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Faecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae and Shiga toxin-producing *Escherichia coli* in asymptomatic nursery children in Lower Saxony (Germany), 2014

M. HARRIES[1,2,3]*, J. DREESMAN[4], S. RETTENBACHER-RIEFLER[5] AND E. MERTENS[6]

[1] *Governmental Institute of Public Health of Lower Saxony, Hanover, Germany*
[2] *Postgraduate Training for Applied Epidemiology (PAE, German Field Epidemiology Training Programme), Robert Koch-Institute, Berlin, Germany*
[3] *European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden*
[4] *Microbiology & Infectious Disease Unit, Governmental Institute of Public Health of Lower Saxony, Hanover, Germany*
[5] *Microbiology & Infectious Disease Unit, Subdivision Virology & Serology, Governmental Institute of Public Health of Lower Saxony, Hanover, Germany*
[6] *Microbiology & Infectious Disease Unit, Subdivision Surveillance and Communicable Disease Epidemiology, Governmental Institute of Public Health of Lower Saxony, Hanover, Germany*

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SUMMARY

Children may be at higher risk for carriage of antimicrobial-resistant bacteria because of higher usage of antimicrobials. They also have higher rates of Shiga toxin-producing *Escherichia coli* (STEC) infections than other population groups. Some infections, particularly in children, are asymptomatic, but still lead to the excretion of large numbers of bacteria and viruses that may cause clinical disease in other individuals. That is one reason why, in Lower Saxony as in other German federal states – asymptomatic carriers of STEC are excluded from nurseries and schools until three consecutive stool samples test negative in order to prevent secondary cases. The prevalence of children who are asymptomatic STEC carriers is unknown. But if it is high, this measure would have substantial socioeconomic effects on families. Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) are an increasing problem for public health, especially for hospitals. However, there are no reliable estimates of the prevalence of asymptomatic ESBL-E carriers in Lower Saxony, as there is no mandatory requirement to report these carriers. In order to discuss the exclusion policies for children attending nurseries and ascertain a baseline of ESBL-E carriers, we conducted a cross-sectional study. The aim was to determine the prevalence of ESBL-E and STEC and identify risk factors for carriage in nursery children without diarrhoea (asymptomatic) aged 0–6 years in four selected districts in Northern Germany. During April–September 2014, we collected stool specimens with the support of voluntarily participating nurseries. We tested for STEC by PCR and for ESBL-E on chromogenic agar. Questionnaires answered by parents contained data on eating and drinking habits, outdoor activities, prior antibiotic treatment and animal contact for each participating child. We compared the epidemiological characteristics of ESBL-E carriers *vs.* non-carriers by

* Author for correspondence: Dr M. Harries, Governmental Institute of Public Health of Lower Saxony, Roesebeckstr. 4–6, D-30449 Hannover, Germany.
(Email: Manuela.Harries@gmx.de)

using univariable analysis (*P* value, odds ratio and 95% confidence interval). We could not perform a statistical analysis for STEC carriers due to the low numbers of positive STEC specimens. Of 224 asymptomatic nursery children, we found a prevalence of 2·3% for ESBL-E carriage and 0·5% for STEC carriage. Asymptomatic ESBL-E carriers were more likely to have consumed raw milk, have had contact with pet rodents, or to have taken antibiotics during the preceding 6 months. We also found a high proportion of raw milk consumption (11%). We suggest that the low STEC prevalence in asymptomatic children supports the current practice of excluding STEC carriers from nurseries. The association between ESBL-E carriage and raw milk consumption and contact with pet rodents needs further investigation.

Key words: Asymptomatic children, extended-spectrum β-lactamase-producing Enterobacteriaceae, Germany, raw milk, Shiga-like toxin-producing *E. coli*.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) are a group of pathogenic *E. coli* strains capable of producing toxins which resemble those produced by *Shigella dysenteriae* [1]. Because of the numerous outbreaks and potential life-threatening complications (haemolytic uraemic syndrome; HUS), STEC are considered an emerging pathogen [2]. As required by the German Protection against Infection Act [3], data on STEC and HUS infections are collected systematically based on reports by laboratories and physicians. Germany registers on average about 1100 STEC cases/year (70 HUS cases/year), with most cases occurring in regions where cattle density is high, such as Bavaria and Lower Saxony [4]. However, in 2011, a serious outbreak of *E. coli* producing both Shiga toxin- and extended-spectrum β-lactamases (ESBL) caused 4907 STEC cases (18 deaths) and 880 HUS cases (32 deaths), mainly in Northern Germany [5].

STEC outbreak management in Germany includes screening close contacts of notified cases to detect further cases that might be asymptomatic. Any child testing positive for STEC needs approval of the local health authorities before they are allowed to return to nursery, kindergarten or school, and must comply with specified protection measures (German Protection against Infection Act §34/2) to prevent transmission to other children. A common measure used by local public health offices is to exclude asymptomatic children who are contacts of confirmed cases from nurseries until three consecutive stool samples test negative for STEC. This measure has substantial socioeconomic effects: STEC is shed for 20 days on average (up to 71 days) [6, 7] and parents often need to take compassionate leave to care for their children at home. In addition, school-age children might fall behind in school.

Similarly, our knowledge about extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) prevalence in asymptomatic children is limited. The number of asymptomatic adults colonized with ESBL-E in the community has also been increasing [8, 9]. Recent estimates of the proportion of ESBL-E carriers in asymptomatic adults vary between countries and range from 5·8% in Switzerland [10] to 65·7% in Thailand [11].

ESBL producers have been found across Enterobacteriaceae, including *Klebsiella* spp. and *E. coli* [8]. ESBL are capable of inactivating β-lactam antimicrobials and they are frequently plasmid encoded, which supports the spread of antimicrobial resistance [12]. Usage of β-lactam antibiotics (e.g. penicillin, first-, second-, and third-generation cephalosporins) increases the conferring of bacterial resistance [13], and the prescription rate of antibiotics in German children aged <6 years is higher than for any other age group [14]. Initially, ESBL-E were only observed and screened in hospitals [15], where they have caused nosocomial outbreaks in neonatal units [16]. The aim of our study was to estimate the prevalence of STEC and ESBL-E in asymptomatic children and identify factors associated with STEC and ESBL-E carriage to guide risk assessment and management.

MATERIAL AND METHODS

Study design and data collection

We performed an anonymous cross-sectional study between April and September 2014 in four districts in Lower Saxony in asymptomatic nursery children

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aged 0–6 years. Lower Saxony is a federal state in northern Germany, which has a population of about eight million and is rather rurally structured with average population density of about 168 inhabitants/km^2. The districts were selected with regard to cattle density. We chose two districts with a high cattle density (range 1·3–1·77 livestock units/ha) and two with low density (range 0·21–0·44 livestock units/ha) [17]. Being asymptomatic was defined as absence of diarrhoea, i.e. if the question 'suffering from diarrhoea within the previous 7 days' in the questionnaire was answered in the negative.

In cooperation with the local public health authorities, we recruited nurseries by using convenience sampling. We provided participating nurseries with study packages containing information material (posters and leaflets in German), questionnaires and sample kits. Parents completed a questionnaire, signed a consent form and collected a stool specimen of their child. The data were linked by using a common survey participant identification number.

Questionnaire

A questionnaire was designed to collected demographic data (age, sex and district) as well as information about food consumed (including meat and milk products), contact with animals, outdoor activities (e.g. swimming), travelling, and symptoms 7 days preceding answering the questionnaire, and antibiotic treatment during the preceding 6 months. The questions were selected from other epidemiological studies focusing on children [18] and were a main part of the ethics committee evaluation. The questionnaire was checked for completeness and plausibility. The inter-questionnaire consistency was tested by comparing conflicting information between two questions. If the answers indicated that the child had suffered from diarrhoea within the previous 7 days, the child was excluded from the study.

Microbiology

The medical laboratory of the Governmental Institute of Public Health of Lower Saxony analysed the stool samples. The stool specimens were enriched in mTSB (tryptone soya broth containing mitomycin C; R-Biopharm AG, Germany) and incubated at 37 °C for 18–24 h. Nucleic acid isolation was performed using the DNA QIAamp Mini kit (Qiagen, Germany) with 200 μl elution volume. Afterwards, the specimens were examined for Shiga toxin (Stx)-producing *E. coli* and Shiga toxin genes (*stx1* and *stx2*) using Shiga toxin-specific DNA sequences by polymerase chain reaction (PCR) [19]. The sensitivities and specificities of the LC-PCR assays were each 100% for the *stx1* gene and 96% and 100%, respectively, for the *stx2* gene [19]. For *stx*-positive tested isolates no follow-up tests (e.g. serotyping) or cultivation were performed.

Enterobacteriaceae producing ESBLs were cultured on selective chromogenic culture media (CHROMagar™ ESBL, MAST Group, Germany). Microorganisms were identified based on their metabolic profile, using an automated system (VITEK 2 compact; bioMérieux, France). No further ESBL confirmatory tests were performed.

Statistical analyses

Microsoft Access2003 (Microsoft Corp., USA) was used as the database system. Descriptive statistics were used to characterise carriers and non-carriers according to age, sex and district. The prevalence of carriage for both STEC and ESBL-E was calculated. To identify factors associated with carriage, we used χ^2 test and Fisher's exact test. Exposures with $P < 0{\cdot}05$ were regarded as statistically significantly associated. The strength of the association was assessed with odds ratios (ORs) and 95% confidence intervals (CIs). We conducted the statistical analyses using Stata/SE12 software (StataCorp., USA). Due to the low numbers of positive specimens (see Results section), a stratified analysis was not performed.

Ethics approval

Ethics approval for the study was granted in March 2014 by the Ethics Commission of the State Chamber of Medicine in Lower Saxony (Landesärztekammer Niedersachsen, Hannover, Germany, BO/45/2013).

RESULTS

We recruited 59 nurseries, of which 30 were from districts with high cattle density and 29 were from districts with low cattle density. In total, 1420 study packages were distributed to parents and 225 stool specimens (15·8%) were returned to the Governmental Institute of Public Health of Lower Saxony together with consent forms and questionnaires. Two participants reported diarrhoea in the preceding 7 days and

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therefore the two stool specimens were subsequently excluded.

The median age of study participants was three years (interquartile range 3 years). Fifty-six percent ($n = 124$) were male and 44% lived in districts with high cattle density.

STEC were found in stool specimens of two children (one with the presence of *stx1* and *stx2* and one with *stx1* only). The latter child reported diarrhoea during the last 7 days and was therefore excluded from the study. Hence, the prevalence of STEC-positive asymptomatic study participants was 0·5% ($n = 1/224$, 95% CI 0–1·3). Due to the low number of STEC carriers, we refrained from further statistical analyses.

Six specimens tested positive for ESBL-E: four samples contained ESBL-producing *E. coli*, one contained both *E. coli* and *Citrobacter* and one ESBL-producing *Klebsiella*. One child with ESBL-producing *E. coli* stated having had diarrhoea during the last 7 days and was excluded consequently from the study. The prevalence of ESBL-E-positive asymptomatic study participants was 2·3% ($n = 5$, 95% CI 0·3–4·2).

Univariable analyses revealed no significant difference in sex, age and district between ESBL-E carriers ($n = 5$) and non-carriers ($n = 219$). One of the ESBL-E carriers had been hospitalized during the previous 6 months. ESBL-E carriers reported a significantly higher use of antibiotics during the preceding 6 months than non-carriers (see Table 1). Children with ESBL-E positive stool specimens were 14 times more likely to have consumed raw milk (milk that has not been pasteurized or cooked), 13 times more likely to have received antibiotic treatment during the preceding 6 months and eight times more likely to have had contact with pet rodents. There were too few observations within some categories to perform a multivariable analysis.

Out of the 224 participants, 24 (11%) reported raw milk consumption and 17 (71%) of the 24 lived in districts with high cattle density.

DISCUSSION

In this cross-sectional study, we identified a low STEC prevalence and an average ESBL-E prevalence in asymptomatic nursery children aged 0–6 years in Lower Saxony. Univariable analysis revealed that raw milk consumption, contact with rodents and antibiotic treatment was more frequent in ESBL-E carriers.

We found a surprisingly high proportion of raw milk consumption in our total study population.

Prevalence of STEC

On some occasions, contact screening has revealed a high proportion of asymptomatic STEC carriers and typing has shown the presence of different strains. This indicates a high prevalence of asymptomatic carriage. Fairly high numbers of asymptomatic carriers have been found during investigation of local outbreaks [20, 21]. For example in 2012, contact screening in 31 farm employees in Lower Saxony identified nine asymptomatic carriers [22]. The virulence pattern of the pathogenic factors deviated widely. If this reflects a general high prevalence of asymptomatic carriage in the population, it would be inadequate to exclude a child who has been identified by chance during a contact investigation. The current policy for exclusion is based on the assumption that asymptomatic carriers are rare. However, the actual prevalence of asymptomatic STEC carriers in German children has not been assessed yet. Considering the current exclusion criteria from nurseries and schools, a known STEC prevalence in asymptomatic children would be informative for policy makers to decide on appropriate control measures.

The observed STEC prevalence of 0·5% in asymptomatic children was lower than expected and not consistent with the proportion of carriers reported in outbreak investigations (screening of contact persons) in Germany and other countries [23–26]. In outbreak situations, contact persons often test positive for other STEC strains than the outbreak strain. Our findings do not support that there is a high prevalence of asymptomatic STEC in the general population. Still, little is known about secondary transmission by asymptomatic carriers [27]. Furthermore, from our own laboratory data (unpublished data) we know that contact screening sometimes detects asymptomatic children carrying STEC strains other than the outbreak strain suggesting that different serotypes circulate during outbreaks. Contact persons of STEC cases might have been exposed to similar risks as the cases, such as having consumed the same food products that constitute a risk for STEC infection.

In Norway, the National Institute of Public Health bases its decision about exclusion from school on the clinical severity of the STEC infection and the age of the child [28]. If a child in a nursery setting is diagnosed with *stx1*-producing *E. coli*, which causes less

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Table 1. *Univariable analysis of covariates for extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) carriage in asymptomatic nursery children (n = 224) in Lower Saxony, 2014*

	ESBL-E carrier		
Covariate	Yes ($n = 5$)	No ($n = 219$)	OR (95% CI)
Demographic characteristic			
Median age (years)	3	2	
Sex			
Male	2	123	
Female	3	96	0·5 (0·04–4·6)
District			
Low cattle density	2	138	
High cattle density	3	81	0·4 (0·03–3·5)
Siblings			
Yes	3	147	
No	2	72	0·7 (0·08–9·0)
Occupation of parents			
Meat sector			
Yes	1	4	
No	4	215	18 (0·3–228)
Healthcare sector			
Yes	0	50	
No	5	169	0·0 (0·0–2·7)
Agriculture sector			
Yes	0	9	
No	5	210	0·0 (0·0–19·7)
Clinical history			
Antibiotic treatment during preceding 6 months			
Yes	4	51	
No	1	168	**13 (1·2–649)**
Hospital stay during preceding 6 months			
Yes	1	10	
No	4	209	5 (0·1–59)
Food consumption			
Raw milk			
Yes	3	21	
No	2	198	**14 (1·5–173)**
Crudités			
Yes	2	196	
No	3	23	**0·1 (0–0·7)**
Pasteurized milk			
Yes	0	148	
No	5	71	**0·0 (0·0–0·4)**
Game			
Yes	3	41	
No	2	178	7 (0·7–79)
Poultry meat			
Yes	4	113	
No	1	106	4 (0–185)
Sheep dairy products			
Yes	1	14	
No	4	205	3 (0·1–40)
Goat dairy products			
Yes	1	17	
No	4	202	3 (0·1–32)
Certified raw milk			
Yes	0	71	
No	5	148	0·0 (0·0–26)

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Table 1 (*cont.*)

Covariate	ESBL-E carrier		
	Yes ($n = 5$)	No ($n = 219$)	OR (95% CI)
Animal contact during preceding 7 days			
Pet rodents (e.g. hamster)			
Yes	3	36	
No	2	183	**8 (0·8–93)**
General animal contact			
Yes	4	152	
No	1	67	2 (0·2–89)
Farm animals			
Yes	2	31	
No	3	188	4 (0·3–36)
Pets			
Yes	4	130	
No	1	89	3 (0·3–135)
Cattle			
Yes	2	22	
No	3	196	6 (0·5–54)
Chicken			
Yes	1	22	
No	4	197	2 (0·1–23)
Other activities			
Sandbox			
Yes	5	207	
No	0	12	—
Travelling			
Yes	0	15	
No	5	209	0·0 (0·0–1·5)
Swimming			
Yes	0	72	
No	5	147	0·0 (0·0–11)

OR, Odds ratio; CI, confidence interval.
Statistically significant associations ($P < 0·05$) appear in bold.

severe symptoms, the child should stay at home until two stool samples test negative. If a child is diagnosed with *stx2*-producing STEC or other serotypes which are more likely to trigger HUS, readmission requires five consecutive negative tests [23]. Snedeker *et al.* [24] showed with an international systematic descriptive and statistical analysis (literature search and generalized linear models) that 19% of *E. coli* O157 outbreak cases were the result of secondary spread. Secondary outbreak cases were significantly younger (median age of <6 years *vs.* 6–16 or 17–59 years) than primary outbreak cases and the number of secondary cases via person-to-person transmission was statistically higher in nurseries than for home or other settings. It might be worth considering a similar approach in Germany, taking into account epidemiological characteristics, such as age of children and microbiological characteristic of the bacteria (*stx* type 1 and/or *stx* type 2, *eae* and sorbitol fermentation).

Prevalence of ESBL-E

Our finding of an ESBL-E prevalence of 2·3% (95% CI 0·3–4·2) in asymptomatic children in Northern Germany corresponds well with the results of other studies performed in Sweden (2·9%) [29], Portugal (2·7%) [30] and France (4·6%) [31].

Several exposures have been shown to increase the risk for ESBL-E carriage including foreign travel, having contact with pets [32], person-to-person transmission [16], hospital stay [33] and antibiotic treatment [12]. A number of studies found ESBL-producing *E. coli* in food production animals and companion animals [34–36].

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Livermore & Woodford [12] showed that intake of antibiotics is a risk factor for ESBL-E carriage. According to the European Antibiotic Resistance Surveillance System (EARS-Net [37]), the proportion of *E. coli* that are resistant to third-generation cephalosporins, an indicator of ESBL production, has been increasing across Europe during the last decade. In 2013, the population-weighted mean percentage for third-generation cephalosporin resistance in Europe was 12·6%, ranging from 5·0% (Iceland) to 39·6% (Bulgaria). In 2014, Germany reported a proportion of 12·6% of isolates resistant to third-generation cephalosporin (cefotaxim) [38]. The proportion for third-generation cephalosporin resistance in Lower Saxony was 11·1% for 2014 and has increased over the last years [39]. Our study showed that ESBL-E carriage was associated with antibiotic treatment in the preceding 6 months. The situation is worrying because of the high proportion (70%) of antibiotic prescription in children aged <5 years [14].

The high frequency of raw milk consumption and its positive association with ESBL-E carriage in asymptomatic children is notable. Raw milk is not heat-treated and may contain pathogens (e.g. *Campylobacter, Listeria, Mycobacterium bovis*, STEC, *Salmonella* or norovirus [40]). In Germany, the sale of raw milk is strongly restricted. Farms that are allowed to sell raw milk need a licence and must undergo strong quality control procedures. Besides this, farms are allowed to sell milk directly to persons in the neighbourhood if they explicitly advise the customers to boil the milk before consumption. However, to date there have been relatively few reports regarding ESBL-E in raw milk. Skočková *et al*. [41] evaluated the presence of ESBL-*E. coli* in bulk tank milk and found only two (0·7%) out of 270 isolates positive. Geser *et al*. [36] did not find ESBL-E in tank bulk raw milk, while Rasheed *et al.* [42] detected a higher proportion of drug-resistant *E. coli* in raw milk (6·7%, 2/30) but did not detect ESBL-*E. coli*.

In our study, we found that ESBL-E carriage was significantly associated with having contact with pet rodents. Knowledge about ESBL-E prevalence in pets is limited but a few studies showed that ESBL-producing *E. coli* strains are commonly found in dogs, cats, horses [43] and cattle [44]. Guenther *et al*. [45] reported that urban rats (16% out of 56) carried an ESBL-producing *E. coli* strain and might be a potential threat for humans acquiring ESBL-E, as the rats might contaminate the environment. Ho *et al.* [46] reported a carriage rate of ESBL-producing *E. coli* of 4·2% (out of 456 rodents) in wild rodents (unspecified). Taken together, these results indicate that pet rodents are a potential source for ESBL-E infection in humans.

Our study has some limitations. All identified potential risk factors should be considered with caution as our numbers are small and the confidence intervals of the corresponding odds ratios are wide. Moreover, there is the possibility of not identifying true risk factors due to small numbers of carriers. It is also possible that different risk factors exist for the three different Enterobacteria strains (e.g. *Klebsiella, Citrobacter, E. coli*). There might also be a selection bias in the recruitment of nursery children as we performed convenience sampling and provided the information material only in the German language.

CONCLUSION

In conclusion, the low STEC prevalence in asymptomatic children suggests that exclusion policies may successfully contribute to reducing transmission in nurseries. Nevertheless, a staged approach to exclusion of asymptomatic children should be considered based on their age and the found *stx* gene type in order to limit unnecessary socioeconomic impact. More research should be conducted on the effect of secondary transmission through asymptomatic carriers in nurseries. This study was performed to gain initial primary data for hypothesis generation.

Our study revealed a low prevalence of ESBL-E. It is, nevertheless, a matter of concern, since there is a reservoir of antibiotic-resistant genes within the community. Further epidemiological studies should focus on the characteristics of ESBL-E in pets and raw milk to gain a better understanding of these potential risk factors in asymptomatic children. Parents should be more intensively informed about the increased risk of foodborne illness from drinking raw milk especially for vulnerable groups, such as infants.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **O'Brien AD, *et al.*** Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. *Journal of Infectious Diseases* 1982; **146**: 763–769.
2. **Pitout JDD, Laupland KB.** Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infectious Diseases* 2008; **8**: 159–166.
3. **Bundesgesetzblatt.** Infectious disease control law in Germany 33 (Teil I-G5702): 1045–77 [in German] (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0025691).
4. **Frank C, *et al.*** Cattle density and Shiga toxin-producing *Escherichia coli* infection in Germany: increased risk for most but not all serogroups. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)* 2008; **8**: 635–643.
5. **Buchholz U, *et al.*** German outbreak of *Escherichia coli* O104: H4 associated with sprouts. *New England Journal of Medicine* 2011; **365**: 1763–1770.
6. **Wahl E, *et al.*** Investigation of an *Escherichia coli* O145 outbreak in a child day-care centre – extensive sampling and characterization of eae- and stx1-positive *E. coli* yields epidemiological and socioeconomic insight. *BMC Infectious Diseases* 2011; **11**: 238.
7. **Dabke G, *et al.*** Duration of shedding of verocytotoxin-producing *Escherichia coli* in children and risk of transmission in childcare facilities in England. *Epidemiology and Infection* 2014; **142**: 327–334.
8. **Kader AA, Kumar A, Kamath KA.** Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in patients and asymptomatic healthy individuals. *Infection Control and Hospital Epidemiology* 2007; **28**: 1114–1146.
9. **Valenza G, *et al.*** Extended-spectrum-β-lactamase-producing *Escherichia coli* as intestinal colonizers in the German community. *Antimicrobial Agents and Chemotherapy* 2014; **58**: 1228–1230.
10. **Geser N, *et al.*** Molecular identification of extended-spectrum-β-lactamase genes from Enterobacteriaceae isolated from healthy human carriers in Switzerland. *Antimicrobial Agents and Chemotherapy* 2012; **56**: 1609–1612.
11. **Luvsansharav U, *et al.*** Prevalence of fecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae among healthy adult people in Japan. *Journal of Infection and Chemotherapy* 2011; **17**: 722–725.
12. **Livermore DM, Woodford N.** The β-lactamase threat in Enterobacteriaceae, *Pseudomonas* and *Acinetobacter*. *Trends in Microbiology* 2006; **14**: 413–420.
13. **Schechner V, *et al.*** Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. *Clinical Microbiology Reviews* 2013; **26**: 289–307.
14. **Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Paul-Ehrlich-Gesellschaft für Chemotherapie e.V., James D.** Report on antibiotic consumption and the extent of resistance against antimicrobials in human and veterinary medicine, Germany, 2014 [in German].
15. **Coque TM, Baquero F, Canton R.** Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. *Eurosurveillance* 2008; **13**: 19044.
16. **Haller S, *et al.*** What caused the outbreak of ESBL-producing *Klebsiella pneumoniae* in a neonatal intensive care unit, Germany 2009 to 2012? Reconstructing transmission with epidemiological analysis and whole-genome sequencing. *BMJ Open* 2015; **5**: e007397.
17. **Thünen Institut.** German maps of agricultural use (beef, veal and agricultural area), 2010 [in German] (https://gdi.ti.bund.de/lr/agraratlas/indexMap.htm?LP=1).
18. **Ziehm D, *et al.*** Risk factors associated with sporadic salmonellosis in children: a case-control study in Lower Saxony, Germany (2008–2011). *Epidemiology and Infection* 2015; **143**: 687–694.
19. **Reischl U, *et al.*** Real-time fluorescence PCR assays for detection and characterization of Shiga toxin, intimin, and enterohemolysin genes from Shiga toxin-producing *Escherichia coli*. *Journal of Clinical Microbiology* 2002; **40**: 2555–2565.
20. **Vogelsang EPM.** Environmental studies of asymptomatic kindergarten children as carriers of enterohemorrhagic *Escherichia coli* (EHEC) in the Ammerland district (Germany). *Das Gesundheitswesen* 1999; **61**: 38–44.
21. **Dreesman J, Röttgers HR, Mellmann APM.** STEC outbreak with 59 cases after raw milk consumption in a summer camp [in German]. *Das Gesundheitswesen* 2007; **69**: 16–18.
22. **Niedersächsisches Landesgesundheitsamt (NLGA).** Screening of STEC in farmers in Lower Saxony 2012, unpublished raw data, 2012.
23. **MacDonald E, *et al.*** Implications of screening and child-care exclusion policies for children with Shiga-toxin producing *Escherichia coli* infections: lessons learned from an outbreak in a daycare centre, Norway, 2012. *BMC Infectious Diseases* 2014; **14**: 673.
24. **Snedeker KG, *et al.*** Primary and secondary cases in *Escherichia coli* O157 outbreaks: a statistical analysis. *BMC Infectious Diseases* 2009; **9**: 144.

25. **Al-Jader L, *et al.*** Outbreak of *Escherichia coli* O157 in a nursery: lessons for prevention. *Archives of Disease in Childhood* 1999; **81**: 60–63.
26. **Allaby MA, Mayon-White R.** *Escherichia coli* O 157: outbreak in a day nursery. *Communicable Disease Report. CDR Review* 1995; **5**: R4–6.
27. **Abu-Sin M, *et al.*** Carrier prevalence, secondary household transmission, and long-term shedding in 2 districts during the *Escherichia coli* O104: H4 outbreak in Germany, 2011. *Journal of Infectious Diseases* 2013; **207**: 432–438.
28. **Norwegian Institute of Public Health.** *Infection Control Book* [in Norwegian], 2009.
29. **Kaarme J, *et al.*** Prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in healthy Swedish preschool children. *Acta Paediatrica* 2013; **102**: 655–660.
30. **Guimaraes B, *et al.*** Genetic detection of extended-spectrum beta-lactamase-containing *Escherichia coli* isolates and vancomycin-resistant enterococci in fecal samples of healthy children. *Microbial Drug Resistance (Larchmont, N.Y.)* 2009; **15**: 211–216.
31. **Birgy A, *et al.*** Community faecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae in french children. *BioMed Central* 2012; **12**: 315.
32. **Meyer E, *et al.*** Pet animals and foreign travel are risk factors for colonisation with extended-spectrum β-lactamase-producing *Escherichia coli*. *Infection* 2012; **40**: 685–687.
33. **Tham J, *et al.*** Risk factors for infections with extended-spectrum beta-lactamase-producing *Escherichia coli* in a county of Southern Sweden. *Infection and Drug Resistance* 2013; **6**: 93–97.
34. **Valentin L, *et al.*** Subgrouping of ESBL-producing *Escherichia coli* from animal and human sources: an approach to quantify the distribution of ESBL types between different reservoirs. *International Journal of Medical Microbiology* 2014; **304**: 805–816.
35. **Eller C, *et al.*** Extended-spectrum beta-lactamase (ESBL) in *Escherichia coli* in hospitals and outpatient sectors and general population [in German]. Abstract book, p. 10. German Medical Science GMS Publishing House, 2013.
36. **Geser N, Stephan R, Hächler H.** Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Veterinary Research* 2012; **8**: 21.
37. **European Centre for Disease Prevention and Control (ECDC).** Surveillance Report: Antimicrobial resistance surveillance in Europe 2013 (http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2013.pdf). Accessed 18 August 2015.
38. **Robert Koch-Institut (RKI).** Antimicrobial resistance surveillance in Germany [in German] (https://ars.rki.de). Accessed 19 August 2015.
39. **Niedersächsisches Landesgesundheitsamt (NLGA).** ARMIN. Antimicrobial resistance monitoring in Lower Saxony [in German] (http://www.armin.nlga.niedersachsen.de). Accessed 19 August 2015.
40. **Headrick M, *et al.*** The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. *American Journal of Public Health* 1998; **88**: 1219–1221.
41. **Skočková A, *et al.*** Antimicrobial-resistant and extended-spectrum β-lactamase-producing *Escherichia coli* in raw cow's milk. *Journal of Food Protection* 2015; **78**: 72–77.
42. **Rasheed MU, *et al.*** Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. *Revista do Instituto de Medicina Tropical de São Paulo* 2014; **56**: 341–346.
43. **Ewers C, *et al.*** Emergence of human pandemic O25: H4-ST131 CTX-M-15 extended-spectrum-lactamase-producing *Escherichia coli* among companion animals. *Journal of Antimicrobial Chemotherapy* 2010; **65**: 651–660.
44. **Schmid A, *et al.*** Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* on Bavarian dairy and beef cattle farms. *Applied and Environmental Microbiology* 2013; **79**: 3027–3032.
45. **Guenther S, *et al.*** Is fecal carriage of extended-spectrum-β-lactamase-producing *Escherichia coli* in urban rats a risk for public health? *Antimicrobial Agents and Chemotherapy* 2013; **57**: 2424–2425.
46. **Ho PL, *et al.*** Extensive dissemination of CTX-M-producing *Escherichia coli* with multidrug resistance to 'critically important' antibiotics among food animals in Hong Kong, 2008–10. *Journal of Antimicrobial Chemotherapy* 2011; **66**: 765–768.