# Cockroaches as Reservoirs and Vectors of Drug Resistant Salmonella spp.

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### **ABSTRACT**

Cockroaches were collected from hospitals, houses and poultry sheds in various parts of Isfahan (Iran) and identified to species. In total, seven species of cockroaches in seven genera were identified: Blatta lateralis, Polyphaga aegyptiaca, Arenivaga roseni and Parcoblatta spp. Three species Periplaneta americana, Blattella germanica and Supella longipalpa (Blattidae and Blattellidae) were more abundant than the others. In another study, forty cockroaches were collected from hospitals in two experiments and were studied for the presence of Salmonella spp. Salmonella (Black colonies on SS agar with urease negative) were isolated from about 70 percent of the cockroaches collected from hospitals. Some of the isolated Salmonella were resistant to antibacterial drugs in a susceptibility test. Isolation of Salmonella from cockroaches collected from hospitals suggests that cockroaches act as natural reservoirs of Salmonella. A second study was conducted to determine if individual B. germanica could transfer Salmonella from an infected food source and then infects uncontaminated colony members. The results showed that the inoculation of 10<sup>6</sup> CFU of Salmonella into cockroaches via their food could infect the uncontaminated cockroaches. These contaminated cockroaches transfer infection to other colony members. Salmonella is stable in cockroaches for more than 10 months. Iran. Biomed. J. 7 (1): 35-38, 2003

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### INTRODUCTION

great varieties of insects are carrier of pathogens [1, 2]. Cockroaches are known to Larry a diverse pathogenic bacteria flora, different protozoan, pathogenic worms, fungus, and viruses [3, 4], but their role in the direct transmission of infection has seldom been established [5-7]. Cockroaches have been established in the past as carriers of Salmonella [8-13]. Cotton et al. [14] suggested that cockroaches are possible vectors of Klebsiella pneumoniae in the hospital environment. Many children are allergic to cockroaches [15, 16]. In some undeveloped countries, cockroaches are so bad that many children do not have any eyebrows. In a study of bronchial asthma, Tsai and Chen [17] showed that 40% of patients in China were allergic to German Isolation of Salmonella cockroaches. cockroaches collected from poultry sheds suggested that cockroaches act as significant reservoirs of in nature. The free Salmonella movements of cockroaches from one location to another and their possible contribution to disease transmission prompted this study. The goal of this study was to determine if *Salmonella* could be isolated from cockroaches and to determine if cockroaches could carry *Salmonella* and infect uncontaminated cockroaches.

## MATERIALS AND METHODS

Collection of specimens. During the period of this study, cockroaches were collected from different houses, animal sheds and hospitals in various parts of Isfahan (Iran) and the species were identified. In addition, forty *B. germanica* were collected in sterile autoclave traps only from hospitals maintained in sterile bottles, and transferred to the laboratory to study Salmonella contamination. This experiment was repeated two times during one summer and the results are the mean of two experiments.

**Processing of specimens.** After transport to the laboratory, the cockroaches were washed in Selenite broth (Merck, http://merck.com/). Then, they were

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killed with diethyl ether and their whole digestive tracts were removed using autoclave-sterilized instruments. The instruments were sterilized with alcohol and flame between dissections. The wholehomogenized guts were processed with and without enrichment, on SS (Salmonella-Shigella) agar, brilliant green agar, and eosin methylene blue (EMB) agar (Merck). Selenite broths were used as enrichment media and were incubated at 37°C for 6-24 h. The culture plates were incubated at 37°C for 24-72 h. The identification of the isolated bacteria was done according to Bergey's manual [18]. The salmonella has black colony on SS agar and white colony on EMB; dose not change the color of brilliant green and urease test is negative. These biochemical tests identified salmonella to genes. More identification test is needed to identify the bacteria to species.

Isolation of uncontaminated cockroach colony. Control cockroaches in this study were obtained from cultures of B. germanica fed on sterile food for two years. The environmental chamber used to maintain the cockroach colonies in these studies was isolated to insure that cockroaches were not disturbed. A photoperiod of L/D 12-12 and 58% the rate of humiditiy was maintained in the chamber. experiments were conducted at room temperature (22-24°C). The cockroaches were maintained in 1-liter glass bottles covered with cotton cloth to allow airflow. Sterile food and water were provided for cockroaches within the bottles. The food (contained wheat straw) was obtained from Parsiran Company (Iran) and sterilized by autoclaving and checked for Salmonella contamination before use.

Ootecas were obtained from twenty subcultures of uncontaminated cockroaches fed sterile autoclaved food. Before the study of transmission, these cockroaches were studied to make sure that they were not contaminated with Salmonella. Six uncontaminated cockroaches were transferred to a chamber with sterile food and another six were transferred to chamber and fed them with contaminated food (5 gram of was contaminated with drug resistant Salmonella). The number of black colonies of bacteria on SS agar was estimated in 0.1-gram contaminated and uncontaminated foods, which used to feed cockroaches (dry and wet contaminated food without cockroaches was also determined for stability of Salmonella). Some bacterial colonies were identified to ensure that the black colonies were Salmonella.

Salmonella culture. Drug resistant Salmonella were obtained from B. germanica and grown to mid-log phase. Five gram of foods were inoculated with 1 ml of culture medium (10<sup>6</sup> CFU/ml) and fed to cockroaches. The number of Salmonella in dry and wet food (without any cockroaches as blanks) and the food of contaminated and uncontaminated cockroaches was estimated.

Antibiotic resistance. The disk diffusion test was used for determining bacteria susceptibility to antimicrobials. In this method, standard paper disks impregnated with known amounts of antibiotics (10-30  $\mu$ g) are placed on Muller-Hinton agar. After 48 h incubation, antibiotic activity was determined by the width of the zone of no growth around the antibiotic disk. In seven experiments, the most resistant *salmonella* was isolated for transmission study [19].

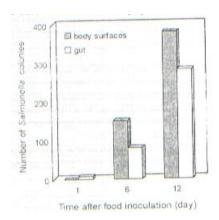
#### RESULTS AND DISCUSSION

In this study, seven cockroach species in seven genera were identified in traps including Periplaneta americana, Blattella germanica Supella longipalpa, Blatta lateralis, Polyphaga aegyptiaca, Arenivaga roseni, Parcoblatta sp., Periplaneta americana (Blattidae), Blattella germanica and Supella longipalpa (Blattellidae). The latter three species were present in the largest numbers.

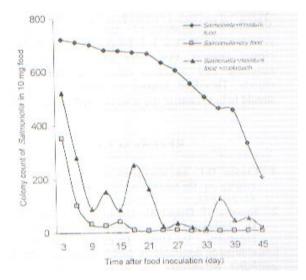
other cockroaches (Blattidae Blattellidae) were collected in sterile traps only from hospitals in each of two separate experiments and examined for the presence of Salmonella. Of the 40 cockroaches, 19 had Salmonella in their guts and body surfaces and nine had Salmonella only on their guts for an infection rate of about 47% contamination in guts together with body surfaces. However, out of 80 cockroaches, 57 were contaminated to Salmonella. This infection rate was unexpectedly high. Because the cockroaches were trapped and held in one sterile container, it is possible that the infection was transmitted between animals in the container.

The isolated bacteria were identified as Gramnegative motile straight rods,  $0.7\text{-}1.5 \times 2\text{-}5 \mu m$ . They were facultatively anaerobic, chemoorganotrophic, having both a respiratory and a fermentative type of metabolism. The optimal temperature for growth was 37°C. D-glucose and other carbohydrates were utilized with the production of acid. Other characteristics were: oxidase negative, catalase positive, indole and

Voges-Proskauer negative, methyl red (MR) and Simmons citrate positive, lysine and ornithine dihydrolase reaction positive, SH2 was produced, urea was not hydrolyzed and growth on SS was positive. All biochemical tests were done according to Bergey's manual [18]. Of the seven bacterial isolates, one was resistant to streptomycin, cotrimoxazole, erythromycin, ampicillin gentamicin, cycloserin and chloramphenicol. The drug resistant Salmonella (which were obtained from B. germanica) were added to food of sterile B. germanica obtained from the sterile culture. This contaminated food could contaminate sterile cockroaches and drug resistant Salmonella were stable for more than 10 months in the cockroaches, whereas in dry food (without cockroaches) the bacteria die in few days. Infection of six uncontaminated and sterile cockroaches, which were obtained from the sterile culture, shows that Salmonella could be transferred to the uncontaminated colony over 12 days from contaminated foods (Fig. 1). Salmonella were not stable in dry food, however in moist food (without cockroaches) they were stable and increased for more than 21 days (Fig. 2). After 21 days, the number of Salmonella decrease, but they were still present in moist food which, used to feed cockroaches (Fig. 2). Significantly, in three different experiments the number of Salmonella in moist food (with present of cockroaches) increased and decreased. This might be the result of regurgitation. The adult and nymph cockroaches regurgitate some of the partially digested food on to the feeding surface, and defecate. These habits increase their potential to contaminate household foods by spreading bacteria [20] to other members of the population.



**Fig. 1.** Contamination of cockroaches to *Salmonella* from contaminated food. Five gram of food was contaminated with 1 ml of  $10^6$  cell forming unit of *Salmonella*. Cockroaches were fed on contaminated food. The number of *Salmonella* in gut and body surfaces was determined.



**Fig. 2.** Stabilities of *Salmonella* in each of dry, moisture and contaminated food, which was used to feed cockroaches. *Salmonella* (10<sup>6</sup> CFU/ml) from German cockroach was added to 5 g food of cockroaches. The stability of this microorganism in each of dry, wet and contaminated food was studied.

Two infected *B. germanica* were transferred into a container with sterile food and four sterile nymphs. After growth of nymphs, they were found to be contaminated by *Salmonella*. One of these cockroaches was maintained in culture and had *Salmonella* 10 months later.

Devi and Murrag [21] studied more than 200 cockroaches and they showed that Salmonella were isolated from 4.1% of them. Cloarec et al. [22] found that 54% of all bacteria identified in cockroaches were pathogenic. Kopanic et al. [23] studied the presence of Salmonella in commercial poultry feed and found that Salmonella contaminated five of 45 food products. In this research, we found more than 70% of cockroaches collected from hospitals were contaminated with Salmonella. This contamination was more severe during the summer. The Salmonella replicated in moist food but survived longer in the cockroaches. Kirby and Davies [24] showed that Salmonella replicated rapidly in mixed foods and on surfaces that have been washed but were not adequately sanitized. These survival times are very long if the organism is not exposed to sunlight. It was shown that Salmonella oranienburg in feces of P. americana for more than 3.25 years on cornflakes, 4.25 years and on crackers, 3.67 years is stable. These feces can contaminate mice and poultry [25]. In this work, it was shown that some of the isolated Salmonella were resistant to antibiotics and Salmonella contaminated almost 71% of B.

germanica collected from hospitals. These findings clearly suggest that cockroaches are capable of acquiring and infecting other cockroaches and objects. Therefore, control of insects in hospitals should help to control the reservoirs of Salmonella.

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