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 10^6 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*

Keywords: *Salmonella*, Cockroach, Drug resistant, Vector

INTRODUCTION

A great varieties of insects are carrier of pathogens [1, 2]. Cockroaches are known to carry 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 cockroaches. Isolation of *Salmonella* from cockroaches collected from poultry sheds suggested that cockroaches act as significant reservoirs of *Salmonella* in nature. The free wandering 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 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 whole-homogenized 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 37ºC for 6-24h . The culture plates were incubated at 37ºC for 24-72h . 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 12-12and 58% the rate of humiditiy was maintained in the chamber. All experiments were conducted at room temperature (22-24ºC). 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 foods 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 (10^6 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 µg) 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.

Forty other cockroaches (Blattidae and 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 Gram-negative motile straight rods, 0.7-1.5 × 2-5 µm. They were facultatively anaerobic, chemo-organotrophic, having both a respiratory and a fermentative type of metabolism. The optimal temperature for growth was 37ºC. 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, SH_2 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 genta-micin, 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 unconta-minated 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* (10^6 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 *Salmonell*a. 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* conta-minated 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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