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In Vitro Antimicrobial Susceptibility of Bacterial Enteropathogens Isolated from International Travelers to Mexico, Guatemala, and India from 2006 to 2008[▽]

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The incidence rates of travelers' diarrhea (TD) have remained high for the last 50 years. More recently, there have been increasing recommendations for self-initiated therapy and use of prophylactic drugs for TD. We last examined the *in vitro* susceptibilities of commonly used antibiotics against TD pathogens in 1997. We now examine 456 enteropathogens isolated from adult travelers to Mexico, India, and Guatemala with diarrhea acquired between 2006 and 2008 to determine changes in susceptibility against 10 different antimicrobials by the agar dilution method. Traditional antibiotics, such as ampicillin, trimethoprim-sulfamethoxazole, and doxycycline, continue to show high levels of resistance. Current first-line antibiotic agents, including fluoroquinolones and azithromycin, showed significantly higher MICs than in our earlier study, and MIC_{90} levels were above the Clinical and Laboratory Standards Institute cutoffs for resistance. There were significant geographical differences in resistance patterns when Central America was compared with India. Entertoxigenic *Escherichia coli* (ETEC) isolates showed increased resistance to ciprofloxacin ($P = 0.023$) and levofloxacin ($P = 0.0078$) in India compared with Central America. Enteroaggregative *E. coli* (EAEC) isolates from Central America showed increased resistance to nearly all of the antibiotics tested. Compared to MICs of isolates 10 years prior, there were 4- to 10-fold increases in MIC_{90} values for ceftriaxone, ciprofloxacin, levofloxacin, and azithromycin for both ETEC and EAEC. There were no significant changes in rifaximin MICs. Rising MICs over time imply the need for continuous surveillance of susceptibility patterns worldwide and geographically specific recommendations in TD therapy.

Globally, 40% of travelers crossing from industrialized to developing countries develop diarrhea (10). The United Nations World Tourism Organization reported 880 million international tourist arrivals in 2009; 45% of these arrivals were in developing countries (28). This results in an estimated 160 million new cases of travelers' diarrhea (TD) annually. Travel to high-risk areas, including southern Asia, has resulted in 2-week incidence rates of over 60% (16). Unfortunately, these incidence rates have remained relatively unchanged over the last half century (3, 10, 24).

Bacterial pathogens have been implicated as the causal agents in more than 80 to 90% of TD cases (1, 2, 23). Entertoxigenic *Escherichia coli* (ETEC) strains have been isolated in the majority of cases, accounting for up to 76% of all isolated pathogens (16). Other bacterial causal agents include enteroaggregative *E. coli* (EAEC), *Campylobacter jejuni*, *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Plesiomonas* spp., *Vibrio* spp., enterotoxigenic *Bacteroides fragilis*, and *Acrobacter* spp. (2, 16, 23).

In the past, antimicrobial therapy was reserved for travelers who had developed acute diarrhea and was initiated by a physician (12). More recently, there has been an emphasis on self-initiated therapy without physician consultation (7). Some experts argue for antimicrobial prophylaxis of TD (6). Antimicrobial prophylaxis is an effective strategy for TD prevention; however, safety, drug resistance, and efficacy against prevalent pathogens must be continuously monitored (7).

Historically, ampicillin, doxycycline, and trimethoprim-sulfamethoxazole were used for treatment of TD, but because of increasing resistance, these drugs have become less effective (11, 20). Currently, ciprofloxacin and azithromycin are the mainstays of antimicrobial therapy for TD and are indicated for moderate to severe disease to reduce the duration of illness (13). More recently, rifaximin, a semisynthetic, poorly absorbed, broad-spectrum antibiotic with minimal effects on gut flora (9), has been added for the treatment of noninvasive forms of TD (8).

For many years, our team has been collecting stool samples from travelers with and without diarrhea while studying abroad in two Mexican cities: Guadalajara and Cuernavaca. In addition, we studied acute TD developed in two cities in India (Goa and Kolkata), and we have worked in Antigua, Guatemala. This study aimed to evaluate antibiotic susceptibility and resistance trends that may have changed over the last decade.

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TABLE 1. Bacterial enteropathogens isolated from subjects with travelers' diarrhea in Mexico, Guatemala, and India and studied for *in vitro* susceptibility to antimicrobial agents, 2006 and 2008

Pathogen	No. of strains				% of total no. of isolates
	Mexico	Guatemala	India	Total	
ETEC	245	25	98	368	81
EAEC	17	3	3	23	5
Aeromonas spp.	1	0	3	4	1
Campylobacter spp.	5	1	17	23	5
Plesiomonas spp.	2	0	8	10	2
Salmonella spp.	10	0	5	15	3
Shigella spp.	2	0	11	13	3
Total	282	29	145	456	

TABLE 2. Susceptibilities (MIC_{50} and MIC_{90}) of 456 enteropathogens isolated from travelers' diarrhea studied in Mexico, Guatemala, and India, 2006 to 2008

Antibiotic	BP[a] (μg/ml)	% R[b]	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
AMP	≥32	45.2	0.5–>1,024	8	>1024
NAL	≥32	41.8	0.015–>1,024	8	>1024
TET	≥16	55.3	0.015–>1,024	64	256
DOX	≥16	50.2	0.015–512	16	128
T/S	≥8/152	50	0.015–>1,024	2	128
CFO	≥32	4.4	0.015–>1,024	0.06	0.25
RIF	≥32	15.3	0.015–>1,024	8	32
CIP	≥4	17.2	0.015–>1,024	0.015	128
LEV	≥8	21	0.015–128	0.06	8
AZM	≥8[c]	16.7	0.015–512	2	32

[a] BP, CLSI breakpoint (resistant strains are at or above the BP, and susceptible strains are below the breakpoint).
[b] % R, percentage of isolates considered resistant based on CLSI breakpoints.
[c] Based on established values for nonenteric bacterial pathogens.

More specifically, this study examined the potential increases in ciprofloxacin, rifaximin, and azithromycin resistance, given that these are currently the three antibiotics most commonly used to treat TD.

MATERIALS AND METHODS

From 2006 to 2008, stool samples were collected from adult (≥18-year-old) travelers with diarrhea acquired in Guatemala (Antigua), India (Goa and Kolkata), and Mexico (Cuernavaca or Guadalajara). TD was defined as ≥3 loose stools in 24 h associated with at least one other symptom of enteric infection, such as nausea, vomiting, abdominal pain or cramps, fecal urgency (tenesmus), or dysentery. A total of 456 bacterial isolates were identified as one of the following: ETEC, EAEC, *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Pleisiomonas* spp., or *Campylobacter* spp. Each isolate was identified by previously described microbiological methods, including DNA hybridization for ETEC (19) and HEp-2 adherence assay for EAEC (18). The distribution of the enteropathogens by geographic site is shown in Table 1.

The following antibiotics were evaluated: ampicillin (AMP) (Sigma-Aldrich, St. Louis, MO), nalidixic acid (NAL) (Sigma-Aldrich), tetracycline (TET) (MP Biomedicals, Solon, OH), doxycycline (DOX) (Sigma-Aldrich), trimethoprim-sulfamethoxazole (T/S) (Sigma-Aldrich), ceftriaxone (CFO) (Sigma-Aldrich), rifaximin (RIF) (Sigma-Aldrich), ciprofloxacin (CIP) (Sigma-Aldrich), levofloxacin (LEV) (Sigma-Aldrich), and azithromycin (AZM) (Pfizer Inc., Brooklyn, NY). T/S was mixed in a trimethoprim-to-sulfamethoxazole ratio of 1 to 19 (4). The MICs of 10 antimicrobial agents were determined by the agar dilution method as standardized by the CLSI (4).

Each isolate was tested at the following dilutions of each antibiotic: 1,024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, and 0.015 μg/ml. Non-*Campylobacter* isolates were incubated on Mueller-Hinton (MH) agar plates at 35°C for 16 h, while *Campylobacter* isolates were incubated on MH agar with 7% lysed sheep blood at 42°C for 48 h under a microaerobic atmosphere, including carbon dioxide. Control strains of *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), and *Staphylococcus aureus* (ATCC 29213) were used for quality control. The MIC_{50} and MIC_{90} were calculated as the MIC at which 50 and 90% of the isolates for each organism were inhibited. Statistical analysis was performed using the *Z* test for comparison of proportions and the chi-square test with the level of significance established at a *P* value of ≤0.05.

RESULTS

In Table 2, the MIC_{50} and MIC_{90}, the range of MICs, and the proportion of each organism that was resistant based on the CLSI breakpoints are shown (breakpoints for AZM have not been established for enteric bacteria). CFO was the only antibiotic that displayed high *in vitro* activity toward the enteropathogens and was below the CLSI breakpoint for both 50% and 90% of all isolates. Conventional antibiotics, including AMP, TET, NAL, and T/S, all had MIC_{90}s greater than 4 times the breakpoint level, placing 40 to 50% of the isolates in a "resistant" category. The fluoroquinolones, CIP and LEV, were highly active when susceptibility of 50% of the organisms (MIC_{50}) was considered. However, the MIC_{90} for CIP and LEV were 128 μg/ml and 8 μg/ml, respectively, both beyond the CLSI breakpoint for susceptibility. RIF had a similar pattern, with the MIC_{50} being below the breakpoint while the MIC_{90} was just at the cutoff of 32 μg/ml. All of the antimicrobial agents had some isolates that were resistant based on CLSI breakpoints, ranging from 4.4% to 55.3%.

Susceptibilities to each of the antibiotics by pathogen are displayed in Table 3 in an attempt to identify any pathogen-specific differences in susceptibilities. The *Campylobacter* isolates were susceptible to all of the antibiotics tested. The MIC_{90} values were all below the CLSI breakpoint levels, except for RIF, where 22% of the isolates had a MIC of ≥32 μg/ml. In addition, the fluoroquinolones showed high activity against all of the *Campylobacter* isolates. For ETEC, traditional antibiotics, AMP, TET, DOX, and T/S, all showed poor *in vitro* activity, with the proportion resistant ranging from 47 to 52%. CIP, LEV, and AZM, all commonly used to treat TD, showed moderate *in vitro* activity, with MIC_{90}s for CIP 5-fold higher than the set breakpoint and the MIC_{90}s for LEV and AZM right at the cutoff. Approximately 1 in 5 (18 to 20%) of the ETEC isolates had MICs greater than the set breakpoint. Rifaximin showed moderate activity, with a MIC_{90} of 32 μg/ml and 16.6% of isolates higher than the breakpoint. Again, CFO was the only antibiotic with low MICs. EAEC displayed a similar pattern of resistance to AMP, NAL, TET, DOX, and T/S. However, there was also greater resistance to the newer agents, CIP, LEV, AZM, and CFO, than ETEC. Only RIF showed complete sensitivity, with an MIC_{90} of 16 μg/ml. *Salmonella* and *Shigella* isolates were both highly resistant to TET and DOX, with 100% of isolates above the breakpoint. *Shigella* isolates also demonstrated high (100% above breakpoint) resistance to T/S. All other antibiotics, most notably CIP and CFO, had high *in vitro* activities for both. Finally, *Aeromonas* sp. isolates were highly susceptible to all antibiotics tested.

Next, the isolates were separated by geography to determine if there were any differences in resistance patterns based on location. Isolates from Mexico and Guatemala were combined to form one group, Central America (Table 4), and compared

TABLE 3. Susceptibilities (MIC_{50} and MIC_{90}) of bacterial enteropathogens isolated from subjects with travelers' diarrhea studied in Mexico, Guatemala, and India, 2006 to 2008

Antibiotic	*C. jejuni* (n = 23)[a]			ETEC (n = 365)			EAEC (n = 23)		
	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)
AMP	4	4	1–4 (0)[b]	64	>1,024	1–>1,024 (52)	16	>1,024	2–>1,024 (52)
NAL	8	16	0.015–16 (0)	8	>1,024	0.015–>1,024 (47)	32	>1,024	1–>1,024 (56)
TET	2	2	0.25–4 (0)	128	256	0.015–>1,024 (58)	2	256	0.5–256 (48)
DOX	2	4	0.015–4 (0)	16	128	0.015–512 (51)	64	256	1–512 (52)
T/S	1	1	0.5–2 (0)	4	128	0.015–>1,024 (49)	>1,024	>1,024	0.5–>1,024 (59)
CFO	0.03	0.06	0.03–0.125 (0)	0.06	0.25	0.015–512 (5)	0.06	64	0.015–1,024 (18)
RIF	16	32	0.015–32 (22)	8	32	0.25–32 (17)	8	16	4–16 (0)
CIP	0.015	0.03	0.015–0.03 (0)	0.06	128	0.015–>1,024 (20)	0.03	512	0.015–512 (30)
LEV	0.06	0.125	0.015–0.125 (0)	0.125	8	0.015–128 (24)	0.25	128	0.015–128 (39)
AZM	0.5	1	0.03–2 (0)	2	32	0.03–512 (18)	1	64	0.25–64 (33)

[a] n, total number of isolates studied.
[b] The numbers in parentheses represent the percentages of isolates with MICs indicating resistance based on the CLSI breakpoints.

to isolates from India. The comparisons were based on geography for the *Campylobacter*, ETEC, and EAEC isolates only. No differences were noted in the remaining isolates. *Campylobacter* isolates were more resistant to RIF in India (29.4%) as opposed to 0% resistant in Central America. None of our *Campylobacter* isolates displayed resistance to either of the fluoroquinolones, as has been described in Thailand (14). There were significantly higher resistance rates for ETEC to NAL ($P < 0.001$), T/S ($P = 0.047$), CIP ($P = 0.023$), and LEV ($P = 0.0078$) in India than in Central America. Furthermore, 15.5% of the ETEC isolates from Central America and 19.6% from India had an MIC_{90} greater than the CLSI cutoff for RIF. Finally, among the EAEC isolates, there was evidence of resistance to all antibiotics except RIF in Central America, whereas in India, EAEC displayed no resistance, except to NAL.

Our data were compared to those for isolates from 1997 (11) to determine if MIC levels have changed over time (Table 5). There was at least a 5-fold increase in the MIC_{90} for CFO susceptibilities for both ETEC (MIC_{90} = 0.25), which is still highly active, and EAEC (MIC_{90} = 64), which is less active. Furthermore, the MIC_{90}s for CIP and AZM for both ETEC and EAEC increased more than 10-fold and for LEV by 4-fold for ETEC and 10-fold for EAEC. Finally, there was no evidence of increasing MIC_{90}s for CIP and LEV for the *Campylobacter* isolates or for RIF for any of the organisms. Interestingly, there was evidence of decreasing MIC_{90}s for T/S by 3-fold over the last 10 years.

DISCUSSION

TD continues to be an important problem, as there are increasing numbers of international travelers to developing countries, where the prevalence of diarrhea has not changed for many years. ETEC continues to be the most common bacterial cause, representing over 80% of the bacteria isolated in this study. Similarly, *Campylobacter* was more commonly isolated in Asia and EAEC more commonly in Latin America, although the total number of each isolated was relatively small.

When the enteropathogens were grouped together, it was

TABLE 4. Susceptibilities (MIC_{90}) of bacterial enteropathogens isolated from subjects with travelers' diarrhea studied in India versus Mexico and Guatemala, 2006 to 2008

Antibiotic	BP[a]	India						Mexico and Guatemala					
		Campylobacter (n = 17)[b]		ETEC (n = 98)		EAEC (n = 3)		*Campylobacter* (n = 6)		ETEC (n = 270)		EAEC (n = 20)	
		MIC_{90} (μg/ml)	% R[c]	MIC_{90} (μg/ml)	% R	MIC_{90} (μg/ml)	% R	MIC_{90} (μg/ml)	% R	MIC_{90} (μg/ml)	% R	MIC_{90} (μg/ml)	% R
AMP	≥32	4	0	>1,024	49.4	4	0	4	0	>1,024	52.8	>1,024	60
NAL	≥32	16	0	>1,024	71.1	128	66.7	16	0	>1,024	38.5	>1,024	55
TET	≥16	2	0	256	52.5	1	0	2	0	256	59.2	256	55
DOX	≥16	4	0	128	48.5	4	0	2	0	64	51.9	256	60
T/S	≥8/152	1	0	64	58.9	1	0	2	0	128	46	>1,024	65
CFO	≥32	0.06	0	0.5	6.2	0.06	0	0.06	0	0.25	4.8	64	20
RIF	≥32	32	29.4	32	19.6	8	0	16	0	32	15.5	16	0
CIP	≥4	0.03	0	256	27.8	0.25	0	0.03	0	64	17.5	512	35
LEV	≥8	0.125	0	8	40.8	0.5	0	0.06	0	8	20.1	128	45
AZM	≥8[d]	2	0	32	24.5	2	0	1	0	32	16.1	64	40

[a] BP, CLSI breakpoint (resistant strains are at or above the BP, and susceptible strains are below the breakpoint).
[b] n, total number of isolates studied.
[c] % R, percent resistant, i.e., the MIC was above the CLSI breakpoint.
[d] Based on established values for nonenteric bacterial pathogens.

TABLE 3—*Continued*

Plesiomonas spp. (n = 10)			*Salmonella* spp. (n = 15)			*Shigella* spp. (n = 13)			*Aeromonas* spp. (n = 4)		
MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)
1	2	0.5–2 (0)	2	2	1–2 (0)	8	128	2–128 (38)	4	4	4 (0)
0.5	1	0.25–1 (0)	4	4	4 (0)	4	64	1–64 (47)	0.125	0.25	0.125–0.25 (0)
0.25	0.25	0.25 (0)	128	128	128 (100)	128	128	64–128 (100)	0.25	0.25	0.25 (0)
0.015	0.06	0.015–64 (10)	128	128	128 (100)	64	64	64–128 (100)	0.015	0.015	0.015 (0)
0.5	0.5	0.5 (0)	0.5	0.5	0.5–1 (0)	64	64	64 (100)	0.5	0.5	0.5 (0)
0.015	0.015	0.015 (0)	0.06	0.06	0.06 (0)	0.015	0.06	0.015–0.125 (0)	0.015	0.015	0.015 (0)
8	8	8 (0)	8	8	8–16 (0)	16	16	8–16 (0)	8	8	8 (0)
0.015	0.015	0.015 (0)	0.015	0.015	0.015 (0)	0.125	0.125	0.015–0.125 (0)	0.015	0.015	0.015 (0)
0.015	0.015	0.015–0.125 (0)	0.03	0.03	0.03 (0)	0.03	0.25	0.015–0.25 (0)	0.015	0.015	0.015 (0)
0.015	0.015	0.015–0.125 (0)	1	1	1 (0)	1	2	1–2 (0)	0.015	0.015	0.015 (0)

evident that there is still high resistance to the antibiotics historically used to treat TD: DOX, TET, T/S, and AMP. More worrisome is the fact that the antibiotics that are currently used to treat TD, the fluoroquinolones and azithromycin, are showing an increase in resistance based on CLSI breakpoints (nonenteric breakpoints for AZM were used). There is still controversy regarding whether CLSI breakpoints, which are based on achievable levels of antibiotics in serum, can be correlated with the clinical response in enteric infections, particularly for the drugs that concentrate in the gut, such as rifaximin. Many antibiotics concentrate in the intestine at much higher levels than are achieved systemically. For instance, intestinal concentrations of fluoroquinolones have been shown to exceed 500 μg/ml (5). Furthermore, after 3 days of therapy, fecal concentrations of RIF up to 8,000 μg/ml have been reported (17). Therefore, *in vitro* resistance based on CLSI cutoffs may not correlate with the clinical response of noninvasive diarrhea. Regardless, the MIC_{90} for CIP was 5-fold greater than the CLSI cutoff, which is higher than that seen in previous studies (11, 14). CFO was the only antimicrobial agent that retained MICs well below the CLSI breakpoint, but this agent, which requires parenteral administration, is not practical for travelers.

Further differentiation of the organisms into individual species revealed similar patterns of resistance. ETEC, which is the most commonly isolated bacterium, displayed surprisingly higher MICs for both the fluoroquinolones and AZM. These antimicrobial drugs are available at local pharmacies without a prescription in many areas of the world. Furthermore, both are used in other clinical settings, such as treatment of urinary tract infections or upper respiratory infections. When stratified by geographical location, a significantly larger proportion of ETEC isolates were resistant in the cases of TD occurring in India as opposed to Central America. Fluoroquinolones are commonly used in India for empirical treatment of typhoid fever and other enteric infections. Furthermore, AZM has been the first-line therapy for TD in Southeast Asia given the growing resistance of *Campylobacter* to fluoroquinolones. It is possible that these factors contributed to the development of higher MICs. Furthermore, it is unclear what effect the use of antibiotics in animal husbandry may have on bacterial resistance. Within the last decade, ETEC strains that were considered CIP resistant increased over 20 times, from 1% (11, 14) to nearly 28% in this study. Clearly, continued aggressive and frequent monitoring of antibiotic sensitivities is crucial, as resistance in enteric bacterial pathogens is increasing at a high rate.

EAEC is one of the newer subtypes of diarrhea-producing *E. coli* that has been implicated in enteric infections; it has been most notably reported among the Latin American countries but is found worldwide (1). Based on this study, overall, EAEC strains are more resistant to the newer agents (CIP, LEV, and

TABLE 5. Susceptibilities (MIC_{90}) of bacterial enteropathogens isolated from subjects with travelers' diarrhea studied in 1997 (11) versus 2006 to 2008

Antibiotic	MIC_{90} (μg/ml)									
	C. jejuni		ETEC		EAEC		*Salmonella* spp.		*Shigella* spp.	
	1997 (n = 9)	2006–2008 (n = 23)	1997 (n = 97)	2006–2008 (n = 368)	1997 (n = 75)	2006–2008 (n = 23)	1997 (n = 46)	2006–2008 (n = 15)	1997 (n = 36)	2006–2008 (n = 13)
AMP	64	4	>1,024	>1,024	>1,024	>1,024	4	2	512	128
NAL	4	16	256	>1,024	64	>1,024	16	4	8	64
DOX	64	4	64	128	128	256	128	128	128	64
T/S	128	1	>1,024	128	>1,024	>1,024	512	0.5	>1,024	64
CFO	2	0.06	≤0.0156	0.25	0.0312	64	0.125	0.06	0.0312	0.06
RIF	32	32	32	32	32	16	64	8	64	16
CIP	0.0625	0.03	0.25	128	0.25	512	0.0312	0.015	0.0312	0.125
LEV	0.25	0.125	1	8	1	128	0.25	0.03	0.25	0.25
AZM	0.25	1	≤0.0156	32	≤0.0156	64	1	1	0.5	2

AZM) than ETEC strains. When stratified by location, all of the resistant strains were from Latin America. Finally, compared to 10 years ago, there was an increase in MIC_{90}s beyond the CLSI breakpoint for all drugs currently used to treat TD. Further study is needed to identify why EAEC strains have consistently higher MICs. Of note, our study included only 23 EAEC samples, and thus, more strains are needed to further determine antimicrobial resistance among EAEC strains. RIF was the only antibiotic that remained active against EAEC strains, and the drug has been shown to effectively treat EAEC diarrhea in travelers (15).

In conclusion, it is clear that continued frequent monitoring of MICs is necessary for the major pathogens causing TD. In the last 10 years, there is evidence of significant increase in MICs for all of the most common antibiotics that are currently used for TD treatment. It is imperative to further evaluate the pharmacokinetics of these antibiotics in the intestine, as CLSI breakpoints do not appear to correlate with clinical failure of TD treatment. As the number of international travelers increases, the number of TD cases will also increase, as will the chronic effects (21, 22, 25–27). Differences in resistance patterns require geographically specific recommendations and surveillance. Increasing fluoroquinolone resistance may make it a less ideal treatment and prophylaxis option. Therefore, nonabsorbable drugs, such as rifaximin, may be a better alternative, but they have limitations in the setting of invasive disease, although strict monitoring of MICs over time is still needed.

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