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Full Length Research Paper

Bacteriological analysis of *Periplaneta americana L.* (Dictyoptera; Blattidae) and *Musca domestica L.* (Diptera; Muscidae) in ten districts of Tangier, Morocco

Bouamama Lamiaa, Lebbadi Mariam and Aarab Ahmed

Center of Mediterranean and Environmental Studies, Laboratory of Applied Biology and Environment Sciences, Department of Life sciences, Faculty of Sciences and Techniques, Abdelmalek Essaâdi University, Tangier, BP 416, Morocco.

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In this study, *Periplaneta americana* and *Musca domestica* were collected from ten districts in Tangier, to isolate and identify some bacteria from their body using selective media. The results indicate that the amounts of bacteria were different between the districts. Moreover, the bacteria isolated from body of American cockroaches differed from those of the houseflies. These findings show that American cockroaches and houseflies may carry pathogenic bacteria in the urban areas of Tangier.

Key words: *Periplaneta americana*, *Musca domestica*, vectors, bacteria, hygiene, Tangier.

INTRODUCTION

American cockroaches (*Periplaneta americana* L.) and houseflies (*Musca domestica* L.) are often found in close association with humans, living in homes, businesses and health care facilities. The intimate association of these insects with humans has resulted in their relegation to pest status (Rau, 1924; Ebeling, 1978; Graczyk et al., 2005). Both American cockroaches and houseflies are abundant in urban and rural areas where unsanitary conditions prevail; they are usually scarce when sanitary conditions are enforced (Greenberg, 1973; Graczyk et al., 2001, 2005). Moreover, because of their association with human environments, both of them may incidentally contact and acquire human pathogens. Pathogens may subsequently be mechanically transmitted to humans or susceptible artefacts or surfaces which may then function as vehicles of indirect transmission (Roth and Willis, 1957).

The American cockroaches are the potential vectors for bacteria (over 100 species of bacteria have been isolated from or passed through cockroaches), fungi and parasites (Cruden and Markovetz, 1987). Because the cockroaches feed on filth and feces, they may disseminate infections with the fecal-oral route (Koura and Kamel, 1990; Fotedar et al., 1992a; Rivault et al., 1993; Pai et al., 2003; Pai et al., 2005).

Houseflies have been reported to be involved in the dissemination of numerous diseases such as typhoid and paratyphoid fevers, bacillary dysentery, cholera, hematics carbuncles, bovine mastitis, conjunctivitis and poliomyelitis (Greenberg, 1970, 1973; Gough and Jorgenson, 1983). Furthermore, houseflies are the important mechanical and/or biological vectors for various pathogenic agents. These pathogens are transmitted on the fly's cuticle, proboscis, by regurgitation or through its faeces (Fotedar et al., 1992b; Senna-Nunes et al., 2002; Banjo et al., 2005).

The city of Tangier records a high demographic growth and a persistent rhythm of urbanisation which create a formation of insalubrious and under-equipped districts. Moreover, in its various districts, there are noticeable differences in density, town planning and social level. The objective of this study was to confirm that *P. americana* and *M. domestica* carry pathogenic bacteria in some districts of the city by bacteriological analysis of these two insects.

*Corresponding author. E-mail: lamiaab1@yahoo.fr. Phone: +212 66375295. Fax: +212 39393953.

MATERIALS AND METHODS

Field survey

Cockroaches and flies were collected randomly from households of 10 districts of Tangier, Morocco in April 2004. The districts were: Bendeban (Bd), Benkiran (Bk), Boukhachkhach (Bkh), Boughaz (Bz), Bnimakada (Bm), Haydadi (Hd), Hayidari (Hi), Student's residences (Sr), Moghogha (Mg) and Tanjabalya (Tb). Benkiran, Bnimakada, Moghogha, Tanjabalya and Bendeban are the popular districts of the city and they are defavorised and under-equipped owing to high density of population and inadequate waste disposal and treatment network.

Collection and identification of cockroaches and flies

Fifty adult of *P. americana* (five per site) and seventy *M. domestica* (seven per site) were collected from houses of the ten selected sites. Flies were caught with sterilised nets from kitchens, living rooms and bathrooms. Each trapped insect (cockroach or fly) was placed in a sterile test tube and was subsequently taken to the laboratory. The insects were immobilised by freezing at 0°C for 5 min. Identification was made by examining the insect under a low power microscope and following standard taxonomic keys.

Isolation and identification of bacteria from external surfaces of insects

Two millilitres of sterile normal saline (0.9%) was added to test tube containing one fly or cockroach, and the tubes were thoroughly shaken for 2 min to isolate micro-organisms from the external surface. Aliquots (0.01 ml) of the washing were then separately inoculated onto the surface of agar plates such as Chapman, Litsky, MacConkey, and Hektoen. Selenite and Roth broths were used as enrichment media successively for *Salmonella* and *Streptococcus*, before culturing. The enrichment broths and agar plates were incubated at 37°C for 24 h. The bacteria growth on the agar media were identified by colonial morphology, Gram staining, and biochemical tests such as oxidase, catalase, motility, coagulase, indole, methyl red, voges proskaeur (MRVP), gelatine hydrolysis, glucose, lactose, mannitol, and gas and H_2S production (Murray 1999).

Statistical analysis

Rates were compared using the ANOVA/MANOVA test. $P<0.05$ was considered to be statistically significant.

RESULTS

All the cockroaches and flies were found to carry several species of bacteria on the external surface. The bacteria isolated from the insects were Gram-positive bacteria (particularly *Staphylococcus* and *Streptococcus*) and Gram-negative belonging to Enterobacteriacae. Morever, most of these bacteria are pathogenic. These bacteria were *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus species, Escherichia coli, Enterobacter* spp*., Klebsiella* spp*., Serratia* spp*., Proteus vulgaris, Proteus* spp*., Shigella* spp*.,* and *Salmonella* spp. The most frequent bacteria isolated from the houseflies and the American cockroaches coming from 10 districts of the city are *Streptocoques*, *S. epidermidis* and *E. coli*, while the least present are *P. vulgaris* and *Klebsiella* spp. (Table 1).

Although, there was no significant difference between the means of Gram-positive bacteria in *P. americana* and *M. domestica* ($p>0.05$) in all the districts, the mean in *P. americana* (93.33%) was significantly higher than that in *M. domestica* coming from Moughogha (61.90%) ($p=0.0003<0.05$) Tables 2 and 3. In the same way, there was no significant difference between the means of Gram-negative bacteria in *P. americana* and *M. domestica* ($p>0.05$), but it was significantly higher in *P. americana* coming from Bnimakada and Tanjabalya (57.50 and 47.50%) than that in *M. domestica* coming from these two sites (28.57 and 17.86%) ($p = 0.033$ and 0.042).

Different species of Gram-positive bacteria were isolated from the two species of insects in different districts. There was no significant difference between the amounts of these bacteria in the different districts (Tables 2 and 3). Eight species of gram-negative Enterobacteriaceae were isolated from *P. americana* and *M. domestica* in these districts (Tables 4 and 5). Moreover, there was a highly significant difference between the amounts of these bacteria in all districts; significantly higher means were found in Bendiban, Benkiran, Bnimakada and student's residences (*M. domestica* 33.93% and *P. americana* 62.5%). The minimum amounts were often observed in Boughaz and Hayidari (*M. domestica* 12.50% and *P. americana* 25%) (Figures 1 and 2).

DISCUSSION

This study showed that eleven species of bacteria were isolated from *M. domestica* and *P. americana* (Tables 3 and 4). Our results are in accordance with other reports which highlight the importance of houseflies and American cockroaches in carrying various enteropathogens (Greeberg, 1973, Echeverria et al., 1983; Fotedar et al., 1992b; Grubel et al., 1997; Kobayashi et al., 1999; Koura and Kamel, 1990; Fotedar et al., 1992a; Rivault et al., 1993; Pai et al., 2003). In this study, more bacterial species were recovered from *P. americana* than those from *M. domestica*. In similar studies, 56 species of bacteria were isolated from cockroaches in France and 14 species were found to be pathogenic or potentially pathogenic for man and animal, with *E. coli* and *K. pneumoniae* being the most important (Rivault et al., 1993; Vythilingam et al., 1997). The role of the cockroaches and houseflies in the transmission of pathogens and gastrointestinal diseases had already been established (Greenberg, 1971; Rueger and Olsen, 1969). Moreover, these two species of insects are the most common ones in Africa because of the favourable environmental and climatic conditions (Boulesteix et al., 2005). These findings and their special habits suggest the importance

Table 1. Average number of insect carrying a given bacterium in ten districts of Tangier.

Insect	Bacteria										
	Sa	***Se***	***St***	***Sm***	***Sh***	***Pv***	***Pr***	***Sr***	***Kl***	***En***	***Ec***
M. domestica (n = 70)	55.7	60	62.8	27.1	24.3	8.6	27.1	20	8.6	17.1	58.6
P. americana (n = 50)	68	50	80	48	52	22	38	40	26	52	62

Sa = Staphylococcus aureus, Se = Staphylocoques epidermidis, St = Streptococcus species, Sm = Salmonella spp., *Sh = Shigella* spp., *Pv = Proteus vulgaris*, *Pr = Proteus* spp., *Sr = Serratia* spp., *Kl = Klebsiella* spp., *En = Enterobacter* spp. And *Ec = Escherichia coli*.

Table 2: Gram-positive bacteria isolated from *Musca domestica* (n= 7 per district) collected from houses.

Districts	***Musca domestica*** **(n= 7)**																	
	Bd		**Bk**		**Bkh**		**Bz**		**Bm**		**Hi**		**Sr**		**Hd**		**Mg**		**Tb**	
Bacteria	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
S. aureus	6	85,71	5	71,43	2	28,57	2	28,57	6	85,71	1	14,29	4	57,14	3	42,86	4	57,14	6	85,71
S. epiderm	2	28,57	3	42,86	4	57,14	4	57,14	6	85,71	3	42,86	6	85,71	5	71,43	4	57,14	4	57,14
Streptococcus	6	85,71	5	71,43	3	42,86	4	57,14	6	85,71	1	14,29	4	57,14	4	57,14	5	71,43	4	57,14
Mean (%)	66,67		61,90		42,86		47,62		85,71		23,81		66,67		57,14		61,90		66,67	

n: one insect may simultaneously harbour more than one species of bacteria.

Table 3: Gram-positive bacteria isolated from *Periplaneta americana* (n= 5 per district) collected from houses.

Districts	***Periplaneta americana*** **(n= 5)**																	
	Bd		**Bk**		**Bkh**		**Bz**		**Bm**		**Hi**		**Sr**		**Hd**		**Mg**		**Tb**	
Bacteria	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
S. aureus	4	80	4	80	2	40	2	40	5	100	1	20	3	60	3	60	5	100	5	100
S. epidermidis	2	40	1	20	3	60	3	60	2	40	4	80	4	80	3	60	4	80	1	20
Streptococcus	5	100	5	100	2	40	3	60	5	100	2	40	5	100	3	60	5	100	5	100
Mean (%)	73,33		66,67		46,67		53,33		80		46,67		80		60		93,33		73,33	

n: one insect may simultaneously harbour more than one species of bacteria.

Table 4: Gram-negative bacteria isolated from *Musca domestica* (n= 7 per district) collected from houses.

Districts	***Musca domestica*** **(n= 7)**																	
	Bd		**Bk**		**Bkh**		**Bz**		**Bm**		**Hi**		**Sr**		**Hd**		**Mg**		**Tb**	
Bacteria	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Salmonella spp	3	42,86	2	28,57	1	14,29	1	14,29	4	57,14	1	14,29	2	28,57	2	28,57	1	14,29	3	42,86
Shigella spp	4	57,14	4	57,14	2	28,57	0	0,00	1	14,29	1	14,29	2	28,57	2	28,57	1	14,29	3	42,86
Proteus vulgaris	2	28,57	1	14,29	1	14,29	1	14,29	1	14,29	0	0,00	0	0,00	0	0,00	0	0,00	1	14,29
Proteus spp	2	28,57	3	42,86	3	42,86	2	28,57	3	42,86	0	0,00	4	57,14	2	28,57	1	14,29	0	0,00
Serratia spp	1	14,29	2	28,57	2	28,57	1	14,29	1	14,29	2	28,57	3	42,86	1	14,29	1	14,29	1	14,29
Klebsiella spp	1	14,29	0	0,00	0	0,00	0	0,00	1	14,29	0	0,00	0	0,00	0	0,00	1	14,29	0	0,00
Enterobacter spp	0	0,00	0	0,00	2	28,57	0	0,00	1	14,29	0	0,00	3	42,86	0	0,00	3	42,86	0	0,00
E. coli	6	85,71	5	71,43	4	57,14	6	85,71	4	57,14	3	42,86	4	57,14	5	71,43	3	42,86	2	28,57
Mean (%)	33,93		30,36		26,79		19,64		28,57		12,50		32,14		21,43		19,64		17,86	

n: one insect may simultaneously harbour more than one species of bacteria.

Table 5: Gram-negative bacteria isolated from *Periplaneta americana* (n= 5 per district) collected from houses.

Districts	*Periplaneta americana* (n= 5)																			
	Bd		Bk		Bkh		Bz		Bm		Hi		Sr		Hd		Mg		Tb	
Bacteria	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Salmonella spp	3	60	4	80	2	40	1	20	4	80	1	20	3	60	2	40	2	40	2	40
Shigella spp	4	80	4	80	3	60	2	40	3	60	2	40	2	40	2	40	1	20	1	20
Proteus vulgaris	3	60	2	40	2	40	1	20	1	20	0	0	1	20	1	20	0	0	0	0
Proteus spp	2	40	2	40	1	20	2	40	3	60	3	60	0	0	2	40	1	20	3	60
Serratia spp	4	80	1	20	1	20	0	0	3	60	0	0	2	40	2	40	4	80	3	60
Klebsiella spp	4	80	1	20	0	0	0	0	2	40	0	0	1	20	0	0	2	40	3	60
Enterobacter spp	2	40	2	40	2	40	1	20	3	60	2	40	1	20	2	40	3	60	3	60
E. coli	3	60	3	60	4	80	3	60	4	80	3	60	2	40	1	20	3	60	4	80
Mean (%)	62,50		47,50		37,50		25,00		57,50		27,50		30,00		30,00		40,00		47,50	

n: one insect may simultaneously harbour more than one species of bacteria.

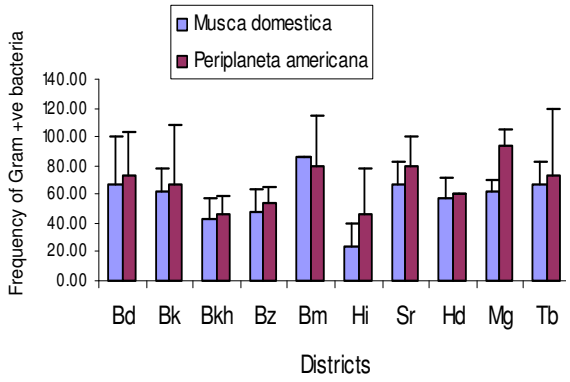

Figure 1. Frequency of Gram +ve bacteria in *P. Americana* and *M. domestica* in various neighbourhoods of Tangier.

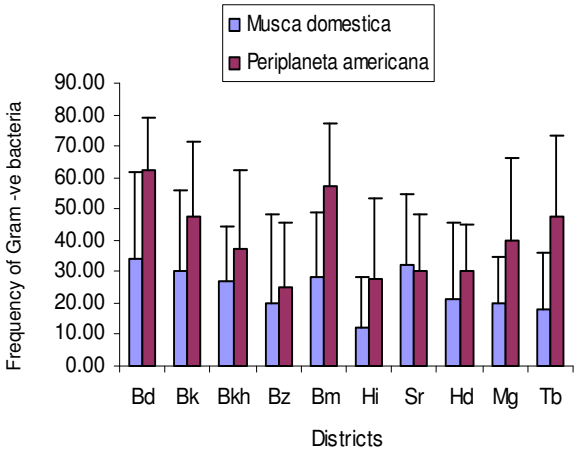

Figure 2. Frequency of Gram -ve bacteria in *P. Americana* and *M. domestica* in various neighbourhoods of Tangier.

of these two species of insects in the transmission of causative agents for food poisoning.

Furthermore, we found that *P. americana* harboured more species of bacteria than *M. domestica*. Although *P. americana* is seven to ten folds larger than *M. domestica* in length, the capability of harbouring micro-organisms in insects is not only related to their size but may also depend on the association of these insects with unsanitary conditions of the environment.

The houseflies and cockroaches caught in Benkiran (Bk), Bendeban (Bd), Bnimakada (Bm), Moghogha (Mg) and Tanja Balya (Tb) carried pathogenic bacteria more often than those caught in Hay Idari (Hi), Boughaz (Bz) and Boukhachkhach (Bkh) (Figures 1 and 2). These results could be attributed to social-economic factors related to each district (kind of population, urbanism and social level) and the insalubrious conditions in some of these districts. The number of areas containing waste refuse and excreta of humans and domestic animals might have provided ideal conditions for the breeding and multiplication of the synanthropic insects (insects associated with the human habitat) as this fly and cockroach species are known to feed and breed most successfully on filth (Fotedar, 2001). This study has established that *P. americana* and *M. domestica* carry pathogenic bacteria in ten districts of Tangier.

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