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Excess body weight and age associated with the carriage of fluoroquinolone and third-generation cephalosporin resistance genes in commensal *Escherichia coli* from a cohort of urban Vietnamese children

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Abstract

Purpose. Antimicrobial-resistant bacterial infections in low- and middle-income countries (LMICs) are a well-established global health issue. We aimed to assess the prevalence of and epidemiological factors associated with the carriage of ciprofloxacin- and ceftriaxone-resistant *Escherichia coli* and associated resistance genes in a cohort of 498 healthy children residing in urban Vietnam.

Methodology. We cultured rectal swabs onto MacConkey agar supplemented with resistant concentrations of ciprofloxacin and ceftriaxone. Additionally, we screened meta-*E. coli* populations by conventional PCR to detect plasmid-mediated quinolone resistance (PMQR)- and extended-spectrum β-lactamase (ESBL)-encoding genes. We measured the associations between phenotypic/genotypic resistance and demographic characteristics using logistic regression.

Results/Key findings. Ciprofloxacin- and ceftriaxone-resistant *E. coli* were cultured from the faecal samples of 67.7 % (337/498) and 80.3 % (400/498) of children, respectively. The prevalence of any associated resistance marker in the individual samples was 86.7 % (432/498) for PMQR genes and 90.6 % (451/498) for β-lactamase genes. Overweight children were significantly more likely to carry *qnr* genes than children with lower weight-for-height z-scores [odds ratios (OR): 1.24; 95 % confidence interval (CI): 10.5–1.48 for each unit increase in weight for height; P=0.01]. Additionally, younger children were significantly more likely to carry ESBL CTX-M genes than older children (OR: 0.97, 95 % CI: 0.94–0.99 for each additional year, P=0.01).

Conclusion. The carriage of genotypic and phenotypic antimicrobial resistance is highly prevalent among *E. coli* in healthy children in the community in Vietnam. Future investigations on the carriage of antimicrobial resistant organisms in LMICs should focus on the progression of carriage from birth and structure of the microbiome in obesity.

INTRODUCTION

Antimicrobial resistance (AMR) has become an increasingly recognized global health problem. The social complexities associated with AMR are multifaceted; however, a major driver of AMR is thought to be non-prescribed use of antimicrobials in the community, which is particularly common in many low- and middle-income countries (LMICs) [1]. Multiple studies have demonstrated a recent dramatic rise in pathogenic bacteria exhibiting resistance against fluoroquinolones and third-generation cephalosporins [2–4], which are two of the most commonly used classes of

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Abbreviations: AMR, antimicrobial resistance; BHI, brain heart infusion media; CI, 95% confidence interval; CLSI, Clinical and Laboratory Standards Institute; ESBL, extended-spectrum β-lactamase; HCMC, Ho Chi Minh City; HTD, Hospital for Tropical Diseases; HVH, Hung Vuong hospital; LMIC, low- to middle-income country; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; OR, odds ratio; PMQR, plasmid-mediated quinolone resistance; WHO, World Health Organization.
Three supplementary tables are available with the online version of this article.

antimicrobials globally [5]. Consistently, cephalosporins and fluoroquinolones are most consumed classes of antimicrobial classes in Vietnam, with prescribed consumption from hospitals equalling consumption from retail pharmacies [1, 6].

Escherichia coli is abundant in the intestine of healthy people and can be used to assess the composition of AMR genes in the human gut [7–9]. *E. coli* are proficient vehicles for AMR gene circulation, and are commonly co-resistant to several clinically important antimicrobials, including cephalosporins, fluoroquinolones and sulfamethoxazole/trimethoprim [10, 11]. Therefore, commensal *E. coli* are sentinel Gram-negative organisms that play a key role in the maintenance and spread of plasmid-associated AMR genes within the *Enterobacteriaceae* [11, 12]. Specifically, *E. coli* are known to be a major reservoir of plasmids carrying both extended-spectrum β-lactamases (ESBLs) and plasmid-mediated quinolone resistance (PMQR) genes [13, 14].

The expression of ESBL genes is a key resistance mechanism against later generation beta-lactams; the circulation of genes encoding these enzymes is of critical importance in human health [13]. ESBLs, which can hydrolyze a wide variety of β-lactamases, are presently classified into several subgroups; the most frequently identified include TEM, SHV and CTX-M [15, 16]. The $bla_{\text{CTX-M}}$ genes in particular have been extremely successful, becoming globally ubiquitous and undergoing extensive diversification (>100 variants have been identified). This important group of ESBL genes is broadly divided into five phylogroups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25. The most dominant global lineage is $bla_{\text{CTX-M-1}}$, which incorporates $bla_{\text{CTX-M-15}}$, a particularly successful and persistent variant that is commonly found in Asia [15].

Fluoroquinolone resistance generally occurs as a result of mutations in the chromosomal drug target genes encoding the DNA gyrase (*gyrA* and *gyrB*) and the topoisomerase IV (*parC* and *parD*) [17]. However, fluoroquinolone resistance can also be induced and enhanced by the PMQR genes. The most common PMQR genes are the *qnr* family (*qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS*), which act by altering the DNA gyrase and topoisomerase IV, thus reducing the interaction between drug and target [18, 19]. The *qnr* genes have been found on a broad range of plasmid structures in various pathogens within the *Enterobacteriaceae*. Notably, PMQR genes frequently co-exist on the same plasmid backbones as ESBL genes, which can be co-transferred during horizontal gene exchange [18, 20, 21].

Several studies have focused on characterizing the carriage of AMR organisms in hospitalized patients and healthy individuals in the community in LMICs [3, 11, 22] However, less is known about the prevalence of AMR bacteria and the diversity of associated AMR genes in the commensal microbiota of healthy children living in an urban community. Here, we used a direct plating method on selective media to measure phenotypic and genotypic resistance against fluoroquinolones and third-generation cephalosporins among a cohort of 498 healthy children resident in Ho Chi Minh City (HCMC) [23]. Further, we aimed to identify the epidemiological risk factors associated with the carriage of phenotypic and genotypic resistance in these urban children.

METHODS

Study population

All rectal swabs were collected from healthy children who were participants in a previously described prospective longitudinal study [24]. In brief, infants were recruited into a birth cohort at Hung Vuong hospital (HVH) in HCMC, the second largest obstetric hospital in south Vietnam, and followed for 12 months for febrile disease through passive surveillance [25]. A subset of these children were followed beyond 12 months and recruited into a continuous cohort for active surveillance for diarrhoeal disease. The children attended scheduled routine follow-up visits at HVH every 6 months. Information on demographics, household water sources, sanitation, health issues in the intervening 6 months between visits and food consumption habits in the 3 days prior to the visit was collected. A rectal and nasopharyngeal swab was collected at the time of the visit. Seven hundred and forty-eight children were enrolled from June 2014 to December 2014 and then followed up to December 2016. To actively monitor diarrhoeal disease episodes, fortnightly SMS messages were sent to the children's parents/care providers. During diarrhoeal episodes nurses visited their homes to confirm the case, collect samples and provide supportive care. Cases were treated according to local standard of care and were required to attend the Hospital for Tropical Diseases (HTD) in central HCMC for medical care.

Identification of antimicrobial-resistant *E. coli*

From March to August 2016, we collected 498 rectal swabs from healthy children (no diarrhoea or other symptoms of illness) at routine follow-up visits for the purposes of this investigation. At the routine follow-up visits, we recorded information regarding the children's general well-being and their antimicrobial practices in the previous 6 months. All rectal swabs were inoculated onto MacConkey agar to isolate suspected *E. coli* colonies (e.g. lactose-fermenting and non-lactose-fermenting, circular, elevated, entire margins, smooth texture, pink or colourless, dry and shiny). Meta-*E. coli* populations (i.e. all *E. coli* from the same rectal swab) were inoculated into brain heart infusion (BHI) media supplemented with 20 % glycerol and stored at −80 °C until being subjected to inoculation onto MacConkey agar supplemented with either ciprofloxacin (4 mg l^{-1}) or ceftriaxone (6 mg l^{-1}) to select for organisms resistant to these antimicrobials. *E. coli* ATCC25922 [ciprofloxacin minimum inhibitory concentration (MIC)]=0.008 mg l^{-1} and ceftriaxone MIC=0.094 mg l^{-1}) and clinical *E. coli* isolate 03_0334 (ciprofloxacin MIC of >256 mg l^{-1} and ceftriaxone MIC of >256 mg l^{-1}) were used as negative and positive culture

controls. All suspected *E. coli* colonies were confirmed using a Bruker biotyper MALDI-TOF bacterial identification system.

Detection of antimicrobial-resistant genes

The meta-*E. coli* populations in frozen BHI were subjected to nucleic acid extraction and multiplex PCR amplification. Approximately 200 µl of meta-*E. coli* in frozen BHI was removed and aliquoted into a 96-well plate and centrifuged for 10 min at 4000 r.p.m. The supernatant was removed and discarded. The pellet was resuspended in 200 µl of ultrapure water and agitated gently to ensure mixing. Nucleic acid was extracted by heating the solution to 100 °C on a heating block for 10 min, followed by cooling to ambient temperature. The solution was subjected to centrifugation for 10 min at 4000 r.p.m. in a microfuge. The supernatant was removed and used as template DNA in all PCR amplifications. We conducted a series of multiple PCR amplifications aiming to detect PMQR genes (*aac(6')-Ib-cr, qepA, qnrA, qnrB*and *qnrS)*, ESBL genes ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} and bla_{OXA}) and AmpC lactamase genes (MOX, DHA, EBC, FOX, ACC and CIT). The primer sequences and the predicted amplification sizes are shown in Table S1 (available in the online version of this article). The amplification conditions were as previously described [12, 14, 26, 27]; a positive PCR result was determined by the detection of a PCR amplicon of an appropriate size (using a UV transilluminator and gel documentation system) for each target in the presence of appropriate positive and negative amplification controls.

Statistical analyses

Categorical and continuous variables were described using frequencies (%) and medians (interquartile range), respectively. Bar plots were used to describe the prevalence of ciprofloxacin- and ceftriaxone-resistant *E. coli* and the various ESBL and PMQR genes. We used univariate and multivariable logistic regression to assess the relationship between the participants' characteristics and the presence of antimicrobial-resistant *E. coli* and AMR-associated genes. These relationships were described in odds ratios (OR) and their corresponding 95 % confidence intervals (CIs). The significance level for the statistical tests was 0.05. All analyses were performed in R v3.4.0 (R Foundation, Vienna, Austria) and companion packages.

RESULTS

Baseline characteristics

Between March and August 2016, we collected rectal swabs from 498 children without diarrhoea attending a routine health check at the designated healthcare facility. All children were from a single district (eight) in HCMC (the same study area assessed for access to pharmacies in this age group [1]) and enrolled in a longitudinal cohort study of 748 individuals. This population was considered to be generally representative of the child population in HCMC. The baseline characteristics of those sampled are shown in Table 1; 269/498 (54 %) were male, 211/498 (42.4 %) were born by caesarean section, and 64/498 (12.9 %) were preterm deliveries (Table 1).

The median age of the children at the point of sampling was 46 months. Therefore, at the time of the health check in which they were sampled, the majority of children were attending nursery/school (67.9 %; 338/498). Only a small fraction of the children were underweight, stunted or wasted. However, more than a quarter of the children were overweight (27.5 %; 137/498), with a similar proportion (27.3 %; 136/498) having a minor non-diarrhoeal illness at the time of visit. A substantial proportion (42.8 %; 213/498) of children had received antimicrobials in the 6 months prior to sampling and 26.9 % (134/498) regularly consumed some form of probiotic as part of their diet (Table 1). A third (29.1 %; 145/498) of the children had taken oral vitamin A supplementation, provided via a government public health nutrition programme. The children had consumed a wide variety of animal products in the 3 days prior to sampling, which included pork (89.6 %, 446/498), shrimp (85.1 %; 424/498), eggs (77.7 %; 387/498), beef (75.9 %; 378/498) and poultry (64.5 %; 321/498) (Table 1).

Ciprofloxacin- and ceftriaxone-resistant *E. coli*

Aiming to assess the prevalence of antimicrobial-resistant *E. coli* carriage in this population, we independently plated the meta-*E. coli* populations onto MacConkey agar supplemented with ceftriaxone and ciprofloxacin to isolate resistant organisms. We cultured *E. coli* colonies that were resistant to ceftriaxone [according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [28]] from 80.3 % (400/498) of the rectal swabs and *E. coli* colonies that were resistant to ciprofloxacin (according to CLSI guidelines [28]) from 67.7 % (337/498) of the rectal swabs. Resistant *E. coli* colonies were cultured from at least one of the antimicrobial supplemented plates (i.e. either ciprofloxacin or ceftriaxone) in 84.7 % (422/498) of the samples; no antimicrobial-resistant *E. coli* colonies were isolated on either plate from the remainder of the samples (76/498; 15.3 %).

Of the 422 samples from which we isolated resistant *E. coli* colonies on at least 1 plate, 315 produced *E. coli* colonies on both the ceftriaxone-supplemented media and the ciprofloxacin-supplemented media. We aimed to identify the epidemiological characteristics associated with the detection of fluoroquinolone- and cephalosporin-resistant *E. coli* in the rectal swabs. However, after investigating multiple factors, including age, sex, antimicrobial usage and other household variables, we found that no factors were significantly associated with their carriage. We suspect that this result either stems from the limited sample size or indicates the ubiquitous nature of antimicrobial-resistant *E. coli* (Table S2).

The prevalence of antimicrobial-resistant genes in rectal swabs from healthy children

The 498 paired MacConkey plates were scraped and aliquoted, DNA was extracted from the meta-*E. coli* populations and all were subjected to PCR amplification to genes

Table 1. Baseline characteristics of the 498 study participants

Characteristics	Frequency (%) or median [interquartile range (IQR)]
Male	269 (54.0 %)
Age at visit (month)	46.4 (35.6, 52.5)
Preterm at birth	64 (12.9 %)
Low birth weight	20 (4.0 %)
Caesarean section	211 (42.4 %)
The only child in the family	271 (54.4 %)
Early breastfeeding at birth	340 (68.3 %)
Feeding at birth	
Only formula	42 (8.4 %)
Exclusive breastfeeding	20 (4.0 %)
Breast milk plus formula	436 (87.6 %)
Breastfeeding duration (month)	4.0 (3.0, 6.0)
Breastfeeding up to 12 months	60 (12.0 %)
Attending school	338 (67.9 %)
Nutritional status*	
Underweight (weight for age)	2 (0.4 %)
Stunting (height for age)	10 (2.0 %)
Overweight (weight for height)	137 (27.5 %)
Wasting (weight for height)	1 (0.2 %)
Probiotic use in the previous 6 months	134 (26.9 %)
Antimicrobial use in the previous 6 months	213 (42.8 %)
Oral vitamin A supplementation in the previous 6 months	145 (29.1 %)
Food consumption in the previous 3 days	
Pork	446 (89.6 %)
Beef	378 (75.9 %)
Shrimp	424 (85.1 %)
Fish	339 (68.1 %)
Egg	387 (77.7 %)
Shellfish	108 (21.7 %)
Pâté (made from live liver)	53 (10.6 %)
Current sickness	136 (27.3 %)
Hospital admission in the previous 6 months	19 (3.8 %)
Low maternal education (<=9 years)	302 (60.6 %)
Mother in paid employment	342 (68.7 %)
Household crowding†	348 (69.9 %)
Main water use at home	
Piped to residence	331 (66.5 %)
Bottled water	163 (32.7 %)
Others	4 (0.8 %)
Ice use at home	82 (16.5 %)
Exposure to flooding	107 (21.5 %)
Exposure to animal around home	300 (60.2 %)
Presence of toilet soap at home	498 (100.0 %)
Handwashing behaviour after toilet	
Always	269 (54.0 %)
Sometimes	227 (45.6 %)
Never	2 (0.4 %)
Handwashing after handling diaper	
Always	420 (84.3 %)
Sometimes	78 (15.7 %)
Never	0 (0.0 %)

*Based on the WHO Child Growth Standards: underweight, weight for age <−2 standard deviations (SD); stunting, height for age <−2 SD; wasting, weight for height <−2 SD; overweight, weight for height >+2 SD.

†Two or more people per room.

associated with resistance to fluoroquinolones and cephalosporins. The overall prevalence for any PMQR gene in the samples was 86.7 % (432/498); the corresponding prevalence for β-lactamase genes was 90.6 % (451/498). Notably, individual *qnr* determinants were the most commonly detected (83.9 %; 418/498) resistance genes, especially *qnrB* (67.3 %; 335/498) and *qnrS* (61.4 %; 306/498) (Fig. 1a). The prevalence of ESBL genes ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} and bla_{OXA}) and AmpC genes (MOX, DHA, EBC, FOX, ACC and CIT) was 83.7 % (417/498) and 67.5 % (336/498), respectively. Alarmingly, the CTX-M class of ESBL gene was the most commonly detected (75.1 %; 374/498), followed by bla_{TEM} (36.9 %; 184/498) (Fig. 1b). Generally, we found that *qnrB*, *qnrS*, $bla_{\text{CTX-M9}}$ and DHA were sequentially the most frequently detected genes, and typically identified in the same sample (Fig. 2a). More than half (52.2 %; 260/498) of the samples had a combination of at least one *qnr*, one AmpC and one $bla_{\text{CTX-M}}$ (Fig. 2b).

Not all antimicrobial-resistant meta-*E. coli* populations were associated with a corresponding AMR gene marker. For the ciprofloxacin-resistant *E. coli*, 37/337 (11 %) samples did not generate an amplicon associated with a PMQR gene, which may potentially be explained by chromosomal DNA gyrase mutations. Correspondingly, from the ceftriaxone-resistant *E. coli*, 20/400 (5 %) samples did not produce an ESBL or AmpC amplicon. Conversely, from the 161 samples with no ciprofloxacin-resistant *E. coli*, 73 % (118/161) generated an amplicon for at least 1 *qnr* gene. Of the 98 rectal swabs containing no *E. coli* exhibiting phenotypic ceftriaxone resistance, a large proportion (72 %; 71/98) generated at least 1 ESBL or AmpC amplicon.

Risk factors for PMQR and β-lactamase gene carriage

We next explored potential epidemiological associations between the participants' characteristics/behaviour and the most common PMQR genes (*qnr* family: 83.9 %; 418/498) (Table 2) and β-lactamase genes ($bla_{\text{CTX-M}}$: 75.1 %; 374/498) (Table 3). We found that the detection of *qnr* genes in meta-*E. coli* populations was significantly more common in children with higher weight-for-height z-scores (OR: 1.24, 95 % CI: 1.05–1.48, for each unit increase in weight-for-height z-score, P=0.01). However,

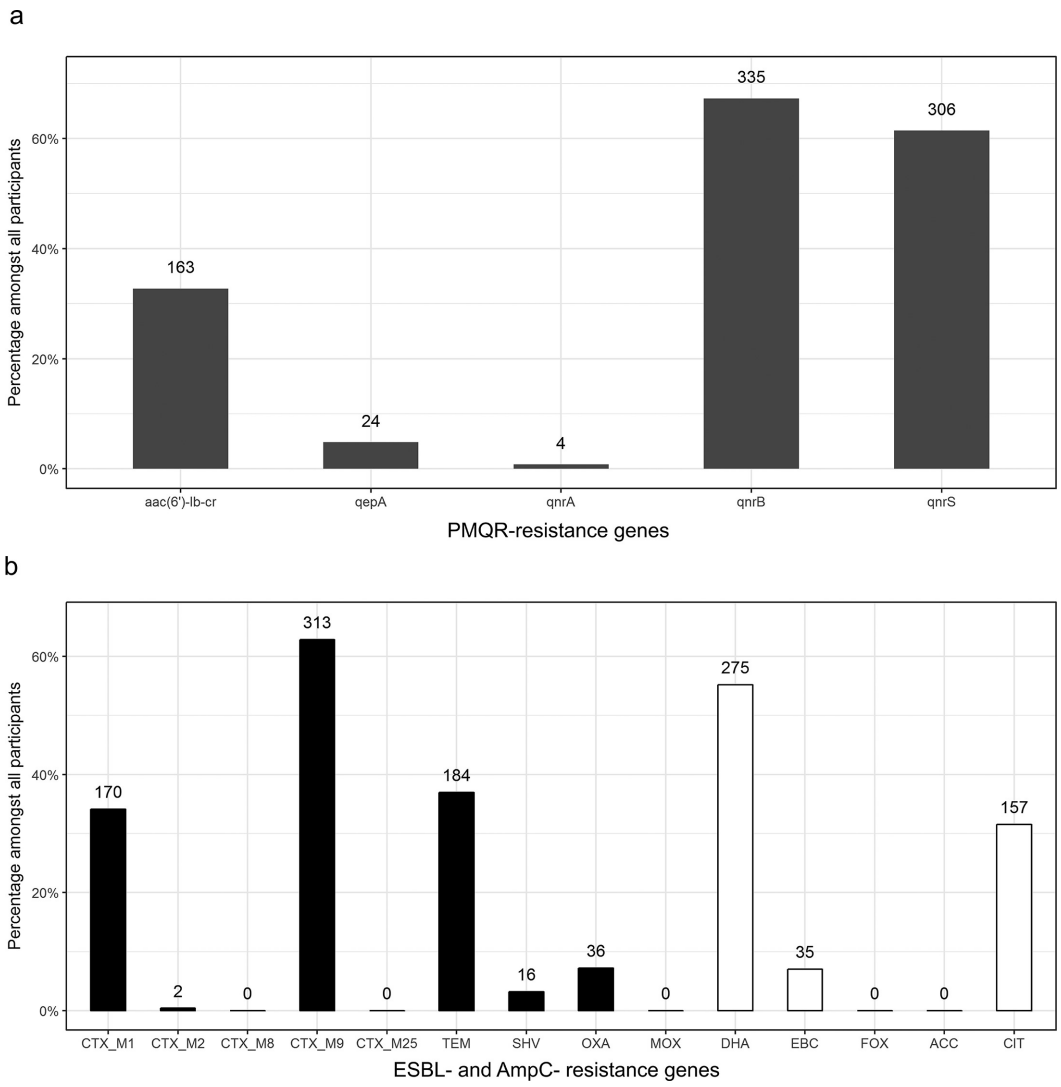

Fig. 1. The prevalence of selected antimicrobial resistance genes in commensal *E. coli* in a cohort of 498 Vietnamese children. Bar charts showing the prevalence of plasmid-mediated quinolone resistance (PMQR) genes (a) and β-lactamase genes (b) in commensal *E. coli* isolated from faecal samples from Vietnamese children.

we did not find any significant association between the age of the children when sampled and the presence of any *qnr* gene (P>0.05). Conversely, we did not identify a relationship between the weight-for-height z-scores and the presence of $bla_{\text{CTX-M}}$ genes (P>0.05). However, $bla_{\text{CTX-M}}$ genes were more commonly detected in the commensal *E. coli* in children of a younger age (OR: 0.97, 95 % CI: 0.94–0.99 for each additional year, P=0.01). Additionally, we investigated potential epidemiological associations between the participants' features and the presence of the most common genes in combination ($bla_{\text{CTX-M}}$ and *qnr*). We found a consistent relationship, in which the co-existence of these genes was significantly higher among younger children (OR: 0.95, 95 % CI: 0.9–1.00, P=0.03) (Table S3).

DISCUSSION

Commensal *E. coli* are important vehicles for the transmission of AMR determinants [9, 11, 12]. Here, we investigated both the phenotypic and genotypic characteristics of AMR in a cohort of urban children in Vietnam using direct plating and conventional PCR, an approach that is sensitive, specific, inexpensive and suitable for screening in LMICs [29, 30]. Our study outlines several new insights into this potential reservoir of AMR genes and their association with demographic characteristics. We found an exceptionally high prevalence of fluoroquinolone- and third-generation cephalosporin-resistant *E. coli* and their associated resistance genes in young children in this location. We report that the most commonly identified PMQR genes were within the *qnr* family (including *qnrB* and *qnrS*) and the

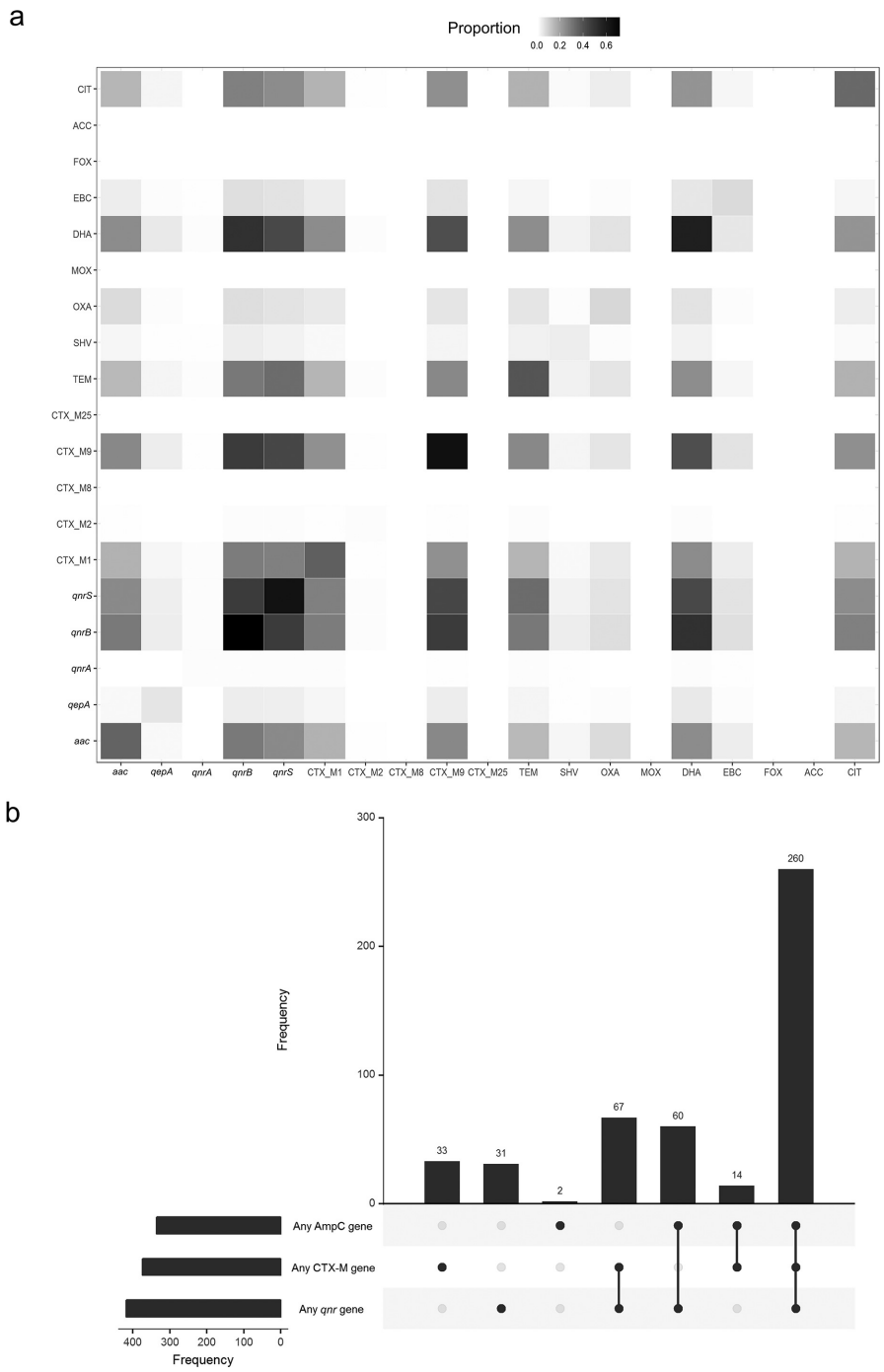

Fig. 2. The co-selection of specific antimicrobial-resistant genes in commensal *E. coli* in a cohort of 498 Vietnamese children. (a) Heat map showing the prevalence of co-selection screened antimicrobial-resistant genes; the greater the intensity, the more common the combination (see key). (b) Two-dimensional bar plots showing the prevalence of the co-existence of various combinations of *qnr*, AmpC and CTX-M genes.

most commonly detected class of ESBL gene was $bla_{\text{CTX-M}}$. We additionally found that the carriage of *qnr* genes was associated with higher weight-for-height z-scores and that younger children were more likely to carry $bla_{\text{CTX-M}}$ ESBL genes in combination with *qnr* PMQR genes.

Previous comparable studies in Vietnam demonstrated a low prevalence of quinolone-resistant commensal *E. coli* [29, 31]. These two studies, conducted in northern Vietnam, suggested that even though the overall prevalence of AMR *E. coli* was low, the isolation of ciprofloxacin-resistant

Table 2. Associations between the presence of *qnr* genes and the characteristics of the study participants

Characteristic	Presence of any *qnr* gene		Univariate analysis			Multivariable analysis		
	Yes (*n*=418)	No (*n*=80)	OR	(95 % CI)	*P* value	OR	(95 % CI)	*P* value
Low maternal education	249 (59.6 %)	53 (66.2 %)	0.75	(0.45, 1.23)	0.259	0.77	(0.45, 1.29)	0.320
Other in paid employment	286 (68.4 %)	56 (70.0 %)	0.93	(0.54, 1.55)	0.780	0.74	(0.42, 1.29)	0.292
Male	229 (54.8 %)	40 (50.0 %)	1.21	(0.75, 1.96)	0.432	1.10	(0.66, 1.83)	0.700
Age* (months)	46.4 (35.6, 52.4)	47.2 (35.8, 53.1)	0.99	(0.96, 1.02)	0.512	0.99	(0.96, 1.02)	0.419
Preterm at birth	54 (12.9 %)	10 (12.5 %)	1.04	(0.52, 2.25)	0.918	0.99	(0.49, 2.20)	0.988
Caesarean section	184 (44.0 %)	27 (33.8 %)	1.54	(0.94, 2.58)	0.086	1.48	(0.88, 2.53)	0.138
Breastfeeding duration* (months)	4.0 (3.0, 6.0)	4.0 (2.8, 6.0)	1.04	(0.99, 1.09)	0.162	1.04	(0.99, 1.10)	0.117
Weight-for-height z-score*	**1.0 (-0.1, 2.3)**	**0.4 (-0.4, 1.5)**	**1.22**	**(1.04, 1.44)**	**0.012**	**1.24**	**(1.05, 1.48)**	**0.011**
Antimicrobial use in the previous 6 months	180 (43.1 %)	33 (41.2 %)	1.08	(0.67, 1.76)	0.764	0.92	(0.54, 1.57)	0.753
Probiotic use in the previous 6 months	116 (27.8 %)	18 (22.5 %)	1.32	(0.76, 2.39)	0.324	1.30	(0.72, 2.41)	0.388
Pork or beef consumption in the previous 3 days	412 (98.6 %)	79 (98.8 %)	0.87	(0.05, 5.18)	0.896	0.90	(0.05, 6.13)	0.929
Poultry or egg consumption in the previous 3 days	385 (92.1 %)	70 (87.5 %)	1.67	(0.75, 3.42)	0.200	1.72	(0.75, 3.68)	0.193
Use of ice in the previous 6 months	65 (15.6 %)	17 (21.2 %)	0.68	(0.38, 1.27)	0.221	0.69	(0.37, 1.32)	0.250
Piped water to residence	278 (66.5 %)	53 (66.2 %)	1.01	(0.60, 1.66)	0.964	0.92	(0.53, 1.58)	0.769
Exposure to flooding	89 (21.3 %)	18 (22.5 %)	0.93	(0.53, 1.69)	0.810	0.91	(0.50, 1.72)	0.773
Exposure to animal	251 (60.0 %)	49 (61.3 %)	0.95	(0.58, 1.55)	0.840	1.03	(0.61, 1.72)	0.917
Attending school	286 (68.4 %)	52 (65.0 %)	1.17	(0.70, 1.92)	0.551	1.30	(0.72, 2.30)	0.383
Household crowding	293 (70.1 %)	55 (68.8 %)	1.07	(0.63, 1.77)	0.811	1.03	(0.59, 1.75)	0.922
Hospital admission in the previous 6 months	18 (4.3 %)	1 (1.2 %)	3.55	(0.72, 64.42)	0.139	3.47	(0.66, 64.31)	0.165
Current sickness	117 (28.0 %)	19 (23.8 %)	1.25	(0.73, 2.23)	0.430	0.99	(0.54, 1.85)	0.970

*Median (IQR); OR, odds ratio of presence of any *qnr* gene; CI, confidence interval.

organisms was higher in the urban area than in the rural area. Here we show a trend towards an increasing prevalence of ciprofloxacin-resistant commensal *E. coli*. This trend may reflect a generic higher degree of resistance to this group of antimicrobials over time, in combination with dramatic increases in antimicrobial use within Vietnam, particularly in urban areas. Using direct plating, we show a higher prevalence of ciprofloxacin-resistant *E. coli* in the commensal microbiota of children in Vietnam compared to those described in Bolivia and Peru (18 % in 2002 and 33 % in 2005) [32, 33]. Additionally, in comparison to a recent study conducted in HCMC among healthy adults [34], we also show a higher prevalence of ciprofloxacin-resistant *E. coli* (15.5 %, 16/103 *E. coli* isolates vs 67.7 %, 337/498 rectal swab samples). However, these differences may be derived from the method of resistance detection – the previous study exploited routine bacterial culture and antimicrobial susceptibility testing – or the population sampled.

Here, we additionally detected a substantially higher prevalence of PMQR and ESBL genes than had been observed in previous investigations in human populations. Our data illustrate a much higher prevalence of PMQR genes among the healthy paediatric population (86.7 %; 432/498) than has been described in other countries. For example, in Mexico in 2013 the prevalence of PMQR genes among *Enterobacteriaceae* isolates from children was 32 % [35]. A global meta-analysis estimated an ESBL positivity rate for *Enterobacteriaceae* among healthy communities of 14 % in 2016 [36]. The prevalence of ESBL-producing *Enteobacteriaceae* among the paediatric community varied according to the level of development: the figures were 2.9 % for Sweden [37], 11.6 % for Tanzania [38], 23 % for Laos [39], 24.8 % for Lebanon [40] and 59 % for Bangui (Central African Republic). Potential explanations for this discrepancy may include the use of differing methods for the detection of PMQR and ESBL determinants (i.e. PCR methods) and a temporal difference of 5 years, which may reflect a natural increase in the circulation of organisms and people carrying such AMR genes. Our data indicate that a high proportion of children in urban Vietnam carry multiple AMR genes, implying high exposure to a range of antimicrobials in this local setting [6], which is comparable to the findings of other reports in Southeast Asia [39, 41–43].

Notably, we found that the prevalence of the $bla_{\text{CTX-M}}$ gene was 75.1 % (374/498), with the majority (62.9 %; 313/498) being $bla_{\text{CTX-M-9}}$ [the next most common was bla_{TEM} (36.9 %; 184/498)]. This distribution is comparable to the patterns observed in a small sample of healthy adults residing in HCMC, where the $bla_{\text{CTX-M}}$ class of genes was the most dominant among ESBL-producing *E. coli* isolates (100 %; 10/10), followed by bla_{TEM} (50 %; 5/10) [34]. We found a relatively consistent prevalence of *qnr* genes, as previously reported in this setting [12]. We further established that *qnrB* and *qnrS* were the most prevalent PMQR genes [67 % (335/498) and 61 % (306/498), respectively]. This prevalence was higher than that found in data from China

Table 3. Associations between the presence of CTX-M genes and characteristics of the study participants

Characteristic	Presence of any CTX-M resistant genes		Univariate analysis			Multivariable analysis		
	Any CTX-M genes (*n*=374)	None CTX_M genes (*n*=124)	OR	(95 % CI)	*P* value	OR	(95 % CI)	*P* value
Low maternal education	224 (59.9 %)	78 (62.9 %)	0.88	(0.58, 1.33)	0.551	0.92	(0.59, 1.42)	0.694
Mother in paid employment	259 (69.3 %)	83 (66.9 %)	1.11	(0.72, 1.71)	0.631	1.12	(0.70, 1.77)	0.643
Male	204 (54.5 %)	65 (52.4 %)	1.09	(0.72, 1.64)	0.681	1.07	(0.70, 1.64)	0.749
Age* (month)	**41.8 (35.6, 52.3)**	**47.3 (36.0, 53.3)**	**0.97**	**(0.94, 0.99)**	**0.006**	**0.97**	**(0.94, 0.99)**	**0.014**
Preterm at birth	45 (12.0 %)	19 (15.3 %)	0.76	(0.43, 1.38)	0.351	0.70	(0.39, 1.30)	0.253
Caesarean section	158 (42.2 %)	53 (42.7 %)	0.98	(0.65, 1.48)	0.923	0.98	(0.64, 1.51)	0.937
Breastfeeding duration* (months)	4.0 (2.0, 6.0)	4.0 (3.0, 6.0)	1.01	(0.98, 1.06)	0.499	1.01	(0.97, 1.05)	0.704
Weight-for-height z-score	0.8 (-0.2, 2.1)	1.0 (-0.2, 2.2)	0.96	(0.85, 1.08)	0.480	0.95	(0.82, 1.09)	0.463
Antimicrobial use in the previous 6 months	167 (44.7 %)	46 (37.1 %)	1.37	(0.90, 2.09)	0.139	1.26	(0.81, 1.98)	0.314
Probiotic use in the previous 6 months	102 (27.3 %)	32 (25.8 %)	1.08	(0.68, 1.73)	0.749	0.94	(0.58, 1.54)	0.789
Pork or beef consumption in the previous 3 days	369 (98.7 %)	122 (98.4 %)	1.21	(0.17, 5.69)	0.824	1.17	(0.16, 5.73)	0.854
Poultry or egg consumption in the previous 3 days	342 (91.4 %)	113 (91.1 %)	1.04	(0.49, 2.07)	0.914	1.04	(0.48, 2.13)	0.914
Use of ice in the previous 6 months	63 (16.8 %)	19 (15.3 %)	1.12	(0.65, 2.00)	0.690	1.18	(0.67, 2.15)	0.581
Piped water to residence	247 (66.0 %)	84 (67.7 %)	0.93	(0.60, 1.42)	0.728	0.85	(0.53, 1.33)	0.473
Exposure to flooding	76 (20.3 %)	31 (25.0 %)	0.77	(0.48, 1.25)	0.277	0.70	(0.42, 1.18)	0.181
Exposure to animal	225 (60.2 %)	75 (60.5 %)	0.99	(0.65, 1.49)	0.949	1.03	(0.67, 1.60)	0.883
Attending school	251 (67.1 %)	87 (70.2 %)	0.87	(0.55, 1.34)	0.527	0.97	(0.59, 1.59)	0.899
Household crowding	260 (69.5 %)	88 (71.0 %)	0.93	(0.59, 1.45)	0.760	0.93	(0.58, 1.47)	0.757
Hospital admission in the previous 6 months	16 (4.3 %)	3 (2.4 %)	1.80	(0.59, 7.84)	0.326	1.48	(0.46, 6.67)	0.535
Current sickness	105 (28.1 %)	31 (25.0 %)	1.17	(0.74, 1.88)	0.503	1.28	(0.76, 2.22)	0.354

*Median (IQR); OR, odds ratio of presence of any CTX-M gene; CI, confidence interval.

and some settings in Latin American [35]. However, this disparity may again be associated with a difference in the population sampled.

We found a significant association between children carrying *qnr* genes and a higher weight-for-height z-score. An explanation for this association could be that overweight children are more likely to be treated with antimicrobials. In this setting, children who are overweight are more commonly from families with a higher socio-economic status, who in turn may be more likely to seek healthcare and, therefore, receive antimicrobials. Furthermore, childhood obesity is known to trigger chronic low-grade inflammation [44], which may lead to their vulnerability to gastrointestinal diseases and diarrhoea [45]. This in turn results in a higher likelihood of antimicrobial consumption prior to hospitalization in cases of diarrhoeal episodes [46]. Quantitative microbiological examination has reported a significant expansion of *E. coli,* coupled with the contraction of *Bifidobacterium*, in obese children in China when compared to age-matched controls [47]. As we screened for PMQR genes, which chiefly circulate within the *Enterobacteriaceae*, we speculate that there is a link between a shift in the gut microbiome during obesity and the selection of genotypic resistance. Fluoroquinolones are amongst the most commonly used antimicrobials worldwide, and their use is particularly common in Vietnam [5, 48]. The association of the carriage of $bla_{\text{CTX-M}}$ genes and younger children may be induced by reducing antimicrobial usage with age. It is difficult to assess the nature of the selective pressure for specific AMR genes during therapeutic use, but we suggest that selection may reduce as children mature.

Our study has limitations. First, the point prevalence of phenotypic and genotypic resistance could identify non-causal associations with the variables assessed in the children. Second, we investigated associations between the carriage of various AMR genes and a wide range of heterogeneous epidemiological variables among a healthy population, which may explain the limited number of significant epidemiological associations. Furthermore, by screening for the presence of PMQR and β-lactamase genes directly from rectal swabs using conventional PCRs, we were unable to assess the direct association between resistant phenotypes and associated genotypes. Notwithstanding these limitations, the results from this, the first study of its type in Vietnam, will provide an important step towards understanding the role of the microbiome in maintaining AMR genes in the community.

We conclude that there is a high prevalence of commensal *E. coli* in children aged under 5 years in an urban setting within Vietnam exhibiting resistance against fluoroquinolones and third-generation cephalosporins. In addition, we have determined that the prevalence of PMQR- and β-lactamase-encoding genes is extremely high among healthy children. We highlight that high weight-to-height z-scores are a major risk for carrying *qnr* genes and that younger children are more likely to carry CTX-M genes in combination with *qnr*. We advocate that future longitudinal studies in Asia assess the maturation of the AMR component of the human microbiome. These data are imperative for understating the ecology of AMR gene carriage and transmission within humans. Our work emphasizes that the role of the commensal bacteria is key for the transmission and maintenance of AMR organisms/genes, especially among children.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The Ethics Committee of the Hospital for Tropical Diseases (HTD) in Vietnam and the Oxford Tropical Research Ethics Committee (OxTREC) of the United Kingdom provided ethical approval for this study. Written informed consent was provided by the parents and care-givers of the children.

References

1. Nhi TQ, Alwis R, Lam PK. *A Mixed-Method Approach Quantifying Antimicrobial Access and Usage for Pediatric Diarrheal Disease in an Urban Community Setting in Asia*. Ho Chi Minh City; 2018.
2. Lautenbach E, Strom BL, Nachamkin I, Bilker WB, Marr AM *et al.* Longitudinal trends in fluoroquinolone resistance among Enterobacteriaceae isolates from inpatients and outpatients, 1989–2000: differences in the emergence and epidemiology of resistance across organisms. *Clin Infect Dis* 2004;38:655–662.
3. Dalhoff A. Global fluoroquinolone resistance epidemiology and implictions for clinical use. *Interdiscip Perspect Infect Dis* 2012; 2012:1–37.
4. Karlowsky JA, Kelly LJ, Thornsberry C, Jones ME, Evangelista AT *et al.* Susceptibility to fluoroquinolones among commonly isolated Gram-negative bacilli in 2000: TRUST and TSN data for the United States. *Int J Antimicrob Agents* 2002;19:21–31.
5. van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT *et al.* Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect Dis* 2014;14: 742–750.
6. Nguyen VK. Situation analysis: antibiotic use and resistance in Vietnam (GARP-VN report). *Cent Dis Dyn Ecnomics Policy* 2010.
7. Lester SC, del Pilar Pla M, Wang F, Perez Schael I, Jiang H *et al.* The carriage of *Escherichia coli* resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China. *N Engl J Med* 1990;323:285–289.
8. Bartoloni A, Cutts F, Leoni S, Austin CC, Mantella A *et al.* Patterns of antimicrobial use and antimicrobial resistance among healthy children in Bolivia. *Trop Med Int Health* 1998;3:116–123.
9. Bailey JK, Pinyon JL, Anantham S, Hall RM. Commensal *Escherichia coli* of healthy humans: a reservoir for antibiotic-resistance determinants. *J Med Microbiol* 2010;59:1331–1339.
10. Tian GB, Wang HN, Zhang AY, Zhang Y, Fan WQ *et al.* Detection of clinically important β-lactamases in commensal *Escherichia coli* of human and swine origin in western China. *J Med Microbiol* 2012;61:233–238.
11. Bryce A, Costelloe C, Hawcroft C, Wootton M, Hay AD. Faecal carriage of antibiotic resistant *Escherichia coli* in asymptomatic children and associations with primary care antibiotic prescribing: a systematic review and meta-analysis. *BMC Infect Dis* 2016;16:359.
12. Le TM, Baker S, Le TP, Le TP, Cao TT *et al.* High prevalence of plasmid-mediated quinolone resistance determinants in commensal members of the *Enterobacteriaceae* in Ho Chi Minh City, Vietnam. *J Med Microbiol* 2009;58:1585–1592.
13. Sommer MOA, Dantas G, Church GM. Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science* 2009;325:1128–1131.
14. Nguyen NT, Ha V, Tran NV, Stabler R, Pham DT *et al.* The sudden dominance of blaCTX-M harbouring plasmids in *Shigella* spp. circulating in southern Vietnam. *PLoS Negl Trop Dis* 2010;4:e702.
15. Bush K. Proliferation and significance of clinically relevant β-lactamases. *Ann N Y Acad Sci* 2013;1277:84–90.
16. Poirel L, Bonnin RA, Nordmann P. Genetic support and diversity of acquired extended-spectrum β-lactamases in Gram-negative rods. *Infect Genet Evol* 2012;12:883–893.
17. Aldred KJ, Kerns RJ, Osheroff N. Mechanism of quinolone action and resistance. *Biochemistry* 2014;53:1565–1574.
18. Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis* 2005;41:S120–S126.
19. Jacoby G, Strahilevitz J, Hooper D. Plasmid-mediated quinolone resistance. *Microbiol Spectr* 2014;2:997–1003.
20. Mammeri H, van de Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. *Antimicrob Agents Chemother* 2005;49:71–76.
21. Wang M, Sahm DF, Jacoby GA, Hooper DC. Emerging plasmid-mediated quinolone resistance associated with the qnr gene in *Klebsiella pneumoniae* clinical isolates in the United States. *Antimicrob Agents Chemother* 2004;48:1295–1299.
22. Lietzau S, Raum E, von Baum H, Marre R, Brenner H. Household contacts were key factor for children's colonization with resistant *Escherichia coli* in community setting. *J Clin Epidemiol* 2007;60: 1149–1155.
23. Liss MA, Nakamura KK, Peterson EM. Comparison of broth enhancement to direct plating for screening of rectal cultures for ciprofloxacin-resistant *Escherichia coli*. *J Clin Microbiol* 2013;51: 249–252.
24. Thompson CN, Anders KL, Nhi Le TQ, Tuyen HT, van Minh P *et al.* A cohort study to define the age-specific incidence and risk factors of *Shigella* diarrhoeal infections in Vietnamese children: a study protocol. *BMC Public Health* 2014;14:1289.
25. Anders KL, Nguyen NM, van Thuy NT, Hieu NT, Nguyen HL *et al.* A birth cohort study of viral infections in Vietnamese infants and children: study design, methods and characteristics of the cohort. *BMC Public Health* 2013;13:937.
26. Xiong Z, Li T, Xu Y, Li J. Detection of CTX-M-14 extended-spectrum β-lactamase in *Shigella sonnei* isolates from China. *J Infect* 2007;55:e125-8.
27. Batchelor M, Hopkins K, Threlfall EJ, Clifton-Hadley FA, Stallwood AD *et al.* bla_{CTX-M} genes in clinical *Salmonella* isolates recovered from humans in England and Wales from 1992 to 2003. *Antimicrob Agents Chemother* 2005;49:1319–1322.
28. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial susceptibility Testing*, Twenty-Third Informational Supplement; 2013.

29. Dyar OJ, Hoa NQ, Trung NV, Phuc HD, Larsson M *et al.* High prevalence of antibiotic resistance in commensal *Escherichia coli* among children in rural Vietnam. *BMC Infect Dis* 2012;12:92.
30. Bui TM, Hirai I, Ueda S, Bui TK, Hamamoto K *et al.* Carriage of *Escherichia coli* producing CTX-M-Type extended-spectrum β-lactamase in healthy Vietnamese individuals. *Antimicrob Agents Chemother* 2015;59:6611–6614.
31. Isenbarger DW, Hoge CW, Srijan A, Pitarangsi C, Vithayasai N *et al.* Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996-1999. *Emerg Infect Dis* 2002;8: 175–180.
32. Bartoloni A, Pallecchi L, Benedetti M, Fernandez C, Vallejos Y *et al.* Multidrug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. *Emerg Infect Dis* 2006;12:907–913.
33. Bartoloni A, Pallecchi L, Fiorelli C, di Maggio T, Fernandez C *et al.* Increasing resistance in commensal *Escherichia coli*, Bolivia and Peru. *Emerg Infect Dis* 2008;14:338–340.
34. Hoang PH, Awasthi SP, Do Nguyen P, Nguyen NL, Nguyen DT *et al.* Antimicrobial resistance profiles and molecular characterization of *Escherichia coli* strains isolated from healthy adults in Ho Chi Minh City, Vietnam. *J Vet Med Sci* 2017;79:479–485.
35. Silva-Sánchez J, Cruz-Trujillo E, Barrios H, Reyna-Flores F, Sánchez-Pérez A *et al.* Characterization of plasmid-mediated quinolone resistance (PMQR) genes in extended-spectrum β-lactamase-producing *Enterobacteriaceae* pediatric clinical isolates in Mexico. *PLoS One* 2013;8:e77968.
36. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal colonization with extended-spectrum β-lactamase-producing *Enterobacteriaceae* and risk factors among healthy individuals: a systematic review and metaanalysis. *Clin Infect Dis* 2016;63: 310–318.
37. Kaarme J, Molin Y, Olsen B, Melhus A. Prevalence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in healthy Swedish preschool children. *Acta Paediatr* 2013;102:655–660.
38. Tellevik MG, Blomberg B, Kommedal Ø, Maselle SY, Langeland N *et al.* High prevalence of faecal carriage of ESBL-producing *Enterobacteriaceae* among children in Dar es Salaam, Tanzania. *PLoS One* 2016;11:e0168024.
39. Stoesser N, Xayaheuang S, Vongsouvath M *et al.* Colonization with *Enterobacteriaceae* producing ESBLs in children attending preschool childcare facilities in the Lao People's Democratic Republic. *J Antimicrob Chemother* 2014;70:1893–1897.
40. Hijazi SM, Fawzi MA, Ali FM, Abd El Galil KH. Prevalence and characterization of extended-spectrum β-lactamases producing *Enterobacteriaceae* in healthy children and associated risk factors. *Ann Clin Microbiol Antimicrob* 2016;15:1–9.
41. Sasaki T, Hirai I, Niki M, Nakamura T, Komalamisra C *et al.* High prevalence of CTX-M β-lactamase-producing Enterobacteriaceae in stool specimens obtained from healthy individuals in Thailand. *J Antimicrob Chemother* 2010;65:666–668.
42. Severin JA, Lestari ES, Kloezen W, Lemmens-den Toom N, Mertaniasih NM *et al.* Faecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae among humans in Java, Indonesia, in 2001–2002. *Trop Med Int Health* 2012;17:455–461.
43. Lai CC, Lee K, Xiao Y, Ahmad N, Veeraraghavan B *et al.* High burden of antimicrobial drug resistance in Asia. *J Glob Antimicrob Resist* 2014;2:141–147.
44. Carolan E, Hogan AE, Corrigan M, Gaotswe G, O'Connell J *et al.* The impact of childhood obesity on inflammation, innate immune cell frequency, and metabolic microRNA expression. *J Clin Endocrinol Metab* 2014;99:E474–E478.
45. Shahunja KM, Ahmed T, Hossain MI, das SK, Faruque AS *et al.* Factors associated With pneumonia among overweight and obese under-five children in an urban hospital of a developing country. *Glob Pediatr Health* 2016;3:2333794X1667252.
46. Das SK, Chisti MJ, Huq S, Malek MA, Vanderlee L *et al.* Clinical characteristics, etiology and antimicrobial susceptibility among overweight and obese individuals with diarrhea: observed at a large diarrheal disease hospital, Bangladesh. *PLoS One* 2013;8: e70402.
47. Gao X, Jia R, Xie L, Kuang L, Feng L *et al.* Obesity in school-aged children and its correlation with gut *E. coli* and Bifidobacteria: a case–control study. *BMC Pediatr* 2015;15:1–4.
48. Nguyen KV, Thi do NT, Chandna A, Nguyen TV, Pham CV *et al.* Antibiotic use and resistance in emerging economies: a situation analysis for Viet Nam. *BMC Public Health* 2013;13:1158.