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# ANTIBIOTIC-RESISTANCE PATTERNS OF ENTERIC BACTERIA OF WILD MAMMALS ON THE KRAKATAU ISLANDS AND WEST JAVA, INDONESIA

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CONTENTS



Apart from feral pigs on Panjang, rats (*Rattus rattus* and *Rattus tiomanicus*) and bats (various families, genera and species) are the only mammals resident on the Krakatau Islands. The two species of rat occur on separate islands, *R. rattus* on Rakata and *R. tiomanicus* on Panjang and Sertung. Both occur on Java. Of the two genera of bats examined, species of *Cynopterus* were found on Java and all the Krakatau islands, whereas *Myotis muricola muricola* was detected only on Java and Rakata. The main faecal bacteria of these mammals were shown to be *Escherichia coli* and species of *Klebsiella*, *Enterobacter* and *Citrobacter*, with other bacteria (e.g. *Proteus* sp., *Pseudomonas* sp., *Aeromonas* sp., *Serratia* sp., *Proteus/Providencia* sp., *Morganella* sp. and *Streptococcus faecalis*) being present in only a minority of individuals

Significant differences were noted between the faecal floras of the two rat species. *E. coli* was always present in *R. rattus*, but was less frequent in *R. tiomanicus* ($p < 0.05$), whereas species of *Klebsiella* and *Citrobacter* were less frequent in *R. rattus* than in *R. tiomanicus* ($p < 0.05$).

Differences between the faecal floras of the two bat genera were less significant, with the four main faecal bacteria being present in each genus. However, some individual *M. muricola muricola* yielded *S. faecalis*, *Morganella* sp. and *Proteus/Providencia* sp. whereas species of *Cynopterus* did not ($p < 0.05$).

*S. faecalis* was detected in rats and the bat *M. muricola muricola* on Java, but not in mammals on the Krakataus. This may be related to the absence of humans and their domesticated animals on the Krakatau islands.

Only on Java did rats carry tetracycline-resistant *E. coli* or tetracycline-resistant species of *Klebsiella*. This may be related to the widespread use of tetracycline by humans on Java, where tetracycline is available without medical supervision.

Isolates of *E. coli* from rats on Panjang were more resistant to chloramphenicol than were *E. coli* isolated from rats on the other islands of the Krakatau group, and isolates of *Klebsiella* from bats on Panjang were more resistant to sulphamethoxazole than were *Klebsiella* species isolated from bats on Rakata. The reason for faecal bacteria from Panjang mammals being more antibiotic resistant than those from mammals on other islands of the group is unclear, but may be related to differences in diet and vegetation, or the presence of feral pigs on Panjang only.

## 1. Introduction

Over a century has elapsed since the cataclysmic volcanic eruption of the Indonesian island of Krakatau (Krakatoa) in 1883 and the destruction of the fauna and flora of its remnant, Rataka, and the adjacent islands Sertung and Panjang. Although the recolonization of the islands by animals has been studied (Thornton 1984; Thornton & Rosengren 1988), there are no records of the gastrointestinal bacteria living within the animals. This study was designed to determine and compare the faecal flora of mammals on the Krakataus with that of mammals on nearby west Java. Specifically, we wished to determine whether the antibiotic-resistance patterns of the bacteria within mammals on Java, where antibiotics are in widespread use by humans, differed from those of bacteria from mammals on the Krakatau islands, where there is no permanent human population.

## 2. Materials and methods

This work was done as part of the 1985 La Trobe University Zoological Expedition to the Krakatau Islands (Thornton & Rosengren 1988). All mammals sampled from west Java were captured in or near the coastal village of Carita, about 10 km north of the port of Labuan.

### (*a*) *Faecal samples*

All samples were taken from live mammals within a few hours of capture. Rats were caught in Elliott traps baited with cheese or peanuts. Bats were caught in nylon mesh nets set up over openings to caves or in clearings in the vegetation. Species identifications were done by P. A. Rawlinson (rats) and C. R. Tidemann (bats).

The skin surrounding the anus was vigorously cleansed with 70% isopropyl alcohol by using a hospital skin cleansing swab, to prevent skin bacteria from contaminating the sampling swab. The sterile sampling swab (Johns Professional Products, Oakleigh, Australia) was of the type used for sampling the human urethra, being 0.1 cm in diameter and 1 cm long, attached to a firm metal handle 18 cm long. It was inserted approximately 2 cm into the rectum of the animal and rotated several times to ensure the transfer of faecal material onto the swab. The swab was immediately transferred to Stuart's transport medium and stored at ambient temperature (approximately 30 °C) for between 2 and 5 weeks (refrigeration was not available).

### (*b*) *Analysis*

On return to Australia all swabs were plated on to MacConkey agar (MCA) (selective for enteric bacteria) and incubated at 37 °C for 48 h. Individual colonies were selected from the MCA plates, eight from each plate. Care was taken to ensure that at least one of all colonial morphologies was selected. Each of the eight colonies was then processed individually to determine its species and antibiotic-resistance pattern, according to standard microbiological diagnostic procedures. Identification by Gram-stain and biochemical characteristics enabled almost all isolates to be classified to species, some to genus only, and a few remained unidentified Gram-negative rods. For the sake of simplicity of nomenclature, bacteria in this study have been grouped according to genus only, with the exception of *Escherichia coli* and *Streptococcus faecalis*.

Individual isolates (except *S. faecalis*, being the only gram-positive bacterium in the study) were examined for resistance to seven antibiotics by means of replica-plating on to seven separate antibiotic-containing agar plates. Each plate contained one of the following: ampicillin 8 μg ml$^{-1}$, chloramphenicol 8 μg ml$^{-1}$, sulphamethoxazole 20 μg ml$^{-1}$, trimethoprim 1 μg ml$^{-1}$, gentamicin 4 μg ml$^{-1}$, cephalothin 8 μg ml$^{-1}$, tetracycline 4 μg ml$^{-1}$.

Either $\chi^2$ or Fisher's exact probability test were used to compare data, depending on sample size.

## 3. Results

A total of 32 rats and 27 bats were sampled. All swabs grew bacteria on return to Australia, but it is not known if more sensitive bacteria failed to survive in the transport medium.

### (*a*) *Faecal flora*

#### (i) *Rats*

The four predominant bacteria isolated from rats were *Escherichia coli*, *Klebsiella*, *Enterobacter* and *Citrobacter*, with lower numbers of rats containing *S. faecalis*, *Proteus*, *Pseudomonas*, *Aeromonas* and *Serratia* (table 1).

Only rats on Java were found to harbour *S. faecalis* (4 out of 12 individuals). This bacterium was not detected from any of the 20 rats sampled on the Krakatau islands.

Significant differences were noted between the faecal floras of the two species of *Rattus* (table 2). *E. coli* was uniformly present in *R. rattus* but not always present in *R. tiomanicus*. *Klebsiella* and *Citrobacter* were more common in *R. tiomanicus* than *R. rattus* ($p < 0.05$).

#### (ii) *Bats*

Two genera of bats were studied: *Cynopterus* (Megachiroptera, Pteropodidae) and *Myotis* (Microchiroptera, Vespertilionidae). Three species of the fruit bat genus *Cynopterus* were examined: *C. tittaecheilus tittaecheilus* (Java and Panjang), *C. brachyotis javanicus* (Java only) and *C. sphinx angulatus* (Rakata, Sertung and Anak Krakatau).

The main faecal bacteria in the *Cynopterus* species were *E. coli*, *Klebsiella* and *Enterobacter*; isolations of *Citrobacter*, *Pseudomonas*, *Serratia* and *Staphylococcus* were also made from some bats (table 3). Of the 21 individuals of *Cynopterus* species examined, 43% contained only one

TABLE 1. FAECAL FLORA OF RATS ON JAVA AND THE KRAKATAU ISLANDS

| site | code number of rat | species of rat | *Escherichia coli*[a] | *Klebsiella*[b] | *Enterobacter*[c] | *Citrobacter*[d] | *Proteus* | other bacteria |
|---|---|---|---|---|---|---|---|---|
| Java | 11J | *R. rattus* | X | X | X | . | X | *S. faecalis* |
| | 12J | | X | . | . | . | . | *S. faecalis* |
| | 13J | | X | X | X | X | . | |
| | 14J | | X | . | . | . | . | |
| | 15J | | X | . | . | . | . | *S. faecalis* |
| | 16J | | X | X | X | . | . | |
| | 17J | | X | . | X | X | . | |
| | 18J | | X | X | . | . | . | |
| | 19J | | X | . | . | . | . | |
| | 20J | | X | X | . | . | . | *Aeromonas* |
| | 21J | | X | . | . | . | . | *S. faecalis* |
| | 22J | | X | X | X | . | . | |
| Rakata | 71R | *R. rattus* | X | . | . | X | . | |
| | 72R | | X | . | X | . | . | |
| | 82R | | X | . | X | X | . | |
| | 83R | | X | . | X | X | . | |
| Panjang | 35P | *R. tiomanicus* | X | X | . | . | . | |
| | 36P | | . | X | X | . | . | |
| | 37P | | X | X | X | X | . | |
| | 38P | | . | X | . | X | . | *Pseudomonas* |
| | 39P | | X | X | . | X | . | unidentified GNR |
| | 40P | | X | X | . | X | X | unidentified GNR |
| Sertung | 43S | *R. tiomanicus* | X | X | X | X | . | |
| | 44S | | X | . | X | X | X | |
| | 45S | | X | . | X | . | X | unidentified GNR |
| | 46S | | X | . | . | . | X | |
| | 47S | | X | X | . | X | . | |
| | 48S | | . | X | X | X | . | |
| | 49S | | . | X | . | X | X | unidentified GNR |
| | 50S | | X | X | . | X | . | *Serratia* |
| | 51S | | X | X | . | X | . | |
| | 52S | | X | X | . | X | . | |

[a] See table 6; [b] see table 9; [c] see table 11; [d] see table 12; X this bacterium isolated from the specified rat; GNR, Gram-negative rod.

TABLE 2. COMPARISON OF THE MICROBIAL FAECAL FLORA OF *RATTUS RATTUS* AND *RATTUS TIOMANICUS*: PERCENTAGE OF RATS HARBOURING THE SPECIFIED BACTERIUM

| rat species[a] | *Escherichia coli* | *Klebsiella* sp. | *Enterobacter* sp. | *Citrobacter* sp. |
|---|---|---|---|---|
| *Rattus rattus* (Java and Rakata) (16) | 100 | 38 | 50 | 31 |
| *Rattus tiomanicus* (Panjang and Sertung) (16) | 75 | 81 | 38 | 75 |
| difference | $p < 0.05$ | $p < 0.05$ | n.s. | $p < 0.05$ |

[a] Number of rats sampled in parentheses; n.s., not significant.

type of bacterium in their faeces. In only 6% of the 32 rats examined was only one type of bacterium present.

Only six individuals of *M. muricola muricola* were examined (table 4). Two of the three individuals from Java contained *S. faecalis*, but none from Rakata did so. Although numbers are small, this parallels the result for rats; only Javan rats contained *S. faecalis* (table 1).

TABLE 3. FAECAL FLORA OF BATS (CYNOPTERUS SPECIES) ON JAVA AND THE KRAKATAU ISLANDS

| site | code number of bat | species of bat | Gram-negative rods: *Escherichia coli*[a] | *Klebsiella*[b] | *Enterobacter*[c] | *Citrobacter*[d] | *Pseudomonas* | *Serratia* | other bacteria |
|---|---|---|---|---|---|---|---|---|---|
| Java | 1J | *C. tittaecheilus tittaecheilus* | . | . | . | X | . | . | . |
| | 2J | *C. brachyotis javanicus* | X | . | . | X | . | . | . |
| | 10J | *C. brachyotis javanicus* | X | . | . | . | . | . | . |
| Rakata | 32R | *C. sphinx angulatus* | X | X | . | . | X | . | . |
| | 33R | | X | X | . | . | . | . | . |
| | 34R | | X | X | . | X | . | . | . |
| | 60R | | X | . | . | . | . | . | . |
| | 69R | | . | . | X | . | . | . | . |
| | 70R | | . | . | X | . | . | . | unGNR |
| Panjang | 42P | *C. tittaecheilus tittaecheilus* | . | X | X | . | . | . | . |
| | 77P | | . | . | X | . | . | . | . |
| | 78P | | . | . | X | . | . | . | . |
| | 79P | | . | . | X | . | . | . | . |
| | 80P | | . | X | X | X | . | . | . |
| | 81P | | X | X | X | . | . | . | . |
| Sertung | 57S | *C. sphinx angulatus* | . | . | X | . | . | . | unGPR |
| | 58S | | . | . | X | . | . | . | . |
| | 59S | | . | . | . | . | . | . | † |
| Anak Krakatau | 25A | *C. sphinx angulatus* | X | . | . | . | X | X | . |
| | 26A | | X | . | . | . | X | . | . |
| | 27A | | . | . | . | . | . | X | . |

[a] See table 13; [b] see table 14; [c] see table 16; [d] all isolates resistant to cephalothin, with 80% resistant to ampicillin also; X, this bacterium is isolated from the specified bat; unGNR, unidentified Gram-negative rod; unGPR, unidentified Gram-positive rod; † *Staphylococcus* sp. coagulase-negative.

TABLE 4. FAECAL FLORA OF BATS (*MYOTIS MURICOLA MURICOLA*) ON JAVA AND THE KRAKATAU ISLANDS

| site | code number of bat | Gram-negative rods: *Escherichia coli* | *Klebsiella* | *Enterobacter* | *Citrobacter* | *Serratia* | *Proteus/Providencia* | *Morganella* | other bacteria |
|---|---|---|---|---|---|---|---|---|---|
| Java | 6J | . | . | . | . | . | . | . | *S. faecalis* |
| | 23J | . | . | . | . | . | X | X | *S. faecalis* |
| | 24J | . | . | . | . | X | . | . | . |
| Rakata | 61R | X[1] | . | X[2] | . | . | . | . | . |
| | 62R | . | X[3] | X[4] | . | . | X | . | . |
| | 63R | . | . | X[2] | X[5] | . | . | X | . |

[1] Antibiotic resistance of *E. coli*: Sul$^r$; [2] antibiotic resistance of *Enterobacter*: Cep$^r$; [3] antibiotic resistance of *Klebsiella*: Amp$^r$; [4] antibiotic resistance of *Enterobacter*: Cep$^r$ and Amp$^r$: Cep$^r$ (two different isolates); [5] antibiotic resistance of *Citrobacter*: Cep$^r$; X, this bacterium isolated from the specified bat.

Table 5. Comparison of microbial faecal flora of bats (*Cynopterus* spp. and *Myotis muricola muricola*): percentage of bats harbouring the specified bacterium

| | Gram-negative rods | | | | | | |
|---|---|---|---|---|---|---|---|
| bat species[1] | *Morganella* | *Proteus/ Providencia* | *Escherichia coli* | *Klebsiella* | *Enterobacter* | *Citrobacter* | other bacteria |
| *Cynopterus* spp. (21)[2] | nil | nil | 43% | 29% | 48% | 19% | nil |
| *Myotis muricola muricola* (6) | 33% | 33% | 17% | 17% | 50% | 17% | 33%[3] |
| difference | $p < 0.05$ | $p < 0.05$ | n.s | n.s. | n.s. | n.s. | $p < 0.05$ |

[1] Number of bats sampled in parentheses; [2] includes *C. tittaecheilus tittaecheilus*, *C. brachyotis javanicus* and *C. sphinx angulatus*; [3] *Streptococcus faecalis*; n.s, not significant.

None of the six bats sampled on Java (*Cynopterus* and *Myotis*) contained *Klebsiella* or *Enterobacter* although these bacteria were common in bats on the Krakatau islands. However, because of small numbers sampled on Java, this may be a statistical artefact.

Significant differences were noted with respect to *S. faecalis*, *Morganella* and *Proteus/Providencia* between the faecal floras of *Cynopterus* species and *M. muricola muricola*. These bacteria were not detected in any of the 21 *Cynopterus* individuals sampled, yet two of the six individuals of *M. muricola muricola* contained one or more of these bacteria (table 5).

### (*b*) *Antibiotic-resistance patterns*

#### (i) *Faecal flora of rats*

*Escherichia coli* (*table* 6). Although many isolates were fully sensitive to all seven antibiotics tested, others were resistant to one, two, three or four antibiotics. Only on Java was tetracycline resistance detected, in 2 of the 20 *E. coli* isolates (table 7) and in 2 of the 12 rats sampled (rats 19J and 21J, table 6). No isolates from the Krakatau islands were tetracycline resistant (table 7). Overall, however, the isolates from Java were no more resistant than those from the Krakatau islands. Isolates from Panjang were apparently more resistant than isolates from other islands of the Krakatau group (table 6), although the difference was not quite statistically significant ($p = 0.08$). When resistance to chloramphenicol alone was considered (table 8), the *E. coli* isolates from Panjang (3 out of 7 resistant) were more resistant than isolates from the other islands (1 out of 4 resistant).

*Klebsiella* (table 9). Very few isolates were fully sensitive to all antibiotics tested. Most were resistant to one, two or three antibiotics. Only on Java was tetracycline resistance detected, in 3 out of 11 *Klebsiella* isolates (table 7) and in 2 of the 6 rats sampled (rats 13J and 20J, table 9). No isolates from the Krakatau islands were tetracycline resistant (table 7). Only rats from Java contained tetracycline-resistant *E. coli* or *Klebsiella* (table 10). However, no rat contained both tetracycline-resistant *E. coli* and tetracycline-resistant *Klebsiella*.

*Enterobacter* (table 11). No isolates were fully sensitive to all antibiotics tested. Most were resistant to one, two, three or four antibiotics. There was no difference in antibiotic resistance between isolates from Java and those from the Krakatau islands.

Table 6. Comparison of antibiotic-resistance patterns of *Escherichia coli* from rectal swabs of rats on Java and the Krakatau Islands

| site | code number of rat | species of rat | number of different *E. coli* isolated from rats | antibiotic-resistance pattern† | average number of antibiotic-resistance factors per *E. coli* isolate | |
|---|---|---|---|---|---|---|
| Java | 11J | *R. rattus* | 2 | Cep$^{r}$ | 1.05 | |
| | | | | Cep$^{r}$; Sul$^{r}$ | | |
| | 12J | | 2 | Cep$^{r}$ | | |
| | | | | Cep$^{r}$; Chl$^{r}$ | | |
| | 13J | | 1 | fully sensitive | | |
| | 14J | | 1 | fully sensitive | | |
| | 15J | | 1 | fully sensitive | | |
| | 16J | | 2 | fully sensitive | | |
| | | | | Tri$^{r}$ | | |
| | 17J | | 1 | fully sensitive | | |
| | 18J | | 1 | fully sensitive | | |
| | 19J | | 4 | fully sensitive | | |
| | | | | Cep$^{r}$ | | |
| | | | | Sul$^{r}$; Tri$^{r}$ | | |
| | | | | Amp$^{r}$; Tet$^{r}$; Sul$^{r}$ | | |
| | 20J | | 1 | Sul$^{r}$; Chl$^{r}$ | | |
| | 21J | | 3 | Chl$^{r}$ | | |
| | | | | Tet$^{r}$; Chl$^{r}$ | | |
| | | | | Sul$^{r}$; Chl$^{r}$ | | |
| | 22J | | 1 | Chl$^{r}$ | | |
| Rataka | 71R | *R. rattus* | 2 | fully sensitive | 0.80 | 0.64‡ |
| | | | | Sul$^{r}$ | | |
| | 72R | | 1 | Cep$^{r}$ | | |
| | 82R | | 1 | Cep$^{r}$ | | |
| | 83R | | 1 | Cep$^{r}$ | | |
| Sertung | 43S | *R. tiomanicus* | 1 | fully sensitive | 0.56 | |
| | 44S | | 1 | fully sensitive | | |
| | 45S | | 1 | fully sensitive | | |
| | 46S | | 1 | fully sensitive | | |
| | 47S | | 2 | Chl$^{r}$ | | |
| | | | | Amp$^{r}$; Cep$^{r}$; Chl$^{r}$ | | |
| | 50S | | 1 | fully sensitive | | |
| | 51S | | 1 | fully sensitive | | |
| | 52S | | 1 | Tri$^{r}$ | | |
| Panjang | 35P | *R. tiomanicus* | 2 | Cep$^{r}$; Sul$^{r}$; Chl$^{r}$ | 1.57‡ | |
| | 37P | | 3 | fully sensitive | | |
| | | | | Cep$^{r}$ | | |
| | | | | Amp$^{r}$; Cep$^{r}$; Chl$^{r}$ | | |
| | 39P | | 1 | fully sensitive | | |
| | 40P | | 1 | fully sensitive | | |

† Amp$^{r}$, resistant to ampicillin; Cap$^{r}$, resistant to cephalothin; Tet$^{r}$, resistant to tetracycline; Sul$^{r}$, resistant to sulphamethoxazole; Tri$^{r}$, resistant to trimethoprim; Chl$^{r}$, resistant to chloramphenicol. ‡ Difference not significant ($p = 0.08$).

*Citrobacter* (table 12). Only one isolate was fully sensitive to all antibiotics tested. Most were resistant to one antibiotic, but a few were resistant to two, three or four antibiotics. It was not possible to compare west Java with the Krakatau islands because only two rats from Java were found with *Citrobacter*. The isolates on Panjang appeared to be more resistant than those from

Table 7. Comparison of *E. coli* and *Klebsiella* strains from rats on Java and the Krakatau Islands: resistance ($Tet^R$ or sensitivity ($Tet^S$) to tetracycline

| | percentage of bacterial strains (numbers in parentheses) isolated from rats on Java | Krakatau Islands |
|---|---|---|
| *E. coli* | | |
| $Tet^r$ | 10% (2) | 0% (0) |
| $Tet^s$ | 90% (18) | 100% (21) |
| *Klebsiella* | | |
| $Tet^r$ | 27% (3)[a] | 0% (0) |
| $Tet^s$ | 73% (8) | 100% (25) |

[a] $p < 0.05$.

Table 8. Comparison of *E. coli* isolates from rats on Panjang and other Krakatau islands: resistance ($Chl^R$) or sensitivity ($Chl^S$) to chloramphenicol

| | Percentage of *E. coli* strains (numbers in parentheses) isolated from rats on Panjang | Rakata and Sertung |
|---|---|---|
| $Chl^r$ | 43% (3) | 7% (1) |
| $Chl^s$ | 57% (4) | 93% (13) |

$p < 0.05$.

Rakata and Sertung, but numbers of isolates were low, and this difference was not statistically significant.

(ii) *Faecal flora of bats*

*Escherichia coli* (table 13). Most isolates were fully sensitive to all antibiotics tested. However, some isolates were resistant to one or two antibiotics. The isolates from Java appeared to be more resistant than those from the Krakatau Islands, but numbers of isolates were low (and only two bats from Java contained *E. coli*), and this difference cannot be regarded as biologically significant.

*Klebsiella* (table 14). Few isolations were made, with only three bats on Rakata and three on Panjang containing this bacterium. The isolates from Panjang appeared to be more antibiotic resistant than those from Rakata, but again low numbers prevented this from being statistically significant. However, when resistance to sulphamethoxazole alone was considered, the Panjang *Klebsiella* isolates were significantly more resistant than those from Rakata (table 15).

*Enterobacter* (table 16). None of the isolates was sensitive to all the antibiotics tested. Most were resistant to between one and six antibiotics. The most resistant isolates were from bats on Sertung rather than Panjang, but as only two bats were sampled on Sertung this difference has no biological significance.

## 4. Discussion

Apart from feral pigs on Panjang, the only mammals on the Krakatau Islands are bats and rats. Bats and rats showed considerable similarities in their faecal flora, with *Escherichia coli*, *Klebsiella* and *Enterobacter* being present in many animals of both groups. *Citrobacter* was also

TABLE 9. COMPARISON OF ANTIBIOTIC-RESISTANCE PATTERNS OF *KLEBSIELLA* FROM RECTAL SWABS OF RATS ON JAVA AND THE KRAKATAU ISLANDS

| site | code number of rat | species of rat | number of different *Klebsiella* isolated from rats† | antibiotic-resistance pattern‡ | average number of antibiotic-resistance factors per *Klebsiella* isolate | |
|---|---|---|---|---|---|---|
| Java | 11J | *R. rattus* | 1 | Amp$^r$ | 1.82§ | |
| | 13J | | 3 | Amp$^r$ | | |
| | | | | Amp$^r$; Tet$^r$; Sul$^r$ | | |
| | | | | Amp$^r$; Cep$^r$; Tet$^r$ | | |
| | 16J | | 1 | Amp$^r$ | | |
| | 18J | | 2 | Amp$^r$ | | |
| | | | | Amp$^r$; Sul$^r$ | | |
| | 20J | | 1 | Amp$^r$; Tet$^r$ | | |
| | 22J | | 3 | Amp$^r$; Sul$^r$; Chl$^r$ | | |
| Panjang | 35P | *R. tiomanicus* | 2 | Amp$^r$ | 1.00 | 1.12§ |
| | | | | Amp$^r$; Sul$^r$ | | |
| | 36P | | 3 | fully sensitive | | |
| | | | | Amp$^r$ | | |
| | | | | Amp$^r$; Sul$^r$ | | |
| | 37P | | 1 | Amp$^r$ | | |
| | 38P | | 1 | Amp$^r$ | | |
| | 39P | | 3 | fully sensitive | | |
| | | | | Amp$^r$ | | |
| | | | | Sul$^r$ | | |
| | 40P | | 1 | Amp$^r$ | | |
| Sertung | 43S | *R. tiomanicus* | 1 | fully sensitive | 1.21 | |
| | 47S | | 2 | Amp$^r$ | | |
| | | | | Amp$^r$; Cep$^r$; Chl$^r$ | | |
| | 48S | | 3 | Amp$^r$ | | |
| | | | | Cep$^r$ | | |
| | | | | Amp$^r$; Chl$^r$ | | |
| | 49S | | 2 | Amp$^r$ | | |
| | | | | Amp$^r$; Chl$^r$ | | |
| | 50S | | 2 | fully sensitive | | |
| | | | | Amp$^r$ | | |
| | 51S | | 1 | Amp$^r$ | | |
| | 52S | | 3 | fully sensitive | | |
| | | | | Amp$^r$ | | |
| | | | | Amp$^r$; Sul$^r$ | | |

† Including *K. pneumoniae*, *K. cloacae* and *K. oxytoca*; ‡ Amp$^r$, resistant to ampicillin; Cep$^r$, resistant to cephalothin; Tet$^r$, resistant to tetracycline; Sul$^r$, resistant to sulphamethoxazole; Tri$^r$, resistant to trimethoprim; Chl$^r$, resistant to chloramphenicol; § difference not significant.

common in rats but not in bats. A variety of other enteric bacteria was isolated, with considerable variation between individual animals.

The two bat genera have very different foraging modes. *Myotis* species are aerial insectivores, whereas *Cynopterus* species are arboreal frugivores, and this may explain, in part, their different faecal flora. *M. muricola* contained a greater variety of bacteria, including some bacteria that were not detected in the *Cynopterus* species (e.g. *Morganella*, *Proteus/Providencia* and *S. faecalis*). The latter bacterium, being of human faecal origin, may indicate that these insectivorous bats ingest copraphagous invertebrates. *Cynopterus* species had a more restricted range of faecal

Table 10. Comparison of rats (*Rattus* spp.) on Java and the Krakatau Islands: resistance of gut flora (*Escherichia coli* and *Klebsiella*) to tetracycline (Tet$^R$)

| | percentage of rats containing *E. coli* or *Klebsiella* with Tet$^r$ factor (number of rats sampled in parentheses) |
|---|---|
| Java | 33 %† (4/12) |
| Krakatau Islands | 0 % (0/20) |

$p < 0.05$.
† Rats 13, 19, 20 and 21.

Table 11. Comparison of antibiotic-resistance patterns of *Enterobacter* from rectal swabs of rats on Java and the Krakatau Islands

| site | code number of rat | species of rat | number of different *Enterobacter* isolated from rats† | antibiotic-resistance pattern‡ | average number of antibiotic-resistance factors per *Enterobacter* isolate | |
|---|---|---|---|---|---|---|
| Java | 11J | *R. rattus* | 1 | Cep$^r$; Tet$^r$; Chl$^r$ | 2.33§ | |
| | 13J | | 1 | Amp$^r$; Tet$^r$; Chl$^r$ | | |
| | 16J | | 1 | Amp$^r$; Cep$^r$ | | |
| | 17J | | 1 | Cep$^r$ | | |
| | 22J | | 2 | Cep$^r$; Chl$^r$ | | |
| | | | | Amp$^r$; Cep$^r$; Chl$^r$ | | |
| Rakata | 72R | *R. rattus* | 1 | Amp$^r$; Cep$^r$; Chl$^r$ | 2.33 | 2.08§ |
| | 82R | | 1 | Cep$^r$; Tet$^r$ | | |
| | 83R | | 1 | Cep$^r$; Tet$^r$ | | |
| Panjang | 36P | *R. tiomanicus* | 2 | Cep$^r$ | 2.00 | |
| | | | | Amp$^r$; Cep$^r$; Sul$^r$; Chl$^r$ | | |
| | 37P | | 1 | Amp$^r$ | | |
| Sertung | 43S | *R. tiomanicus* | 1 | Cep$^r$ | 2.00 | |
| | 44S | | 1 | Amp$^r$; Cep$^r$ | | |
| | 45S | | 2 | Cep$^r$ | | |
| | | | | Amp$^r$; Cep$^r$; Tet$^r$ | | |
| | 48S | | 2 | Cep$^r$; Chl$^r$ | | |
| | | | | Cep$^r$; Tet$^r$; Chl$^r$ | | |

† Including *E. aerogenes*, *E. cloacae*, *E. agglomerans*; ‡ Amp$^r$, resistant to ampicillin; Cep$^r$, resistant to cephalothin; Tet$^r$, resistant to tetracycline; Sul$^r$, resistant to sulphamethoxazole; Chl$^r$, resistant to chloramphenicol; § difference not significant.

bacteria, and 43 % of individuals contained but a single species of bacterium (table 3). This may be related to a restricted range of bacteria on the fruit eaten by these bats. *Pseudomonas* was detected in *Cynopterus* species (3 out of 21 individuals), but not from any of the six *M. muricola* sampled.

The observed differences between the enteric flora of the two species of rats on the Krakataus (table 2) may represent a basic physiological difference in their gastrointestinal tracts, rather than differences in their diet. Species of *Rattus* are arboreal scavengers or terrestrial scavengers or both, and also eat fruit, both fallen and on trees. It is unlikely that the diets of the two *Rattus* species are significantly different. However, the species are allopatric on

TABLE 12. COMPARISON OF ANTIBIOTIC-RESISTANCE PATTERNS OF *CITROBACTER* FROM RECTAL SWABS OF RATS ON JAVA AND THE KRAKATAU ISLANDS

| site | code number of rat | species of rat | number of different *Citrobacter* isolated from rats† | antibiotic-resistance pattern‡ | average number of antibiotic-resistance factors per *Citrobacter* isolate | |
|---|---|---|---|---|---|---|
| Java | 13J | *R. rattus* | 1 | Cep$^{r}$ | 1.00 | 1.23§ |
| | 17J | | 1 | Cep$^{r}$ | | |
| Rakata | 71R | *R. rattus* | 1 | Cep$^{r}$ | 1.33 | |
| | 82R | | 1 | Amp$^{r}$; Sul$^{r}$ | | |
| | 83R | | 1 | Cep$^{r}$ | | |
| Sertung | 43S | *R. tiomanicus* | 2 | fully sensitive | 1.20 | |
| | | | | Cep$^{r}$ | | |
| | 44S | | 2 | Cep$^{r}$; Chl$^{r}$ | | |
| | | | | Cep$^{r}$ | | |
| | 47S | | 1 | Cep$^{r}$ | | |
| | 48S | | 1 | Amp$^{r}$; Cep$^{r}$; Chl$^{r}$ | | |
| | 49S | | 1 | Cep$^{r}$ | | |
| | 50S | | 1 | Cep$^{r}$ | | |
| | 51S | | 1 | Cep$^{r}$ | | |
| | 52S | | 1 | Cep$^{r}$ | | |
| Panjang | 37P | *R. tiomanicus* | 2 | Amp$^{r}$; Cep$^{r}$; Sul$^{r}$ | 2.00§ | |
| | | | | Amp$^{r}$; Cep$^{r}$; Sul$^{r}$; Tri$^{r}$ | | |
| | 38P | | 1 | Cep$^{r}$ | | |
| | 39P | | 1 | Cep$^{r}$ | | |
| | 40P | | 1 | Cep$^{r}$ | | |

† Including *C. freundii*, *C. diversus* and unspeciated isolates; ‡ Amp$^{r}$, resistant to ampicillin; Cep$^{r}$, resistant to cephalothin; Sul$^{r}$, resistant to sulphamethoxazole; Tri$^{r}$, resistant to trimethoprim; Chl$^{r}$, resistant to chloramphenicol; § difference not significant.

TABLE 13. COMPARISON OF ANTIBIOTIC-REISTANCE PATTERNS OF *ESCHERICHIA COLI* FROM RECTAL SWABS OF BATS (*CYNOPTERUS* SPP.) ON JAVA AND THE KRAKATAU ISLANDS

| site | code number of bat | species of bat | number of different *E. coli* isolated | antibiotic-resistance pattern† | average number of antibiotic-resistance factors per *E. coli* isolate |
|---|---|---|---|---|---|
| Java | 2J | *C. brachyotis javanicus* | 3 | fully sensitive | 0.83‡ |
| | | | | Tri$^{r}$ | |
| | | | | Sul$^{r}$ | |
| | 10J | | 3 | fully sensitive | |
| | | | | Cep$^{r}$ | |
| | | | | Cep$^{r}$; Sul$^{r}$ | |
| Anak Krakatau | 26A | *C. sphinx angulatus* | 2 | fully sensitive | 0.25‡ |
| | | | | Cep$^{r}$ | |
| Rakata | 32R | *C. sphinx angulatus* | 1 | fully sensitive | |
| | 33R | | 1 | fully sensitive | |
| | 34R | | 1 | fully sensitive | |
| | 60R | | 2 | fully sensitive | |
| | | | | Sul$^{r}$ | |
| Panjang | 81P | *C. tittaecheilus tittaecheilus* | 1 | fully sensitive | |

† Tri$^{r}$, resistant to trimethoprim; Sul$^{r}$, resistant to sulphamethoxazole; Cep$^{r}$, resistant to cephalothin.
§ Difference not significant.

Table 14. Comparison of antibiotic-resistance patterns of *Klebsiella* from rectal swabs of bats (*Cynopterus* spp.) on Rakata and Panjang

| site | code number of bat | species of bat | number of different *Klebsiella* isolated† | antibiotics-resistance pattern‡ | average number of antibiotic-resistance factors per *Klebsiella* isolate |
|---|---|---|---|---|---|
| Rakata | 32R | *C. sphinx angulatus* | 1 | Amp$^r$ | 0.6§ |
| | 33R | | 2 | fully sensitive | |
| | | | | Amp$^r$ | |
| | 34R | | 2 | fully sensitive | |
| | | | | Amp$^r$ | |
| Panjang | 42P | *C. tittaecheilus tittaecheilus* | 2 | Amp$^r$; Sul$^r$ | 2.0§ |
| | | | | Amp$^r$; Cep$^r$; Sul$^r$ | |
| | 80P | | 1 | Amp$^r$ | |
| | 81P | | 2 | Amp$^r$ | |
| | | | | Amp$^r$; Cep$^r$; Sul$^r$ | |

† Including *K. pneumoniae*, *K. ozanae*, *K. aerogenes* and *K. oxytoca*. ‡ Amp$^r$, resistant to ampicillin; Cep$^r$, resistant to cephalothin; Tet$^r$, resistant to tetracycline; Sul$^r$, resistant to sulphamethoxazole. § Difference not significant ($p = 0.08$).

Table 15. Comparison of *Klebsiella* isolates from bats on Panjang and Rakata: resistance (Sul$^R$) or sensitivity (Sul$^S$) to sulphamethoxazole

| | percentage of *Klebsiella* strains (numbers in parentheses) isolated from bats on: Panjang | Rakata |
|---|---|---|
| Sul$^r$ | 60% (3) | 0% (0) |
| Sul$^s$ | 40% (2) | 100% (5) |
| | $p < 0.05$ | |

the archipelago (table 1), and this may account for their different faecal floras. It will be interesting to see which species eventually colonizes Anak Krakatau and whether it has a distinctive faecal flora. Up to 1985, only one individual (*R. rattus*) has been found on Anak Krakatau (Thornton & Rosengren 1988).

In comparing the mammals caught on West Java with those from the Krakatau islands, the most outstanding difference was the total absence of *S. faecalis* from animals on the Krakataus. Several rats and bats from west Java contained this bacterium, which is a common enteric bacterium of humans. It is a Gram-positive coccus, unlike most other enteric bacteria which are Gram-negative rods, yet grows on MacConkey agar because of its resistance to bile salts. It is generally considered to be a good indicator of faecal pollution of the environment. Considering the large human population of Java and the inadequate sewage disposal systems, it is not surprising that faecal release into the environment occurs. Rats and bats are apparently ingesting these faecal bacteria, which are then establishing themselves in the animals' intestines. The mammals on the uninhabited Krakatau islands 44 km away appear to have not yet been colonized by *S. faecalis*. This situation may change if large scale exposure to human faeces occurs in the future. *S. faecalis* is not of itself a dangerous bacterium but it does indicate an effect of humans on the environment in west Java.

The detection of tetracycline-resistant *E. coli* and *Klebsiella* in the gastrointestinal tract of rats

Table 16. Comparison of antibiotic-resistance patterns of *Enterobacter* from rectal swabs of bats (*Cynopterus* spp.) on Rakata, Panjang and Sertun.

| site | code number of bat | species of bat | number of different *Enterobacter* isolated† | antibiotic-resistance patterns‡ | average number of antibiotic-resistance factors per *Enterobacter* isolate |
|---|---|---|---|---|---|
| Rakata | 69R | *C. sphinx angulatus* | 1 | Amp$^{r}$; Cep$^{r}$ | 2.0 |
| | 70R | | 1 | Amp$^{r}$; Cep$^{r}$ | |
| Panjang | 42P | *C. tittaecheilus tittaecheilus* | 2 | Cep$^{r}$<br>Amp$^{r}$; Cep$^{r}$ | 2.5§ |
| | 77P | | 3 | Amp$^{r}$; Cep$^{r}$; Tet$^{r}$; Sul$^{r}$; Tri$^{r}$<br>Amp$^{r}$; Cep$^{r}$; Tet$^{r}$; Gen$^{r}$; Tri$^{r}$<br>Amp$^{r}$; Cep$^{r}$; Tet$^{r}$ | |
| | 78P | | 3 | Cep$^{r}$<br>Amp$^{r}$; Cep$^{r}$; Tet$^{r}$<br>Amp$^{r}$; Cep$^{r}$; Tet$^{r}$; Tri$^{r}$ | |
| | 79P | | 1 | Cep$^{r}$ | |
| | 80P | | 4 | Cep$^{r}$<br>Amp$^{r}$; Cep$^{r}$<br>Cep$^{r}$; Tet$^{r}$<br>Amp$^{r}$; Cep$^{r}$; Tet$^{r}$ | |
| | 81P | | 1 | Amp$^{r}$; Tet$^{r}$ | |
| Sertung | 57S | *C. sphinx angulatus* | 3 | Amp$^{r}$; Cep$^{r}$; Tet$^{r}$<br>Amp$^{r}$; Cep$^{r}$; Tet$^{r}$; Chl$^{r}$<br>Amp$^{r}$; Cep$^{r}$; Tet$^{r}$; Sul$^{r}$; Tri$^{r}$; Chl$^{r}$ | 4.0§ |
| | 58S | | 1 | Amp$^{r}$; Cep$^{r}$; Tet$^{r}$ | |

† Including *E. aerogenes*, *E. cloacae* and unspeciated isolates. ‡ Amp$^{r}$ resistant to ampicillin; Cep$^{r}$ resistant to cephalothin; Tet$^{r}$ resistant to tetracycline; Sul$^{r}$ resistant to sulphamethoxazole; Tri$^{r}$, resistant to trimethoprim; Gen$^{r}$, resistant to gentamicin; Chl$^{r}$, resistant to chloramphenicol. § Difference not significant.

on west Java and their complete absence from the rats on Krakatau islands is noteworthy. *E. coli* isolates (10%) and 27% of *Klebsiella* isolates from rats on Java were tetracycline resistant; 33% of rats from Java contained either *E. coli* or *Klebsiella* that were tetracycline resistant. These significant differences probably indicate a real difference in tetracycline resistance between these bacteria in rats on Java and the Krakatau islands. The reason may well be the presence of tetracycline-resistant human faecal bacteria in the Javan environment.

Rats may ingest these bacteria, as we believe they also ingest *S. faecalis* after contamination of the environment with human faeces. Why human faeces should contain tetracycline-resistant bacteria is an important question and is almost certainly because the human diet contains tetracycline. This substance, being a wide-spectrum antibiotic, is generally taken to treat a variety of infections, and is available in west Java without prescription, along with chloramphenicol, several penicillins (including procaine penicillin for intramuscular injection), sulphonamides, polymyxin, neomycin, streptomycin and fansidar. Erythromycin, gentamicin, trimethoprim, amoxycillin and cephalexin evidently are only sold with a prescription. It is likely that people purchase tetracycline tablets for therapeutic or prophylactic self-medication, and so select tetracycline-resistant *E. coli* and *Klebsiella* in their own intestines. With inadequate faecal disposal systems these bacteria are then excreted into the environment, where rats ingest them. The tetracycline-resistant bacteria establish themselves in the rats' intestines, as a result of some unknown selective advantage, and are then excreted by the rat.

Another possible explanation may have nothing whatever to do with humans; tetracycline-resistant bacteria may occur naturally in the environment of Java, but not on the Krakatau islands. In this regard, it is noteworthy that we detected significantly more antibiotic-resistant faecal bacteria on Panjang than on the other islands of the Krakatau group. For example, *E. coli* from rats on Panjang were more resistant to chloramphenicol than *E. coli* from rats on Rakata and Sertung (43% compared with 7%). Also *Klebsiella* from bats on Panjang were more resistant to sulphamethoxazole than *Klebsiella* from bats on Rakata (60% compared with 0%). These differences are unlikely to be due to the release of antibiotics into the environment by humans, since Panjang is uninhabited and very rarely visited. Local fishermen sometimes camp on the shore but rarely if ever penetrate far inland. The presence of feral pigs on Panjang may be responsible for these differences. Alternatively, vulcanologists, who lived on Panjang from 1896–97, 1927–35 and 1937–40 may have influenced the ecology of the island. Although they would not have exposed the island to human-derived antibiotics, because antibiotics were not generally available until after World War II, they did introduce plants, e.g. mangos and coconut palms (Thornton & Rosengren 1988).

The most likely reason for the difference in antibiotic resistance between faecal bacteria of mammals on Panjang and those on other islands is the diet of the mammals. This in turn is probably related to the vegetation present on the island and the antibiotic resistance of the bacteria associated with the plants eaten by these mammals. We have previously shown that antibiotic-resistant Gram-negative rods in the soil are associated with plant growth (Graves *et al.* 1988). The faecal bacteria may be derived from fruit and nuts of these plants. Certainly, antibiotic-resistant bacteria have been demonstrated on fruit and vegetables elsewhere (Remington & Schimpff 1981).

Whatever the correct explanation for the differences observed between faecal bacteria from mammals on Java and the Krakatau Islands and between Panjang and the other islands of the Krakatau group, there is ample evidence that the level of antibiotic resistance of human enteric bacteria is increasing, particularly in countries where antibiotics are readily available and thus often misused, leading to widespread disease (Farrer 1985). Antibiotic resistance can be coded on plasmids and transferred to other bacteria of the same or related species, leading to enhanced spread of the resistant phenotype(s) (Tenover 1986). Recently, *E. coli* from some developing countries has shown increasing resistance to trimethoprim and sulphamethoxazole (Murray *et al.* 1985), and so has *Shigella dysenteriae* in Bangladesh (Shahid *et al.* 1985). In both cases this change is linked to the increased usage of antibiotics by humans. Routman *et al.* (1985), in studying *E. coli* from African baboons, found the antibiotic resistance of *E. coli* to be similar to that of human *E. coli* from the pre-antibiotic era, but significantly less than the antibiotic resistance of recent human *E. coli* isolates. Rolland *et al.* (1985), studying a different group of African baboons, demonstrated that those baboons living close to a tourist park and feeding from human refuse harboured more antibiotic-resistant enteric bacteria than baboons living elsewhere.

Our study appears to have confirmed that excessive or uncontrolled use of antibiotics by human populations in areas where faecal contamination of the environment is inevitable leads to changes in the enteric flora of wild mammals living in these areas. *S. faecalis* and tetracycline-resistant *E. coli* and *Klebsiella* have become established in rats in west Java, whereas rats on the nearby uninhabited Krakatau Islands do not contain these bacteria. To a lesser extent, Javan bats have also been affected by human faecal contamination of the environment.

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