OXFORD

Vector-Borne Diseases, Surveillance, Prevention

Conjugation-Mediated Transfer of Antibiotic-Resistance Plasmids Between Enterobacteriaceae in the Digestive Tract of *Blaberus craniifer* (Blattodea: Blaberidae)

I. Anacarso, R. Iseppi, C. Sabia, P. Messi, C. Condò, M. Bondi,[1] and S. de Niederhäusern

Department of Life Sciences, University of Modena and Reggio Emilia, Via Campi, 287, 41125 Modena, Italy (immacolata.anacarso@unimore.it; ramona.iseppi@unimore.it; carla.sabia@unimore.it; patrizia.messi@unimore.it; carla.condo@unimore.it; bondi.moreno@unimore.it; denieder.simona@unimore.it) and [1]Corresponding author, e-mail: bondi.moreno@unimore.it

Abstract

Cockroaches, insects of the order Blattodea, seem to play a crucial role in the possible conjugation-mediated genetic exchanges that occur among bacteria that harbor in the cockroach intestinal tract. The gut of these insects can be thought of as an effective in vivo model for the natural transfer of antimicrobial resistance plasmids among bacteria. In our study, we evaluated the conjugation-mediated horizontal transfer of resistance genes between *Escherichia coli* and other microorganisms of the same Enterobacteriaceae family within the intestinal tract of *Blaberus craniifer* Burmeister, 1838 (Blattodea: Blaberidae). Different in vivo mating experiments were performed using *E. coli* RP4 harboring the RP4 plasmid carrying ampicillin, kanamycin, and tetracycline resistance genes as the donor and *E. coli* K12 resistant to nalidixic acid or *Salmonella enterica* serovar Enteritidis IMM39 resistant to streptomycin as the recipients. The RP4 plasmid was successfully transferred to both recipients, producing *E. coli* K12-RP4 and *S.* Enteritidis IMM39-RP4 transconjugants. Conjugation frequencies in vivo were similar to those previously observed in vitro. The transfer of the RP4 plasmid in all transconjugants was confirmed by small-scale plasmid isolation and agar gel electrophoresis, suggesting that the intestinal tract of cockroaches is an effective in vivo model for natural gene transfer. Our results confirm that cockroaches allow for the exchange of antimicrobial resistance plasmids among bacteria and may represent a potential reservoir for the dissemination of antibiotic-resistant bacteria in different environments. These findings are particularly significant to human health in the context of health care settings such as hospitals.

Key words: *Blaberus craniifer*, cockroach, gut, conjugation, antibiotic resistance

Acquisition of antimicrobial resistance and other important characteristics (virulence genes, toxin genes, etc.) has been frequently demonstrated in bacterial populations by in vitro horizontal gene transfer methods using plasmids and transposons (Elsas and Bailey 2002; de Niederhäusern et al. 2004, 2011; Hall 2004; Warnes et al. 2012). In nature, exchange of genetic information occurs preferentially among the intestinal bacteria of different classes of living organisms (Huddleston 2014) that, consequently, spread the bacteria in the environment through shedding of feces. In this context, arthropods, especially cockroaches, insects of the order Blattodea, seem to play a crucial role because their behavior is versatile and adaptable to different environmental conditions and because they live in close cohabitation with humans (Poole and Crippen 2009). These insects move freely through many environments to find conditions favorable for survival (i.e., suitable temperature, humidity, and available food sources), thus facilitating the possible spread of bacteria. Institutions such as hospitals, rehabilitation and nursing home facilities, and community centers may occasionally contain a small number of cockroaches or be subject to infestations (Fotedar et al. 1991a, Pai et al. 2004, Pampiglione and Velo 2010, García et al. 2012, Tilahun et al. 2012). Many studies have demonstrated the possible involvement of cockroaches as vectors of pathogenic microorganisms in different environments (Fotedar et al. 1989, Kim et al. 1995, Pai et al. 2004, García et al. 2012). In particular, cockroaches have been shown to be able to serve as reservoirs for *Salmonella* in livestock premises and in poultry processing environments (Kopanic et al. 1994, Marty 1998, Mpuchane et al. 2006) and have been associated with potential nosocomial infections (Cotton et al. 2000, Prado et al. 2002, Gliniewicz et al. 2003, Fakoorziba et al. 2010). Although person-to-person contact remains the main route of transmission for nosocomial infections, the possible role of cockroaches cannot be excluded. These insects, in fact, can harbor several million potential pathogens on their body and in their fecal droppings, including bacteria, protozoa, fungi, viruses, and helminths, that can

infect both humans and animals (Bennett, 1993, Pai et al. 2004). Even if the direct involvement of cockroaches in the transmission of infectious diseases remains difficult to prove, their presence in hospital environments is nonetheless worrisome because of the possible genetic exchange of virulence traits among bacteria in the cockroach intestinal microbiota (Fotedar et al. 1991b, Pai et al. 2004, Fakoorziba et al. 2010). The high concentration of microorganisms in the insect gut and their close proximity may in fact create conditions that favor genetic exchange by conjugation and allow the acquisition or transfer of virulence and resistance factors (Armstrong et al. 1990, Axtell 1999, Petridis et al. 2006, Poole and Crippen 2009). Cockroaches captured in hospitals and households have been found to harbor multidrug-resistant bacteria, and those carrying drug-resistant *Klebsiella* spp., for example, have been suggested to play a role in the epidemiology of nosocomial infections (Fotedar et al. 1991, Pai et al. 2005). The spread of multidrug-resistant (MDR) pathogens, in part due to the transfer of plasmids between bacteria, has become an important emerging public health concern. Therefore, the purpose of our study was to determine whether horizontal resistance gene transfer could occur via conjugation between *Escherichia coli* and other microorganisms of the Enterobacteriaceae family in the digestive tract of the *Blaberus craniifer* Burmeister, 1838 species of cockroaches.

Materials and Methods

Choice of Insect Species and Intestinal Microbial Flora

To select the most suitable insect for the study, three groups of eight specimens each, belonging to three different species of cockroaches (*B. craniifer*, *Gromphadorina portentosa*, and *Blatta lateralis*) provided by a private breeder, were tested to determine which had the greatest attraction to food. Specifically, we directly observed the insects and counted how many times they made contact with food in a specific period of time (6 h a day for 3 d). The cockroaches were fed a diet consisting of commercial dry dog food purchased at the supermarket (croquettes, Friskies Dog with meat, diameter 10–12 mm, weight 0.6–0.7 g, Nestlé Italiana S.p.A. Assago, MI, Italy) during the maintenance phase prior to the experiment. During experiments, the same food was soaked with bacterial suspensions. The croquettes (one for each parental strain) were replaced twice a day. Cockroaches were reared individually in small plastic containers (10 by 16 by 15 cm^3) under laboratory conditions (a photoperiod of 16:8 [L:D] h at 22°C). The cockroaches of three species used for experimentation were all adult females. Specifically, *B. craniifer* and *G. portentosa* were ~9 mo old and ~6 and 8 cm in size, and *Bl. lateralis* were 5 mo old and ~3 cm in size. The insects were not subjected to any ill treatment, and after the end of each experiment, they were given food and water daily and were left to live in captivity until the end of their days. Careful observations of the three cockroach species revealed that *B. craniifer* had the greatest attraction to the above-mentioned diet (it made contact with the food ~20% more frequently than the other two species), so it was chosen for the experiment. Before the beginning of the experiment, we assessed the composition of the intestinal microbial flora of all the insects of *B. craniifer* species used for the experiment (task 1 and task 2) to confirm the absence of *E. coli* and *Salmonella* spp., microorganisms that could interfere with the determination of possible transconjugants after conjugation. Fecal samples of each insect (20 mg) were collected for two consecutive days, homogenized in 1 ml of sterile saline solution and, following a twofold serial dilution, spread onto MacConkey agar plates (Oxoid, Milan, Italy) until

a plate count of ~100 CFU (colony-forming unit) was obtained. After a 24-h incubation at 37°C, colonies with different characteristics (morphology, size, and colour), those that were most abundant, and those with morphological characteristics consistent with Enterobacteriaceae were isolated. We identified the isolated bacterial species using microscopic observation, Gram staining, the API system 20E (bioMérieux, Marcy-l'Étoile, France), and Enterotube II (Becton Dickinson Diagnostic System, Pont de Claix, France).

Bacterial Strains

The strains used and their properties are shown in Table 1.

Bacterial Conjugation in the Cockroach Gut

Task 1. Horizontal Transfer of Antibiotic-Resistance Genes by Conjugation From *E. coli* to *E. coli*

In vivo mating experiments were performed using *E. coli* RP4 as the donor and *E. coli* K12 as the recipient. Eight *B. craniifer* adults, individually housed in eight plastic containers, were fed dry dog food daily (two croquettes for every plastic container). Each croquette was soaked with a suspension (500 µl) of one of the two parental strains and replaced twice a day.

To normalize the bacterial suspensions, the McFarland Scale was used. Briefly, cultures of each parental strain were grown in tryptic soy broth (TSB, Oxoid) and incubated at 37°C until they reached a turbidity of 4 McFarland, as determined using a nephelometer (~1.2×10^9 CFU/ml). CFUs were determined by twofold serial dilutions of cultures plated onto MacConkey agar and incubated under the previous conditions. Each culture was separately transferred into 10-ml Eppendorf tubes and was centrifuged ($10,000 \times g$ for 10 min at 4°C). The supernatant was discarded, and cells were harvested, washed twice, and suspended in 1 ml of phosphate-buffered saline (PBS, pH 7.4), resulting in a solution of ~10^{10} CFU.

Twice a day (at 8 a.m. and 8 p.m.), for each insect, two croquettes soaked with separate suspensions of either the donor or the recipient strains were placed at opposite ends of the plastic container to prevent direct contact of the two parental strains. In addition, to further limit cross-contamination, the plastic container was surface-sterilized using 0.05% sodium hypochlorite and 70% ethanol and then washed with sterile water to remove the residual disinfectant. The insect was collected gently with sterile gloves and temporarily placed inside a sterile glass Petri dish (12-cm diameter) during sterilization of the container. The efficiency of the sanitation and washing technique for the plastic container were determined using the contact plate method (25 cm^2 tryptic soy agar plate). With this technique in the containers, a residual microbial load has never been found.

The experiment lasted two weeks. During this period, the insects were fed treated food, making sure that donors (*E. coli* RP4) and recipients (*E. coli* K12) did not come into contact with one another. From the first day of the experiment, the feces of the cockroaches were collected in a sterile container using sterile forceps, weighed (20 mg), homogenized in 1 ml of sterile saline solution, and spread at an appropriate dilution on selective MacConkey agar (50 mcg/ml nalidixic acid and 35 mcg/ml kanamycin). Feces were collected every day as soon as possible after their emission (up to the 20 mg necessary) before sanitization of the container, and remaining feces were immediately discarded each day during the experiment.

Table 1. Names and properties of strains used

Recombinant strains	Antibiotic resistances	Other properties
Escherichia coli RP4	Ap^R, Km^R, Tc^R	Lac− used as RP4 plasmid donor (60 kb) (Sabia et al. 2009)
Escherichia coli K12	Nal^R	Lac+, Plasmid-free, used as recipient (Sabia et al. 2009)
Salmonella enterica serovar Enteritidis IMM39	S^R mutant	Lac− used as recipient (belongs to our collection)

Ap, ampicillin; Km, kanamycin; Tc, tetracycline; Nal, nalidixic acid; S, streptomycin; Lac+/−, lactose fermentation.

Task 2. Horizontal Transfer of Antibiotic-Resistance Genes by Conjugation From *E. coli* to *S.* Enteritidis

Using a group of eight different insects, the same experiment was performed using *E. coli* RP4 as the donor and *S.* Enteritidis IMM39 as the recipient. As described in the previous experimental design, from the first day of experimentation, the feces of the insects were collected, homogenized in sterile saline solution, and spread on MacConkey agar supplemented with selective antibiotics (500 mcg/ml streptomycin and 35 mcg/ml kanamycin).

For both tasks, insects were fed untreated food starting from the end of the second week of the experiment.

Colonies of putative transconjugants grown on selective medium were counted and identified using morphological and biochemical methods (API system 20E and Enterotube II). Simultaneously, the conjugation frequency was determined for each sample of feces and expressed as the number of transconjugants per recipient cell. To determine the number of recipient strains in the feces of the insects, twofold serial dilutions of samples were spread on MacConkey agar supplemented with 50 mcg/ml nalidixic acid for *E. coli* K12 or 500 mcg/ml streptomycin for *S.* Enteritidis IMM39 until a plate count of ∼100 CFU was reached. For each sample, the number of recipients was determined by subtracting the number of transconjugants from the total number of colonies, determined by replica plating on MacConkey agar supplemented with the selective agents (50 mcg/ml nalidixic acid, 35 mcg/ml kanamycin or 500 mcg/ml streptomycin, 35 mcg/ml kanamycin). As a control, the number of donors was also evaluated on plates supplemented with kanamycin (35 mcg/ml); donors were recognized because they do not ferment lactose (Lac-), while the transconjugants do.

Viability of Transconjugants in Feces of *B. craniifer*

After the second week, insects were fed food with no bacterial supplementation. To assess how long the transconjugants were present in the insects, suspensions of 20 mg of stool samples, collected daily before plastic container sterilization, were treated as previously described. One hundred microliters of the diluted suspensions was plated onto MacConkey agar supplemented with the specific selective agents. When necessary, the sample was plated without dilution or enriched in peptone water for 24 h at 37°C prior to plating. The evaluation was carried out until transconjugants were no longer detected (zero CFU on MacConkey agar).

Plasmid Isolation

To confirm the transfer of the 60 kb plasmid carrying Ap, Km, and Tc resistances in *E. coli* k12 and *S.* Enteritidis IMM39 recipients, we compared the plasmid profile of the parental strains with those of putative transconjugants. Small-scale plasmid isolation was performed using the Qiagen Plasmid Mini Kit according to the manufacturer's guidelines (Qiagen, Hilden, Germany), and DNA plasmids from donors, recipients, and transconjugants were analyzed using gel electrophoresis with 0.7% agarose at 35 V/cm for 8 h in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Plasmids purified from *E. coli* V517 (Macrina et al. 1978) were used as size reference plasmids for molecular weight determinations.

Results

Intestinal Microbial Flora Evaluation of *B. craniifer*

There were no *E. coli* or *Salmonella* spp. isolated from midguts of the 16 cockroaches used in task 1 and task 2 before the experiment commenced.

Bacterial Conjugation in the Cockroach Gut

During the first experiment using donor strain *E. coli* RP4 and recipient strain *E. coli* K12, transconjugants were isolated from fecal samples of several of the cockroaches by the middle of the first week (Fig. 1). In particular, *E. coli* K12-RP4 transconjugants were detected in two of the eight insects by the 4th day after the start of the experiment and in four other insects at the end of the first week (7th day). In the remaining two insects, *E. coli* K12-RP4 transconjugants emerged only after the 4th day of the second week. In all cases, the conjugation frequency ranged from a minimum of 10^{-4} to a maximum of 10^{-3}, with recipient viable counts ranging from 6×10^5 to 4×10^6 CFU/ml and donor viable counts ranging from 3×10^5 to 2×10^6 CFU/ml (1 ml corresponds to 20 mg of feces).

In the second conjugation experiment (using *E. coli* RP4 and *S.* Enteritidis IMM39), *S.* Enteritidis IMM39-RP4 transconjugants were observed in two of the eight insects on the 6th day and in three other insects after the 5th day of the second week. In the remaining insects, transconjugants were not detected (Fig. 2). In all of the experiments, the conjugation frequency ranged from a minimum of 10^{-5} to a maximum 10^{-4} (an average of 5×10^{-5}), with recipient viable counts ranging from 1×10^5 to 2×10^6 CFU/ml and donor viable counts ranging from 4×10^5 to 3×10^6 CFU/ml.

Viability of Transconjugants in Feces of *B. craniifer*

After the insects were fed food with no bacterial supplementation, they still kept the transconjugants of *E. coli* and *S.* Enteritidis in the gut and were able to shed them in the feces for a period not longer than 8 and 5 d, respectively, and they shed in their feces the corresponding parental strains for a few days more.
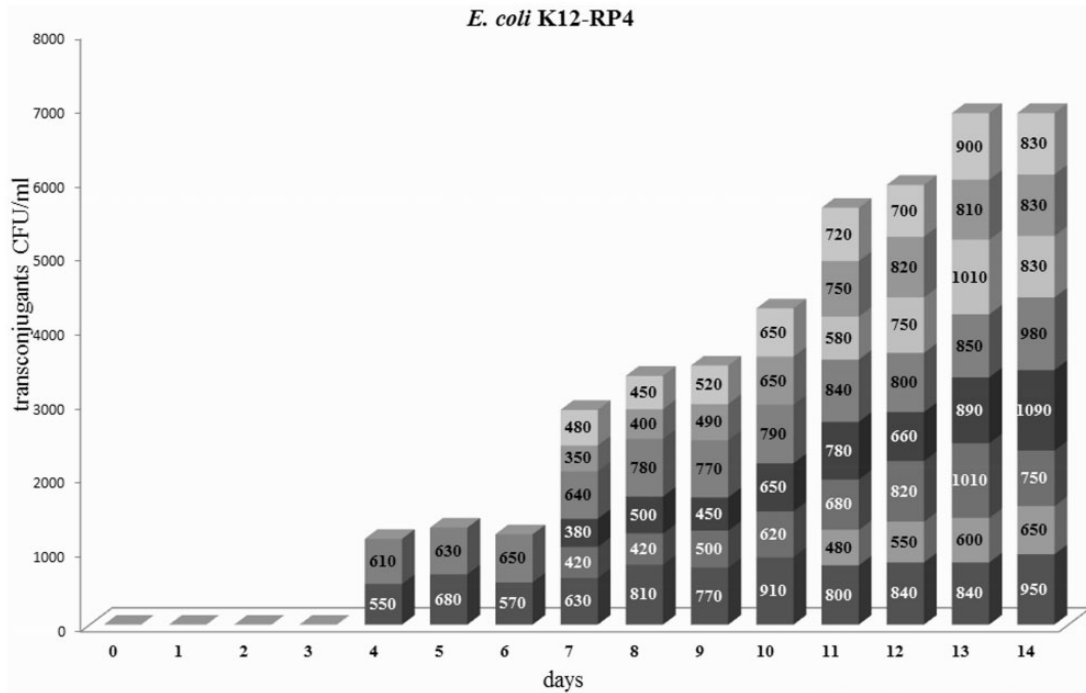
Fig. 1. Conjugation process from *E. coli* RP4 to *E. coli* K12. Each shade of gray represents a different insect, and the numbers in the histogram indicate the CFU/ml of *E. coli* K12-RP4 transconjugants detected for each day of the experiment during the 14 d of bacterial supplementation of the food. Feces from all eight insects were examined daily and CFUs/ml are only included for those in which transconjugants were detected. The 0 CFU/ml is not included.
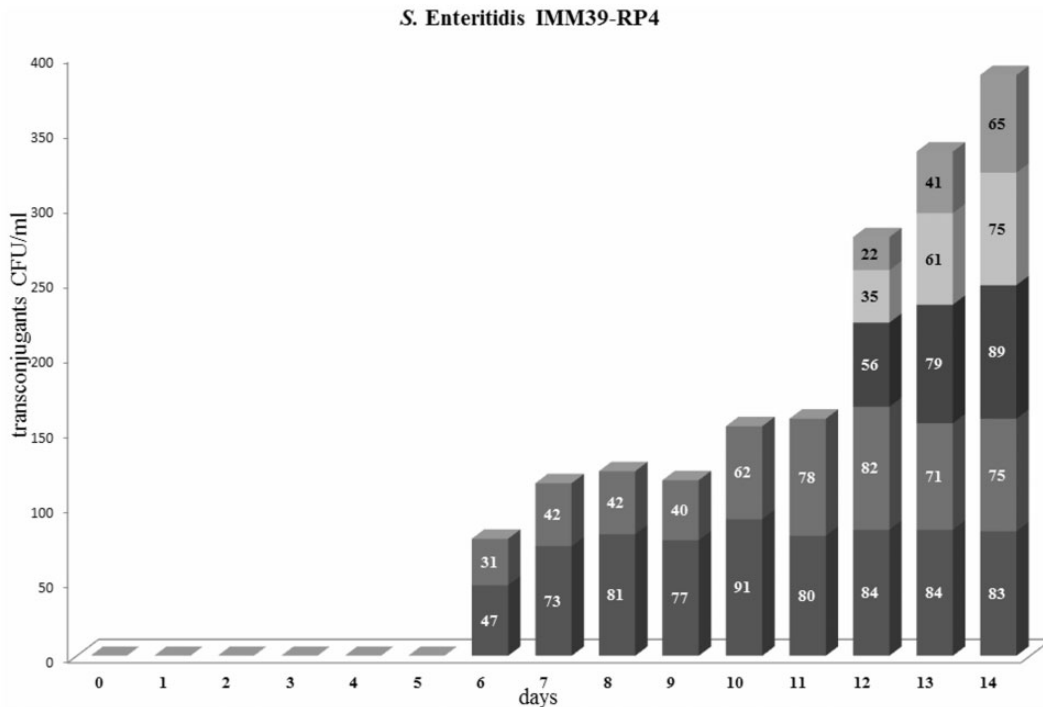

Fig. 2. Conjugation process from *E. coli* RP4 to *S.* Enteritidis IMM39. Each shade of gray represents a different insect, and the numbers in the histogram indicate the CFU/ml of *S.* Enteritidis IMM39-RP4 transconjugants detected for each day of the experiment during the 14 d of bacterial supplementation of the food. Feces from all eight insects were examined daily and CFUs/ml are only included for those in which transconjugants were detected. The 0 CFU/ml is not included.

Plasmid DNA Analysis

Fig. 3a and b shows the plasmid profiles of parental and transconjugant strains. Whereas no large plasmids were harbored in the recipient strains, the same 60-kb plasmid band was present in the donor and in transconjugants profiles. The transfer of this 60-kb plasmid by conjugation to the recipient strains (*E. coli* K12 or *S.* Enteritidis IMM39) was demonstrated in all randomly selected transconjugants among those grown on MacConkey agar supplemented with the
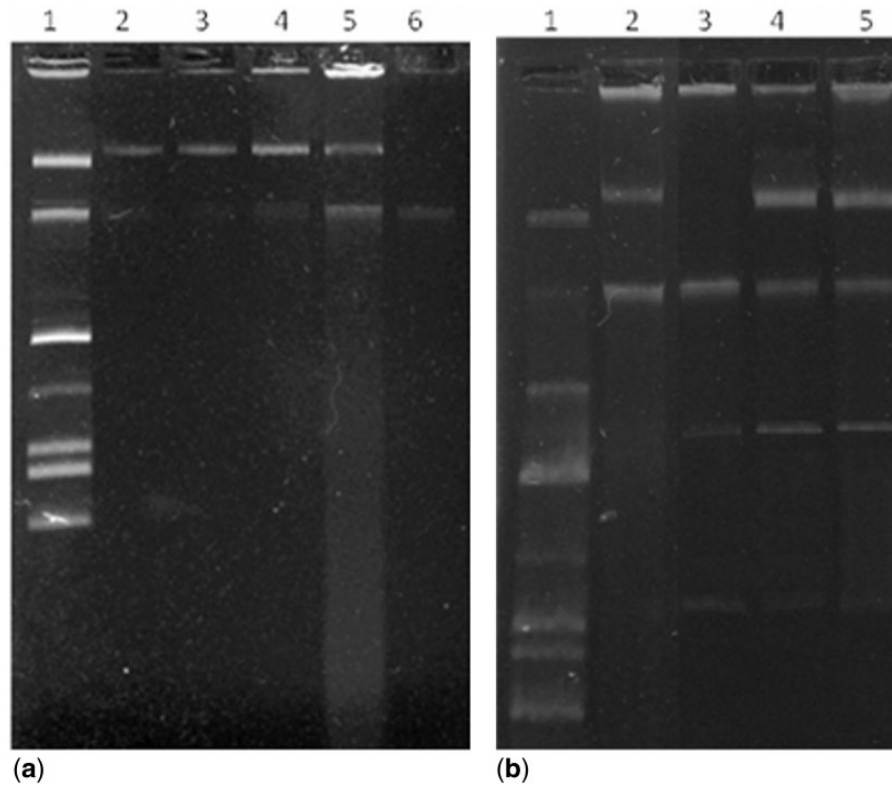
Fig. 3. Plasmid profiles of the parental strains and their transconjugants obtained from conjugation. (**a**) Lane 1: molecular size markers prepared from *E. coli* V517 (54.2; 5.6; 5.1; 3.9; 3.0; 2.7; 2.1 kb); Lanes 2, 3, and 4: plasmid profiles of *E. coli* K12-RP4 (transconjugants); Lane 5: plasmid profile of *E. coli* RP4 (donor); Lane 6: plasmid profile of *E. coli* K12 (recipient). (**b**) Lane 1: molecular size markers prepared from *E. coli* V517; Lane 2: plasmid profile of *E. coli* RP4 (donor); Lane 3: plasmid profile of *S.* Enteritidis IMM39 (recipient); Lanes 4 and 5: plasmid profile of *S.* Enteritidis IMM39-RP4 (transconjugant).

selective agents, confirming the acquisition of the ampicillin, kanamycin, and tetracycline resistances by the recipients.

Discussion

Cockroaches are insects that are widely distributed in the environment, including houses, urban areas, food industries, kitchens, and hospitals. Many studies have demonstrated the role of these insects as vectors of pathogenic microorganisms (Fotedar et al. 1991a, Kitae et al. 1995, Pai et al. 2004, Pampiglione and Velo 2010, Akinjogunla et al. 2012, García et al. 2012, Tilahun et al. 2012) even if their direct involvement in the transmission of infectious diseases and, in particular, in nosocomial infections has not been fully proved. Nosocomial infections, particularly those caused by drug-resistant bacteria, represent worldwide an important public health concern and have encouraged health authorities to implement prevention programs and epidemiological surveillance. In this context, it is very important that we are able to limit the spread of the involved etiological agents through the adoption of good hygiene practices and changes in behavior. Therefore, effective management strategies aimed at reducing insect pest populations, especially cockroaches, should be an important component of safety efforts in the future. While the issue of insects as vectors of human diseases is often undervalued, many researchers consider these organisms to be a serious threat in hospitals. In this environment, the presence of cockroaches could be contributing to the spread of multi-resistant strains (Fotedar et al. 1991b, Sramova et al. 1992, Cotton et al. 2000, Prado et al. 2002, Gliniewicz et al. 2003). Despite the fact that the transfer of plasmid-mediated resistance in the intestines of

cockroaches has rarely been documented, our results demonstrated that the exchange of genetic information among bacteria, even those of different genera, can occur in the guts of cockroaches, possibly because the small surface area of the cockroach gut provides an optimal environment for successful conjugation (Poole and Crippen 2009).

Under the conditions used in our study, antibiotic resistance genes were able to transfer from one *E. coli* strain to another or from *E. coli* to *S.* Enteritidis, and the frequency of horizontal plasmid transfer was similar to those observed during in vitro conjugation (Sabia et al. 2009), even if the exact frequency of conjugation is not easy to define. Indeed, as observed by Poole and Crippen (2009) during a conjugation experiment between *S.* Enterica and *E coli* in the gut of the lesser mealworm beetle, *Alphitobius diaperinus*, the exact amount of parental strains that each insect acquires from the artificially contaminated foods and their degree of development in the gut is unknown. Moreover, as discussed by Armstrong et al. (1990), it is not known whether transconjugants from a single insect result from multiple transfer events or from one event followed by the reproduction of the transconjugants. It is also difficult to understand why, as occurred in our study, different insects eliminate the transconjugants at different times or why the transconjugants are sometimes not observed. These differences are likely due to fluctuations in the number of parental strains ingested by the insect. In the study reported by Petridis et al. (2006), the insect (house fly) was immobilized and force-fed. This method may result in a higher ingestion of the two parental strains and thus a greater number of opportunities for contact so that the conjugation occurred within 1 h of feeding. We also observed that *B. craniifer* was able to keep in

the gut and shed in the feces transconjugants of both *E. coli* and salmonella and their respective parental strains for a short period of time. The increasing difficulty of isolating salmonella and *E. coli* from the fecal samples over time indicated that these microorganisms do not multiply easily in the gut of our cockroaches. This result is in disagreement with the results of Ash and Greenberg (1980), who found that their species of cockroaches (Dictyoptera: Blattellidae), which were artificially contaminated with Enterobacteriaceae, kept the bacteria in their intestinal tract for a longer time with no recoveries from the gut or feces after a month. This different outcome may be due to the different species of insects studied, to differences in the composition of the intestinal microbiota, to different diets, or to other causes. Regardless, it is important to remember that pathogenic bacteria shed by cockroaches in their feces remain viable and potentially infectious for a long time. According to experimental infections (Devj and Murray 1991), *Salmonella oranienberg* was shown to remain viable in the feces of *Periplaneta americana* for as long as 85 d to 6 mo under humid conditions. Therefore, the insects in the order Blattodea may be considered to be a potential reservoir for microorganisms able to facilitate the dissemination of antimicrobial resistance plasmids among commensal and pathogenic bacteria in different environments, including hospitals. Bacterial antimicrobial resistance is particularly relevant to a patient with an infection because the degree of virulence of the bacteria is likely also affected by the therapeutic possibilities. Finally, as recently reported by Zurek and Ghosh (2014), bacteria can transfer other important traits in addition to antimicrobial resistance genes, such as virulence and toxicity factors by conjugation in the guts of cockroaches. Bacteria can be quickly selected from the environment, and a possible mechanism of acquisition of new characteristics is the exchange of mobile DNA elements. Therefore, cockroaches can be thought of as small living laboratories where bacteria can undergo significant changes before being released into the environment.

In conclusion, our study, combined with other related studies, suggests that the intestinal tract of insects, including cockroaches, is an important and effective in vivo model for natural gene transfer. The acquisition of foreign DNA from unrelated organisms is one of the major sources of variability leading to the development of new bacterial strains (Ochman et al. 2000), and in many cases, it can contribute to an evolutionary leap of the same microorganisms.

References Cited

Akinjogunla, O. J., A. T. Odeyemi, and E. P. Udoinyang. 2012. Cockroaches (*Periplaneta americana* and *Blattella germanica*): Reservoirs of multi drug resistant (MDR) bacteria in Uyo, Akwa Ibom State. Sci. J. Biol. Sci. 1: 19–30.

Armstrong, J. L., N. D. Wood, and L. A. Porteous. 1990. Transconjugation between bacteria in the digestive tract of cutworm *Peridroma saucia*. Appl. Environ. Microbiol. 56: 1492–1493.

Ash, N., and B. Greenberg. 1980. Vector potential of the German cockroach (Dictyoptera: Blattellidae) in dissemination of *Salmonella enteritidis* serotype *typhimurium*. J. Med. Entomol. 17: 417–423.

Axtell, R. C. 1999. Poultry integrated pest management: Status and future. Integr. Pest Manage. Rev. 4: 53–73.

Cotton, M. F., E. Wasserman, C. H. Pieper, D. C. Theron, D. van Tubbergh, G. Campbell, F. C. Fang, and J. Barnes. 2000. Invasive disease due to extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal unit: The possible role of cockroaches. J. Hosp. Infect. 44: 13–17.

de Niederhäusern, S., C. Sabia, P. Messi, E. Guerrieri, G. Manicardi, and M. Bondi. 2004. Glycopeptide-resistance transferability from vancomycin-

resistant enterococci (VRE) of human and animal source to *Listeria* spp. Lett. Appl. Microbiol. 39: 483–489.

de Niederhäusern, S., M. Bondi, P. Messi, R. Iseppi, C. Sabia, G. Manicardi, and I. Anacarso. 2011. Vancomycin-resistance transferability from VanA enterococci to *Staphylococcus aureus*. Curr. Microbiol. 62: 1363–1367.

Devj, S. G. N., and C. J. Murray. 1991. Cockroaches (*Blatta* and *Periplaneta* species) as reservoirs of drug-resistant salmonellas. Epidemiol. Infect. 107: 357–361.

Elsas, J. D., and M. J. Bailey. 2002. The ecology of transfer of mobile genetic elements. FEMS Microbiol. Ecol. 42: 187–197.

Fakoorziba, M. R., F. Eghbal, J. Hassanzadeh, and M. G. Moemenbellah-Fard. 2010. Cockroaches (*Periplaneta americana* and *Blattella germanica*) as potential vectors of the pathogenic bacteria found in nosocomial infections. Ann. Trop. Med. Parasitol. 104: 521–528.

Fotedar, R., E. Nayar, J. C. Samantray, U. B. Shriniwas, V. Dogra, and A. Kumar. 1989. Cockroaches as vectors of pathogenic bacteria. J. Commun. Dis. 21: 318–322.

Fotedar, R., U. B. Shriniwas, and A. Verma. 1991a. Cockroaches (*Blattella germanica*) as carriers of microorganisms of medical importance in hospitals. Epidemiol. Infect. 107: 181–187.

Fotedar, R., U. B. Shriniwas, U. Banerjee, J. C. Samantray, E. Nayar, and A. Verma. 1991b. Nosocomial infections: Cockroaches as possible vectors of drug-resistant *Klebsiella*. J. Hosp. Infect. 18: 155–159.

García, F., M. J., Notario, J. M. Cabanás, R. Jordano, and L. M. Medina. 2012. Incidence of bacteria of public health interest carried by cockroaches in different food-related environments. J. Med. Entomol. 49: 1481–1484.

Gliniewicz, A., E. Czajka, A. E. Laudy, M. Kochman, K. Crzegorzak, K. Ziolkowska, B. Sawicka, H. Stypulkowska-Misiurewicz, and J. Hopskins. 2003. German cockroaches (*Blattella germanica* L.) as a potential source of pathogens causing nosocomial infections. Indoor Built Environ. 12: 55–60.

Hall, B. G. 2004. Predicting the evolution of antibiotic resistance genes. Nat. Rev. Microbiol. 2: 430–435.

Huddleston, J. R. 2014. Horizontal gene transfer in the human gastrointestinal tract: Potential spread of antibiotic resistance genes. Infect. Drug Resist. 7: 167–176.

Kim, K. T., J. H. Jeon, and D. K. Lee. 1995. Various pathogenic bacteria on German cockroaches (Blattellidae, Blattaria) collected from general hospitals. Korean J. Entomol. 25: 85–88.

Kopanic, R. J., Jr, B. W. Sheldon, and C. G. Wright. 1994. Cockroaches as vectors of salmonella: Laboratory and field trials. J. Food Prot. 57: 125–132.

Macrina, F. L, D. J., Kopecko, K. R. Jones, D. J. Ayers, and S. M. McCowen. 1978. A multiple plasmid-containing *Escherichia coli* strain: Convenient source of size reference plasmid molecules. Plasmid 1: 417–420.

Marty, A. M. 1998. Cockroaches can vector human diseases. Int. J. Dermatol. 37: 639–640.

Mpuchane, S., J. Allotey, I. Matsheka, M. Simpanya, S. Coetzee, A. Jordaan, N. Mrema, and B. A. Gashe. 2006. Carriage of micro-organisms by domestic cockroaches and implications on food safety. Int. J. Trop. Insect. Sci. 26: 166–175.

Ochman, H., J. G. Lawrence, and E. A. Groisman. 2000. Lateral gene transfer and the nature of bacterial innovation. Nature 405: 299–304.

Pai, H. H., W. C. Chen, and C. F. Peng. 2004. Cockroaches as potential vectors of nosocomial infections. Infect. Control Hosp. Epidemiol. 25: 979–984.

Pai, H. H., W. C. Chen, and C. F. Peng. 2005. Isolation of bacteria with antibiotic resistance from household cockroaches (*Periplaneta americana* and *Blattella germanica*). Acta Trop. 93: 259–265.

Pampiglione, G., and E. Velo. 2010. Pest control in Albania: An example of collaboration in technical and scientific development in public health. Vet. Ital. 46: 37–43.

Petridis, M., M. Bagdasarian, M. K. Waldor, and E. Walker. 2006. Horizontal transfer of Shiga toxin and antibiotic resistance genes among *Escherichia coli* strains in house fly (Diptera: Muscidae) gut. J. Med. Entomol. 43: 288–295.

Poole, T., and T. Crippen. 2009. Conjugative plasmid transfer between *Salmonella enterica* Newport and *Escherichia coli* within the

gastrointestinal tract of the lesser mealworm beetle, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Poult. Sci. 88: 1553–1558.

Prado, M. A., F. C., Pimenta, M. Hayashid, P. R. Souza, M. S. Pereira, and E. Gir. 2002. Enterobacteria isolated from cockroaches (*Periplaneta americana*) captured in a Brazilian hospital. Rev. Panam. Salud Publica 11: 93–98.

Sabia, C., S. de Niederhäusern, E. Guerrieri, M. Bondi, I. Anacarso, R. Iseppi, and P. Messi. 2009. Interference of *Lactobacillus plantarum* strains in the "in vitro" conjugative transfer of R-plasmids. Curr. Microbiol. 58: 101–105.

Sramova, H., M. Daniel, V. Absolonova, D. Dedicova, Z. Jedlickova, H. Lhotova, P. Petras, and V. Subertova. 1992. Epidemiological role of arthropods detectable in health facilities. J. Hosp. Infect. 20: 281–292.

Tilahun, B., B. Worku, E. Tachbele, S. Terefe, H. Kloos, and W. Legesse. 2012. High load of multi-drug resistant nosocomial neonatal pathogens carried by cockroaches in a neonatal intensive care unit at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. Antimicrob. Resist. Infect. Control 1: 12.

Warnes, S. L., C. J. Highmore, and C. W. Keevil. 2012. Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: Implications for public health. mBio 3: e00489–e00512.

Zurek, L., and A. Ghosh. 2014. Insects represent a link between food animal farms and the urban environment for antibiotic resistance traits. Appl. Environ. Microbiol. 80: 3562–3567.