear. It remains possible that the role of various MDR pumps in quinolone resistance may be more insidious, with physiologic increased expression under certain conditions of growth in vivo that results in reductions in the quinolone activity that would not be apparent by usual testing in vitro. Since the frequency of selection of resistant mutants decreases with the increasing ratio of quinolone concentration to MIC, physiologic increases in MIC in vivo due to increased MDR pump expression might contribute to higher frequencies of resistance selection in vivo (38).

Transmissability of quinolone resistance

The mechanisms of quinolone resistance described above all result from chromosomal mutations and not from acquired genes carried on plasmids. In merodiploid strains constructed in the laboratory quinolone-resistance alleles of *gyrA*, *gyrB*, *parC*, and *parE* are generally either recessive or codominant to their wildtype (susceptible) counterparts, but hyperexpression of resistance alleles on plasmids may confer some level of resistance (17; 18; 39). The *norA* gene cloned on a plasmid in the laboratory can also confer quinolone resistance (31). Hyperexpression of any of these genes, however, may be toxic to the cell, and plasmid-mediated resistance by any of these mechanisms has not been described in clinical isolates. Recently, however, plasmid-mediated quinolone resistance was described for the first time in clinical isolates of *Klebsiella pneumoniae* (40). Resistance was also expressed upon transfer to *E. coli* in the laboratory. The mechanism of this resistance is not yet known nor is it clear the extent to which this type of resistance occurs in clinical resistant isolates.

Thus, in most cases a high prevalence of quinolone resistance appears to represent either selection of resistance due to chromosomal mutations in endogenous flora associated with intense quinolone exposure or spread of resistant strains, rather than spread of plasmids. In clinical isolates, the occurrence of high levels of resistance caused by multiple mutations implies the opportunity for repetitive drug exposures and the presence of reservoirs for organisms in which strains with the initial mutations may persist and be the source for selection of incrementally resistant strains with additional mutations upon further exposure to quinolones.

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