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Air exposure as a possible route for ESBL in pig farmers☆

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ABSTRACT

Livestock can carry extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, with $bla_{CTX\text{-}M\text{-}1}$ being most prevalent. ESBL carriage in farmers is associated with ESBL carriage in animals, with direct animal-human contact considered as the dominant route of transmission. However, inhalation of stable air might represent another route of transmission. We, therefore, quantified presence of $bla_{CTX\text{-}M}$ group 1 genes (CTX-M-gr1) in dust and the association with CTX-M-gr1 carriage in pig farmers, family members and employees. We included 131 people living and/or working on 32 conventional Dutch pig production farms (farmers, family members and employees) during two sampling moments over a 12-month interval. Human stool samples, rectal swabs from 60 pigs per farm, and 2–5 dust samples collected using an electrostatic dust collector (EDC) (as a proxy for presence of viable CTX-M-gr1 carrying bacteria in air) were obtained per farm. Presence of ESBL-producing *Escherichia Coli* (*E. coli*) in stool samples and rectal swabs was determined by selective plating and CTX-M-gr1 was identified by PCR. Dust samples were analyzed directly by PCR for presence of CTX-M-gr1. Questionnaires were used to collect information on nature, intensity and duration of animal contact. Overall human prevalence of CTX-M-gr1 carriage was 3.6%. CTX-M-gr1 was detected in dust on 26% of the farms and in pigs on 35% of the farms, on at least one sampling moment. Human CTX-M-gr1 carriage and presence of CTX-M-gr1 in dust were associated univariately (OR=12.4, 95% CI=2.7–57.1). In multivariate analysis human CTX-M-gr1 carriage was associated with the number of working hours per week (OR=1.03, 95% CI=1.00–1.06), presence of CTX-M-gr1 carrying pigs on the farm (OR=7.4, 95% CI=1.1–49.7) and presence of CTX-M-gr1 in dust (OR=3.5, 95% CI=0.6–20.9). These results leave open the possibility of airborne CTX-M-gr1 transmission from animals to humans next to direct contact.

1. Introduction

Livestock can carry extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, with CTX-M-1 ESBL as the most important enzyme representatives in pigs in Europe (Ewers et al., 2012; Schmithausen et al., 2015; Dahms et al., 2015; Mesa et al., 2006; Hammerum et al., 2014). Transmission of ESBL genes from animals to humans has been shown on pig farms (Hammerum et al., 2014; Moodley and Guardabassi, 2009; Dohmen et al., 2015; de Been et al., 2014). Moreover, ESBL carriage in farmers is associated with ESBL carriage in pigs on the same farm (Dohmen et al., 2015). Direct contact with pigs has been assumed as the dominant route of transmission. However, ESBL has also been detected in dust and air within pig farms (Schmithausen et al., 2015; Moodley and Guardabassi, 2009; Garcia-Cobos et al., 2015; Hering et al., 2014; von Salviati et al., 2015). Two German studies detected ESBL-producing Enterobacteriaceae in air on 6 out of 35 and 4 out of 7 farms respectively (Schmithausen et al., 2015; von Salviati et al., 2015). In the latter study, no ESBL-producing Enterobacteriaceae were found in dust, while another German study did detect ESBL-producing bacteria in 11% of 282 collected dust samples on 47 pig farms (Garcia-Cobos et al., 2015). Also in Germany, cefotaxime resistant *Escherichia coli* (*E. coli*) were detected in 11% of 95 dust samples collected on 48 farms (Hering et al., 2014). Considering the presence of ESBL genes in air or dust, inhalation of stable air might represent a second route of transmission. The role of ESBL in air (and dust) as a risk factor for human ESBL carriage and potential route of transmission has not been explored yet. This might be important because transmission through air will involve a different spectrum of potential preventive measures than those related to direct contact. In a longitudinal study, we investigated the presence of ESBL genes belonging to $bla_{CTX\text{-}M}$ group 1 (CTX-M-gr1) in dust (as a proxy for exposure through air) in pig farms and the association with CTX-M-gr1 carriage in pig farmers, family members and employees.

☆ The Medical Ethical Committee of the University Medical Center Utrecht approved the study protocol (No. 10-471/K). All participants gave written informed consent.

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2. Materials and methods

2.1. Study design

The design of the study has been described partially in previous studies (Dohmen et al., 2015; Dorado-Garcia et al., 2015; Bos et al., 2016). In short, 40 conventional Dutch pig production farms were enrolled in the study between March and October 2011. At two sampling moments, over a 12-month interval, human stool samples were obtained from pig farmers, family members and employees using feces cups (Minigrip®) and sent to the laboratory by mail. Dust samples were obtained from the stables and the family home by using electrostatic dust collectors (EDC's). EDC's consist of two sterilized electrostatic dust cloths in a polypropylene sampler, which passively capture airborne settled dust (Noss et al., 2008). Four EDC's were placed at different locations in the stables out of reach from pigs and at least one meter above the ground (for example on a windowsill) and one EDC was placed in the house (usually on the highest cupboard in the living room or kitchen of the house). The EDC's were left in place for a period of 2 weeks, this collection time was needed to gather a sufficient amount of dust for DNA extraction. Afterwards they were send to the laboratory by mail where they were stored at −80 °C until analysis. Rectal samples from 60 pigs were collected on each farm by the farm veterinarian, using sterile cotton-wool swabs (Cultiplast®) and sent refrigerated to the laboratory by courier. All animal age groups present were sampled (sows, gilts, suckling piglets, weaning piglets and finishing pigs). Rectal swabs were combined in 10 pools of 6 pigs. Each pool consisted of an age group in the same pen. Participants filled out questionnaires on general characteristics, farm activities, intensity and duration of animal contact and other risk factors for ESBL carriage such as traveling, hospitalization and antimicrobial use. Questionnaires on farm characteristics were filled out by veterinarians and farmers. The Medical Ethical Committee of the University Medical Center Utrecht approved the study protocol (No. 10–471/K). All participants gave written informed consent.

2.2. Laboratory analysis

2.2.1. Human and pig samples

All faecal samples from humans and pooled swabs from pigs were analyzed for the presence of ESBL-producing Enterobacteriaceae by selective plating as described previously (Dohmen et al., 2015). Samples were suspended in 10 ml buffered peptone water and incubated overnight at 37 °C. For screening of ESBL-producing Enterobacteriaceae, suspensions were cultured on selective agar plates (*Brilliance*™ ESBL Agar, Oxoid®) and incubated overnight at 37 °C aerobically. In absence of growth, plates were incubated another night at 37 °C. Morphologically different colonies suspected of ESBL production were cultured individually on a blood agar plate (Oxoid®) and incubated overnight at 37 °C. In case of morphological uncertainty an oxidase test was performed before culturing. Bacterial species identification of the isolates was performed by MALDI/TOF (Bruker®). For phenotypical confirmation of ESBL-producing Enterobacteriaceae, a 0.5 McFarland suspension was inoculated on a Mueller Hinton agar and a combination disc test (ROSCO®) including cefotaxime, cefotaxime+clavulanate, ceftazidime, ceftazidime+clavulanate, cefepime, and cefepime+clavulanate (*Neo-Sensitabs*™) was used (according to the guidelines of the manufacturer (http://www.rosco.dk)). Isolates were stored at −80 °C before molecular analysis.

In all ESBL-suspected *E. coli* the presence of CTX-M-gr1 was identified by PCR. Due to logistic reasons, the molecular analysis was performed in two laboratories. In the first laboratory, DNA from human isolates from both sampling moments and pig isolates from the first sampling moment was isolated using UltraClean® Microbial DNA Isolation Kit (MO BIO Laboratories, Inc.). A CTX-M-gr1 specific PCR was used to detect presence of CTX-M-gr1. DNA from CTX-M-gr1 positive isolates from humans was sequenced using the same primers to determine the presence of CTX-M-gr1 (Paauw et al., 2006). In the second laboratory, DNA from pig isolates from the second sampling moment was isolated using DNeasy 96 Blood & Tissue Kit (Qiagen). Real-Time PCR (SybrGreen, Life Technologies) was used to detect presence of CTX-M-gr1. PCR was repeated for a part of the DNA samples using a conventional PCR (BioMix Red, Bioline), since the Real-Time PCR wasn't optimized fully unfortunately for all DNA samples.

2.2.2. Dust samples

Dust samples were analyzed by qPCR for presence and quantification of CTX-M-gr1. First, dust was removed by homogenizing an EDC with 10 ml of pyrogen-free water with 0,05% Tween20 in a stomacher bag (VWR StarBlender) for 10 min. After repeating the homogenizing process with 10 ml of sample suspension, the resulting 16 ml were stored at −80 °C for at least one night. Samples were freeze dried for 2–4 days to remove the extraction liquid. Extracted dust was stored at −80 °C until DNA extraction. Fourteen extraction blanks were included, these consisted of EDC's that were not exposed to air.

Second, DNA was extracted from dust. DNA extraction was performed using the Macherey-Nagel NucleoSpin® 8 Plant II kit (http://www.mn-net.com). The standard protocol was slightly modified by performing an initial bead beating step using a dry weight of 40 mg where possible (with double the amount of PL1 and RNase A, and 500 mg glass beads with a diameter of 212–300 µm in a FastPrep FP120 Cell disrupter at a speed of 6.5 for 45 s). For samples with > 20 mg of dust extracted, the wash step with buffer PW1 was repeated. Finally, a single DNA elution step was performed.

Third, presence and amount of CTX-M-gr1 was determined. A SybrGreen qPCR assay using published primers (Xu et al., 2005), at an annealing temperature of 57.5 °C, by use of the CFX384 system (Biorad) was performed. Dilutions of a plasmid extraction of a clone of the PCR product of a CTX-M-gr1 positive *E. coli* strain (E54) was used as calibration curve. DNA was diluted 1:100 times as PCR inhibition was still occasionally observed at a dilution of 1:10. Quantification of the CTX-M-gr1 was not possible as the Ct values of the positive samples fell out of the linear range of the calibration curve, and the assay was thus treated as a qualitative assay. As the amount of positive signals was low, samples that showed one or more positive PCR triplicates with a correct melt peak (87.5–88 °C) in a 100× DNA dilution were confirmed in a second PCR on a 1:10 dilution of the DNA. If two or more of the three replicates of the second PCR were positive, and if melt curve analysis and gel electrophoresis confirmed the presence of correct amplicons, the sample was deemed positive. The great majority of the samples that showed 1 or more positive triplicate in the 1:100 diluted DNA (16 out of 19) was confirmed to be positive in the second PCR. None of the extraction blanks gave positive results, neither showed the non-template controls amplification.

2.3. Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Simple descriptive analyses were used to explore presence of CTX-M-gr1 in humans, dust and pigs over time. Farms were classified as CTX-M-gr1 positive in dust when CTX-M-gr1 was determined in at least one EDC sample. Farms were classified as CTX-M-gr1 positive in animals if CTX-M-gr1 was detected in an isolate from at least one pooled pig sample.

Generalized linear mixed models (PROC GLIMIX; SAS Institute, Inc.) adjusted for clustering at farm level and repeated measurements were used to calculate associations with CTX-M-gr1 carriage in humans. Our main determinant of interest was presence of CTX-M-gr1 in dust. Presence of CTX-M-gr1 in pigs and average number of hours working per week were also considered, since they were shown to have an effect by previous work based on cross-sectional data from this

Table 1
Baseline characteristics of participants who completed both sampling moments (n=131).

Human characteristics[a]	Frequency (%)
Gender	
Male	76 (58)
Female	55 (42)
Category	
Farmer	45 (34)
Family of farmer	70 (53)
Employee	16 (12)
Age	131 (mean 36, range 6–79)
Age < 18 years	30 (23)
Age 18–65 years	98 (75)
Age > 65 years	3 (2)
Average number of hours working on the farm per week	125 (mean 25, range 0–80)
0	39 (30)
1–20	25 (19)
≥20	61 (47)
Smoking	
Yes	11 (8)
No	120 (92)

[a] Measured at the start of the study period (first sampling moment). Differences in characteristics between the two sampling moments were minor.

study (Dohmen et al., 2015). All were analyzed separately as well as together in a model. Potential confounders age, gender, and smoking were analyzed univariately and selected for multivariate analysis when p-value was below 0.2. Model assumptions were checked with diagnostic plots.

3. Results

3.1. Human characteristics

During the first sampling moment, 142 people living and/or working on 34 of the 40 included pig farms participated. During the second sampling moment, 135 people living and/or working on 32 out of 39 participating pig farms were measured. In total, 131 people living and/or working on 32 pig farms participated in both sampling moments. Baseline characteristics of these 131 participants are presented in Table 1.

3.2. CTX-M-gr1 in humans

During the first sampling moment, 6 out of 142 participants carried CTX-M-gr1 versus 4 out of 135 participants during the second sampling moment. From the total of 131 participants who completed both sampling moments, only 1 person was a CTX-M-gr1 carrier during both sampling moments. In all human CTX-M-gr1 carriers, $bla_{\text{CTX-M-1}}$ was the determined gene type. Of the total of 9 carriers (6 farmers, 3 family members), 8 participants reported to work at least 20 h per week on the farm. During 8 out of the 10 positive human observations, pooled pig samples that originated from the corresponding farm were CTX-M-gr1 positive as well. All seven human carriers living on a farm with CTX-M-gr1 positive pigs reported to have daily contact with pigs. During 6 out of the 10 positive human observations, dust samples originated from the corresponding farm were CTX-M-gr1 positive. None of the carriers reported to have used antimicrobials, been hospitalized or have traveled to risk countries in the previous 12 months before both sampling moments.

Table 2
CTX-M-gr1 in humans, dust and pigs.

		First sampling moment			Second sampling moment	
Farm	**CTX-M-gr1 in humans**	**CTX-M-gr1 in dust**	**CTX-M-gr1 in pooled pig samples**	**CTX-M-gr1 in humans**	**CTX-M-gr1 in dust**	**CTX-M-gr1 in pooled pig samples**
1	0/5	0/4	0/10	0/6	0/4	0/10
2	0/1	1/2	9/10	1/1	2/4	3/10
3	0/1	0/4	1/10	0/1	0/4	0/10
4	0/9	0/2	1/10	0/9	0/4	1/10
5	2/4	2/4	5/10	0/4	1/4	3/10
6	NA	0/4	0/10	NA	0/4	0/10
7	0/2	0/4	1/10	0/2	0/4	1/10
8	0/4	0/4	0/10	0/4	0/4	0/10
9	NA	1/4	4/10	NA	0/4	0/10
10	0/2	0/3	0/10	0/2	0/4	0/10
11	0/1	0/2	0/10	0/1	0/4	0/10
12	0/3	NA	10/10	NA	NA	8/10
13	1/3	1/3	9/10	0/3	0/3	6/10
14	0/4	1/4	0/10	1/4	0/3	7/10
15	0/8	1/4	0/10	0/8	0/4	0/10
16	0/6	0/4	0/10	0/6	0/4	0/10
17	NA	1/4	9/10	NA	NA	0/9
18	0/4	0/3	0/10	0/4	0/3	0/10
19	0/9	0/2	0/10	0/8	0/4	0/10
20	0/3	0/4	0/10	0/2	0/4	0/10
21	0/3	0/3	0/10	0/3	0/3	0/10
22	0/2	1/2*	5/10	0/2	1/3	6/10
23	NA	0/2	0/10	NA	0/3	0/10
24	0/11	0/3[a]	0/10	1/11	0/4	0/10
25	0/1	NA	0/10	NA	NA	NA
26	0/5	0/4	1/10	0/5	0/4	1/10
27	0/2	0/1	0/10	0/2	0/4	0/10
28	0/2	0/2	0/10	0/1	0/6	0/10
29	NA	0/4	0/10	NA	0/4	0/10
30	0/7	0/2	0/10	0/8	0/3	0/10
31	2/5	1/3	8/10	1/5	0/4	5/10
32	NA	1/4	7/10	NA	0/4	6/10
33	0/5	0/3	0/10	0/3	0/3	0/10
34	0/7	0/4	0/10	0/7	NA	0/10
35	0/1	0/4	0/10	0/1	0/4	0/10
36	0/9	0/4	0/10	0/9	0/4	0/10
37	0/2	0/2	0/10	0/2	0/4	0/10
38	0/3	0/4	0/10	0/3	0/4	0/10
39	0/2	0/4	0/10	0/2	0/4	0/10
40	1/6	0/3	0/10	0/6	0/4	0/10

NA=no samples were collected from these farms
* CTX-M-gr1 was also determined in house EDC.
[a] Two living houses were sampled

3.3. CTX-M-gr1 in dust and pigs

The presence of CTX-M-gr1 in dust and pigs is listed in Table 2. During the first sampling moment, CTX-M-gr1 was detected in 11 out of 123 EDC's placed in the stables of 38 farms and in 1 of 34 EDC's placed in the house. During the second sampling moment, CTX-M-gr1 was detected in 4 out of 138 EDC's placed in the stables of 36 farms and in none of the 35 house EDC's. During the first and second sampling moment, 10 out of 38 and 3 out of 36 farms were positive for CTX-M-gr1 in dust. All 3 positive farms in the second sampling moment, were also positive in the first sampling moment. Quantification of CTX-M-gr1 was not possible, therefore we only presented binary results.

On 13 out of 40 and 11 out of 39 farms CTX-M-gr1 was present in pigs in the first and second sampling moment respectively. On 8 out of 10 farms (and 11 out of 13 farm observations) where CTX-M-gr1 positive farm EDC's were found, CTX-M-gr1 was detected in pigs simultaneously in time.

3.4. Association between ESBL carriage in humans and dust

Human CTX-M-gr1 carriage and presence of CTX-M-gr1 in dust were associated univariately (OR=12.4, 95% CI=2.7–57.1). Other determinants, univariately associated with human CTX-M-gr1 carriage, were presence of CTX-M-gr1 in pigs (OR=14.4, 95% CI=2.5–82.9) and average number of hours working on the pig farm per week (OR=1.04, 95% CI=1.01–1.07). When the three determinants were mutually adjusted in a multivariate analysis, the effect of working hours per week only changed marginally (OR=1.03, 95% CI=1.00–1.06). However, the effect sizes of presence of CTX-M-gr1 carrying pigs on the farm and presence of CTX-M-gr1 in dust were greatly reduced in the final model (OR=7.4, 95% CI=1.1–49.7 and OR=3.5, 95% CI=0.6–20.9 respectively). Gender was the only confounder considered for multivariate analysis (OR=4.6, 95% CI=0.8–25.9) but was not retained in the final model after adjustment for working hours. To evaluate dependency between the different effects in the final model, bivariate analyses were performed. Both presence of CTX-M-gr1 in dust and presence of CTX-M-gr1 in pigs declined in effect size when bi-variately analyzed (OR=5.3, 95% CI=1.1–26.2 and OR=7.2, 95% CI=1.2–41.5 respectively). In bivariate models together with the effect of working hours per week, there was a modest drop in the effect size of presence of CTX-M-gr1 in dust (OR=9.2, 95% CI=1.9–45.9). The change in effect size of presence of CTX-M-gr1 in pigs was minor (OR=12.8, 95% CI=2.2–74.4). The effect of average number of hours working on the pig farm per week hardly changed in any of the bivariate models. All associations are presented in Table 3.

4. Discussion

In pig farmers, CTX-M-gr1 carriage was associated with exposure to CTX-M-gr1 containing dust and exposure to CTX-M-gr1 positive pigs. The observation that both variables were associated with human carriage, after mutual adjustment, leaves open the possibility that next to direct contact, airborne transmission plays a role as well. However, the number of human carriers was small and these findings need replication in a larger population sample. Moreover, presence of CTX-M-gr1 in dust and pigs were partially dependent, which is plausible because CTX-M-gr1 carrying pigs are shedding CTX-M-gr1 producing Enterobacteriaceae into the environment and CTX-M-gr1 might be picked up from the environment as well, which complicates the analysis.

Overall prevalence of human $bla_{CTX\text{-}M\text{-}1}$ carriage was 3.6%, which is more or less comparable to numbers found in two recent Dutch studies. In residents of Amsterdam and residents living in the vicinity of livestock farms 26 out of 1695 (1.5%) and 13 out of 2432 (0.5%) carried $bla_{CTX\text{-}M\text{-}1}$ respectively (Reuland et al., 2016; Wielders et al., 2017). However, in pig farmers a $bla_{CTX\text{-}M\text{-}1}$ prevalence of 8% was seen. The number of farms where CTX-M-gr1 was detected in dust decreased from 10 out of 38 farms to 3 out of 36 farms during the study period. The prevalence of CTX-M-gr1 in dust samples collected from the stables declined from 9% to 3%. This is in the same order of magnitude as a German study where 29 out of 282 dust samples harbored ESBL-producing *E. coli* (10.3%) of which most of the isolates (86 out of 106) belonged to CTX-M-gr1 (Garcia-Cobos et al., 2015). In a German study, cefotaxime resistant *E. coli* were present on 10 out of 48 farms and 11% of all dust samples (Hering et al., 2014). However, the number of farms with cefotaxime resistant *E. coli* in manure collected from the floor was much higher (40 out of 48 farms) than in the present study. In one out of the total of 69 EDC's placed in living houses, presence of CTX-M-gr1 was determined. Considering air exposure as a potentially relevant transmission route, this could partially explain the low carriership in people only living on the pig farm and not working in the stables (i.e. family members).

There was a considerable decrease in the number of farms where CTX-M-gr1 was detected in dust during the study period. At the same time, the number of farms where CTX-M-gr1 carrying pigs were present hardly changed over time. However, a reduction in pig prevalence (in terms of number of pooled pig samples) from 18% till 12% was seen. The proportion of positive EDC's on a farm was lower when the number of positive pooled pig samples was lower. Therefore, it seems likely that the amount of CTX-M-gr1 in dust must have been smaller during the second sampling round. It seems reasonable to assume that a lower sample prevalence is accompanied by a lower load of CTX-M-gr1 in the farm environment. As a consequence, more non-detects of CTX-M-gr1 in dust might have occurred during the second sampling moment. Since the qPCR signals were close to the detection limit, CTX-M-gr1 levels in dust could have been too low to be detected. Possibly, this might have diluted the effect of air exposure, mostly due to the second sampling moment. The occurrence of non-detects is less

Table 3
Longitudinal univariate and multivariate analyses for CTX-M-gr1 carriage in pig farmers, family members and employees.

Determinant	No[a] or mean	Univariate OR (CI)	Bivariate OR (CI)		Multivariate OR (CI)
Presence of CTX-M-gr1 in farm dust					
Yes	34	12.4 (2.7–57.1)	5.3 (1.1–26.2)	9.2 (1.9–45.9)	3.5 (0.6–20.9)
No	222	Ref.	Ref.	Ref.	Ref.
Presence of CTX-M-gr1 in pigs					
Yes	67	14.4 (2.5–82.9)	7.2 (1.2–41.5)	12.8 (2.2–74.4)	7.4 (1.1–49.7)
No	195	Ref.	Ref.	Ref.	Ref.
Average number of hours working on pig farm per week (per hour)	24 ± 25	1.04 (1.01–1.07)	1.03 (1.00–1.06)	1.03 (1.00–1.06)	1.03 (1.00–1.06)
Per 10 h		1.4 (1.1–1.9)	1.3 (1.0–1.8)	1.4 (1.0–1.8)	1.3 (1.0–1.8)
Age (per year)	37 ± 17	1.02 (0.98–1.06)			
Gender					
Male	152	4.6 (0.8–25.9)			
Female	110	Ref.			
Smoking					
Yes	21	1.5 (0.2–14.9)			
No	239	Ref.			

Ref=reference category
[a] Based on total no of observations.

likely for human and pooled pig samples since these were analyzed by culturing after using pre-enrichment. This approach has a very low detection limit, lower than qPCR applied for the EDC's. However, the low CTX-M-gr1 prevalence in humans together with the sample size is creating an issue of power. Overall, more conclusive evidence for the association between ESBL carriage in pig farmers and presence of CTX-M-gr1 in dust may have been conducted by increased power and quantitative results. In addition, fixed static spot measurements might be underestimating real exposure compared to mobile equipment. The EDC's were placed out of reach from pigs, while air levels of ESBL might be higher in the direct surroundings of pigs.

Exposure to CTX-M-gr1 was measured by analyzing dust and pig feces. No measurements were performed on hands, mouth, face or in the nose. Therefore, the significance of the exact transmission route is hard to determine. In a German short report no ESBL-producing Enterobacteriaceae were detected in the nares of pig farmers (Fischer et al., 2016). This result is rather difficult to interpret, since intestinal carriage in pig farmers nor presence of ESBL-producing Enterobacteriaceae in animals or the environment was assessed. Since dust particles are relatively large, CTX-M-gr1 containing dust might be ingested instead of inhaled, which further complicates the differentiation between transmission routes. In two Dutch studies, personal inhalable dust samples were obtained from pig farmers. Both studies showed an average exposure to dust of approximately 2.6 mg/m^3 (Preller et al., 1995; Spaan et al., 2006). Inhalable dust is the dust fraction that can penetrate the respiratory organ. Because most of the particulates are relatively large, they will be deposited mainly in the (upper) airways and ingested after deposition. Assuming an average working day of eight hours and respiratory minute volume of 6 L/m, it can be estimated that pig farmers inhale ~7.5 mg dust per day on average. Since ESBL genes have been detected in dust, transmission of ESBL through dust in air is not unlikely. Quantitative information about viable ESBL-producing Enterobacteriaceae content of dust is required to use this information for a quantitative risk analysis and explore the plausibility of this hypothesis relative to other transmission routes such as uptake through hand mouth contact.

Since qPCR detects DNA directly, there was no information available on the viability of the Enterobacteriaceae that produce the CTX-M-gr1 enzymes. However, ESBL-producing Enterobacteriaceae have been cultured from dust samples in other studies (Garcia-Cobos et al., 2015; Hering et al., 2014). Furthermore, this study showed epidemiological associations between the presence of CTX-M-gr1 in dust, pigs and humans, but didn't take into account the molecular complexity of ESBL transmission fully, i.e. clonal transmission or horizontal gene transfer through plasmids. However, previous work has shown that clonal transmission is relevant between pigs and humans on farms, although horizontal transfer can occur as well (Dohmen et al., 2015; de Been et al., 2014).

Results leave open the possibility of transmission through air as a relevant transmission route potentially leading to human ESBL carriage. If these results are confirmed in additional studies, personal preventive measures for pig farmers might need to involve general hygiene measures (changing clothing, hand washing) as well as reducing airborne particulate exposure. In addition, air exposure to ESBL might be involved in human to human transmission in other (clinical) settings as well. For improved exposure assessment and to gain more insight in potential transmission routes, quantified personal exposure measurements should be implemented in future research.

5. Conclusions

Results from this study suggest the possibility of airborne transmission of CTX-M-gr1 from pigs to humans.

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Transparency declarations

None to declare.

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