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Bacterial load of cockroaches in relation to urban environment

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SUMMARY

Sanitation is an important problem in relation to the control of pests in urban environments. This investigation analysed the potential risk related to the presence of cockroaches and their capacity for disseminating bacteria in six different types of buildings: hospital nursing area and out-patient area, swimming-pool pool-side and toilet area, low-income flats and food-handling places. Fifty-six species of bacteria were identified from 157 samples, 14 of these have previously been reported as potentially pathogenic for man and vertebrates. Similarities were found between samples collected in (*a*) the hospital out-patient area and food-handling establishments and (*b*) the hospital nursing area and flats. Pool-sides possessed a poorer bacterial flora. There was a greater bacterial specific diversity in food-handling establishments, flats and swimming-bath toilet area. *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* were dominant species in flats and the hospital nursing area. Therefore, cockroaches can play a role in disseminating bacteria, which they can carry passively on their cuticle.

INTRODUCTION

Sanitation is an important problem in relation to the control of many pest species in an urban environment. The range of problems that can result from insanitary environmental conditions depends, amongst others, on the specific use of the building. Sanitary standards and practices vary between hospitals, food-handling establishments, public institutions and multi-family dwellings. All these types of buildings in an urban environment can be faced with problems caused by the presence of cockroaches.

Cockroaches can be a real sanitary hazard as they are known to carry bacteria, fungi, helminths and viruses. In addition, cockroach populations are highly variable in size. Among the bacteria they may carry, some are potentially pathogenic for vertebrates and man. Roth and Willis [1] and Story [2] have given well-documented lists of bacteria, including pathogenic species, which contaminate cockroaches naturally. Bacteria have also been introduced experimentally into the cockroach diet and thus proved to be viable after a more or less durable stay in their guts [3–6].

The aim of this investigation was to analyse the potential risk related to the presence of cockroaches and their capacity for disseminating bacteria. We compared the species richness and relative abundance of each bacterial species carried by cockroaches between several types of urban buildings including: hospital, swimming-pool, low-income flats, food-handling places like restaurants and bakehouses. These types of buildings have sanitary practices and goals which are different enough to give an interesting comparison and to allow us to reveal in which type of building cockroaches are the more likely to act as reservoirs of bacterial species and therefore diseases.

MATERIAL AND METHODS

Sampling

In the urban environments we studied, there were three predominant species of domestic cockroaches: *Blattella germanica* (L.) (Blattellidae), *Supella longipalpa* (F.) (Blattellidae) and *Blatta orientalis* (L.) (Blattidae). All the samples were analysed in the same way so the cockroach species did not influence the results.

Cockroaches were caught in food-baited pit-fall traps following the method described previously by Rivault [7]. Each sample was composed of 5–10 adults or old larvae, depending on how many animals were caught. Enough cockroaches were caught to make 157 samples for bacterial analyses.

We selected six types of areas in several types of buildings in Rennes, France.

Hospital. The main hospital is a large 10-floor L-shaped building covering a ground surface of approximately 3600 m². This environment is far from homogeneous; the structure and function of rooms vary greatly from floor to floor and from room to room. This building is divided into separate independent medical units. Each unit usually occupies part of one floor and is limited to one medical speciality. Very few exchanges occurred between units. Risks of bacterial contamination are high and their consequences can be serious because of the decline of some patients' resistance.

Two types of area were selected in this building, nursing areas (HSE) and out-patient areas (HCO). In the nursing area, cockroaches were caught in patients' rooms, main corridors and various technical rooms in different units. In the out-patient area, cockroaches were trapped in consultation rooms, laboratories, offices, kitchens, toilets and corridors in different units.

Public swimming-pool. The building housing this public swimming-pool has a floor surface of approximately 3700 m². Two types of areas were selected in this large building, the toilet area (PWC) and the pool-sides (PBA). A tiled bench was placed along the walls all around the pool-side. Heating and aeration grids opened under the bench.

Food-handling establishments (ALI). Here insects were trapped in several different kitchens of private restaurants and bakehouses. These surfaces could not be evaluated precisely, but the total surface investigated was less than in the two previous cases.

Low-income flats (APP). These flats were in 15 large 15-floor buildings. Each tower included approximately 100 flats. Insects were usually caught in the kitchens, bathrooms and toilets.

The number of samples collected and analysed for each type of area is given in Table 1.

Bacteriological analyses

Only aerobic and facultative anaerobic bacteria were investigated; the typical anaerobic cockroach bacterial intestinal flora was not studied. Once collected, the insects were killed with diethyl ether. Insects were ground with a pestle in an alcohol-sterilized mortar.

Serial dilutions of each sample in sterile water were inoculated on various bacteriological nutritive media (AES Laboratory, France) and incubated for 48 h at 37 °C. Samples were not studied quantitatively as non-selective media were not used. The identification of Gram-negative bacteria was made after incubation on Drigalski medium by use of standard methods (API System, France). *Staphylococcus aureus* were incubated on Chapman medium and identified by slide agglutination and respiratory tests using Staphyslide tests (Bio-Mérieux, France). *Salmonella* spp. were incubated on Mueller–Kaufman culture medium then inoculated on Hektoen medium. *Streptococcus* spp. were incubated on bile–esculine medium. *Pseudomonas aeruginosa* were incubated on cetrimide agar.

Statistical analyses

As one of the aims of this study was to compare bacterial diversity in relation to type of building infested by cockroaches, the data sets were pooled in a single matrix including type of building in columns and bacterial species in rows. Multivariate analysis has proved to be an efficient tool to study complex data sets like this one and to give a simplified picture of the observations by reducing the data sets to their main components, with minimal information loss and without prior hypothesis [8]. Correspondence analysis (FCA) gives several simultaneous ordinations of rows (bacterial species) and columns (type of building) which have known properties [9]. Although FCA is only a descriptive method and not a statistical method, it reveals discrepancies between species diversity in different types of building.

RESULTS

Fifty-six species of bacteria were identified from the 157 samples analysed; 30% of the species were recorded only once and only 10% of them appeared in more than 20 samples (Table 1). Amongst the bacterial species identified in this series of cockroach samples were 14 species that have previously been reported to be pathogenic or potentially pathogenic for man and animals [1, 2, 10] (Table 1).

Bacterial species diversity was analysed in more detail in relation to the type of building. A factorial correspondence analysis (FCA) [8, 9] was computed on the total data matrix (157 samples) including the six types of buildings previously described in columns and frequency of bacterial species (frequency of occurrence of each species in each sample) in rows. The first two axes of the correspondence analysis extracted 57% of total variability of the data. The first axis denoted an increase in specific diversity and clearly separated the samples collected on the pool-side (PBA) from the samples collected in all the other types of buildings. That

Table 1. *Occurrence of different bacterial species in the different urban environments studied*

Environment...	ALI	APP	HCO	HSE	PBA	PWC	Tot
Name of bacteria							
Achromobacter sp.	0	0	0	1	0	0	1
Acinetobacter calcoaceticus	2	7	1	0	9	2	21
Acinetobacter laumanii	0	0	0	0	2	0	2
Acinetobacter sp.	0	3	0	0	2	2	7
Aeromonas hydrophila	0	1	1	1	1	2	6
Aeromonas sobria	0	0	0	0	0	2	2
Alcaligenes denitrificans	0	0	0	0	3	1	4
Alcaligenes faecalis	0	0	1	0	0	2	3
Buttiauxella agrestis	1	2	1	0	5	1	10
Cedecea sp.	0	0	0	1	2	3	6
**Citrobacter diversus*	3	1	1	0	0	0	5
**Citrobacter freundii*	2	5	10	9	3	3	32
**Enterobacter agglomerans*	5	2	2	5	2	5	21
**Enterobacter cloacae*	5	19	9	15	2	6	56
**Enterobacter aerogenes*	4	5	1	0	0	1	11
Enterobacter gergoviae	1	0	0	0	0	0	1
Enterobacter intermedium	0	3	0	0	0	0	3
Enterobacter sakazaki	1	1	0	2	0	1	5
Enterobacter amnigenus	0	0	0	1	0	0	1
Erwinia amylovora	1	0	0	0	0	0	1
Escherichia adecarboxylata	0	1	3	3	0	0	7
**Escherichia coli*	4	0	0	2	0	3	9
Escherichia hermanii	1	0	0	0	0	0	1
Escherichia vulneris	0	1	0	0	0	0	1
Ewingella americana	0	1	0	0	0	0	1
Hafnia alvei	2	0	0	0	0	1	3
**Klebsiella oxytoca*	2	9	5	11	0	1	28
**Klebsiella pneumoniae*	1	13	3	12	0	4	33
Kluyvera sp.	1	3	0	1	0	1	6
Morganella morganii	0	2	0	0	0	2	4
**Pasteurella* sp.	0	0	0	0	3	1	4
Providencia alcalifaciens	0	1	0	0	0	0	1
**Proteus mirabilis*	0	0	0	2	0	0	2
**Pseudomonas aeruginosa*	1	0	0	1	0	0	2
Pseudomonas cepacia	0	1	0	1	0	0	2
Pseudomonas diminuta	0	0	0	0	0	1	1
Pseudomonas mendocina	0	0	0	0	0	1	1
**Pseudomonas fluorescens*	1	1	0	1	0	0	3
Pseudomonas oryzihabitans	0	0	0	0	1	0	1
Pseudomonas putida	0	0	0	0	0	2	2
Pseudomonas maltophilia	1	2	0	1	0	2	6
Pseudomonas pseudomallei	0	0	0	0	0	1	1
Pseudomonas paucimobilis	1	1	1	0	0	1	4
Pseudomonas sp.	0	2	0	1	0	0	3
Pseudomonas testosteroni	1	0	0	0	0	0	1
Pseudomonas stutzeri	1	0	1	0	0	0	2
Pseudomonas vesicularis	0	0	0	0	1	0	1
Rahnella agnatilis	0	0	0	0	0	1	1
Serratia liquefaciens	4	6	3	0	0	0	13
**Serratia marcescens*	6	2	5	0	0	1	14
Serratia odorifera	0	6	0	0	0	0	6
Serratia plymuthica	0	2	0	0	0	0	2

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Table 1. (*cont.*)

Environment... Name of bacteria	ALI	APP	HCO	HSE	PBA	PWC	Tot
Serratia rubibaea	0	1	0	0	0	0	1
**Staphylococcus aureus*	0	0	1	3	1	2	6
Staphylococcus non aureus	0	0	0	0	0	1	1
Vibrio fluvialis	0	1	0	0	0	2	3
Number of cockroach samples in each environment	12	52	29	40	12	12	157
Number of different species identified in each environment	24	30	17	20	15	30	

ALI, food-handling establishments; APP, low-income flats; HCO, hospital out-patient area; HSE, hospital nursing area; PBA, pool-side of swimming-pool; PWC, toilet areas of swimming-pool; Tot, total number of occurrences of each bacterial species.

* Pathogenic species carried by cockroaches according to Roth and Willis [1] and Story [2].

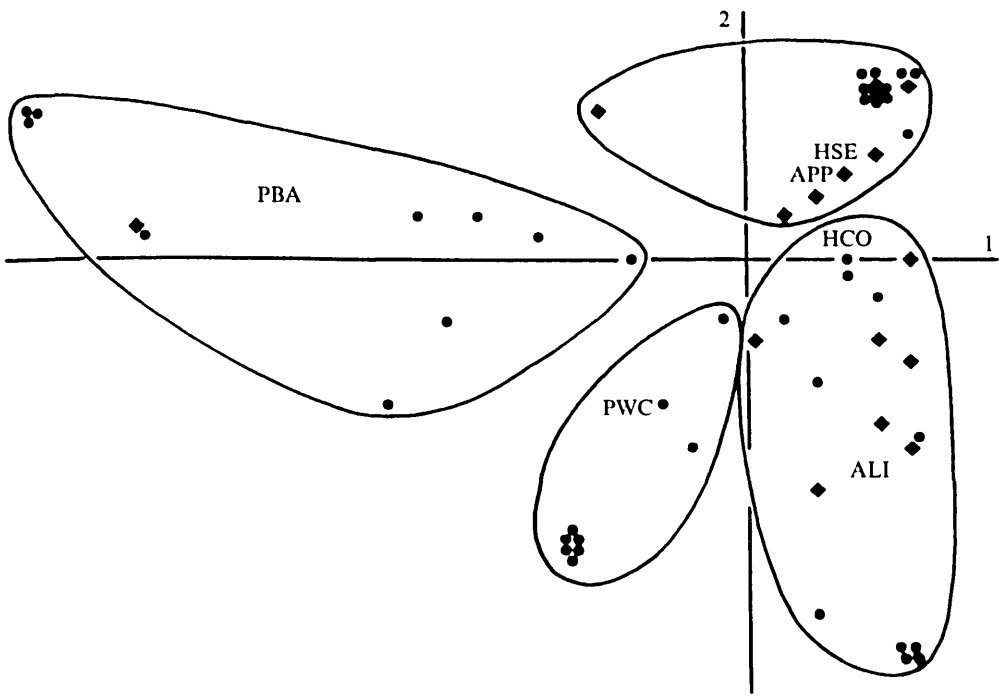

Fig. 1. Factorial Correspondence Analysis (FCA) computed on the data matrix including the six types of buildings described in the text in columns (ALI, APP, HCO, HSE, PBA and PWC) and frequency of bacterial species (frequency of occurrence of each species in each sample) in rows. Clouds of data points on the first two factorial axes (1 and 2). The four main clusters of bacterial species were determined by a hierarchical cluster analysis performed on the coordinates of the points on the first two axes of the FCA. Diamonds: potentially pathogenic bacteria; circles: other bacterial species.

means that PBA possessed a much poorer bacterial flora than the other areas. The second axis enabled us to separate the different types of buildings according to their bacterial flora (Fig. 1).

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Table 2. *Pianka's overlap indices comparing bacterial flora between urban environments. All possible pair-wise comparisons were calculated*

Environment	APP	HCO	HSE	PBA	PWC
ALI	0·59	0·65	0·50	0·29	0·62
APP		0·74	0·83	0·37	0·68
HCO			0·82	0·32	0·61
HSE				0·23	0·69
PBA					0·46

See Table 1 for abbreviations of different environments.

A hierarchical cluster analysis was then performed on the coordinates of the data points on these first two axes as variables [11]. This hierarchical analysis allowed us to divide the different bacterial species into four main clusters according to type of building.

The first group of bacterial species characterized the pool-side (PBA) which was already well individualized by the first two FCA axes. The second group was centred on the centres of gravity of data points representing the samples collected in the toilet area of the swimming pool (PWC). The third group was centred on the data points of the samples collected in the hospital out-patient area (HCO) and in food-handling establishments (ALI). The fourth and last group was centred on the data points for the samples collected in the hospital nursing area (HSE) and in the low-income flats (APP).

The FCA shows that the centres of gravity of the data points for potentially pathogenic bacteria on the factorial plane defined by the first two axes all fall within the APP, HSE, HCO and ALI clouds, that is all the points except the centre of gravity for one species (*Pasteurella* sp.) found in the cloud of points characterizing the pool-sides (PBA).

This analysis stressed similarities on the one hand between samples collected in the hospital out-patient area and in food-handling establishments and on the other hand between samples collected in the hospital nursing area and in the flats.

In order to test the FCA results, Pianka's [12] niche overlap index, R, which evaluates the degree of overlap between two factors on one dimension of the ecological niche, was used to compare bacterial diversity between types of buildings.

$$R_x = \frac{\sum (x_{ij} \times x_{ik})}{\sqrt{\sum (x_{ij})^2 \times \sum (x_{ik})^2}}$$

where x_{ij} and x_{ik} are relative frequencies of presence of bacterial species i in type of building j or k. This index varies from 0 to +1, 0 indicating no overlap and +1 complete overlap.

After calculating all possible pair-wise comparisons, these indices revealed a high level of overlap between the bacterial flora collected from cockroaches (*a*) in the hospital nursing area and in the flats, (*b*) in the flats and in the hospital out-patient area, and (*c*) in the two parts of the hospital, the nursing area and the out-patient area (Table 2). Overlap between other types of buildings was much lower. The lowest overlap indices included the pool-side.

These results confirm the trends described by the correspondence analysis. The

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centres of gravity of data points representing the samples collected in the flats, in the hospital nursing area and in the out-patient area were all very close on the FCA plane defined by the first two axes, whereas the data points for the pool-side were further away (Fig. 1).

DISCUSSION

Sanitation is an important concept relative to the control of many pest species in urban environments [13]. A wide range of problems can result from insanitary environmental conditions and that includes proliferating cockroaches.

Cockroaches always carry species collected in the environment where they live, as well as their specific anaerobic flora, even if the bacterial species collected have no effect on the cockroach [3, 4, 14, 15]. The fewer the bacterial species there are in the environment, the fewer the bacteria cockroaches will carry, as our data for the swimming pool-sides indicate. Far more bacterial species were found in the samples collected in all the other types of buildings. It seems improbable that the high level of chlorination in the swimming pool could influence the low frequency of bacteria on the pool-sides as the same level of chlorination was kept in the toilet area.

We identified 14 potentially pathogenic bacterial species carried by cockroaches from the different environments. *Escherichia coli* is a key-stone species in environmental surveillance as a measure of faecal contamination. *Staphylococcus aureus* is a serious pathogen even though healthy carriage is observed. The other bacteria are either opportunist pathogens, like *Enterobacter agglomerans*, *E. cloacae*, *E. aerogenes*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Ps. fluorescens*, or potential pathogens, like *Citrobacter* sp., *Pasteurella* sp. and *Serratia marcescens*. These species can cause sepsis, gastroenteritis, urinary, biliary and peritoneal infections, pneumonia or wound infections when the required developmental conditions are encountered [1, 2]. Their frequency of occurrence varied with type of building. The pool-side samples presented only five potentially pathogenic bacterial species. However, between 9 and 11 different potentially pathogenic species were identified from cockroach samples from all the other types of buildings.

It appears from our data (Fig. 1) that the potentially pathogenic bacterial floras carried by cockroaches and collected in the flats and in the hospital nursing areas present a high degree of similarity. The question arising from this apparent similarity concerns the level of antibiotic resistance of bacteria from these two environments. According to Fotedar and coworkers [16], it would seem that bacterial resistance to antibioties was higher in samples from hospitals than in samples from flats. These authors considered the samples collected in flats as controls. However, cockroach potentiality for carrying resistant, pathogenic bacteria is not neglible in multi-family dwellings, even if it is probably lower there than in hospitals.

The importance of cockroaches as potential vectors of potentially pathogenic bacteria in private flats appears to have been overlooked and underestimated. Similarly, the possibility that cockroaches may contact food in food-handling establishments could be dangerous if the bacteria they carry are pathogenic. We

did not find any *Salmonella* sp. in our samples, but these bacteria can be carried by cockroaches [17].

Cockroaches can therefore present a real hazard for human health because of the bacteria and other micro-organisms they may carry and not only because people are afraid of them and consider them to be 'dirty creatures'. The presence of cockroaches is never desirable, but it is very frequent and it must be taken into consideration. The presence of cockroaches in a bacteria-rich environment is more serious than in a bacteria-poor environment, especially if there are potentially pathogenic bacteria present, and the people have a low level of immunity.

In hospitals and in public swimming-pools, people are aware of bacterial problems and a high sanitation level is kept. Cockroaches are rarely considered to be a problem and bacterial contamination is no doubt much lower in swimming-pools than in hospitals [18, 20] and this agrees with the low level of contamination of our samples.

In the food-handling establishments and in the flats we are confronted with private and individual hygiene standards. People are not always aware of bacterial contamination problems or do not know how to solve them. The situation becomes more complex when larger areas such as industrial buildings or multi-unit housing are considered.

Practices and regulations differ between public institutions and private dwellings. Each situation is unique and should be evaluated and treated as such. A substantial effort is necessary to educate and to inform the general public in France before urban pest management can be developed.

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