

Study of Pathogenic Microorganisms in the External Body Parts of American Cockroach (*Periplaneta americana*) Collected from different Kitchens.

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Abstract: American cockroach (*Periplaneta americana*) harbor and disseminate the microorganisms to the environment. They play important role in the transmission of different diseases either mechanically, occasionally and biologically. The presence of these cockroaches in the kitchens is an indicator of poor food hygiene. These pathogenic bacteria along with their vector not only deteriorate our environment but also contaminate our food. Cockroaches may spread a range of diseases. Cockroaches are a health hazard. They carry bacteria on their bodies from sewers, drains and garbage areas and then transmit it to individuals by visiting their kitchens. The study was attempted to isolate the pathogenic bacteria from the household kitchens or the kitchen areas of different hotels/restaurants. Cockroaches were collected from kitchens by using sticky traps or direct collection. All the collected cockroaches were selected for the presence of pathogenic bacteria. From the collected *Periplaneta americana*, pathogenic species of bacteria like *E.coli*, salmonella, staphylococcus and streptococcus were isolated and identified by growth in their selective media using microbial techniques. The external body parts of all the collected American cockroaches were found contaminated with above mention species of bacteria. These findings proved potential role of *Periplaneta americana* in the transmission of pathogenic bacteria to the humans. Results showed that presence of cockroaches in the kitchen due to unhygienic conditions can threaten the health of individuals and cause serious diseases.

Key words: Pathogenic microorganisms, *Periplaneta americana*, *E.coli*, salmonella, staphylococcus.

I. Introduction

Food consumers in developing countries suffer from food-borne bacterial illnesses, especially from those of *Salmonella sp.*, *Shigella sp.*, *Staphylococcus aureus* and *Bacillus cereus*. Unhygienic food handling results in food contaminated by pathogens. One possible source of food contaminations could be dissemination of the pathogens to foods and/or utensils of catering centres through small animals such as cockroaches that live closely with humans in urban environments. Various investigations around the world revealed that cockroaches living close to human dwellings were important carriers of etiologic agents belonging to all groups of potential pathogens: viral, bacterial, protozoan and helminthes (Burgess and Chetwyn 1981, Agbodaze and Owusu 1989, Fotedar *et al.*, 1991, Cloarec *et al.*, 1992, Pai *et al.*, 2003a).

The cockroach poses one of the greatest health hazards of all households' pests. Millions of them live in our homes, hotels, restaurant kitchens, shops, supermarkets and bars, spreading diseases which can prove fatal to humans. Many cases of food poisoning are known to be as a result of cockroach contamination. Cockroaches have survived on the earth for more than 300 million years virtually without change (Zurek and Schal, 2004). There are approximately 3500 species of cockroaches worldwide (Kopanic, 1994). 50 species of them have been reported living in or around human structures (Kinfua and Erkob, 2008). The majority of these species live in tropical and subtropical area but are not pests (Vazirianzadeh *et al.*, 2009), so they found in abundance near areas where there is frequently standing water or areas where continued moist is usually available such as toilets, kitchen and drainages water frequently serve as a migration routes from place to place (Siachua *et al.*, 2008). Cockroaches frequently feed on human feces, garbage and sewage, therefore they have copious opportunity to disseminate pathogenic agents (Uckay *et al.*, 2009). They are known as one of the most important agents in transmission and distribution of many different bacteria, viruses, protozoa and fungi to human life, and they are intermediate host for some pathogenic intestinal worms (Cloarec *et al.*, 1992). In addition to the presence of some bacteria, parasites and fungi in external surfaces of cockroaches they have been found in internal parts of their body, therefore these insects are considered important diseases vectors transmitted by both mechanical and biological routes (Thyssen *et al.*, 2004).

The fact that cockroaches carry *pathogenic bacteria* has been reported for many years. Longfellow in 1913 showed that cockroaches carried pathogens, including *Proteus vulgaris*, *Staphylococcus aureus*, *Citrus* and *Bacillus of the subtilis type 9*. Fungi of medical importance such as *Candida*, *Rhizopus*, *Mucor*, *Alternaria* and *Aspergillus spp.* were isolated from cockroaches in hospital wards and residential areas (Fotedar *et al.*, 1992). A recent study in central Tehran, Iran reported that at least 25 different species of medically important bacteria, the most frequent being *Klebsiella sp.*, were isolated from *Blattella germanica* and *Periplaneta americana* collected from public hospitals and a residential house. Cockroaches from hospitals were more likely to be contaminated with medically important *bacteria*. A study by Cotton *et al.* from South Africa compared pathogenic strains from cockroaches and those which colonize infants and found no difference (Cotton *et al.*, 2000).

Periplaneta Americana are the carriers of agent of bacterial diarrhea. 208 cockroaches were collected from the Accra and they were cultured for enteric bacterial pathogen. Six of them had *salmonella species*, one had *shigella dysenteriae*, 64 had *coliforms*, 13 had *proteus species* and 2 had *pseudomonas species*. Cockroaches are one of those insects which are responsible for the transmission of pathogenic bacteria. (Aqbodaze and Owusu, 1989).

Cockroaches were trapped from food dispensing areas (kitchens). 104 cockroaches were caught, consisting of *Periplaneta americana* (67.3%), *Blattella germanica* (26%), *P. brunnea* (4.8%), and *Supella longipalpa* (1.9%). Bacteria were isolated from all cockroaches except 3 *P. americana*. Many bacterial species were identified, including the pathogenic and potentially pathogenic species *Shigella boydii*, *S. dysenteriae*, *Salmonella typhimurium*, *Klebsiella oxytoca*, *K. ozaena* and *Serratia marcescens* (Oothuman *et al.*, 1989).

II. Materials and methods

Total 300 samples were collected from kitchens of different localities of Lahore to test the presence of pathogenic bacteria. All these samples were used for the culturing of microorganisms.

To make nutrient broth medium 3.25g of nutrient broth mixture was added into the 250ml of distilled water. After 24 hours the collected samples of cockroaches were dipped into the nutrient broth by using the autoclaved forceps and placed them in the incubator-shaker for 16 - 18 hours at 37 °C (120 rpm).

2. Preparation of nutrient agar medium

To make a nutrient agar medium 6.8 g of nutrient agar was added in the 250ml of distilled water. After 18 hours of incubation of test tubes, clear turbidity ensured the bacterial presence. After overnight incubation of plates, samples from the liquid media is inoculated into the nutrient agar plates.

3. Preparation of eosine methylene blue agar

Eosine methylene blue agar medium is the selective and differential media to identify *E. coli* from others coliform bacteria (*salmonella*, *shigella*). For this 37.7g of EMB was added into one litre of distilled water. After the preparation of Eosine methylene blue agar plates, those plates were streaked with the culture in the nutrient broth and were placed as inverted in an incubator for 18 hours at 30°C.

4. Preparation of sorbitol macconkey agar medium

Prepared sorbitol MacConkey Agar plates by mixing 50 g of the medium in one liter of purified water. Then these plates were streaked with the culture grown in the nutrient broth medium and were placed in an incubator for 18-24 hours at 30 °C.

5. Ec media modified with novobiocin

To make medium 5g of novobiocin containing media was dissolved in 150ml of distilled water. Plates containing nutrient agar media were streaked by the bacterial culture grown in novobiocin containing media. These plates were then incubated for one day at 37°C.

Mannitol salt agar is the selective and differential media which was used to differentiate the *staphylococcus species*. For this 111g of mannitol salt agar medium was added into the one litre of distilled water. These plates were streaked with the culture grown in the nutrient broth medium.

7. Preparation of hektoen enteric agar medium

Hektoen enteric agar medium is selective and differential medium which is used to isolate and differentiate the *Salmonella sp.* and *shigella sp.* Add 75g of medium in one litre of distilled water. Streak these

plates of with the help of inoculating loop with the culture grown in the nutrient broth and were placed in the incubator.

III. Results

After 18-24 hours of inoculation, the nutrient broth medium become turbid, turbidity ensured the presence of pathogenic bacteria. Nutrient agar plates showed growth like, (i) the opaque, smooth, and glistening colonies with grape like clusters appeared on the nutrient agar plate which detected the presence of *staphylococcus spp.* (ii) Small, discrete, circular, smooth, translucent and colorless colonies appeared on the nutrient agar plate which detected the presence of *Salmonella spp.* (iii) Small, smooth, round and colorless colonies appeared on the nutrient agar plate which detected the presence of *E.coli*.

Sorbitol MacConkey agar (SMAC) plates showed the fermentation of sorbitol. Sorbitol fermenting strains of *E.coli* produce pink red colonies which ensured the presence of pathogenic *E.coli O157:H7*. EC media modified with Novobiocin were also ensured the presence of *E.coli O157:H7* by heavy growth on nutrient agar plates.

Mannitol salt agar or MSA is selective for the growth of *Staphylococcus spp.* Mannitol is fermented by a bacterium, acid is produced, which lowers the pH and results in the formation of a yellow area surrounding an isolated colony on MSA.

Eosine Methylene blue media were used for confirmation of *E.coli*. Incubated plates presented the metallic sheen colonies which ensured the presence of pathogenic *E.coli*.

Hektoen enteric Agar plates showed the green colonies which ensured the presences of pathogenic *Shigella* and green colonies with black centered ensured the presence of pathogenic *Salmonella*.

IV. Discussion

The main objective of this study was to examine human pathogenic microorganisms (bacteria) on the external body parts of American cockroach (*Periplaneta americana*) from kitchens, collected from different localities of Lahore, Pakistan. The study was attempted for the detection of pathogenic bacteria, to raise the public awareness about these pathogenic bacteria and the diseases they would cause. The presence of these cockroaches in the kitchens is an indicator of poor food hygiene. The literature reviewed in the present study provided evidences that *E.coli*, *salmonella*, *shigella*, *staphylococcus*, *streptococcus species* are frequently occurring organisms on the external body parts of American cockroaches. These pathogenic bacteria along with their vector not only detoriate our environment but also contaminate our food. Cockroaches may spread a range of diseases. Cockroaches are a health hazard. They carry bacteria on their bodies from sewers, drains and garbage areas and then transmit it to individuals by visiting their kitchens and bathroom areas. Feeding in such areas as sewers, drains and garbage areas brings them in contact with disease organisms including *E.coli*, *salmonella shigella*, *staphylococcus*, *streptococcus* and other organisms associated with dysentery, typhoid, hepatitis and tuberculosis. All these bacteria make the food contaminated and eventually it causes the illness in humans which may be fatal. Cockroaches are one of the most appalling sights, especially when they are in our home, kitchen nearby foods, and bathrooms. Any bug indoors will scare someone, but cockroaches are far worse than other household pests. They have been identified as one of the filthiest pests to encounter in Florida. Cockroaches carry many pathogens, they pick up from contaminated places such as: sewers, drains, garbage, landfills, bathrooms, and toilets. Many health risks come along with a cockroach infestation. These cockroaches are typical to causes many intestinal diseases and illnesses.

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