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A geospatial analysis of flies and the spread of antimicrobial resistant bacteria

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ABSTRACT

Livestock is often colonized with ESBL-producing *Enterobacteriaceae* (ESBL-E) and *Staphylococcus aureus*. There is a risk that flies spread antimicrobial resistant bacteria from livestock to humans. Here, we screened flies from urban and rural areas near the city of Münster, Germany, for the presence of ESBL-E and *S. aureus* and compared molecular characteristics of these isolates with those isolated from humans in the same region.

In total, 1346 single flies were grinded and cultured overnight in BHI-broth. The broth was cultured on Columbia blood agar and selective media for the detection of *S. aureus* and ESBL-E. Human isolates from routine diagnostics at the University Hospital Münster were used for comparison. Antimicrobial susceptibility, phylogroups (*Escherichia coli*), *spa* types/multilocus sequence types (*S. aureus*) and selected antimicrobial resistance genes were determined for each isolate. GPS data of the sampling sites were used to map flies carrying ESBL-E and *S. aureus*.

Overall, *Serratia fonticola* (123/1346; 9.1%) was the most prevalent ESBL-E in flies, followed by *E. coli* (44/1346; 3.3%). A high proportion of flies was positive for ESBL-producing *E. coli* (15.0–22.2%) in a rural area. CTX-M-1 was the most prevalent beta-lactamase in *E. coli* (38.6%). One livestock-associated methicillin resistant *S. aureus* (LA-MRSA, t011/ST398) was found in the city centre of Münster. Overall, a substantial part of ESBL-producing *E. coli* and *S. aureus* from flies and humans showed a similar genetic background.

In conclusion, flies can carry ESBL-E and LA-MRSA in urban and rural areas. The similar genetic background of isolates from humans and flies points towards a common source.

1. Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase- and carbapenemase-producing *Enterobacteriaceae* (ESBL-E, CP-E) are amongst the major challenges in human healthcare. Huge efforts were undertaken to prevent the spread of these "super bugs". However, effective infection control is hampered by reservoirs that are difficult to reach. For instance, livestock, such as pigs, is frequently colonized with ESBL-E (30.2%) and MRSA (20.7%) (Schmithausen et al., 2015).

In Germany, livestock-associated MRSA (LA-MRSA) mostly belong to multilocus sequence typing (MLST) clonal complex 398 (CC 398), which has crossed the species barrier causing up to one third of all human MRSA infection/colonization in some regions (Köck et al., 2013; van Alen et al., 2016). While direct contact to livestock is the major risk factor to acquire LA-MRSA, inhalation of contaminated dust can increase LA-MRSA colonization rates in exposed personnel (Bos et al., 2016). Colonization of livestock with ESBL-E can be associated with ESBL-E colonization in humans pointing towards a direct transmission (Dohmen et al., 2015; Kluytmans et al., 2013). Recent studies reported ESBL-E on flies in the vicinity of livestock farms in the Netherlands and Germany (Blaak et al., 2014; von Salviati et al., 2015). Colonization of flies with ESBL-E might promote the spread of antibiotic resistant bacteria from farms to urban places. This could be feasible as flight

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distance of house flies (*Musca domestica*) ranges between 5 and 7 km.

The Northwestern part of Germany (including the area around the city of Münster) has the highest density of pigs (between 600 and >900 pigs/ha) and cattle (150 and >200 cattle/ha, http://www.atlas-agrarstatistik.nrw.de) in Germany. Our hypothesis was that flies ingest ESBL-E and *S. aureus* in the livestock environment and disperse it even in urban areas.

The objective of the study was to screen flies from the city of Münster and the rural region in its surrounding for ESBL-E and *S. aureus* and to compare these isolates with ESBL-E and *S. aureus* isolated from humans.

2. Materials and methods

2.1. Ethical approval

Ethical approval to report patient-related data was granted by the Ethical Committee of the University of Münster (2016-008-f-S).

2.2. Study area

The study area comprised urban (city of Münster) and rural areas (villages, farmland) around Münster (Fig. 1). Münster has approximately 300,000 inhabitants and is the cultural capital of the region of Westphalia in Northwestern Germany. The North-South extension of the study area was 15 km, the East-West extension was 18 km resulting in an overall size of approximately 270 km^2 (Fig. 1).

2.3. Flies

Flies (Diptera) were collected between April and July 2015 in a gaze trap placed over a bait containing proteins, lipids and minerals (Feldner, Waldsee, Germany). Flies were killed in 50 ml tubes filled with 10 ml 70% ethanol. Ethanol sanitizes the body surface of the flies to rule out cross-contamination in the trap without killing the intestinal microbiome (Gupta et al., 2012). Not only the exoskeleton but also more importantly the gastrointestinal tract is the source of pathogen transmission from flies to humans (i.e. regurgitation, faecal deposition on food items) (Graczyk et al., 2001).

Flies were dried on silica gel (2–5 mm, Carl Roth, Karlsruhe, Germany) to remove the ethanol off the exoskeleton and were cultured within 2 h. Species identification of flies was done phenotypically.

Single flies were grinded with a sterile pestle in 1.5 ml tubes and incubated in 1 ml BHI-broth overnight at 35 °C. 10 μl of the broth suspension were cultured on Columbia blood agar and selective media for the detection of *S. aureus* (SAID, bioMérieux, Marcy l'Etoile, France) and ESBL-E (chromID, bioMérieux).

For each sampling point, environmental data (i.e. sky cover, presence of refuse dump, decomposing organic matters, livestock faeces in a 10 m radius), the setting (urban, rural) and weather conditions (temperature, humidity, windforce, air pressure, sunshine hours/day, as reported by the "Deutscher Wetterdienst" for the region Münster/Osnabrück) were recorded.

2.4. Bacterial isolates from humans

Bacterial isolates from humans were consecutively collected in the routine microbiology laboratory of the University Hospital Münster (January–May 2015). All isolates were recovered from clinical and screening specimens of hospital in- and outpatients. Only one isolate per patient was included. All patient-related data (date of birth, sex, date of admission, infection/colonization) were extracted from the laboratory software.

2.5. Identification

Species identification of isolates from flies and human was done using MALDI-TOF (microflex LT, Bruker Daltonics, Bremen, Germany). Species confirmation of *Enterobacteriaceae* was done using VITEK2 automated systems (bioMérieux). Species of *S. aureus* was confirmed by the PCR-detection of the *S. aureus* specific thermostable nuclease *nuc* (Brakstad et al., 1992).

2.6. Antimicrobial resistance

Antimicrobial susceptibility testing was done with VITEK2 automated systems (bioMérieux) using EUCAST clinical breakpoints. The ESBL-phenotype in *Enterobacteriaceae* was confirmed by the double disk diffusion test (Mast discs, Mast Diagnostics, Bootle, UK). In addition, ESBL-E were screened for the presence of $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM} and $bla_{CMY\text{-}2}$ (Monstein et al., 2007; Souna et al., 2014). PCR amplicons were sequenced to type the respective *bla* gene. Carbapenem resistance was assessed by the isothermal amplification of genes encoding KPC, VIM, NDM, and OXA-48- and OXA-181-group carbapenemases (eazyplex SuperBug CRE, Amplex, Gießen, Germany).

The methicillin-resistance in *S. aureus* was confirmed by PCR targeting *mecA* (Murakami et al., 1991).

2.7. Genotyping

ESBL-producing *Escherichia coli* were phylogrouped following the revised scheme by Clermont et al. (Clermont et al., 2013). Presence of clonal group A among isolates belonging to phylogroup D was tested using a PCR-based assay (Johnson et al., 2004). A minimum spanning tree was constructed for all ESBL-producing *E. coli* using SeqSphere$^+$ (Ridom, Münster, Germany) based on categorical data derived from resistance against piperacillin/tazobactam, cefotaxime, ceftazidime, ertapenem, ciprofloxacin, trimethoprim/sulfamethoxazole, CTX-M- and TEM-type as well as *E. coli* phylogroups.

All *S. aureus* were *spa* typed and one isolate per *spa* type of each group (flies, humans) was randomly selected for MLST (Enright et al., 2000; Mellmann et al., 2006).

2.8. Mapping

GPS data from all sampling points were collected and the proportion of ESBL-E colonized flies at each sampling point was mapped to "Google maps" (https://maps.google.de) using "R" statistical software and the "plotGoogleMaps" (version 2.2) package.

2.9. Statistical analysis

Categorical variables were compared with chi^2 or Fisher's exact test and the Odds Ratio was calculated to assess the strength of association. The significance level was set at 0.05. Analyses were done with "R" statistical software.

3. Results

3.1. Sampling

In total, 1346 flies including *Musca domestica* (n = 895), *Calliphora* sp. (n = 447) and others (n = 4) were collected at 80 sampling sites (Fig. 1).

The sampling sites were rural (n = 32, 40%) or urban (n = 48, 60%). At some sampling sites, refuse dumps (n = 22, 27.5%), decomposing organic matters (n = 34, 42.5%) and animal faeces (n = 17, 21.2%) were seen within a 10 m radius around the flytrap. A mean number