Insects represent a link between food animal farms and the urban environment for antibiotic resistance traits

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Antibiotic resistant bacterial infections result in higher patient mortality rates, prolonged hospitalization, and increased healthcare costs. Extensive use of antibiotics as growth promoters in the animal industry represents great pressure for evolution and selection of antibiotic resistant bacteria on farms. Despite growing evidence showing that antibiotic use and bacterial resistance in food animals correlate with resistance in human pathogens, the proof for direct transmission of antibiotic resistance is difficult to provide. In this review, we make a case that insects commonly associated with food animals likely represent a direct and important link between animal farms and urban communities for antibiotic resistance traits. Houseflies and cockroaches have been shown to carry multi-drug resistant clonal lineages of bacteria identical to those found in animal manure. Furthermore, several studies demonstrated proliferation of bacteria and horizontal transfer of resistance genes in the insect digestive tract as well as transmission of resistant bacteria by insects to new substrates. We propose that insect management should be an integral part of pre- and post-harvest food safety strategies to minimize spread of zoonotic pathogens and antibiotic resistance traits from animal farms. Furthermore, the insect link between the agricultural and urban environment presents an additional argument for adopting prudent use of antibiotics in the food animal industry.
Antibiotic resistance has become a serious global public health problem; reduced effectiveness of antibiotics results in higher patient mortality rates, prolonged hospitalization, and increased healthcare costs (1-4). The annual cost to the U.S. health care system from antibiotic resistant infections is estimated between $21 and $34 billion which includes more than 8 million additional hospital days (5). In a recent report entitled “Antibiotic resistance threats in the United States” published by Centers for Disease Control and Prevention (6), it is estimated that 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die as a direct result of these infections in the United States each year. This problem has been recognized in the clinical community and efforts for more prudent use of antibiotics are under way (7, 8).

**ANTIBIOTICS AND ANIMAL AGRICULTURE**

Antibiotic producing and antibiotic resistant bacteria are commonly found in various soil environments (9). However, extensive use of antibiotics, especially as growth promoters, in the animal industry has resulted in great pressure for evolution and selection of antibiotic resistant bacteria in the food animal environment (10-15). As a result, food animals and animal production environments have become reservoirs for antibiotic resistant strains that are released to the environment in animal feces and then potentially spread to surrounding habitats (16-23). Despite a growing body of evidence that antibiotic use in animals correlates with resistance in human pathogens (24-30), direct proof for movement of antibiotic resistance traits between the agricultural and urban environment is difficult to provide. Nonetheless, all countries in the European Union adopted the precautionary principle and banned the use of all antibiotics as growth promoters in animal agriculture in 2006 (31). Although the U.S. Food and Drug
Administration has recently taken the first step to reduce the use of medically important
antibiotics for enhancing animal growth (32), with one notable exception (ban on use of
fluoroquinolones in poultry in 2005) (33), no such policy has been implemented in the United
States yet, partly because of the argument made by the food animal industry pointing to the lack
of data that demonstrate a direct connection between animal farms and public health for
antibiotic resistant strains.

ANTIBIOTIC RESISTANCE AND INSECTS

While insects are a numerous and diverse group in many environments, their potential to
play a role in the ecology of antibiotic resistance traits has not been recognized, with a few
exceptions. Tian et al. (34) used a metagenomic approach to screen for antibiotic resistance in
bacteria from the gut of honeybees (Apis mellifera L.) and showed an accumulation of mobile
genes coding for resistance to tetracycline and oxytetracycline which were closely related to genes
from human pathogenic strains. Allen et al. (35) reported several antibiotic resistance
determinants from the midgut bacteria of the gypsy moth larvae (Lymantria dispar L.) and Lowe
and Romney (36) authored a highly publicized but rather limited study where they isolated
vancomycin resistant Enterococcus faecium (VRE) and methicillin resistant Staphylococcus
aureus (MRSA) from five human bedbugs (Cimex lectularius L.) in Vancouver, Canada.
Antibiotic resistant enterococci were also isolated from stored-product beetles collected from a
feed mill, a grain storage silo, and a retail store (37). A few other studies showed the Mexican
fruit flies (Anastrepha ludens L.) from laboratory-reared colonies (38), the oil fly (Helaeomyia
petrolei L.) larvae from asphalt seeps (39), and cockroaches (Periplaneta americana L. and
Blattella germanica L.) from food-handling facilities, households, and a hospital (40, 41) as carriers of antibiotic resistance traits.

Livestock insects and food-borne pathogens

With continuing urban expansion into agriculturally-zoned areas, the concern in the public health community about insect pests, such as flies and cockroaches, associated with animal productions, has increased because of the capacity of these insects to spread zoonotic food-borne pathogens (reviewed in 42, 43). For example, in Japan, houseflies (Musca domestica L.) were implicated in transmission of Escherichia coli O157:H7 from reservoir animals to other animals and humans (44). Houseflies and blow flies collected from dumpsters of urban restaurants were shown to carry Cronobacter spp., Salmonella spp., and Listeria monocytogenes (45). Alam and Zurek (46) reported E. coli O157:H7 from the digestive tract of houseflies collected in a cattle feedlot from feed bunks and cattle-feed storage and suggested that houseflies in cattle farms play a role in the dissemination of this food-borne pathogen. In the same study, they also showed that 95% of houseflies sampled were positive for fecal coliforms in their gut in the level ranging from 3.0 x 10^1 to 3.0 x 10^6 CFU/fly. The large number of fecal coliforms in houseflies indicates a potential to harbor other zoonotic pathogens. In a subsequent study, calves were individually exposed for 48 h to houseflies that were orally inoculated with a mixture of four strains of nalidixic acid-resistant E. coli O157:H7 (Nal^R EcO157) (47). Rectal sampling of fresh cattle feces showed the presence of Nal^R EcO157 strains until the end of the study (11 days after fly exposure), with a concentration as high as 10^6 CFU/g, demonstrating the capability of houseflies not only to carry this pathogen but actually transmit E. coli O157:H7 to the cattle digestive tract through contamination of feed and water and/or direct contact with animals (47).

Livestock insects as carriers of antibiotic resistance traits
a) **Food animal environment.** Many antibiotics used as growth promoters are poorly absorbed in the animal digestive tract and are therefore released to the environment in animal feces (19, 20, 22). At the same time, organic waste in and around animal productions provides an excellent habitat for the development of insects such as houseflies and stable flies (*Stomoxys calcitrans* L.). In addition, some animal facilities (e.g. confined swine productions) provide a new and ideal habitat for insects that are typically considered urban pests, particularly German cockroaches (*Blattella germanica* L.) (48). As a consequence, the likelihood that the livestock insect pests acquire and carry bacteria with antibiotic resistance traits is high (Table 1). Insects such as houseflies and German cockroaches have a great potential to disseminate fecal bacteria because of their developmental habitat, unrestricted movement, mode of feeding, strong attraction to human food, and synanthropic nature (42, 43).

The first report on the potential of flies to acquire antibiotic resistant *Escherichia coli* from food animals (swine and cattle) was published in 1990 by Marshall et al. (49). The Australian bush fly (*Musca vetustissima*) was reported as a carrier of multi-drug resistant *Salmonella* sp. and *Shigella* sp. on a cattle farm and in urban areas in Australia (50). Literak et al. (51) found that houseflies from two swine operations in the Czech Republic carried *E. coli* with the same antibiotic resistance patterns and genotypic profiles as those from swine manure. The same group isolated *E. coli* with the same antibiotic resistance phenotypes and genetic backgrounds from both, flies and manure, from a dairy farm (52). Usui et al. (53) sampled flies (houseflies and false stable flies) and cattle feces from a cattle farm in Japan and found 14.3% (13/91) of houseflies, 10.3% (7/68) of false stable flies and 7.5% (7/93) of cattle feces were positive for a third-generation cephalosporin resistant strains of *E. coli* that contained transferrable plasmids encoding the *bla*CTX-M-15 gene. Pulsed-field gel electrophoresis (PFGE)-
based genotypic analysis indicated that the flies carried the same *E. coli* clones that were detected in cattle feces. Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* were also isolated from houseflies and blowflies from two poultry farms in Netherlands, and the genetic background of these isolates was identical to that of ESBL-producing *E. coli* isolates from the chicken manure (54). In a study from poultry farms in the U.S., houseflies collected at and near confined chicken operations carried antibiotic resistant enterococci that matched genotypically and phenotypically those from poultry litter (55).

Our research team has focused on the association of insects and antibiotic resistant enterococci in several studies. We compared enterococci from houseflies, German cockroaches, and pig feces from two commercial swine operations in Kansas and North Carolina (56). Enterococci were detected in the majority (>89%) of all samples and multi-drug (mainly tetracycline and erythromycin) resistant enterococci were common from all three sources. Genotypic PFGE analysis of selected *Enterococcus faecalis* and *E. faecium* isolates demonstrated that cockroaches and houseflies shared the same enterococcal clones that were detected in the swine manure, indicating that insects acquired enterococci from swine manure (56). The above studies demonstrate that insects on farms commonly carry the same clonal lineages of multi-drug resistant bacteria that are found in animal feces.

b) Urban environment. Previous studies using fly traps and multilocus DNA fingerprinting reported random dispersal (up to 125 km) of houseflies from poultry and cattle farms (57, 58). We screened the digestive tract of houseflies collected at five fast-food restaurants in a town in northeastern Kansas and found that antibiotic resistant enterococci were common (59). The majority (97%) of flies were positive for enterococci with a mean CFU of $10^3$ per fly. *Enterococcus faecalis* was found as the most abundant species (88.2%) harboring
resistance to tetracycline (66.3% of isolates), erythromycin (23.8%), streptomycin (11.6%),
ciprofloxacin (9.9%), and kanamycin (8.3%). In addition, the conjugative transposon Tn916 and
members of the Tn916/Tn1545 family that are frequently involved in the horizontal transfer of
antibiotic resistance traits during bacterial conjugation were common and detected in 30.2% and
34.6% of the identified isolates, respectively (59). Our subsequent study showed that ready-to-
eat food from the same restaurants was commonly contaminated with antibiotic resistant
enterococci (60). Overall concentration of enterococci throughout the year averaged ~10^3 CFU/g
with greater prevalence during the summer than the winter. The higher prevalence of
enterococcal contamination among food samples in summer correlated with housefly activity.

Enterococci from summer samples were resistant to tetracycline (22.8% of isolates),
erythromycin (22.1%), and kanamycin (13.0%) (60). These studies implied that food served in
restaurants is commonly contaminated with antibiotic resistant enterococci and that houseflies
may play a role in this contamination.

Most recently, we assessed the prevalence of enterococci in houseflies collected from
four municipal wastewater treatment facilities (WWTF) as these sites are another potential
source of antibiotic resistant strains. Interestingly, the highest prevalence of multi-drug resistant
enterococci was detected from a WWTF (sludge and associated houseflies) that processed the
waste from a nearby sausage factory, pointing again to animal agriculture as a source of these
bacteria (61). Genotypic analysis (PFGE) revealed the same clones of *E. faecalis* present in the
waste and the housefly digestive tract. Doud et al. (61) also collected houseflies from the
residential environment (restaurant, apartment complex, mobile homes) close (0.7 – 2.0 km) to
one of the WWTF and found similar antibiotic resistance profiles in *E. faecalis* and *E. faecium*
although in lower prevalence and with no clonal matches to enterococci isolated directly from
the WWTF environment.

**Bacterial proliferation in the insect digestive tract and transmission of bacteria by insects**

Bacterial proliferation and transfer during insect feeding has been demonstrated
previously in houseflies for *E. coli* (62, 63). We used a GFP-labeled *E. faecalis* OG1RF:pMV158
to track the fate of this bacterium in the digestive tract of houseflies and to assess the vector
potential of this insect for *E. faecalis* (64). Analysis of viable fluorescing cells within various gut
components over several time points revealed the highest bacterial count in the midgut in first
few hours (1-4 h) after feeding and that declined gradually; while the CFU peaked in the fly
foregut (crop) after 48 h and remained high until the end (96 h) of the experiment. This
suggested that *E. faecalis* was digested in the midgut but proliferated in the crop (64). Bacterial
proliferation in the housefly crop and digestion in the midgut have also been reported for
*Aeromonas hydrophila* and *Pseudomonas aeruginosa* (65, 66). This is important because the
content of the crop, including associated bacteria, is typically released on a food source by
housefly regurgitation during feeding (42, 67). Both drinking water and feed (flaked corn)
sampled at the end of the assay were contaminated by fluorescing *E. faecalis*, demonstrating that
the flies disseminated *E. faecalis* to their surroundings (64). Furthermore, we also directly
assessed the ability of houseflies to contaminate ready-to-eat food with enterococci under
laboratory conditions (68). Within 30 minutes, exposure of as few as five flies collected from a
cattle feedlot resulted in an average of ~10^3 CFU/g of enterococcal deposit on the food (beef
patty from a hamburger) (68). These studies further support the notion that houseflies can act not
only as a mechanical but also as a bioenhanced vector for bacteria, and have great potential to
contaminate substrates by microbes during feeding and by defecation.
Livestock insects and horizontal transfer of antibiotic resistance traits

In addition to bacterial proliferation in the digestive tract of houseflies, the potential for horizontal transfer of genes coding for toxins and antibiotic resistance among bacteria was also evaluated. Petridis et al. (69) observed relatively frequent ($10^{-3}$ to $10^{-2}$ transconjugant per donor) transfer of genes for chloramphenicol resistance and the Shiga-toxin among strains of *E. coli* in both the midgut and crop of houseflies after 1 h of post-feeding. Our study showed that the tetracycline resistance gene (*tetM*) on a pheromone-responsive plasmid pCF10 was frequently transferred between *E. faecalis* strains in the housefly mouthparts and digestive tract (70). The transfer occurred within 24 h after exposure with a transconjugant/donor rate from $8.6 \times 10^5$ to $4.5 \times 10^1$. The implications of these studies are significant to public and animal health as they point to the ability of bacteria to actively share toxins and antibiotic resistance genes within the housefly gut beyond what is consumed initially by the fly and beyond simple bacterial proliferation.

CONCLUSIONS

The above studies demonstrate: a) the association of multi-drug resistant bacterial strains of food animal origin with flies and cockroaches; b) bacterial proliferation and horizontal transfer of antibiotic resistance genes in the insect digestive tract; and c) potential of these insects to transmit multi-drug resistant bacteria from food animals to the urban environment. We propose that integrated pest management should be incorporated into pre- and post-harvest food safety programs to minimize spread of antibiotic resistant bacterial strains. In addition, the insect link between agricultural and urban environments presents another reason for implementation of prudent use of antibiotics in the food animal industry.
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REFERENCES


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Ludek Zurek is a Professor of microbial ecology with a dual appointment in Department of Entomology and Department of Diagnostic Medicine and Pathobiology at Kansas State University. He received B.S. degree from Mendel University and Ph.D. from the University of Alberta. He was a postdoctoral fellow at North Carolina State University from 1999 to 2002. His interests and expertise are in the ecology of antibiotic resistance traits in clinical and non-clinical environments, microbial ecology and homeostasis of the insect digestive tract, and the role of insect gut microbial communities in vector competence for animal and zoonotic pathogens.

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* AMP, ampicillin; AMX, amoxicillin; CAZ, ceftazidime; CED, cefpodoxime; CEF, cefotaxime; CEP, cefapin; CER, cefteror; CEZ, cefazolin; CHL, chloramphenicol; CIP, ciprofloxacin; CLN, clindamycin; CLR, cefaclor; CTE, chlorotetracycline; DOX, doxycycline; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NEO, neomycin; OXY, oxytetracycline; PEN, penicillin; ROX, roxithromycin; SPC, spectinomycin; STR, streptomycin; SUL, sulfonamides; SXT, sulfamethoxazole/trimethoprim; SYN, quinupristin-dalfopristin; TET, tetracycline; TRM, trimethoprim.