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Risk Factors for Fecal Quinolone-Resistant *Escherichia coli* in Mexican Children

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We determined the prevalence of, and risk factors for, fecal quinolone-resistant *Escherichia coli* (QREC) in 324 children from Yucatan, Mexico. QREC was higher in children with recent *Salmonella* infection (100%) than in children with diarrhea (61%) or healthy children (54%) ($P = 0.007$). Multivariate analysis identified recent hospitalization of a family member ($P = 0.011$, odds ratio [OR] = 5.1) and carriage of *Salmonella* ($P = 0.004$, OR = 3.7) as independent risk factors for QREC.

The use of antibiotics for food animal production has stirred great controversy in the scientific community (13, 15, 19, 20). It is widely accepted that antibiotics used in veterinary medicine present cross-resistance with those of the same class used for human therapeutics and that antibiotic-resistant food-borne bacteria, or their resistance determinants, may be transferred from animal reservoirs to humans (17, 18, 19). However, there is considerable debate on the frequency of such transfer and the human health impact of such an event (13, 17). Moreover, studies that measure the individual contribution of human and veterinary antibiotic usage to antibiotic resistance in human food-borne disease are lacking (13, 20).

Poultry and swine production are major economic activities in Yucatan, Mexico. Fluoroquinolones, such as enrofloxacin, are widely used, but precise information on the indications, dosage, and total quantity of antimicrobials are not available. *Escherichia coli* is a useful indicator of the selective pressure exerted by antibiotic usage in a particular environment (14, 17). In this study, we used fecal quinolone-resistant *E. coli* (QREC) in children as an indicator of the selective pressure from quinolone and fluoroquinolone use in Yucatan. Children under 13 years of age were selected since they rarely, if ever, receive quinolones or fluoroquinolones for treatment.

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The study was conducted from September to December 2000 in Yucatan, Mexico. Fecal samples were collected from children with acute community-acquired diarrhea (group 1), healthy children from five day care centers or kindergartens in three different cities (group 2), and children with a history of *Salmonella* infection in the last 6 months (group 3). A standard questionnaire was administered to each child's mother or guardian from groups 1 and 2. Data was collected on the child's age, antibiotics administered to the child and/or close family members during the previous 90 days, and history of recent hospitalization and occupational exposure to food animals or raw meat in close family members. The study was approved by the Hospital General O'Horan Internal Review Board, and informed consent was obtained from all participants.

For *E. coli*, 1 g or 1 ml of feces was diluted in 4 ml of phosphate-buffered saline and plated onto MacConkey agar with 32 μg of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.)/ml. Isolation of *Salmonella* was performed with previously described methods (5). Susceptibility testing was performed by disk diffusion according to NCCLS guidelines (9), and data was analyzed with WHONET, version 5.2, software. Univariate analyses with the chi-square test and multivariate logistic regression analysis were conducted with SPSS, version 6.01.

Fecal samples were collected from 324 children (1 month to 12 years). QREC isolates were recovered more frequently from children with a recent *Salmonella* infection than from children with acute diarrhea of any etiology or from healthy children ($P = 0.007$) (Table 1). All mothers answered the questionnaire; only 6% of them were unable to answer certain questions. In all three cities, ampicillin, amoxicillin, and trimethoprim-sulfamethoxazole comprised 70 to 80% of the antibiotics used by children and close family members.

QREC isolates were resistant to antibiotics used in Yucatan for treating diarrhea (ampicillin, 62%; chloramphenicol, 25%;

TABLE 1. Recovery of fecal QREC and *Salmonella* from ill and healthy children

Source group (*n*)	% Recovery		
	NA-R[a] *E. coli*	CIP-R[b] *E. coli*	*Salmonella*
3[c] (12)	100	50	25
1[d] (36)	61	30.6	22
2[e] (276)	54	18.5	11.6

[a] NA-R, nalidixic acid resistant.
[b] CIP-R, ciprofloxacin resistant.
[c] Healthy children with *Salmonella* infection in the previous 6 months.
[d] Children with diarrhea of any etiology.
[e] Healthy children.

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TABLE 2. Risk factors for fecal QREC in children[a]

Risk factor	No. (%) of NA-R^{+}[e] isolates (n = 172)	No. (%) of NA-R^{-} isolates (n = 140)	P	OR (95% CI)[f]
Recovery of *Salmonella* from feces	31 (18)	9 (6)	0.004	3.2 (1.5–7.0)
Personal history of antibiotic treatment in last 90 days[b]	77 (48)	44 (34)	0.022	1.8 (1.1–2.9)
History of antibiotic treatment of close family member in last 90 days[b]	47 (29)	24 (19)	0.067	1.7 (1.0–3.0)
History of hospitalization of close family member in last 90 days[c]	20 (12)	4 (3)	0.007	4.5 (1.5–13.5)
Close family member works on farm	28 (17)	19 (14)	0.69	
Close family member with occupational exposure to raw meat[d]	17 (13)	13 (12)	0.97	

[a] Healthy asymptomatic children and children with diarrhea of any etiology.
[b] No child or close family member reportedly received outpatient treatment with quinolones or fluoroquinolones.
[c] Classes of antibiotics received in the hospital are unknown.
[d] Exposure includes work in a butchery, restaurant, or cafeteria.
[e] NA-R, nalidixic acid resistant.
[f] CI, confidence interval.

trimethoprim-sulfamethoxazole, 71%) and severe *Salmonella* infection (ceftriaxone, 8%). QREC isolates resistant to all four of these antibiotics were found more frequently in children with diarrhea or recent *Salmonella* infection than in healthy children (10 and 8%, respectively, versus 1%; $P = 0.01$). *Salmonella* isolated from children with QREC were more frequently resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, or nalidixic acid than those isolated from children without QREC (13 of 34 versus 3 of 9), but the difference was not statistically significant. No *Salmonella* isolates were resistant to ciprofloxacin or ceftriaxone.

Univariate analysis identified antibiotic exposure, hospitalization of a close family member, and concomitant presence of *Salmonella* in the feces as risk factors for QREC (Table 2). These variables, as well as outpatient antibiotic treatment in family members ($P = 0.067$), were entered into a stepwise logistic regression analysis. Only recent hospitalization of a close family member ($P = 0.011$, odds ratio [OR] = 5.1) and fecal shedding of *Salmonella* ($P = 0.004$, OR= 3.7) remained significant after multivariate analysis.

The carriage of fecal QREC in our study population was extremely high, particularly in children with recent *Salmonella* infection. Calva et al. (1) reported that only 1% of children in Mexico City carried norfloxacin-resistant *E. coli* during 1990 and 1991, and van de Mortel et al. (16) found fecal Cip^{r} *E. coli* in only 1% of adults and children in Venezuela in 1996. Results similar to those of our study were reported by Garau et al. (7) in Spain. These investigators isolated fecal Cip^{r} *E. coli* from 24 and 26% of healthy adults and children, respectively, and from 90% of live poultry in 1998. They also found a much higher prevalence in children with diarrhea (40%). Since all these studies used selective media with antibiotic to recover QREC, the discrepancies could be due to different selection pressures exerted at the human and veterinary levels. More importantly, it suggests that QREC is a recently emerging problem.

Other studies (4, 7) have found QREC to be multiple-drug resistant, which is in agreement with our findings. A *marRAB* operon that confers increased levels of resistance to quinolones and other antibiotics and that is inducible by nonquinolone drugs such as tetracycline, chloramphenicol, and salicylates has been described (3, 8). High-level fluoroquinolone resistance is usually attained through multiple mutations in target genes as well as enhanced efflux (4, 10). Repeated exposure to antibiotics in animal and human hosts could provide the necessary setting for such mutants (4).

The association between hospitalization of a close family member and QREC can be attributed to the heavy use of fluoroquinolones at our hospitals, where resistance in nosocomial pathogens is a major problem (21). Prior treatment with fluoroquinolones is a major risk factor for the emergence of fluoroquinolone-resistant *E. coli* in the gastrointestinal flora of hospitalized patients (2, 11). Transmission of trimethoprim-resistant *E. coli* among family members has been reported (6, 12), and we may reasonably assume that QREC is as easily transferred in the household setting. The association between QREC and fecal carriage of *Salmonella* suggest acquisition from a common source such as contaminated food. Surveillance data for 2000 and 2001 detected a higher prevalence of *Salmonella* and Cip^{r} *E. coli* in retail meats than in ill and healthy children (M. Zaidi, E. Zamora, P. J. Fedorka-Cray, J. Hermosillo, M. Headrick, and L. Tollefson, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-53, 2001). Given their high prevalence in meat, transmission of these organisms from food to humans is likely to be a frequent event.

Our study identified a high prevalence of fecal QREC in children that appears to be associated with both human and veterinary usage of antibiotics. Novel strategies are needed to measure the selection pressure of each for quinolone resistance in *E. coli* and the impact of QREC on human health. Human QREC infections should be closely monitored in the future.

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REFERENCES

1. **Calva, J. J., J. Sifuentes-Osornio, and C. Ceron.** 1996. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. Antimicrob. Agents Chemother. **40:**1699–1702.
2. **Carratala, J., A. Fernandez-Sevilla, F. Tubau, M. A. Dominguez, and F. Gudiol.** 1996. Emergence of fluoroquinolone-resistant *Escherichia coli* in fecal flora of cancer patients receiving norfloxacin prophylaxis. Antimicrob. Agents Chemother. **40:**503–505.
3. **Cohen, S. P., M. McMurry, D. C. Hooper, J. S. Wofson, and S. B. Levy.** 1989. Cross-resistance to fluoroquinolones in multiple antibiotic resistant (*mar*) *Escherichia coli* selected by tetracycline or chloramphenicol: decreased drug accumulation associated with membrane changes in addition to OmpF reduction. Antimicrob. Agents Chemother. **33:**1318–1325.
4. **Everett, M. J., Y. F. Jin, V. Ricci, and L. J. V. Piddock.** 1996. Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. Antimicrob. Agents Chemother. **40:**2380–2386.

5. **Fedorka-Cray, P. J., D. A. Dargatz, L. A. Thomas, and J. T. Gray.** 1998. Survey of *Salmonella* serotypes in feedlot cattle. J. Food Prot. **61:**525–530.
6. **Fornasini, M., R. R. Reves, B. E. Murray, A. L. Morrow, and L. K. Pickering.** 1992. Trimethoprim-resistant *Escherichia coli* in households of children attending day care centers. J. Infect. Dis. **166:**326–330.
7. **Garau, J., M. Xercavins, M. Rodríguez-Carballeira, J. R. Gómez-Vera, I. Coll, D. Vidal, T. Llovet, and A. Ruíz-Bremón.** 1999. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob. Agents Chemother. **43:**2736–2741.
8. **Maneewannakul, K., and S. Levy.** 1996. Identification of *mar* mutants among quinolone-resistant clinical isolates of *Escherichia coli*. Antimicrob. Agents Chemother. **40:**1695–1698.
9. **National Committee for Clinical Laboratory Standards.** 2000. Performance standards for antimicrobial disk susceptibility tests; approved standard, 7th ed. NCCLS document M2-A7. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. **Poole, K.** 2000. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. Antimicrob. Agents Chemother. **44:**2233–2241.
11. **Richard, P., M. H. Delangle, F. Raffi, E. Espaze, and H. Richet.** 2001. Impact of fluoroquinolone administration on the emergence of fluoroquinolone-resistant gram-negative bacilli from gastrointestinal flora. Clin. Infect. Dis. **32:**162–166.
12. **Rydberg, J., and A. Cederberg.** 1986. Intrafamilial spreading of *Escherichia coli* resistant to trimethoprim. Scand. J. Infect. Dis. **18:**457–460.
13. **Singer, R. S., R. Finch, H. C. Wegener, R. Bywater, J. Walters, and M. Lipsitch.** 2003. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. Lancet Infect. Dis. **3:**47–51.
14. **Sunde, M., K. Fossum, A. Solberg, and H. Sorum.** 1998. Antibiotic resistance in *Escherichia coli* of the normal intestinal flora of the swine. Microb. Drug Resist. **4:**289–299.
15. **Teuber, M.** 1999. Spread of antibiotic resistance with food-borne pathogens. Cell. Mol. Life Sci. **56:**755–763.
16. **van de Mortel, H. J. E., E. J. P. Jansen, G. J. Dinant, N. London, E. Palacios Prü, and E. E. Sobberingh.** 1998. The prevalence of antibiotic-resistant faecal *Escherichia coli* in healthy volunteers in Venezuela. Infection **26:**292–297.
17. **van den Bogaard, A. E., and E. E. Stobberingh.** 2000. Epidemiology of resistance to antibiotics: links between animals and humans. Int. J. Antimicrob. Agents **14:**327–335.
18. **Witte, W.** 2000. Selective pressure by antibiotic use in livestock. Int. J. Antimicrob. Agents **16:**S19–S24.
19. **World Health Organization.** 1997. The medical impact of the use of antimicrobials in food animals. Report and proceedings of a W. H. O. meeting. W. H. O./EMC/ZOO/97.4, 13 to 17 October. World Health Organization, Berlin, Germany.
20. **World Health Organization.** 1998. Use of quinolones in food animals and potential impact on human health. Report of a W. H. O. Meeting. W. H. O./EMC/ZDI/98.10, 2 to 5 June. World Health Organization, Geneva, Switzerland.
21. **Zaidi, M., J. Sifuentes-Osornio, A. L. Rolon, G. Vazquez, R. Rosado, M. Sanchez, J. J. Calva, and S. Ponce de Leon-Rosales.** 2002. Inadequate therapy and antibiotic resistance. Risk factors for mortality in the intensive care unit. Arch. Med. Res. **33:**290–294.