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ABSTRACT

Objective: **To determine if antimicrobial resistance occurs in various bacterial species isolated from rats.**

Method: **Two hundred and fifteen rats were trapped from areas in and around Nairobi, Kenya. They were sacrificed and their intestinal, liver and spleen specimens obtained. Various bacterial species were isolated from these specimens. The species were analysed for antimicrobial susceptibility to 12 commonly used antimicrobials using the disc diffusion technique.**

Results: **The bacterial species isolated included pathogenic and potentially pathogenic ones such as *Escherichia coli* 137, *Salmonella typhimurium* 1, *Klebsiella pneumoniae* 2, *Enterobacter cloacae* 4, *Enterobacter sakazakii* 2, *Citrobacter freundii* 3, *Morganella morganii* (2), *Pseudomonas aeruginosa* 2 *and Burkhoddria cepacia* 6. Depending on the species, the resistance to the various antimicrobials were: 0-100% for cefotaxime, nalidixic acid, cefuroxime, tetracycline, chloramphenicol, co-amoxyclav, sulfamethoxazole, ampicillin, trimethoprim and cephradine, 0-66.6% for gentamicin and 0-25% for apramycin.**

Conclusion: **The results showed that, rats from the study area harboured bacterial species with antimicrobial resistance. These micro-organisms may form an important reservoir for antibiotic resistance which could pose a public health hazard. Control of rat populations, better management of sewer systems and waste dumping sites are recommended in order to reduce occurrence of these drug resistance reservoirs.**

INTRODUCTION

Rats are of great importance as reservoirs of various bacterial organisms causing diseases in humans and other animal species, and further they cause huge losses to stored foods, crops and property(1). Their tendency to invade houses with subsequent contamination of foods and feeds may play a major role in the epidemiology of diseases in both humans and other animal species(2).

Although rats have been shown to harbour various organisms pathogenic to humans, most of these organisms have not been shown to be directly responsible for epidemics. Some bacteria isolated from rats such as *Yersinia pestis, Streptococcus moniliformis* and *Salmonella typhimurium* have been shown to be responsible for outbreaks of plague, Haverhill and rat-bite fevers and salmonellosis respectively(1,3,4). Other bacteria isolated from rats such as *E. coli* and *Yersinia enterocolitica* have been suspected to cause gastroenteritis in humans though the direct epidemiological evidence linking rats to the outbreaks has not been well documented(4,5).

Resistance to antibiotics is an increasingly common problem in both veterinary and human medicine(6). Increased antibiotic resistance in animals may have great impact on human health especially in an environment where animals and humans share the same ecosystem(7). Efforts to reduce resistance are based on the assumption that it is maintained in bacterial populations as a result of exposure to antibiotics and that restricting the use of antibiotics should therefore restrain the spread of resistance. This, however, may not generally apply as antibiotic resistance has been found to be prevalent in populations of wild rodents that have not been exposed to antibiotics(8).

The extensive use of antibiotics for the treatment of bacterial infections in humans and animals selects for resistant micro-organism which may in turn transfer resistance to other bacteria thereby enhancing their spread(9). The transfer of resistant bacteria has been shown to occur among different animal species, between humans and from animals to humans and vice versa(10,11).

The aim of the present study was to investigate if antimicrobial resistance occurs in bacteria isolated from rats trapped in homes and bushes around homes at two sites in Nairobi.

MATERIALS AND METHODS

Study sites: Rats were trapped in the densely populated areas (slums) of Kibera and the less populated areas of Kabete in Nairobi, Kenya. The rats were trapped alive and transported to the laboratory.

Collection and processing of specimens: In the laboratory, the rats were sacrificed using chloroform (Oxoid, Unipath Ltd, Basingstoke, England) and their surfaces disinfected with 70% alcohol. Dissection of the abdomen was performed and intestinal contents and scrapings, pieces of the liver and spleen were obtained aseptically. The specimens were separately inoculated into peptone water (Oxoid) and incubated at 37°C for 18 hours for enrichment. A loopful of each specimen was then subcultured onto MacConkey agar (Oxoid) and blood agar and incubated in air at 37°C overnight. Identification of bacteria was confirmed using biochemical tests on analytical profile index (API) strips (Bio Merieux, Marcy, France) and microscopic examination. Slide agglutination test using group specific sera was done to confirm species of *Salmonella*. All isolates were stored at -80°C until analysed.

Antimicrobial susceptibility testing: All the bacterial isolates were tested for their susceptibility to 12 antimicrobials using the disk diffusion technique according to the National Committee for Clinical Laboratory Standards(12) criteria on Isosensitest agar plates (Oxoid). These antimicrobials and their disc strengths were: cefotaxime (30 µg), nalidixic acid (30 µg), cefuroxime (30 µg), tetracycline (30 µg), chloramphenicol (30 µg), co-amoxyclav (20:10 µg), sulphamethoxazole (100 µg), ampicillin (10 µg), trimethoprim (5 µg), cephradine (30 µg), gentamicin (10 µg) and apramycin (10 µg). A separate plate was inoculated with *E. coli* ATCC 25922 to control for bacterial growth and antibiotic disk potency.

RESULTS

Bacterial isolates: A total of 142 bacterial isolates were obtained from the intestinal specimens and 86 from liver and spleen specimens. *Escherichia coli* were isolated in the greatest numbers (137) and highest prevalence (47.6%) and were isolated from both the intestinal, liver and spleen specimens. Other bacteria isolated in great numbers and high prevalences were *Pseudomonas paucimobilis (31, 10.8%)* and *Chryseomonas luteola (13, 4.51%).* The rest of the bacteria were isolated in low numbers and prevalences as shown in Table 1.

Susceptibility testing: Two bacterial isolates (*Salmonella typhimurium* and *Agrobacter radiobacter)* were susceptible to all the antimicrobials tested. All the other isolates were resistant to one or more antimicrobials. Only *Aeromonas salmonicida* was resistant to all antimicrobials tested. Overall, the highest resistance was for nalidixic acid where seven isolates were resistant (100%). The isolates were also highly resistant to co-amoxyclav where six isolates were resistant (100%). In addition three isolates were multiply resistant to ampicillin, cefuroxime, cephradine and trimethoprim while two isolates were resistant to both sulfamethoxazole and chloramphenicol and one was resistant to tetracycline and cefotaxime. The isolates were most susceptible to apramycin and gentamicin as only three and five isolates showed resistance to these antimicrobials respectively and the most resistant bacterial spp. having 25% resistance

Table 1

Bacteria isolated from intestinal and liver and spleen specimens isolated from rats trapped in and outside houses, in and around Nairobi, Kenya

Bacteria isolate	Intestinal contents and scrapping specimens	Liver and spleen specimens	Total (% prevalence)
Escherichia coli	131	6	137 (47.56)
Enterobacter cloacae	4	–	4 (1.38)
E. sakazakii	2	–	2 (0.69)
Citrobacter freudii	3	–	3 (1.04)
Morganella morganii	2	–	2 (0.69)
Salmonella typhimurium	1	–	1 (0.34)
Klebsiella pneumoniae	2	–	2 (0.69)
Pseudomonas paucimobilis	–	31	31 (10.76)
P. aeruginosa	–	2	2 (0.69)
Chryseomonas luteola	–	13	13 (4.51)
C. indologenes	–	1	1 (0.34)
Aeromonas caviae	–	8	8 (2.77)
A. sobria	–	2	2 (0.69)
A. salmonicida	–	4	4 (1.38)
Burkhoddria cepacia	–	6	6 (2.08)
Comamonas spp.	–	4	4 (1.38)
Vibrio alginolyticus	–	2	2 (0.69)
Alcaligenes spp.	–	2	2 (0.69)
Mycoplasms putrifaciens	–	2	2 (0.69)
Onchobacterium anthropi	–	2	2 (0.69)
Agrobacter radiobacter	–	1	1 (0.34)
Total	204	86	290 (100)

Table 2

Antimicrobial susceptibility of various bacteria organisms isolated from rat samples

Isolate	Specimens		% resistance											
	IN	L/S	TE	CN	APR	S3	C	CTX	CRD	AMC	CXM	AML	NA	W
Escherichia coli	131	0	3.3	0	0	25	0	0	0	1.7	0	23.3	0	6.6
Escherichia coli	0	6	16.7	16.7	16.7	66.6	83.3	0	66.6	0	33.3	50	50	50
Salmonella typhimurium	1	0	0	0	0	0	0	0	0	0	0	0	0	
Enterobacter cloacae	4	0	0	0	0	0	0	0	0	100	0	25	0	0
Enterobacter sakazakii	2	0	0	0	0	50	0	0	0	100	0	0	0	0
Citrobacter freudii	2	0	0	0	0	0	0	0	0	66.7	0	0	0	0
Klebsiella pneumoniae	2	0	0	0	0	50	100	0	100	100	50	100	0	100
Morganella morganii	2	0	0	0	0	0	0	0	0	100	0	0	0	0
Pseudomonas paucimobilis	0	31	12.9	0	0	9.7	12.9	67.7	3.2	9.7	16.1	6.5	41.9	6.5
Chryseomonas luteola	0	13	15.4	7.7	7.7	23.1	0	92.3	53.8	46.2	53.8	4.2	77	46.2
Aeromonas caviae	0	8	37.5	25	0	50	0	50	25	37.5	62.5	62.5	87.5	87.5
Burkhoddria cepacia	0	6	66.6	66.6	0	66.6	0	66.6	0	16.7	83.3	66.6	100	66.6
Comamonas spp.	0	4	0	0	0	50	0	50	25	50	50	25	100	75
Aeromonas salmonicida	0	4	75	25	25	75	25	100	100	100	100	100	100	100
Pseudomonas aeruginosa	0	2	100	0	0	100	100	100	100	100	100	100	100	100
Aeromonas sobria	0	2	0	0	0	0	0	100	0	0	0	0	100	0
Vibrio alginolyticus	0	2	50	0	0	0	0	100	50	50	100	50	100	50
Alcaligenes spp.	0	2	0	0	0	0	0	50	0	0	50	0	50	50
Mycoplasms purifaciens	0	2	0	0	0	0	0	50	0	0	0	0	0	0
Onchrobacterium anthropi	0	2	0	0	0	0	0	0	0	0	0	0	100	0
Agrobacter radiobacter	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomonas indologenes	0	1	0	0	0	0	0	0	0	0	0	0	50	0

Key: TE=Tetracylcine, CN=Gentamicin, APR=Apramycin, S3=Sulfomethoxazole, C=Chloramphenicol, CTX=Cefotaxime, CRD=Cephradine, AMC=Co-amoxyclav, CXM=Cefuroxime, AML=Ampicillin, NA=Nalidixic acid, W=Trimethoprim
IN=Intestines, L/S=Liver and Spleen

to apramycin and 66.6% resistance to gentamicin. Table 2 shows the distribution of bacterial isolates and their susceptibility to various antimicrobial agents.

DISCUSSION

The results of this study have revealed that rats harbour bacterial organisms, which are pathogenic or potentially pathogenic to humans. Although rats are yet to be unequivocally linked to epidemics arising from the bacteria we isolated, potential occurrences of epidemics cannot be ruled out. Rats have been incriminated as the carriers of *Yersinia pestis* the cause of plague(4) and the same sylvatic mode of transmission as occurs in plague can arise for the pathogenic and potentially pathogenic bacteria we isolated.

The isolation of such pathogenic bacteria as *Escherichia coli, Salmonella typhimurium* and *Pseudomonas aeruginosa* raises public health concern. Although *E. coli* is a normal microflora of the gut and has been isolated from rats previously(5) its role as a cause of gastroenteritis in children and the immunocompromised individuals(13,14) is of importance. *Salmonella typhimurium* is incriminated as an important cause of non-typhi salmonellosis(15) and although it has been reported to occur in rats previously in Kenya(1), its isolation in the present study is important due to its potential transmission in humans through contamination of grain in storage. *Pseudomonas aeruginosa* is an important cause of burn wound infections and nosocomial infections(16). Bacteria such as *Klebsiella pneumoniae, Eneterobacter cloacae, Enterobacter sakazakii, Citrobacter freundii, Morganella morganii* and *Burkhoddria cepacia* that were isolated from rats are important as opportunistic pathogens in wound and nosocomial infections. The rest of the bacteria isolated from rats including *Proteus vulgaris, Pseudomonas paucimobilis, Chryseomonas luteola, Aeromonas caviae, Aeromonas sobria, Aeromonas salmonicida, Comamonas spp., Vibrio alginolyticus, Alcaligens, spp., Mycoplasma putrifaciens, Onchobacterium anthropi, Agrobacter radiata* and *Chryseomonas indolegenes* are normal environmental contaminants.

Antimicrobial susceptibility data from this study showed that rats harbour various bacterial isolates resistant to various antimicrobials used in both humans and animals. Although there is controversy over the natural ecology of resistant genes in a bacterial population(9), these results can incriminate rats as an important source of antimicrobial resistant bacteria which may infect humans and other animals.

In this study resistance was observed in all antimicrobials tested. Resistance to these antimicrobials could have arisen from the fact that rats get into contact with these antimicrobials through various sources in the environment, for example, food, water and sewer systems. Interestingly, higher resistance was found for nalidixic acid though it is not commonly used for treatment of infection in humans and animals, as opposed to tetracycline, ampicillin, co-amoxyclav and sulfomethoxazole which are more commonly used. This can support the idea that antimicrobial resistance can occur naturally in a bacterial population(17,18), not exposed to antimicrobials.

The actual mechanism by which the transfer of antimicrobial resistant bacteria and their genes from humans to rats and vice versa occurs is still debatable. Considering that in Kenya, antibiotics are used widely in clinical practice and that antibiotics are sold over the counter for treatment of various infections in humans and animals(14), the need to avert the spread of resistance is important. It appears that rats can act as reservoirs of genetic pools of antimicrobial resistance genes which could be transferred to humans and other animals. On the other hand, humans and domestic animals may act as reservoirs of genetic pools of antimicrobial resistance genes which could be transferred to rats. The rats can thus act as foci of multiplication of these genes with subsequent transmission to humans and other animals.

In conclusion, this study demonstrated that rats in the wild may have been exposed to materials containing antimicrobial residues and that rats carry antimicrobial resistant bacterial organisms which can pose a public health hazard. Control of rat population is crucial so as to reduce the foci of antimicrobial resistant bacterial organisms and their genes and decrease the possibility of dissemination to humans and other animals.

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