High Prevalence of Acquired Antimicrobial Resistance Unrelated to Heavy Antimicrobial Consumption

Alessandro Bartoloni,[1] Filippo Bartalesi,[1] Antonia Mantella,[1] Emanuela Dell'Amico,[2] Mimmo Roselli,[1] Marianne Strohmeyer,[1] Herlan Gamboa Barahona,[3] Virgilio Prieto Barrón,[4] Franco Paradisi,[1] and Gian Maria Rossolini[2]

[1]Dipartimento di Area Critica Medico Chirurgica, Clinica di Malattie Infettive, Università di Firenze, Florence, and [2]Dipartimento di Biologia Molecolare, Sezione di Microbiologia, Università di Siena, Siena, Italy; [3]Distrito de Salud Cordillera, Departamento Santa Cruz, Camiri, and [4]Ministerio de Salud de Bolivia, La Paz, Bolivia

In a very remote rural Bolivian community where the use of antimicrobials has been minimal and where exchanges with the exterior are very limited, 67% of subjects were found to be carriers of fecal *Escherichia coli* with acquired resistance to ⩾1 antimicrobial agent(s); the highest rates were observed for tetracycline (64%), ampicillin (58%), trimethoprim-sulfamethoxazole (50%), and chloramphenicol (41%). The most relevant implication of these findings is that, in certain settings, the spread and maintenance of antimicrobial resistance can occur, regardless of whether selective pressure generated by the use of antimicrobials is present.

Microbial resistance to drugs has become a global public-health problem compromising the efficacy of antimicrobial chemotherapy. The magnitude of this problem has recently been acknowledged by the World Health Organization, which has launched a global strategy for the containment of antimicrobial resistance [1]. The emergence and spread of resistance are universally acknowledged to be associated with heavy consumption of antimicrobial agents in clinical and veterinary practices, and the prudent use of antibiotics is considered to be mandatory for preservation of their therapeutic effectiveness for as long as possible [2]. Within this perspective, a combination of misuse and overuse of antimicrobial agents, along with overcrowding and poor sanitation, is among the reasons given to explain the exceedingly high resistance rates observed in low-income countries [3]. However, several aspects of the impact of antimicrobial use remain poorly understood [4], and this issue is difficult to investigate, because, for several decades, the use of antimicrobial agents has been almost universal and because there is a lack of representative bacterial collections from the preantibiotic era.

The phenomenon of drug resistance is not restricted to pathogenetic bacteria; it also involves the commensal microbiota of humans and animals, which, although not specific targets, are continuously exposed to the selective pressure generated by antimicrobial chemotherapy and may become a major reservoir of resistant strains and resistance determinants [5]. For this reason, some commensal microbiota, such as fecal *Escherichia coli*, have been exploited as sensitive indicators in surveillance regarding antimicrobial resistance [6–9].

In the present study, we investigated antimicrobial resistance in the commensal *E. coli* microbiota of the population of a very remote rural Bolivian community where the use of antimicrobials has been minimal. Unexpectedly, remarkably high resistance rates were detected, a finding suggesting that, in some cases, the spread and maintenance of resistant strains and resistance determinants may not be directly related to antimicrobial consumption.

Subjects, materials, and methods. Alto Los Zarzos, a very remote rural Bolivian community of 130 Guaraní Indians that is located in the Gran Chaco region of the Tarija department, was investigated in the present study. This community is at an altitude of ~1700 m and is accessible from the closest health-care post only via a 3-h climb up a steep slope in the forest. The population lives in huts with no sanitary or hygienic facilities. Locally collected rainwater is the only source of water. The principal activities are agriculture and animal breeding. A health-care worker visits the community approximately every 3 months. This community was chosen because local health-care authorities considered it to be one of the most remote, having little exchange with the exterior and, thus far, very limited access to health-care services.

Before fieldwork was started, a meeting was held with the community leader and adults, to explain the purposes and procedures of the survey. The study design, including its ethical aspects, was reviewed and approved in advance by the Bolivian Ministry of Social Welfare and Public Health and by local health-care authorities. Informed consent was obtained from all adult participants and from the parents or legal guardians of minors.

Received 16 June 2003; accepted 19 September 2003; electronically published 16 March 2004.

Financial support: Ente Cassa di Risparmio, Florence, Italy.

Reprints or correspondence: Prof. Alessandro Bartoloni, Dipartimento di Area Critica Medico Chirurgica, Clinica di Malattie Infettive, Università di Firenze, Policlinico Careggi, viale Morgagni 85, 50134 Florence, Italy (bartolonia@ao-careggi.toscana.it).

The Journal of Infectious Diseases 2004; 189:1291–4

0022-1899/2004/18907-0021$15.00

Information about previous drug use, visits to the health-care post, travel outside the community, and previous hospitalization was obtained from each adult participant (parents or legal guardians spoke on behalf of the children). Specially prepared forms were used to record the data collected. The interviews were conducted by trained Guaraní health-care workers. The local health-care workers were interviewed with regard to the same information, separately from the villagers. Data on veterinary and agricultural use of antimicrobial agents were obtained from the community leader.

A total of 108 individuals (61 male and 47 female), ages 1–77 years (mean age, 22.7 years; median age, 14 years), were investigated. This group included all the inhabitants present on the day (20 September 1999) when the survey was conducted.

A stool sample was collected from each subject, and a fecal swab obtained from each sample was stored in Amies transport medium (Oxoid) and, within 24 h, was transferred, in a cold box, to the laboratory of the Camiri District Hospital. Screening for resistant isolates was performed by a rapid method, essentially as described elsewhere [7]. This approach was preferred because it is known to correlate well with those based on testing of randomly collected colonies from primary stool culture and because it is more sensitive [6]. The swabs were plated on McConkey agar, and disks containing antimicrobial agents (ampicillin, chloramphenicol, gentamicin, amikacin, kanamycin, tobramycin, nalidixic acid, nitrofurantoin, trimethoprim-sulfamethoxazole, and tetracycline [Becton Dickinson]) were applied to the seeded plates. After incubation at 37°C for 18 h, the plates were inspected for growth. One of the following types of results was observed for each disk: (1) the presence of a substantial zone of growth inhibition (with a diameter generally larger than that which the NCCLS recommends as the susceptibility breakpoint for disk-diffusion testing); (2) the presence of a substantial zone of growth inhibition but with isolated colonies growing inside it; or (3) either no or a very small zone of growth inhibition. The first type of result (i.e., presence of a substantial zone of growth inhibition) was interpreted as indicative of susceptibility to the agent contained by the disk, because this correlation had always been found in preliminary tests performed on similar samples (A.B., unpublished data); the other 2 types of results were interpreted as being presumptive for resistance, and either a pool of the colonies grown inside the zone of inhibition or a loopful of the microbial lawn that had grown in the proximity of a disk was preserved in cystine-trypticase agar tubes (Becton Dickinson), for use in confirmatory studies in which these preserved cultures were isolated on McConkey agar and in which lactose-fermenting oxidase-negative colonies were identified by use of the API 20E identification system (bioMérieux). All confirmed *E. coli* isolates were then tested for antimicrobial susceptibility, by the standard disk-diffusion method [10]. *E. coli* ATCC 25922 was used as a reference strain for quality control in susceptibility testing.

Colony blot hybridization was performed as described elsewhere, by use of a bla_{TEM} probe labeled with [^{32}P] by the random-priming technique [11]. The probe was a polymerase chain reaction–generated amplicon containing the entire $bla_{TEM\text{-}1A}$ gene (GenBank accession number J01749). *E. coli* ATCC 25922 and *E. coli* DH5α(pBR322) were used as negative and positive controls, respectively. Differences between proportions were statistically assessed by χ^2 test.

Results. According to interviews, during the 12 months preceding the study, 41 subjects (38%) reported a history of travel outside the community (mostly to either the district capital, Entre-Rios, or the department capital, Tarija); 6 subjects (6%) and 35 subjects (32%), respectively, reported either visiting the nearest health-care post or seeking the aid of a traditional healer (mostly for trauma); and 8 subjects (7%) reported previous use of antimicrobial agents (6 reported use of ampicillin, and 2 reported use of trimethoprim-sulfamethoxazole). Only 4 subjects (4%) reported a history of hospitalization in the district capital (3 women had been hospitalized during delivery of children, and 1 man had been hospitalized for treatment of trauma). The health-care worker who visited the community approximately every 3 months confirmed that the use of antimicrobial agents was very limited. Veterinary and agricultural use of antimicrobial agents was totally absent.

Confluent bacterial growth was confirmed in all fecal swabs. Samples from 29 subjects (27%) did not yield isolates with presumptive resistance to any of the tested drugs; samples from the other 79 subjects (73%) yielded isolates with presumptive resistance to $\geqslant$1 drugs, and these isolates were subjected to confirmatory analysis, including species identification and, for isolates confirmed to be *E. coli,* to conventional susceptibility testing on the basis of disk diffusion. Multiple isolates from the same subject that showed the same resistance profile were considered to be replicates. Confirmatory analysis resulted in a total of 113 nonreplicate antibiotic-resistant *E. coli* isolates from 72 (67%) of these 79 subjects (the isolates from 7 subjects belonged to species other than *E. coli*); of these 72 subjects, 43 had *E. coli* isolates with 1 resistance profile, 21 had isolates with 2 resistance profiles, 4 had isolates with 3 resistance profiles, and 4 had isolates with 4 resistance profiles. The prevalence of drug resistance detected in isolates from the studied population is reported in table 1. Remarkably high rates were observed for resistance to tetracycline, ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. A relationship between the prevalence of drug resistance in an isolate and the age of the source subject was observed only for trimethoprim-sulfamethoxazole.

Multidrug resistance (MDR) characterized isolates from the majority (64/72) of the subjects yielding antibiotic-resistant *E. coli*. The most common MDR, observed in 37 subjects, included

Table 1. Prevalence of fecal carriage of antimicrobial-resistant *Escherichia coli* among 108 subjects from a remote rural community in Bolivia.

Type of drug resistance[a]	Prevalence, by age range			
	1–6 years (n = 17)	7–14 years (n = 37)	⩾15 years (n = 54)	Total (n = 108)
Tetracycline	9 (53)	26 (70)	34 (63)	69 (64)
Ampicillin	7 (41)	24 (65)	32 (59)	63 (58)
Trimethoprim-sulfamethoxazole[b]	4 (24)	23 (62)	27 (50)	54 (50)
Chloramphenicol	4 (24)	18 (49)	22 (41)	44 (41)
Kanamycin	1 (6)	1 (3)	3 (6)	5 (5)
Tobramycin	0	0	1 (2)	1 (1)
⩾1 Drug	10 (59)	27 (73)	35 (65)	72 (67)

NOTE. Data are no. (%) of subjects.

[a] No isolates resistant to gentamicin, amikacin, nalidixic acid, or nitrofurantoin were detected.

[b] The prevalence of resistance to trimethoprim-sulfamethoxazole was significantly (P = .017) lower among the youngest subjects (those ⩽6 years of age) than among older subjects (those ⩾7 years of age).

resistance to tetracycline, ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. Most of the subjects yielding MDR isolates yielded ⩾1 isolate in which all of the corresponding traits were simultaneously present (table 2). Individual *E. coli* isolates exhibited variable drug-resistance patterns: 32 (28%) were resistant to 1 drug, whereas the remaining 81 (72%) were resistant to >1 drug. The most common MDR pattern included tetracycline, ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol and was observed in almost one-third of the isolates. Of the 81 ampicillin-resistant isolates, 78 were recognized by a bla_{TEM} probe in a colony blot hybridization.

Discussion. In the present study, we detected, in the population of a very remote rural Bolivian community exhibiting a relatively high degree of isolation and a history of very limited use of antimicrobial agents, a high rate of fecal carriage of *E. coli* with acquired resistance to antimicrobial agents. The fact that 96% (78/81) of the ampicillin-resistant isolates carried a bla_{TEM} gene [12] corroborates this finding. Because the use of antimicrobial agents has been claimed to be the driving force in the emergence and diffusion of bacterial resistance, our findings were quite unexpected: even if we cannot consider the study population as virgin in terms of antimicrobial use, antimicrobial consumption seems to be far too low to account for the high prevalence of antimicrobial resistance in the resident microbiota, especially when compared with the prevalences measured in settings characterized by overuse of antimicrobial agents [8]. As far as nonhuman use of antimicrobials is concerned, veterinary and agricultural use has been totally absent in the area that we studied. The acquisition of resistant bacteria from water sources contaminated by other human populations can be excluded because of the geographical location of the community. The hypothesis most likely to explain such a scenario is that resistant strains have occasionally been introduced into the community after exchanges, although limited, between its population and that of other areas—and that resistant strains and resistance determinants have efficiently spread, facilitated by unhygienic conditions. Nevertheless, the persistence and spread of similar strains in the community, in the absence of significant selective pressure generated by antimicrobial consumption, remains to be explained, unless these strains exhibit additional selective advantages linked to the re-

Table 2. Single-drug resistance and multidrug resistance (MDR) in isolates from 72 subjects with fecal carriage of antimicrobial-resistant *Escherichia coli*.

Resistance profile	No. (%) of subjects[a]	No. (%) of isolates	
		MDR for all drugs in category[b]	Nonreplicate drug resistant
Single drug	8[c] (7)	NA	8
Tet/Amp/Tsx/Cm	37 (34)	35 (95)	67
Tet/Amp/Tsx	11 (10)	11 (100)	14
Tet/Amp	6 (6)	6 (100)	6
Tet/Amp/Tsx/Cm/Km	3 (3)	3 (100)	7
Tet/Amp/Cm	2 (2)	1 (50)	5
Tet/Cm	2 (2)	2 (100)	2
Amp/Tsx	1 (1)	1 (100)	1
Tet/Amp/Tsx/Km	1 (1)	1 (100)	2
Amp/Tsx/Km/Tob	1 (1)	1 (100)	1
Total	72 (67)	61 (85)	113

NOTE. Amp, ampicillin; Cm, chloramphenicol; Km, kanamycin; NA, not applicable; Tet, tetracycline; Tob, tobramycin; Tsx, trimethoprim-sulfamethoxazole.

[a] Data in parentheses are percentage of the 108 subjects enrolled in the study.

[b] Data in parentheses are percentage of subjects in resistance-profile group (no. of subjects with MDR profile/no. in resistance-profile group).

[c] Isolates from 7 subjects had resistance to Tet, and an isolate from 1 subject had resistance to Amp.

sistance determinants [4]. Another possibility might be environmental exposure to antibiotic-producing organisms (e.g., stored food contaminated by molds producing antibiotic substances). Yet another possibility would be the maintenance of antimicrobial resistance due to the associated linkage selection resulting from exposure to heavy metals. In fact, heavy metals and antimicrobial coresistance have been evidenced in the microbial population of ecosystems contaminated by metals in the absence of antimicrobial selection [13–15]. Although the clonality of resistant isolates was not specifically investigated in the present study, the resistance patterns were quite heterogeneous, suggesting that it is unlikely that this phenomenon was due to efficient dissemination of a single resistant strain in the population.

One limitation of the present study is that quantitative data on antimicrobial consumption were not available and that information regarding population mobility, exposure to antimicrobial agents, and health-care practices could be obtained only through retrospective interviews. However, the community's awareness of the importance of this study, demonstrated by its willingness to participate and by the involvement of trained Guaraní health-care workers in the interviews, implies that the data collected are reliable. Another limitation is represented by the fact that clonality was not specifically investigated in resistant isolates. The most relevant implication of the present study's findings is that, in certain settings, the spread and maintenance of antimicrobial resistance can occur regardless of the selective pressure generated by the use of antimicrobial agents. Further investigation will be necessary for an understanding of the mechanisms underlying similar phenomena, mechanisms that may be relevant to any program aimed at the control of antimicrobial resistance.

References

1. World Health Organization. Global strategy for containment of antimicrobial resistance. Geneva: WHO, **2001**. Available at: http://www.who.int/csr/drugresist/guidance/en. Accessed 12 March 2004.
2. Avorn JL, Barrett JF, Davey PG, McEwen SA, O'Brien TF, Levy SB. Antibiotic resistance: synthesis of recommendations by expert policy groups. Alliance for the Prudent Use of Antibiotics [WHO/CDS/CSR/DRS/2001.10]. Geneva: World Health Organization, **2001**. Available at: http://www.who.int/csr/drugresist//Antimicrobial_resistance_recommendations_of_expert_polic.pdf. Accessed 12 March 2004.
3. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioural factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg Infect Dis **1999**; 5:18–27.
4. Salyers AA, Amábile-Cuevas CF. Why are antibiotic resistance genes so resistant to elimination? Antimicrob Agents Chemother **1997**; 41:2321–5.
5. Alliance for the Prudent Use of Antibiotics (APUA). Reservoirs of Antibiotic Resistance (ROAR). Cemmensal bacteria are reservoirs of resistance. Available at: http://www.tufts.edu/med/apua/ROAR/project.htm. Accessed 12 March 2004.
6. Lester SC, Del Pilar Pla M, Wang F, et al. The carriage of *Escherichia coli* resistant to antibiotic agents by healthy children in Boston, in Caracas, Venezuela and in Qin Pu, China. N Engl J Med **1990**; 323:285–9.
7. Bartoloni A, Cutts F, Leoni S, et al. Patterns of antimicrobial use and antimicrobial resistance among healthy children in Bolivia. Trop Med Int Health **1998**; 3:116–23.
8. Österblad M, Hakanen A, Manninen R, et al. A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. Antimicrob Agents Chemother **2000**; 44:1479–84.
9. Calva JJ, Sifuentes-Osornio J, Cerón C. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. Antimicrob Agents Chemother **1996**; 40:1699–702.
10. NCCLS. Performance standards for antimicrobial disk susceptibility tests; approved standard. 7th ed. Supplement M2-A7, vol. 20, no. 1. Wayne, PA: NCCLS, **2000**.
11. Rossolini GM, Zanchi A, Chiesurin A, Amicosante G, Satta G, Guglielmetti P. Distribution of *cphA* or related carbapenemase-encoding genes and production of carbapenemase actvity in members of genus *Aeromonas.* Antimicrob Agents Chemother **1995**; 39:346–9.
12. Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev **1995**; 8:557–84.
13. Levin BR, Lipsitch M, Perrot V, et al. The population genetics of antibiotic resistance. Clin Infect Dis **1997**; 24(Suppl 1):S9–16.
14. Timoney JF, Port J, Giles J, Spanier J. Heavy-metal and antibiotic resistance in the bacterial flora of sediments of New York bight. Appl Environ Microbiol **1978**; 36:465–72.
15. Calomiris JJ, Armstrong JL, Seidler RJ. Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. Appl Environ Microbiol **1984**; 47:1238–42.