

SHORT REPORT

Low-level antimicrobial resistance of Enterobacteriaceae isolated from the nares of pig-exposed persons

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SUMMARY

Extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae (ESBL-E) have recently emerged in livestock and humans. Therefore, this study assessed the carriage of Enterobacteriaceae in the anterior nares and associated antimicrobial resistance in pig-exposed persons. Nasal swabs were enriched in non-selective broth and then plated on MacConkey and ESBL-selective agars. Species was confirmed by matrix-assisted laser-desorption ionization–time-of-flight mass spectrometry (MALDI-ToF MS). Antimicrobial susceptibility testing was performed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Of 114 pig-exposed persons tested, Enterobacteriaceae were detected in the nares of 76 (66.7%) participants. The predominant species were *Proteus mirabilis* ($n = 17$, 14.9%), *Pantoea agglomerans* ($n = 13$, 11.4%), *Morganella morganii* ($n = 9$, 7.9%), *Citrobacter koseri* ($n = 9$, 7.9%), *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus vulgaris* (each $n = 8$, 7.0%). ESBL-E were not detected. Of all isolates tested, 3.4% were resistant against ciprofloxacin, 2.3% against gentamicin, 23.9% against trimethoprim-sulfamethoxazole and 44.3% against tigecycline. Despite the high prevalence of ESBL-E in livestock, pig-exposed persons did not carry ESBL-E in their nares. This finding is important, because colonization of the nasal reservoir might cause endogenous infections or facilitate transmission of ESBL-E in the general population.

Key words: ESBL, *Escherichia coli*, Germany, livestock, farmers.

Recently, extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae (ESBL-E) have been detected in livestock animals including poultry, pigs and cattle [1]. In Germany, ESBL-E, mostly belonging to the species *Escherichia coli* and *Klebsiella pneumoniae*, were detected on 44% and 56% of pig fattening

and breeding farms, respectively [1]. Moreover, ESBL-E were found in samples from 100% of broiler farms [1]. Of note, the occurrence of enterobacterial isolates that were able to produce carbapenemases in addition to ESBL was recently reported from a German poultry farm [2].

The emergence of ESBL-E in livestock may have important implications for humans. In the past years, intestinal carriage of ESBL-E has increased in the general population [3]. In Germany, the prevalence of rectal ESBL-E carriage reached 6.3% in

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healthy persons [3]. As enterobacteria (including ESBL-producing *E. coli* and *Klebsiella* spp.) are among the most important causes of community-acquired and endogenous healthcare-associated infections of humans, this development raises concern. However, it is still a matter of discussion if and to what extent increasing intestinal ESBL carriage in humans is due to ingestion of contaminated food items or animals in general [4].

For methicillin-resistant *Staphylococcus aureus* (MRSA), another facultative pathogen that has emerged in livestock husbandries during the past 10 years [5], multiple investigations found that persons with direct livestock exposure or frequent exposure to dust from livestock holdings are very often colonized with MRSA in the nares. Hence, in European countries, 24–100% of pig farmers, 37% of poultry farmers and 30–38% of cattle farmers are nasally colonized with the same MRSA genotypes that are also predominant in the animals [5]. Moreover, it was shown that short-time exposure to dust, only leads to transient nasal MRSA carriage, while regular exposure leads to stable colonization which persists when contact to the animals is interrupted during holidays [5].

Interestingly, there is little data assessing occupational contact with livestock as a risk factor for ESBL-E carriage. In a Dutch investigation, it was found that 33% of chicken farmers exhibited rectal ESBL-E carriage [6]. Although it is well known that Enterobacteriaceae are found not only in the intestinal tract, there is currently no information on nasal carriage of Enterobacteriaceae (and ESBL-E in particular) in farmers. Since this might be a very relevant factor facilitating transmission of ESBL-E in the general population (possibly even more relevant than rectal colonization) via hands and contaminated surfaces, we performed a nasal screening of persons with regular occupational pig exposure. We assessed the prevalence of nasal Enterobacteriaceae carriage, associated species and antimicrobial susceptibility patterns of these isolates.

The investigation was performed between July and December 2014. Pig-exposed persons were sampled (i) at educational training courses for pig farmers in the federal state of North Rhine-Westphalia, Germany and (ii) within the framework of an ongoing cross-sectional study on livestock farms in this federal state. The area (districts DEA33–38 according to the European Nomenclature for Territorial Units) is one of the regions with the highest densities of pig production in Europe.

Swabs (Transwab, Check Diagnostics, Germany) from the anterior nares were obtained from persons having contact with pigs. For every participant, we assessed profession, age, sex, hours of weekly animal contact and farm type using a standardized questionnaire.

Swabs were enriched in nutrient broth (Heipha Diagnostics, Germany, without antibiotic pre-selection) for 24 h at 36 °C and then streaked on MacConkey agar (Oxoid, Germany) and a colorimetric medium for ESBL-screening (bioMérieux, France), which were both incubated for 24 h. Phenotypically different colonies from MacConkey agar and all colonies from ESBL-screening agar were subcultured on Columbia blood agar. Species identification was performed using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-ToF MS, Bruker Daltonik, Germany). For all Enterobacteriaceae, antimicrobial susceptibility testing was done by agar disc diffusion as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) applying EUCAST clinical breakpoints for categorization of susceptible, intermediate and resistant isolates. If an isolate tested intermediate or resistant to either cefotaxime or ceftazidime, a double-disk diffusion test (MAST Laboratories, UK) including third-generation cephalosporins (cefepodoxime, ceftazidime, cefotaxime) with and without combination of clavulanic acid was performed to confirm ESBL production.

Pearson's χ^2 test and Mann–Whitney *U* test were used as a first description of statistical differences (SPSS v. 22, SPSS Inc., USA). Ethical agreement was obtained from the Chamber of Physicians of Westfalen-Lippe and the Medical Faculty of the University of Münster (2014-143-f-S). Written informed consent was obtained from each participant prior to enrolment.

Nasal swabs from 114 persons were analysed. Of these, 110 (96.5%) were men. Median age was 42 years (mean 42 years, range 18–77 years). Of all participants, 114/114 (100%) had contact with pigs, and 18/114 (15.8%) had contact with cattle in addition. The average exposure time to pigs was as follows: <20 h/week ($n = 40$ persons, 35.1%), 20–39 h/week ($n = 49$, 43.0%), ≥ 40 h/week ($n = 25$, 21.9%). For all participants pig contact was due to work on pig fattening farms ($n = 54$, 47.4%), on farrow-to-finishing farms ($n = 47$, 41.2%) or on farrow-to-weaning farms ($n = 7$, 6.1%). Six (5.3%) participants were not farmers, but worked as pig-care veterinarians or farm consultants for the optimization of the pig production system.

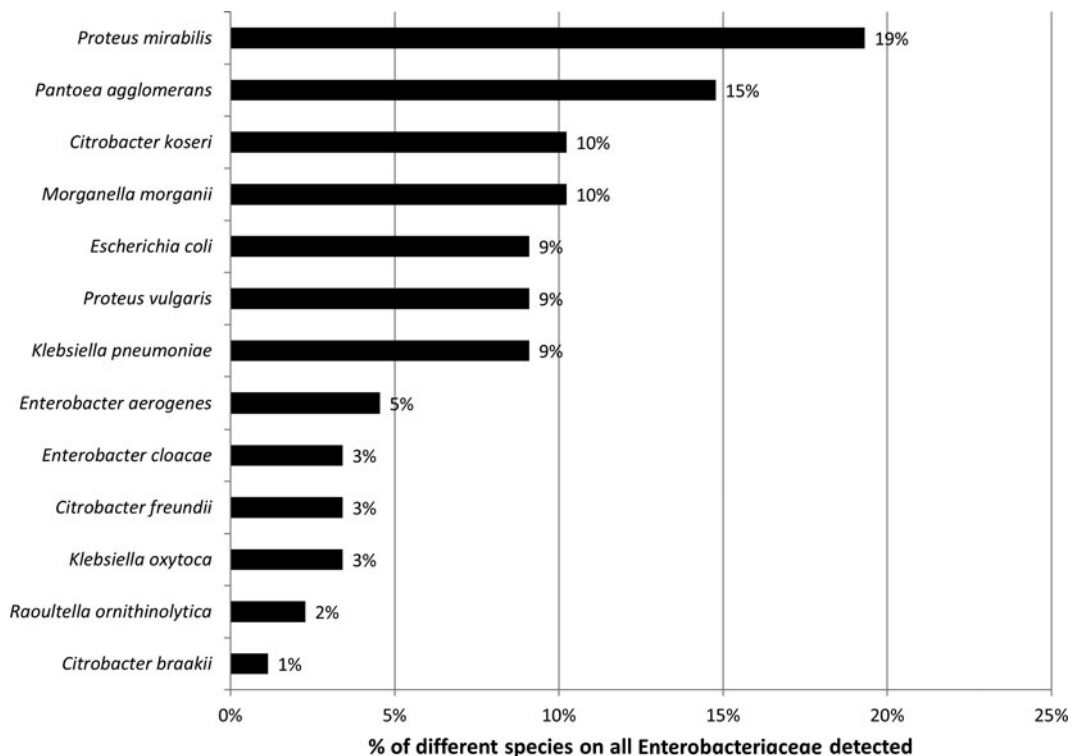


Fig. 1. Proportion of different species in Enterobacteriaceae ($n = 88$ isolates) from the anterior nares of 76 pig-exposed individuals.

Enterobacteriaceae were detected in the nares of 76/114 participants (66.7%). Weekly pig contact time did not influence the nasal prevalence of Enterobacteriaceae with a prevalence of 0.7 for <20 h/week (28 persons colonized vs. 12 persons not colonized), 0.64 for 20–39 h/week (32 vs. 17 persons), and 0.65 for ≥ 40 h/week (16 vs. 9 persons, $P = 0.85$). Carriage of Enterobacteriaceae was 72% in persons working on farrow-to-finishing farms, 61% on fattening farms and 57% on farrow-to-weaning farms, and was 83% in the six persons who were not farmers ($P = 0.48$). The median age of persons with Enterobacteriaceae carriage vs. persons without carriage (44 years vs. 38 years) did not differ significantly ($P = 0.11$). Farmers who had contact with cattle in addition to pigs had enterobacterial carriage rates similar to those who were only pig-exposed (13/18 vs. 63/96, $P = 0.79$).

Seventeen (14.9%) of the 114 different participants carried *Proteus mirabilis*, 13 (11.4%) *Pantoea agglomerans*, nine (7.9%) *Morganella morganii*, nine (7.9%) *Citrobacter koseri*, eight (7.0%) *K. pneumoniae*, eight (7.0%) *E. coli*, eight (7.0%) *Proteus vulgaris*, four (3.5%) *Enterobacter aerogenes*, three *Enterobacter cloacae* complex (2.6%), three *Citrobacter freundii* (2.6%), three *Klebsiella oxytoca* (2.6%), two

Raoultella ornithinolytica (1.8%) and one participant *Citrobacter braakii* (0.9%).

Twelve of the 76 persons (15.8%) carrying Enterobacteriaceae in the anterior nares, carried two different enterobacterial species [*P. mirabilis*/*K. pneumoniae* ($n = 3$), *P. vulgaris*/*E. coli* ($n = 2$), *P. mirabilis*/*E. coli* ($n = 2$), and *E. aerogenes*/*C. braakii*, *M. morganii*/*K. oxytoca*, *M. morganii*/*K. pneumoniae*, *P. mirabilis*/*C. koseri*, *P. vulgaris*/*K. pneumoniae* (each $n = 1$)].

Overall, we retrieved 88 nasal bacterial isolates with the species distribution shown in Figure 1. The antimicrobial susceptibility pattern of all isolates belonging to different species is shown in Table 1. Resistance against carbapenems was not detected. Of all Enterobacteriaceae, only one isolate of *M. morganii* and one isolate of *C. koseri* grew on ESBL screening agar. These isolates were intermediate or resistant to third-generation cephalosporins indicative of ESBL production. However, ESBL production was not confirmed by double-disk diffusion test for these two isolates. The overall rates of resistance of all isolates tested were: ciprofloxacin (3.4%), gentamicin (2.3%), trimethoprim-sulfamethoxazole (23.9%) and tigecycline (44.3%), respectively. However, tigecycline was considered intrinsically resistant for all

Table 1. Antimicrobial susceptibility of Enterobacteriaceae isolated from the anterior nares of pig-exposed persons

Bacterium	Proportion of susceptible isolates (%)						
	CTX	CTZ	MER	CIP	SXT	GEN	TGC
<i>Proteus mirabilis</i> (n = 17)	100	100	100	94.1	64.7	94.1	0
<i>Pantoea agglomerans</i> (n = 13)	100	100	100	100	100	100	100
<i>Morganella morganii</i> (n = 9)	88.9	88.9	100	88.9	66.7	100	0
<i>Citrobacter koseri</i> (n = 9)	88.9	88.9	100	100	77.8	100	100
<i>Klebsiella pneumoniae</i> (n = 8)	100	100	100	100	100	100	75
<i>Escherichia coli</i> (n = 8)	100	100	100	87.5	62.5	100	100
<i>Proteus vulgaris</i> (n = 8)	100	100	100	100	50	88.9	0
<i>Enterobacter aerogenes</i> (n = 4)	100	100	100	100	75	100	75
<i>Enterobacter cloacae</i> (n = 3)	100	100	100	100	100	100	100
<i>Citrobacter freundii</i> (n = 3)	100	100	100	100	66.7	100	100
<i>Klebsiella oxytoca</i> (n = 3)	100	100	100	100	100	100	100
<i>Raoultella ornithinolytica</i> (n = 2)	100	100	100	100	50	100	0
<i>Citrobacter braakii</i> (n = 1)	100	100	100	100	100	100	100

CTX, Cefotaxime; CTZ, ceftazidime; MER, meropenem; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; GEN, gentamicin; TGC, tigecycline.

Percentage of susceptible isolates for the respective antibiotic. Intermediate test results are counted as non-susceptible. Table sorted by numbers of isolates (n) tested per species.

Morganella spp. and *Proteus* spp. isolates according to EUCAST test standards. Tigecycline resistance in isolates of enterobacterial species other than *Proteus* spp. and *Morganella* spp. was only 9.3% (5/54).

Data on the occurrence of Enterobacteriaceae in the anterior nares is rare. Single reports found that this affects 4–38% of the population [7–9]. We are not aware of any reports on occupation-related ESBL-E carriage of the nares. Hence, this study was planned to evaluate whether the nares represent a potential reservoir for ESBL-E, which is neglected by current investigations. As multiple studies found ESBL-E in dust samples from livestock farms [10], we hypothesized that inhalation of dust directly exposes the anterior nares, which could lead to colonization or contamination with ESBL-E. Besides the classically considered oral transmission route facilitating intestinal colonization this may represent an additional route of transmission, which has not yet been studied.

Indeed, we found that 67% of farmers and other pig-exposed persons carried Enterobacteriaceae in their nares. This prevalence was higher than expected compared to the few data assessments elucidating this issue: In Switzerland, *Klebsiella* spp., *E. coli*, *E. cloacae* and *Citrobacter diversus* were isolated from nasal specimens in 38% of healthy men [7].

However, when considering the antibiotic susceptibility profiles of the nasal isolates, we did not identify any person who carried ESBL-E. This was surprising, because ESBL-E was detected in high frequencies in

the environment of pig farms in Germany [1] and rectal ESBL-E carriage involves up to 6.3% of persons in the human general population [3]. However, ESBL-E distributed both on farms and in faecal samples from the general population are mostly *E. coli* or *K. pneumoniae* or *K. oxytoca* [1, 3]. These species were also detected in the nares of participants included in this study, but represented only 22% (n = 19 isolates) of the nasal Enterobacteriaceae. The majority of isolates belonged to other species such as *Proteus* spp., *Pantoea* sp., *Citrobacter* spp. or *Morganella* spp. Hence, it can be concluded that ESBLs were not found in these four predominating species and that ESBLs were not detected in a limited number of isolates associated with ‘classical’ ESBL-bearing species tested. This argues against a wide distribution of ESBL-E as nasal colonizers, which is in contrast to one study suggesting rather high (33%) rectal ESBL-E carriage in Dutch poultry farmers [6]. For the two isolates in which cefotaxime and ceftazidime resistance was detected (*Morganella* spp. and *Citrobacter* spp.), phenotypic confirmatory tests indicated that this resistance was most likely not due to ESBL production, but related to other mechanisms such as *ampC* β -lactamase production, which is known to be frequent in isolates of these two species [11].

Resistance to antibiotics other than third-generation cephalosporins also differed from clinical data: nasal Enterobacteriaceae showed very low resistance rates to ciprofloxacin (3.4% of all isolates) and to

gentamicin (2·3%). This is in contrast to isolates from diagnostic specimens related to infections. German national surveillance data demonstrated that ciprofloxacin and gentamicin resistance involved 9·0% and 4·1% of isolates in 2013 in *K. pneumoniae*, or 16·5% and 5·4% in *E. coli*, respectively (<http://ars.rki>; ARS.RKI reference data for ambulatory care, 2013, data assessed 16 December 2014). Resistance to trimethoprim-sulfamethoxazole was more frequent in nasal enterobacterial isolates (23·9%) and was comparable to susceptibility levels of clinical isolates included in the reference database (11·5% of *K. pneumoniae*, 25·3% for *E. coli*). Of note, carbapenem resistance was not detected, which is important considering the first report of carbapenemase-producing Enterobacteriaceae in German livestock holdings [2].

This study has three important limitations. First, we have no information on the occurrence of ESBL-E in animals, dust or manure samples from the farms, where the persons included in this study were working. However, other recent studies performed in the same geographical area have demonstrated that almost all (85%) pig farms were affected by ESBL-E [10]. Second, we did not simultaneously assess rectal carriage of ESBL-E in the farmers. We focused on nasal carriage since we considered this body site as highly relevant for the transmission to other persons (not working with livestock) via the hands or indirectly via contamination of surfaces (in households, public transport, healthcare facilities, etc.). However, there is a need to address the topic of occupation-related rectal ESBL-E carriage in farmers or veterinarians in further studies, as there is only very limited data regarding this issue and rectal colonization can be a source for endogenous infections. The third limitation is that the study design does not allow for differentiating between persistent colonization and short-term contamination or carriage of the nares. To answer this question consecutive sampling in a cohort of pig-exposed persons over time is needed.

In conclusion, we found high rates of nasal carriage of Enterobacteriaceae in German pig farmers. However, ESBL-E was not detected. This data suggests that, in contrast to MRSA, the nares are not a major reservoir for carriage of ESBL-E in pig-exposed persons.

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DECLARATION OF INTEREST

None.

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