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ESBL carriage in pig slaughterhouse workers is associated with occupational exposure

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SUMMARY

We investigated the prevalence of extended-spectrum β-lactamase (ESBL) carriage in slaughterhouse workers and the association with occupational exposure to slaughter animals and products. Stool samples from 334 employees in a Dutch pig slaughterhouse were obtained. Presence of ESBL was determined by selective plating, microarray analysis, and gene sequencing. Questionnaires were used to collect personal and occupational information. The overall prevalence of ESBL carriage was 4·8% (16/334). All ESBL-producing isolates were *Escherichia coli*. The ESBL genes detected were $bla_{\text{CTX-M-1}}$ ($n = 8$), $bla_{\text{CTX-M-15}}$ ($n = 3$), $bla_{\text{CTX-M-27}}$ ($n = 2$), $bla_{\text{CTX-M-24}}$ ($n = 1$), $bla_{\text{CTX-M-55}}$ ($n = 1$), and $bla_{\text{SHV-12}}$ ($n = 1$). A higher prevalence of ESBL was seen in workers in jobs with as tasks 'removal of lungs, heart, liver, tongue' (33%), and 'removal of head and spinal cord' (25%). For further analysis, participants were divided in two groups based on potential exposure to ESBL as related to their job title. One group with an assumed higher exposure to ESBL (e.g. stable work, stabbing, dehairing, removal of organs) and another group with an assumed lower exposure to ESBL (e.g. refrigeration, packaging and expedition). In the 'higher exposure' group, ten out of 95 (10·5%) were carrying ESBL *vs.* six out of 233 (2·6%) in the 'lower exposure' group. Human ESBL carriage was significantly associated with job exposure in the slaughterhouse (OR 4·5, CI 1·6–12·6). Results suggest that ESBL carriage in slaughterhouse workers overall is comparable with the Dutch population. Within the slaughterhouse population a difference in carriage exists depending on their position along the slaughter line and tasks involved.

Key words: Antimicrobial resistance, $bla_{\text{CTX-M-1}}$, occupational epidemiology, pig abattoir workers, zoonosis.

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INTRODUCTION

In humans, infections with extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* are associated with increased burden of disease and costs [1]. Livestock can carry ESBL-producing *Enterobacteriaceae* with $bla_{\text{CTX-M-1}}$ as the most prevalent ESBL gene found in Europe [2]. ESBL-producing *Enterobacteriaceae* can be transferred from animals to humans through food or direct contact [3, 4]. Direct contact with livestock mainly occurs in an occupational setting. In farmers, carriage of ESBL-producing *Enterobacteriaceae* is associated with the presence of ESBL-producing *Enterobacteriaceae* in animals [3, 5, 6]. Slaughterhouse workers might also be occupationally exposed to ESBL-producing *Enterobacteriaceae*. Depending on the job task, slaughterhouse workers have frequent contact with live animals, animal carcasses or animal products. ESBL-producing *Enterobacteriaceae* have frequently been found in intestinal content of pigs at the slaughter level [7–9]. Besides that, ESBL-producing *Enterobacteriaceae* can also be detected on pig carcasses at the slaughterhouse [10]. A higher prevalence of antimicrobial resistant *Escherichia coli* in pig slaughterhouse workers has been reported [11–13]. However, carriage of ESBL-producing *Enterobacteriaceae* in slaughterhouse workers has not been established yet. In addition, *E. coli* contamination on carcasses seems to be reduced during the slaughter process [14, 15]. Therefore exposure, and as a consequence prevalence of carriage, of ESBL-producing *Enterobacteriaceae* might be dependent on the working area and job task within a slaughterhouse.

We investigated the prevalence of ESBL carriage in pig slaughterhouse workers and the association with occupational exposure.

MATERIALS AND METHODS

Study design

A large pig slaughterhouse in the southern part of the Netherlands (Vion, Boxtel) was visited by the researchers during 1 week in June 2015. All slaughterhouse workers with a slaughter process related job were asked to participate in the study by the use of flyers and information on screens. In total, 1781 eligible slaughterhouse workers were employed by the slaughterhouse at the time of the study. Participants were asked to provide a faecal specimen and to fill out a consent form and a questionnaire containing items on personal and occupational information, including job function in the slaughterhouse. Due to the heterogeneity in nationality of the slaughterhouse workers (14 different nationalities), the flyer, questionnaire and consent form were provided in 11 different languages. Participants were motivated for participation with a voucher worth of 25 euro. Faeces samples and documents were handed in to the researchers at the slaughterhouse within the same week. At the same day, for the purpose of detecting ESBL-producing *Enterobacteriaceae*, <1 g faeces was collected from the tube by the use of a swab and stored in the refrigerator immediately. All swabs were sent refrigerated in one batch to the laboratory. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The Medical Ethical Committee of the University Medical Centre Utrecht confirmed that the Medical Research Involving Human Subjects Act did not apply for this study and that therefore an official approval by the Medical Ethical Committee was not required (Protocol no. 14–346/C). All participants gave written informed consent.

Laboratory analysis

At the day of arrival in the laboratory, all faecal specimens were analysed for the presence of ESBL-producing *Enterobacteriaceae* by selective plating. Samples were suspended in 10 ml LB-medium with cefotaxime (1 µg/ml) and incubated overnight at 37°C. Approximately 10 µl of each suspension was streaked on MacConkey agar plates with cefotaxime (1 µg/ml) and incubated overnight at 37°C. Individual colonies with different morphology were selected for bacterial species identification performed by MALDI-TOF MS. All isolates suspected of producing ESBLs were selected for further molecular analysis to confirm the presence of ESBL genes. DNA was isolated using DNeasy 96 Blood & Tissue Kit (Qiagen, Hilden, Germany). A β-lactamase microarray assay (Check-MDR CT101, Checkpoints, Wageningen, the Netherlands) was used to detect genes encoding carbapenemases (KPC and NDM), ESBLs (CTX-M groups 1, 2, 8/25 and 9, TEM, SHV) and AmpCs (CMY-1 /MOX, ACC, DHA, ACT/MIR, CMY-2, FOX). DNA from ESBL/AmpC microarray positive isolates was amplified and sequenced with group-specific primers to determine

Table 1. *Distribution of slaughterhouse workers and ESBL carriage over different job tasks*

	Job task	Frequency		
		Total	ESBL carriers	Non-ESBL carriers
'Higher exposure' group	Stable/lairage part, stunning part	12	0	12
	Stabbing and bleeding part	5	0	5
	Dehairing and hanging part	6	1	5
	Removal of penis, stab wound and loosing/ligation anus (anal duct)	6	0	6
	Evisceration	10	1	9
	Removal of lungs, hart, liver, tongue	12	4	8
	Inspection platform	8	0	8
	Dressing (first, second), removal abdominal fat, diaphragm	17	1	16
	Removal of head, spinal cord	8	2	6
	Organs (tongues, hearts, livers, kidneys)	11	1	10
'Lower exposure' group	Refrigeration and cooling area	26	0	26
	Cutting room	75	2	73
	Deboning area	63	1	62
	Packaging	30	2	28
	Expedition (moving and shipment)	8	0	8
	Other (e.g. technical services)	31	1	30

the exact gene type [16]. DNA sequences were interpreted with Basic Local Alignment Search Tool (National Center for Biotechnology Information). Plasmids encoding the ESBL (or AmpC) genes were determined using a transformation-based approach. Purified plasmid DNA was electro-transformed into *E. coli* DH10B cells (Invitrogen, Van Allen Way, CA, USA) under the following conditions: 1·25 kV/cm, 200 Ω, 25 μFar [17]. When strains recovered from workers were not giving transformants (workers 12 and 15), conjugation was performed using the plasmid-free rifampin-resistant *E. coli* E3110 as a recipient strain for liquid-mating assays in 1:1 ratio as previously described [17]. Transformants were selected on lysogeny broth (LB) agar supplemented with cefotaxime (1 μg/ml), whereas transconjugants on LB agar with rifampin (100 μg/ml) and cefotaxime (1 μg/ml). PBRT (PCR-based replicon typing) on the transformants and multilocus sequence typing (MLST) on the parental strains were performed to analyse genetic similarities of isolates and plasmid content [17].

Statistical analysis

Slaughterhouse workers were classified as ESBL positive, if any ESBL gene was detected in their faeces sample. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The association between carriage of ESBL-producing *Enterobacteriaceae* in humans and job function was calculated with logistic regression analysis (proc GENMOD). For this analysis, participants were divided in two groups based on potential exposure within their specific job task. One group with an assumed higher exposure to ESBL-producing *Enterobacteriaceae* consisted of job tasks in the early stages of the slaughter process (e.g. stable work, stabbing, dehairing, removal of organs, i.e. direct contact with pigs and pig carcasses). The other group, assumed to have lower exposure to ESBL-producing *Enterobacteriaceae*, consisted of job tasks in the latter stages of the slaughter process (e.g. refrigeration, packaging, expedition, i.e. contact with meat or meat products) (see Table 1). When participants reported to have more than one job function, they were assigned to the first job function at the slaughter line. Other potential risk factors such as nationality, consumption of raw meat, hospitalization, use of antimicrobials, living on a farm, travelling, and potential confounder's age, gender and smoking were analysed univariately as well and selected for multivariate analysis when *P*-value was below 0·2. Model selection was performed by a backward procedure. Model fit was checked with the QIC-statistic (Quasi-likelihood under the Independence model Criterion). The final model retained variables significant at $P \leqslant 0{\cdot}05$.

RESULTS

Human stool samples from 334 employees were obtained. Most of the participants were male and did

Table 2. *Overview of participant characteristics and Odds Ratios from the univariate analysis for the probability of ESBL carriage*

Characteristics of participants	Frequency			OR (95% CI)
	Total	ESBL carriers	Non-ESBL carriers	
Exposure group in slaughter process ($n = 328$)				
'Higher exposure' group	95	10	85	4·45 (1·57–12·62)
'Lower exposure' group	233	6	227	Ref.
Age (mean) ($n = 328$)	39·8	38·1 ($n = 16$)	39·9 ($n = 312$)	0·99 (0·94–1·03)*
Gender ($n = 324$)				
Male	276	14	262	2·51 (0·32–19·54)
Female	48	1	47	Ref.
Nationality ($n = 305$)				
Non-Dutch†	245	12	233	0·98 (0·27–3·58)
Dutch	60	3	57	Ref.
Consumption of raw meat ($n = 294$)				
Yes	106	4	102	0·70 (0·21–2·28)
No	188	10	178	Ref.
Hospitalization in the past 12 months ($n = 295$)				
Yes	32	2	30	1·28 (0·28–5·96)
No	263	13	250	Ref.
Use of antimicrobials in the past 12 months ($n = 323$)				
Yes	48	3	45	1·34 (0·37–4·90)
No	275	13	262	Ref.
Living on a farm ($n = 297$)				
Yes	17	0	17	NE
No	280	13	267	
Travelling in the past 12 months ($n = 311$)				
Yes	216	7	209	0·36 (0·13–1·04)
No	95	8	87	Ref.
Smoking ($n = 321$)				
Yes	140	3	137	0·31 (0·09–1·12)
No	181	12	169	Ref.

Ref., Reference category; NE, Not estimated due to empty cells.
* Per 1 year increase.
† Not from the Netherlands: Cape Verdean ($n = 1$), Ghanese ($n = 1$), Hungarian ($n = 46$), Latvian ($n = 3$), Polish ($n = 119$), Portugese ($n = 12$), Romanian ($n = 41$), Slovakian ($n = 20$), Turkish ($n = 2$).

not originate from the Netherlands (Table 2). The overall prevalence of ESBL carriage was 4·9% (16/334), and all ESBL-producing isolates were *E. coli*. The ESBL genes detected were $bla_{\text{CTX-M-1}}$ ($n = 8$), $bla_{\text{CTX-M-15}}$ ($n = 3$), $bla_{\text{CTX-M-27}}$ ($n = 2$), $bla_{\text{CTX-M-24}}$ ($n = 1$), $bla_{\text{CTX-M-55}}$ ($n = 1$) and $bla_{\text{SHV-12}}$ ($n = 1$). In addition, three other slaughterhouse workers carried an AmpC gene [$bla_{\text{CMY-2}}$ ($n = 2$) and $bla_{\text{DHA-1}}$ ($n = 1$)]. Plasmids encoding ESBL genes were assigned to multi-F ($n = 6$), I1α ($n = 4$), N ($n = 3$) and K ($n = 1$) replicon types. For two isolates, transformation and conjugation experiments failed to give transformants and transconjugants suggesting the chromosomal location of these genes. MLST revealed that ESBL-producing *E. coli* belonged to 11 different sequence types (STs), with ST131 ($n = 4$), ST88 ($n = 3$) and ST93 ($n = 3$) as the most frequent ones. All molecular aspects are listed in Table 3.

ESBL carriage was not equally distributed across different jobs in the slaughterhouse. A higher prevalence of ESBL seemed to appear in workers involved in 'removal of lungs, heart, liver, tongue' (33%) and 'removal of head and spinal cord' (25%) (see Table 1). In the 'higher exposure' group, ten out of 95 (10·5%) were carrying ESBL *vs.* six out of 233 (2·6%) in the 'lower exposure' group. Human ESBL carriage was significantly associated with job exposure in the slaughterhouse (odds ratio (OR) 4·45, CI 1·57–12·62). Smoking was univariately and negatively borderline significantly associated with ESBL carriage

Table 3. *Molecular aspects of ESBL/AmpC positive E. coli isolates from slaughterhouse workers*

Slaughterhouse worker	ESBL/AmpC gene	Plasmid rep/ inc-type	Sequence types (ST)	Job task
1	$bla_{\text{CTX-M-1}}$	IncI1α	88	Evisceration
2	$bla_{\text{CTX-M-1}}$	IncI1α	88	Removal of lungs, hart, liver, tongue
3	$bla_{\text{CTX-M-1}}$	IncI1α	88	Removal of lungs, hart, liver, tongue
4	$bla_{\text{CTX-M-1}}$	IncI1α	10	Dressing (first, second), removal abdominal fat, diaphragm
5	$bla_{\text{CTX-M-1}}$	IncN	93	Removal of head, spinal cord
6	$bla_{\text{CTX-M-1}}$	IncN	93	Removal of head, spinal cord
7	$bla_{\text{CTX-M-1}}$	IncN	93	Organs (tongues, hearts, livers, kidneys)
8	$bla_{\text{CTX-M-1}}$	IncFIA-FII	131	Packaging
9	$bla_{\text{CTX-M-15}}$	IncK	156	Cutting room
10	$bla_{\text{CTX-M-15}}$	incFIB-FII	410	Deboning area
11	$bla_{\text{CTX-M-15}}$	IncFIA-FII	131	Dehairing and hanging part
12	$bla_{\text{CTX-M-24}}$	−*	354	Packaging
13	$bla_{\text{CTX-M-27}}$	IncFIA-FIB-FII	131	Other (e.g. technical services)
14	$bla_{\text{CTX-M-27}}$	IncFIB-FII	131	Cutting room
15	$bla_{\text{CTX-M-55}}$	−*	95	Removal of lungs, hart, liver, tongue
16	$bla_{\text{SHV-12}}$	IncFIB-FIC-FII	665	Removal of lungs, hart, liver, tongue
17	$bla_{\text{CMY-2}}$	IncI1α	752	Cutting room
18	$bla_{\text{CMY-2}}$	IncI1α	752	Cutting room
19	$bla_{\text{DHA-1}}$	IncFIA-FII	38	Stabbing and bleeding part

* No successful transformation or conjugation.

(OR 0·31, CI 0·09–1·12) as well as travelling (OR 0·36, CI 0·13–1·04). None of the other potential risk factors (nationality, consumption of raw meat, hospitalization, use of antimicrobials, living on a farm) or confounders (age, gender) were significantly associated with ESBL carriage. In multivariate analysis, the effects of smoking and travelling appeared independent of job exposure (only a slight change in OR) and did not remain in the final model. Therefore the final model only contained job exposure as a risk factor for ESBL carriage.

When looking at ESBL carriage by nationality, Romanian workers had a higher prevalence (five out of 41 (12·2%)) compared with the other three well-represented nationalities: Hungarian (one out of 46 (2·2%)), Dutch (three out of 60 (5·0%)) and Polish (five out of 119 (4·2%)). However, 23 out of 41 (56·1%) of the Romanians worked in the 'higher exposure' group, which is a considerably higher percentage than within Hungarian, Dutch and Polish slaughterhouse workers (seven out of 46 (15·2%), nine out of 60 (15·0%) and 30 out of 119 (25·2%) respectively). When in the final model the effect of job exposure was adjusted for Romanian nationality, the association between ESBL carriage and job exposure hardly changed (OR 4·85, CI 1·54–15·24). No significant interaction between Romanian nationality and job exposure was observed for ESBL carriage.

Focusing on $bla_{\text{CTX-M-1}}$ gene solely, the prevalence was 7·4% (7/95) in the 'higher exposure' group and 0·4% (1/233) in the other group. When analysis was restricted for $bla_{\text{CTX-M-1}}$ alone, the association between ESBL carriage and exposure group was confirmed (OR 18·46, CI 2·24–152·16).

From the 74 participants who reported to have more than one job title, 11 participants mentioned job titles not within the same exposure group. This misclassification of exposure might have changed the association between exposure and ESBL carriage. When a sensitivity analysis was performed by switching these 11 participants from the 'higher exposure' to the 'lower exposure' group, the observed association between ESBL carriage and occupational exposure did not change significantly and had a similar point estimate (OR 5·36, CI 1·89–15·25).

DISCUSSION

The study revealed that slaughterhouse workers were more likely to carry ESBL when working in the early slaughtering steps (before chilling of the pig carcasses) than slaughterhouse workers working from

this slaughter step forward, i.e. working in the cooling, cutting and deboning area. Overall prevalence of ESBL carriage was 4·8%, which is more or less comparable to numbers found in two recent Dutch studies in residents of Amsterdam (8·6%) and residents living in the vicinity of livestock farms (4·5%) [18, 19]. However, the ESBL prevalence differed between the assumed higher and lower exposed groups (10·5 *vs.* 2·6%) pointing at higher occupational exposures during specific tasks performed by individual slaughterhouse workers.

A previous study in the same slaughterhouse showed a decline in presence and counts of *Salmonella* on skin surface and the exterior of carcasses from bleeding till chilling [20]. In addition, a decline in presence of *E. coli* during the slaughter process, especially after chilling of the pig carcasses, has been described in literature [14, 15, 21, 22]. It is likely that ESBL-producing *E. coli* shows the same decline as *E. coli* in general, although we can only assume since exposure to ESBL was not measured in this study. This could explain the higher carriage of ESBL among slaughterhouse workers when involved in the slaughter steps before chilling. For some slaughter steps only low numbers of participants could be included. As a consequence, it was not possible to estimate differences in ESBL carriage in more detail. However, when focusing on the descriptive figures, ESBL carriage is considerably higher when working in the slaughter steps 'removal of lungs, heart, liver, tongue' and 'removal of head and spinal cord'. Exposure to ESBL might be higher for workers in these slaughter steps, because both steps involve handling of the throat area, including the pharyngeal tonsils which are colonized with high counts of several bacteria (potentially including ESBL-producing *E. coli*) [23, 24].

In this study, $bla_{\text{CTX-M-1}}$ was the most predominantly detected ESBL gene in pig slaughterhouse workers. Out of the ESBL-positive slaughterhouse workers, eight were carrying $bla_{\text{CTX-M-1}}$ (50%), which is significantly higher than the proportions of $bla_{\text{CTX-M-1}}$ reported in two Dutch studies. In ESBL-positive residents of Amsterdam and ESBL-positive residents living in the vicinity of livestock farms 26 out of 145 (18%) and 13 out of 99 (13%) carried a $bla_{\text{CTX-M-1}}$ gene, respectively [18, 19]. In addition, $bla_{\text{CTX-M-1}}$ is also the most frequently found ESBL gene in slaughter pigs in the Netherlands [25]. Moreover, pig farmers were found to carry the same ESBL gene type as exclusively or predominantly detected in their pigs, of which $bla_{\text{CTX-M-1}}$ was most frequently found in both of them [3]. In contrast, $bla_{\text{CTX-M-15}}$ has been found mostly in humans in a clinical setting and in the Dutch human population, but not that often in livestock [2, 18, 26]. A great diversity was seen in STs; therefore clonal transmission between slaughterhouse workers is probably not the dominant route of transmission. Besides, half of the STs (ST10, ST38, ST88, ST93, ST354) have been previously associated with *E. coli* from pig origin [8, 27, 28]. In addition, IncN and IncI1 plasmids are known to play a role in the dissemination of $bla_{\text{CTX-M-1}}$ in *E. coli* in livestock, including pigs [29–31]. Although no samples from carcasses or slaughter products were obtained, these results regarding ESBL gene types, plasmids and STs, suggesting transmission from animals to humans.

Besides occupational exposure, none of the analysed determinants (nationality, consumption of raw meat, hospitalization, use of antimicrobials, living on a farm, travelling, age, gender and smoking) were found to be risk factors for ESBL carriage in slaughterhouse workers. ESBL carriage was higher in slaughterhouse workers with a Romanian nationality compared with other nationalities, although the association between ESBL carriage and occupational exposure remained when adjusting for Romanian nationality. Besides that, the effect of being in the 'higher exposure' group is similar for Romanian slaughterhouse workers as for the other nationalities, considering the non-significant interaction. Also, in three out of five Romanian ESBL carriers the ESBL gene type detected was $bla_{\text{CTX-M-1}}$. Smoking and travelling were protective factors for ESBL carriage univariately, although they did not retain in the final model. From the 173 non-Dutch slaughterhouse workers who reported their travelling destiny, 88% travelled to their country of origin. Although travelling frequencies were not assessed, travelling might be interpreted as long distance commuting. To our knowledge, there is no explanation described in literature for any effect of smoking on ESBL carriage.

Although ESBL carriage was detected in slaughterhouse workers, the risk of acquiring ESBL-producing *E. coli* when handling meat intensively seems to be limited. When this is extrapolated to consumers, exposure to ESBL in pork through handling might not be of a high public health concern. In combination with environmental exposure data, this information can be used in formal risk assessments. Although not addressed in this study design, duration and persistence of ESBL carriage can vary greatly [32, 33].

Duration of ESBL carriage is not only important for assessing the risk of infection, but for the risk of transmission of ESBLs from slaughterhouse workers into the general population as well.

The results suggest that the overall carriage in slaughterhouse workers is comparable with the Dutch population, but the proportion of the $bla_{\text{CTX-M-1}}$ gene (commonly found in livestock) is higher. Differences exist between slaughterhouse workers depending on their job tasks. A higher prevalence of ESBL carriage was found in slaughterhouse workers working before the chilling process of the carcasses compared to workers in the cooling area, cutting and deboning departments.

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DECLARATION OF INTEREST

None.

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