# High prevalence of antimicrobial resistance in commensal bacteria from human populations living in urban and rural areas of Bolivia

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**Summary -** Bacterial resistance to antimicrobials represents a global public health problem, that undermines the efficacy of antimicrobial chemotherapy. The emergence and spread of resistance are universally acknowledged to be associated with heavy consumption of antimicrobials in clinical and veterinary practices. A combination of misuse and overuse of antimicrobial agents, along with poor sanitation, are among the claimed reasons for the exceedingly high resistance rates observed in low-resource countries. However, several aspects concerning the impact of antimicrobial usage remain poorly understood.
Some studies conducted in Bolivia in '80-'90 years evidenced a high prevalence of antimicrobial resistance in commensal bacteria from human populations living in urban and rural areas. The selective pressure generated by widespread and inappropriate usage was considered the main responsible for these findings. However, an unexpected remarkable high prevalence of antimicrobial resistance detected in the commensal *Escherichia coli* microbiota of the human population of a very remote rural community, raises the question about the origin of the emergence and spreading of bacterial resistance. These findings suggest that, at least in certain settings and for some resistance determinants, a significant spread of antimicrobial resistance can take place regardless of the overuse/misuse of antimicrobial agents in clinical practice.

*Elevata prevalenza di resistenza agli antimicrobici nella flora batterica commensale umana in aree urbane e rurali della Bolivia*
**Riassunto -** La resistenza batterica agli antimicrobici costituisce un problema globale di salute pubblica, che mina l'efficacia della chemioterapia antimicrobica. E' universalmente noto come l'emergenza e la diffusione delle resistenze sia associata al massiccio consumo di antimicrobici in campo medico e veterinario. Un incongruo ed eccessivo utilizzo degli antimicrobici, unitamente a condizioni igienico-sanitarie scarse, costituiscono le ragioni più immediate di un alto tasso di resistenza agli antimicrobici nei paesi a limitate risorse. Tuttavia, molti aspetti inerenti l'effettivo peso dell'uso degli agenti antimicrobici rimangono ancora poco compresi.
Alcuni studi condotti in Bolivia negli anni '80-'90 hanno evidenziato un'elevata prevalenza di resistenza ai chemioantibiotici nella flora batterica commensale umana in aree urbane e rurali. La pressione selettiva generata da un diffuso e inappropriato uso di antimicrobici è stata considerata il fattore principale responsabile di questi risultati. Tuttavia, l'estremamente inatteso riscontro di un elevato tasso di resistenza agli antimicrobici in *Escherichia coli* commensale in una popolazione di una comunità rurale molto isolata, suscitano l'interrogativo circa l'origine dell'emergenza e diffusione delle resistenze batteriche. Questi dati suggeriscono come, almeno in alcuni contesti e per alcuni determinanti di resistenza, possa esserci una significativa diffusione di resistenze batteriche anche a prescindere da un eccessivo/incongruo uso nella pratica clinica di agenti antimicrobici.

**Key words**: antimicrobial resistance, commensal bacteria, *Escherichia coli*, low-resource countries, Bolivia

## INTRODUCTION

Despite of the big progresses in health system, the infectious diseases are responsible of the 25% of deaths in the world and of the 45% in low income countries (SHEARS, 2001, WORLD HEALTH ORGANISATION, 2002).

Antimicrobials have deeply changed the treatment of common bacterial infections and play a crucial role in reducing morbidity and mortality all over the world, especially in low-resource countries. Since antimicrobials were first introduced, their use has increased steadily throughout the world and nowadays they constitute a large proportion of the cost of

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the health care systems. Antimicrobial drugs are the most commonly purchased class of drugs in low-resource countries, where the infectious diseases are extremely frequent and the bacterial infections are the major cause of death, especially in childhood (COL-O'CONNOR, 1987, WORLD HEALTH ORGANISATION, 1988, GUNDERSSEN, 1992).

Antimicrobial chemotherapy exerts a selective pressure on microorganisms and the emergence and spread of resistance are universally acknowledged to be associated with the heavy consumption of antimicrobial agents. Bacterial resistance has represented one of the most important problem limiting the chemotherapy efficacy since their introduction in clinical practice (FARRAR, 1985, WORLD HEALTH ORGANISATION, 2001), and now poses a global public health problem threatening to undermine many of the advances made in the past 50 years in the treatment of infections (SHEARS, 2001).

Worldwide there are reports about the presence of antimicrobial resistance in important pathogens. The high-resource countries are going to face the emerging of infections, and the choice of antimicrobials to manage them has becoming more and more difficult, because of the appearance of glycopeptide-intermediate and vancomycin-resistant *S. aureus* (GISA, VRSA), glycopeptide-resistant enterococci, and multiple drug-resistant *Acinetobacter*, *Stenotrophomonas*, *Klebsiella*, and *Pseudomonas* (GILLESPIE, 2004).

Resistance to antimicrobials is particularly serious in low-resource countries, where resistance rates are even higher than in industrialized countries, and where therapeutical options are often unavailable or too expensive (O'BRIEN, 1992, WORLD HEALTH ORGANISATION, 1997).

From most low-resource countries there are reports of resistance to penicillin in clinical isolates of *Neisseria meningitidis*, resistance to different antimicrobial agents in organisms producing community-acquired respiratory tract infections (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*), as well as in organisms causing enteric infections (including *Shigella* spp., *Escherichia coli*, *Vibrio* spp.) and urinary tract infections (GUZMÁN BLANCO *et al.,* 2000).

In these countries there is also evidence of spreading of multiple resistant strains of *Salmonella* spp., *Mycobacterium tuberculosis* and *Neisseria gonorrhoeae*. An epidemic of *Shigella dysenteriae* type 1 infections affected Burundi in 1994 and all the isolates were resistant to ampicillin, chloramphenicol, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole (TMP/SXT) (RIES *et al.,* 1994). A high incidence of resistance to multiple antimicrobials in clinical isolates of *S. pneumoniae* has been reported in an university hospital in Korea in 1991-1993: seventy percent of isolates were not susceptible to penicillin, exhibiting either intermediate resistance (37%) or high-level resistance (33%) (LEE *et al.,* 1995). The first report of epidemic ciprofloxacin-resistant *Salmonella typhi* was in the central Asian republic of Tajikistan (located to the north of Afghanistan) during the 1997 epidemic of typhoid fever (MURDOCH *et al.,* 1998).

The phenomenon of drug resistance is not restricted to pathogenic bacteria, and there is increasing evidence that non-clinical isolates such as commensal bacteria of humans and animals, plant and soil bacteria may be playing an important role in bacterial resistance (RAO, 1998, APUA, 2004).

Commensal bacteria, although not being a specific target, are continuously exposed to the selective pressure generated by antimicrobial chemotherapy and may become a potential reservoir of resistant strains and resistance determinants that can be transferred to pathogenic bacteria (LESTER *et al*., 1990, AMYES *et al*., 1992, CALVA *et al*., 1996, LEVIN *et al*., 1997 ECONOMIC AND SOCIAL COMMITTEE OF EUROPEAN COMMUNITIES, 1998, KARIUKI-HART, 2001, APUA, 2004).

The commensal flora of the gut is considered the most important reservoir for antimicrobial resistance genes in both the community and hospital environments. It has been well documented that intestinal bacteria represent a major reservoir for resistance genes (LEVY *et al*., 1988, LESTER *et al*., 1990, APUA, 2004) which can be transferred to more pathogenic organisms such as *Shigella* spp. and *Salmonella* spp. (TAUXE *et al*., 1989) and to non-intestinal organisms, such as *H. influenzae* (LEVY, 1986). For this reason, some members of the commensal microbiota, such as the faecal *E. coli*, are considered sensitive indicator bacteria for surveillance of antimicrobial resistance, to measure the selective pressure generated by antimicrobial usage, to evaluate the impact of modifications in antimicrobial prescription policies and to predict the emergence of resistance in pathogens (MOORHOUSE, 1969, DATTA, 1969, LINTON *et al.,* 1972, SHANAHAN *et al.,* 1993, CALVA *et al.,* 1996, BARTOLONI *et al.,* 1998, OKEKE *et al.,* 2000, ÖSTERBLAD *et al.,* 2001).

**Antimicrobial resistance in commensal bacteria from healthy subjects in Bolivia**

The Infectious Diseases Division of the University of Florence, Italy, has been carrying out scientific research and health co-operation in Latinamerican countries since 1986. The main study area has been the Chaco region, south-eastern Bolivia.

One of the aims of the research activity conducted in agreement with the local health authorities and the Bolivian Ministry of Health was to investigate the patterns of antimicrobial use and the antimicrobial susceptibility of the commensal bacteria from healthy subjects living in urban and rural communities.

In 1987, in 1992, and in 1999, bacterial resistance to antimicrobial agents was evaluated among isolates

of staphylococci and *E. coli* from healthy subjects living in urban and rural areas (BARTOLONI *et al.,* 1990, BARTOLONI *et al.,* 1998, BARTOLONI *et al.,* 2004).

**Antimicrobial resistance in commensal staphylococci**

The first study was carried out in November-December 1987 in three different localities of the Chaco Region (Camiri, Boyuibe and Javillo), to evaluate the *in-vitro* antimicrobial susceptibility of commensal staphylococci isolated from healthy people (BARTOLONI *et al.,* 1990).

At the time of the study, Camiri was a city of about 25,000 inhabitants with two hospitals: the District Hospital, run by the Bolivian Ministry of Health, and a private hospital. Boyuibe was a poor town of 2,500 inhabitants with no infrastructure, not even a potable water supply or sewerage system, situated approximately 3 h by jeep south of Camiri. In Boyuibe there was an area hospital run by the Bolivian Ministry of Health. The third locality, Javillo, was a small community of an ethnically homogeneous group of about 100 Guaraní Indians, situated in the jungle north-east of Camiri. The population lived in huts without any hygienic-sanitary facilities or services.

Staphylococcal isolates were obtained from nasal, axillary, and perineal swabs from hospital staff of the Camiri District Hospital and of the Boyuibe Hospital, from school students in Camiri and Boyuibe, and from apparently healthy people living in Javillo.

A total of 519 staphylococcal isolates (50 of *S. aureus* and 469 of coagulase-negative staphylococci) were collected in the three localities. All the isolates were tested for susceptibility to penicillin G using the agar diffusion method and, for isolates recorded as resistant or moderately susceptible, the minimum inhibitory concentrations of nine antibiotics (methicillin, ampicillin, cephalotin, gentamicin, erythromycin, chloramphenicol, clindamycin, tetracycline, vancomycin) were determined by broth micro-dilution technique. The results are shown in Tables 1 and 2.

The highest resistance rates were observed for the staphylococci isolated from the two groups of hospital personnel. All the *S. aureus* isolates collected from the hospital personnel exhibited resistance to penicillin. None of these was recorded as methicillin resistant. Cephalotin, gentamicin, clindamycin and vancomycin were active against all the isolates. No statistically significant differences were observed between the resistance patterns of the two populations studied ($P>0.05$). The penicillin resistance prevalences of the coagulase-negative staphylococci

**Table 1 - Penicillin sensitivity of commensal staphylococci isolated from human populations in the Chaco region, Bolivia, by the agar diffusion method (1987)**

| | *Staphylococcus aureus* | | | | Coagulase-negative staphylococci | | | |
|---|---|---|---|---|---|---|---|---|
| | *n* | S (%) | I (%) | R (%) | *n* | S (%) | I (%) | R (%) |
| Camiri | | | | | | | | |
| hospital staff | 7 | 0 | 0 | 100 | 83 | 15.7 | 10.8 | 73.5 |
| school students | 24 | 0 | 0 | 100 | 104 | 23.1 | 26.9 | 50.0 |
| Boyuibe | | | | | | | | |
| hospital staff | 2 | 0 | 0 | 100 | 12 | 0 | 8.3 | 91.7 |
| school students | 17 | 17.6 | 5.9 | 76.5 | 178 | 37.6 | 45.5 | 16.9 |
| Javillo | | | | | | | | |
| healthy subjects | - | - | - | - | 92 | 65.2 | 34.8 | 0 |

S, Susceptible; I, Intermediate; R, Resistant.

**Table 2 - Percentage of antimicrobial resistance of commensal staphylococci isolated from human populations in the Chaco region, Bolivia, by the microdilution method (1987)**

| | Methicillin | | Ampicillin | | Cephalotin | | Gentamicin | | Erythromycin | | Chloramphenicol | | Clindamycin | | Tetracycline | | Vancomycin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SA | CNS | SA | CNS | SA | CNS | SA | CNS | SA | CNS | SA | CNS | SA | CNS | SA | CNS | SA | CNS |
| Camiri | | | | | | | | | | | | | | | | | | |
| hospital staff | 0 | 2.9 | 85.7 | 32.8 | 0 | 1.4 | 0 | 8.6 | 14.3 | 18.6 | 42.9 | 38.6 | 0 | 7.1 | 14.3 | 45.7 | 0 | 0 |
| school students | 0 | 1.2 | 66.7 | 18.7 | 0 | 0 | 0 | 2.5 | 20.8 | 16.3 | 25.0 | 22.5 | 0 | 0 | 16.7 | 52.5 | 0 | 0 |
| Boyuibe | | | | | | | | | | | | | | | | | | |
| hospital staff | 0 | 8.3 | 100 | 58.3 | 0 | 0 | 0 | 8.3 | 0 | 0 | 100 | 16.7 | 0 | 8.3 | 0 | 33.3 | 0 | 0 |
| school students | 0 | 0 | 71.4 | 20.7 | 0 | 0 | 0 | 0.9 | 0 | 9.0 | 14.3 | 22.5 | 7.1 | 1.8 | 14.3 | 38.7 | 0 | 0 |
| Javillo | | | | | | | | | | | | | | | | | | |
| healthy subjects | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 6.3 | - | 0 | - | 9.4 | - | 0 |

SA, *Staphylococcus aureus*; CNS, coagulase-negative staphylococci.

in the Camiri and Boyuibe hospital staffs were 73.5 and 91.7%, respectively. Two isolates (2.9%) from Camiri and one (8.3%) from Boyuibe were resistant to methicillin. Cephalotin, clindamycin and gentamicin were the most active drugs. Vancomycin was the only drug found effective against all the coagulase-negative staphylococci isolated from both hospital staffs and exhibiting a multidrug resistant phenotype. There were no statistically significant differences between the resistance patterns of the coagulase-negative staphylococcal isolates from the two studied population ($P>0.05$).

As far as *S. aureus* isolates from the school students were concerned, the penicillin resistance rate was 100% and 76.5% in the Camiri and Boyuibe school groups, respectively. None of the isolates were recorded as methicillin resistant. Cephalotin, gentamicin and vancomycin were the most active drugs (100% of the isolates were susceptible). Regarding the coagulase-negative staphylococci, 50% and 16.9% resulted resistant to penicillin in the Camiri and Boyuibe school children, respectively. Only one isolate in Camiri was recorded as methicillin resistant, and vancomycin, cephalotin, gentamicin and clindamycin were the most active drugs. Comparison of the resistance patterns for coagulase-negative staphylococci isolated from the two groups of students did not show any statistically significant difference ($P>0.05$).

The staphylococci isolated from the rural population of Javillo were highly susceptible to all the antibiotics tested. None of them was resistant to penicillin.

With the help of the medical staff and nurses responsible for the delivery of drugs we collected information about the pattern of use of antimicrobial agents in the two hospitals. Moreover, we obtained data, by using a questionnaire distributed to the pharmacists, concerning the distribution of antimicrobials by the pharmacies of Camiri and Boyuibe. This investigation showed that the drugs most used, with or without medical prescription, were penicillin and ampicillin, and, less commonly, TMP/SXT and chloramphenicol. The distribution of the other drugs, such as erythromycin, amoxicillin, tetracycline and gentamicin was much more limited. In all cases we noted that the cycles of treatment were short, from 3 to 5 days.

The results from this study confirmed the remarkable capacity of staphylococci to adapt to antimicrobial agents, and a close correlation between use of antimicrobial agents and development of resistance to them was hypothized. In the rural community of Javillo, visited by a doctor approximately once a year, or even less often, and where antimicrobials were not used, staphylococci were still highly susceptible to antimicrobial agents. In Camiri, where antibiotics were commonly used, we found various antimicrobial resistant staphylococcal isolates. On the basis of the obtained results, the selective pressure generated by antimicrobial usage was considered the main responsible for the high rate of antimicrobial resistance in the urban areas.

### Antimicrobial resistance in commensal *Escherichia coli*

The Infectious Diseases Division of the University of Florence kept on studying the phenomenon of antimicrobial resistance in the same area and extended the research to the commensal flora of the gut. In 1992, a study was carried out to evaluate patterns of antimicrobial availability and use in Camiri, and the faecal carriage of antimicrobial resistant *E. coli* by healthy children living in Camiri and Javillo (BARTOLONI *et al.*, 1998).

The antimicrobial availability was investigated by a questionnaire survey of hospital and private pharmacies of Camiri. For each hospital, data were also collected on the annual expenditure for all drugs and for antimicrobials. All private pharmacies stocked ampicillin, amoxycillin, penicillin and erytromycin, all but one stocked first-generation cephalosporins, chloramphenicol and tetracycline; most stocked isoxazolilpenicillin and gentamicin; half had TMP/SXT, lincomycin and rifampicin; and a minority stocked amikacin, streptomycin and doxycycline. Ciprofloxacin was stocked by two private pharmacies. Broad-spectrum penicillins and benzylpenicillin were the most commonly used antimicrobials. Erythromycin, cloramphenicol, first-generation cephalosporin, TMP/SXT, tetracyclines, and streptomycin were also frequently used. For the hospitals, antimicrobials represented an average of 40% of the total pharmaceutical budget.

An additional arm of the study examined antibiotic usage patterns using simulated patients scenarios. Study personnel presented one of the six clinical scenarios at each of the private pharmacies in Camiri. In each case they recorded the name and quantity of the medications dispensed by the pharmacist and any advice given. The six scenarios included: 1) a 6-month old child with acute watery diarrhea without fever, 2) an adult with acute watery diarrhea without fever, 3) a 2-year old child with low grade fever and rhinorrhea, 4) an 8-year old child with fever and sore throat, 5) an adult female with acute dysuria and fever, and 6) an adult male with purulent urethral discharge. In this simulated patients study, over two-thirds of the pharmacies dispensed antimicrobials without a medical prescription, and the quantity dispensed varied according to the patient's ability to pay.

Antimicrobials were dispensed inappropriately for 92% of adults and 40% of children with watery diarrhea. Sixty percent of the pharmacies also gave antidiarrhoeals to children while none gave oral rehydration salts. Tetracycline was the most commonly dispensed antimicrobial in adults with diarrhea and in one case it was given to a six-month old child. Furazolidone, sulphoguanide, sulphathiazole, neomycin, and streptomycin were the other antimicrobials dispensed. All pharmacies only

dispensed enough antibiotics for two days or less. For the mock illnesses for which antimicrobial therapy is indicated, antimicrobials were dispensed to 67% of males with purulent urethral discharge. Ciprofloxacin was the most commonly dispensed drug. Benzathine penicillin, procaine penicillin, TMP/SXT, and spectinomycin were also dispensed. Only spectinomycin was given in an appropriate dose. Only 58% percent of pharmacies dispensed antimicrobials (pipemidic acid, sulfametizole, nitrofurantoin, nalidixic acid, norfloxacin) to women with fever and dysuria. The recommended duration of therapy ranged from 1 to 5 days but in all cases the quantity of tablets dispensed was four or less. Forty-two percent of pharmacies dispensed only phenazopyridine, a urinary analgesic with no antibacterial activity.

The ready availability of antimicrobials at pharmacies was mirrored by the high utilization of antimicrobials by children in the community survey, even in the remote rural community of Javillo. In Camiri 296 children and in Javillo 25 children were enrolled. The mean age was 35 months in Camiri and 41 months in Javillo. Fifteen percent of the children in Camiri and 4% in Javillo had a history of previous travel outside the community; 58% and 64%, respectively from Camiri and Javillo, were said to have received an antimicrobial more than four months prior to the study. The most common reasons for antimicrobial use in children were upper respiratory tract infections, followed by diarrhoea. In the survey most families stated that antimicrobials had been purchased at the advice of a physician, though approximately one-third of families in Camiri had obtained the antimicrobial from a pharmacist. The study did not evaluate prescribing habits of physicians, but other studies have shown that the majority of antimicrobial agents prescribed for hospitalised and ambulatory patients were inappropriate (STEIN *et al*., 1984, ISLAM, 1985).

A selective screening method employed in earlier studies was used to detect resistant *E. coli* in stool samples (DATTA, 1969, LESTER *et al*., 1990). Presumptive resistant isolates were subjected to confirmatory analysis, including species identification and conventional susceptibility according to the standard disk diffusion method. The results are shown in Table 3.

A very high rate of faecal carriage of antimicrobial resistant *E. coli* was found in children from both urban and rural areas. The prevalence of resistance to the commonly dispensed antimicrobials in Camiri was 97% to ampicillin, 94% to TMP/SXT, and 92% to tetracycline. Resistance was also high to chloramphenicol (69%). The prevalence of resistance to other antimicrobials was much lower. In Javillo, resistance was also frequently found to commonly used antimicrobials, although the prevalence was lower than that in Camiri for ampicillin (87%), tetracycline (80%), TMP/SXT (71%), and chloramphenicol (57%).

In both communities, multidrug resistant *E. coli* isolates were detected in most faecal samples. The most frequently occurring multidrug resistant pattern included resistance to ampicillin, TMP/SXT, tetracycline, and chloramphenicol. The mean number of antimicrobials to which an *E. coli* strain was resistant by disk diffusion was 4.3 in Camiri and 3.3 in Javillo ($P<0.01$). Younger children carried strains resistant to more antimicrobial agents than older children.

Further studies were conducted on a random sample of ampicillin resistant isolates to investigate the production of β-lactamase activity, and the nature of β-lactamase determinants (BARTOLONI *et al*., 2000). Production of β-lactamase was observed in all the isolates and in most of them (over 90%) acquired *bla*TEM determinants were detected. AmpC production was detectable in some isolates but only in a minority provided a significant contribution to the resistance phenotype.

The prevalence of resistance to ampicillin and TMP/SXT found in this study was higher than that previously reported in low-resource countries. Reports from Chile, Honduras, Costa Rica, Brazil, and Thailand have reported 38-50% prevalence of

**Table 3 - Prevalence of faecal carriage of antimicrobial-resistant *Escherichia coli* strains isolated in 1992 from children in Camiri and Javillo, Bolivia, by disk diffusion.**

| | Percentage of resistant strains | |
|---|---|---|
| | Camiri (95% CI) | Javillo |
| Amikacin | 00 | |
| Ampicillin | 97 (95-99)[a] | 87[a] |
| Cefotaxime | 0 | 0 |
| Cephalothin | 10 (6-14) | 25 |
| Chloramphenicol | 69 (64-74) | 57 |
| Ciprofloxacin | 0 | 0 |
| Trimethoprim-sulphamethoxazole | 94 (91-97)[b] | 71[b] |
| Gentamicin | 5 (2-8) | 0.6 |
| Nalidixic acid | 4 (2-6) | 0.6 |
| Nitrofurantoin | 5 (2-8) | 0 |
| Tetracycline | 92 (89-95) | 80 |

[a] $P<0.05$, [b] $P<0.01$

resistance to TMP/SMX (MURRAY *et al*, 1985). Findings for ampicillin resistance are as high or higher than those reported from India, Venezuela, and China, which have previously documented some of the highest frequencies of antimicrobial resistant commensal bacteria within a healthy low-resource country population (LESTER *et al*, 1990, AMYES *et al*, 1992).

Carriage of resistant faecal *E. coli* was significantly associated with younger age, which may reflect both the increased use of antimicrobials in both communities in recent years (BARTOLONI *et al*., 1990) and the higher incidence of infections, leading to more frequent use of antimicrobials in younger children (GRANT, 1993). The high prevalence of antimicrobial resistance and the widespread poor practice in pharmacies, with inappropriate use of antimicrobials for those who do not need them and ineffective treatment regimens for those who do, highlighted the magnitude of this public health problem. In conclusion, this study documented several problems associated with the use of antimicrobials, including inappropriate and ineffective regimes dispensed at pharmacies, widespread use of antimicrobials in young children even in a remote rural area of Bolivia, and widespread carriage of resistant commensal gut bacteria in urban and rural areas.

In 1999, a new study was conducted with the aim to investigate antimicrobial resistance in the commensal *E. coli* microbiota of the human population of a very remote rural community of Bolivia, where the use of antimicrobials had been minimal (BARTOLONI *et al*., 2004). The local health authorities identified the community of Alto Los Zarzos as one of the remotest communities, whose population had few exchanges with the exterior and, thus far, a very limited access to the health services. The community is located in the Gran Chaco region of the Tarija Department, on the foothills of the Andes at an altitude of about 1700 m, and is only reachable on foot from the closest health post by climbing a slope in the forest that requires about three hours of walking. The population was constituted by approximately 130 Guaraní Indians whose principal activities were agriculture and animal breeding. They lived in huts with no sanitary or hygienic facilities. The community was visited by a health worker approximately every three months. From each adult participant information was obtained on previous drug use, visits to the health care post, travels outside the community, and previous hospitalization. A stool sample was collected from each subject and the presence of resistant isolates was screened for by the rapid method used in the previous studies (BARTOLONI *et al*., 1998). Presumptive resistant *E. coli* isolates were subjected to confirmatory analysis, including species identification and conventional susceptibility testing on the basis of disk diffusion. The nature of beta-lactamase determinants was investigated by colony blot hybridization by using a *bla*$_{TEM}$ probe. A total of 108 individuals, aged 1-77 years, were investigated. This group included all the inhabitants present on the day when the survey was conducted.

According to interviews, during the 12 months preceding the study, 38% subjects reported a history of travel outside the community, 6% and 32% subjects, respectively, reported either visiting the nearest health-care post or seeking the aid of a traditional healer (mostly for trauma), 7% subjects reported previous use of antimicrobial agents, and 4% subjects reported, during lifetime, a previous history of hospitalisation in the district capital. The health-care worker confirmed that the use of antimicrobial agents was very limited. Veterinary and agricultural use of antimicrobial agents was totally absent.

The results of the microbiological study are shown in Table 4 and 5. Sixty-seven percent of the subjects were found to be carriers of faecal *E. coli* with acquired resistance to ≥1 antimicrobial agent(s). The highest rates were observed for tetracycline (64%), ampicillin (58%), TMP/SXT (50%) and chloramphenicol (41%). The majority of subjects in which antimicrobial-resistant isolates were detected, harboured a faecal *E. coli* population with multiple resistance traits. The most common clustering of resistance traits included resistance to tetracycline, ampicillin, TMP/SXT and chloramphenicol. Most of the subjects harbouring a multidrug resistant faecal population, yielded at least one isolate in which all of the corresponding traits were simultaneously present. Of the 81 ampicillin-resistant isolates, 78 were recognized by a *bla*$_{TEM}$ probe.

Considering that the use of antimicrobial agents is claimed to be the driving force for the emerging and diffusion of bacterial resistance, our findings were highly unexpected. Even if we cannot consider the surveyed human population as virgin in terms of antimicrobial use, antimicrobial consumption seemed to be far too low to account for the high prevalence of antimicrobial resistance in the resident microbiota, especially when compared with the prevalence measured in other settings characterized by overuse of antimicrobial agents (ÖSTERBLAD *et al*., 2000).

Overall, the studied community exhibited a relatively high degree of isolation, with rare exchanges with the exterior and access to health services, and a history of very limited use of antimicrobial agents. Considering that non-human (veterinary and agricultural) use of antimicrobials was totally absent in that area, and that the role of a faecal contamination of water sources contaminated by other human populations could be excluded due to the geographical situation of the community, other hypothesis have been formulated to explain a similar scenario. The likeliest hypothesis is that resistant strains had occasionally been introduced in the community following exchanges, although limited, of the population with the exterior, and that resistant strains and/or resistance determinants had efficiently spread, facilitated

**Table 4 - Prevalence of faecal carriage of antimicrobial-resistant *Escherichia coli* in 108 subjects from a remote rural community of Bolivia (1999).**

| Resistance trait[a] | Age groups ≤6 years (17 subjects) | | 7-14 years (37 subjects) | | ≥15 years (54 subjects) | | Total (108 subjects) | |
|---|---|---|---|---|---|---|---|---|
| | n° | (%) | n° | (%) | n° | (%) | n° | (%) |
| Tetracycline | 9 | (53) | 26 | (70) | 34 | (63) | 69 | (64) |
| Ampicillin | 7 | (41) | 24 | (65) | 32 | (59) | 63 | (58) |
| Trimethoprim-sulphamethoxazole[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) |
| At least one trait | 10 | (59) | 27 | (73) | 35 | (65) | 72 | (67) |

[a]Isolates resistant to gentamicin, amikacin, nalidixic acid, and nitrofurantoin were never detected.
[b]The prevalence of resistance to trimethoprim-sulphamethoxazole was significantly lower ($P$=0.017) in the youngest subjects (≤6 years) than in the older ones (≥7 years) considered as a whole.

**Table 5 - Clustering of resistance traits in the 72 subjects with faecal carriage of antimicrobial-resistant Escherichia coli, and distribution of multidrug-resistant (MDR) isolates from those subjects (1999).**

| Resistance traits* | No. of subjects (%)‡ | No. of isolates | |
|---|---|---|---|
| | | MDR isolates (%)§ | Total n |
| Single resistance trait | 8† (7) | N.A.^ | 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 |

*Tet, tetracycline; Amp, ampicillin; Tsx, trimethoprim-sulphamethoxazole; Cm, chloramphenicol; Km, kanamycin; Tob, tobramycin
‡Percentages refer to the total of 108 subjects enrolled in the study.
§Isolates showing a multidrug resistant (MDR) phenotype including all the resistant traits detected in the corresponding group of subjects. The percentage refers to the number of subjects in each group.
°Total number of nonreplicate antimicrobial-resistant isolates from the corresponding group of subjects.
†Single resistance traits included 7 to Tet, and 1 to Amp.
^N.A., not applicable.

by unhygienic conditions. The occasional travels of some members of the community to the nearest town might have resulted in the acquisition of resistant bacteria through contaminated food. In a previous study, carried out in urban and rural communities of the same area of Bolivia, a high prevalence of antimicrobial resistance was detected in the commensal *E. coli* microbiota, and a widespread use of antimicrobials associated with documented inappropriate administration policies offered the most plausible explanation for the high prevalence of resistance rates to the most commonly dispensed antimicrobial agents (BARTOLONI *et al*., 1998). Resistance patterns found in the population of Alto Los Zarzos were similar to those reported in urban communities of the same area. Another possibility could be represented by environmental exposure to antibiotic-producing organisms, in particular, a role could be speculated for stored food contaminated by moulds producing antibiotic substances. Yet another possibility could be represented by the maintenance of antimicrobial resistance due to the associated linkage selection resulting from the exposure to heavy metals. In fact, heavy metals and antimicrobial co-resistance have been evidenced in the microbial population of ecosystems contaminated by metals in the absence of antimicrobial selection (TIMONEY *et al*., 1978, CALOMIRIS *et al*., 1984, LEVIN *et al*., 1997). Nevertheless, the long-term persistence and spreading of similar strains in the community in the absence of a significant selective pressure generated by antimicrobial consumption remain to be explained, unless these strains exhibit additional selective advantages that are somewhat linked to the resistance determinants (SALYERS–AMABILE CUEVAS, 1997). On the

other hand, the resistance patterns observed in resistant isolates were quite variable, suggesting that this phenomenon was unlike to be due to efficient dissemination of a single resistant strain in the population. In conclusion, the most relevant implication of the study's findings is that, in certain settings, spread and maintenance of antimicrobial resistance can take place regardless of a selective pressure generated by the use of antimicrobial agents.

## DISCUSSION

Bacterial resistance to antimicrobial agents represents a global public health problem, that undermines the efficacy of antimicrobial chemotherapy, particularly serious in low-resource countries where bacterial infections are a major cause of morbidity and mortality, especially in childhood (WORLD HEALTH ORGANISATION, 2002). The emergence and spread of resistance are universally acknowledged to be associated with heavy consumption of antimicrobial agents in clinical and veterinary practices, and a prudent use of antibiotics is considered mandatory for preserving their therapeutic efficacy as long as possible (AVORN *et al.*, 2001). In this perspective, a combination of misuse and overuse of antimicrobial agents, along with crowding and poor sanitation, are among the claimed reasons for the exceedingly high resistance rates observed in low-resource countries (OKEKE *et al.*, 1999). However, several aspects concerning the impact of antimicrobial usage remain poorly understood (SALYERS–AMABILE CUEVAS, 1997), and this issue is difficult to investigate due to the almost universal use of antimicrobial agents for several decades, and to the lack of representative bacterial collections from the pre-antibiotic era.

A study conducted in Bolivia in 1987 showed that no cutaneous staphylococcal isolates obtained from the human population of a rural community were resistant to penicillin and only 10% to tetracycline, while 50% and 53% resistance, respectively, were observed in staphylococci isolated from the population of an urban area. The relevant difference in antimicrobial susceptibility patterns was attributed to the different pattern of antimicrobial use in the two settings. At that time, the antimicrobial consumption in the rural community was practically absent while in the urban area antimicrobial agents were commonly used. The selective pressure generated by antimicrobial usage was considered the main responsible for the high resistance rate observed in staphylococci isolated from the urban population, especially from the hospital personnel.

A disturbingly high prevalence of faecal carriage of antimicrobial resistant *E.coli* among healthy children was evidenced in a study conducted five years after in the same communities. The documented widespread and inappropriate use of antimicrobials offered an explanation to the study's findings. In the urban area poor practice of pharmacies was documented. Antimicrobials were dispensed without a medical prescription, inappropriately to "simulated patients" with conditions that did not warrant this therapy, and the quantity dispensed varied according to client's ability to pay. A widespread use of antimicrobials in young children was even documented in the remote rural community, as a result of the recent institution of regular visits by hospital physicians.

The study findings highlighted the magnitude and the complexity of two related major challenges posed to low-resource countries: widespread and inappropriate use of antimicrobials and bacterial drug resistance. The need for effective intervention to address inappropriate drug use is evident in the study area, as well as in many other low-resource countries. Efforts should be concentrated at both the provider and consumer levels (CHRISTIANSEN *et al.*, 2002). Interventions targeted at providers should include education programmes, provision of tools to improve diagnosis, implementation of practice guidelines. Consumer interventions should be based on information, education and communication (IEC) campaigns aimed at improving public's knowledge about the risk and benefits of antimicrobial therapy. Controls by means of regulatory interventions, if efficiently enforced, may help further to reduce inappropriate antimicrobial use and thereby decrease the selective pressure for development of resistance. In order to achieve lasting change, interventions should be multifaceted, long-term, and based on solid understanding of the socio-cultural rationality of antimicrobial usage (RADYOWYJATI-HAAK, 2003). Antimicrobial resistance surveillance is a key tool to provide current information on the magnitude and trends in resistance, and to plan and monitor intervention strategies aimed at preserving the therapeutic efficacy of antimicrobial agents. In low-resource countries effective surveillance programs are difficult to be implemented due to the lack of laboratory facilities, and, where they do exist, to the lack of quality control, reliable reagents and adequate supervision (SHEARS *et al.,* 2001). Rapid screening methods able to detect resistance in indicator organisms, such as faecal *E. coli*, represent an useful tool to conduct large-scale resistance surveillance studies and to monitor resistance-control programs.

The unexpected remarkable high prevalence of antimicrobial resistance detected in 1999 in the commensal *E. coli* microbiota of the human population of a very remote rural Bolivian community, where the use of antimicrobials has been minimal, raises the question about the origin of the emergence and spreading of bacterial resistance. These findings suggest that, at least in certain settings and for some resistance determinants, a significant spread of antimicrobial resistance can take place regardless of the overuse/misuse of antimicrobial agents in clinical practice. Additional investigation will be necessary to better understand the mechanisms underlying similar phenomena, mechanisms that can be relevant

to any program aimed at the control of antimicrobial resistance. The analysis of genetic basis of resistance is pivotal to try to fill some knowledge gaps on the basis of the complex phenomenon of emergence, spreading and maintenance of bacterial resistance, as well as to interpret the impact of interventions (PFALLER *et al.,* 2001).

A continuation of the research and co-operation activity addressing this important public health problem is represented by the ongoing project entitled "Towards controlling antimicrobial use and resistance in low-income countries - an intervention study in Latinamerica (ANTRES)" (www.unifi.it/infdis/antres). The project, financed from the European Commission within the Fifth Framework Programme (INCO-DEV), involves twelve Institutions from Bolivia, Peru, Italy and Sweden.

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