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 hospitalizations, and increased health care 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 multidrug-resistant clonal lineages of bacteria identical to those found in animal manure. Furthermore, several studies have 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 postharvest 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 hospitalizations, and increased health care costs (1–4). The annual cost to the U.S. health care system from antibiotic-resistant infections is estimated to be 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 the Centers for Disease Control and Prevention (6), it was 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 agricultural and urban environments 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 (a ban on use of fluoroquinolo-

nes 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 numerous and diverse 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 oxytetracyline that were closely related to genes from strains pathogenic to humans. Allen et al. (35) reported several antibiotic resistance determinants from the midgut bacteria of 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 that Mexican fruit flies (*Anastrepha ludens* L.) from laboratory-reared colonies (38), oil fly (*Helaeomyia petrolei* L.) larvae from asphalt

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seeps (39), and cockroaches (*Periplaneta americana* L. and *Blattella germanica* L.) from food-handling facilities, households, and a hospital (40, 41) are 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 production has increased because of the capacity of these insects to spread zoonotic food-borne pathogens (reviewed in references 42 and 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 blowflies 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 they suggested that houseflies in cattle farms play a role in the dissemination of this food-borne pathogen. In the same study, those authors also showed that 95% of houseflies sampled were positive for fecal coliforms in their gut at levels ranging from 3.0×10^1 to 3.0×10^6 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 (47). Rectal sampling of fresh cattle feces showed the presence of nalidixic acid-resistant *E. coli* O157:H7 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. (i) Food animal environments. 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 production facilities 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 production facilities) 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 to be a carrier of multidrug-resistant *Salmonella* spp. and *Shigella* spp. 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* organisms 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 strains of *E. coli* that were resistant to a third-generation cephalosporin and that contained transferable plasmids carrying the $bla_{\text{CTX-M-15}}$ 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* was 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 United States, 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 multidrug-resistant (mainly to tetracycline and erythromycin) 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 demonstrated that insects on farms commonly carry the same clonal lineages of multidrug-resistant bacteria that are found in animal feces.

(ii) Urban environments. 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 tracts 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 the most abundant species (88.2%) and harbored 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, which 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, the concentration of enterococci throughout the year averaged ∼10^3 CFU/g, with a 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

TABLE 1 Insects with antibiotic-resistant bacteria from food animal production farms and surrounding urban environments

Insect	Bacterial species	Antibiotic resistance profile[a]	Environment(s)	Reference
Cockroaches (Dictyoptera)				
German cockroach (*Blattella germanica*)	*Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	56
Flies (Diptera)				
Housefly (*Musca domestica*)	*Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus casseliflavus*	CIP, ERY, KAN, STR, TET	Fast-food restaurants	59
Housefly (*Musca domestica*) Blowfly (*Lucilia* spp.) Bottle fly (*Phaenicia* spp.)	*Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus* spp.	CLN, ERY, PEN, SYN, TET	Poultry farms	55
Housefly (*Musca domestica*)	*Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	56
Housefly (*Musca domestica*)	*Enterococcus faecalis*, *Enterococcus faecium*	DOX, ERY, GEN, STR, TET	Wastewater treatment facilities	61
Housefly (*Musca domestica*)	*Escherichia coli* O157:H7	AMP, CER, CTE, GEN, NEO, OXY, SPC, SXT	Cattle farm	46
Housefly (*Musca domestica*)	*Escherichia coli*	AMP, STR, SUL, TET	Swine farms	51
Housefly (*Musca domestica*) Stable fly (*Stomoxys calcitrans*)	*Escherichia coli*	AMP, AMX, CHL, CEP, CIP, GEN, NAL, SUL, STR, SXT, TET	Dairy cattle farm	52
Housefly (*Musca domestica*) False stable fly (*Muscina stabulans*)	*Escherichia coli*	AMP, CED, CEZ, STR, TET, TRM	Cattle farm	53
Housefly (*Musca domestica*) Blowfly (*Lucilia* spp.)	*Escherichia coli*	CAZ, CEF	Poultry farms	54
Australian bush fly (*Musca vetustissima*)	*Escherichia coli*, *Salmonella* spp., *Shigella* spp.	AMX, CLR, ROX	Cattle farm, urban area, outdoor eateries	50

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

facilities (WWTF), as these sites are another potential source of antibiotic-resistant strains. Interestingly, the highest prevalence of multidrug-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 residential environments (restaurant, apartment complex, mobile homes) close to (0.7 to 2.0 km) one of the WWTF and found similar antibiotic resistance profiles in *E. faecalis* and *E. faecium*, although at a 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 green fluorescent protein-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 across several time points revealed the highest bacterial count in the midgut in the first few hours (1 to 4 h) after feeding and this count 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 fly 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 re-

leased on a food source via 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 min, exposure of as few as five flies collected from a cattle feedlot resulted in an average $\sim 10^3$ 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 they have great potential to contaminate substrates with 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 transfer (10^{-3} to 10^{-2} transconjugants per donor) of genes for chloramphenicol resistance and the Shiga toxin among strains of *E. coli* in both the midgut and crop of houseflies 1 h postfeeding. Our study showed that the tetracycline resistance gene [*tet*(M)] on a pheromone-responsive plasmid, pCF10, was frequently transferred between *E. faecalis* strains in the housefly mouth parts and digestive tract ([70](#)). The transfer occurred within 24 h after exposure, with a transconjugant/donor rate from 8.6×10^{-5} to 4.5×10^1. 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 the following: (i) the association of multidrug-resistant bacterial strains of food animal origin with flies and cockroaches, (ii) bacterial proliferation and horizontal transfer of antibiotic resistance genes in the insect digestive tract, and (iii) the potential of these insects to transmit multidrug-resistant bacteria from food animals to the urban environment. We propose that integrated pest management should be incorporated into pre- and postharvest 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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