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ESBL/AmpC-producing Enterobacteriaceae in households with children of preschool age: prevalence, risk factors and co-carriage

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Objectives: ESBL/AmpC-producing Enterobacteriaceae are an emerging public health concern. As households with preschool children may substantially contribute to the community burden of antimicrobial resistance, we determined the prevalence, risk factors and co-carriage of ESBL/AmpC-producing bacteria in preschool children and their parents.

Methods: From April 2013 to January 2015, each month 2000 preschool children were randomly selected from Dutch population registries. The parents were invited to complete an epidemiological questionnaire and to obtain and send a faecal sample from the selected child and from one parent. Samples were tested for ESBL/AmpC-producing bacteria. Logistic regression was used to identify risk factors for ESBL/AmpC carriage in children and parents, and findings were internally validated by bootstrapping.

Results: In total, 1016 families were included and ESBL/AmpC prevalence was 4.0% (95% CI 3.2%–5.0%); 3.5% (95% CI 2.5%–4.8%) in children and 4.5% (95% CI 3.4%–6.0%) in parents. Attending a daycare centre (DCC) was the only significant risk factor for children (OR 2.1, 95% CI 1.0–4.3). For parents, the only significant risk factor was having one or more children attending DCCs (OR 2.2, 95% CI 1.2–4.8). For parents of ESBL/AmpC-positive children the OR for ESBL/AmpC carriage was 19.7 (95% CI 9.2–42.4). Co-carriage of specific ESBL/AmpC genotypes in child and parent occurred more often than expected by chance (14.6% versus 1.1%, $P<0.001$).

Conclusions: In this study, intestinal carriage with ESBL/AmpCs was detected in ~4% of households with preschool children. DCC attendance was a risk factor in both children and parents and co-carriage of specific genotypes frequently occurred in child–parent pairs. These findings suggest household transmission or/and family-specific exposure to common sources of ESBL/AmpC-producing bacteria.

Introduction

The global spread of AmpC and ESBL-producing Enterobacteriaceae conferring resistance to extended-spectrum cephalosporins represents an emerging public health threat.[1] Bacteria harbouring ESBLs may cause both community-onset bacteraemia and healthcare-associated infections.[2] Moreover, infections caused by such bacteria have been associated with increased morbidity and mortality,[3] most likely because of delays in administering appropriate therapy.[4] In the Netherlands, infections caused by ESBL-producing bacteria seem to increase in hospitalized patients, in residents of long-term care facilities (LTCFs) and in those consulting their general practitioner (GP) (www.ISIS-web.nl).[5]

ESBL/AmpC-producing bacteria can be detected in patients in healthcare settings and among healthy subjects in the community.[6–9] Yet, risk factors for carriage have mainly been investigated in adult hospitalized patients.[10–14] Frequently reported risk factors for carriage of ESBL/AmpC-producing bacteria are prior antibiotic use, hospitalization,[15,16] prior ESBL carriage, nursing home residency,[15] exposure to farm and companion animals,[17] and foreign travel.[8,9,17–19]

Empirical data on carriage of ESBL/AmpC-producing bacteria (ESBL/AmpC carriage) in healthy children are scarce. In a recent Dutch study[20] the reported overall ESBL/AmpC carriage prevalence was 4.5% among children attending daycare centres (DCCs), being as high as 8.0% among those ≤1 year old, and

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with risk factors being mainly related to the hygiene practices enforced in the DCC. ESBL/AmpC prevalence in non-DCC-attending children of preschool age and the occurrence of co-carriage of ESBL/AmpC-producing bacteria in children and their parents were not determined. In a single study the prevalence of carriage of ESBL-producing bacteria was 96% among adoptees from Mali on arrival in France and within-family transmission was observed in 23% of the families.[21]

As transmission of ESBL/AmpC resistance in households with preschool children may substantially contribute to the community burden of antimicrobial resistance, we aimed to quantify the prevalence of carriage and of co-carriage of ESBL/AmpC-producing bacteria in preschool children and their parents and risk factors for carriage to better inform public health policy makers.

Methods

Study design and epidemiological data collection

We performed each month from April 2013 to January 2015 a cross-sectional survey of ESBL/AmpC-producing bacteria in the stool of preschool children (≤4 year-old) and their parents, in the Netherlands. During the 2 years of study, 2000 children per month, 1 per family, were randomly selected (without replacement) from Dutch population registries of 335 (out of 415) municipalities, covering 78% of the general population of the Netherlands (~16.9 million inhabitants). The parents of the selected children were invited by regular mail to complete a web-based questionnaire addressing household characteristics, DCC attendance, chronic disease, medication use, clinical symptoms, medical care, occupation, animal contact, leisure activities and eating habits. The questionnaire was developed based on previous population-based studies conducted in the Netherlands[22–25] and referred to the addressed child and to one parent chosen freely within the household; questions referred to the prior 4 weeks. The degree of urbanization (<500, 500–2500, >2500 addresses/km^2) and the socio-economic status [SES; a normalized score ranging from −6.8 (low SES area) to 3.1 (high SES area), based on income, employment and education] were obtained at the postcode level from Statistics Netherlands (http://www.cbs.nl/en-GB/menu/home/default.htm). A detailed explanation of the study design can be found in a previous paper.[26]

Faecal sample collection

Upon completion of the questionnaire, parents were asked to provide a faecal sample from the sampled child and from the participating parent, regardless of the answers given in the questionnaire. If willing to submit the samples, we provided a stool sample collection kit containing two pre-labelled sterile tubes in order to collect the samples from the child and the parent. In addition, information was given on how to collect and send back the samples to the laboratory using a pre-stamped envelope. An additional questionnaire was also completed by the participants to check whether new symptoms had occurred in the 2 weeks prior to stool sampling.

Ethics

This study received ethics approval from the Medical Research Ethics Committee of Utrecht University (WAG/om/13/048247). Informed consent was obtained from all subjects.

ESBL/AmpC gene detection

All stool samples were enriched in 3 mL of LB broth supplemented with 1 mg/L cefotaxime. MacConkey agar plates with 1 mg/L cefotaxime were used as a selective medium for extended-spectrum cephalosporin-resistant (ESCR) isolates. In case of growth, up to five colonies based on different morphologies were picked from agar plates, and speciated using a MALDI-TOF MS method. *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates were screened for ESBL/AmpC genes by micro-array analysis (Check-MDR CT-101, Check-points, the Netherlands), PCR and subsequent sequencing as described previously.[27]

Semi-quantitative culture

The number of cfu/g of faeces present in an *E. coli*-positive stool sample was determined semi-quantitatively with the 'running-drop method'. Tenfold dilutions were made from faecal suspension of which 10 μL per suspension was allowed to run down the surface of Tryptone Bile X-glucuronide agar (TBX) plates and incubated overnight at a temperature of 37°C. Consequently, the cfu were based on the highest dilution where growth was still visible. In order to determine the ratio of ESBL/AmpC-positive and -susceptible bacteria, two plates were used, one with 1 mg/L cefotaxime and one without cefotaxime. The detection limit was 10^2 cfu/g of stool.

Data analysis

Logistic regression analysis was performed to identify factors associated with ESBL/AmpC carriage in children and their parents. A total of 29 and 41 putative risk factors for ESBL/AmpC carriage in children and parents (Table 1) were assessed univariately. Based on the number of observations, robustness to reporting/prevarication bias and biological plausibility with regard to ESBL/AmpC carriage, variables with a P value ≤0.20 in univariate analysis were selected for inclusion in a multivariable model using a manual backward stepwise approach in which variables having a $P<0.05$ remained in the model. Potential confounding effects were addressed by retaining variables whose exclusion from the models changed the effect of the other covariates by ≥10%. Interactions between independent variables were also tested and the final multivariable models were expanded to include the significant ($P<0.05$) interaction terms, if any. Collinearities between independent variables were checked prior to multivariable analysis and selection between collinear variables was made based on an improved model fit as revealed by the Akaike information criterion (AIC). In addition, sensitivity analysis was performed by including only ESBL-producing bacteria as outcome variable in the models (i.e. AmpC-producing bacteria were excluded). Associations were expressed as ORs and corresponding 95% CIs.

Co-carriage of ESBL/AmpC-producing bacteria in child–parent pairs was assessed independently of risk factors for ESBL/AmpC carriage in children and parents. Subsequently, to determine whether the number (cfu) of ESBL/AmpC-producing bacteria in children influenced the risk of the respective parent being a carrier, a logistic regression model was built in which the cfu in the children was included as predictor of ESBL/AmpC carriage in the parents (outcome variable).

Because of the limited number of outcome events, to cross-validate the models, bootstrapped ORs and bias-corrected bootstrap 95% CIs were also calculated (1000 replications) and presented with the standard ones as suggested elsewhere.[28] The bootstrap procedure consisted of drawing 1000 random samples with replacement from the observed data and using these samples to feed back into the models. All multivariable models showed an overall statistical significance (likelihood-ratio χ^2-test, $P<0.05$) and goodness-of-fit (Hosmer–Lemeshow test, $P>0.05$). Statistical analysis was performed using STATA 13.

We also calculated the expected probability (P) of randomly finding the same ESBL/AmpC genotype (GT) x in a child–parent pair as performed previously,[29] with P being given by:

$$\begin{aligned}&P(\text{child GT} = \text{parent GT})\\&= \Sigma_x\{P(\text{child GT} = x|\text{child sample}\\&= \text{positive for ESBL/AmpC producing bacteria})\\&\times[P(\text{parent GT} = x|\text{parent sample}\\&= \text{positive for ESBL/AmpC producing bacteria}) \times M]\},\end{aligned}$$

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Table 1. Adjusted ESBL/AmpC prevalence in children and parents

Children		Parents	
Variable	Prevalence, % (95% CI)	Variable	Prevalence, % (95% CI)
SES[a]		SES[a]	
low	2.2 (0.6–3.9)	low	4.7 (2.4–7.1)
intermediate	3.7 (1.5–5.8)	intermediate	4.6 (2.2–6.9)
high	4.4 (2.3–6.5)	high	4.4 (2.2–6.5)
Urbanization degree		Urbanization degree	
urbanized	3.2 (0.3–0.6)	urbanized	3.8 (0.7–6.8)
intermediate urbanized	3.6 (2.1–5.0)	intermediate urbanized	4.9 (3.2–6.6)
rural	3.4 (0.8–5.9)	rural	4.1 (1.4–6.7)
Child's age		Parent's age	
≤12 months	3.1 (0.4–5.7)	≤30	3.5 (0.5–6.6)
13–36 months	4.0 (2.4–5.6)	31–34	5.5 (2.9–8.1)
37–48 months	2.5 (0.5–4.4)	35–37	4.4 (1.8–6.9)
>48 months	5.0 (0.0–15.0)	>38	4.2 (2.0–6.5)
Attending daycare		Children attending daycare in the household	
yes	4.6 (2.7–6.4)	yes	5.8 (3.9–7.8)
no	2.2 (0.8–3.6)	no	2.8 (1.2–4.4)
Nationality		Nationality	
non-Dutch	9.0 (0.0–18.9)	non-Dutch	8.8 (0.0–18.3)
Dutch	3.3 (2.1–4.4)	Dutch	4.4 (3.1–5.7)

[a]Normalized score ranging from −6.8 (low SES area) to 3.1 (high SES area), based on income, employment and educational level per postal code area, which is categorized based on tertiles.

where Σ_x is the summation over all GTs found in both children and parents and M is the overall prevalence of ESBL/AmpC carriage.

Results

In total, 49 732 households were invited and 10 109 (20.3%) completed the questionnaire, of which 3376 (33.4%) were willing to and of which 1016 did provide a faecal sample. We received and tested for ESBL/AmpC-producing bacteria a total of 1999 samples (1004 from children and 995 from parents). Median ages were 29 months (IQR 18–40 months) for children (50.4% males) and 34 years (IQR 31–37) for parents (14.1% males). In children, 8.4% had a chronic gastrointestinal disease and 31.1% had—in the 2 weeks prior to faecal sample collection—one or more gastrointestinal complaints [diarrhoea (n=197; 17.8%), stomach cramps (n=122; 12.2%), blood (n=1; 0.1%) or mucous in stool (n=32; 3.2%), pale stool (n=56; 5.6%), nausea (n=34; 3.4%) or vomiting (n=81; 8.1%)]. Among parents 6.6% reported chronic gastrointestinal complaints and 33.8% reported one or more gastrointestinal complaints in the 2 weeks prior to sample collection [diarrhoea (n=139; 13.9%), stomach cramps (n=190; 19.1%), blood (n=21; 2.1%) or mucous in stool (n=45; 4.5%), pale stool (n=29; 2.9%), nausea (n=100; 10.1%) or vomiting (n=43; 4.3%)]. Antimicrobials in the past 6 months were reported in 7.7% of the children and 3.2% of parents. In samples growing ESBL/AmpC-producing bacteria semi-quantitative counts were >0 to <10^3 cfu/g in 24 samples (30%) and ≥10^3 cfu/g in 56 samples (70%). Proportions were comparable for children and parents. General baseline characteristics are presented in Table S1 (available as Supplementary data at *JAC* Online).

Prevalence

Overall, 80 (4.0%, 95% CI 3.2%–5.0%) samples were ESBL/AmpC positive: 35 (3.5% 95% CI 2.5%–4.8%) from children (5 were only AmpC-producing bacteria) and 45 (4.5% 95% CI 3.4%–6.0%) from parents (3 were only AmpC-producing bacteria). Adjusted prevalence estimates stratified by SES, urbanization degree, DCC attendance, age and nationality are presented in Table 1. From April 2013 to December 2013, the prevalence in children was 3.3% (95% CI 2.0%–5.4%) and 5.2% (95% CI 3.5%–7.6%) in parents. From January 2014 to January 2015, the prevalence in children was 3.6% (95% CI 2.2%–5.8%) and 3.9% (95% CI 2.4%–6.1%) in parents.

In children, *E. coli* was the predominant species (n=32; 91.4%), followed by *E. cloacae* (n=2; 5.7%) and *K. pneumoniae* (n=1; 2.9%). In parents, *E. coli* was also the most prevalent (n=43; 95.6%) followed by *K. pneumoniae* (n=2; 4.4%).

The most prevalent genotypes found in children were $bla_{\text{CTX-M-15}}$ (n=10; 27.8%), $bla_{\text{SHV-12}}$ (n=5; 13.9%), $bla_{\text{CMY-2}}$ (n=4; 11.1%) and $bla_{\text{CTX-M-14}}$ (n=4; 11.1%), and in parents were $bla_{\text{CTX-M-15}}$ (n=16; 34.0%), $bla_{\text{CTX-M-1}}$ (n=8; 17.0%) and $bla_{\text{CTX-M-14}}$ (n=6; 12.8%) (Figure 1 and Table S2). In children attending DCCs the most prevalent genotypes were $bla_{\text{CTX-M-15}}$ (n=6; 25.0%) and $bla_{\text{SHV-12}}$ (n=5; 20.8%) and in

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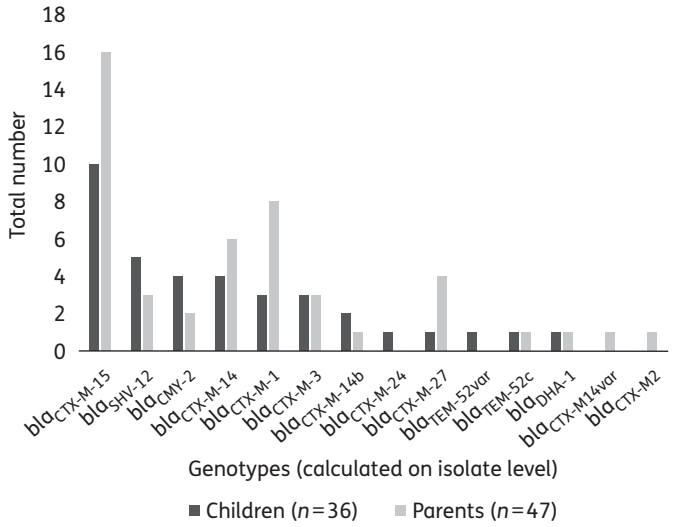

Figure 1. Prevalence of the genotypes present in children and parents.

Table 2. Prevalence of ESBL/AmpC genotypes in children attending and not attending DCC

Genotype	Attending DCC (N=24[a]), n (%)	Not attending DCC (N=11[a]), n (%)
$bla_{CTX\text{-}M\text{-}15}$	6 (25.0)	3 (27.3)
$bla_{SHV\text{-}12}$	5 (20.8)	
$bla_{CMY\text{-}2}$	2 (16.7)	2 (18.2)
$bla_{CTX\text{-}M\text{-}14}$	2 (16.7)	2 (18.2)
$bla_{CTX\text{-}M\text{-}1}$	3 (12.5)	
$bla_{CTX\text{-}M\text{-}3}$	3 (12.5)	
$bla_{CTX\text{-}M\text{-}14b}$	1 (4.2)	1 (9.1)
$bla_{CTX\text{-}M\text{-}24}$		1 (9.1)
$bla_{CTX\text{-}M\text{-}27}$	1 (4.2)	
$bla_{TEM\text{-}52var}$		1 (9.1)
$bla_{TEM\text{-}52c}$		1 (9.1)
$bla_{DHA\text{-}1}$	1 (4.2)	

[a]Calculated at the isolate level.

children not-attending DCCs $bla_{CTX\text{-}M\text{-}15}$ (n=3; 27.3%), $bla_{CMY\text{-}2}$ (n=2; 18.2%) and $bla_{CTX\text{-}M\text{-}14}$ (n=2; 18.2%) (Table 2).

Risk factors

Having a child carrying ESBL/AmpC-producing bacteria was a risk factor for ESBL/AmpC carriage in the parents (OR 19.7, 95% CI 9.2–42.4). In addition, having a parent carrying ESBL/AmpC-producing bacteria was a risk factor for children as well and the same effect estimates were observed.

In the final multivariable model for ESBL/AmpC carriage in children, attending a DCC (OR 2.1 95% CI 1.0–4.3) was the only other significant risk factor retained, independent from the risk factor of having an ESBL/AmpC-positive parent (Table 3).

In the final multivariable model for ESBL/AmpC carriage in parents (Table 4), the only significant risk factor was having one or more children attending a DCC (OR 2.2 95% CI 1.1–4.2). Bootstrap analysis confirmed the significance of this association (Table 4). In both models for children and parents, neither confounders nor interactions show any significant effect and were therefore excluded from the models. Logistic regression models of children and parents with only ESBL- and not AmpC-producing bacteria yielded the same risk factors, even after bootstrapping (data not shown).

Co-carriage

There was a trend towards an association between the semi-quantitative ESBL/AmpC count in the children's faecal samples and the risk of co-carriage in the parents. Compared with the absence of detectable ESBL/AmpC-producing bacteria, an ESBL/AmpC cfu count of >0 to $\leq 10^2$/g in children was associated with an OR of 12.7 (95% CI 3.1–51.4), and a cfu count of $\geq 10^3$/g was associated with an OR of 23.2 (95% CI 9.8–55.3) for ESBL/AmpC carriage in parents. Genotypes $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}3}$, $bla_{CTX\text{-}M\text{-}14b}$ and $bla_{TEM\text{-}52c}$ co-occurred in the child–parent pairs significantly more often than expected by chance (Table 5).

Discussion

In this study, the prevalence of ESBL/AmpC carriage in households was 4.0%, with comparable carriage prevalence among children and parents and with co-occurrence of resistance genotypes between children and parents. Attendance at DCCs was a risk factor for carriage for both children and parents.

The observed prevalence of 4.0% was lower than previous findings in the Dutch community.[6–9,20,30] Yet direct comparison of these studies is hampered by differences in study design, populations under study and analytical methods used. Most studies were conducted in adult populations, and healthy children attending DCCs were studied only in one study.[20] In that study, children ≤1 year old had a higher prevalence (8.0%) compared with the older age groups (4.5%). The prevalence of carriage of ESBL/AmpC-producing bacteria was 3.1% in the ≤1 year olds in the current study, and in children attending a DCC the ESBL/AmpC prevalence was lower (2.8%) as compared with the older age groups (3.6%). In a similar study conducted in France the reported prevalence of carriage with ESBL-producing bacteria was 6.4% in children attending DCCs.[31] In another French study, performed between 2010 and 2015, the observed overall ESBL prevalence was 7.6% (95% CI: 6.5%–9.0%) in children <24 months old seeking paediatric attention, which is somewhat higher than the prevalence observed here.[32] In 2010, a Swedish study found a prevalence of 2.9% (95% CI: 1.4%–5.6%) in preschool children from the city of Uppsala, which is comparable to our findings.[33]

$bla_{CTX\text{-}M\text{-}15}$ was the most prevalent genotype in both children and parents, followed by $bla_{CMY\text{-}2}$ and $bla_{CTX\text{-}M\text{-}1}$. The dominance of $bla_{CTX\text{-}M\text{-}15}$ in the Netherlands is in line with the global human epidemiology of ESBL-producing bacteria.[34] The current study confirms the relevance of $bla_{CMY\text{-}2}$ in children, accounting for 11.1% of all ESBL/AmpC-producing bacteria. Koningstein *et al.*[20] reported previously that in children attending DCCs in the Netherlands $bla_{CMY\text{-}2}$ was the most prevalent genotype, accounting for 26% of all resistant isolates. The comparable distribution of genotypes in children and parents reflects the high rate of co-occurrence of genotypes in both groups.

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Table 3. Risk factors for ESBL/AmpC carriage in children

Variable	ESBL/AmpC+, $n=35$ (%)	ESBL/AmpC−, $n=970$ (%)	Univariable OR (95% CI)	Multivariable OR (95% CI)	Bootstrapped multivariable OR (bias-corrected bootstrap 95% CI)[a]
Attending DCC	24 (68.6)	496 (51.3)	2.1 (1.0–4.3)	2.1 (1.0–4.3)	2.1 (1.0–4.8)
Nationality (non-Dutch versus Dutch)	4 (11.5)	32 (3.3)	3.8 (1.3–11.3)		
Someone vegetarian in the household	4 (11.4)	54 (5.6)	2.2 (0.7–6.4)		
Breastfed infant	24 (68.6)	758 (78.4)	0.6 (0.3–1.2)		
Pets in the household	14 (40.0)	489 (50.5)	0.7 (0.3–1.3)		
Household close to wooded areas, urban parks, meadows or croplands	11 (31.4)	431 (44.6)	0.6 (0.3–1.2)		

[a]ORs and bias-corrected bootstrap 95% CIs based upon 1000 replications.

Table 4. Risk factors for ESBL/AmpC carriage in parents

Variable	ESBL/AmpC+, $n=45$ (%)	ESBL/AmpC−, $n=950$ (%)	Univariable OR (95% CI)	Multivariable OR (95% CI)	Bootstrapped multivariable OR (bias-corrected bootstrap 95% CI)[a]
One or more DCC-attending children in the house	33 (73.3)	532 (56.0)	2.2 (1.1–4.2)	2.2 (1.1–4.2)	2.2 (1.2–4.8)
Eating chicken (>once a week/<4 times a month)	35 (77.8)	640 (67.4)	1.7 (0.8–3.5)		
Eating raw and/or undercooked meat (>once a week/<4 times a month)	8 (17.8)	255 (26.8)	0.6 (0.3–1.3)		

[a]ORs and bias-corrected bootstrap 95% CIs based upon 1000 replications.

Table 5. Co-occurrence of ESBL/AmpC genotypes in child–parent pairs[a]

Genotype	Observed co-occurrence (%)	Expected co-occurrence (%)	*P* (binomial probability test)
$bla_{CTX\text{-}M\text{-}14}$	3.7	0.2	<0.001
$bla_{CTX\text{-}M\text{-}15}$	3.7	1.2	0.072
$bla_{CTX\text{-}M\text{-}27}$	1.2	0.0	0.024
$bla_{CTX\text{-}M\text{-}3}$	2.4	0.1	0.001
$bla_{CTX\text{-}M\text{-}14b}$	1.2	0.0	0.012
$bla_{SHV\text{-}12}$	1.2	0.1	0.086
$bla_{TEM\text{-}52c}$	1.2	0.0	0.006
Overall	14.6	1.1	<0.001

[a]Calculated at the isolate level ($n=83$).

DCC attendance was the only significant risk factor for carriage of ESBL/AmpC-producing bacteria in both children and parents, and the absolute difference between children attending (4.6%) and not attending DCC (2.2%) was 2.4%. Moreover, there was a trend towards an association between the semi-quantitative level of ESBL/AmpC carriage in children and the likelihood of carriage in parents, and co-carriage of specific genotypes in child–parent pairs occurred more frequently than expected based on chance alone.

To the best of our knowledge these associations have not been reported before. The higher prevalence may result from the intensity of contacts among children in DCC, also resulting in higher carriage rates of enteropathogens[35] and increased risks for gastrointestinal and respiratory infections.[26,36–38] DCCs, therefore, may play a role in the spread of ESBL/AmpC-producing bacteria among children, and also facilitate further spread within households. This is also supported by other studies in similar study populations where parents of children with influenza-like-illness,[38] gastroenteritis[26] and in the presence of enteropathogens (bacteria, viruses and parasites)[39] were at increased risk of experiencing the same symptoms as their children during the same 4 week period. The possibility of transmission of ESBL-producing bacteria within families was previously demonstrated in families that adopted a child,[21] after community-acquired infections[40,41] and after hospital-acquired carriage of ESBL-producing bacteria.[42] In another study infants became colonized with $bla_{CTX\text{-}M\text{-}15}$-producing *K. pneumoniae* during a neonatal ICU outbreak and the same bacteria were subsequently detected in 32% of the households.[43] An alternative explanation for the observed associations would be differences in exposure to external sources, such as contaminated foods or environment. More studies are needed to disentangle the relevance of these different acquisition routes in the epidemiology of ESBL/AmpC-producing bacteria.

Few other risk factors for carriage of ESBL/AmpC-producing bacteria in healthy children have been described. A recent French study investigated ESBL occurrence and risk factors in children <24 months of age visiting 18 paediatricians who participated in the study and observed that those children cared for at

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home were at increased risk for carriage of ESBL-producing bacteria.[32] This is different from our finding where DCC attendance is a risk factor for carriage of ESBL/AmpC-producing bacteria. Several explanations are possible and may be related to the different populations under investigation (i.e. 'healthy' children selected at random from the general population in the present study versus paediatrician-attending children in the French study), the different age groups considered (i.e. 6–24 months in the French study versus 0–48 months in the present one), and different (hitherto unknown) factors associated with home-based childcare in France versus the Netherlands, e.g. socio-economic status, ethnic background, etc. Another study, conducted in Lebanese children, observed that regular consumption of all types of meat (including chicken) was a risk factor for ESBL/AmpC carriage.[44] In the current study, we determined associations between food consumption practices and ESBL/AmpC carriage in parents only, but significant associations were not detected.

This study has some limitations. The relatively low number of carriers of ESBL/AmpC-producing bacteria limited statistical power to investigating some relatively less prevalent exposures in our (predominantly healthy) population, such as hospitalization or antibiotic use. In addition, due to the study design, we could not investigate some established risk factors, e.g. travelling abroad. Moreover, although potential confounding effects were addressed, the possibility of residual confounding is always there. Furthermore, the 20.3% response rate may have caused bias. For instance, subjects experiencing (gastrointestinal) complaints may have been more motivated to submit a faecal sample. However, the response rate was comparable to other studies on ESBL epidemiology in the Netherlands.[7,9] Recall bias may also have occurred, but it is unlikely that this led to differential misclassification, as there is no reason to assume different recall between carriers and non-carriers.

In conclusion, carriage of ESBL/AmpC-producing bacteria was detected in ∼4% of households with preschool children, with DCC attendance being the most important risk factor for carriage in both children and parents. The high co-carriage of resistance genes between children and their parents, the semi-quantitative carriage load in children and the risk of carriage among parents suggest the occurrence of household transmission.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Euro Surveill* 2008; **13**: pii=19044.

2 Lee J, Kang S. Epidemiology and clinical features of community-onset bacteremia caused by extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*. *Microb Drug Resist* 2011; **17**: 267–73.

3 de Kraker MEA, Wolkewitz M, Davey PG *et al*. Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to *Escherichia coli* resistant to third-generation cephalosporins. *J Antimicrob Chemother* 2011; **66**: 398–407.

4 Rottier WC, Ammerlaan HSM, Bonten MJM. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum β-lactamase-producing Enterobacteriaceae and patient outcome: a meta-analysis. *J Antimicrob Chemother* 2012; **67**: 1311–20.

5 van der Steen M, Leenstra T, Kluytmans JAJW *et al*. Trends in expanded-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* among Dutch clinical isolates, from 2008 to 2012. *PLoS One* 2015; **10**: e0138088.

6 Reuland EA, Overdevest ITMA, Al Naiemi N *et al*. High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints. *Clin Microbiol Infect* 2013; **19**: 542–9.

7 Huijbers PMC, de Kraker M, Graat EAM *et al*. Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in humans living in municipalities with high and low broiler density. *Clin Microbiol Infect* 2013; **19**: E256–9.

8 Paltansing S, Vlot JA, Kraakman MEM *et al*. Extended-spectrum β-lactamase-producing Enterobacteriaceae among travelers from the Netherlands. *Emerg Infect Dis* 2013; **19**: 1206–13.

9 Reuland EA, Al Naiemi N, Kaiser AM *et al*. Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam. *J Antimicrob Chemother* 2016; **71**: 1076–82.

10 Pessoa-Silva C, Meurer Moreira B, Câmara Almeida V *et al*. Extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit: risk factors for infection and colonization. *J Hosp Infect* 2003; **53**: 198–206.

11 Boo N-Y, Ng S-F, Lim VKE. A case–control study of risk factors associated with rectal colonization of extended-spectrum β-lactamase producing *Klebsiella* sp. in newborn infants. *J Hosp Infect* 2005; **61**: 68–74.

12 Ko YJ, Moon H-W, Hur M *et al*. Risk factors of fecal carriage with extended-spectrum β-lactamase-producing Enterobacteriaceae in hospitalized patients. *Am J Infect Control* 2013; **41**: 1241–3.

13 Denkel La, Schwab F, Kola A *et al*. The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E). *J Antimicrob Chemother* 2014; **69**: 2230–7.

14 Tham J, Odenholt I, Walder M *et al*. Risk factors for infections with extended-spectrum β-lactamase-producing *Escherichia coli* in a county of Southern Sweden. *Infect Drug Resist* 2013; **6**: 93–7.

15 Shitrit P, Reisfeld S, Paitan Y *et al*. Extended-spectrum β-lactamase-producing Enterobacteriaceae carriage upon hospital admission: prevalence and risk factors. *J Hosp Infect* 2013; **85**: 230–2.

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16 Colodner R, Rock W, Chazan B *et al*. Risk factors for the development of extended-spectrum β-lactamase-producing bacteria in nonhospitalized patients. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 163–7.

17 Meyer E, Gastmeier P, Kola A *et al*. Pet animals and foreign travel are risk factors for colonisation with extended-spectrum β-lactamase-producing *Escherichia coli*. *Infection* 2012; **40**: 685–7.

18 Tängdén T, Cars O, Melhus A *et al*. Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum β-lactamases: a prospective study with Swedish volunteers. *Antimicrob Agents Chemother* 2010; **54**: 3564–8.

19 Von Wintersdorff CJH, Penders J, Stobberingh EE *et al*. High rates of antimicrobial drug resistance gene acquisition after international travel, the Netherlands. *Emerg Infect Dis* 2014; **20**: 649–57.

20 Koningstein M, Leenen MA, Mughini-Gras L *et al*. Prevalence and risk factors for colonization with extended-spectrum cephalosporin-resistant *Escherichia coli* in children attending daycare centers: a cohort study in the Netherlands. *J Pediatric Infect Dis Soc* 2015; **4**: e93–9.

21 Tandé D, Boisramé-Gastrin S, Münck MR *et al*. Intrafamilial transmission of extended-spectrum-β-lactamase-producing *Escherichia coli* and *Salmonella enterica* Babelsberg among the families of internationally adopted children. *J Antimicrob Chemother* 2010; **65**: 859–65.

22 Mughini-Gras L, Enserink R, Friesema I *et al*. Risk factors for human salmonellosis originating from pigs, cattle, broiler chickens and egg laying hens: a combined case–control and source attribution analysis. *PLoS One* 2014; **9**: e87933.

23 Mughini Gras L, Smid JH, Wagenaar JA *et al*. Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case–control and source attribution analysis. *PLoS One* 2012; **7**: e42599.

24 Enserink R, Duizer E, Kortbeek T. Risk factors for gastroenteritis in child day care. *Epidemiol Infect* 2015; **143**: 2707–20.

25 Doorduyn Y, van Pelt W, Havelaar A. The burden of infectious intestinal disease (IID) in the community: a survey of self-reported IID in The Netherlands. *Epidemiol Infect* 2012; **140**: 1185–92.

26 Mughini-Gras L, Pijnacker R, Heusinkveld M *et al*. Societal burden and correlates of acute gastroenteritis in families with preschool children. *Sci Rep* 2016; **6**: 1–10.

27 Liakopoulos A, Geurts Y, Dierikx C *et al*. Extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg strains, the Netherlands. *Emerg Infect Dis* 2016; **22**: 1257–61.

28 Vittinghoff E, Mcculloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol* 2007; **165**: 710–8.

29 Mughini-Gras L, Smid JH, Wagenaar JA *et al*. Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. *Epidemiol Infect* 2013; **141**: 2526–35.

30 Platteel TN, Leverstein-van Hall MA, Cohen Stuart JW *et al*. Predicting carriage with extended-spectrum β-lactamase-producing bacteria at hospital admission: a cross-sectional study. *Clin Microbiol Infect* 2015; **21**: 141–6.

31 Blanc V, Leflon-Guibout V, Blanco J *et al*. Prevalence of day-care centre children (France) with faecal CTX-M-producing *Escherichia coli* comprising O25b:H4 and O16:H5 ST131 strains. *J Antimicrob Chemother* 2014; **69**: 1231–7.

32 Birgy A, Levy C, Bidet P *et al*. ESBL-producing *Escherichia coli* ST131 versus non-ST131: evolution and risk factors of carriage among French children in the community between 2010 and 2015. *J Antimicrob Chemother* 2016; **71**: 2949–56.

33 Kaarme J, Molin Y. Prevalence of extended-spectrum β-lactamase-producing Enterobacteriaceae in healthy Swedish preschool children. *Acta Paediatr* 2013; **102**: 655–60.

34 Woerther P-L, Burdet C, Chachaty E *et al*. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* 2013; **26**: 744–58.

35 Enserink R, Scholts R, Bruijning-Verhagen P *et al*. High detection rates of enteropathogens in asymptomatic children attending day care. *PLoS One* 2014; **9**: e89496.

36 Enserink R, Lugnér A, Suijkerbuijk A *et al*. Gastrointestinal and respiratory illness in children that do and do not attend child day care centers: a cost-of-illness study. *PLoS One* 2014; **9**: e104940.

37 Lu N, Samuels ME, Shi L *et al*. Child day care risks of common infectious diseases revisited. *Child Care Health Dev* 2004; **30**: 361–8.

38 Mughini-Gras L, Enserink R, Heusinkveld M *et al*. Influenza-like illness in households with children of preschool age. *Pediatr Infect Dis J* 2016; **35**: 242–8.

39 Heusinkveld M, Mughini-Gras L, Pijnacker R *et al*. Potential causative agents of acute gastroenteritis in households with preschool children: prevalence, risk factors, clinical relevance and household transmission. *Eur J Clin Microbiol Infect Dis* 2016; doi:10.1007/s10096-016-2714-9.

40 Rodriguez-Bano J, Lopez-Cerero L, Navarro MD *et al*. Faecal carriage of extended-spectrum β-lactamase-producing *Escherichia coli*: prevalence, risk factors and molecular epidemiology. *J Antimicrob Chemother* 2008; **62**: 1142–9.

41 Valverde A, Grill F, Coque TM *et al*. High rate of intestinal colonization with extended-spectrum-β-lactamase-producing organisms in household contacts of infected community patients. *J Clin Microbiol* 2008; **46**: 2796–9.

42 Haverkate MR, Platteel TN, Fluit AC *et al*. Quantifying within-household transmission of ESBL-producing bacteria. *Clin Microbiol Infect* 2016; doi:10.1016/j.cmi.2016.08.021.

43 Löhr IH, Rettedal S, Natås OB *et al*. Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing *Klebsiella pneumoniae* following a nosocomial outbreak. *J Antimicrob Chemother* 2013; **68**: 1043–8.

44 Hijazi SM, Fawzi MA, Ali FM *et al*. Prevalence and characterization of extended-spectrum β-lactamases producing Enterobacteriaceae in healthy children and associated risk factors. *Ann Clin Microbiol Antimicrob* 2016; **15**: 3.

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