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Cockroaches act as a reservoirs and vectors of drug resistant *pseudomonas* spp.

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ABSTRACT

Cockroaches are among the most notorious pests of premises, which not only contaminate food by leaving droppings and bacteria that can cause food poisoning but also they transmit bacteria, fungi and other pathogenic microorganisms in infected areas. Cockroaches feed indiscriminately on garbage and sewage and so have copious opportunity to disseminate human pathogens. Cockroaches were collected from different sites of tertiary care hospitals. In total 20 cockroaches were collected and processed on Blood agar, Macconkey agar and Nutrient agar by using spread plate method. Microorganisms were isolated and identification both internal and external surface of cockroaches. The identification test was carried out by using micro morphological and cultural characteristics. Antibiotic sensitivity test was conducted on MHA plates. Four isolates were identified from external surface such as *Escherichia coli*, *Bacillus sps*, *Staphylococcus sps*, and *Pseudomonas sps* and three different isolates were identified in internal surface such as *Escherichia coli*, *Pseudomonas sps* and *Salmonella sps*. In the present study revealed that cockroaches carried 75% of *Pseudomonas sps*, 25% of *Salmonella sps*. In the cockroaches carrying *Pseudomonas sps* had a high infection rate when compared to others.

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KEYWORDS

Cockroaches;
Pseudomonas spp;
Drug resistant;
Internal surface;
External surface.

INTRODUCTION

Cockroaches are the most predominant residential, commercial, Institutional and Industrial pests in the world today. Approximately 3500 species in the world have become adapted to living in human habitations and are thus referred to as domestic species[4]. Since Cockroaches spent a lot of time indoors they can remain active throughout the year. They adversely affect human health by biting/gnawing the fingernails of sleeping children, by contaminating food, importing an unpleasant odour and taste, and transmitting disease organisms attached on their body parts[8].

They are proven or suspected carriers of the organisms causing diarrhea, dysentery, cholera, plaque, typhoid fever and viral disease such as poliomyelitis. In addition they carry the eggs of parasitic worms and may cause allergic reactions, including dermatitis, itching, swelling of the eyelids and more serious respiratory conditions. Its not only contaminate food by leaving

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droppings and bacteria that can cause food poisoning but also they transmit bacteria, fungi and other pathogens in ingested areas and pathogenic helminthes and to carry helminthes eggs, viruses, protozoa and fungi affecting man other vertebrate animals.

The hospital environments provide them with suitable temperature, humidity and ready source of food. The drug resistance bacteria are of great importance in hospitals, which are potential carriers of microorganisms, and their presence makes the problem more significant. The numerous pathogenic bacteria such as *Salmonella spp, Shigella spp, Campylobacter sp, Pseudomonas aeruginosa* and *Klebsiella pneumonia*[3], *Bacillus spp, Staphylococcus spp, Escherichia coli, Enterobacter spp, Erwinia spp* and *Serratia spp*. Yeast like *Pichia spp, Candida spp* and *Torulopsis spp* were associated with food spoilage[12]. Molds that known to produce Mycotxins such as *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus parasiticus* was isolated[14]. Helminthes, some Parasites were also found in external or internal parts of Cockroaches[6,15]. And German Cockroaches are capable of transmitting some of Hepatitis viruses. Among the microbial contaminants the *salmonella* is stable in cockroaches for more than 10 months and some parasites and fungal antigens play an important role in Asthma related health problem[2].

In addition to the external and internal source of disease causing pathogens, Cockroach's excrement and discarded skin contains many allergens to which sensitive people may develop allergies. In fact, exposure to Cockroach allergen early in life may actually contribute to the development of Asthma in susceptible children[9]. In hospital they may feed readily on a variety of human foodstuffs and also on patient's excreta, sputum, skin scrapings, boiled surgical swabs and other patient's wastes. They are ideally equipped to carry pathogenic organisms from the infected source of the hospital to uncontaminated material.

The activities and number of specific gut bacteria varied significantly with the insect's diet and developmental stage. Acetate and Lactate were the principle organic acids present in the gut fluid of adult Cockroaches. The acids were greatest amount were found in foregut and midgut regions of Cockroaches and it contains abundant populations of lactic acid bacteria.

MATERIALS AND METHODS

Collection of samples

The Cockroaches were collected at random manually from different sites of Tertiary Care hospitals, using rubber gloves. The cockroaches were collected twice a month and were brought to the laboratory.

Isolation of microorganisms from external surfaces

A total of 20 Cockroaches were collected from different region of hospital area. Each Cockroach was collected in a sterile test tube, transported to the laboratory and 2ml of sterile normal saline (0.9%) was added to the test tube and the Cockroaches were thoroughly shaken for 2 minutes.

A fixed volume (0.1ml) of the washing was cultured on Nutrient agar, Blood agar, MacConkey agar and Cetrimide agar separately by using spread plate method and incubated at 37°C for 24-48 hours and the colonies were identified by its cultural morphology, Gram staining, Motility and Biochemical tests.

Isolation of microorganisms from internal surface

After external washings, Cockroaches were placed in flasks rinsed with 70% alcohol for 5minutes (to decontaminate external surfaces as 70% alcohol is bactericidal), transferred to the sterile flasks and allowed to dry at room temperature at sterile condition.

Cockroaches were then washed with sterile for 2-3 minutes to remove traces of alcohol. Only Cockroaches captured whole and live were utilized for this study.

After the saline treatment the gut of the cockroach was dissected out and macerated aseptically in a sterile pestle and mortar in 2ml of sterile normal saline. The resulting 0.1ml of macerate was cultured on MacConkey agar, SS agar and Cetrimide agar, Nutrient agar plates separately, incubated at 37°C for 24-48 hours and the colonies were identified by cultural morphology, Gram staining, Motility and Biochemical tests.

Mannitol motility agar test

The medium was prepared with the following ingredients, (i.e.) Beef extract 1.0g, sodium chloride 75.0g, D- Mannitol 10.0g, phenol red 0.75g put in to the 1000ml of distilled water, the range of pH 7.2 ± 7.4. the above ingredients were dissolved and steril-

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TABLE 1 : Microorganisms from external surface of cockroach

S. No.	Name of the organism	Positive samples (Mixed isolates) N=17	Percentage 100%
1	*Escherichia coli and Pseudomonas spp.*	11	55%
2	*Pseudomonas spp and Staphylococcus spp.*	04	20%
3	*Bacillus and E.coli.*	02	10%

TABLE 2 : Microorganisms from internal surface of cockroach

S. No.	Name of the organisms	Positive samples N=16	Percentage 100%
1	*Pseudomonas aeruginosa*	12	75%
2	*Salmonella spp*	04	25%

TABLE 3 : Morphological characteristics

S. No.	Name of the organisms	Gram's staining	Motility
1	*Salmonella spp*	Gram negative rod	Motile (peritrichous flagella)
2	*Pseudomonas spp*	Gram negative Bacilli	Motile (polar flagella)
3	*Bacillus spp*	Gram positive rod	Motile (peritrichous flagella)
4	*Escherichia coli*	Gram negative rod	Motile (peritrichous flagella)
5	*Staphylococcus spp*	Gram positive cocci	Non-motile

TABLE 4 : Biochemical characteristics

S. No.	Name of the organisms	I	MR	VP	Citrate	TSI	Catalase	Oxidase	Urease
1	*Salmonella spp*	-Ve	+Ve	-Ve	+Ve	Acid & Gas	-Ve	-Ve	-Ve
2	*Pseudomonas spp*	-Ve	-Ve	-Ve	+Ve	Acid, no gas & H_2S	+Ve	+Ve	-Ve
3	*Bacillus spp*	+Ve	+Ve	-Ve	+Ve	Acid & no gas	+Ve	-Ve	-Ve
4	*Escherichia coli*	+Ve	+Ve	-Ve	-Ve	Acid & gas	-Ve	-Ve	-Ve
5	*Staphylococcus aureus*	-Ve	+Ve	+Ve	+Ve	Acid & gas	+Ve	-Ve	+Ve

ized by using autoclave at 121°C for 15 minutes, then poured in to sterile screw cap tubes and then inoculated the isolates. Incubated at 37°C for 24 hours, after the incubation the results were noted.

Antibiotic sensitivity test

All the clinical isolates were checked for the susceptibility pattern by "Disc Diffusion Technique" (Bauer *et al*., 1986). The single colony isolated from the SS agar, MacConkey agar, Cetrimide agar and Nutrient agar plates were inoculated in to peptone water. Then the suspension ii uniformly spread into the solidified Mueller Hinton Agar (MHA) plates by using standard sterile swabs and the following antibiotics such as (5-30 mcg/ml) Erythromycin, Gentamycin, Streptomycin, Ampicillin, chloramphenicol and Vancomycin were carefully placed over the spreaded culture. All the plates were incubated at 37°C for 24-48 hours. After the incubation the diameter of inhibited zones were measured and the results were interpreted based on National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

RESULT AND DISCUSSION

The experiment was conducted that the disease awareness in hospital roaming Cockroaches.

A total number of three different hospital sites were selected for sample collection such as Medical ward, skin ward and Canteen. A total number of three different hospital sites were collection such as Medical ward, Skin ward and Canteen. A total of 20 Cockroaches were collected from various sites of a Tertiary care Hospital from January to February 2009. All the samples were processed and incubated. After incubation numbers of colonies were observed from which four isolates were identified from external surface such as *Escherichia coli, Bacillus, Staphylococcus spp* and *Pseudomonas spp* (TABLE 1) were as three different isolates were identified in internal surface such as *Escherichia coli Pseudomonas aeruginosa* and *Salmonella spp* (TABLE 2). From the 20 Cockroaches 12 had single isolate of *Pseudomonas* in their internal regions of guts and mixed isolates of *Pseudomonas spp, Salmonella spp, Staphylococcus spp* and *Bacillus spp* in the body surface (external region) of Cockroaches. Total of 4 had both *Pseudomonas* and *Salmonella* on their guts for an infection rate of about 75% and 25% of contamination in guts together with body surfaces. 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.

All isolated strains were identified by micro morphological and biochemical test results were showed in (TABLE 3 & 4).

One from the isolates of Gut associate *Pseudomonas* was resistant to Erythromycin, Gentamycin, Streptomycin, Ampicillin, Chloramphinicol and Vancomycin. The *Salmonella* were resistant to Ampicillin, Chloramphenicol, Nitrofuration, Erythromycin and Gentamycin.

The drug resistant *Pseudomonas* was added to the food of Cockroaches obtained from sterile culture. This

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contaminated food could contaminate sterile Cockroach and drug resistant *Pseudomonas* was stable for more than two months in Cockroaches whereas in dry food the bacteria die in few days. The *Pseudomonas* was not stable in dry food, however in moist food they were stable and increased for more then ten days. These habits increase their potential to contaminate household foods by spreading bacteria[13] to other members of the population.

CONCLUSION

Drug resistant *Pseudomonas aeruginosa* in Cockroaches was found to be high among the hospitals and its may transmit the infections in hospitals to be included in the study.

The data from the study emphasis the importance of Cockroaches as potential vectors of medically important microorganisms such as Pathogenic Drug Resistant bacteria in Hospital environments. So, we should take care of Cockroaches in hospitals.

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REFERENCES

[1] G.Arakere, S.Nagdig, G.Swedberg, R.Macaden, S.K.Amarnath, D.Raghunath; J.Clin.Microbiol., **43**, 3198-202 **(2003)**.
[2] L.K.Arruda; Curr.Allergy Asthma Rep., **5**, 411-6 **(2005)**.
[3] M.F.Cotton, E.Wasserman, C.H.Pieper, D.Van Tubbegh, G.Cambell, F.C.Fang, J.Barnes; J.Hosp.Infect., **44**, 13-7 **(2000)**.
[4] W.Ebeling; Urban entomology. Berkeley: University of California, Division of Agricultural Sciences, **(1978)**.
[5] R.Fotedar, U.B.Shriniwass, A.Verna; Epidemiol.Infect., **107**, 181-187 **(1991)**.
[6] R.Fotedar, U.Banerjee; Acta.Trop., **50**, 339-43 **(1992)**.
[7] E.Galdiero, P.Villari, V.Onofrio, M.G.Pisciotla, A.Lucariello, L.Sommense, G.Liguori; New.Microbiol., **28**, 171-176 **(2005)**.
[8] J.Goddard; Infectious diseases and arthropods. Totowa, New Jersy, Humana Press, **(2000)**.
[9] A.A.Litonjua, V.J.Carey, H.A.Burge, S.T.Weiss, D.R.Gold; J.Allergy Clin.Immunol., **107**, 41-7 **(2001)**.
[10] D.Matthew, A.Kane, John, Breznak; Applied and Environmental Microbiology, **57**, 2628-2634 **(1991)**.
[11] H.Pai, Y.C.KO, E.R.Chen; Acta.Trop., **87**, 355-359 **(2003)**.
[12] M.F.Rueger. T.A.Olson; J.Med.Entomol., **6**, 185-9 **(1969)**.
[13] W.H.Robinson; Urban entomology chapman and Hall publiching, London, 131-138 **(1996)**.
[14] S.Mpuchne, M.Ignatius, Matsheka, A.Berhanu, Gashe, J.Allotey, G.Murindamombe, N.Mrema; African Journal of Food, Agriculture, Nutrition and Development, **6**, 1684-5358 **(2006)**.
[15] P.J.Thyssen, T.Moretti, M.T.Ueta, O.B.Ribeiro; 'The Role of Insects (Blattodea, Diptera and Nymenoptera) as Possible Mechanical Vectors of Helmenths in the Domiciliary and Peri Domiciliary Environment', Cad.Saude Publica., **20**, 1096-102 **(2004)**.