ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1996, p. 1699–1702 Vol. 40, No. 7
0066-4804/96/$04.00+0

Antimicrobial Resistance in Fecal Flora: Longitudinal Community-Based Surveillance of Children from Urban Mexico

JUAN J. CALVA,* JOSÉ SIFUENTES-OSORNIO, AND CECILIA CERÓN

Department of Infectious Diseases, Instituto Nacional de la Nutrición "Salvador Zubirán," Tlalpan, México Distrito Federal, México

Received 7 November 1995/Returned for modification 9 February 1996/Accepted 7 May 1996

We assessed the colonization patterns, over time, of three sentinel drug-resistant enteric bacterial genera in samples from a cohort of 20 healthy small children in a periurban community in Mexico. The children were monitored during a 13-week period by means of weekly home visits and examinations of stool collections. These specimens were tested for the presence of *Escherichia coli*, *Klebsiella* species, and *Shigella* species resistant to one or more of seven antimicrobial agents. Ninety, 77, and 62% of the stool specimens had *E. coli* isolates resistant to ampicillin, trimethoprim, and tetracycline, respectively. Simultaneous resistance to more than one antibiotic by an *E. coli* isolate was observed in 88.5% of stool samples. Persistent fecal shedding of ampicillin-, trimethoprim-, and tetracycline-resistant *E. coli* occurred during the study period in the majority of children. We detected colonization by *E. coli* resistant to chloramphenicol, gentamicin, nitrofurantoin, or norfloxacin, as well as by *Klebsiella* species and *Shigella* species resistant to one of these antibiotics, in fewer children and for shorter periods. These data suggest the common and persistent intestinal shedding of multidrug-resistant *E. coli* strains by small healthy children.

During the last several years an alarming worldwide increase in the incidence of community-acquired infections with bacteria resistant to multiple antibiotics of common use has been observed (7, 12). The fecal flora represents a large potential reservoir for sources of antimicrobial resistance, as well as the site where resistance genes can be transferred from the commensal flora to virulent microorganisms (9, 20). Thus, a better knowledge of the frequency of occurrence of antibiotic-resistant bacteria carried by healthy individuals in the community is urgently needed.

There are few studies addressing this issue, and they have shown a high prevalence of commensal fecal coliforms resistant to various antibiotics (3, 6, 13, 14, 17, 21). Yet, these have been cross-sectional surveys which have not allowed an evaluation of the carriage of these microorganisms in stools over time.

During the last decade, our group has carried out cohort studies on childhood diarrheal diseases (5, 11, 16, 23), as well as on antibiotic misuse (2, 4), in a periurban community in Mexico City. Taking advantage of a sample of healthy children less than 2 years of age who were under surveillance for infections by enteropathogens, we designed a substudy to longitudinally assess the pattern of intestinal colonization by gram-negative bacilli resistant to one or more of seven antimicrobial agents.

MATERIALS AND METHODS

Study area. This study was conducted in San Pedro Mártir, a periurban area on the southwestern outskirts of Mexico City, approximately 2.5 miles (ca. 4 km) from the Instituto Nacional de la Nutrición. Household and pharmacy surveys of antibiotic usage in this community have been previously published (4).

Sample population and epidemiologic surveillance. A subsample of 20 healthy children under 2 years of age was randomly selected and enrolled in this study. All were participating in a longitudinal surveillance of diarrheal morbidity, feeding practices, and colonization with enteropathogens; children from this cohort were those whose mothers volunteered to participate in the study (11, 23). These 20 children were monitored during 13 weeks; 10 were monitored during the 3-month period of July to October 1990, and the remaining 10 were monitored during the period of October 1990 to January 1991. Trained field workers visited every household once a week to interview each child's mother and to collect stool specimens.

Stool collection and processing. Freshly passed stool specimens from each child were collected weekly in the homes regardless of symptom status, placed in a plastic container, kept on ice, and transported to the laboratory at the Institute within 3 h after collection. Thus, 13 consecutive weekly fecal samples were taken from each child.

Antibiotic resistance screening test. A sterile cotton swab was immersed in a stool sample and rubbed until brown. The soiled swab was then squeezed into 2 ml of sterile saline solution, yielding what was considered to be a suspension of approximately 250 mg of stool specimen per ml (10). Aliquots (0.1 ml) were streaked on seven antibiotic-containing MacConkey agar plates, each containing one of the following antimicrobial agents in the specified amount (in micrograms per milliliter): ampicillin (64), trimethoprim (16), tetracycline (32), chloramphenicol (50), gentamicin (16), nitrofurantoin (200), and norfloxacin (16). These antibiotic concentrations were chosen after three consecutive tests of 0.1-ml aliquots of a broth suspension of *Escherichia coli* ATCC 25922 (equivalent to standard 1 of McFarland). These suspensions were plated in media supplemented with decreasing concentrations of each of the studied antibiotics; the minimal drug concentration showing the absence of growth was selected as the concentration used for the screening method. A control MacConkey plate containing no antibiotic was also streaked. After incubation at 37°C for 24 h, the plates were examined for the presence of colony growth and compared with the control plate. One colony of each morphological type observed on each plate was selected for further study. Isolates were identified by the API 20E system (Analytab Products, Plainview, N.Y.).

Antibiotic resistance confirmatory test. MICs of each of the seven antibiotics were determined for *E. coli*, *Klebsiella* spp., and *Shigella* spp. isolated from the antibiotic-containing plates. CFU (10^4 to 10^5 per ml) were inoculated into Mueller-Hinton broth supplemented with $CaCl_2$ (25 mg of calcium per liter) and $MgCl_2$ (12.5 mg of magnesium per liter) and containing various serial twofold-decreasing antibiotic concentrations (19). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

The presence of an antibiotic-resistant isolate was defined as growth of at least one colony on the antibiotic-containing plate. An antibiotic-susceptible isolate exhibited the growth of at least one colony on the plate containing no antibiotic (control) but no growth in the antibiotic-containing plate. The majority of the isolates from the antibiotic-containing plates were confirmed to be drug resistant since they showed MICs above the resistance cutoff values determined by the National Committee for Clinical Laboratory Standards (positive predictive value

* Corresponding author. Mailing address: Department of Infectious Diseases, Instituto Nacional de la Nutrición "Salvador Zubirán," Vasco de Quiroga 15, Tlalpan, México 14000 D.F., México. Phone: (52) (5) 655-9675. Fax: (52) (5) 513-0010.

TABLE 1. Frequency of occurrence of stool specimens with at least one colony of *E. coli* or *Klebsiella* spp. resistant to any of the tested antimicrobial agents[a]

Antibiotic	No. of stool samples (%)[b] with resistant:	
	E. coli[c]	*Klebsiella* spp.[d]
Ampicillin	235 (90)	NR[e] (NR)
Trimethoprim	201 (77)	20 (8)
Tetracycline	161 (62)	13 (5)
Chloramphenicol	102 (39)	10 (4)
Gentamicin	12 (5)	1 (<1)
Nitrofurantoin	4 (2)	1 (<1)
Norfloxacin	2 (1)	0 (0)

[a] Resistance was confirmed by the broth microdilution test of isolates from the antibiotic-containing plate.
[b] Percentages were determined from a total of 260 stool samples from 20 children.
[c] From all 260 stool samples, *E. coli* was isolated on the plates containing no antibiotic (control).
[d] From 54 of a total 260 stool samples, *Klebsiella* spp. were isolated on the plates containing no antibiotic (control).
[e] NR, not reported, as bacteria were intrinsically resistant to ampicillin.

of the screening test, >90%). Bacteria isolated only from the plates containing no antibiotic (controls) were not further tested by the broth microdilution assay.

Colonization patterns. Intestinal colonization by antibiotic-resistant bacteria during the 13-week study period was classified as follows: continuous colonization was uninterrupted shedding of a resistant isolate of a given species lasting more than 8 weeks; recurrent colonization consisted of two episodes of excretion of a resistant isolate of a given species separated by at least two consecutive negative stool samples; intermittent colonization consisted of more than two episodes of shedding of a resistant organism of a given species separated by at least two consecutive negative specimens; and sporadic colonization was a single episode of excretion of a resistant isolate, lasting 8 weeks or less.

RESULTS

Antibiotic-resistant enteric isolates. Two hundred and sixty stool samples from the 20 children were obtained and processed in the microbiology laboratory (13 specimens per child). In the no-antibiotic (control) plates, *E. coli* was isolated from all fecal samples, *Klebsiella* spp. were isolated from 54 samples (21%), and *Shigella* spp. were isolated from 4 samples (1.5%). Table 1 shows the relative frequencies of occurrence of stool samples with at least 1 CFU of *E. coli* or of *Klebsiella* spp. resistant to any of the tested antimicrobial agents (ampicillin-resistant *Klebsiella* spp. are not reported, as these microorganisms are intrinsically resistant to the drug). Table 2 shows the frequency of stool specimens with *E. coli* resistant to only one or, simultaneously, to more than one of the seven antibiotics. Eighty-four percent of stool samples had an *E. coli* isolate simultaneously resistant to two, three, or four different antimicrobial agents.

Colonization by antibiotic-resistant *E. coli*. All 20 children were colonized at some point by *E. coli* resistant to ampicillin, trimethoprim, tetracycline, and chloramphenicol (Table 3). In less than half of the 20 children, *E. coli* organisms resistant to gentamicin (9 children), nitrofurantoin (3 children), and norfloxacin (1 child) were identified. As determined by specific colonization patterns, the majority of children who shed *E. coli* resistant to ampicillin, trimethoprim, tetracycline, and chloramphenicol had a continuous or recurrent colonization and, except for isolates resistant to chloramphenicol, in the majority of children, resistant *E. coli* organisms were excreted during most of the study period. In contrast, all but one child with *E. coli* resistant to gentamicin, nitrofurantoin, and norfloxacin had sporadic colonization patterns of short duration (less than 7 weeks).

Colonization by antibiotic-resistant *Klebsiella* spp. Most children were colonized at some point by klebsiellas resistant to trimethoprim (14 children). Less than half of the 20 children were colonized by isolates resistant to tetracycline (9 children), chloramphenicol (7 children), gentamicin (a single child), or nitrofurantoin (a single child); no child shed any isolate resistant to norfloxacin. In contrast to what was found with *E. coli*, the great majority of the children colonized by antibiotic (including trimethoprim, tetracycline, and chloramphenicol)-resistant isolates had sporadic colonization patterns of short duration.

Colonization by antibiotic-resistant *Shigella* spp. Only two children were colonized by resistant isolates of *Shigella* spp.: one child had two episodes of colonization (of 1 week's duration each), one by a trimethoprim- and one by a tetracycline-resistant isolate of *Shigella boydii*; and the second child had a single (2-week) episode of colonization by *Shigella sonnei*, resistant to ampicillin, trimethoprim, and tetracycline.

DISCUSSION

There is worldwide concern about the emergence of antibiotic resistance in common pathogens of community- as well as hospital-acquired infections. Normal nonpathogenic floras of ambulatory and hospitalized individuals may represent an enormous and constant reservoir of resistant genes, potentially transferable to virulent microorganisms; yet, data on the frequency of antibiotic-resistant bacteria colonizing the bowels of healthy individuals are scarce.

Surveys conducted with hospitalized and healthy individuals in diverse areas from developed and developing countries have shown a high frequency of naturally occurring aerobic gram-negative bacteria in fecal floras with resistance to one or several antimicrobial agents (3, 6, 13, 14, 17, 21, 22). Cross-sectional studies of small children attending day-care centers in Houston, Tex., have shown that up to 37 and 70% of infants are colonized with fecal *E. coli* resistant to trimethoprim and ampicillin, respectively (17); also, transmission of trimethoprim-resistant *E. coli* among children within day-care centers, and from day-care children to household members, is common (8). Carriage of *E. coli* resistant to antimicrobial agents has been found far more often in healthy children from Venezuela and China than from North America (13). Another study, in a children's emergency hospital in Sudan, showed that more than 39% of the children had enteric isolates resistant to all six

TABLE 2. Frequency of occurrence of stool specimens with *E. coli* resistant to antimicrobial agents[a]

No. of antibiotics to which *E. coli* was resistant	No. of stool samples (%)[b]	Cumulative frequency (%)[c]
0	4 (1.5)	
1	26 (10)	256 (98.5)
2	84 (32.3)	230 (88.5)
3	73 (28.1)	146 (56.2)
4	62 (23.8)	73 (28.1)
5	9 (3.5)	11 (4.2)
6	2 (0.8)	

[a] Ampicillin, trimethoprim, tetracycline, chloramphenicol, gentamicin, nitrofurantoin, and norfloxacin.
[b] Percentages were calculated from a total of 260 stool samples from 20 children.
[c] Cumulative frequency is the number of specimens remaining from the 260 original samples from which numbers of samples with *E. coli* resistant to additional antimicrobial agents could be determined.

TABLE 3. Number of children under 2 years of age colonized by *E. coli* resistant to any of seven antimicrobial agents

Antibiotic	No. of children colonized by resistant *E. coli* with:					
	Colonization pattern[a]:					Fecal shedding of 7 weeks' or longer duration[b]
	Continuous	Recurrent	Intermittent	Sporadic	Any	
Ampicillin	18	2	0	0	20	20
Trimethoprim	11	9	0	0	20	19
Tetracycline	9	6	0	5	20	11
Chloramphenicol	1	11	2	6	20	7
Gentamicin	0	1	0	8	9	0
Nitrofurantoin	0	0	0	3	3	0
Norfloxacin	0	0	0	1	1	0

[a] For definitions, see Materials and Methods.
[b] Regardless of the colonization pattern.

antibiotics studied and 80% had enteric isolates resistant to at least four of these drugs (22).

Despite this evidence of the high prevalence of potentially transferable antibiotic resistance in gut commensal organisms of children, especially children from developing countries, to our knowledge there has been no assessment of the pattern of the intestinal carriage of drug-resistant coliforms, over time, among healthy children in a community. The longer the period of shedding of these microorganisms in feces, the greater the chances of interpersonal transmission and contamination of drinking water and food with these bacteria (18). A recent study in two southern cities of The Netherlands showed that the high degree of drug resistance of members of the family *Enterobacteriaceae* in fecal samples from 124 healthy, presumably adult, volunteers remained more or less constant over time during a 15-week follow-up period (15).

Through the longitudinal surveillance of our cohort of 20 healthy children, we were able to define the patterns of intestinal colonization, during a 13-week period, of three sentinel bacterial genera resistant to any of seven antibiotics commonly used in clinical practice. Interestingly, we documented that the majority of these children were continuously shedding in their stools *E. coli* resistant to ampicillin, trimethoprim, and tetracycline throughout the observational period. On the contrary, colonization by *E. coli* organisms resistant to gentamicin, nitrofurantoin, and norfloxacin was observed in only a few children and excretion of these gram-negative bacilli was of short duration.

We have previously reported the pattern of antibiotic usage in the same urban community (4). On the basis of a survey of 1,659 randomly selected households, we have estimated that approximately 5% of the individuals use at least one antimicrobial agent in a 2-week period and that the most commonly used antibiotics are ampicillin, trimethoprim, and tetracycline, which constituted one-third of all reported drugs (20, 7, and 6% of all consumed antibiotics, respectively). In contrast, chloramphenicol, gentamicin, nitrofurantoin, and norfloxacin represented only 2% of all antibiotics used by these individuals. There seems to be an association between the common consumption of the former group of drugs and the continuous shedding of *E. coli* resistant to these antibiotics in most of the studied children in this community; yet, a firm conclusion cannot be drawn from the present study as data on antibiotic usage by the children or their household contacts were not gathered.

These observations further support the hypothesis that the overuse and misuse of these drugs in the management of common illnesses (2) may represent an important environmental pressure for the selection, in the bowels of these individuals, of bacteria possessing genes encoding drug resistance. In turn, these microorganisms might be continuously shed in feces and widely spread to other household members, including small children, by fecal-oral transmission routes commonly seen in this kind of community with crowding and poor sanitary conditions. Thus, the colons of these individuals present numerous opportunities for fecal floras to exchange genetic material; in fact, the possibility of acquisition of resistance determinants by pathogens, from commensal bowel bacteria, was suggested after an epidemic of enteritis by multidrug-resistant *Shigella dysenteriae* in central Africa (9).

In contrast to colonization by *E. coli*, colonization by strains of antibiotic-resistant *Klebsiella* spp. occurred less frequently among studied children and for shorter periods. A possible explanation for this is the lack of optimal sensitivity of our method to isolate these microorganisms; it might be that the antibiotic concentrations in media used for selection in the screening method were rather high and/or that our ability to detect antibiotic-resistant *Klebsiella* isolates was overshadowed by the relative abundance of *E. coli* organisms in stools.

We identified only two children who shed *Shigella* spp. in their stools; this low frequency of isolation could be explained by the relatively low incidence of infections with these microorganism in this age group as well as by the fact that a *Shigella*-selective medium was not used in this study. All five isolates showed resistance to one or more of the antibiotics of common use in the treatment of shigellosis. The alarming increase in multidrug-resistant strains observed in many areas of the world (1, 9), as well as the recent description of a high incidence of symptom-free carriers of *Shigella* spp. other than *S. dysenteriae* (11) (as a potential source of a wide dissemination of these microorganisms), has raised concerns about perspectives of the optimal antibiotic management of inflammatory diarrhea.

In conclusion, data from this longitudinal study confirm that in certain communities small children can be colonized by *E. coli* strains resistant to multiple commonly used antimicrobial agents. Furthermore, fecal shedding of these microorganisms persistently occurs for periods of at least a few months, which could represent an important hazard for the dissemination of antibiotic-resistant bacteria to other members of the community.

ACKNOWLEDGMENTS

We thank Edmundo Calva-Mercado for reviewing the manuscript and María Consuelo Ontiveros for her technical support.

This study was financially supported by the INCLEN Pharmacoepidemiology Task Force and the National Institutes of Health (grant HD-13021).

REFERENCES

1. **Bennish, M. L., and M. A. Salam.** 1992. Rethinking options for the treatment of shigellosis. J. Antimicrob. Chemother. **30:**243–247.
2. **Bojalil, R., and J. J. Calva.** 1994. Antibiotic misuse in diarrhea. A household survey in a Mexican community. J. Clin. Epidemiol. **47:**147–156.
3. **Bonten, M., E. Stobberingh, J. Philips, and A. Houben.** 1992. Antibiotic resistance of *Escherichia coli* in fecal samples of healthy people in two different areas in an industrialized country. Infection **20:**258–262.
4. **Calva, J. J., and R. Bojalil.** 1996. Antibiotic use in a periurban community in Mexico: a household and drugstore survey. Soc. Sci. Med. **42:**1121–1128.
5. **Calva, J. J., G. M. Ruiz-Palacios, A. B. López-Vidal, A. Ramos, and R. Bojalil.** 1988. Cohort study of intestinal infection with Campylobacter in Mexican children. Lancet **i:**503–506.
6. **Chambers, S. T., C. Steele, and C. M. Kunin.** 1987. Enteric colonization with antibiotic resistant bacteria in nurses working in intensive care units. J. Antimicrob. Chemother. **19:**685–693.
7. **Cohen, M. L.** 1992. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science **257:**1050–1055.
8. **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.
9. **Frost, J. A., B. Rowe, and J. Vandepitte.** 1982. Acquisition of trimethoprim resistance in epidemic strain of *Shigella dysenteriae* from Zaire. Lancet **i:**963.
10. **Guerrant, R. L., S. J. Wood, L. Krongaard, R. A. Reid, and R. H. Hodge.** 1981. Resistance among fecal flora of patients taking sulfamethoxazole-trimethoprim or trimethoprim alone. Antimicrob. Agents Chemother. **19:** 33–38.
11. **Guerrero, L., J. J. Calva, A. L. Morrow, et al.** 1994. Asymptomatic *Shigella* infections in a cohort of Mexican children younger than two years of age. Pediatr. Infect. Dis. J. **13:**597–602.
12. **Kunin, C. M.** 1993. Resistance to antimicrobial drugs—a worldwide calamity. Ann. Intern. Med. **118:**557–561.
13. **Lester, S. C., M. P. Pla, F. Wang, I. P. Schael, H. Jiang, and T. F. O'Brien.** 1990. The carriage of *Escherichia coli* resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China. N. Engl. J. Med. **323:**285–289.
14. **Levy, S. B., B. Marshall, S. Schluederberg, D. Rowse, and J. Davis.** 1988. High frequency of antimicrobial resistance in human fecal flora. Antimicrob. Agents Chemother. **32:**1801–1806.
15. **London, N., R. Nijsten, A. V. D. Bogaard, and E. Stobberingh.** 1993. Antibiotic resistance of faecal Enterobacteriaceae isolated from healthy volunteers, a 15-week follow-up study. J. Antimicrob. Chemother. **32:**83–91.
16. **López-Vidal, Y., J. J. Calva, A. Trujillo, A. Ponce de León, A. Ramos, A. M. Svennerholm, and G. M. Ruiz-Palacios.** 1990. Enterotoxins and adhesins of enterotoxigenic *Escherichia coli*: are they risk factors for acute diarrhea in the community? J. Infect. Dis. **162:**442–447.
17. **Reves, R. R., B. E. Murray, L. K. Pickering, D. Prado, M. Maddock, and A. V. Bartlett.** 1987. Children with trimethoprim- and ampicillin-resistant fecal *Escherichia coli* in day care centers. J. Infect. Dis. **156:**758–762.
18. **Rydberg, J., and A. Cederberg.** 1986. Intrafamilial spreading of *Escherichia coli* resistant to trimethoprim. Scand. J. Infect. Dis. **18:**457–460.
19. **Sahm, D. F., and J. A. Washington II.** 1991. Antibacterial susceptibility tests: dilution methods, p. 1105–1116. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
20. **Shanahan, P. M. A., C. J. Thomson, and S. G. B. Amyes.** 1995. β-Lactam resistance in normal faecal flora from South Africa. Epidemiol. Infect. **115:** 243–253.
21. **Shanahan, P. M. A., B. A. Wylie, P. V. Adrian, H. J. Koornhof, C. J. Thomson, and S. G. B. Amyes.** 1993. The prevalence of antimicrobial resistance in human faecal flora in South Africa. Epidemiol. Infect. **111:**221–228.
22. **Shears, P., G. Suliman, and C. A. Hart.** 1988. Occurrence of multiple antibiotic resistance and R plasmids in Enterobacteriaceae isolated from children in the Sudan. Epidemiol. Infect. **100:**73–81.
23. **Velázquez, F. R., J. J. Calva, M. L. Guerrero, D. Mass, R. I. Glass, L. K. Pickering, and G. M. Ruiz-Palacios.** 1993. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. Pediatr. Infect. Dis. J. **12:**54–61.