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# ANTIBIOTIC RESISTANT BACTERIA IN RODENTS, BIRDS, AND INSECTS

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**Abstract** The aim of this study was to find out whether pests on the Utrecht University campus and environs were vectors for ESBL/AmpC-producing bacteria and/or methicillin resistant *Staphylococcus aureus* (MRSA). Samples were collected on and around campus in educational buildings, an aviary, the university pig farm, botanical gardens, the university hospital, and community buildings. Samples were analysed for the presence of ESBL/AmpC-producing *Enterobacteriaceae* and MRSA and for susceptibility to five antibiotics. Of the samples 5/78 (6.4%) were positive for ESBL *E. coli* and/or MRSA. All fly samples and bird samples originated on the farm. Antibiotic susceptibility testing revealed that one of the isolates from flies was confirmed ESBL-producing *E. coli* according to CLSM guidelines. In conclusion, it is possible that pests on the university farm could be a vector for ESBL- producing *E. coli* and MRSA.

**Key words** ESBL, AmpC, *E. coli*, MRSA, flies.

## INTRODUCTION

Antibiotic resistant bacteria represent an increasing problem for public health and animal health because infections caused by them are more difficult to treat (Adegoke et al., 2016). Extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamases are enzymes produced by resistant bacteria to counteract the effect of antibiotics (Pitout & Laupland, 2008). Resistance to methicillin has been found in *Staphylococcus aureus* (Tiemersma et al., 2004). Strict hygiene measures and limiting the use of antibiotics are important strategies for limiting the spread of resistant bacteria. However, rodents, flies and birds travel between locations and may act as vectors for transmission of bacteria, including resistant strains (Burt et al., 2012; Davies et al., 2016; Guenther et al., 2010; Meerburg et al., 2007; Veldman et al., 2013). The aim of this study was to find out whether pests collected on a university campus could be vectors for the ESBL/AmpC-producing *Enterobacteriaceae* and methicillin resistant *Staphylococcus aureus* (MRSA).

## MATERIALS AND METHODS

Samples were collected on and around the Utrecht University campus during May – November 2014. Locations included campus educational buildings, the university pig farm, botanical gardens, an aviary, the university hospital, and various houses and community buildings in Utrecht. Snap-traps and flypapers placed on the walls or under furniture were used to trap rodents, flies and cockroaches. Fly samples were pooled samples of all flies attached to a fly paper on a particular day. Other small animals found dead such as a wood mouse, a shrew and birds were also collected and examined.

Detection of ESBL/AmpC-producing *Enterobacteriaceae* was carried out using the method of Dierikx et al. (2013) using selective enrichment and plating out onto MacConkey agar (Oxoid) and TBX agar (Oxoid), both containing 1mg/L cefotaxime. For MAC-CTX: sharply defined pink colonies were scored as *E. coli*; slimy pink colonies were scored as *Klebsiella*; other pink colonies were scored as *Enterobacter*. For TBX-CTX green colonies were scored as *E. coli*. All isolates were tested for the indole reaction and oxidase reaction. MRSA was determined according to the method of Graveland et al. (2009) by selective enrichments and plating out onto Brilliance 2 MRSA agar (Oxoid). Dark purple colonies on agar typical for MRSA were subjected to coagulase and catalase tests.

For suspected ESBL/AmpC-producers susceptibility to five antibiotics was determined with the BD Sensi Disk® method (Figure 1). A fresh culture was adjusted to turbidity of 0.5 McFarland in physiological saline and spread onto a Müller-Hinton agar plate (Oxoid). Five discs containing antibiotics were dispensed by the Sensi Disk® apparatus and incubation was 18 hours at 37°C. Inhibition zones were measured with a digital calliper and the phenotype (ESBL- or AmpC-producing) was determined by reference to breakpoints according to the CLSI standards (CLSI, 2010). Strains *E. coli* ATCC 25922 and *Klebsiella pneumonia* SHV-18 were used as controls. All isolates were stored at -80°C for later confirmation by PCR analysis.

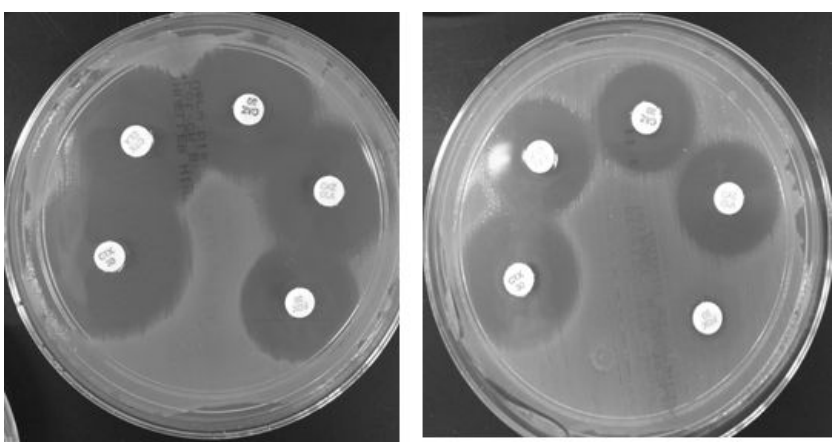

**Figure 1.** The assessment of antibiotic resistance phenotype using BD Sensi Disk®. Left, A bacterial strain sensitive to all five antibiotics; Right: A less sensitive strain.

## RESULTS AND DISCUSSION

Preliminary identification, pending PCR confirmation, indicates that 5/78 (6.4%) of the samples were positive for ESBL *E. coli* and/or MRSA, as presented in Table 1.

**Table 1.** Preliminary identification of antibiotic resistant bacteria in samples collected on and around the university campus May – July 2014.

| Sample | n | ESBL/AmpC *E. coli* | ESBL/AmpC *Klebsiella* | ESBL/AmpC *Enterobacter* | MRSA |
|---|---|---|---|---|---|
| House mice | 34 | - | - | - | - |
| Birds | 21 | 1 | - | - | 1 |
| Flies (pool) | 20 | 2 | - | - | 2 |
| American cockroach | 1 | - | - | - | - |
| Common shrew | 1 | - | - | - | - |
| Wood mouse | 1 | - | - | - | - |

Birds collected were mostly tree sparrows but also 1 jackdaw, 2 starlings, 2 blackbirds and a greenfinch. The pooled fly samples included mostly the housefly, lesser housefly and drain flies. All of the samples found positive (three fly samples and two bird samples) originated on the university pig farm. Antibiotic susceptibility testing on the isolated *Enterobacteriaceae* confirmed that one of the isolates from a pooled fly sample had an ESBL-phenotype according to CLSM guidelines, as shown in Table 2.

**Table 2.** Bacterial isolate from flies classified as ESBL-producing phenotype according to CLSI guidelines (CLSI, 2010).

| Sample | Bacterial species | Inhibition zone (diameter in mm) per antibiotic | | | | |
|---|---|---|---|---|---|---|
| | | cefotaxime | cefotaxime + clavulanic acid | ceftazidime | ceftazidime + clavulanic acid | cefoxitin |
| Flies (pool) | *E. coli* | 7 | 13 | 13 | 30 | 26 |

## DISCUSSION

Our findings suggest that flies and birds on the university farm may be vectors for MRSA and ESBL-producing *E. coli*. These results confirm those of previous studies in which flies on pig and poultry farms were found positive for ESBL-producing *E. coli* (Blaak et al., 2014; Von Salviati et al., 2015). Samples of houseflies caught in a hospital were shown to be carriers of *Enterobacteriaceae* 15/67 (22.4%), although the presence of ESBL/AmpC phenotypes were not determined (Davies et al., 2014). Our findings for birds are comparable with the low prevalence of MRSA in wild birds found in central Europe (0.3%) (Konicek et al., 2016). An earlier Dutch study of faecal swab samples from dead wild birds positive for ESBL/AmpC phenotype *E. coli* found a prevalence of 65/414 (15.7%) (Veldman et al., 2013), which is higher than found in the present study. This difference could be due to the fact that our bird samples concerned chiefly starlings and sparrows whereas the study by Veldman et al. took samples from waders and seabirds, which come into contact with contaminated surface waters.

In conclusion, flies and birds on the university farm may be a vector for ESBL-producing *E. coli* and MRSA. Whether the bacteria originate outside the campus or are picked up from animals on the university farm has yet to be determined.

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