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Research Article

P. AMERICANA AS AN INTESTINAL CARRIER OF NOSOCOMIAL AND FOOD BORNE BACTERIAL PATHOGENS

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ABSTRACT

The study was undertaken to search the carrier potential of *P. americana*, the predominant cockroach species in India, of various human bacterial pathogen including *L. monocytogenes* in their intestine. Cockroaches were collected from tertiary care hospitals, domestic environments, market places and restaurants. Identification of the bacterial isolates from the aseptically removed intestine was carried out by standard bacteriological procedures (Cowan and Steel, 1974) including morphological, cultural, biochemical and physiological studies. *Listeria* species were isolated following USDA method. The bacteriological examination of the intestinal content of *P. americana* revealed the presence of various potential human bacterial pathogens including *Listeria* spp. The major bacteria with epidemiological significance in nosocomial infection isolated from the intestinal contents of *P. americana* were *Enterococcus* spp. (95.6%), *Klebsiella* spp. (39.6%) and *Proteus* spp. (36%).51.2% of cockroaches under study were found to be harboured with *Listeria* spp. The observations made in this study establish the possible role of the insect *P. americana* in carrying and transmitting human pathogens especially in nosocomial and food borne infections and goes to suggest that the pest cannot be ignored as a casual harmless inhabitant of the human environments.

Keywords: P. americana, intestine, bacterial pathogens

INTRODUCTION

Being an omnipresent insect with filth feeding habits, it is likely that cockroaches acquire and harbour human pathogens in their intestine and may subsequently be transmitted to human environments through faecal matter raising concern on health in human beings[1]. In spite of the potential of this insect to carry and disseminate pathogenic organisms, the intestinal flora of this insect is not adequately explored particularly in India. *L. monocytogenes* is a food-borne human pathogen responsible for listeriosis particularly in pregnant women and immunocompromised individuals. Owing to the omnipresence and filth feeding habits of *P. americana*, it is likely that they can be a possible carrier of *Listeria* species. Despite the potential of *L. monocytogenes* to cause human infection, the probable role of cockroaches as an intestinal carrier of this bacterial species has not been studied in detail and the literature regarding its isolation from the insect is very scanty.

This study deals with the isolation and identification of various bacterial pathogens with special emphasis on *Listeria* species from the intestinal contents of *P. americana*

MATERIALS AND METHODS

Collection and dissection of cockroaches

Cockroaches were collected from tertiary care hospitals, domestic environments, market places and restaurants in central Kerala. The captured insects were placed in previously decontaminated bottles, transported alive to the laboratory and immobilized at a temperature of 0^0 C for 5 to 20 min. Using standard criteria outlined by Cochran[2], 500 of them (130 each from hospitals and domestic environments and 120 each from market places and restaurants) were identified to be belonging to *Periplaneta americana.* The external surface of each insect was wiped with 70 % ethanol to ensure that the gut content was not contaminated by the surface microflora of the body during dissection. The insect was then transferred and fixed on to a disinfected dissection board and the intestine was exposed from the ventral side with the aid of sterile scissors and forceps. The aseptically removed intestine was then placed in 2mL of sterile phosphate buffered saline (PBS) in a test tube and emulsified.

Isolation and identification of bacteria

For the isolation of bacteria, a loopful (0.01 mL) of the emulsified intestinal content of each cockroach was plated onto nutrient agar (Hi- Media), MacConkey agar (Hi- Media) and blood agar.The plates were then incubated at 37^0C for 24 hr. Simultaneously 0.5 mL of the emulsion was also inoculated into selenite F broth (Hi-Media) and after incubation for 6-8 hr, a loopful of the broth was plated onto xylose lysine deoxycholate (XLD) agar (Hi-Media) and incubated at 37^0 C for 24 hr. Representative pure colonies of bacteria on the agar plates were then sub cultured onto nutrient agar slants and identification of the isolates was carried out by standard bacteriological procedures[3] including morphological, cultural, biochemical and physiological studies.

For the isolation of *Listeria* spp., the aseptically removed intestine was emulsified in 2 mL phosphate buffered saline and then transferred into 10 mL *Listeria* primary enrichment medium broth (UVM –1) (Oxoid) and incubated aerobically at 28 ± 2^0C for 18 to 24 hr. After incubation, 1 mL of the primary enrichment broth was inoculated into 10 mL of Fraser's broth (Oxoid) and incubated at 36 ± 1^0C for 18 to 24 hr. A dark or black coloured growth indicates the hydrolysis of esculin showing the possible presence of *Listeria*. A loopful of the Fraser's broth culture was then streaked on to Listeria selective agar (Oxoid) and incubated at 36 ± 1^0C for 48-72 hr. After incubation, the plates were checked for typical *Listeria* colonies with black/dark zone around. Fraser's broth giving no growth was refrigerated further at 4^0C to obtain growth, if any. Typical *Listeria* colonies (three from each plate) were then streaked onto trypticase soy agar (TSA) (Hi-Media) and incubated at 37^0C for 24 to 48 hr. Colonies demonstrating a characteristic blue colour under Henry's illumination were subjected to further biochemical characterization using a battery of tests including Gram's reaction, production of catalase, haemolytic activity on blood agar, umbrella motility in semisolid nutrient agar (Hi-Media), tumbling motility at room temperature, nitrate test, fermentation of glucose, D-mannitol, L-rhamnose, alpha methyl D-mannoside, D-xylose and hydrolysis of esculin. Identification of *L. monocytogenes* isolates was done by 16S rDNA sequencing.

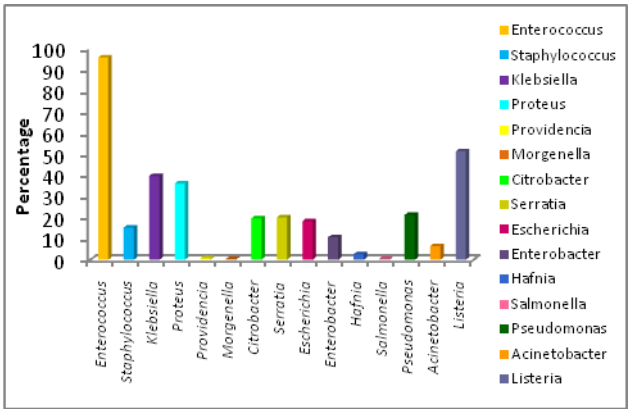
Statistical analysis

Source-wise variation of the bacterial isolates was analysed by proportion test. The level of significance was set up at $p<0.05$.

RESULTS

The bacteriological examination of the intestinal content of *P. americana* revealed the presence of various potential human bacterial pathogens including *Listeria*.

The overall incidence of bacteria isolated



The overall incidence of different bacterial pathogens isolated from the gut content of *P. americana* is shown in Figure 1. The major groups of bacteria with epidemiological significance in nosocomial infection isolated from the intestinal contents of *P. americana* were *Enterococcus* spp. (95.6%), *Klebsiella* spp. (39.6%) and *Proteus* spp. (36%). *Pseudomonas* spp., *Serratia* spp., *Ctrobacter* spp. and *E. coli* were noticed in 21.2%, 20%, 19.6% and 18.2% of the cockroaches respectively. The incidence of isolation of *S. epidermidis* was found to be 15.2%. 51.2% of cockroaches under study were found to be harboured with *Listeria* spp. *Morgenella* and *Salmonella* were noticed to be least isolated (0.4% each).

Table 1: The incidence of various bacterial species isolated- source-wise break up

Bacterial isolates	No. and percentage (*) of samples positive			
	H(**130)	D(**130)	M(**120)	R (**120)
E. faecium	128(98.4)	108(83)	82(68.3)	80(66.6)
E. faecalis	61(46.9)	3(2.3)	3(2.5)	6(5)
E. casseliflavus	2(1.5)	4(3)	1(0.8)	0
S. epidermidis	32(24.6)	15(11.5)	15(12.5)	14(11.6)
K. pneumoniae	72(55.3)	47(36.1)	38(31.6)	29(24.1)
K. oxytoca	2(1.5)	5(3.8)	0	0
K. rhinoscleromatis	3(2.3)	0	2(1.6)	0
P. mirabilis	34(26.1)	63 (48.4)	63(52.5)	8(6.6)
P. vulgaris	2(1.5)	7(5.3)	3(2.5)	0
Prov. rettgeri	0	3 (2.3)	0	0
M. morganii	0	1(0.7)	1 (0.8)	0
C. freundii	18(13.8)	14 (10.7)	5 (4.1)	8(6.6)
C. diversus	10(7.6)	40(30.7)	3(2.5)	0
S. marcescens	32(24.6)	43(33)	7(5.8)	18(15)
E. coli	27(20.7)	38(29.2)	16(13.3)	10(8.3)
E. cloacae	0	14(10.7)	18(9)	0
E. agglomerans	1(0.7)	0	12(10)	8(6.6)
H. alveoli	4(3)	6(4.6)	1(0.8)	2(1.6)
Salmonella spp.	1(0.7)	1(0.7)	0	0
P. aeruginosa	61 (46.9)	18(13.8)	21(17.5)	3(2.5)
P. fluorescens	2 (1.5)	0	1(0.8)	0
A. baumanii	7(5.3)	6(4.6)	0	1(0.8)
A. lwoffi	0	0	12(10)	6(5)
L. monocytogenes	2(1.5)	0	0	0
L. innocua	4(3)	2(1.5)	0	0
L. grayi	62(47.6)	92(70.7)	50(41.6)	44(36.6)

H – Hospital, D – Domestic environment, M – Market, R – Restaurant *(percentage is given in brackets) ** (No. of samples processed)

Table 1 shows the incidence of isolation of various bacterial species from different sources viz. hospitals, domestic environments, markets, restaurants. The isolation of bacterial pathogens viz. *E. faecium* (98.4%), *E. faecalis* (46.9%), *S. epidermidis* (24.6%), *K. pneumoniae* (55.3%), *P. aeruginosa* (46.9%) was found to be more in cockroaches captured from hospital sources in comparison with other sources which is statistically found to be significant by two sample proportion test ($p<0.001$). Hospital cockroaches also formed the source for the only 2 isolates of *L. monocytogenes*. However, *C. diversus* (30.7%), *S. marcescens* (33%), *E. coli* (29.2%) dominated in domestic samples ($p<0.01$).

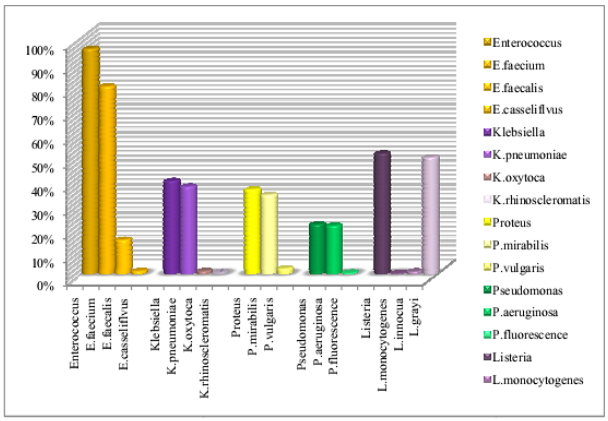


Figure 2: Species-wise break up of predominant bacterial isolates

Species – wise break up of predominant bacterial isolates obtained from the gut content of *P. americana* is shown in Figure 2. Of the 95.6% of *Enterococcus* isolates obtained from 500 cockroach samples, the major species was found to be *E. faecium* (79.6%) followed by *E. faecalis* (14.6%) and *E. casseliflavus* (1.4%). The *Klebsiella* were isolated from 39.6% of samples of which *K. pneumoniae* makes 37.2%. The other species were *K. oxytoca* (1.4%) and *K. rhinoscleromatis* (1%). Among the *Proteus* spp., *P. mirabilis* predominated (33.6%) with *P. vulgaris* making 2.4%. *P. aeruginosa* represented 20.6% of *Pseudomonas* isolates and *P. fluorescens,* 0.6%. *L. grayi* (49.6%) was the major species of the genus *Listeria* isolated with other species being *L. innocua* (1.2%) and *L. monocytogenes* (0.4%).

DISCUSSION

The importance of cockroaches as a potential carrier of pathogenic micro organisms appears to have been overlooked. Even if it is difficult to prove the direct involvement of cockroaches in the transmission of bacterial diseases, their role in the spread of pathogens is strongly suspected. A wide range of bacteria including both Gram positive and Gram negative have been recorded to be carried by cockroaches. *S. aureus*, *E. faecalis*, *Salmonella* spp., *Shigella* spp., *P. aeruginosa*, *Enterobacter* spp., and *Klebsiella* spp. were a few among them[1]. The possible mechanisms of spread of micro organisms by this insect may be by mechanical transmission by the adherence of organisms to the surface cuticle or by colonizing the pathogens in the intestinal tract and then disseminating while defaecation. The bacteria carried in the intestinal tract of this insect were proved to be viable and can multiply for several days before being expelled[4]. The developmental sites of the insect, its dependence on the microbial communities, feeding mechanism (regurgitation), its attraction to human food and wandering habits make it a suitable carrier of bacteria that are associated with human infections.

The present study noticed *Enterococcus* spp. as the major bacterial species inhabiting the gut of cockroaches. Ahmad et al. [5] reported a

similar finding of high prevalence of *Enterococcus* spp. in cockroach's gut contents. The enterococci are ubiquitous Gram positive bacteria found in various habitats including the intestinal tract of insects, animals, humans and in environment contaminated by animal or human faecal material[6]. The two major species, *E*. faecalis and *E. faecium* are considered as opportunistic and nosocomial pathogens of humans causing urinary tract infections, bacteraemia, intraabdominal and pelvic infections, wound and tissue infections and endocarditis[6, 7].The current observation of *K. pneumoniae* as the dominant *Klebsiella* species isolated is in agreement with earlier reports [8 , 9,10]. *K. pneumoniae* strains are often considered to be opportunistic rather than true pathogens since they mostly affect debilitated patients, infants and the elderly. According to Podschun and Ullmann [11], *K. pneumoniae* accounts for a significant proportion of hospital acquired urinary infection, pneumonia, septicaemia and soft tissue infection. The current study observed *Proteus* spp. as the second most common Gram negative bacterial isolate in the gut of the insect. It is not surprising to find the saprophytic bacteria like Proteus in the intestine of an omnivorous insect like cockroach. Chaichanawongsaroj et al. [12]; Elgderi et al [13] and others noticed *Proteus* spp. such as *P. mirabilis* and *P. vulgaris* in the gut contents of cockroaches. *Proteus* spp. are found to be associated with infections of urinary tract , surgical wounds and lower respiratory tract mostly in children whose immune system is underdeveloped[14]. Of the 21.2% of Pseudomonas isolates obtained in the current study *P. aeruginosa* was noticed as the frequently isolated species (20.6%). Fotedar et al. [8]; Salehzadeh et al. [15] also recorded *P. aeruginosa* as one among the commonly isolated bacterial pathogens from cockroaches. *P. aeruginosa* is noticed as the second most common Gram negative pathogen in health care institutions as recorded by United States national nosocomial infection surveillance system contributing substantially to wound associated morbidity and mortality [16]. Saitou et al. [17] observed the biofilm formation potential of *P. aeruginosa* obtained from hospital inhabiting cockroaches. S. marcescens isolated from cockroaches can be involved in disease breakouts and nosocomial infections particularly in immunocompromised as notticed by earlier studies [9,13]. Tilahun et al. [10] observed *Citrobacter* species predominantly *C. diversus* as one of the common isolate from cockroaches captured from ICU, a finding in accordance with the current study. Apart from *Enterococcus*, *Klebsiella, Proteus*, *Pseudomonas*, *Serratia* and *Citrobacter*, other dominating Gram negative bacteria isolated include *E. coli*, *Enterobacter* spp. and *Acinetobacter* spp. These bacteria are frequently associated with urinary tract infections, sepsis, gastroenteritis, biliary and peritoneal infections, pneumonia or wound infections in intensive care units (ICU) and medical clinics, surgical and orthopedic units[18, 1]. In a study conducted in cockroaches [18], the presence of various opportunistic as well as potentially pathogenic Gram negative bacterial pathogens in the gut contents. Though the incidence of isolation of *E. coli* from the insect was not as high as expected (18.2%), its detection reflects its filth feeding habits particularly with faecal matter [20]. The *S. epidermidis* isolates from the insect, though generally dismissed as contaminants, are found to have significance in intravascular or catheter related infections[21], cerebrospinal fluid shunt infections [22] and bacterial endocarditis [23]. Moreover, being a resistant organism it may also survive on inanimate objects for prolonged periods after being deposited by the insect through faecal matter. *A. baumanii* detected in the current study has been found to be surviving for several weeks even under dry conditions serving as a constant source of secondary infection in the health care facilities [24]. The isolation of Salmonella spp. from cockroaches captured from hospitals and houses indicate the potential of these pests in posturing food borne infections. According to Devi and Murray [25], cockroaches acquire *Salmonella* naturally from environment polluted with the animal and human wastes. Tachbele et al. [26] also noticed cockroaches as a reservoir and vehicle of food-borne pathogens like *Salmonella* and suspect the role of these insect in spreading multiple drug resistant *Salmonella* in hospitals and food catering establishments. Source to source variation regarding the isolation of bacterial species was obvious with certain bacterial isolates (Table 1). Predominance of *E. faecium*, *E. faecalis*, *K. pneumoniae, S. epidermidis and P. aeruginosa* in samples captured from hospitals indicates the potential of *P. americana* inhabiting in heath care facilities to act as reservoirs for these bacteria which can cause serious infections in neonates and in immunocompromised patients.

The overall prevalence of *Listeria* spp. in the insect's gut was 51.2 %. Of the 256 *Listeria* isolates, *L. grayi* was found to be the most frequently isolated species (49.6%) followed by *L. innocua* (1.2%) and *L. monocytogenes* (0.4%). The ability of *L. monocytogenes* to survive and multiply in many habitats coupled with its easy mode of transmission may contribute to its survival in an omnipresent insect like cockroach. Though the overall incidence of isolation of this pathogen in the current study is less, its presence in hospital samples may not be disposed as unimportant.

CONCLUSION

The observations made in this study establish the possible role of the insect *P. americana* in carrying and transmitting human pathogens especially in nosocomial and food borne infections and goes to suggest that the pest cannot be ignored as a casual harmless inhabitant of the human environments. Thus, even though there is no direct proof there is mounting evidence that cockroaches inhabiting in human environments serve as a vehicle of potential bacterial pathogens and therefore as a source of possible infection.

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