MICROBIAL DRUG RESISTANCE
Volume 18, Number 1, 2012
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DOI: 10.1089/mdr.2011.0003

Carriage of Antibiotic-Resistant *Escherichia coli* Among Healthy Children and Home-Raised Chickens: A Household Study in a Resource-Limited Setting

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We have previously observed high rates of acquired antibiotic resistance in commensal *Escherichia coli* from healthy children living in urban areas of Bolivia and Peru, including resistance to tetracycline and quinolones, which are not routinely used in childhood. In this work we investigated acquired resistance in commensal *E. coli* from healthy children and home-raised chickens in 12 households from one of the previously surveyed urban area in Bolivia, to ascertain the possibility of human–animal exchange of resistant strains in similar settings. The resistance rates to ampicillin, tetracycline, chloramphenicol, streptomycin, and trimethoprim-sulphametoxazole were overall high ($\geq$50%) and comparable between children and chickens, whereas those to quinolones were significantly higher in chickens (81% vs. 29% for nalidixic acid; 43% vs. 10% for ciprofloxacin). Molecular characterization of tetracycline- and quinolone-resistant isolates ($n = 66$) from children and chickens of three selected households revealed a remarkable clonal diversity and, in some cases, the presence of the same resistant strains among children or among chickens living in the same household, but not between children and chickens. Several resistance plasmids were characterized, but inter-clonal plasmid dissemination was not detected. Overall, the results from the present study suggested that cross-transmission between children and home-raised chickens could not represent a major spreading mechanism for resistant *E. coli* in households of resource-limited settings with high human–animal promiscuity.

Introduction

Transmission of bacteria between animals and humans has increasingly been recognized as a factor contributing to the emergence and spread of antibiotic resistance among human pathogens. The use of antibiotics in animal husbandry and veterinary medicine provides favorable conditions for selection of resistant bacteria, potentially transferred to humans. Routes of transmission may include the food chain and direct or indirect contacts (*e.g.*, pet owners and occupational exposure). Resistant bacteria of animal origin may cause human infections (*e.g.*, food-borne pathogens) or may colonize humans, acting in turn as potential donors of resistance genes to the commensal human microbiota, even in case of a temporary colonization.[1,11]

In a previous study we detected high rates of acquired resistance in commensal *Escherichia coli* from healthy children living in urban areas of Bolivia and Peru, including resistance to quinolones and tetracycline, which are not routinely used in childhood.[3] This suggested that acquisition of resistant bacteria by children might be related, at least in part, to cross-transmission (*e.g.*, adults to children or animals to children) favored by the conditions of poor sanitation typical of households of resource-limited settings.

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These results were presented in part at the 20th ECCMID, 10–13 April 2010, Vienna, Austria.

In this work we investigated acquired resistance in commensal *E. coli* from healthy children and home-raised chickens in households from one of the previously surveyed urban areas in Bolivia, where chickens represented the most common domestic animals and were reared in close household proximity. To ascertain the possible transmission of resistant strains or resistance plasmids between children and home-raised chickens, resistant isolates of both human and animal origin were subjected to detailed molecular characterization.

Materials and Methods

Study population

The study was carried out in a medium-sized urban area (approximately 30,000 inhabitants) of the Bolivian Chaco (Villa Montes, Tarija Department, Bolivia) and involved 12 households, randomly selected among those that had been found to rear domestic chickens in a previous survey (ANTRES study, see www.unifi.it/infdis/antres/default.htm). In that setting, chickens were reared in close household proximity, in conditions of high human–animal promiscuity.

For each household, rectal swabs were obtained from one or two healthy children aged 6–72 months ($n = 21$), and from one or two chickens ($n = 21$). Full ethics clearance was obtained from the qualified local authorities that had revised and approved the study design. Informed consent was obtained from children's parents or other legal guardians before collecting the samples.

Screening for fecal carriage of antibiotic-resistant E. coli

Screening for fecal carriage of antibiotic-resistant *E. coli* was carried out by a direct-plating method (DPM), as described previously.[3,4] Briefly, each rectal swab was spread on a MacConkey Agar No. 3 (MCA) plate (Oxoid) to yield uniform growth, and antibiotic-containing disks were directly placed onto the seeded plate. Antibiotics tested included ampicillin, ceftriaxone, tetracycline, chloramphenicol, streptomycin, kanamycin, gentamicin, amikacin, trimethoprim-sulphametoxazole, nalidixic acid, and ciprofloxacin (Oxoid). After incubation at 37°C for 12–14 hours, DPM plates were inspected for growth, and inhibition zone diameters were measured and interpreted according to previously described breakpoints. Only bacterial growth exhibiting an *E. coli*–like morphology was considered valid for the analysis.[3,4]

E. coli *isolates selected for molecular analysis*

Molecular analysis was focused on isolates exhibiting a resistance phenotype to tetracycline or quinolones, neither of which is routinely used for children. Moreover, resistance to these two classes of drugs is mainly plasmid-mediated or mutational, respectively.[9,10]

Three households in which carriage of tetracycline- or quinolone-resistant isolates had been observed in both humans and animals were selected. Rectal swabs from children ($n = 6$) and chickens ($n = 6$) were streaked on two MCA plates containing tetracycline 5 μg/mL (TET-MCA) or nalidixic acid 40 μg/mL (NAL-MCA), respectively. After incubation at 37°C for 12–14 hours, three isolated colonies from each plate were picked up and subjected to biochemical identification by the API20E system (BioMérieux) and *in vitro* susceptibility testing by the disk-diffusion method.[7,8] A total of 66 *E. coli* isolates were collected, of which 36 from TET-MCA (18 from chickens and 18 from children) and 30 from NAL-MCA (18 from chickens and 12 from children, as children from one of the three selected households did not carry nalidixic acid-resistant *E. coli*).

Genotyping of E. coli *isolates*

E. coli phylogroups (A, B1, B2, and D) were determined by the multiplex polymerase chain reaction (PCR) methods developed by Clermont *et al.*[6] Random amplification of polymorphic DNA (RAPD) genotyping was performed using, separately, the decamer primers 1290 and 1254, as previously described.[13] RAPD patterns were considered to be different when the profiles differed by at least one band.

Characterization of plasmids and of plasmid-mediated resistance genes

Tetracycline resistance genes [(*tet*(A), *tet*(B), *tet*(C), and *tet*(D)] were investigated by PCR, as described previously.[10] Plasmid-mediated quinolone resistance (PMQR) genes were investigated by PCR and sequencing as described previously (*qnrA*, *qnrB*, and *qnrS*[15]; *aac(6′)-Ib-cr*[16]; *qepA*[18]). Controls for *qnr* and *qepA* genes were kindly provided by Prof. Patrice Nordmann and Dr. Laurent Poirel (Université Paris-Sud, K.-Bicêtre, France), and by Prof. Patrice Courvalin (Institut Pasteur, Paris, France), respectively. Nucleotide sequences were determined on both strands of PCR amplification products at the Macrogen sequencing facility (Macrogen Inc.).

Plasmid replicon typing was carried out by a multiplex PCR method,[5] and by Southern blotting using a probe specific for ColE-like replicons (not included in the multiplex PCR approach), as described elsewhere.[15] Plasmid restriction profiles were analyzed by agarose gel electrophoresis after digestion with *Hae*III and *Eco*RI (Promega). Conjugal transfer of *tet* genes was assayed by mating experiments in Mueller Hinton (MH) broth (Difco Laboratories), using *E. coli* J53 (*pro met* [Rif^r] [Nal^r]) as the recipient and MH agar plates containing rifampin (400 μg/mL) and tetracycline (5 μg/mL) for selection of transconjugants, as described previously.[14] *qnrB* genes were transferred by electroporation into *E. coli* HB101 (F$^-$ *hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20* [Str^r] *xyl-5 mtl-1 supE44 leuB6 thi-1*), using MH agar plates containing nalidixic acid (8 μg/mL) for selection of transformants. Sequence analysis of *gyrA* and *parC* of transformants was carried out as described previously,[15] to exclude the occurrence of chromosomal mutations after selection on nalidixic acid.

Statistical analysis

Statistical differences in the prevalence of antibiotic resistance were determined by the Chi-square test. Confidence intervals were calculated by Stata Software release 8.0 (StataCorp., 2003).

Results

Prevalence of fecal carriage of antibiotic-resistant E. coli

All children ($n = 21$) and chickens ($n = 21$) of the 12 households investigated were found to carry commensal

Table 1. Carriage of Antibiotic-Resistant *Escherichia coli* in Healthy Children and Home-Raised Chickens from 12 Households of an Urban Area of the Bolivian Chaco (Villa Montes)

No. positive isolates (%; 95% confidence interval)

Antibiotic	*Children* (n = 21)	*Chickens* (n = 21)	p-*values*[a]
Ampicillin	21 (100%; 84–100)	18 (86%; 64–97)	NS
Ceftriaxone	0 (0%; 0–16)	1 (5%; 0–24)	NS
Tetracycline	20 (95%; 76–100)	18 (86%; 64–97)	NS
Chloramphenicol	12 (57%; 34–78)	11 (52%; 30–74)	NS
Streptomycin	20 (95%; 76–100)	17 (81% 58–94)	NS
Kanamycin	7 (33%; 15–57)	7 (33%; 15–57)	NS
Gentamicin	4 (19%; 5–41)	6 (29%; 11–52)	NS
Amikacin	1 (5%; 0–24)	0 (0%; 0–16)	NS
Trimethoprim-sulphametoxazole	21 (100%; 84–100)	18 (86%; 64–97)	NS
Nalidixic acid	6 (29%; 11–52)	17 (81%; 58–94)	<0.001
Ciprofloxacin	2 (10%; 1–30)	9 (43%; 22–66)	0.01

[a]NS, not significant ($p > 0.05$).

E. coli with at least one acquired resistance trait. Except for resistance to quinolones, which was significantly higher in chickens ($p < 0.001$ and $p = 0.01$ for nalidixic acid and ciprofloxacin, respectively), resistance prevalence detected in children and chickens was overall comparable and characterized by very high resistance rates (≥50%) to ampicillin, tetracycline, chloramphenicol, streptomycin, and trimethoprim-sulphametoxazole (Table 1). Remarkable rates of resistance to these drugs have been previously reported in commensal *E. coli* from healthy individuals living in urban and rural areas of the Bolivian Chaco.[2,3]

Phenotypic and genotypic characterization of E. coli *isolates with acquired resistance to tetracycline or quinolones*

To investigate the possible spread of resistant strains between children and home-raised chickens, *E. coli* isolates of human and animal origin exhibiting acquired resistance to tetracycline or quinolones were characterized. A total of 66 isolates were obtained by streaking rectal swabs from children ($n = 6$) and chickens ($n = 6$) of three selected households on TET-MCA and NAL-MCA plates, and picking up three isolated colonies from each plate (36 and 30 isolates from TET-MCA and NAL-MCA, respectively) (Table 2).

Susceptibility testing showed that all isolates selected onto NAL-MCA were also resistant to tetracycline, whereas 50% of those selected onto TET-MCA were also resistant to nalidixic acid. Moreover, a multidrug-resistant phenotype (resistance to more than three different antibiotic classes) was more common among isolates from children (70%) than among those from chickens (42%) ($p = 0.02$) (Table 2).

Resistant isolates belonged to phylogenetic group A (67%), B1 (18%), and D (15%): group A was significantly more prevalent in children than in chickens (80% vs. 56%, $p = 0.03$), group B1 was present only in chickens (33%, $p < 0.001$), whereas group D was equally distributed (20% vs. 11%, $p = 0.3$) (Table 2). A diverse distribution of the four main phylogenetic groups among human and animal commensal *E. coli* has been observed in a number of studies, and reflects adaptation to both host characteristics (*e.g.*, diet, gut morphology, and body mass) and environmental forces.[17]

RAPD genotyping demonstrated a remarkable clonal diversity of resistant isolates of both human and animal origin (12 and 14 different RAPD types, respectively), with most children ($n = 4$) and chickens ($n = 5$) being colonized by more than one resistant clone. Transmission of resistant strains was observed between children (family number 2, RAPD type 12) or chickens (family number 1, RAPD type 8) living in the same household, but in no case the same resistant strain was found to be shared by both humans and animals, or by members of different households (Table 2).

The overall genetic heterogeneity of resistant isolates and the observed intra host diversity of resistant *E. coli* suggested a possible role of horizontal gene transfer in resistance dissemination. To verify this hypothesis, the 66 isolates were investigated for the nature and transferability of plasmid-mediated tetracycline and quinolone resistance genes.

Nature and transferability of plasmid-mediated tetracycline and quinolone resistance genes

Resistance to tetracycline was related to carriage of either *tet*(A) or *tet*(B) genes (46% and 54% of RAPD types, respectively), with no significant association between nature of *tet* genes and human or animal origin of resistant isolates ($p = 0.71$ for *tet*(A) and *tet*(B)) (Table 2). *tet* genes were often located on conjugative multidrug resistance plasmids harboring different replicon combinations (F, HI1, I1, Iγ, and N). In no case inter-clonal plasmid dissemination was documented, based on replicon typing and RFLP analysis.

PMQR genes, investigated in all the 66 isolates regardless of their susceptibility phenotype, were detected only in isolates collected from children: *aac(6′)-Ib-cr* was found in a ciprofloxacin-resistant clone from a single child (RAPD type 2), and *qnrB19* was found in two different ciprofloxacin-resistant clones from children living in the same household (RAPD type 11, colonizing only one child; RAPD type 12, shared by the two children) (Table 2). Analysis of transformants obtained with plasmid DNA from the *qnrB19*-positive clones revealed two small ColE-like plasmids (8 kb in RAPD type 11 and 2.7 kb in RAPD type 12), harboring *qnrB19* as the sole antibiotic resistance gene. By restriction mapping with *Hae*III, the 2.7 kb plasmid was found to be identical to pECY6-7, a *qnrB19* harboring ColE-like plasmid that has

Table 2. Features of Resistant Isolates from Healthy Children and Home-Raised Chickens of Three Selected Households, Villa Montes, Bolivia

Family	Origin	RAPD type	Number of isolates (total = 66)	Phylogenetic group	Resistance phenotype[a]	*tet* and PMQR genes	*Conjugal transfer of tet-harboring plasmids into* Escherichia coli *J53*	
							Resistance phenotype	Replicon type[b]
Family number 1	Child 1	1	3	D	Amp/Tet/Str/Sxt	*tetB*	Amp/Tet/Str/Sxt	repF
		2	3	A	Amp/Tet/Str/Kan/Sxt/Nal/Cip	*tetB*, *aac(6′)-Ib-cr*	-	-
	Child 2	3	1	A	Tet	*tetB*	-	-
		4	1	A	Amp/Tet	*tetB*	-	-
		5	1	A	Amp/Tet/Nal	*tetB*	-	-
		6	3	A	Tet/Chl/Str/Kan/Gen/Nal	*tetB*	-	-
	Chicken 1	7	3	B1	Amp/Tet/Str	*tetA*	Amp/Tet	Not identified
		8	3	B1	Tet/Sxt/Nal	*tetA*	-	-
	Chicken 2	8	3	B1	Tet/Sxt/Nal	*tetA*	-	-
		9	3	A	Amp/Tet/Str/Sxt	*tetB*	Amp/Tet/Str/Sxt	HI1
Family number 2	Child 1	10	1	D	Amp/Tet/Str/Sxt	*tetB*	Amp/Tet/Str/Sxt	FIB, repF
		11	2	A	Tet/Sxt/Nal/Cip	*tetA, qnrB19*	-	-
		12	3	A	Tet/Chl/Kan/Nal/Cip	*tetA, qnrB19*	-	-
	Child 2	12	6	A	Tet/Chl/Kan/Nal/Cip	*tetA, qnrB19*	-	-
	Chicken 1	13	1	A	Tet	*tetB*	-	-
		14	1	A	Amp/Tet/Str/Sxt	*tetA*	-	-
		15	1	A	Amp/Tet/Str/Sxt	*tetB*	Amp/Tet/Sxt	HI
		16	2	A	Amp/Tet/Nal	*tetB*	-	-
		17	1	D	Amp/Tet/Sxt/Nal	*tetA*	-	-
	Chicken 2	18	3	A	Amp/Tet/Str	*tetB*	Amp/Tet/Str	HI1, FIB, repF
		19	3	D	Amp/Tet/Chl/Str/Nal	*tetB*	Amp/Tet/Chl/Str	HI1, repF
Family number 3	Child 1	20	3	A	Tet	*tetA*	Tet	repF
	Child 2	21	2	D	Amp/Tet/Str/Sxt	*tetA*	Amp/Tet/Str/Sxt	I1, Iγ, repF
		22	1	A	Tet	*tetA*	Tet	N, repF
	Chicken 1	23	2	A	Tet/Nal	*tetA*	-	-
		24	1	A	Tet/Sxt/Nal	*tetB*	-	-
		25	3	B1	Amp/Tet/Nal	*tetA*	-	-
	Chicken 2	26	6	A	Tet/Chl/Kan/Nal	*tetA*	Tet/Chl/Kan	N, repF

[a]AMP, ampicillin; TET, tetracycline; CHL, chloramphenicol; STR, streptomycin; KAN, kanamycin; GEN, gentamicin; SXT, trimethoprim-sulphametoxazole; NAL, nalidixic acid; CIP, ciprofloxacin.
[b]repF, different types of F replicons.
PMQR, plasmid-mediated quinolone resistance; RAPD, random amplification of polymorphic DNA.

been recently found to be widespread among commensal enterobacteria of healthy children in Bolivia and Peru.[15]

Dissemination of PMQR genes is believed to be an important promoter for evolution of fluoroquinolone resistance in *Enterobacteriaceae*.[12] The finding of PMQR genes in all the isolates exhibiting a resistance phenotype to ciprofloxacin would support this hypothesis.

Discussion

Although the limited number of isolates subjected to molecular characterization (a total of 66 isolates, collected from six children and six chickens of three different households) could have missed the identification of low frequency chicken-to-child transmission of resistant strains, the results of the present study overall suggested that home-raised chickens could not represent a major source of contamination responsible for the high prevalence of tetracycline- and quinolone-resistant *E. coli* detected in healthy children from that setting. The high rates of resistance to antibiotics not routinely used in childhood could more likely reflect adult-to-child transmission or acquisition of resistant strains by contaminated food and water sources. Further studies are warranted to clarify these points.

Acknowledgments

We wish to thank Cinthya Fatima Illescas Gallardo, Jenny Cossío Cuellar, and Mary Vargas (Servicio Departamental de Salud Santa Cruz, Red de Salud Cordillera, Camiri, Bolivia) for their support for the study.

This study was carried as a follow-up of research activities of the ANTRES project (a project on antibiotic use and resistance in Latin America, supported by the European Commission within the INCO-DEV program of the FP5), and was partially supported by grants from the Italian Ministry for Foreign Affairs ("Fortalecimiento de la red de salud del Chaco Boliviano: una perspectiva comunitaria"), and by the Ente Cassa di Risparmio di Firenze (Florence, Italy).

Disclosure Statement

No conflict of interest to be declared.

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