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Bacterial, fungal and parasitic contamination of cockroaches in public hospitals of Hamadan, Iran

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Abstract

Background & objectives: To determine the possible role of cockroaches in dissemination of medically important microorganisms, a study was carried out in public hospitals and residential areas of Hamadan city, west of Iran. Bacteria, fungi and parasites of medical importance were isolated and identified. The total number of *Blattella germanica* collected from hospitals were 133 as the case group. The cockroaches collected from residential areas were 45 as the control group.

Methods: A total of 178 cockroaches were collected, over a period of two years (133 from hospitals; and 45 from residential areas) in Hamadan. Medically important microorganisms were isolated from external and internal surfaces using standard methods.

Results: In the case group, 130 out of 133 (98%) *Blattella germanica* showed contamination with high bacterial load (more than 1×10^3) whereas only 2 out of 45 (4.45%) cockroaches of the control group were carrying medically important bacteria with high bacterial load. Bacteriological examinations revealed that almost all test cockroaches had at least one of the following microorganisms either in their body surface or digestive tract. *Enterobacter* (22.6%), *Klebsiela* (21%), *Enterococcus* (17.3%), *Staphylococcus* (16.5%), *Esherichia coli* and *Streptococcus* (8.3%), *Pseudomonas* (3%), and also *Shigella*, *Haemophilus* and group A ß-hemolytic *Streptococcus* each less than 1%. In addition the results showed (74.4.%) of test cockroaches harboured fungi—*Candida* (48.9%), *Mucor* (10.5%), *Aspergillus niger* (7.5%), *Rhizopus* (4.5) and also *Penicillium* and *Aspergillus fumigans* each 1.5%. Some parasitic worms of medical importance were also isolated from the test cockroaches, but carriage rates were low.

Interpretation & conclusion: The data from this study emphasise the importance of cockroaches as potential vectors of medically important microorganisms such as pathogenic bacteria and fungi in hospital environments.

Key words Bacteria – cockroach – fungi – nosocomial infection – parasite

Introduction

Cockroaches are among the most notorious pests of premises, which not only contaminate food by leaving droppings and bacteria that can cause food poisoning[1] but also they transmit bacteria, fungi and other pathogenic microorganisms in infested areas[2,3]. Cockroaches feed indiscriminately on garbage and sewage and so have copious opportunity to disseminate human pathogens[4,5]. Also their nocturnal and filthy habits[6] make them ideal carriers of various pathogenic microorganisms[7].

So far numerous pathogenic bacteria, including *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, *Pseudomonas aeruginosa* and *K. pneumoniae* have been isolated from cockroaches[4]. In addition some parasites and fungi have been found in external surfaces or internal parts of body of cockroaches[8,9] and some study have shown that exposure to cockroach antigens may play an important role in asthma-related health problems[10,11].

Since the hospital environments provide them with suitable temperature, humidity and a ready source of food, presence of cockroaches there is not uncommon[12]. Many researches in recent years have shown that drug resistant bacteria are of great importance in hospitals[13,14] which are potential carriers of microorganisms and their presence makes the problem more significant.

The present study was conducted to isolate and identify microorganisms from external surfaces and digestive tract of the cockroaches (*Blattella germanica*), which were collected from different parts of public hospitals of Hamadan.

Material & Methods

One hundred and seventy-eight cockroaches were collected, over a period of two years, 133 from different wards of hospitals of Hamadan (Hamadan, Iran) as the test group and 45 from residential areas, situated within 4 km premises from the hospitals as the control group. The test group of insects captured (mostly at night time or in the early morning) from the floor of wards and kitchens.

In order to compare the contamination rates of cockroaches from hospitals (test) and residential dwellings (control), during same period, cockroaches were also collected from kitchens, basements or bathrooms of residential area and their microbial flora was studied.

Each cockroach was collected in a sterile test tube, transported to the laboratory and anaesthetised by putting at 0°C for 5 min, examined under the dissecting microscope and identified using standard taxonomic keys for Blattidae of Iran[15]. For comparing control and test groups, chi-square test was applied.

Isolation and identification of microorganisms from external surfaces: After identification, 2 ml of sterile normal saline (0.9%) was added to the test tube and the cockroaches were thoroughly shaken for 2 min.

A fixed volume (0.01 ml each) of the washing was cultured on blood agar, MacCoonky agar, and desoxycholate citrate agar plates separately, incubated overnight at 37°C and the colonies identified by standard bacteriological procedures[16]. In each case a representative colony was studied by its macroscopic morphology, Gram's stain, various biochemical and other specific characters. In addition 0.5 ml of the washings was also inoculated in thioglycolate and Selenite-broths, simultaneously and incubated for 24 h at 37°C and subcultured in the same media. The results were read and colonies identified after overnight incubation at 37°C.

For isolation of fungi, the washing was cultured in Sabouraud's dextrose agar with 0.5% chloramphenicol[17]. The tubes were incubated at 25°C and the resulting growth (if any) was identified by standard mycological methods[18]. Isolation of parasitic ova/cyst was carried out by using 1 ml of washing which was centrifuged at 2000 for 5 min. The deposit examined after staining with 1% Lugols iodine under light microscopy and identified[19].

Isolation and identification of microorganisms from internal surfaces: After external washings, cockroaches were placed in flasks rinsed with 70% alcohol for 5 min (to decontaminate external surfaces as 70% alcohol is bactericidal), transferred to sterilised flasks, and allowed to dry at room temperature under sterile conditions. Cockroaches were then washed

with sterile normal saline for 2–3 min to remove traces of alcohol. Only cockroaches captured whole and live were utilised for the study. After being immobilised at 0ºC the gut of the cockroach was dissected out and macerated aseptically in a sterile pestle and mortar in 2 ml of sterile normal saline. The resulting macerate was then processed in a similar way as described previously and the results recorded.

The cultures were examined using a stereomicroscope, and colony-forming units were counted. The disk diffusion test was used to determine antimicrobial susceptibility. For parasitic ova/cyst, about 1 ml of washing was centrifuged at 2000 rpm for 5 min and the deposit examined after staining with 1% Lugols iodine under light microscopy and identified[19].

Quantitative estimation of bacterial isolates: Quantitative analysis of medically important bacteria (*Klebsiella* spp, *Esherichia. coli*, *Proteus* spp, *Ps. aeruginosa* and *S. aureus*) isolated from external and internal surfaces of each insect was calculated by Mile and Misra's method[17]. In each case 0.05 ml undiluted and two ten-fold dilutions of 0.05 ml of washings (external and internal) were cultured on blood agar and MacConkey agar plates in duplicate. Colony-forming units (c.f.u.) were counted after overnight incubation at 37ºC and mean count of plates was taken. From this, viable count of a particular bacteria was calculated in 2 ml of washings. The overall load of bacteria carried by each insect was counted by taking into consideration both external and internal c.f.u. together.

Antibiograms: Using the Stokes disk diffusion method, antimicrobial sensitivity tests were carried out for all the strains of *Klebsiella* spp and group A ß-hemolytic *Streptococcus* by bacitracin (0.04U/disk) and cotrimoxazole (25 µg/disk).

Results

Medically important microorganisms were isolated from external and internal surfaces of 98% of test cockroaches and 8.9% of control cockroaches, the difference being statistically significant ($p < 0.01$). Fig. 1 presents the quantitative estimation of bacteria isolated from cockroaches in Hamadan, i.e. *Klebsiella* spp, *E. coli*, *Enterobacter* spp, *Enterococcus* spp, *Staphylococcus* spp, *Streptococcus* spp, *Pseudomonas*, *Haemophilus* spp and *Streptococcus* (ß group A).

A high bacterial load, greater than 10^3 c.f.u. was carried out by 96% of test cockroaches, whereas only 4.45% of control were shown to have bacterial load higher than 10^3 c.f.u. The difference being statistically significant ($p < 0.001$). All common bacterial pathogens encountered in hospitals, were isolated in higher numbers from test cockroaches as compared to control cockroaches (Fig. 2). Resistance to antibiotic only detected in *Streptococcus* isolated from test cockroaches. Other bacteria either from test or control cockroaches were showed to be susceptible to both antibiotics.

In addition to bacteria, human parasites were also isolated from test group of cockroaches. In this case

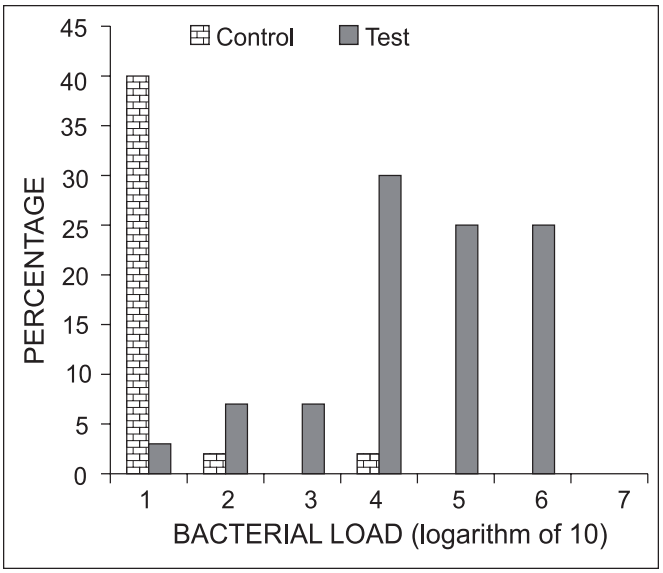

Fig. 1: Comparative bacterial loads carried by cockroaches (Bacterial load equal to 10^1 means practically no growth)

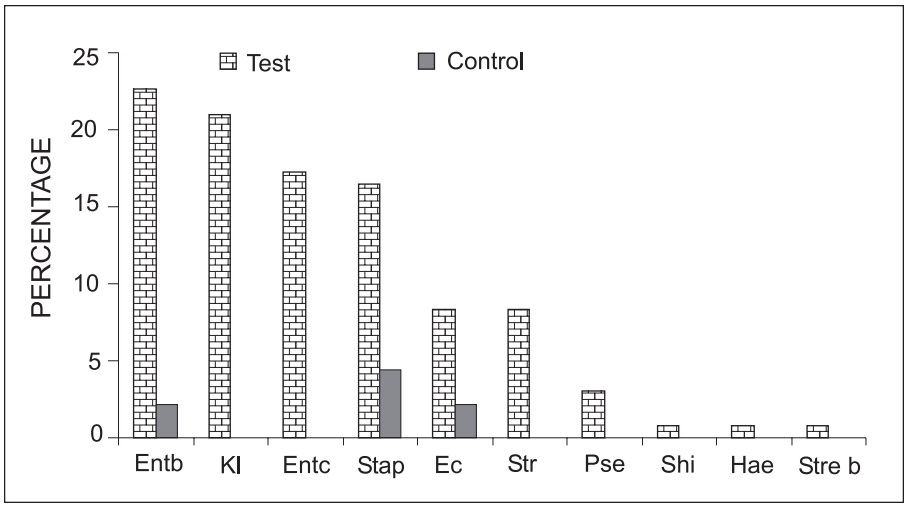

Fig. 2: Bacterial carriage rate of cockroaches. Entb – *Enterobacter*; Kl – *Klebsiella*; Ent – *Enterococcus*; Stap – *Staphylococcus*; Ec – *Esherichia coli*; Str – *Streptococcus*; Pse – *Pseudomonas*; Shi – *Shigella* sp; Hae – *Haemophilus*; Stre b– *Streptococcus* (β group A)

the experiments showed 4 adult *Enterobius vermicularis* and 8 *Ascaris* eggs in two cockroaches from hospital but observation of control group did not show any parasitic contamination.

Also the experiments showed some fungal contamination (some of them of medical importance) on test and control cockroaches which only the contamination with *Candida* sp was highly significant in test group (48%) in comparison to the control group (2.2%) ($p < 0.001$). The other isolated fungi from test group were *Mucor*, *Aspergillus niger*, *Rhizopus* sp, *Penicillium* sp and *Aspergillus fumigans* with 10.5, 7.5, 4.5 and 1.5% (for last tree fungi) contamination respectively. In this case most contaminations were related to surface of body (63%) and the rest (34%) was from gut of cockroaches. There was no other fungal contamination in control group other than *Candida* spp and *Aspergillus niger*.

Discussion

The results of the present study revealed contamination of almost all cockroaches collected from hospitals with different microorganism which is significantly higher in comparison to control group. So far a large number of microorganisms have been isolated from cockroaches captured either from housing, hospitals or other buildings[4,17,20–22]. In the present study, also a high percentage of test cockroaches (98%) were showed to carry various microorganisms (bacteria, fungi and parasites), some of them of medical importance. However, only few numbers of cockroaches collected from residential areas (8.9%) showed bacterial contamination and none of them showed to have parasitic contamination. High bacterial load in test cockroaches in comparison to control group (96% and 4.45% respectively) also suggested that in hospital environments there was more possibility for cockroaches to come in contact with contaminated objects. In addition, isolation of *E. coli* in cockroaches which had been detected in both test and control groups mean that they have been in contact with human faeces or faeces contaminated objects[21].

There have been a number of reports, which support the presence of drug resistance bacteria in hospitals[23,24]. Although in the present study only one isolate showed drug resistance, but reports from other studies indicate that resistance can be seen in more

bacteria. For instance, Fotedar *et al*[17] showed that bacterial pathogens like *Klebsiella* spp, *Ps. aeruginosa* and *S. aureus* were resistant to more than four antimicrobial agents. So due to widespread use of antibiotics[25] it is predictable that in future such problems can be seen in hospitals of Hamadan as well. Moreover, the tendency of cockroaches to move freely and inhabit toilets, sewers and drains can help to make the problem worse.

Regarding the importance of cockroaches as carriers of parasitic worm, cyst or eggs, there are some reports of the presence of parasitic forms on or in cockroaches[26,27]. The finding of the present study also showed the parasitic contamination in low numbers, which makes comment difficult nevertheless the presence of *Enterobius* infestation indicates that these cockroaches had opportunity to get touch with infested patients or contaminated cloths which emphasises their vectorial potential for parasitic diseases.

In case of fungal contamination in comparison to control the experiments showed that the presence of *Candida* spp in test cockroaches was significantly high ($p < 0.001$). It was the most prevalent fungi that is in accord with the findings of Fotedar & Banerjee[9].

The nosocominal infectious disease due to candida is of great importance and hospital-related blood stream infections has been known as the fourth most common cause of this kind of disease[28]. Therefore, the isolation of such fungi from cockroaches in hospital is alarming especially for patients such as recipients of bone marrow or organ transplants whose immune systems have been weakened[29].

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