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Research Article

# PERIPLANATA AMERICANA - A CARRIER OF MULTI RESISTANT LISTERIA SPECIES

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**ABSTRACT**

Objectives : The study was undertaken to search whether *P.americana* could act as carriers of *Listeria* spp. in their intestine and to determine the antibiotic susceptibility of *Listeria* spp. obtained . The resistance transfer, if any, between L.grayi and *E. faecalis* , the co inhabitants in the insect's intestine , was also under the per view of the study.

Methods: *Listeria* spp. were isolated from the intestinal contents of *P.americana* using enrichment and selective media. The antibiotic susceptibility of the isolates was determined by disk diffusion and agar dilution methods. Gene transfer study was carried out by conjugation.

Result: 254 *Listeria* isolates were obtained from the intestinal contents of 500 cockroaches collected from different sources with *L.grayi* constituting 49.6% and *L.innocua* 1.2 % showing multiple resistance to penicillin, cloxacillin, new generation cephalosporins and nalidixic acid. *L. grayi* strains under gene transfer study were found to be harbored with plasmid. 2 strains of *L. grayi* having plasmid were resistant to ampicillin. The ampicillin resistance was found to be transferrable to *E. faecalis* through conjugation.

Conclusion: The isolation of *L. grayi and L. innocua* from the gut content of *P. americana* indicates the possibility that the insect could also act as a carrier of pathogenic species of *Listeria*. Transfer of plasmid between L.grayi and *E. faecalis* in the current study suggests the possibility of such gene transfer between bacteria in the gut of the insect and thereby facilitating the dissemination of resistance genes among them .

**Keywords:** *Periplanata americana; Listeria* spp; antibiotics

## INTRODUCTION

Although it is difficult to prove the direct involvement of cockroaches in the transmission of pathogenic microbial agents to humans, the importance of these insects in the spread of bacteria cannot be ruled out. The omnivorous feeding habits of *P. americana* make them ideal agents for harbouring and transmitting bacterial pathogens in their intestinal tract. Species of *Citrobacter, Enterobacter, Klebsiella, Serratia Pseudomonas, Proteus, Acinetobacter, Escherichia and Enterococcus* have been isolated from the gut contents of cockroaches inhabiting the hospital environment[1]. Although intensive research has been done to ascertain the role of cockroach as a carrier various microbial human pathogens, its role as a carrier of *Listeria species* has been seldom established. All *Listeria* species are ubiquitous in the environment and are potential food contaminants. Of its six species , *L. monocytogenes* is gaining recognition as a human pathogen which can cause listeriosis, a disease primarily affecting women and the neonates, patients who are immunocompromised and the elderly. However, rare cases of infections have been described with *L. seeliger*[2] and *L.innocua*[3] and *L. grayi*[4-5] .

Most cases of human listereosis appear to be sporadic. However, association of *L. monocytogenes* with several large food borne outbreaks suggests that contaminated food may be the primary source of the organism [6-7]. The presence of any *Listeria* species in food indicates poor hygiene. Standard antibacterial therapy for the effective treatment of listeriosis consists of administration of ampicillin, penicillin or rifampin in combination with gentamicin[8]. But recently, resistance to various antibiotics has been demonstrated in many strains of *L. monocytogenes*[9].

The transfer of plasmid encoded resistance to antimicrobial agents between bacteria is a significant public health concern. Conjugation is the most important mechanism of gene transfer among bacteria whereby the strains which lack virulence may acquire it.

The present study was conducted to gain insight into the prevalence of *Listeria species* in the intestinal contents of *P. americana and* to determine its antibiotic resistance pattern . The mode of antibiotic resistance transfer of *Listeria species* was also a part of this study.

## MATERIALS AND METHODS

### Isolation of *Listeria*

Cockroaches were captured from clinical and supplementary areas of three multi-speciality hospitals including a medical college hospital, houses, restaurants and market places in Kerala, India.

Cockroaches captured were put in sterile bottles, transported to the laboratory, immobilized at $0^0$C, and were confirmed as *P americana* using standard taxonomic keys. The gut was aseptically removed and placed in sterile test tube containing 2ml sterile phosphate buffered saline. An emulsion of the gut was prepared and transferred to 10 ml. of *Listeria* primary enrichment medium broth (UVM-1 broth base) (Oxoid) and incubated at $28 \pm 2^0$C for 18 to 24 hours. One ml. of the primary enrichment broth was inoculated into 10 ml of Frazer's broth (UVM broth base 1 L, lithium chloride 3g, ferric ammonium citrate 0.5g, Oxoid supplement) and incubated at $36 \pm 1^0$C for 18 to 24 hours. A black or dark coloured growth due to hydrolysis of esculin indicated the possible presence of *Listeria*. Loopful of the culture from the Frazer's broth was streaked on to Listeria selective agar (Oxoid) and incubated at $36 \pm 1^0$C for 48-72 hours. Plates were examined for typical colonies.

### Identification of isolate

Isolates were tentatively identified as *Listeria* species by studying the morphological, cultural and biochemical properties. Guidelines of Bergey's manual of systematic bacteriology[10] were followed. Confirmation was made by PCR amplification.

### Antibiotic susceptibility testing

The antimicrobial susceptibility of *Listeria isolates* was performed by disk diffusion method on Muellar Hinton Agar (MHA) plates by using commercially available discs (Himedia laboratories). The diameter (in millimeters) of zone around each antibiotic disc was measured and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendation[11]. Minimal inhibitory concentration was determined against 0.25 – 248 µg penicillin $mL^{-1}$, 0.25 – 186 µg ampicillin $mL^{-1}$, .25 – 64 µg tetracycline $mL^{-1}$ , 0.25 – 64 µg erythromycin $mL^{-1,}$ and 0.25-32 µg gentamicin $mL^{-1.}$ The result was interpreted according to NCCLS guidelines.

### Conjugation studies

To examine the conjugative transfer of ampicillin resistance, broth mating (BM) was performed by using a modification of technique described by Ike *et al*[12.] *Listeria grayi* isolate (plasmid harbouring , ampicillin resistant, rifampicin sensitive) was used as donor strain and *E.faecals* (plasmid free, ampicillin sensitive,rifampicin resistant) as recipient. Briefly , 0.5 ml. of donor and 0.5 ml. of recipient cell cultures were mixed and added to 5 ml. of Luria Bertani broth ( Hi

media laboratories) .The mixture was incubated statically at 35°C ± 2 for 5 h., serially diluted and spread plated on agar plates supplemented with 64 µg/ml ampicillin and 20 µg/ml rifampicin . After incubation at 37° C for 48 h , the transconjugants were selected based on their phenotypic properties and plasmid detection. The conjugation efficiency was expressed relative to the number of donor cells.

## RESULTS

254 *Listeria* isolates were obtained from the intestinal contents of 500 cockroaches collected from different sources. Data was analysed statistically employing the Sigma stat software version 7.5 (M/s sigma Aldrich, USA ) The highest rate of isolation was from the samples collected from houses followed by those from hospitals. Rate of isolation of *Listeria* species from different sources in shown in table 1. When analyzed statistically no significant difference in the rate of isolation of *Listeria* species from different sources was observed (Chisquare: 4.635 and p = 0.236) Table – 2 shows the species wise break-up of the isolates. The resistance towards different antibiotics by *L. grayi* is shown in table 3.

Of the total 254 *Listeria* isolates, 248 were belonging to the species *grayi* and 6 isolates were *L.innocua.* Statistical analysis of the data in table 2 indicated no significant difference in the proportion of isolation between the two species of Listeria (Chi-square 3.105, p = 0.928) Resistance to nalidixic acid was shown by 100% of *L. grayi* isolates. Resistance towards penicillin, cefepime, cloxacillin and cefuroxime were 85.4%, 79%, 77.4% and 77.4% respectively. However, the isolates showed comparatively lower resistance percentage towards vancomycin (6.4%), ciprofloxacin (9.6%), imipenam (27.4%), tetracycline (19.3%), erythromycin (25%), gentamicin (24.1%) and ampicillin (20.9%). *L. innocua presented* 100% of resistance towards nalidixic acid, penicillin, cloxacillin, cefepime and cefuroxime.

Conjugation study noticed the transfer of plasmid with ampicillin resistant genes from *L. grayi* to *E. faecalis* with the conjugation frequency $10^{-6}$ – $10^{-7}$.

**Table 1: Rate of isolation of *Listeria* species from the intestinal contents of cockroaches**

| Source of cockroach | No. of samples processed | No.& Percentage* of samples positive for *Listeria* |
|---|---|---|
| Hospitals | 130 | 66 (50.76) |
| Houses | 130 | 94 (72.30) |
| Markets | 120 | 50 (41.66) |
| Restaurants | 120 | 44 (36.66) |

*** Percentage is given in brackets**

**Table 2: Species wise break up of *Listeria* isolates from cockroaches.**

| *Listeria* species | Prevalence (%) | Number of isolates harboured by the cockroaches from different sources | | | |
|---|---|---|---|---|---|
| | | Hospital | House | Restaurant | Market |
| *L. grayi* | 49.6 | 62 | 92 | 44 | 50 |
| *L innocua* | 1.2 | 4 | 2 | - | - |
| Total | 50.8 | 66 | 94 | 44 | 50 |

**Table 3: MICs of antibiotics against *Listeria* spp.**

| *Listeria* species | No.of isolates | MIC (µg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | P | AMP | TC | EM | GM |
| *L grayi* | 25 | 0.25-124 | 0.4-64 | 2-32 | 0.25-16 | 0.25-16 |
| *L. innocua* | 6 | 32-64 | 0.25-1 | 1-8 | 0.25-1 | 0.64-4 |

**P, Penicillin; AMP, Ampicillin; TC, Tetracycline; EM, Erythromycin; GM, Gentamicin**

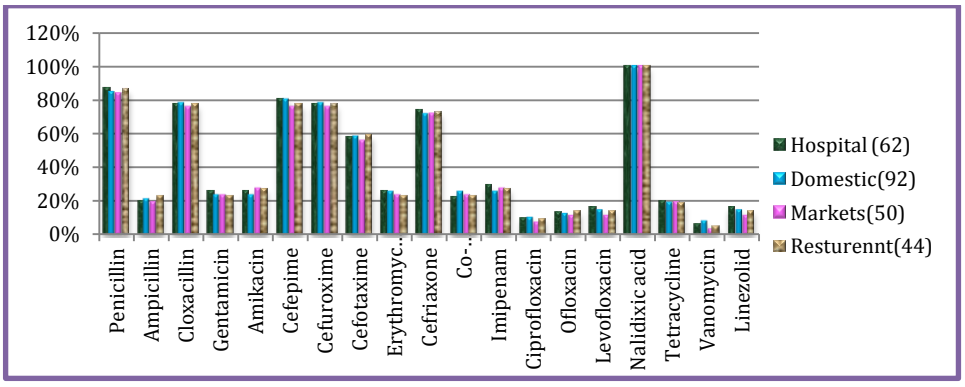


**Figure 1: Comparison of resistance towards different antibiotic shown by *L.grayi* isolates from different sources.**

## DISCUSSION

Although cockroaches are yet to be linked with epidemics of bacterial diseases, their involvement has been strongly suspected[13]. Even though an impressive array of pathogenic or potentially pathogenic micro-organisms have been isolated and documented previously [14], the role of cockroach as a carrier of *Listeria* species is seldom reported .

In the present study, 50.8% of cockroaches were found to harbour *Listeria* species. Cockroaches from household environments yielded higher percentage of *Listeria* isolates (72.3%) followed by those from hospitals (50.76%). The isolation rate was comparatively lesser in the insects caught from market places (41.66%) and food establishments (36.66%). As *Listeria* species were not isolated in the remaining 49.2%, it is assumed that *Listeria* is not a regular inhabitant of the gut of cockroach. The moderate prevalence rate (50.8%) of *Listeria* species in cockroach may reflect the ubiquitous distribution of *Listeria* species in the environment as well as the filthy feeding habits of the insect.

Of the 254 *Listeria* isolates, *L.grayi* was found to be the most predominant species isolated followed by *L. innocua* . The species viz. *L. monocytogenes*, *L.welshimeri*, *L. seeligeri* or *L. ivanovii* could not be recovered during the study. Although *Listeria monocytogenes* is widely distributed , the number of organisms present in most environmental habitats are low[15] . The higher percentage of isolation of *L. grayi* may be related to the differential selection during the enrichment and recovery procedure or simply that it is more common in the environment than other *Listeria* species. Certain species of *Listeriae* may also have a competitive advantage over others in certain media[16].An alternative explanation is that *L. grayi* could be inhibitory to other species, possibly by the production of bacteriocin like substance which is to be investigated further. Many authors [17-18] demonstrated inhibitory components such as bacteriocin produced by some *Listeria* spp. that are active against other *Listeria* Spp.

**Table 4: Resistance to different antibiotics shown by the *L.grayi* isolates. (Total numbers of isolates tested: 248).**

| Antibiotics tested and potency | No. and percentage * of isolates resistant. |
|---|---|
| Penicillin (10 units) | 212 (85.4) |
| Ampicillin (10 µg/ml) | 52 (20.9) |
| Cloxacillin (30 µg/ml) | 192 (77.4) |
| Cefepime (30 µg/ml) | 196 (79) |
| Cefuroxime (30 µg/ml) | 192 (77.4) |
| Cefotaxime (30 µg/ml) | 144 (58) |
| Cefriaxone (30 µg/ml) | 180 (72.5) |
| Gentamicin (30 µg/ml) | 60 (24.1) |
| Amikacin (30 µg/ml) | 64 (25.8) |
| Nalidixic acid (30 µg/ml) | 248 (100) |
| Tetracycline (30 µg/ml) | 48 (19.3) |
| Erythromycin (15 µg/ml) | 62 (25) |
| Imipenam (10 µg/ml) | 68 (27.4) |
| Vancomycin (30 µg/ml) | 16 (6.4) |
| Ciprofloxacin (5 µg/ml) | 24 (9.6) |
| Ofloxacin (5 µg/ml) | 32(12.9) |
| Levofloxacin (5 µg/ml) | 36 (14.5) |
| Co-trimoxazole (25 µg/ml) | 60 (24.1) |
| Linezolid (30 µg/ml) | 36 (14.5) |

The antibiogram pattern of *Listeria* isolates obtained in the current study reiterates the emergence of multiple resistances in *Listeria* species [19-22]. Resistance to more than two antibiotics was observed in 80.64% of *L. grayi* isolates. Except for a few reports of bacteraemia, *L. grayi* is generally considered to be a non pathogenic species. But the multidrug resistance exhibited by it poses a threat of transferring the same through plasmid to other bacteria including the pathogenic *L. monocytogenes* or *ivanovii*. The three *L. innocua* isolates obtained in the present study presented resistance to penicillin, cloxacillin , cefuroxime, cefepime and nalidixic acid. It has been reported that the occurance of resistance is seen not only in *L.monocytogenes* but also in other *Listeria* species[23].

Intestinal ecosystem is the most probable meeting point of different bacterial species and hence the site for the intergeneric transfer of genetic information. The presence of bacterial species such as *Entercocci* in the intestinal tract of *P.americana* along with *Listeria* species is of significance because of its ability to acquire and transfer resistance to other bacterial inhabitants in the intestine. Conjugative mobilization of self transferable plasmids have been demonstated between *L.monocytogenes* and *E.faecalis* [24]. *Enterococci* and *Streptococci* act as the reservoirs of genes and with their extraordinary host range , genes could be transferred between bacterial species through conjugation [25]. In the conjugation experiment conducted in the present study between *L. grayi* and *E. faecalis* where the transfer of ampicillin resistance through plasmid was taken into consideration, transfer of plasmids containing resistance at a frequency of $10^{-6}$ – $10^{-7}$ was noticed. *L.grayi* was found to be a better donor or a recipient of plasmid in comparison with *L. monocytogenes*[26]. The reason for the better donor or receipient status of *L,grayi* could be a subject of future study.

The isolation of *L.grayi* from the gut contents of *Periplanata americana* indicates the possibility that it could also act as a carrier of other pathogenic species viz. *L.monocytogenes* or *ivanovii.* This *Listeria* carriage potential of the insect is of utmost importance as far as hospital environment is concerned .Though the pathogenic potential of *L. grayi* is not well documented in healthy individuals , the two reported cases of bacteraemia in transplant patients indicates its ability in causing infection in persons with depressed immune function. Moreover , *L. grayi*, a frequent inhabitant of the intestine of *P. americana* where different *Entercoccus* species also co-exist, the possibility of *in vivo* transfer of plasmid from *Listeria* and *Enterococcus* and vice-versa poses a threat for the dissemination of antibiotic resistance in bacteria. . The finding in the present study is an additional support to the notion that intestinal ecosystem is the most probable site of drug resistance determinants transfer between the co-existing bacterial flora.

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