Clinical Microbiology and Infection 23 (2017) 120.e1–120.e8

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Extended-spectrum β-lactamase- and pAmpC-producing Enterobacteriaceae among the general population in a livestock-dense area

C.C.H. Wielders [1, *], A.H.A.M. van Hoek [1], P.D. Hengeveld [1], C. Veenman [1], C.M. Dierikx [1], T.P. Zomer [1], L.A.M. Smit [2], W. van der Hoek [1], D.J. Heederik [2], S.C. de Greeff [1], C.B.M. Maassen [1], E. van Duijkeren [1]

[1] Centre for Infectious Disease Control (Clb), National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[2] Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form
3 October 2016
Accepted 13 October 2016
Available online 20 October 2016

Editor: P. T. Tassios

Keywords:
AmpC
Antimicrobial resistance
Environment
Extended-spectrum β-lactamases
β-lactam resistance
Livestock farming
Prevalence
Risk factors

ABSTRACT

Objectives: In the Netherlands there is an ongoing debate regarding environmental health risks of livestock farming for neighbouring residents. This explorative study aims to determine the prevalence of carriage of extended-spectrum β-lactamase and/or plasmid-mediated AmpC-producing Enterobacteriaceae (ESBL/pAmpC-E) in the general population living in a livestock-dense area, and to study associations between determinants, including exposure through contact with animals and the environment, and human carriage of ESBL/pAmpC-E.

Methods: A cross-sectional study was performed among 2432 adults (aged 20–72 years) in 12 temporary research centres in the south of the Netherlands, consisting of a questionnaire and analysis of a faecal sample to assess carriage of ESBL/pAmpC-E. Risk factors were analysed using logistic regression.

Results: The prevalence for carriage of ESBL/pAmpC-E was 4.5% (109/2432; 95% CI 3.7–5.4) ranging from 1.4% to 10.9% among the research centres. ESBL/pAmpC resistance genes were detected in *Escherichia coli* and *Klebsiella pneumoniae* isolates obtained from these 109 persons and the most common ESBL-resistance genes were $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14/17}}$ and $bla_{\text{CTX-M-1}}$, originating from 76 participants. Travel in the previous 12 months to Africa, Asia or Latin America (OR 2.82; 95% CI 1.71–4.63), having kept cows for a hobby in the previous 5 years (OR 3.77; 95% CI 1.22–11.64), usage of proton-pump inhibitors (OR 1.84; 95% CI 1.05–3.23), and living within 1000 m of a mink farm (OR 2.26; 95% CI 1.28–3.98) were identified as risk factors. Exposure to poultry was not identified as a risk factor.

Conclusions: Overall, living in close proximity to livestock animals and farms does not seem to be a risk factor for carriage of ESBL/pAmpC-E. **C.C.H. Wielders, CMI 2017;23:120.e1–120.e8**

© 2016 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Introduction

Enterobacteriaceae that produce extended-spectrum β-lactamases (ESBLs) and/or plasmid-mediated AmpC (pAmpC) are an important reason for therapy failure with β-lactam antibiotics [1]. ESBL/pAmpC-producing Enterobacteriaceae (ESBL/pAmpC-E) were initially only observed in human health care [2,3], but they are increasingly detected in the community [2,4–8] as well as in companion animals [9,10], livestock [2–4,9,11,12] and meat [2,3,13]. Potential routes of transmission of ESBL/pAmpC-E to humans are via the food chain [2,3], by direct contact with animals [4] or indirectly via the environment [11,14].

In the Netherlands there is a debate regarding the environmental health risks of livestock farming. Neighbouring residents are concerned about these potential health risks. In the Netherlands the animal farm density is the highest in the world and also the population density is one of the highest [15]: on a surface of 41 000 km^2 live 17 million people together with 107 million chickens, 12 million pigs, 4 million cows, 1.5 million goats and sheep, and 1 million mink [16] (Fig. 1 and Supplementary material, Fig. S1).

* Corresponding author. C.C.H. (Lieke) Wielders, Centre for Infectious Disease Control (Clb), National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands
E-mail address: lieke.wielders@rivm.nl (C.C.H. Wielders).

http://dx.doi.org/10.1016/j.cmi.2016.10.013
1198-743X/© 2016 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

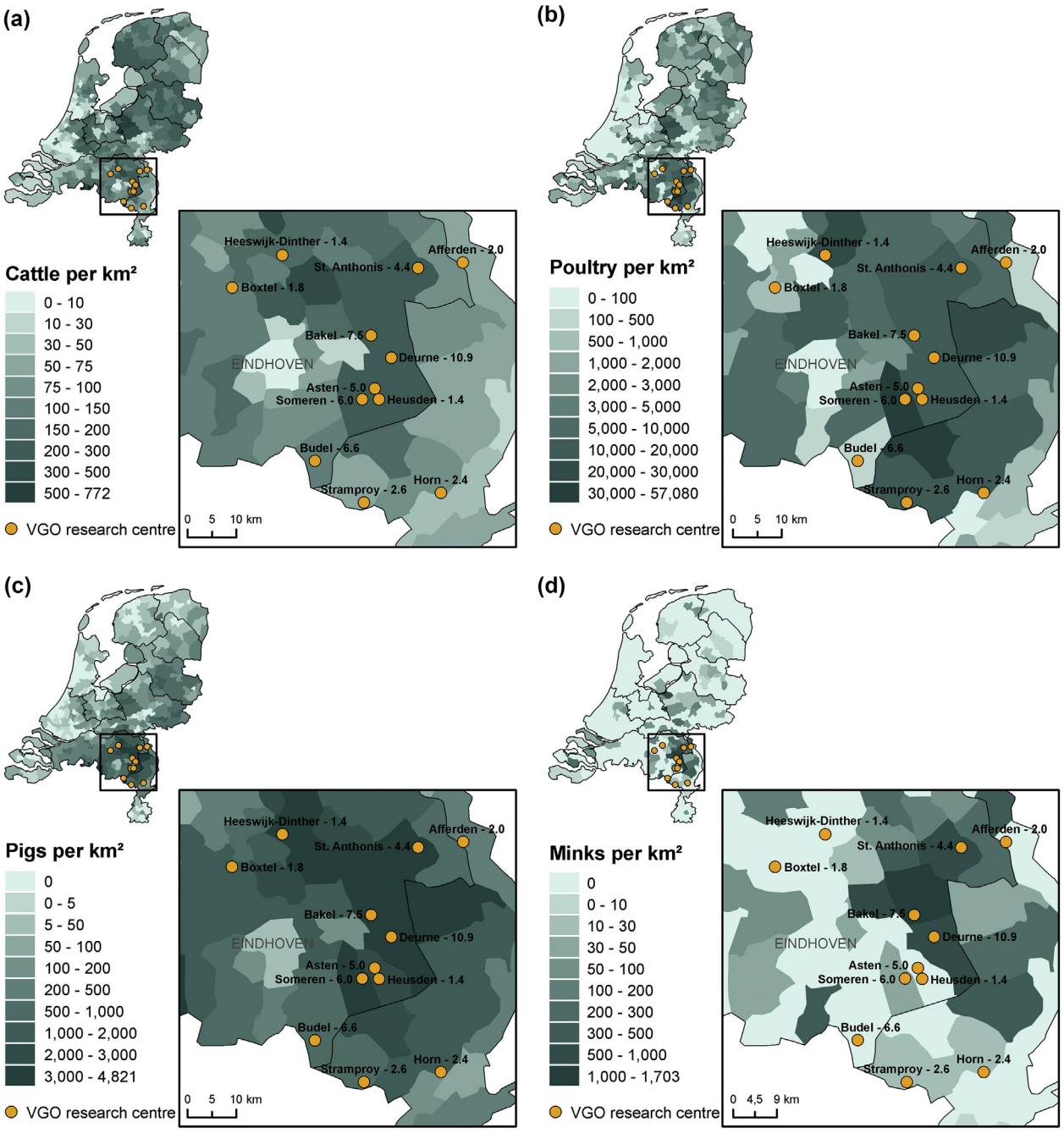

Fig. 1. Map with the locations of the twelve temporary VGO research centres (located in the provinces Noord-Brabant and Limburg) and the density of livestock on municipality level for cattle (a), poultry (b), pigs (c) and mink (d). The numbers behind the names of the research centre show the observed extended-spectrum β-lactamase and/or plasmid-mediated AmpC (ESBL/pAmpC-E) prevalence. Data source of livestock densities: the annual agricultural census (*Landbouwtelling*), 2014, Statistics Netherlands.

Enteric bacteria are introduced into the environment with human and animal faeces and residents of rural areas may be exposed to ESBL/pAmpC-E through the air, through contact with contaminated surface water or soil, or through consumption of home grown fresh produce [14]. Usually, people working on a farm have a higher carriage rate of ESBL/pAmpC-E than the general population [4,6]. Although several studies hypothesize that exposure to ESBL/pAmpC-E is elevated in close proximity around animal farms [17,18], the risk of this exposure is not clear [14].

Previous studies on the carriage of ESBL-producing bacteria in the Dutch general population found prevalences of 5.1% and 8.6% [5,7]. The latter estimate was found in an urban setting [7], whereas the 5.1% prevalence was observed in the general population living in high- and low-density poultry areas. No increased risk was found for the population in the high-density area [5], but the average distance to the nearest broiler farm was relatively large. The present study focused on various kinds of livestock animals and the average distance of a substantial part of the participants to livestock farms was considerably shorter, contributing to higher discriminatory power of the study.

The aim of the present explorative cross-sectional study was first, to determine the prevalence of carriage of ESBL/pAmpC-E in the general population living in a livestock-dense area, and second, to study associations between determinants, including exposure

through contact with animals and the environment, and human carriage of ESBL/pAmpC-E in the same population.

Methods

Study population

This study is part of the Livestock Farming and Neighbouring Residents' Health study (Dutch acronym: VGO). The methodology is described in detail by Borlée *et al.* [19]. Participants were selected in a two-step procedure. First, participants were recruited via their general practitioner (GP) through a short questionnaire survey in November 2012; eligible participants were 18–70 years old and were living in a municipality with <30 000 inhabitants in the eastern part of the province of Noord-Brabant or northern part of the province of Limburg. One person per home address was randomly selected. Second, people who indicated that they were willing to participate in further studies, and who were not working or living on a livestock farm, were invited for the present cross-sectional population-based study, which was conducted between March 2014 and February 2015. Twelve temporary research centres were established (Fig. 1). People were only invited if they lived within 10 km of one of the research centres.

Participants had to fill out a detailed questionnaire including items on demographics, hospitalization, profession (history), current and past animal contact (pets/farm animals), and travel, and bring it to the research centre. Current medication was registered during the research centre visit. Previous use of antibiotics (according to the Anatomical Therapeutic and Chemical classification) and co-morbidities were available from the GP electronic medical records through the NIVEL Primary Care Database [20]. These GP data were only included when the GP registered prescriptions and morbidity (International Classification of Primary Care codes) for ≥46 weeks during the calendar year and if the patient was registered at the particular GP for at least three-quarters of the year. A faecal sample was taken by the participants themselves and was sent to the laboratory by regular mail.

The medical ethics committee of the University Medical Centre Utrecht, Utrecht, the Netherlands (NL), approved the VGO study (number 13/533). All participants signed informed consent.

Environmental exposure (livestock farms)

Based on the participants' home address, several exposure variables were computed using a geographic information system (ArcGis 10.1; Esri, Redlands, CA, USA) [21]. These included distance to nearest farm (irrespective of animal type and specifically for cattle, goat, horse, mink, pig, poultry and sheep farms), and the presence of a specific type of livestock farm, the total number of specific farm animal species, and number of farms within 500 or 1000 metres of the residential address. Farm characteristic information (type and number of farm animals and geographic coordinates) was derived from the provincial databases of mandatory environmental licenses for keeping livestock for 2012.

Laboratory tests

To determine the presence of ESBL/pAmpC-E, faecal samples were incubated overnight in selective enrichment broth (Luria–Bertani broth; MP Biomedicals, Amsterdam, NL), supplemented with 1 mg/L cefotaxime (Sigma-Aldrich, Zwijndrecht, NL) and isolated on MacConkey no. 3 supplemented with 1 mg/L cefotaxime (MacConkey$^+$; Oxoid, Badhoevedorp, NL). When growth was visible, the selective enrichment was also cultured on Brilliance™ *E. coli*/Coliform Selective Agars (BECSA$^+$; Oxoid). Five colonies per person, depending on the total number of colonies and their diversity in morphology, were selected and tested for oxidase production (BBL Dryslide Oxidase; Becton Dickinson BV, Breda, NL). All oxidase-negative isolates were analysed for ESBL/pAmpC-production. Morphologies on both MacConkey$^+$ and BECSA$^+$ from oxidase-negative isolates were used to detect *E. coli*. Non-*E. coli* isolates were further analysed by API® (bioMérieux Benelux BV, Zaltbommel, NL). Phenotypical detection of ESBL/pAmpC production was done by combination disc-diffusion test according to CLSI guidelines [22]. Phenotypically confirmed ESBL and/or pAmpC-producing *E. coli* or *Klebsiella* isolates were screened for the presence of CTX-M and/or CMY and DHA β-lactamase genes, and if negative for OXA, SHV and TEM in PCR tests as described earlier [23] or for ACC-, ACT-, FOX-, MIR-, MOX-genes by microarray (Check-MDR CT101; Checkpoints, Wageningen, NL)). Isolates from other Enterobacteriaceae species were only analysed further if they displayed an ESBL phenotype. If they displayed an AmpC phenotype that is normal for the species concerned (*Enterobacter* spp., *Citrobacter freundii*, *Hafnia alvei*, *Morganella morganii*) it was considered as chromosomal resistance and these isolates were excluded from further analysis. The complete ESBL and/or pAmpC gene sequence (one per person, depending on the multiplex PCR results) was determined as described by van Hoek *et al.* [24]. A faecal sample was considered positive for ESBL/pAmpC when at least one Enterobacteriaceae isolate was cultured in which an ESBL and/or pAmpC gene was found.

Statistical analysis

The overall prevalence with a 95% exact mid-p confidence interval (95% CI) of ESBL/pAmpC-E was calculated.

For the environmental exposure analysis, the median distance to livestock farms (irrespective of animal type and animal specific) between participants testing positive and negative for carriage of ESBL/pAmpC-E were compared using the Mann–Whitney *U* test (corrected for ties). The number of farm animals (cattle, goats, horses, mink, pigs, poultry and sheep) and farms within 1 000 m of the home address were categorized and were analysed with a chi-square test (Fisher's exact test when the expected count was <5) and a chi-square test for linear trend.

Logistic regression analysis was performed to study potential risk factors for carriage of ESBL/pAmpC-E, and ORs with 95% CIs were obtained. Univariate analyses were performed for potential risk factors being gender, age, educational level, birth country, hospitalization, specific diet, smoking status, use of antibiotics and proton-pump inhibitors (PPIs), co-morbidities, childhood spent in the study region or on a farm, performing jobs on a farm, contact with animals during work/study, keeping pets/farm animals for a hobby, travel history, farm visit with/without farm animal contact, and living within 1000 m of a specific farm type. Variables with a p-value <0.20 (chi-square test or Fisher's exact test) and gender and age were included in a multiple logistic regression analysis. Adjusted ORs and 95% CIs were calculated for cases without missing values ($n = 2176$) and a p-value <0.05 was used to determine significance. In addition, the Benjamini–Hochberg procedure with a 10% false discovery rate was used to correct for the number of univariate tests performed [25]. Data were analysed using IBM SPSS Statistics version 22.0.0.0. (IBM Corp., Armonk, NY, USA).

Results

Of the 7180 invited persons, 2494 participated in the study (response rate: 34.7%). ESBL/pAmpC test results were available for 2432 participants (97.5%): median age 59 years (range 20–72 years; interquartile range 49–66) and 45.2% were male. Overall

prevalence of ESBL/pAmpC-E was 4.5% (109/2432; 95% CI 3.7–5.4), ranging from 1.4% among participants at research centres in Heusden (95% CI 0.1–6.7) and Heeswijk-Dinther (95% CI 0.5–3.1) to 10.9% (95% CI 6.4–17.3) in Deurne (Table 1).

ESBL/pAmpC resistance genes

Enterobacteriaceae with an ESBL/pAmpC resistance gene were isolated from 109 participants: 102 carried *E. coli*, five carried *Klebsiella pneumoniae*, and two harboured both *E. coli* and *K. pneumoniae* (see Supplementary material, Table S1). The most common ESBL-resistance genes were $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14/17}}$ and $bla_{\text{CTX-M-1}}$, found in samples originating from 46, 19 and 13 participants, respectively. pAmpC genes were found in ten persons (nine $bla_{\text{CMY-2}}$ and one $bla_{\text{DHA-1}}$).

Environmental exposure (livestock farms)

No relation was found between the distance of the residential address to the nearest farm (irrespective of the animal species) and carriage of ESBL/pAmpC-E (p 0.806) (Table 2). However, carriers lived on average farther from goat farms than non-carriers (p <0.001), as opposed to mink farms, which were located closer to carriers (p <0.001). In addition, there was no difference in the average number of farms within 500 and 1000 m of the residential address between carriers and non-carriers (one farm within 500 m, p 0.492, and nine farms within 1000 m, p 0.520), irrespective of the type of farming. Likewise, there was no clear association between number of animals and specific farm types within 1000 m and carriage of ESBL/pAmpC-E (see Supplementary material, Table S2). Only for mink and mink farms there seemed to be a possible association consistent over the different variables assessed—distance (Table 2), number of animals and farms (see Supplementary material, Table S2) and presence within 1000 m (Table 3). Although there was no significant relation between the distance to a pig farm and carriage (p 0.102; Table 2), there seemed to be an association with living nearby a large or several pig farms (see Supplementary material, Table A2). The number of goats showed a non-linear association with carriage of ESBL-E. Poultry and poultry farms near the residential address did not show any relationship with carriage.

Table 1
Prevalence of ESBL/pAmpC-producing Enterobacteriaceae (*Escherichia coli*/*Klebsiella pneumoniae*) per research centre among people living near livestock farms in the Netherlands ($n = 2432$)

Research centre[a]	Number of participants	Number of participants with ESBL/pAmpC-producing Enterobacteriaceae detected	Prevalence of ESBL/pAmpC (%)	95% CI
Afferden	49	1	2.0	0.1–9.7
Asten	282	14	5.0	2.9–8.0
Bakel	308	23	7.5	5.0–10.8
Boxtel[b]	167	3	1.8	0.5–4.8
Budel	197	13	6.6	3.7–10.7
Deurne[c]	128	14	10.9	6.4–17.3
Heeswijk-Dinther[b]	357	5	1.4	0.5–3.1
Heusden	72	1	1.4	0.1–6.7
Horn	84	2	2.4	0.4–7.6
Someren	166	10	6.0	3.1–10.5
St. Anthonis	389	17	4.4	2.7–6.8
Stramproy[b]	233	6	2.6	1.1–5.3
Total[b]	***2,432***	***109***	***4.5***	***3.7–5.4***

ESBL/pAmpC, extended-spectrum β-lactamase and/or plasmid-mediated AmpC.
[a] The locations of the research centres in the Netherlands are shown in Fig. 1.
[b] Significantly lower prevalence of ESBL/pAmpC-E than observed in Deurne.
[c] Research centre Deurne versus all other centres: OR 2.86; 95% CI 1.58–5.16.

Table 2
Distance from residential address to the nearest farm and carriage of ESBL/pAmpC-producing Enterobacteriaceae (*Escherichia coli*/*Klebsiella pneumoniae*) among people living near livestock farms in the Netherlands ($n = 2432$)

Determinant	ESBL/pAmpC-positive persons ($n = 109$)	ESBL/pAmpC-negative persons ($n = 2323$)	p-value[a]
	Median (IQR)	Median (IQR)	
Distance to the nearest farm in metres			
All farm types combined	363 (278–549)	402 (241–585)	0.806
Cattle farm (≥5 cows)	484 (314–687)	482 (305–671)	0.743
Goat farm (≥50 goats)	3258 (2207–4106)	2465 (1608–3482)	<0.001
Horse farm (≥5 horses)	756 (509–1000)	761 (522–1061)	0.577
Mink farm (≥400 mink)	2696 (1354–4828)	3958 (2019–6018)	<0.001
Pig farm (≥25 pigs)	616 (421–862)	691 (466–938)	0.102
Poultry farm (≥250 chickens)	977 (598–1261)	928 (644–1311)	0.961
Sheep farm (≥50 sheep)	1451 (937–1916)	1281 (874–1841)	0.177

ESBL/pAmpC, extended-spectrum β-lactamase and/or plasmid-mediated AmpC; IQR, inter-quartile range.
[a] Mann–Whitney *U* test corrected for ties

Risk factor analysis

Statistically significant risk factors in univariate logistic regression analysis were: travel in the previous 12 months to Africa, Asia or Latin America; not having been raised in the study area; PPI usage; having kept cows for a hobby during the previous 5 years; and living within 1000 m of at least one mink farm (Table 3). All five univariate risk factors except place of residence during childhood remained statistically significantly associated in the multiple logistic regression analysis. The Benjamini–Hochberg procedure showed that travelling, PPI usage and living close to mink farms were significant determinants. When adjusting the results from the environmental exposure analysis for travel, having kept cows and PPI usage, the results were similar: only the number of pigs within 1000 m was no longer statistically significant (data not shown). Usage of antibiotics (data available for $n = 2032$ participants of whom 80 were carriers) during the last 3 or 6 months was not statistically significantly associated with carriage (Table 3).

The risk factor and environmental exposure analyses were repeated for the three most commonly detected genotypes ($bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14/17}}$, $bla_{\text{CTX-M-1}}$; $n = 76$) and genes associated with poultry ($bla_{\text{CMY-2}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$; $n = 31$), but results similar to those described above were found, and there was no association with living near or contact with poultry.

Discussion

An increased environmental exposure due to livestock did not cause a higher prevalence of carriage of ESBL/pAmpC-E in the population: the prevalence of 4.5% (95% CI 3.7–5.4) is comparable to the prevalence previously reported in the general population living in areas with high- as well as low-broiler densities [5], but lower than the prevalence observed among the general population in Amsterdam, although this urban population had a higher travel frequency, which may explain the difference [7]. The most common ESBL genes detected were $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14/17}}$ and $bla_{\text{CTX-M-1}}$,

Table 3
Univariate and multiple logistic regression analyses of determinants for ESBL/pAmpC-producing Enterobacteriaceae (*Escherichia coli*/*Klebsiella pneumoniae*) carriage among people living near livestock farms in the Netherlands ($n = 2432$)

Determinant	Total number		Prevalence of persons carrying isolates with ESBL/pAmpC-E	ESBL/pAmpC-positive persons ($n = 109$)	ESBL/pAmpC-negative persons ($n = 2323$)	Univariate odds ratio	Multivariate odds ratio ($n = 2176$ of whom 94 ESBL/pAmpC-positive cases)
	n	Missing	%	*n* (%)	*n* (%)	OR (95% CI)	OR (95% CI)
Demographics							
Gender							
Female	1332		4.3	57 (52.3)	1275 (54.9)	Ref.	Ref.
Male	1100		4.7	52 (47.7)	1048 (45.1)	1.11 (0.76–1.63)	1.05 (0.67–1.63)
Age							
20–29 years	50		6.0	3 (2.8)	47 (2.0)	-	
30–39 years	160		3.8	6 (5.5)	154 (6.6)	-	
40–49 years	431		3.2	14 (12.8)	417 (18.0)	-	
50–59 years	666		3.9	26 (23.9)	640 (27.6)	-	
≥60 years	1125		5.3	60 (55.0)	1065 (45.8)	-	
Age increase per 10 years	-		-	-	-	1.12 (0.93–1.35)	1.10 (0.89–1.38)
Country of birth		17					
The Netherlands	2354		4.5	105 (96.3)	2249 (97.5)	Ref.	
Other	61		6.6	4 (3.7)	57 (2.5)	1.50 (0.54–4.22)	
Place of residence during youth not in the study area	580	26	6.4	37 (33.9)	543 (23.6)	**1.66 (1.10–2.50)**	1.12 (0.67–1.83)
Educational level[a]							
Low	623		4	25 (22.9)	598 (25.7)	0.85 (0.50–1.44)	
Medium	1086		4.6	50 (45.9)	1036 (44.6)	0.98 (0.63–1.53)	
High	723		4.7	34 (31.2)	689 (29.7)	Ref.	
Health							
Smoking							
Never smoked	1024		4.0	41 (37.6)	983 (42.3)	Ref.	
Current or ex-smoker	1408		4.8	68 (62.4)	1340 (57.6)	1.22 (0.82–1.81)	
Co-morbidity[b]	412	400	4.6	19 (23.8)	393 (20.1)	1.24 (0.73–2.09)	
Antibiotic use during last 3 months	98	400	4.1	4 (5.0)	94 (4.8)	1.04 (0.37–2.90)	
Antibiotic use during last 6 months	211	400	4.7	10 (4.7)	201 (10.3)	1.24 (0.63–2.45)	
Proton-pump inhibitor use (current)	288	18	8.0	23 (21.3)	265 (11.5)	**2.08 (1.29–3.36)***	**1.84 (1.05–3.23)**
Hospitalized during last 12 months[c]	294	21	4.4	13 (11.9)	281 (12.2)	0.97 (0.54–1.76)	
Specific diet (without meat or fish or animal products)	135	13	4.4	6 (5.5)	129 (5.6)	0.99 (0.43–2.29)	
Travel							
Travel during last 12 months		28					
No travel, travel to Western/Northern Europe, North America, Australia or New Zealand	1348		3.6	49 (45.0)	1299 (56.6)	Ref.	Ref.
Travel to Southern/Eastern Europe	712		4.1	29 (26.6)	683 (29.8)	1.13 (0.71–1.80)	1.12 (0.67–1.89)
Travel to Africa, Asia (West/ South/ Southeast/ East/ Central, including Turkey) or Latin America	344		9.0	31 (28.4)	313 (13.6)	**2.63 (1.65–4.19)***	**2.91 (1.73–4.76**)
Exposure at work/study/home							
During work/study contact with patients	282	123	5.0	14 (14.0)	268 (12.1)	1.18 (0.66–2.10)	
During work/study contact with residents of nursing homes	362	123	4.4	16 (16.0)	346 (15.7)	1.02 (0.60–1.77)	
During work/study contact with children	293	123	3.1	9 (9.0)	284 (12.9)	0.67 (0.33–1.35)	
During work/study contact with animals	143	123	7.0	10 (10.0)	133 (6.0)	1.73 (0.88–3.41)	1.90 (0.92–3.93)
Lived on a farm during childhood	821	24	3.7	30 (27.5)	791 (34.4)	0.72 (0.47–1.11)	0.72 (0.40–1.28)
Performed jobs on a farm during childhood	1226	110	3.9	48 (46.6)	1178 (53.1)	0.77 (0.52–1.15)	0.91 (0.54–1.52)
Had holiday on a farm during childhood	1151	65	4.4	51 (47.7)	1100 (48.7)	0.96 (0.65–1.42)	

Table 3 (*continued*)

Determinant	Total number		Prevalence of persons carrying isolates with ESBL/pAmpC-E	ESBL/pAmpC-positive persons ($n = 109$)	ESBL/pAmpC-negative persons ($n = 2323$)	Univariate odds ratio	Multivariate odds ratio ($n = 2176$ of whom 94 ESBL/pAmpC-positive cases)
	n	Missing	%	*n* (%)	*n* (%)	OR (95% CI)	OR (95% CI)
Kept pets during the last 5 years[d]	1255	17	4.6	58 (53.2)	1197 (51.9)	1.05 (0.72–1.55)	
Kept a dog	785	23	5.5	43 (39.4)	742 (32.3)	1.37 (0.92–2.03)	1.41 (0.90–2.20)
Kept farm animals for a hobby during the last 5 years[e]	439	34	4.3	19 (17.9)	420 (18.3)	0.97 (0.59–1.62)	
Kept pigs	18	45	0	0 (0.0)	18 (0.8)	NA	
Kept cows	32	45	12.5	4 (3.8)	28 (1.2)	**3.19 (1.10–9.26)**	**3.56 (1.12–11.34)**
Kept horses	149	44	6.7	10 (9.5)	139 (6.1)	1.62 (0.83–3.19)	1.13 (0.50–2.55)
Kept poultry	317	44	3.8	12 (11.4)	305 (13.4)	0.84 (0.45–1.55)	
Visit to a farm last 12 months	1512	13	3.9	59 (54.1)	1453 (62.9)	0.70 (0.47–1.02)	0.72 (0.46–1.14)
Contact with animals during farm visit	773	43	4.4	34 (31.5)	739 (32.5)	0.96 (0.63–1.45)	
Environmental exposure (livestock farms)							
Living within 1000 m of one or more farms (based on environmental license)							
All farm types combined	2332		4.5	106 (97.2)	2226 (95.8)	1.54 (0.48–4.94)	
Cattle farm(s)	2277		4.6	104 (95.4)	2173 (93.5)	1.44 (0.58–3.58)	
Goat farm(s)	167		2.4	4 (3.7)	163 (7.0)	0.51 (0.18–1.39)	0.40 (0.12–1.29)
Horse farm(s)	1604		4.7	76 (69.7)	1528 (65.8)	1.20 (0.79–1.82)	
Mink farm(s)	225		8.4	19 (17.4)	206 (8.9)	**2.17 (1.30–3.63)***	**2.26 (1.28–3.98)**
Pig farm(s)	1733		4.8	84 (77.1)	1649 (71.0)	1.37 (0.87–2.17)	1.37 (0.83–2.26)
Poultry farm(s)	1241		4.1	51 (46.8)	1190 (51.2)	0.84 (0.57–1.23)	
Sheep farm(s)	415		3.6	15 (13.8)	400 (17.2)	0.77 (0.44–1.34)	

ESBL/pAmpC, extended-spectrum β-lactamase and/or plasmid-mediated AmpC; GP, general practitioner; NA, not applicable; Ref, reference.

* Result remained statistically significant after performing the Benjamini–Hochberg procedure with a 10% false discovery rate [25].

[a] Education level: Low, no education, primary school or preparatory vocational education; Medium, secondary school or intermediate vocational education; High, higher vocational education or university level.

[b] Co-morbidity includes cerebrovascular disease, chronic cardiovascular disease, liver disease, chronic lung disease, chronic renal disease, autoimmune disease, neurological co-morbidity, diabetes and malignancy.

[c] Hospitalized in the Netherlands and/or abroad.

[d] Bird, cat, dog, fish, guinea pig, hamster, mouse, rabbit, rat, or turtle.

[e] Chicken, cow, donkey, duck, goat, goose, horse, pig, pony, sheep or turkey.

which is in accordance with other recent population-based studies in the Netherlands [7,24].

In general, the assessed proxy variables for environmental exposure to livestock animals and farms were not a risk factor for carriage of ESBL/pAmpC-E. The risk related to pig farms was inconsistent: although there was no significant relation between the distance to a pig farm and carriage, there seemed to be an association with living near to a large or several pig farms, suggesting that there may be a dose–response relationship. An unexpected finding was the observed association with exposure to mink and mink farms. Literature regarding resistant bacteria and antibiotic use in mink is scarce [26]. No data are available concerning antibiotic use in mink in the Netherlands, but it is the only animal species where antibiotics are still administered via medical food prescribed by the veterinarian (i.e. on attest). In contrast to the other types of animals studied, mink are carnivores; they eat raw residual products from the poultry and fish industry at least once a day [27]. Given the fact that ESBL/pAmpC-E have been frequently found in poultry [2,4,6,17], it is likely that mink carry ESBL/pAmpC-E. It is still unclear if, and how, the presence of mink leads to an increased risk for carriage in the community. We observed the lowest mink density in the municipalities with a low ESBL/pAmpC-E prevalence (Fig. 1). It might be that other factors, which were not assessed, are also associated with ESBL/pAmpC-E carriage.

Multiple logistic regression analysis showed that travel in the previous 12 months to Africa, Asia or Latin America is a risk factor for carriage of ESBL/pAmpC-E, which is in agreement with findings of others [7,28,29]. The findings of Reuland *et al.* showing that the use of PPIs is a risk factor were confirmed [7]. In the present study, previous use of antibiotics and close contact with horses were not confirmed as being risk factors, although these determinants have been identified as risk factors in other studies [5,30].

To justify the observed increased prevalence in Deurne, the available variables were assessed to find any differences in determinants between Deurne and the other research centres, but no explanation was found. In addition, a binary variable 'Deurne

versus other centres' was added to the multivariable model to check if any of the significant risk factors became non-significant after correcting for the high rate in Deurne. However, this did not affect the results either. Therefore, we have to conclude that this finding of the higher rate in Deurne is a coincidence or that another carriage rate increasing factor is involved, which was not measured in the present study.

People who worked or lived on a farm were excluded from this study. However, it is already known that broiler and pig farmers and their family members have a higher prevalence of ESBL/pAmpC carriage, and contact with animals was identified as risk factor in two studies [4,6,12]. Prevalence was 27%–33% for broiler farmers [4,6] and 13% for persons living or working on ESBL/pAmpC-positive pig farms [12].

The distances of 500 and 1000 m between farms and residential address were chosen to be consistent throughout the different studies that were performed within the VGO project and to facilitate communication of research results to policy-makers. Analyses with distances <500 m would often result in analyses with very small numbers, leading to inaccurate and non-significant results. Therefore, we did not include any analyses with smaller distances.

With the large sample size and the objective assessment of the presence of livestock farms at the individual level, a robust estimate of the prevalence of carriage in relation to living in a livestock-dense area was possible. Such a large study among the general population in the Netherlands and carriage of ESBL/pAmpC-E has not been performed before.

Limitations

We lacked information on the differences in animal housing systems, type of farm (closed or open farm, breeding or fattening farm, animals kept indoors or outdoors), management on the farm, such as ventilation and manure handling systems, and practices of land application of manure that may affect local exposure levels. There are large differences in the housing systems of livestock: pigs and poultry are usually kept indoors, although some poultry farms have open-air areas; cattle are kept in somewhat open barns and most farms let their animals graze in the meadow during daytime, at least for a part of the year; goats are usually housed within fairly open stalls; sheep are kept outside; and mink live in open cages. Besides the housing systems, the usage of manure is also an important factor. Manure is, depending on the animal type, spread out on the land or processed otherwise, and may also cause environmental stresses of gases and microorganisms. In addition, it was unknown which farms were ESBL/pAmpC-positive. In the Netherlands, in the period just before this study (2009–2011), 100% of broiler farms [4,6] and 45% of pig farms were ESBL/pAmpC-positive [12], but for other types of farms, this prevalence is unknown. Finally, selection bias may have occurred due to the two-step selection process of participants. Responders were in general older, more often women and lived closer to livestock farms than non-responders (data not shown).

Conclusions

In conclusion, the overall prevalence of 4.5% for carriage of ESBL/pAmpC-E in a livestock-dense area was not higher than previous estimates in the general population, suggesting that living in close proximity to livestock animals and farms does not increase the risk for carriage. Other factors, such as travelling and PPI usage, were associated with carriage of ESBL/pAmpC-E. Future studies on this topic should not only determine the prevalence, but should also investigate possible interventions to decrease the prevalence.

Transparency declaration

The authors declare that there are no conflicts of interest.

Some of the results of this manuscript were presented as a poster presentation at ECCMID 2016 in Amsterdam, the Netherlands.

Acknowledgements

The authors would like to thank Floor Borlée and Esmeralda Krop (Institute for Risk Assessment Sciences, Utrecht University) for their contribution to the data collection and Joris Yzermans and Christel van Dijk (Netherlands Institute for Health Services Research (NIVEL)) for the data from the NIVEL Primary Care Database. We thank Lianne van Ruitenbeek and Ramón Noomen (Centre for Infectious Disease Control, National Institute for Public Health and the Environment) for their contribution in the laboratory testing of the faecal samples. The Livestock Farming and Neighbouring Residents' Health (VGO) study was funded by the Ministry of Health, Welfare and Sports and the Ministry of Economic Affairs of The Netherlands, and supported by a grant from the Lung Foundation Netherlands (Grant number: 3.2.11.022).

Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article at http://dx.doi.org/10.1016/j.cmi.2016.10.013.

References

[1] Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis 2008;8:159–66.
[2] Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 2011;17:873–80.
[3] Overdevest I, Willemsen I, Rijnsburger M, et al. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg Infect Dis 2011;17:1216–22.
[4] Dierikx C, van der Goot J, Fabri T, van Essen-Zandbergen A, Smith H, Mevius D. Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J Antimicrob Chemother 2013;68:60–7.
[5] Huijbers PM, de Kraker M, Graat EA, 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.
[6] Huijbers PM, Graat EA, Haenen AP, et al. Extended-spectrum and AmpC β-lactamase-producing *Escherichia coli* in broilers and people living and/or working on broiler farms: prevalence, risk factors and molecular characteristics. J Antimicrob Chemother 2014;69:2669–75.
[7] 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.
[8] Reuland EA, Halaby T, Hays JP, et al. Plasmid-mediated AmpC: prevalence in community-acquired isolates in Amsterdam, the Netherlands, and risk factors for carriage. PLoS One 2015;10:e0113033.
[9] Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 2012;18:646–55.
[10] Dierikx CM, van Duijkeren E, Schoormans AH, et al. Occurrence and characteristics of extended-spectrum-β-lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. J Antimicrob Chemother 2012;67:1368–74.
[11] Hartmann A, Locatelli A, Amoureux L, et al. Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in France (Burgundy region). Front Microbiol 2012;3:83.
[12] Dohmen W, Bonten MJ, Bos ME, et al. Carriage of extended-spectrum beta-lactamases in pig farmers is associated with occurrence in pigs. Clin Microbiol Infect 2015;21:917–23.
[13] European Food Safety Authority (EFSA) Panel on Biological Hazards (BIOHAZ). Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamase and/or AmpC β-lactamases in food and food-producing animals. EFSA J 2011;9:2322.

[14] Huijbers PM, Blaak H, de Jong MC, Graat EA, Vandenbroucke-Grauls CM, de Roda Husman AM. Role of the environment in the transmission of antimicrobial resistance to humans: a review. Environ Sci Technol 2015;49:11993–2004.
[15] Food and Agriculture Organization of the United Nations Regional Office for Europe and Central Asia. FAO Statistical yearbook 2014. Budapest: Europe and Central Asia food and agriculture; 2014.
[16] Statistics Netherlands. Agriculture; crops, livestock and land use by general farm type, region. The Hague/Heerlen, the Netherlands: Statistics Netherlands; 2015. Available at: http://statline.cbs.nl/Statweb.
[17] Blaak H, van Hoek AH, Hamidjaja RA, et al. Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment. PLoS One 2015;10:e0135402.
[18] von Salviati C, Laube H, Guerra B, Roesler U, Friese A. Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. Vet Microbiol 2015;175:77–84.
[19] Borlée F, Yzermans CJ, van Dijk CE, Heederik D, Smit LA. Increased respiratory symptoms in COPD patients living in the vicinity of livestock farms. Eur Respir J 2015;46:1605–14.
[20] Prins M, Verheij R. NIVEL Zorgregistraties eerste lijn: Huisarts (Netherlands Primary Care Database: General Practitioners). Utrecht, the Netherlands: NIVEL; 2015. Available at: www.nivel.nl/node/4346.
[21] Smit LA, van der Sman-de Beer F, Opstal-van Winden AW, et al. Q fever and pneumonia in an area with a high livestock density: a large population-based study. PLoS One 2012;7:e38843.
[22] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. Wayne, PA, USA: CLSI; 2012.
[23] Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother 2010;65:490–5.
[24] van Hoek AH, Schouls L, van Santen MG, Florijn A, de Greeff SC, van Duijkeren E. Molecular characteristics of extended-spectrum cephalosporin-resistant Enterobacteriaceae from humans in the community. PLoS One 2015;10:e0129085.
[25] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodological) 1995;57:289–300.
[26] Pedersen K, Hammer AS, Sorensen CM, Heuer OE. Usage of antimicrobials and occurrence of antimicrobial resistance among bacteria from mink. Vet Microbiol 2009;133:115–22.
[27] Dutch Federation of Fur-bearing animals (NFE). Food. Available at: http://nfe.nl/pelsdierenhouderij/voeding/.
[28] Tangden T, Cars O, Melhus A, Lowdin E. 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.
[29] Peirano G, Laupland KB, Gregson DB, Pitout JD. Colonization of returning travelers with CTX-M-producing *Escherichia coli*. J Travel Med 2011;18:299–303.
[30] Ben-Ami R, Rodriguez-Bano J, Arslan H, et al. A multinational survey of risk factors for infection with extended-spectrum β-lactamase-producing enterobacteriaceae in nonhospitalized patients. Clin Infect Dis 2009;49:682–90.