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Risk factors for antibiotic-resistant *E. coli* in children in a rural area

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SUMMARY

We surveyed antimicrobial susceptibility in faecal *Escherichia coli* in primary schoolchildren in rural Tamil Nadu, India. Resistance profiles of *E. coli* samples from local water sources were also obtained. We investigated sociodemographic characteristics as risk factors for resistance and local paediatric prescription patterns. In 119 stool samples, carriage of resistance to $\geqslant 1$ antibiotic was 63% and multiple drug resistance was 32%. Resistance outcomes were associated with school of attendance, having a sibling attend the same school, younger age, and less crowded households. Eight of nine water samples were resistant to $\geqslant 1$ antibiotic. Recent history of medication use was not associated with resistance carriage. Resistance patterns may have been influenced by local paediatric prescription patterns and veterinary antibiotic use. Frequent, low-cost surveillance of commensal resistance can guide development of locally appropriate treatment guidelines. School-based hygiene programmes should be considered as means of limiting the spread of antibiotic resistance.

Key words: Antibiotic resistance, children, *E. coli*, epidemiology, India.

INTRODUCTION

The global increase in antibiotic resistance has lead to greater morbidity and mortality due to bacterial infections, delayed administration of effective therapies, longer courses of illness, enhanced transmission, and increased treatment costs [1]. Much of the research on antibiotic resistance has focused on pathogens; however, commensal flora can also be an important reservoir for antimicrobial resistance. Although intestinal flora are composed of many bacterial species, *Escherichia coli* has been identified as the main carrier of antibiotic resistance in a between-species comparison of Enterobacteria isolated from faecal samples [2]. The genes that confer drug resistance are stable, persistent, and have been shown experimentally to be easily transferable between normal flora and pathogenic *E. coli* and *Salmonella* [3]. Resistance in commensal *E. coli* may reflect resistance in circulating pathogens as demonstrated by an observational study that showed recent infection with *Salmonella* was a risk factor for carriage of quinolone-resistant *E. coli* [4].

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Antimicrobial resistance in commensal bacteria is widely prevalent in both developed and developing countries [4–9]. There are conflicting results regarding the association between antibiotic use in humans and rates of resistance in both pathogenic and commensal bacteria [10–13]. Environmental and agricultural sources are also important reservoirs for resistance determinants that are transferable to human strains [14, 15]. Overall, the relative contribution of social, medical, environmental and agricultural factors to rates of antibiotic resistance remains unclear.

Recent studies have highlighted the importance of community-based monitoring of antibiotic resistance carriage in gauging the local resistance patterns of pathogenic bacteria [5, 6]. Such studies have identified household size, younger age, water contamination, and unregulated consumption of antibiotics as risk factors for resistance in faecal flora [16–21]. The primary aim of this study is to describe the prevalence of antibiotic-resistant *E. coli* carried by primary schoolchildren and in local water sources, in two rural South Indian villages. We investigated sociodemographic and participant characteristics as risk factors for carriage of antibiotic resistance, and also examined the relationship between local antibiotic prescription patterns and resistance prevalence. We hypothesized that: (i) recent history of medication use would be a risk factor for antibiotic resistance; (ii) carriage of resistant *E. coli* would be associated with proximity to a local commercial poultry operation; and (iii) the resistance patterns in the stool and water samples would be similar.

METHODS

Study population and enrolment procedures

The study took place in two rural villages in the state of Tamil Nadu in southern India, within 10 km of the Pondicherry Institute of Medical Sciences (PIMS). We surveyed the prevalence of and risk factors for antibiotic resistance in faecal *E. coli* in children attending primary school in Mathur (~2500 inhabitants in 600 households) and Kozhuvari (~1200 inhabitants in 300 households). These villages were selected for proximity to PIMS and the presence of a primary school within each village. Both schools had a single classroom for instruction of standards 1–5. Agricultural labour was the primary income source for most households. The larger village, Mathur, was comprised of two clusters of households (denoted as 'big' and 'small' colonies) with separate water sources. Small colony Mathur contained a small commercial poultry operation using tetracycline, gentamicin, and enrofloxacin as feed additives.

Survey and sample collection occurred between November 2005 and January 2006. Subjects were identified by registration at the primary school in each village: a total of 90 and 31 students attended school in Mathur and Kozhuvari, respectively. All students aged 5–10 years were eligible; participants provided a stool sample and answered a household survey. Samples from community water taps were collected. A questionnaire was administered to local health-care workers to assess the most frequently dispensed antibiotics and most common illnesses for which treatment was sought.

This study was approved by the Committee for Human Research at the Johns Hopkins Bloomberg School of Public Health (CHR no. H.22.05.07.28.B2) and the Institutional Review Board for Research Projects at PIMS. Written informed consent was obtained from a parent or guardian of each participant and written assent was obtained from each study subject. Prior to obtaining consent, the study was explained to parents and participants.

Sample collection and processing

Study subjects were instructed to collect freshly passed stool prior to arriving at school. Stool samples were collected by subjects, placed into sealable plastic containers, and transported to the Microbiology Laboratory at PIMS for processing within 2–4 h of collection. Stool samples were streaked for isolation on MacConkey agar (Difco; Becton Dickinson & Co., Sparks, MD, USA). Lactose-fermenting colonies were confirmed as *E. coli* by 3–5 of the following biochemical assays depending on reagent availability: indole production, fermentation of glucose, sucrose, lactose; motility and fermentation of mannitol; citrate utilization; and urease production. Reagents were prepared and tests performed in accordance with standard procedures [22]. We modified the Kirby–Bauer disk diffusion method to test pools of colonies from each stool sample for antibiotic susceptibility [23]. A pool of five *E. coli* colonies was diluted in saline and plated onto Mueller–Hinton agar (Difco). Pools of colonies were tested in order to increase the chance of identifying carriage of resistance to multiple antibiotics by each subject while conserving

resources. *E. coli* samples were tested for resistance to: ampicillin, 10 μg; aztreonam, 30 μg; ceftriaxone, 30 μg; ciprofloxacin, 5 μg; gentamicin, 10 μg; nalidixic acid, 30 μg; tetracycline, 30 μg; trimethoprim/sulfamethoxazole (cotrimoxazole), 1·25/23·75 μg (Remel, Lenexa, KS, USA); cefazoline 30 μg (HiMedia, Mumbai, India); and cefotaxime 30 μg (HiMedia, prepared at PIMS). Testing for extended-spectrum β-lactamases (ESBL) was performed by the double-disk screening test using 30 μg ceftazidime and 30 μg cefotaxime placed 20 cm centre-to-centre from 30/10 μg amoxycillin/clavulanic acid (HiMedia). Antibiotic potency was assessed using the reference strain *E. coli* ATCC 25922. Pooled colonies were classified as 'susceptible', 'intermediate', or 'resistant' according to the CLSI guidelines and as multidrug resistant (MDR) if resistant to $\geqslant$3 antibiotics [24].

Water samples were collected from community taps in Mathur and Kozhuvari. Sources included a municipal water supply, ground water pumped into a central holding tank and piped to neighbourhood taps, and ground water directly pumped from tube wells. Both schools had tanks to store water used for preparation of school lunches. None of the water was chemically treated prior to reaching the tap. Each tap yielded two specimens – water collected in sterile containers and a swab of the inside of the tap. Samples were transported to the laboratory at PIMS within 2–4 h of collection and processed the same day. For each tap, 100 ml water was filtered through a 22-μm pore-size filter (Millipore Corp., Bedford, MA, USA). Each filter was removed and placed onto the upper half of a MacConkey agar plate, incubated at room temperature for 15 min then moved to the lower half of the plate and incubated overnight at 37 °C. Swabs were streaked directly onto MacConkey agar. Lactose-fermenting colonies were processed in the same manner as stool samples to identify *E. coli* and antimicrobial susceptibility.

Survey collection

The head teacher at each school administered a questionnaire regarding household characteristics to study participants and recorded responses in Tamil on pre-printed forms; answers were later translated into English. School records provided the birthdate of each participant. The questionnaire enquired about family composition (age and gender of siblings), socioeconomic status (SES) indicators (parental occupation and years of education, presence of electricity in the household, ownership of a bicycle, motor scooter, radio, television, and refrigerator, and number of rooms in the house), animal ownership (cow, chicken), and household water purification methods. The questionnaire also asked about recent history of illness (diarrhoea, respiratory or other illness within the last 2 weeks) and medication use (any antibiotic within the last 30 days; name and duration of antibiotic use). We surveyed the prescription patterns of several local medicine providers (i.e. the PIMS pharmacy, two local medical shops, the nearest government primary health-care centre, a privately run free clinic for nearby villages, the government health-care worker assigned to both villages, and the doctor at the weekly clinic in Mathur). They were asked to identify the five most frequently dispensed antibiotics for treatment of paediatric infections and three most common childhood illnesses for which people sought medication. Surveys were conducted with the aid of a translator and answers were recorded in English.

Data analysis

Differences in proportion of demographic characteristics and antibiotic resistance prevalence between the two schools were analysed using two-tailed χ^2 tests. *E. coli* samples classified as 'intermediate' were considered resistant. Logistic regression was used to study the associations between two outcome measures, resistance to $\geqslant$1 antibiotics and MDR, and participant characteristics and household variables derived from the household survey. Both age and household crowding (total number of people in the household/number of rooms in the house) were analysed as continuous variables. Variables approaching statistical significance for either outcome in the bivariate analysis were considered in multivariate analysis. Age was considered a variable of interest because of previously published associations between younger age and carriage of antibiotic resistance [20, 21]. Because of collinearity with school (all of the subjects who reported using no purification method resided in Kozhuvari), the water purification variable was not used in multivariate analysis. Generalized Estimating Equations (GEE) were used to adjust for clustered data since siblings shared the same household, using household as the clustering unit. Odds ratios (OR) and 95% confidence intervals (CI) were calculated and $\alpha = 0{\cdot}05$ was designated as the significance level for statistical testing. All statistical analyses were

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Table 1. *Sociodemographic characteristics of 119 South Indian primary schoolchildren*

	n	(%)
Village		
Mathur, big colony	67	(56)
Mathur, small colony	21	(18)
Kozhuvari	31	(26)
School		
Kozhuvari	31	(26)
Mathur	88	(74)
Female	66	(56)
Mothers with $\geqslant 1$ years of school	62	(52)
Fathers with $\geqslant 1$ years of school	75	(63)
Mode of conveyance (bicycle/scooter)	88	(74)
Livestock ownership	62	(52)
Electricity in household	105	(88)
Television ownership	73	(61)
Age, years (mean $\pm$ S.D.)	7·60 $\pm$ 1·50	
Household size (mean $\pm$ S.D.)	4·98 $\pm$ 1·09	
Number of rooms in house (mean $\pm$ S.D.)	1·73 $\pm$ 0·74	

conducted using Stata 10.0 (Stata Corp., College Station, TX, USA).

RESULTS

Study population and enrolment

All 121 students identified through registration records at the Mathur and Kozhuvari primary schools were enrolled and submitted completed household questionnaires. One student was excluded when it was determined that he was aged 11 years. A total of 120 stool samples were collected; the overall participation rate was 100%. *E. coli* could not be isolated from one stool sample, thus we analysed 119 *E. coli* colony pools for antibiotic susceptibility. In all, 85 households participated in the study with 67 (56%) subjects residing in big colony Mathur, 21 (18%) residing in small colony Mathur, and 31 (26%) residing in Kozhuvari. A total of 88 (74%) participants attended Mathur primary school; fewer than half were male (44%) (Table 1). The average household size was 4·98 ($\pm$1·09), and the mean number of children aged <10 years was 1·93 ($\pm$0·74) per household. Most households had electricity but there were no latrines in either village.

Residents of both villages were similar on characteristics such as mothers' education, fathers' education, animal ownership, and presence of electricity in the household. Participating households in Mathur were of higher SES than those of Kozhuvari as assessed by ownership of a mode of conveyance (bicycle, scooter) (83% *vs.* 48%, $P<0·01$), and a television (77% *vs.* 43%, $P<0·01$). Significantly more fathers in Kozhuvari had no years of schooling compared to Mathur ($P=0·05$).

Prevalence of antibiotic resistance in stool samples

Of the 119 *E. coli* colony pools tested, 75 (63%) samples were resistant to $\geqslant 1$ antibiotic and 38 (32%) were MDR, comprising 29 different resistance patterns (Table 2). Resistance was most frequently observed to nalidixic acid (42%), ampicillin (39%), cotrimoxazole (37%), and tetracycline (35%) (Table 3). Resistance to $\geqslant 1$ antibiotic was 1·5 times more prevalent in *E. coli* samples from Mathur than Kozhuvari (69% *vs.* 45%, $P=0·02$); MDR was 2·4 times more prevalent in samples from Mathur (38% *vs.* 16%, $P=0·03$). Two samples, from siblings, were resistant to all antibiotics tested; both were ESBL positive. Resistance to nalidixic acid was similar between students residing in both colonies of Mathur (48%), and significantly greater than in Kozhuvari students (26%, $P=0·03$). Resistance to tetracycline was not statistically significantly different in the three geographical areas (48% in small colony Mathur, 36% in big colony Mathur, 23% in Kozhuvari, $P=0·33$). Resistance to gentamicin and cephalosporins occurred only in isolates from Mathur.

Prevalence of antibiotic resistance in water samples

We collected 22 water samples [big colony Mathur (eight), small colony Mathur (four), Kozhuvari (ten)] from the two school taps, six tube wells, and 14 taps supplying centrally stored water. Bacteria were cultured from all samples (swab, filter or both), but *E. coli* was only isolated from nine (41%) samples. One tap in Mathur yielded *E. coli* samples with different resistance patterns from the swab and filter. Eight of nine samples (88%; from six taps of centrally stored water and one tube well) contained *E. coli* that were resistant to $\geqslant 1$ antibiotic. The prevalence and patterns of resistance in the water-sample *E. coli* colony pools are shown in Tables 2 and 3. Resistance was most frequently observed to ampicillin (56%), nalidixic acid (33%), and cefazoline (33%). Similarly to the stool samples, resistance to cefazoline was limited to Mathur isolates; however, resistance to ciprofloxacin, tetracycline, and cotrimoxazole occurred only in Kozhuvari.

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Table 2. *Resistance patterns of stool and water* E. coli *samples*

Antibiotic resistance status	Antibiotic resistance patterns										No. with resistance pattern	
	AMP	ATM	CIP	CRO	CN	NA	TET	SXT	CZ	CE	Stool	Water
Susceptible to all	−	−	−	−	−	−	−	−	−	−	44	1
Resistant to 1	−	−	−	−	−	+	−	−	−	−	10	1
	−	−	−	−	−	−	+	−	−	−	6	1
	−	−	−	−	−	−	−	+	−	−	4	1
	+	−	−	−	−	−	−	−	−	−	2	1
	−	+	−	−	−	−	−	−	−	−	1	
Resistant to 2	+	−	−	−	−	−	−	+	−	−	4	
	−	−	−	−	−	+	+	−	−	−	3	
	−	−	−	−	−	+	−	+	−	−	2	
	+	−	−	−	−	+	−	−	−	−	2	
	+	−	−	−	−	−	−	−	+	−	1	2
	−	−	+	−	−	+	−	−	−	−	1	
	−	+	−	−	−	−	+	−	−	−	1	
Resistant to 3	+	−	−	−	−	−	+	+	−	−	6	
	+	−	−	−	−	+	−	+	−	−	4	
	−	−	−	−	+	+	+	−	−	−	1	
	+	−	+	−	−	+	−	−	−	−	1	
	+	−	−	−	−	+	+	−	−	−	1	
	+	−	−	−	−	+	−	−	+	−		1
Resistant to 4	+	−	−	−	−	+	+	+	−	−	8	
	+	−	+	−	−	+	−	+	−	−	1	
	+	−	+	−	−	+	+	−	−	−		1
Resistant to 5	+	−	+	−	−	+	+	+	−	−	7	
	+	−	+	−	−	+	+	−	+	−	1	
	+	+	−	−	−	+	+	+	−	−	1	
Resistant to 6	+	+	+	−	−	+	+	+	−	−	1	
Resistant to 7	+	+	−	+	−	+	−	+	+	+	1	
	+	−	+	−	+	+	+	+	+	−	1	
Resistant to 8	+	+	−	+	−	+	+	+	+	+	2	
Resistant to 10	+	+	+	+	+	+	+	+	+	+	2	
Total number of stool and water samples analysed											119	9

AMP, Ampicillin; ATM, aztreonam; CRO, ceftriaxone; CIP, ciprofloxacin; CN, gentamicin; NA, nalidixic acid; TET, tetracycline; SXT, cotrimoxazole; CZ, cefazoline; CE, cefotaxime; −, sensitive; +, resistant.

Survey of local prescription patterns

Of the seven medicine providers surveyed, six agreed to complete the survey; a participation rate of 86%. The most commonly dispensed antibiotics were amoxicillin, cotrimoxazole, and ampicillin (reported on five, four and three of six surveys respectively), in line with the patterns of resistance in stool samples. Cephalosporins were reported as commonly dispensed on three surveys; ciprofloxacin and a tetracycline family antibiotic (doxycycline) were each only identified by a single survey. Respiratory infections, followed by skin and enteric infections were the most commonly identified illnesses for which people sought treatment.

Risk factors for antibiotic resistance in stool samples

The odds of resistance to $\geqslant 1$ antibiotic and MDR were about three times greater for Mathur primary school students compared to Kozhuvari students. The prevalence and bivariate analysis of potential risk factors is shown in Table 4. All of the participants reporting being 'sick in the last 2 weeks' indicated respiratory symptoms; none reported diarrhoeal

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Table 3. *Prevalence of antibiotic resistance in* E. coli *from stool and water samples*

	Big colony Mathur		Small colony Mathur*		Kozhuvari	
Antibiotic	Stool *n* (%)	Water *n* (%)	Stool *n* (%)	Water *n* (%)	Stool *n* (%)	Water *n* (%)
Nalidixic acid	32 (48)	1 (25)	10 (48)	0	8 (26)	2 (50)
Ampicillin	24 (39)	4 (100)	14 (67)	0	8 (26)	1 (25)
Cotrimoxazole	26 (39)	0	9 (43)	0	9 (29)	1 (25)
Tetracycline	24 (36)	0	10 (48)	0	7 (23)	2 (50)
Ciprofloxacin	10 (15)	0	2 (10)	0	3 (10)	1 (25)
Aztreonam	8 (12)	0	0	0	1 (3)	0
Ceftriaxone	5 (7)	0	0	0	0	0
Gentamicin	3 (4)	0	1 (5)	0	0	0
Cefazoline	6 (9)	3 (75)	2 (10)	0	0	0
Cefotaxime	5 (7)	0	0	0	0	0
Total no. of samples	67	4	21	1	31	4

* Commercial poultry operation using enrofloxacin, tetracycline and gentamicin located in small colony Mathur.

symptoms. In multivariate analysis, resistance to $\geqslant 1$ antibiotic was significantly associated with school attendance in Mathur and having a sibling attending the same school (Table 5). In bivariate analysis, the presence of children aged <5 years in the household was a significant risk factor for resistance to $\geqslant 1$ antibiotic; this relationship did not hold in the multivariate model. In a subanalysis of resistance to specific antibiotics in sibling pairs, participants with a sibling resistant to a specific antibiotic were not more likely to carry resistance to that antibiotic (data not shown). In multivariate analyses with MDR as the outcome, attendance at school in Mathur, older age, and less crowding in the household remained statistically significant. Sociodemographic variables (i.e. gender, presence of children aged <5 years in the household, parental education level, ownership of livestock, a mode of transport or a television) and recent history of illness or medication use were not significantly associated with either resistance outcome in any analysis.

DISCUSSION

This study demonstrates the diversity of antibiotic resistance found in faecal *E. coli* circulating in a rural South Indian community. Over half of the stool *E. coli* samples were resistant to $\geqslant 1$ antibiotic and one third were MDR. We found high levels of antimicrobial resistance to nalidixic acid, ampicillin, cotrimoxazole, and tetracycline, levels considerably higher than those seen in a population of women attending antenatal clinics in South India [6]. These differences may reflect geographic variation or the differences in study population age.

The observed prevalence was lower than that reported in other studies of healthy children in developing countries [4, 20]. Bartoloni *et al*. [20] observed prevalences of resistance to ampicillin, tetracycline, and cotrimoxazole $>90\%$ in Bolivian and Peruvian children; Zaidi *et al*. [4] found that 54% of Mexican children carried *E. coli* resistant to nalidixic acid. The pattern of drug resistance identified through our survey is consistent with that in children admitted to the PIMS hospital for severe diarrhoea during 2006–2007. The majority of these infections were caused by *E. coli*, *Salmonella typhi*, and *Shigella* spp. About 60% of isolates were resistant to nalidixic acid, 50% were resistant to ampicillin, and a single *E. coli* isolate was resistant to ampicillin, ciprofloxacin, and nalidixic acid. Ciprofloxacin resistance was highly prevalent in *E. coli* isolated from paediatric blood (3/3, 100%) and urinary tract (13/23, 57%) infections during the same time period (R. Kanungo, personal communication).

In the risk factor analysis, students with a sibling at school were more likely to be resistant to $\geqslant 1$ antibiotic, consistent with previously documented transmission within families, between day-care attendees, and within households of attendees [12, 13]. Having a sibling at school may increase an individual's exposure to resistant strains circulating within the school. The association between younger age and MDR has been seen previously [19, 25]. It is conceivable that younger children may be more exposed to resistant strains from their peer group, possibly as a function of greater faecal–oral transmission. *E. coli*

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Table 4. *Prevalence and bivariate analysis of potential risk factors for antibiotic resistance in primary schoolchildren in rural Tamil Nadu, India* (*n* = *119*)

	Resistance to ⩾1 antibiotic				Multiple drug resistance*			
Potential risk factor	*n*	%	OR (95% CI)†	*P* value	*n*	%	OR (95% CI)	*P* value
Age (years)			1·02 (0·79–1·30)	0·89			0·77 (0·59–1·01)	0·06
Household crowding‡ (mean 3·36 ± 1·42)			1·02 (0·79–1·31)	0·88			0·79 (0·60–1·03)	0·09
Village cluster								
Kozhuvari	14	45	1		5	16	1	
Mathur, big colony	45	67	2·42 (0·97–6·01)	0·06	23	34	2·89 (1·04–8·05)	0·04
Mathur, small colony	16	76	3·88 (1·18–12·81)	0·03	10	48	3·84 (1·01– 14·66)	0·05
School								
Kozhuvari	14	45	1		5	16	1	
Mathur	61	69	2·69 (1·13–6·41)	0·03	33	38	3·10 (1·13–8·50)	0·03
Sibling at school								
No	26	50	1		16	31	1	
Yes	49	73	2·69 (1·22–5·96)	0·01	22	33	1·08 (0·51–2·28)	0·84
Children <5 years in the household								
No	62	68	1		31	34	1	
Yes	13	46	0·42 (0·18–0·98)	0·05	7	25	0·60 (0·23–1·56)	0·29
Sick in the last 2 weeks								
No	58	62	1		31	33	1	
Yes	16	67	1·24 (0·53–2·91)	0·62	7	29	0·76 (0·28–2·07)	0·59
Medicine taken in the last 30 days								
No	62	63	1		32	32	1	
Yes	13	65	1·09 (0·44–2·65)	0·86	6	30	0·89 (0·31–2·55)	0·83
Water purification method								
None	5	38	1		3	23	1	
Boil	59	64	2·80 (0·80–9·79)	0·11	33	36	2·01 (0·48–8·34)	0·34
Filter	10	77	5·12 (0·88–29·64)	0·07	2	15	0·73 (0·10–5·20)	0·75

* Resistance to ⩾3 antibiotics.
† Odds ratios (OR) and 95% confidence intervals (CI) were adjusted for clustering at the household level by Generalized Estimating Equations.
‡ Total number of people in the household/number of rooms in the house (mean ± standard deviation).

Table 5. *Multivariate analysis of risk factors for antibiotic resistance outcomes*

	Resistance to ⩾1 antibiotic		Multiple drug resistance*	
	OR (95% CI)†	*P* value	OR (95% CI)	*P* value
School (Kozhuvari = 1)	2·72 (1·07–6·93)	0·04	4·46 (1·60–12·42)	<0·01
Sibling at school	2·67 (1·20–5·96)	0·02	1·22 (0·54–2·79)	0·63
Age (years)	0·99 (0·76–1·30)	0·95	0·74 (0·55–0·99)	0·04
Household crowding‡	0·96 (0·72–1·28)	0·78	0·70 (0·52–0·94)	0·02

* Resistance to ⩾3 antibiotics.
† Odds ratios (OR) and 95% confidence intervals (CI) were adjusted for clustering at the household level by Generalized Estimating Equations.
‡ Total number of people in the household/number of rooms in the house.

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samples from more crowded households had lower odds of MDR. We were unable to understand this association. Although this may be a chance finding, one could also speculate that the household crowding variable serves as an SES indicator, with houses having more rooms and families with fewer members receiving lower crowding scores. It is conceivable that higher SES households have greater purchasing power for antibiotics and may be more likely to seek medical care and thus have greater exposure to antimicrobials.

Attendance at school in Mathur was the most significant risk factor for both resistance to $\geqslant 1$ antibiotic and MDR. The student body at Mathur primary school was three times the size of that in Kozhuvari. This may have provided exposure to more *E. coli* strains and classroom crowding may have led to greater faecal–oral transmission. Larger day-care facilities for children have been shown to have higher rates of transmission of infectious diseases; this is likely to be true for the transmission of commensal organisms as well [26, 27]. The association of only a few household characteristics with resistance outcomes may be further indication that school crowding affects transmission. Schools could be a target for hygiene education programmes that aim to interrupt the transmission cycle.

Interestingly, recent history of medication use was not a significant risk factor for resistance to $\geqslant 1$ antibiotic or MDR. Previous studies have also shown no correlation between antibiotic intake and carriage of resistance [12, 13]. A potential source of bias in this study is non-specific or inaccurate responses to the survey questions on recent history of illness and medication use; the majority answer to the type of medication used was 'don't know'. Anecdotal evidence suggests cotrimoxazole in syrup form was the most commonly used medication (for respiratory infections) in both villages. The survey of prescription patterns indicated frequent paediatric use of ampicillin and cotrimoxazole in the study area. These findings were consistent with the high carriage level of ampicillin and cotrimoxazole resistance by the participants. The high prevalence of fluroquinolone and tetracycline resistance seen in the stool samples, which were not among the most frequently identified paediatric antibiotics and are contra-indicated for paediatric use, suggests that factors other than direct exposure to medications may play a role in the transmission of resistance. One potential source of resistance determinants may be locally circulating enteric pathogens such as quinolone-resistant *Salmonella* paratyphi A, *Shigella*, and *Vibrio cholerae* [28–30]. Other factors such as agricultural use of antibiotics may also lead to the development of resistance in human commensal organisms [31, 32].

The higher prevalence of strains resistant to fluoroquinolones, tetracycline, and gentamicin in participants in Mathur may be a byproduct of their use at the poultry farm in the small colony. The fact that there were significant differences in antibiotic resistance prevalence between Mathur and Kozhuvari despite their close proximity suggests that there may be limited intra-village transmission. There were few differences in the prevalence of the antibiotic resistance between the big and small colonies of Mathur; however, since children from both geographic clusters attended the same primary school, this supports the idea that school may be an important locus of transmission.

Although we could not directly test the relationships between the stool and water samples in this survey, they were qualitatively similar with respect to the high prevalence of nalidixic acid and ampicillin resistance. Additionally, cefazoline resistance occurred only in Mathur. One limitation of this study was our inability to assess genetic relationships between the stool and water samples. Such genetic analysis of the strain relationships could help to determine whether the *E. coli* in the water samples merely reflects contamination of the water taps with faecal *E. coli* or if water sources are actively involved in the transmission cycle. Participants and water sources were sampled only once, so the duration of resistance in commensal organisms is unknown.

This study highlights the silent presence of antimicrobial resistance in commensal bacteria circulating widely even in a rural community. Global antibiotic resistance surveillance networks like the SENTRY Programme which monitor global antibiotic resistance are important, but they focus exclusively on clinical pathogenic isolates and there is little coverage in Asia and Africa [33]. Even simple, low-cost surveys of resistance in commensal bacteria similar to this study could provide information crucial to developing locally appropriate guidelines for efficacious treatment of *E. coli* and other bacterial infections. The development of vaccines against enteric pathogens may also reduce transmission of resistant pathogenic strains and limit opportunities for resistance to be transferred between commensal and pathogenic bacteria. The results of several pneumococcal

vaccine studies indicate that the vaccines can help decrease carriage of strains resistant to several antibiotics [34, 35]. Future studies using genetic analysis to characterize the relationships between stool and water *E. coli* samples and within households should be conducted to investigate the importance of environmental reservoirs of resistance and determine how resistant strains are passed through the population. A better understanding of the relationship between circulating commensal and pathogen resistance could help to determine how resistance flows between non-pathogenic and pathogenic bacterial species in this community.

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DECLARATION OF INTEREST

None.

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