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Research Article

ANTIBIOTIC RESISTANCE PROFILE OF BACTERIAL PATHOGENS IN THE GUT OF *P. AMERICANA*

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ABSTRACT

The study was conducted to determine the antibiotic sensitivity of various bacterial isolates including *L. monocytogenes* obtained from the intestinal content of *P. americana* captured from hospitals, domestic environments, restaurants and market places. The antimicrobial susceptibility of the bacterial isolates was determined by Kirby-Bauer disk diffusion method. Among the different groups of antibiotics, cephalosporins resistance was obvious in all the bacterial isolates under study. *E. faecium*, the most predominant isolate in the study, showed noticeable resistance to penicillin (39%), erythromycin (35%) and cloxacillin (32%) apart from its cephalosporin resistance. Among the Gram negative isolates, though resistance to quinolones was not as apparent as cephalosprins, tendency to resist nalidixic acid was evident particularly in *P. aeruginosa* (79%). Resistance to penicillin, nalidixic acid was noticed in all the *Listeria* species under study. The multidrug resistant bacteria carried by the omnipresent insect cockroach in their intestine as noticed in this study urges the necessity of further epidemiological studies for revealing the role of this insect in nosocomial infection and food spoilage.

Keywords: *P. americana*, bacterial pathogens, antibiotic sensitivity

INTRODUCTION

P. americana are often found to carry pathogenic microorganisms on their cuticle and in the intestinal tract. Pechal *et al.,* highlighted the importance of cockroaches in the spread of pathogens to various surfaces creating a public health concern[1]. It has been observed that it can act as a carrier of different multidrug resistant bacteria[2] and spreading them through faecal pellets to inanimate objects of various environments such as hospital or domestic environment or food establishments. Lemmen *et al.,* noticed the significance of inanimate objects serving as a secondary reservoir of multi resistant bacterial pathogens for cross transmission[3].

This study deals with the antibiogram pattern of various pathogenic bacterial isolates obtained from the intestinal contents of *P. americana*.

MATERIALS AND METHODS

The bacterial strains used in this study were isolated from the intestinal contents of cockroaches captured from hospitals, domestic environments, restaurants and market places. The strains were maintained on trypticase soy agar slopes at refrigeration temperature and recovered on TSA prior to examination.

The antimicrobial susceptibility of the bacterial isolates was determined by Kirby-Bauer disk diffusion method[4] following the recommended procedures according to NCCLS recommendations[5] .The bacterial isolates were submitted to the following antibiotics supplied by Hi-Media Laboratories: penicillin (10units), cloxacillin (30mcg), ampicillin (10mcg), erythromycin (15mcg), linezolid (30mcg), co-trimoxazole (1.25mcg), vancomycin (30 mcg), tetracycline (30mcg), cefuroxime (30mcg), cephotaxime (30mcg), cefepime (30mcg), ceftriaxone (30mcg), ciprofloxacin (5mcg), ofloxacin (5mcg), levofloxacin (5mcg), nalidixic acid (30mcg), gentamicin (10mcg), amikacin (30mcg) and imipenam (10mcg). After incubation for 24 hr at 37^{0}C, the diameter (mm) of the zone around each disc in the medium was measured and interpreted in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines to classify the antibiotic sensitivity of each isolate as susceptible or resistant.

Statistical analysis

Two way ANOVA test was carried out to show the variation in resistance among different antibiotics by the predominant bacterial isolates (F test with 5% significant level).

Results

Table 1: The antibiogram pattern of bacterial isolates obtained from *P. americana*

Bacteria	No. of isolates tested	No. and percentage * of isolates showing resistance to different antibiotics																		
		P	Cx	A	E	L3	Q	Va	T	Cu	Ce	Cpm	Ci	Cf	Of	Na	Le	G	Ak	I
E. faecium	398	155 (39)	127 (32)	42 (11)	139 (35)	8 (2)	59 (15)	64 (16)	88 (22)	127 (32)	119 (30)	155 (39)	167 (42)	41 (10)	76 (19)	107 (27)	57 (14)	67 (17)	36 (9)	56 (14)
E. faecalis	73	23 (31)	11 (15)	16 (22)	16 (22)	6 (8)	10 (14)	14 (19)	26 (36)	15 (20)	23 (32)	31 (43)	23 (32)	16 (22)	9 (13)	28 (38)	21 (29)	7 (10)	9 (12)	4 (6)
E. casseliflavus	7	3 (43)	0	1 (14)	2 (29)	0	1 (14)	0	1 (14)	1 (14)	3 (43)	2 (29)	2 (29)	0	0	0	1 (14)	1 (14)	0	0
S. epidermidis	76	38 (50)	24 (32)	30 (39)	16 (21)	6 (8)	14 (19)	5 (7)	14 (19)	22 (29)	24 (32)	30 (39)	17 (23)	28 (37)	12 (16)	15 (20)	17 (22)	16 (21)	11 (14)	5 (7)
K. pneumoniae	186	32 (17)	54 (29)	62 (33)	42 (23)	15 (8)	58 (31)	13 (7)	82 (44)	55 (30)	138 (74)	95 (51)	63 (34)	48 (26)	45 (24)	73 (39)	33 (18)	56 (30)	60 (32)	4 (2)
K. oxytoca	7	1 (14)	1 (14)	0	1 (14)	0	1 (14)	0	1 (14)	2 (29)	1 (14)	4 (57)	4 (57)	0	0	2 (29)	0	0	3 (43)	0
K.	5	0	0	0	0	0	0	0	0	1	1	1	3	0	0	0	0	2	0	0

rhinoscleromatis										20)	(20)	(20)	(60)					(40)		
P. aeruginosa	103	40 (39)	35 (34)	39 (38)	26 (25)	21 (20)	18 (17)	5 (4)	30 (29)	65 (63)	59 (57)	52 (50)	38 (37)	30 (29)	26 (25)	80 (79)	28 (27)	47 (46)	34 (33)	18 (17)
P. fluorescens	3	0	1 (33)	1 (33)	0	0	0	0	33	1 (33)	1 (33)	67	1 (33)	0	0	2 (67)	1 (33)	2 (67)	0	0
P. mirabilis	168	25 (15)	29 (17)	49 (29)	28 (17)	17 (10)	40 (24)	6 (4)	57 (34)	47 (28)	72 (43)	49 (29)	121 (72)	37 (22)	26 (14)	81 (48)	24 (14)	27 (16)	18 (11)	17 (10)
P. vulgaris	12	2 (16)	5 (42)	2 (16)	2 (16)	1(8)	1(8)	0	4 (33)	6 (50)	4 (33)	2 (16)	6 (50)	1(8)	1(8)	7 (58)	4 (33)	3 (25)	4 (33)	0
Prov. rettgeri	0	0	0	0	0	0	1 (33)	0	0	0	0	1 (33)	1 (33)	0	0	1 (33)	0	0	0	0
M. morganii	0	0	0	0	0	0	0	0	1 (50)	0	1 (50)	0	0	0	0	0	0	0	0	0
C. diversus	53	21 (40)	8 (15)	18 (34)	10 (19)	9 (17)	16 (30)	3 (6)	19 (36)	35 (66)	26 (49)	42 (79)	30 (57)	14 (26)	15 (28)	23 (43)	22 (42)	14 (26)	9 (17)	2 (4)
C. freundi	45	11 (24)	16 (36)	6 (13)	9 (20)	6 (13)	7 (16)	30 (67)	15 (33)	22 (49)	17 (38)	22 (49)	20 (44)	11 (24)	10 (22)	19 (42)	13 (29)	10 (22)	10 (22)	5 (11)
S. marcescens	100	8 (8)	6 (6)	18 (18)	12 (12)	8 (8)	22 (22)	2 (2)	12 (12)	28 (28)	32 (32)	31 (31)	23 (23)	19 (19)	14 (14)	25 (25)	12 (12)	26 (26)	8(8)	1 (1)
E. coli	91	12 (13)	6 (7)	21 (23)	15 (17)	8 (9)	11 (12)	2 (2)	35 (38)	22 (24)	21 (23)	24 (26)	24 (26)	19 (21)	15 (16)	26 (29)	17 (19)	16 (18)	4 (4)	0
E. cloacae.	32	4 (12)	3 (10)	20 (61)	1(3)	4 (13)	5 (16)	0	4 (12)	4 (13)	13 (40)	11 (33)	6 (19)	8 (24)	5 (15)	5 (16)	4 (13)	1(4)	1(3)	2(6)
E. agglomerans	21	12 (57)	3 (14)	5 (24)	10 (47)	2 (10)	6 (29)	1 (5)	8 (38)	7 (33)	9 (42)	8 (38)	7 (33)	3 (14)	1 (5)	7 (33)	5 (24)	1 (5)	4 (19)	4 (19)
H.alveoli	(13)	2 (15)	2 (15)	3 (23)	1 (8)	0	2 (15)	0	3 (23)	6 (46)	4 (31)	5 (38)	4 (31)	3 (23)	2 (15)	6 (46)	2 (15)	2 (15)	2 (15)	0
Salmonella spp.	(2)	0	0	1 (50)	0	0	1 (50)	0	0	1 (50)	0	1 (50)	1 (50)	0	0	1 (50)	0	1 (50)	1 (50)	0
A. lwoffi	18	4 (22)	3 (17)	2 (11)	1 (6)	2 (11)	3 (17)	0	6 (33)	5 (28)	6 (33)	6 (31)	5 (28)	1 (6)	2 (11)	4 (23)	3 (17)	1 (6)	2 (11)	5 (28)
A. buamanii	14	3 (21)	3 (21)	2 (14)	2 (14)	1 (7)	1 (7)	0	5 (36)	4 (29)	5 (33)	9 (64)	4 (27)	1(7)	2 (14)	4 (29)	3 (21)	3 (21)	4 (29)	1 (7)
L. monocytogenes	2	2 (100)	0	0	0	0	0	0	0	1 (50)	0	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	0	0	0	0
L. innocua	6	6 (100)	6 (100)	0	1 (17)	0	2 (33)	0	2 (33)	6 (100)	0	6 (100)	4 (67)	2 (33)	1 (17)	6 (100)	1 (17)	0	1 (17)	2 (33)
L. grayi	248	212 (85)	192 (77)	52 (21)	62 (25)	36 (15)	60 (24)	16 (6)	48 (19)	192 (77)	144 (58)	196 (79)	180 (73)	24 (10)	32 (13)	248 (100)	36 (15)	60 (24)	64 (26)	68 (27)

***percentage is given in brackets**

P- penicillin; Cx- cloxacillin; A- ampicillin; E- erythromycin; Lz- linezolid; Va- vancomycin; T -tetracycline; Cu- cefuroxime; Ce- cephotaxime; Cpm- cefepime; Ci- cefriaxone; Cf- ciprofloxacin; Of- ofloxacin; Na- nalidixic acid; Le- levofloxacin; G- gentamicin; Ak- amikacin; I- imipenam.

Among the different groups of antibiotics, cephalosporins resistance was obvious in all the bacterial isolates under study. E. faecium, the most predominant isolate in the study, showed noticeable resistance to penicillin (39%), erythromycin (35%) and cloxacillin (32%) apart from its cephalosporin resistance. 16% of E. faecium were noticed to be showing resistance to vancomycin. However, its susceptibility to linezolid was excellent with only 2% of isolates showing resistance. Among the Gram negative isolates, though resistance to quinolones was not as apparent as cephalosprins, tendency to resist nalidixic acid was evident particularly in P. aeruginosa (79%).

Multiple drug resistance in predominant bacterial isolates

Bacteria	No. of isolates	No & percentage* of isolates showing resistance		
		<3 antibiotics	3 - 5 antibiotics	>5 antibiotics
E. faecium	398	27 (6.7)	155 (38.9)	216 (54.2)
K. pneumoniae	186	33 (17.7)	35 (18.8)	118 (63.4)
P. mirabilis	168	45 (26.7)	42 (25)	81 (48.2)
P. aeruginosa	103	11 (10.6)	18 (17.4)	74 (71.8)
L. grayi	248	18 (7.2)	22 (8.8)	208 (83.8)
Total	1103	134 (12.1)	272 (25)	697 (63.1)

***percentage is given in brackets**

Of the 1103 strains of predominant bacterial isolates tested, 12.1% of the total was resistant to less than 3 drugs while 63.1% were resistant to more than 5 drugs. 25% of the predominant bacteria were noticed to be resisting 3-5 antibiotics. Multiple resistance was predominant in *P. aeruginosa* (71.8%) as anticipated, followed by *K. pneumoniae* (63.4%). 54.2% of *E. faecium* presented multiple resistance; *P. mirabilis* showing relatively less multiple resistance (48.2%). Multiple resistance was noticed to be frequent in *L. grayi* isolates (83.8%).

The mean resistance shown by predominant isolates viz. *E. faecium, K. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *L. grayi* obtained from different sources towards 19 antibiotics was analysed by two way ANOVA test. Of the 19 antibiotics under study, *E. faecium* showed highest resistance to cefriaxone and lowest to linezolid. Cephotaxime was the antibiotic against which *K. pneumoniae* presented most resistance and imipenam the least. *P. mirabilis* showed highest level of resistance to cefriaxone and the lowest level to vancomycin. The resistance exhibited by *P. aeruginosa* was the highest towards nalidixic acid and the lowest to vancomycin. *L. grayi*, the most common species of the genus *Listeria* isolated from *P. americana* presented highest tolerance to nalidixic acid followed by cefepime and the lowest towards vancomycin.

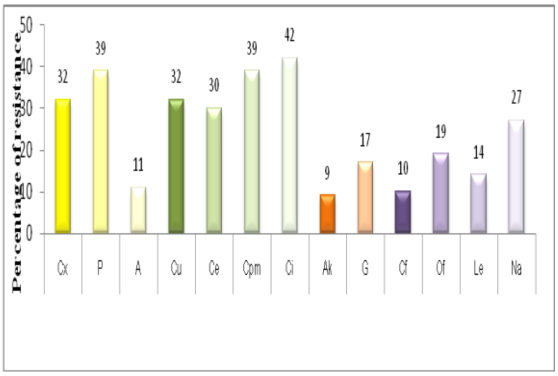

Figure 1: Comparison of resistance towards different classes of antibiotics - *E. faecium*

Cx – cloxacillin P- penicillin A - ampicillin Cu – cefuroxime Ce – cephotaxime Cpm – cefepime Ci – cefriaxone

Ak - amikacin G – gentamicin Cf - ciprofloxacin Of – ofloxacin Le– levofloxacin Na – nalidixic acid

Within the penicillin group of antibiotics, three antibiotics were tested viz. cloxacillin, penicillin and ampicillin. 39% of *E. faecium* isolates exhibited resistance towards penicillin with ampicillin resistance in 11%. On considering the cephalosporin group of antibiotics, 42% of this bacterial species showed resistance to cefriaxone and 30% to cephotaxime. The two antibiotics tested under aminoglycoside group were amikacin and gentamicin. Among them more resistance was noticed towards gentamicin (17%). Of the different quinolones, 27% of the *E. faecium* isolates were found to be resisting nalidixic acid with only 10% resisting ciprofloxacin.

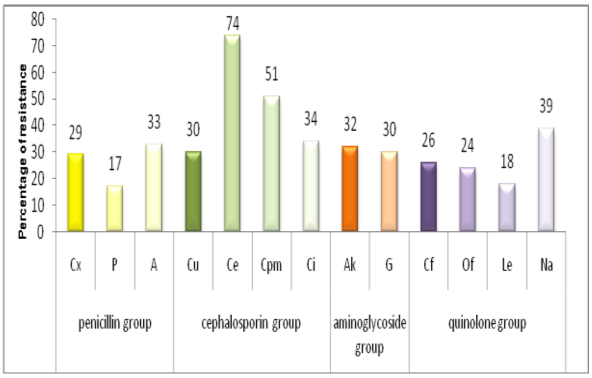

Figure 2: Comparison of resistance towards different classes of antibiotics - *K. pneumoniae*

Cx – cloxacillin P- penicillin A - ampicillin Cu – cefuroxime Ce – cephotaxime Cpm – cefepime Ci – cefriaxone

Ak - amikacin G – gentamicin Cf - ciprofloxacin Of – ofloxacin Le – levofloxacin Na – nalidixic acid

33% of *K. pneumoniae* presented resistance to ampicillin whereas only 17% of the tested isolates resisted penicillin. Within the cephalosporins, the highest resistance was noticed towards cephotaxime with 74% of the isolates showing resistance and lowest to cefuroxime (30%). 32% of *K. pneumoniae* isolates were found to be resisting amikacin and 30% resisting gentamicin. Within the quinolone group, nalidixic acid was found to be the most resistant antibiotic with 39% of isolates showing resistance.

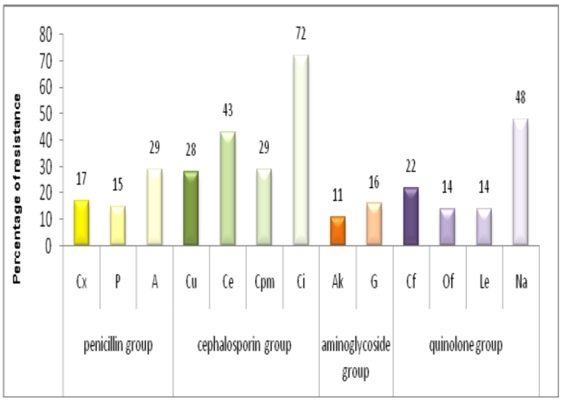

Figure 3: Comparison of resistance towards different classes of antibiotics - *P. mirabilis*

Cx – cloxacillin P- penicillin A - ampicillin Cu – cefuroxime Ce – cephotaxime Cpm – cefepime Ci – cefriaxone

Ak - amikacin G – gentamicin Cf - ciprofloxacin Of – ofloxacin Le – levofloxacin Na – nalidixic acid

29% of *P. mirabilis* exhibited resistance to ampicillin. The resistance towards penicillin was rather low (15%). Cefriaxone resistance was noticed in 72% of this bacterial species with 28% resisting cefuroxime. No noticeable difference in resistance among the aminoglycoside group was noticed; 16% of isolates resisting gentamicin with amikacin resistance 11%. Within the quinolone group resistance was more pronounced towards nalidixic acid (48%). Only 14% of isolates resisted ofloxacin and levofloxacin.

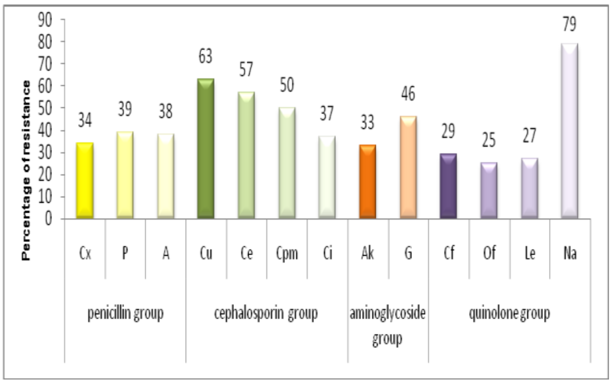

Figure 4: Comparison of resistance towards different classes of antibiotics - *P. aeruginosa*

Cx – cloxacillin P- penicillin A - ampicillin Cu – cefuroxime Ce – cephotaxime Cpm – cefepime Ci – cefriaxone

Ak - amikacin G – gentamicin Cf - ciprofloxacin Of – ofloxacin Le– levofloxacin Na – nalidixic acid

On considering resistance of *P. aeruginosa* towards different members of penicillin group, more resistance was noticed towards penicillin with 39% of isolates showing resistance. Cloxacillin resistance was noticed in 34% of the isolates. When the resistance of this bacterial species to different cephalosporin members was analyzed, 63% of isolates presented resistance to cefuroxime with 37% resisting cefriaxone. Among the quinolones, 79% of *P. aeruginosa* resisted nalidixic acid with only 25% resisting ofloxacin.

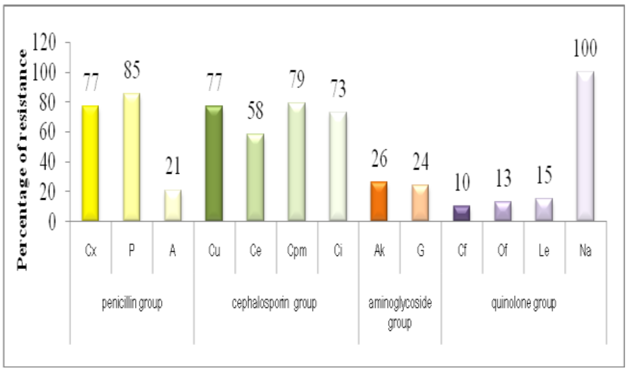

Figure 5: Comparison of resistance towards different classes of antibiotics - *L. grayi*

Cx – cloxacillin P- penicillin A - ampicillin Cu – cefuroxime Ce – cephotaxime Cpm – cefepime Ci – cefriaxone Ak - amikacin G – gentamicin Cf - ciprofloxacin Of – ofloxacin Le – levofloxacin Na – nalidixic acid

85% of *L. grayi* were found to be resisting penicillin. The resistance towards ampicillin was noticed in 21% of isolates. Of the different cephalosporin members, resistance was predominant towards cefepime (79%). Comparatively less resistance was noticed towards cephotaxime (58%). Among the aminoglycosides, no noticeable difference in resistance was observed between amikacin and gentamicin. 100% resistance to nalidixic acid shown by this *Listeria* species is noteworthy.

DISCUSSION

Although resistance to antimicrobials is an inevitable consequence of the evolutionary adaptation of microbes, its use and misuse has driven a rapid emergence of resistance in pathogenic and non pathogenic bacteria[6]. Certain bacteria show intrinsic resistance when an entire species show resistance to an antibiotic based on inherent and inherited characteristics where as acquired resistance arise either through mutation or horizontal gene transfer. Earlier, concern over resistance was restricted only to clinically relevant microorganisms. However recently, antibiotic resistance among bacteria becomes so common that a pool of resistance is emerging in non pathogenic organisms found in humans, animals and in the environment.

Of the different bacterial species, resistance to various antibiotics appeared to be more pronounced in *E. faecium*, *K. pneumoniae, P. mirabilis, P. aeruginosa* and *L. grayi*. As a predominant cause of nosocomial infections, antibiotic resistant enterococci particularly *E. faecalis* and *E. faecium* represent a serious public health problem. The antimicrobial susceptibility profile of enterococci as evaluated in the current study reinforces the concept of this bacteria being a reservoir of multiple resistance genes. In addition to the intrinsic resistance to several antibiotics, the ease with which they acquire and transfer resistance genes [7]could be the reason for the high level resistance particularly to penicillin, erythromycin, tetracycline and cephalosporins showed by these bacterial isolates. Resistance to vancomycin (16%) presented by the isolates is in agreement with Karmarkar *et al*.,[8] who noted an upsurge of vancomycin resistance in clinical isolates of *Enterococcus*.

In the present study, *K. pneumoniae* were showing noticeable resistance to ampicillin, gentamicin, tetracycline and various cephalosporins. *K. pneumoniae* resisting multiple antibiotics has been reported from cockroaches [9, 10]. Resistance to ceftazidime and cefotaxime in *Klebsiella* and *E. coli* may be considered as a marker for the presence of extended spectrum β lactamases (ESBL) [11]. The resistance to cephalosporins (second, third and fourth generation) shown by the *K. pneumoniae* isolates noticed in the current study and its reported ability of plasmid mediated transfer to other co existing bacterial flora poses a threat as far as treatment of patients especially those who are immunocompromised are concerned[12,13,14].

Proteus species are frequently encountered in nosocomial as well as community acquired infections. The resistance of *P. mirabilis* isolates towards ampicillin as noticed in the current study was in accordance with Pagani *et al.*, [15]. The current finding of tetracycline resistance in *P. mirabilis* isolates may be correlated with the intrinsic resistance of this bacterial species to tetracycline[16].

P. aeruginosa isolates presented considerable resistance to ampicillin, gentamicin, amikacin and nalidixic acid in addition to its high level resistance to cephalosporins, an observation in confluence with the studies on *Pseudomonas* isolates from cockroaches[10]. The resistance of the *Pseudomonas* spp. to imipenam was, however comparatively of low level (17 %). *Psuedomonas* isolates were also presented commentable resistance to fluoroquinolones ssuch as ciprofloxacin and ofloxacin. Similar resistance pattern in clinical isolates of *P. aeruginosa* to ciprofloxacin, cephalosporins, gentamicin and imipenam was observed[17, 18, 19]. Though *Pseudomonas* spp. rarely affects healthy adults it is increasingly been recognized as the etiological agent of infection in hospitalized patients especially in immunocompromised. The emergence of resistance to antimicrobial agents with reliable activity against *Psuedomonas* such as cephalosporins and fluoroquinolones as noticed in the current study has been recognized as a cause of treatment failure[20].

Widespread use of tetracycline and cephalosporins as well as plasmid-mediated acquired resistance to tetracycline and third-generation cephalosporins as reported earlier[21] might be the reason for the resistance presented by the *E. coli* isolates towards these antimicrobials. Moreover, both tetracycline and cephalosporins are naturally derived compounds and therefore bacteria can be exposed to them in nature which may ultimately enter in the insect during feeding.

Among the *S. epidermidis* isolates resistance to cephalosporins, penicillin, cloxacillin, ampicillin, ciprofloxacin was predominant. Staphylococci are ubiquitous bacteria widely distributed in the environment showing high tolerance to drying and dehydration. Like many other environmental bacteria, the coagulase negative staphylococci (CNS) behave as opportunistic pathogens and in the recent years the risk of infection with the CNS has been on a rise particularly due to an increase in immunodeficiencies. Moreover, the escalade of antibiotic resistance observed in the CNS make their presence highly undesirable in hospital environment[22, 23].

Although multi resistant strains of *Listeria* spp. are rare in nature, in recent years there have been reports of the emergence of resistance in *L. monocytogenes* strains obtained from various sources[24,25] . The results observed in the current study provide an additional evidence of the appearance of *Listeria* strains with multiple resistance in nature. Both the *L. monocytogenes* isolates tested in the current study presented resistance to penicillin. Prazak *et al.*,[24] also reported a parallel finding of penicillin resistance in an environmental isolate of *L. monocytogenes*. It was not surprising to observe the resistance shown by *L. monocytogenes* to cefepime, cefriaxone and cefuroxime since natural resistance to cephalosporins in this bacterial species is common[26]. However, the susceptibility of *L. monocytogenes* towards ampicillin and gentamicin noticed in this study has to be emphasized as this combination is the treatment of choice for listeriosis. A similar finding of sensitivity of clinical isolates of *L. monocytogenes* to ampicillin and gentamicin was made by Reis *et al* [27]. Though the incidence of tetracycline resistance is reported to be high in *Listeria* species[28, 29], the current study noticed both *L. monocytogenes* isolates as sensitive to this drug.

This study demonstrates the possible role of cockroaches in the dissemination of multi resistant bacterial pathogens including *Listeria* species in domestic and peridomestic environments.

CONCLUSION

The study noticed cockroaches inhabiting in human environments serving as a vehicle of potential bacterial pathogens with antibiotic resistance. The multidrug resistant bacteria carried by the omnipresent insect cockroach as noticed in this study urges the necessity of further epidemiological studies for revealing the role of this insect in nosocomial infection and food spoilage.

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