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Microbial organisms carried by brown-banded cockroaches in relation to their spatial distribution in a hospital

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SUMMARY

A simultaneous study of cockroach (*Supella supellectilium*) distribution and of associated carried bacterial flora has been made in the main hospital in Rennes (France).

Wild cockroaches carry a high number of bacterial species that can be related to the normal environmental flora and a contaminant flora acquired from particular environments. The diversity of carried bacterial species reveals a proximity factor between continguous floors of the building which leads us to suppose that cockroaches are able to forage from one floor to the other.

INTRODUCTION

The medical importance of cockroaches is probably underestimated. It has been proved that they carry a large flora of pathogenic bacteria, but their direct involvement in disease transmission is difficult to demonstrate (Cochran, Grayson & Gurney, 1975; Burgess, McDermott & Whiting, 1973, 1981). Nevertheless the presence of cockroaches in hospitals and their ability to carry pathogenic organisms suggest their involvement in the transmission of some infectious diseases.

Cockroaches are resident in garbage cans, sewers and toilets as well as kitchens, restaurant working-tables or sterile instruments in hospitals. Furthermore, their alimentary habits are such that they can forage and feed on faeces and rubbish prior to encountering human food which make them potentially dangerous to human health.

Roth & Willis (1957) and Cornwell & Mendes (1981) gave a large list of pathogenic bacteria which naturally contaminate cockroaches; but other species experimentally introduced in their diet may be carried as well (Ash & Greenberg, 1980; Burgess, McDermott & Whiting, 1973).

We studied simultaneously the distribution of the cockroach *Supella supellectilium* in different areas of the hospital (by a trapping programme), and the different bacterial species carried on their body or housed in their guts, in relation with their capture area. In general, the species and numbers of microorganisms

associated with cockroaches are reflective of the environment in which the arthropod lived or through which it passed (Alcamo & Frishman, 1980).

MATERIALS AND METHODS

Cockroaches were trapped in the main hospital in Rennes (France). It is a 20-year-old building with ten floors. Each floor has three vertically distributed wings covering a surface of 3600 m^2.

Areas studied

Nursing area

Five nursing departments situated from the third to the seventh floor were selected. They will be referred to as S3, S4, S5, S6 and S7.

These departments are composed of a main corridor with about 30 patient rooms on one side and technical rooms on the other side. The total area studied measures 5057 m^2; 2671 traps have been used during the five trapping periods (one every other week).

Out-patient area

This area is situated in an opposite wing of the building and lies from the sixth to ninth floors. The total area studied measures 2240 m^2. The area will be referred to as C6, C7, C8 and C9. The departments are composed of consultation rooms, laboratories, offices, kitchens, toilets and corridors. A total of 1011 traps was used during the whole sampling period.

Trapping

A baby-food jar closed with an inverted funnel is baited with bread and beer. When insects step in, they generally cannot get out.

Traps are deposited at night and collected the next morning. Traps are deposited in the clean linen cupboard and the washroom of each patient room, in the corridors and in the different technical rooms, kitchens, offices and bathrooms.

Bacteriology

Collected cockroaches were killed with diethyl ether, then ground in an alcohol-sterilized mortar. The number of insects analysed varied with their age (adult – small larvae – medium larvae – large larvae) and with the trap contents; serial dilutions of mixture in sterile water were inoculated on various bacteriological media and incubated at 37 °C for 48 h.

The identification of Gram-negative bacteria is achieved by use of standard methods (API system, France). *Staphylococcus aureus* were identified as yellow colonies on Chapman agar (Bio-merieux) and confirmed by slide agglutination (Bio-merieux; Staphyslide test).

After incubation the number of colonies was counted on the various plates to determine the concentration of bacteria per mg of cockroach. The results are expressed in c.f.u. (colony forming units) per mg of cockroach weight.

The grinding method used results in the recovery of bacteria from both cuticle and the gut of the cockroach.

Table 1. *Insects collected in the nursing area and in the out-patient area*

		Larvae			
		Small	Medium	Large	Adults
Nursing area	S4	37	10	7	21
	S5	63	16	38	27
	S6	119	31	48	64
	Total	291	57	93	112
Out-patient area	C6	2	9	10	3
	C7	27	12	29	32
	C8	27	7	6	20
	C9	11	8	5	6

A total of 532 cockroaches was investigated: 120 adult cockroaches, 131 large and medium larvae, 281 small larvae.

RESULTS

Population survey

Trapped cockroaches were characterized by age and sex. Larvae are grouped as small, medium and large.

Nursing area

All insects were totalled and each floor analysed separately (Table 1). The population structure of the different floors did not reveal significant differences ($\chi^2 = 10{\cdot}42$, $P < 0{\cdot}05$). The analysis could not be extended to S3 because of the small number of insects caught or to S7 which could not be studied until the end of the survey because of rebuilding.

It is important to note that the population size is higher on the sixth floor than on the fifth and fourth floors.

During the survey, the population seemed rather stable. But the developmental cycle lasts about 6 months at 28 °C under laboratory conditions, so our survey was too short to allow us to observe any development of the population.

Out-patient area

The population size was also stable over the survey (Table 1). But the population structure varied on each floor level ($\chi^2 = 32{\cdot}45$, $P < 0{\cdot}05$). C8 and C9 populations did not differ from the nursing area population ($P < 0{\cdot}05$). On the contrary C6 was characterized by few small larvae, many medium larvae and few large ones ($\chi^2 = 25{\cdot}45$, $P < 0{\cdot}05$). This seemed to be a very young population where the observed larvae were the first generation born on this site. C7, which is also different from the nursing areas, was characterized by many adults, few small larvae and many large ones; in this case the larvae collected probably did not belong to the first generation born on the site; and in fact, a non-controlled usage of insecticide sprays had artificially modified the population structure.

Table 2. *Numeration of Gram-negative bacteria (c.f.u./mg)*

	Small larvae		Medium larvae		Large larvae		Adults	
Areas	*n*	Numeration c.f.u./mg	*n*	Numeration c.f.u./mg	*n*	Numeration c.f.u./mg	*n*	Numeration c.f.u./mg
S3	—	—	—	—	3	$5{\cdot}43 \times 10^5$	—	—
S4	24	$1{\cdot}42 \times 10^4$	—	—	11	$2{\cdot}57 \times 10^5$	17	$1{\cdot}68 \times 10^5$
S5	53	$6{\cdot}93 \times 10^6$	—	—	24	$2{\cdot}58 \times 10^5$	23	$1{\cdot}86 \times 10^5$
S6	69	$1{\cdot}25 \times 10^5$	—	—	27	$4{\cdot}18 \times 10^5$	25	$2{\cdot}60 \times 10^5$
S7	22	$1{\cdot}56 \times 10^5$	—	—	6	$6{\cdot}5 \times 10^4$	5	28
C6	69	$1{\cdot}22 \times 10^6$	8	$5{\cdot}84 \times 10^4$	10	$3{\cdot}33 \times 10^5$	4	$2{\cdot}03 \times 10^5$
C7	12	$8{\cdot}29 \times 10^4$	11	$4{\cdot}31 \times 10^6$	21	$9{\cdot}47 \times 10^6$	21	$2{\cdot}14 \times 10^7$
C8	20	$1{\cdot}27 \times 10^5$	4	$2{\cdot}77 \times 10^6$	5	$3{\cdot}11 \times 10^6$	16	$7{\cdot}70 \times 10^5$
C9	12	$1{\cdot}25 \times 10^4$	7	$5{\cdot}96 \times 10^5$	4	$9{\cdot}87 \times 10^3$	9	$5{\cdot}16 \times 10^5$
Totals	281		30		111		120	

n, number of insects.

Bacteriology

The c.f.u./mg cockroach weight did not vary with the insect age or with time during the course of the survey.

The average colonization was about 10^5 c.f.u./mg on adult cockroaches with the highest number (10^7 c.f.u.) on the seventh floor (C7) which coincides with the highest population (Table 2).

The number of c.f.u./mg for larvae ranged from 10^4 to 10^6; this does not reveal any significant difference.

Many environmental factors are able to modify the development of bacteria. In order to discriminate among some factors responsible for spatial distributions in the area studied, we made a factorial correspondence analysis (FCA). The analysis is based on bacterial numbers (c.f.u./mg) in the different areas (Fig. 1). The two first factors comprise 72% of the total variability. Its representation on the two first axes shows a 'V' shape (Guttman effect, Benzecri *et al.* 1973). The first axis opposes the high to the low numbers. C7 and S7, which are located on two different wings on the same floor, are on opposite sides of this diagram.

Species diversity

Qualitative results led us to separate bacteria into several groups.

First, bacteria present in nearly all areas: *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*. These bacteria have already been described by Burgess, Macdermott & Whiting (1973); they seem to represent the cockroach normal flora. The same bacterial species have also been found in *Eublaberus posticus* and *Periplaneta americana* guts by Cruden & Markovetz (1987).

Second, bacteria present in three or four areas: *Enterobacter agglomerans*, *Escherichia adecarboxylata*, *Serratia marcescens*, *Serratia liquefaciens*. These species, which were found less often, are also present in the normal cockroach flora but are less numerous. These bacteria are generally opportunist species which are able to produce secondary infection in hospitals.

Third, bacteria present in only one or two areas. These species probably give

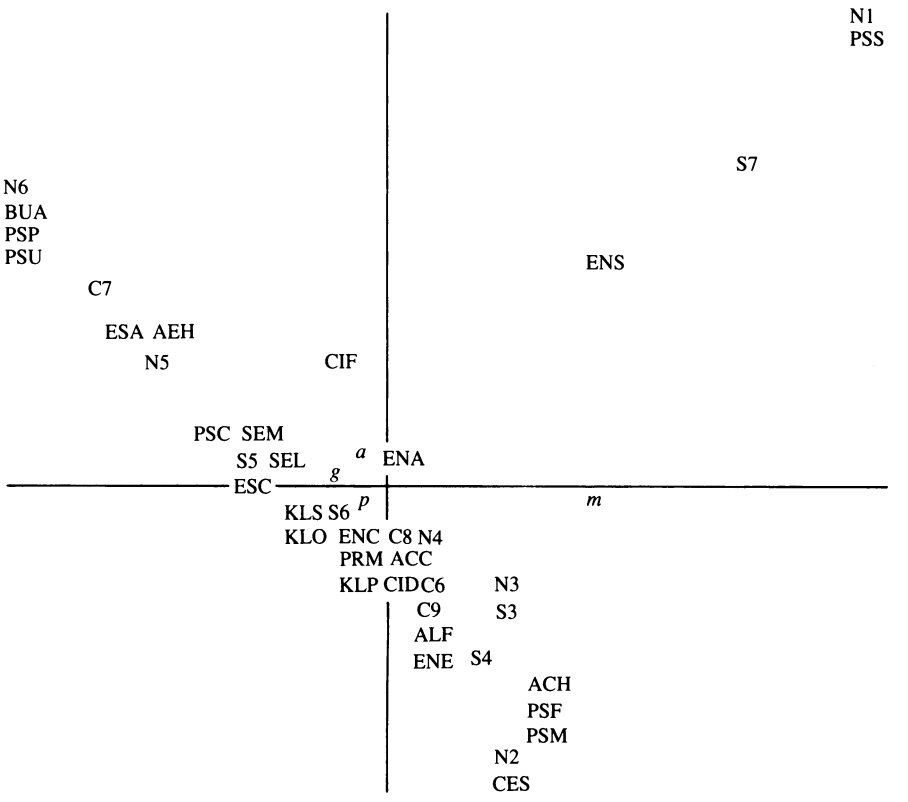

Fig. 1. Distribution of bacteria numeration on the two first axes (FCA analysis). *a*, adult; *g*, large larvae; *m*, medium larvae; *p*, small larvae; S3 to S7, C6 to C9, different areas; bacteria species, see Table 3; N1 to N6, numeration classes.

evidence of cockroach environmental contamination. In this group, were found species usually encountered in hospitals, such as *Acinetobacter*, *Pseudomonas fluorescens*, *P. putida*, and *P. aeruginosa*. The presence of *Escherichia coli* in our data means, according to Frishman & Alcamo (1977), that cockroaches have been in contact with human faeces or faeces-contaminated objects.

Staphylococcus aureus was not rare in our investigations but we cannot say if this bacterium belongs to the insect normal flora or to the environment.

Many published results suggest that cockroaches are salmonella carriers (Ash & Greenberg, 1980; MacKerras & Pope, 1948), but salmonellas were not recovered in this study.

Diversity of bacteria species as a function of cockroach age and areas studied have been analysed with an FCA. Species found only once (16 out of 29) have been removed from the analysis and used as supplementary variables (Table 3). The two first axes represent 56% of total variability. The first factor opposes areas with high and low species diversity (Fig. 2). The second factor reveals geographical proximity of the different areas studied; departments are ordered according to their floor position. S6 and C6, which are on the same floor, are very near to each other on the two first axes plan. S7 and C7 are slightly more separated; these two

Table 3. *List of prospective Gram-negative bacteria and* Staphylococcus aureus. *Presence in the different areas*

		S3	S4	S5	S6	S7	C6	C7	C8	C9
Citrobacter freundii	CIF	—	2	2	3	3	2	6	2	—
Citrobacter diversus	CID	—	—	—	—	—	—	—	1	—
Enterobacter sakazakii	ENS	—	—	—	1	1	—	—	—	—
Enterobacter cloacae	ENC	1	5	5	3	1	1	2	6	
Enterobacter agglomerans	ENA			2	2	1	2			
Enterobacter amnigenus	ENT		1							
Enterobacter aerogenes	ENE									1
Escherichia adecarboxylata	ESA			1	2			3		
Escherichia coli	ESC			1	1					
Klebsiella oxytoca	KLO		1	1	9		3	1	1	
Klebsiella pneumoniae	KLP	1	4	3	4		1		2	
Proteus mirabilis	PRM	1		1						
Serratia marcescens	SEM						1	2	1	1
Serratia liquefaciens	SEL							1	1	1
Buttiauxella agrestis	BUA							1		
Cedecea species	CES		1							
Kluyrera species	KLS				1					
Acinetobacter calcoaceticus (variety *haemolyticus*)	ACC								1	
Achromobacter species	ACH	1								
Aeromonas hydrophila	AEH				1			1		
Alcaligenes faecalis	ALF						1			
Pseudomonas aeruginosa	PSA				1					
Pseudomonas cepacia	PSC			1						
Pseudomonas paucimobilis	PSP							1		
Pseudomonas species	PSS					1				
Pseudomonas fluorescens	PSF	1								
Pseudomonas maltophilia	PSM	1								
Pseudomonas stutzeri	PSU							1		
Staphylococcus aureus	STA		1	1	1			1		

areas were temporarily separated because of rebuilding. This was also indicated by the first analysis. S7 which has recently been reopened after a complete rebuilding seems to have a poorer bacterial flora.

The centre of gravity of the points which represents the different cockroach age classes is very near to the origin of the two first factorial axes. The only point set apart is tied to medium larvae; the simplest explanation for that lies in the small size of the sampling.

The bacterial floras collected on the different cockroach age classes are similar. This could mean that all the cockroaches, adults and larvae, are settled and forage in the same places.

Correlations between bacterial population of two contiguous areas reveal a possible contamination due to proximity. Two hypotheses can be put forward. First, species carried by cockroaches are representative of the bacterial flora; they characterize the flora encountered in the contiguous areas which are exposed to frequent staff or equipment exchanges. Second, bacteria carried by cockroaches reveal their movements between two contiguous areas from one floor to another or between departments on the same floor which means that they can contribute to cross-contamination between two areas.

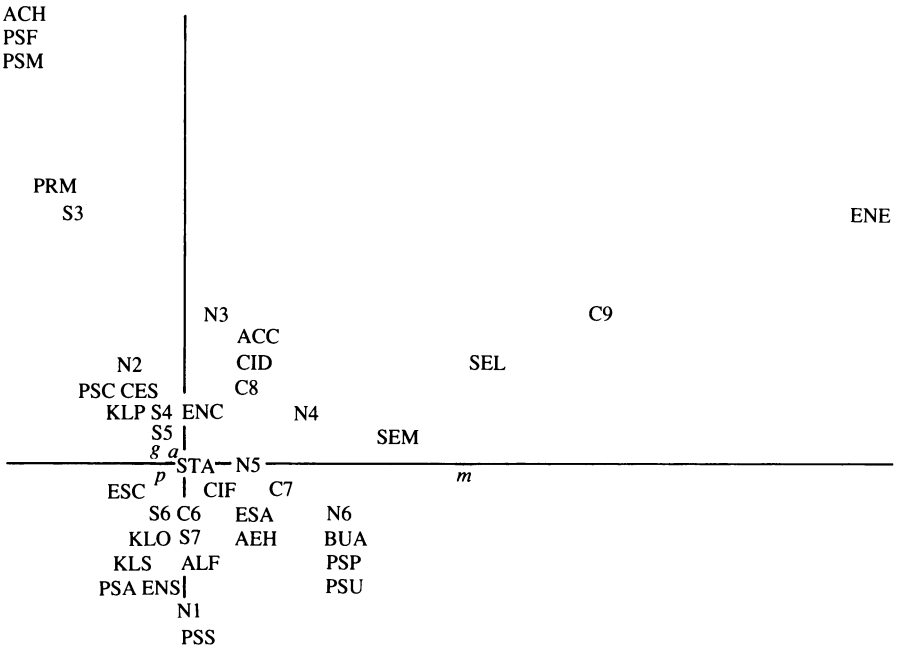

Fig. 2. Distribution of bacteria species on the two first axes (FCA analysis). Same legend as Fig. 1.

This first hypothesis does not seem to be very likely because each department is totally independent of the others; furthermore, members of staff and patients move by lifts inside the building which reduces the effects of geographical proximity. In the second hypothesis, the data give us circumstantial evidence of cockroach movements inside the building. Unfortunately we have not been able to get direct evidence of these movements, for example, with marked animals.

In conclusion 29 bacterial species were isolated from cockroaches (*Supella supellectilium*) caught in the hospital.

As already demonstrated by Burgess, McDermott & Whiting (1973) and Roth & Willis (1957), the part that cockroaches may play in bacterial contamination is not to be neglected.

Nevertheless, most of the results reported in the published literature have been obtained with the species *Blatta orientalis* whose ecology and behaviour are very different from *Supella supellectilium*. Even though they infest human habitats, their ecological needs lead them to invest separate places which might induce different bacterial species carriage. Anyhow, *Supella supellectilium* movements from one department to the other inside the hospital increases potential bacterial contamination risks, for some of the species such as *Acinetobacter* and *Pseudomonas* are dangerous for some kind of patients.

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