

Isolation and Identification of Pathogenic Microorganisms on the External Body Parts of Carpenter Ant (*Camponotus pennsylvanicus*) Collected from Kitchens.

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Abstract: Carpenter ants (*Camponotus pennsylvanicus*) are not only found in kitchens but they are also present in gutters, woods, and the places which are poorly adapted to healthy environment. The presence of these ants in the kitchen makes all the utensils and the food entirely unhygienic as a result when they are used by humans make them ill or may cause death. Carpenter ants are the very important source of spreading these pathogenic bacteria to humans and spreads food borne illness. The study was attempted to isolate the pathogenic bacteria from the external body parts of *Camponotus pennsylvanicus* collected from kitchen of different hotels, houses and restaurants located in different areas of district Lahore. To isolate these pathogenic microorganisms, carpenter ants external body parts were dipped in the nutrient broth medium and the turbidity produced in the medium, (after 24 hours incubation with shaking) indicated the presence of certain microorganisms. Among the collected samples, bacteria like *E.coli*, salmonella, staphylococcus and streptococcus pathogenic species were isolated and identified by the help of selective media. Total 300 samples were collected and all were found to be contaminated with these pathogenic bacteria mentioned above. Results concluded showed and confirmed that the presence of carpenter ants (*Camponotus pennsylvanicus*) in the kitchen of houses, hotels and restaurants are the important source of spreading different pathogenic bacteria which are very lethal to human health and may cause even death.

Key words: *Camponotus pennsylvanicus*, pathogenic microorganisms, *E.coli*, staphylococcus, salmonella.

I. Introduction

Many ants show mutualism and parasitic relationships with other insects or plants, some of which are classical examples of the mutual benefit of symbiosis. Usually ants are considered to be harmless and small creature. We have no problem picking them out of our tea cups or sugar jars, throwing them away and continuing to consume the food. The presence of *Camponotus pennsylvanicus* (Hymenoptera: Formicidae) in our surroundings may constitute a public health problem, especially since they are mechanical vectors for pathogenic microorganisms. Social insects, usually occupy microbe-rich environment and densely populated colonies of related individuals and are most susceptible to infection and rapid disease transmission. These insects have evolved a variety of adaptations to cope with pathogenic pressures, including behavioural, biochemical and immunological responses.

Camponotus pennsylvanicus, commonly known as carpenter ant is black in color and (75-150mm long). It is usually found in United States and utilize the wood structures to make satellite nests, and exacerbate damage done by water or rot. They have a property of breaking down old dead and rotting trees and hence are the great contributors to ecosystem. Carpenter ants are not able to digest cellulose; as a result they do not eat wood (Jacobs, 2008).

Microorganisms are a large and diverse form of life. Many of them live in association with large multicellular organisms, developing symbiotic relations with the host and some have even evolved to form obligate endosymbiosis. *Escherichia coli* was named by a German pediatrician and bacteriologist named Theodor Von Escherich (1857-1977). He was the first person who isolates this bacteria belonging to the family Enterobacteriaceae. *E.coli* is a common inhabitant of warm blooded animals as well as of human intestine. They reached the victim through food or water or through the individuals handling the child. It adheres to the mucus of large intestine in bowl. This bacterium possesses the ability to transfer DNA through bacterial conjugation, transduction or transformation. This process led to the spread of the gene encoding *Shiga* toxin from *Shigella* to *E. coli* O157:H7, carried by bacteriophage (Vogt and Dippold, 2005). *E. coli* is usually anaerobic, Gram-negative and is non-sporulating bacterium. Cells are typically rod shaped with the diameter of 0.5µm and are 2µm long. The cell volume is 0.6-0.7 µm³ (Madigan and Martinko, 2006).

Staphylococcus aureus is a member of the Micrococcaceae family and appear as gram-positive cocci in clusters. *S. aureus* is distinguished from other staphylococcal species on the basis of the gold pigmentation of colonies and positive results of coagulase, mannitol-fermentation, and deoxyribonuclease tests (Wilkinson, 1997). Humans are considered to be a natural reservoir of *S. aureus*. Thirty to 50 percent of healthy adults are

colonized, with 10 to 20 percent persistently colonized (Noble *et al.*, 1967). Both methicillin-sensitive and methicillin-resistant isolates are persistent colonizers (Casewell and Hill, 1986).

Salmonella typhi has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation, crowding, and social chaos. It may have responsible for the Great Plague of Athens at the end of the Peloponnesian War (Papagrigorakis *et al.*, 2007). The name *Salmonella typhi* is derived from the ancient Greek typhos, an ethereal smoke or cloud that was believed to cause disease and madness. In the advanced stages of typhoid fever, the patient's level of consciousness is truly clouded. Although antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries (Christie, 1987).

II. Materials and Methods

Collection of Samples

Total 300 samples were collected from kitchens of different localities of Lahore to test the presence of pathogenic bacteria. First group of 150 carpenter ants were collected from October 2011 to December 2011. And second group of 150 carpenter ants were collected from March 2012 to June 2012.

Preparation of Nutrient Broth Medium

About 3.25g of nutrient broth medium was added into the 250ml of distilled water and mixed well with stirrer. After complete mixing, poured into the conical flask. Mouth of the conical flask was covered with a cotton plug and aluminum foil and autoclaved it at 15psi pressure and temperature of 121°C for at least 30 minutes.

Preparation of Nutrient Agar Medium

About 6.8g of nutrient agar was added in the 250 ml of distilled water and mixed the ingredients by using the stirrer. When the components of the media were completely dissolved, poured the media in the conical flask. Covered the mouth of the conical flask with cotton plug and aluminum foil and autoclaved it at 15psi pressure and temperature of 121°C for at least 30 minutes.

Preparation of Eosine Methylene Blue Agar

Eosine methylene blue agar medium is the selective and differential media for identify *E.coli* and other bacteria e.g. *Salmonella* and *Staphylococcus aureus*. It also differentiates the bacteria that ferment lactose from those that do not ferment lactose. 37.7g of EMB was added into the one liter of distilled water in a beaker and mixed the media with the help of stirrer. After the complete dissolve of media into the water, poured the media into the conical flask. Covered the mouth of the conical flask with cotton plug and aluminum foil and autoclaved it.

Preparation of Mannitol Salt Agar

Mannitol salt agar is the selective and differential media which is used to differentiate the *staphylococcus species*. And also differentiate between the bacteria that ferment the mannitol from those that do not ferment the mannitol. 111g of mannitol salt agar medium was added into the one liter of distilled water in a beaker and mixed it with the help of stirrer. Then, poured the media into the conical flask. Covered the mouth of the conical flask with cotton plug and aluminum foil and autoclaved it.

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.* added 75g of medium in one liter of distilled water in a beaker and mixed with the help of stirrer. Then, poured the media into the conical flask. Covered the mouth of the conical flask with the cotton plug and aluminum foil and autoclaved it.

Preparation of Novobiocin Containing Media

About 5g of novobiocin containing media was dissolved in 150ml of distilled water in a beaker. It was mixed on magnetic stirrer, autoclaved for 2-3 hours and then cooled at room temperature. 1ml of autoclaved media was poured in each appendrof and was incubated for 16-18 hrs.

III. Results

The present study was conducted in order to isolate and identify pathogenic microorganisms on the external body parts of carpenter ant (*Camponotuspennsylvanicus*). For this purpose, total 300 samples were collected from kitchens situated in different localities of Lahore, Pakistan. The samples collected were cultured

with nutrient broth, nutrient agar and for further confirmation they were cultured on the selective media. All 300 samples were found contaminated with pathogenic bacteria (*E.coli*, *salmonella*, *shigella*, and *staphylococcus*).

To check the presence of pathogenic bacteria nutrient broth medium was prepared and transferred into the test tubes. After 18-24 hours of inoculation the nutrient broth medium became turbid, this turbidity ensured the presence of certain bacteria in the samples. For colony morphology identification of bacteria which were present on the external body parts of carpenter ant, nutrient agar plates were prepared. Small amount of culture was taken with the help of inoculating loop from the nutrient broth medium and were streaked on the agar plates.

- The opaque, smooth, and glistening colonies with grape like clusters appeared on the nutrient agar plate which detected the presence of *staphylococcus spp.*
- Small, discrete, circular, smooth, translucent and colorless colonies appeared on the nutrient agar plate which detected the presence of *Salmonella spp.*
- Small, smooth, round and colorless colonies appeared on the nutrient agar plate which detected the presence of *E.coli*.

Sorbitol MacConkey agar (SMAC) is the selective media used for *E.coli* detection. This medium contains sorbitol instead of lactose and is recommended for the detection of enteropathogenic strains of *E.coli* O157:H7 which ferments lactose but does not ferment sorbitol, therefore produce colorless or light pink color colonies. Sorbitol fermenting strains of *E.coli* produce pink red colonies.

Mannitol salt agar or MSA is a commonly used growth medium in microbiology. This media is selective for the growth of *Staphylococcus species*. Coagulase-positive *Staphylococci* produce yellow colonies with yellow zones, whereas coagulase-negative *Staphylococci* produce small pink or red colonies with no color change to the medium.

Eosine methylene blue agar was used to clearly differentiate between the colonies of lactose fermenting and nonfermenting microbes. On EMB *E.coli* gives a distinctive metallic green sheen color (due to the metachromatic Microorganisms that ferment lactose display "nucleated colonies" -- colonies with dark centers properties of the dyes, *E.coli* movement using flagella, and strong acid end-products of fermentation. This medium has been specifically designed to discourage the growth of gram positive bacteria.

EC medium modified with novobiocin (antimicrobial supplement) used in the detection of *E.coli* O157:H7. Growth in EC medium modified with novobiocin is demonstrated as an increase in turbidity.

Hektoen Enteric Agar (HEA) is used to isolate and differentiate *Salmonella sp* and *Shigella sp*. The growth of *E.coli* is inhibited on this medium while *Shigella* showed a very good growth with green colonies and *Salmonella* appeared as green colonies with black centers.

IV. Discussion

The main objective of the present microbial study was to isolate and identify the pathogenic microorganisms on the external body parts of carpenter ants (*Camponotus pennsylvanicus*) and to create public awareness about the health hazards resulting from these pathogenic microorganisms. To conduct this study, total 300 samples were collected from the kitchen of houses, hotels and restaurants located in Lahore, Pakistan. All the samples were first cultured on nutrient broth and nutrient agar, which ensured the presence of certain pathogenic microorganisms. For further confirmation and identification, the culture from the nutrient agar was streaked on different selective media and the presence of pathogenic bacteria like *E.coli*, *staphylococcus*, *salmonella*, *shigella*, and *streptococcus* was confirmed.

The presence of carpenter ants in the kitchen indicates not only the poor environmental conditions but also the poor food hygiene. The literature reviewed for the present study provides clear evidence that *E.coli*, *salmonella sp*, *shigella sp* and *staphylococcus sp* are frequently occurring bacteria in our environment, which not only make our environment unhealthy but also spread number of diseases. Carpenter ants are not only found in kitchens but they are also present in gutters, woods, and the places which are poorly adapted to healthy environment. The presence of these ants in the kitchen makes all the utensils and the food entirely unhygienic as a result when they are used by humans make them ill or may cause death. Carpenter ants are the very important source of spreading these pathogenic bacteria to humans and spreads food borne illness.

Environment of Pakistan is favorable for the growth of pathogenic microorganisms. It provides all the suitable conditions for them to nourish and prosper. To make our environment free of these bacteria, certain preventive measures should be taken.

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Isolation and Identification of Pathogenic Microorganisms on the External Body Parts of Carpenter

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