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Aerobic bacteria occurring in the hind-gut of the cockroach, *Blatta orientalis*

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SUMMARY

Methods are described for the isolation and identification of aerobic bacteria occurring naturally in the hind-gut of the cockroach *Blatta orientalis* captured from a number of wild sources, to establish whether or not human pathogens occurred naturally within the gut. During the investigation an organism was frequently found which could not be classified in any described species, and for which we propose the name *Escherichia blattae*.

INTRODUCTION

Some 3500 species of cockroach have been described, the vast majority of which are of little or no significance to man. There are, however, perhaps seven species which are closely associated with man and commonly found breeding in buildings (Cornwell, 1968). Three of these, *Blatta orientalis*, the oriental cockroach or 'black beetle', *Blatella germanica* the German cockroach or 'steam fly', and to a lesser extent *Periplaneta americana*, the American cockroach, are spread throughout the British Isles.

These domestic cockroaches are all of tropical, probably African, origin (Rehn, 1945) and, in contrast to the three outdoor species of *Ectobius* found in Southern England, can survive only in a warm and a fairly humid environment such as is provided in kitchens, boiler-rooms and stores. Being essentially nocturnal in their feeding habits, cockroaches are often present in large numbers in buildings used during the day, and remain unnoticed, since they will secrete themselves in any available crack or crevice, behind furniture, underneath fixtures and around water pipes. It is only the occasional inadvertent wanderer, a dead body, a cast skin, or more dramatically a major structural repair to the building which reveals their presence.

The cockroach has been shown to feed readily on faeces, sputum, skin scrapings and other human waste, and on a wide variety of human foodstuffs (Roth & Willis, 1967). Cockroaches are ideally equipped to carry pathogenic organisms from an infected source to uncontaminated material, since they have hairs and bristles on legs and body, grasping claws and pads on their feet (although in our work this purely mechanical means of transmission was found to be of little significance), and their feeding habits involve considerable use of saliva, and indiscriminate defaecation. An impressive array of pathogenic organisms have been isolated from cockroaches living under natural conditions including *Escherichia*

coli, a number of *Salmonella* species, *Staphylococcus aureus* and the poliomyelitis virus (Roth & Willis, 1967). On at least three occasions we have found cockroaches in hospital kitchens, only a few yards away from refuse bins and surgical operating theatres, and we have isolated *E. coli* from these insects.

Under experimental conditions cockroaches have been shown to carry numerous pathogenic organisms externally, without multiplication of the organisms. But, more significantly, a variety of organisms, having been ingested, have been shown to multiply in the gut and appear in the faeces over a period of several days without loss of virulence. Examples of these are the cholera vibrio and plague bacillus (Barber, 1912), and *E. coli* (Steinhaus, 1941; Bitter & Williams, 1949). *Entamoeba histolytica* has also been isolated from cockroaches (Frye & Meleney, 1936) and the ova of a number of parasitic worms, for instance *Ancylostoma duodenale*, *Taenia saginata* and *Ascaris lumbricoides* (MacFie, 1922).

It is much more difficult to associate known outbreaks of disease with cockroach vectors since by their nature these diseases may be, and usually are, transmitted in a variety of ways. The evidence incriminating cockroaches may be purely circumstantial. In one striking incident an epidemic of food-poisoning in the children's nursery of a Brussels hospital subsided immediately an infestation of the German cockroach was controlled. *Salmonella typhimurium* was isolated from the insect (Graffar & Mertens, 1950). Other instances are reported by Antonelli (1943) and Mackerras & Mackerras (1948, 1949).

The natural infection of the cockroach with pathogenic organisms, and experimental evidence, coupled with the known domestication and unhygienic habits of the cockroach, make it, in the words of Roth & Willis, 'impossible to accept cockroaches as only minor annoyances of little medical importance'.

METHODS AND MATERIALS

Capturing the cockroaches

Forty cockroaches were investigated from six different sources as follows: twelve from the 7th floor kitchens of a large London hospital; five from the boiler-room of a smaller hospital; two from a paved yard and five from the staff canteen of the same hospital; six from a teaching college and ten from a long-established insectary culture. The wild cockroaches were caught on site between 11 p.m. and 2 a.m. using specially modified Petri dishes. The smaller half of a sterile dish was placed over the insect and the larger half, from which the rim over half the circumference had been removed, was slipped underneath, trapping the insect in the dish.

Dissection of gut and isolation of organisms

Dissection of the cockroach was carried out the next morning under sterile conditions. The legs, and wings if present, were removed, followed by the head. The sides of the abdomen were cut on either side of the anus, and the complete gut removed posteriorly. In four of the cultured insects, the fore-gut, mid-gut and hind-gut were treated separately, but in all the others only the hind-gut was investigated, the relevant portion being ruptured and emulsified in $\frac{1}{4}$ strength Ringer's solution. The emulsion was plated out on normal blood agar, 6 % blood

Table 1. *Gram positive bacteria isolated from cockroaches*

Organism	Number of isolates		Number of cockroaches in which organism occurred	Percentage of cockroaches with organism
Bacillus spp.	76		37	93
B. cereus gp.		1		
B. firmus		3		
B. licheniformis		6		
B. megaterium		4		
B. subtilis gp.		1		
B. coagulans		1		
B. pulvifaciens		5		
B. pantothenticus		2		
B. brevis		2		
B. circulans		5		
B. polymyxa		3		
Bacillus sp.		43		
Streptococcus spp.	53		28	70
S. bovis		2		
S. equinus		4		
S. durans		3		
S. faecalis		5		
S. faecium		1		
S. sanguis		5		
S. lactis		4		
S. cremoris		16		
Streptococcus sp.		13		
Staphylococcus spp.	11		9	23
Baird-Parker gp. II		3		
Baird-Parker gp. III		1		
Baird-Parker gp. IV		1		
Baird-Parker gp. V		4		
Baird-Parker gp. VI		2		
Micrococcus spp.	3		3	8
Baird-Parker gp. 6		1		
Baird-Parker gp. 7		2		
Aerococcus viridans	6		6	15
Unknown genera	8			

agar, MacConkey's agar and deoxycholate-citrate agar (DCA). After 24 hours' incubation the aerobic organisms were grouped, further tests carried out and identification achieved by use of Cowan and Steel's methods and tables (Cowan & Steel, 1965), except for the staphylococci where Baird-Parker's technique was used (Baird-Parker, 1963).

Biochemical tests were carried out using the following specific techniques: decarboxylase (Møller, 1955), citrate (modified Simmons, 1926), triple sugar iron agar (Report, 1958), indole (Kovacs, 1928), Hugh and Leifson's O-F medium (Hugh & Leifson, 1953), oxidase test (Kovacs, 1956), Voges-Proskauer (V-P) reaction (Barritt, 1936), malonate-phenylalanine medium (Shaw & Clarke, 1955) and gluconate (Shaw & Clarke, 1955).

Table 2. *Gram negative bacteria isolated from cockroaches*

Organism	Number of isolates		Number of cockroaches in which organism occurred	Percentage of cockroaches with organism
Enterobacteriaceae	54		30	75
Citrobacter freundii		11		
Enterobacter aerogenes		1		
E. cloacae		12		
Escherichia coli		2		
E. blattae		16		
Klebsiella edwardsii		2		
K. ozaenae		6		
Proteus vulgaris		1		
Serratia marcescens		3		
Acinetobacter anitratus	3		3	8
Pseudomonas aeruginosa	3		3	8
Unknown genera	2			

RESULTS

A total of 219 isolations was made, of which 157 were Gram-positive bacteria which were placed in 28 different species, and 62 were Gram-negative, placed in 11 species, including a new species which we have named *Escherichia blattae*. Results are shown in Tables 1 and 2.

DISCUSSION

It is often difficult to define the term 'normal flora', and to decide if it is made up of those organisms which occur most frequently and in the greatest numbers in a healthy animal, or whether it should include all organisms found in the healthy animal, ill-health being caused by abnormal flora. Again, the term 'healthy' is equally hard to describe when referring to an insect. If an insect is behaving normally, should we presume it is healthy?

None of the bacteria isolated from the cockroaches in this series of experiments appeared to have any deleterious effect on the animal. Indeed, in later experiments where human pathogenic Enterobacteriaceae were passaged through the cockroach, the insect survived admirably. The only fatalities occurred when *Serratia marcescens* was allowed to accumulate on MacConkey agar on which the cockroaches fed. From our work it appears that the insect will take up any organism in its environment. This was apparent when a series of *Klebsiella edwardsii* and *Proteus vulgaris* was isolated from insects from one locality but not from any other. *Acinetobacter anitratus* was isolated only from insects in an insectary culture. Species of *Hafnia*, *Enterobacter* and *Citrobacter* were isolated from the majority of insects investigated, and *Streptococcus cremoris* was very common.

In four cockroaches in which the bacteria of the fore-gut, mid-gut and hind-gut were investigated the variety of species increased from fore-gut to hind-gut, there being, on average, two species in the fore-gut, three in the mid-gut and five in the

Table 3. *Correlation between* Escherichia blattae *and similar organisms*

	Esch. spp.	*Esch. blattae* 1	*Esch. blattae* 2	*Hafnia alvei*	*Entero-bacter* spp.	*Serratia marcescens*
Citrate	− (0·8 % +)	−	+	+	+	+
Motility	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Indole	+ (1·0 % −)	−	−	−	−	−
H_2S	−	−	−	−	−	−
Gluconate	−*	+	+	+	+	+
PPA	−	−	−	−	−	−
Gelatin	−	−	−	−	+	+
Lactose	+ (9·5 % −)	−	−	−	+	d
ONPG	+	−	−	+	+	+
Sucrose	d	−	−	d	+	+
Mannitol	+ (1·0 % −)	−	−	+	+	+
VP	−	−	−	d	+	+
Arginine	d	+	+	−	d	−
Malonate	−	−	+	+	d	d
KCN	−	−	−	+	+	+

Percentage variations in parentheses.
* Except *Esch. adecarboxylata* and *Esch. blattae*.

Table 4. *Biochemical reactions of three species of* Escherichia

	*Esch. coli**	*Esch. blattae*	*Esch. adecarb-oxylata*†
Motility	+	+	+
KCN	−	−	+
Glucose (gas)	+	+	+
Lactose (acid)	+	−	+
Mannitol (acid)	+	−	+
Sucrose (acid)	d	−	+
Dulcitol (acid)	d	−	+
Inositol (acid)	−	−	.
Adonitol (acid)	−	−	.
Arabinose (acid)	+	+	.
Malonate	−	d	+
Indole	+	−	+
MR	+	+	+
VP	−	−	−
Citrate (Simmons)	−	d	−
Urea	−	−	+
H_2S (TSI)	−	−	−
Gelatin	−	−	+
Phenylalanine	−	−	−
Lysine decarboxylase	d	+	−
Ornithine decarboxylase	d	+	−
Arginine dihydrolase	d	+	−
Gluconate	−	+	+

* Cowan & Steel (1965). † Leclerc (1962).

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hind-gut. As the pH value is known to increase from fore-gut to hind-gut increased acidity may have a bactericidal effect.

Escherichia blattae

Sixteen biochemically similar organisms of the 54 Enterobacteriaceae isolated could not be placed in any accepted group. The organism which we have named *Escherichia blattae* appeared as two biotypes, one of which was citrate and malonate positive, the other negative. In every other respect the two varieties were identical. The organism closely resembled *E. coli*, except for a positive gluconate reaction. However, a gluconate positive *Escherichia*, namely *E. adecarboxylata*, has been described (Leclerc, 1962). Apart from *E. coli*, the organism resembled most closely the following motile gluconate positive species: *Hafnia alvei*, *Enterobacter* spp. and *Serratia marcescens*. Correlation with all the above organisms was strengthened by negative PPA and H_2S reactions.

As can be seen from selected tests shown in Table 3, correlation between *E. blattae* and *Escherichia* spp. is greater than that between *E. blattae* and the other organisms shown, bearing in mind especially the modern view that lactose-negative strains of *Escherichia* are acceptable. Although there was a close correlation between *E. blattae* and *Hafnia alvei*, numerical identification confirmed a closer relationship to *Escherichia*. The organism was tested against all known *E. coli* sera and no cross-reactions were detected. Percentage variations of reactions of *Escherichia* spp. shown in Table 3 are quoted from Edwards & Ewing (1962).

Table 4 shows biochemical reactions of *E. blattae* compared with *E. coli* and *E. adecarboxylata*.

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