Cockroach-associated food-borne bacterial pathogens from some hospitals and restaurants in Addis Ababa, Ethiopia: Distribution and antibiograms.

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Abstract

The association of cockroaches with various pathogens is well documented and this study assessed the role of cockroaches as potential vectors of food-borne bacterial pathogens in Addis Ababa, Ethiopia. A total of 1600 adult cockroaches, captured aseptically from four hospitals and four restaurants, were identified as *Blattela germanica*. Culturing external surface wash and gut homogenates by pooling cockroaches in batches of ten resulted in the isolation of 12 *Salmonella* spp., two *Shigella flexneri*, two *Escherichia coli* O157, 17 *Staphylococcus aureus*, and 25 *Bacillus cereus*. The analysis of isolates for antimicrobial susceptibility demonstrated that most of the isolates, belonging to the various genera, developed multiple drug resistance to up to 12 antimicrobials. To evaluate survival in and shedding of pathogens by *B. germanica*, *Salmonella* Group B, *S. flexneri* and *S. aureus* were separately fed to *B. germanica* at a level of 106 cfu/g of contaminated food. Cultural examination of faecal pellets from *B. germanica* showed that *Salmonella* and *S. aureus* could be excreted for 35 and 14 days, respectively. *Shigella flexneri* was not shed by cockroaches during the experiment. The results indicated that *B. germanica* is a possible reservoir and potential vector of some food-borne pathogens and may spread multiple drug resistance in hospitals and food catering establishments.

Key words: *E. coli* O157, other food-borne pathogens, cockroach, drug resistance

Introduction

Food consumers in developing countries suffer from food-borne bacterial illnesses, especially from those of *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Bacillus cereus*. Unhygienic food handling results in food contaminated by pathogens. One possible source of food contaminations could be dissemination of the pathogens to foods and/or utensils of catering centres through small animals such as cockroaches that live closely with humans in urban environments. Various investigations around the world revealed that cockroaches living close to human dwellings were important carriers of etiologic agents belonging to all groups of potential pathogens: viral, bacterial, protozoan and helminthes (Burgess and Chetwyn 1981, Agbodaze and Owusu 1989, Fotedar et al. 1991a, Cloarec et al. 1992, Pai et al. 2003ab).

Over 4% of cockroaches collected from hospitals, houses, animal sheds, grocery stores, and restaurants in India harboured multiple drug resistant *Salmonella* (Devi and Murray 1991). According to the studies of Fotedar et al. (1991ab), almost all cockroaches isolated from hospital and residential areas carried medically important microorganisms. In addition, similar strains of a particular pathogen were isolated from hospital cockroaches and patients with wounds. A study from South Africa showed that pathogenic strains isolated from cockroaches were indistinguishable from those colonizing infants (Cotton et al. 2000). Oothuman et al. (1989) isolated *Shigella boydii, Shigella dysenteriae,* and *Salmonella typhimurium*, from cockroaches collected from hospital kitchens in Malaysia. In a recent study, 70% of cockroaches collected from hospitals in Iran yielded *Salmonella* spp. and some of the isolates were resistant to antimicrobial drugs (Fathpour et al. 2003). Various food-borne pathogens were isolated from cockroaches collected from kitchens in Ghana (Agbodaze and Owusu 1989) and in Nigeria (Umunnabuike and Irokamulo 1986). In Bangladesh, *Salmonella, Shigella, S. aureus, B. cereus,* and *E. coli* were isolated from cockroaches (Paul et al. 1992). Survival of pathogens in experimentally infected cockroaches was reported by Fotedar et al. (1993) and Imamura et al. (2003).

The number of immuno-compromised people and bacterial drug resistance is on the increase in Ethiopia. The role of cockroaches as mechanical vectors and/or reservoir hosts to pathogens and their drug resistance is unknown. The aim of this study is, therefore, to identify the major cockroach species in hospital and restaurant environments in Addis Ababa, to isolate the common food-borne pathogens from the cockroaches, to assess the drug susceptibility pattern of the isolates and to determine survival and shedding of the pathogens in experimentally infected cockroaches.

Materials and methods

Collection and identification of cockroaches

Four hospitals and four food catering centres in Addis Ababa were considered in this study. The hospitals were among the largest public health institutions in the city. The food catering centres represented medium level eating centres which served about 200 customers per day. Samples of cockroaches were collected from all study sites once a week for twenty weeks. Cockroaches were collected using sterile screw-capped 250 ml jars and sterile hand-gloves (Paul et al. 1992). Each time 10 cockroaches were caught from each of the eight sampling areas, they were pooled as one sample. Only cockroaches caught whole and alive were considered in the study. Identification of cockroaches was performed in accordance with Burgess (1993).

Processing of cockroaches for isolation of pathogens

The collected cockroaches were brought to the laboratory and killed in a sterile jar using chloroform soaked cotton. The external body surface was washed by vortexing in 5 ml sterile physiological saline for two minutes, and the wash was taken as external body homogenate sample. After external body washing, the cockroaches were soaked in 90% ethanol for five minutes to decontaminate their external surfaces and were dried. They were then re-washed with sterile saline to remove traces of ethanol, and the alimentary tract was aseptically dissected out using autoclave-sterilized entomological dissecting needles under a dissecting microscope. The instruments were dipped in ethanol and flamed between dissections. The excised gut was then homogenized in 5 ml of sterile normal saline water. A total of 320 specimens consisting of 160 external body surface and 160 gut homogenates of the cockroaches were analysed. For primary enrichment, 1 ml of each homogenate was inoculated separately into 9 ml of buffered peptone water (BPW) (OXOID, Basingstoke, UK) and incubated at 37°C for 18-24 h.

Isolation and identification of pathogens

For the isolation of *Salmonella* and *Shigella*, a volume of 0.1 ml of growth from BPW was inoculated in to 10 ml of Rappaport-Vassilidias(RV) broth (OXOID, Basingstoke, UK) and incubated at 42°C for 24-48 h for secondary enrichment. This was streaked on Xylose Lysine Deoxycholate (XLD) agar (OXOID, Basingstoke, UK). After 18-24 h of incubation at 37°C, *Salmonella* and *Shigella* were distinguished by their characteristic appearance on the XLD Agar (Collins et al. 1991). Colonies typical of *Salmonella* and *Shigella* were picked from each plate, characterized biochemically following standard methods (Farmer 1999) and confirmed by serogrouping with slide agglutination using BBL antisera.

Escherichia coli O157 was isolated by streaking a loopful of overnight growth from BPW on Sorbitol MacConkey Agar (SMAC) (OXOID, Basingstoke, UK). This was incubated at 37°C for 18-24 h. After 18-24 h incubation, non-sorbitol fermenting presumptive *E. coli* colonies were characterized biochemically and confirmed by Dry spot *E. coli* O157 latex agglutination test (OXOID, Basingstoke, UK) and *E.coli* H7 antiserum (DIFCO, Detroit, USA). Positive controls were included during diagnostic testing.

For the isolation of *S. aureus, g*rowth from BPW was heavily plated on Mannitol Salt Agar (MSA) (OXOID, Basingstoke, UK) and incubated at 37°C for 48 h. Mannitol fermenting colonies were further characterized by microscopic examinations and biochemical tests. Further conformation of *S. aureus* was done using DNAase and coagulase tests (Collins et al. 1991).

Bacillus cereus was isolated after heat-treating BPW culture for 10 min at 80°C in a water bath. A loopful was streaked on *Bacillus cereus* Selective Medium and incubated at 37°C for 18-24 h. Lecithinase positive pink colonies were picked and further characterized by morphology, Gram reaction, and catalase production and presence of spore.

Survival and excretion of pathogenic bacteria from experimentally infected cockroaches

The cockroaches used for the challenge experiment were those collected from the same study sites considered in this work. Each cockroach was transferred to a sterile test tube containing sterile food. Faecal pellets were collected and checked for pathogens to rule out previous contamination with *Salmonella, Shigella or S. aureus*. Each cockroach was checked three times at three-day intervals for the presence or absence of the bacterium in question. Cockroaches free of *Salmonella, Shigella or S. aureus* were selected for the challenge study and were starved for 5 days at room temperature as described in Fotedar et al, *(*1993). *Salmonella* group B, *Shigella flexneri a*nd *S. aureus*, previously isolated from cockroaches in this study, were used as test pathogens. Each test strain was separately grown in Tryptose Soy Broth (OXOID, Basingstoke, UK) at 37°C for 36 h. Four uncontaminated and starved cockroaches were transferred aseptically to a 100 ml test tube containing 1 g of food (a mixture of milk, wheat powder and sucrose) contaminated with 0.1 ml of test bacterial culture containing 10^6 cfu/ml and allowed to feed on it for one hour. Another group of four uncontaminated cockroaches was

transferred to a similar test tube containing only sterile food and allowed to feed for an hour as a negative control. Each group of cockroaches was transferred aseptically to a sterile wire mesh vessel plugged with wet cotton wool, hung over in a sterile 250 ml jar and kept at room temperature for 24 h. The exposed portion of the mesh was wrapped with sterile aluminium foil to prevent contamination from an external source. The set up allowed faecal pellets to be collected from the bottom of the jar passed through the mesh without contamination from external body of cockroaches. During this period, no further food was given to prevent regurgitation (Fotedar et al. 1993). The next day, each group of cockroaches (test and control) was transferred aseptically to a new sterile container of the same set up but with sterile semisolid food composed of milk, wheat powder, sucrose and water coated on the cotton wool plug of the mesh. The cockroaches were transferred to new sterile containers containing sterile food at three-day intervals. The experiments continued until excretion of test strains ceased or, if excretion continued, until all test cockroaches were dead. Excreted faecal pellets were processed for isolation of the respective test strains. The faecal pellets were aseptically picked at intervals of 72 h and placed in 2 ml of nutrient broth. The broth was incubated overnight at 37°C. For isolation of *S. aureus,* broth culture was streaked on MSA. For isolation and identification of *Salmonella* and *Shigella flexneri,* direct plating of growth was made on XLD, and a volume of 0.1 ml of growth was dispensed into RV broth. After 48 h incubation at 42°C, enriched culture was streaked on XLD agar. The faeces of control insects not exposed to the test pathogens were analysed in the same way. All confirmatory biochemical and serological tests were done as indicated previously.

In-vitro drug susceptibility testing

Susceptibility testing was done on Mueller-Hinton agar plates following the standardized disk diffusion technique (Bauer et al., 1966) with Oxoid (Basingstoke, UK) drug discs: ampicillin (Amp), (30µg); sulfamethoxazole (SXT), (25 µg); polymyxin B (Pol), (30 µg); carbenicillin (Car), (10µg); cephalothin (Cep), (30 µg); chloramphenicol (Chl), (30 µg); gentamicin (Gen), (10 µg); kanamycin (Kan), (30 µg); streptomycin, (Str) (10 µg); tetracycline (Tet), (30 µg) augmentin (Aug), (30 µg); clindamycin (Cli),(2 µg); oxacillin (Oxa), (5 µg); erythromycin, (15 µg); penicillin-G, (Pen), (10 µg); vancomycin (Van), (30 µg); and mupirocin (Mup), (5 µg). The reference strains, *S. aureus* (ATCC 6538) and *E. coli* (ATCC 25922), sensitive to all the drugs used in this study, were routinely tested. Interpretation of readings as sensitive, intermediate or resistant was made according to a chart (Jorgenson et al. 1999). Intermediate readings were few and therefore considered as sensitive for the purpose of assessing the data.

Statistical analysis

Comparisons of isolation rates and drug resistance between collection sites and body parts were made using Student's t-test. Significant differences were at the $p<0.05$ level.

Results

A total of 1600 cockroaches were collected In this study. A batch of ten cockroaches was separately collected from each source for 20 consecutive weeks. All cockroaches were identified as *Blattella germanica*. A total of 12 *Salmonella*, two each of *Shigella* and *E. coli* O157, 17 *Staphylococcus aureus* and 24 *Bacillus cereus* were isolated from cockroaches in this study (Table 1). Thirty-four isolates were obtained from hospital and 23 from restaurant environments. Gut and external surface samples yielded 34 and 23 isolates, respectively. There was no significant difference in the distribution of potential pathogens between source locations or body parts. Based on serological tests by group antisera, the *Salmonella* isolates consisted of four each of *Salmonella B*, *Salmonella D*, and *Salmonella E*. Both *Shigella* isolates were *Shigella flexneri*. Of the 13 non-sorbitol-fermenting isolates from SMAC, which were identified as *E. coli* using biochemical tests, two belonged to *E. coli* O157 but failed to react with the H7 antiserum.

Table 1: Distribution of pathogens isolated from *Blattela germanica*

Isolate	Hospital		Restaurant		
	External	Gut	External	Gut	Total
Salmonella B	2	2	-	-	4
Salmonella D	-	3	-	1	4
Salmonella E	-	4	-	-	4
Shigella flexneri	-	-	1	1	2
E. coli O157	-	2	-	-	2
S. aureus	3	8	4	2	17
B. cereus	5	5	8	6	24
Total	10	24	13	10	57

Six individual batches of cockroaches (five from restaurant, one from hospital) yielded *B. cereus* from gut and external surface samples. One individual batch from a hospital contained *Salmonella* group E in gut and external surface samples. Multiple carriage of pathogens was also noted for *B. cereus* and *Staphylococcus aureus.* One individual batch of an external surface sample from a restaurant harboured both pathogens. Similarly, both pathogens were isolated from two individual batches (one each of gut and external surface sample) from hospital samples. This, however, would not mean that a single insect was infected with two different pathogens or carried the same pathogen in the gut and the external parts at the same time.

Salmonella isolates were sensitive to polymyxin B, gentamicin and kanamycin. Over a third of the isolates were, however, resistant to the other drugs used in this study (Table 2). Similarly, *Shigella* and *E. coli* O157 isolates were sensitive to polymyxin B, cephalothin, gentamicin, and kanamycin. *E. coli* O157 isolates were, in addition, sensitive to tetracycline. Less than half of the *Staphylococcus aureus* isolates were sensitive to cephalotin, chloramphenicol, gentamicin, and tetracycline. Resistance to vancomycin was observed in 16 of the 17 isolates. Most of the *Bacillus cereus* isolates were sensitive to nine of the 13 drugs tested except to augmentin, oxacillin, penicillin, and mupirocin. The number of *Staphylococcus aureus* isolates resistant to the various antimicrobials used in this study was significantly higher in restaurant isolates than in the hospital ones. On the other hand, a significantly higher number of resistant *B. cereus* isolates were obtained from hospitals than from restaurants. There was only one *Salmonella* isolate from a restaurant to make a comparison.

Table 2: Susceptibility/resistance of pathogens isolated from *Blattela germanica*

Isolate	No. of isolates	Amp	Sxt	Pol	Car	Cep	Chl	Gen	Kan	Str	Tet	Aug	Cli	Oxa	Ery	Pen	Van	Mup
Salmonella	12	10	9	0	8	10	8	0	1	8	8	10	ND	ND	ND	ND	ND	ND
Shigella	2	2	2	0	2	0	2	0	0	2	2	2	ND	ND	ND	ND	ND	ND
E. coli O157	2	2	2	0	2	0	2	0	0	2	0	2	ND	ND	ND	ND	ND	ND
Staphylococcus aureus	17	ND	ND	ND	ND	8	5	0	5	10	8	17	15	16	14	17	16	17
Bacillus cereus	24	ND	ND	ND	ND	1	1	0	0	1	4	17	6	22	4	23	5	17

ND, not determined; Amp, ampicillin; SXT, sulfamethoxazole; Pol, polymyxin B; Car, carbenicillin; Cep, cephalothin; Chl, chloramphenicol; Gen, gentamicin; Kan, kanamycin; Str, streptomycin; Tet, tetracycline; Aug, augmentin; Cli, clindamycin; Oxa, oxacillin; Ery, erythromycin; Pen, penicillin-G; Van, vancomycin; Mup, mupirocin.

The antimicrobial susceptibility analysis of all *Salmonella* isolates showed four patterns of multiple resistance to the antimicrobial drugs used in this study (Table 3). The commonest resistance pattern (Amp, Sxt, Car, Cep, Chl, Str, Tet, Aug) was noted in six of the 11 hospital isolates and the single restaurant isolate, and one hospital isolate was resistant to nine drugs. A single resistance pattern to 7 drugs (Amp, Sxt, Car, Chl, Str, Tet, Aug) was seen in the two *Shigella* isolates. The two *E. coli* O157 isolates also had a resistance pattern to six drugs (Amp, Sxt, Cep, Chl, Str, Aug). All *S. aureus* isolates obtained from both sources were multiply resistant to various drugs. Fifteen different multiple resistance patterns were seen ranging from resistance to three drugs to resistance to 12 drugs. No particular pattern was dominant. Nine different patterns of multiple resistance were detected among the *Bacillus cereus* isolates ranging from resistance to 4 drugs to resistance to 12 drugs. The commonest pattern was Oxa, Pen, Aug, Mup and this was seen in 16 of the 24 isolates. This pattern was evenly distributed among hospital and restaurant isolates.

In survival and shedding experiments, all excreta samples of cockroaches fed with *Salmonella* were shed *Salmonella* for 35 days, after which all cockroaches were dead. *Salmonella* B was also isolated from the gut content of dead cockroaches. All *Salmonella* isolates were obtained from samples enriched in RV and later streaked on XLD plates. All direct streaks on XLD from an overnight broth failed to yield *Salmonella* throughout the study period (Table 4). All excreta samples from negative control group were found to be negative both with and without RV enrichment throughout the study period. Culture examinations of faecal samples on MSA shed *S. aureus* for 14 days, after which three of the four cockroaches were dead in the test group. None of the cockroaches in the negative control excreted *S. aureus* (Table 4). Gut contents of dead cockroaches yielded *S. aureus* within a day of their death.

Culture examination of faecal pellets of *B. germanica* after exposure to *Shigella flexneri* contaminated food failed to yield the bacterium on XLD agar plates both with and without RV enrichment for 30 days. All challenged cockroaches were found dead after 30 days. However, it was possible to recover *Shigella flexneri* on XLD agar plates, after direct and RV enrichment, from the contaminated feed. The gut contents of dead cockroaches were negative for *Shigella flexneri* both after primary and RV enrichment. The negative control group was treated similarly and found negative for the bacterium.

Table 3: Frequency of multiple resistance pattern among various pathogens isolated from *Blattela germanica*

Isolate	No.	Pattern
Salmonella B	3	
Salmonella D	3	Amp, Sxt, Car, Cep, Chl, Str, Tet, Aug
Salmonella E	1	
Salmonella B	1	Amp, Cep, Aug
Salmonella D	1	Amp, Sxt, Car, Cep, Chl, Kan, Str, Tet, Aug
Salmonella E	1	Amp, Sxt, Cep, Aug
Shigella B	2	Amp, Sxt, Car, Chl, Str, Tet, Aug
E. coli O157	2	Amp, Sxt, Cep, Chl, Str, Aug
Staph. aureus	2	Cli, Oxa, Pen, Van, Aug, Mup
	2	Cep, Chl, Kan, Str, Tet, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	2	Kan, Tet, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	2	Cep, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Pen, Aug, Mup
	1	Chl, Str, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Kan, Str, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Cep, Str, Tet, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Chl, Str, Tet, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Kan, Str, Tet, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Cep, Chl, Str, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Cep, Chl, Str, Tet, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Cep, Chl, Kan, Str, Cli, Oxa, Ery, Pen, Van, Aug, Mup
Bacillus cereus	16	Oxa, Pen, Aug, Mup
	1	Cli, Pen, Aug, Mup
	1	Clin, Oxa, Pen, Aug, Mup
	1	Tet, Oxa, Pen, Aug, Mup
	1	Tet, Oxa, Pen, Van, Aug, Mup
	1	Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Cep, Tet, Cli, Oxa, Ery, Van, Aug, Mup
	1	Gen, Cli, Oxa, Ery, Pen, Van, Aug, Mup
	1	Chl, Str, Tet, Cli, Oxa, Ery, Pen, Van, Aug, Mup

Amp, ampicillin; SXT, sulfamethoxazole; Pol, polymyxin B; Car, carbenicillin; Cep, cephalothin; Chl, chloramphenicol; Gen, gentamicin; Kan, kanamycin; Str, streptomycin; Tet, tetracycline; Aug, augmentin; Cli, clindamycin; Oxa, oxacillin; Ery, erythromycin; Pen, penicillin-G; Van, vancomycin; Mup, mupirocin.

Table 4. Excretion of *Salmonella* B and *S. aureus* by *Blattela germanica*

	Salmonella B		*S. aureus*
Time (days)	Primary enrichment	Secondary enrichment	
2	++	++++	+++
5	-	++++	+++
8	-	++++	++
11	-	++++	++
14	-	+++	++
17	-	+++	CD
20	-	+++	
23	-	++	
26	-	++	
29	-	+	
32	-	+	
35	-	+	
38	CD	CD	

Key: ++++, 250-300 colonies; +++, 100-250 colonies; ++, 50-100 colonies; +, <50 colonies; -, no colonies; CD, cockroaches dead

Discussion

All cockroaches collected in this study were identified as *Blattella germanica*. This species was commonly found in out-patient rooms, wards and staff resting rooms, cafeteria, and food handling establishment of the hospitals. Tea/coffee machines, food and drink service cabinet, feeding and processing units, drawers and even the ceilings of the restaurants were found to be infested with cockroaches. *Blattella germanica* is the most abundant and closely associated with humans worldwide. Since there was no prior work done on the identification, prevalence, and vector potential of cockroach species in Ethiopia, it was not possible to compare our data with local works.

The isolation of *Salmonella* spp., *S. aureus*, *Shigella*, *E. coli O157* and *B. cereus* from this cockroach species indicated that domestic pests could pose health problem to humans. Based on the available literature, *E. coli* O157 was isolated from *B. germanica* for the first time, which may indicate the potential role of cockroaches to spread rare and emerging pathogens into the community. Considering the abundance of cockroaches and the very low infective dose of *E. coli* O157, its presence in *B. germanica* might enable high rates of transmission through foods, which might result in outbreaks. As both *E. coli* O157 isolates were recovered from the gut of hospital cockroaches, the multi-drug resistant feature indicated the importance of hospital cockroaches in nosocomial infection.

Salmonella has been isolated from different species of cockroaches found in hospitals, restaurants, residents, schools, animal shelters etc. throughout the world (Agbodaze and Owusu 1989, Devi and Murray 1991, Fotedar et al. 1991, Rivault et al. 1993, Cotton et al. 2000, Prado et al. 2002, Fathpouer et al. 2003). The fact that 10 of the 12 *Salmonella* isolates were from the gut suggested that cockroach intestine served as a major reservoir of *Salmonella*. Moreover, 11 of the isolates were from hospital cockroaches and were found to be resistant to 3 or more drugs, suggesting the possible role of cockroaches as reservoir and vectors of drug resistant *Salmonella* in health facilities that may contribute to nosocomial infections. Isolation of drug resistant salmonellae and other pathogens from hospital cockroaches has been reported by various workers elsewhere (Fotedar et al. 1991b, Rivault et al. 1993, Cotton et al. 2000, Prado et al. 2002, Fathpour et al. 2003).

Although food handlers are claimed to play the major role in the transmission of *Shigella*, different authors have reported the presence of *Shigella* spp. in cockroaches found in hospitals, restaurants and residences indicating their importance in the dissemination of the bacterium (Oothuman 1989, Paul 1992, Agbodaze and Oswusu 1989). Our *Shigella flexneri* isolates, obtained from restaurant cockroaches, were resistant to 7 antimicrobials. This indicated that *B. germanica* could be a potential vector in spreading multi-drug resistant *Shigella* in food establishment areas in Ethiopia.

Our isolation of *S. aureus* from *B. germanica* collected from hospitals and restaurants is in agreement with other findings elsewhere (Paul et al. 1992, Oothuman et al. 1989, Burgess 1984, Le Guyader et al. 1989, Prado et al. 2002). The isolation of almost proportional numbers of *S. aureus* from hospital and restaurant cockroaches is indicative of the potential role of cockroaches in the dissemination of *S. aureus* in hospitals and food catering centres alike. Furthermore, all *S. aureus* isolates were resistant to all antibiotics tested except gentamicin suggesting the wide-spread distribution of multiple drug resistant *S. aureus* in the environment and the potential role of cockroaches in spreading such strains. In this study, although the number of isolates tested was small, an increase in resistance was seen when compared to previous studies in Tikur Anbessa Hospital and elsewhere in Ethiopia (Gedebou 1982, Gedebou et al. 1987, Lindtjorn et al. 1989, Wolday and Erge 1997, Mengistu et al. 1999).

Considering the fact that *S. aureus* is the most frequent cause of nosocomial infections caused by Gram-positive bacteria, the detection of high numbers of multiple drug resistant isolates, including resistance to vancomycin, is cause for concern. Vancomycin is one of the few reserved drugs used for the treatment of serious bacterial infections. Strains of vancomycin resistant *S. aureus* have been reported from various developed countries (Tenover et al. 2001). Its resistance to mupirocin might make the drug of less use in topical treatment of nostril infections due to *S. aureus.*

We could not demonstrate whether our *B. cereus* isolates from cockroach intestine or external parts were vegetative forms or spores. Their introduction onto appropriate type of food by cockroaches would, however, result in their proliferation and production of enterotoxins. The observation of various resistance patterns among our *B. cereus* isolates agreed with that in another study (Drobniewski 1993). The commonest pattern was, however, resistance to four particular drugs (Oxa, Pen, Aug, Mup). Since *B. cereus* produces β-lactamase, it is expected to be resistant to penicillin and oxacillin. *B. cereus* is more of a toxin producer than infectious. Thus, its observed resistance to various drugs may not be of great concern.

Although the mechanical transmission of pathogens has received considerable attention among researchers, few attempts have been made to determine whether cockroaches sustain internal infections after ingesting them for

an extended period of time. Various workers have shown that cockroaches could maintain and excrete viable pathogens for many days (Burgess et al. 1973, Allen 1987). In our study, cockroaches fed with *Salmonella* could shed the pathogen for 35 days after which all the test cockroaches were dead. It is evident from this result that *B. germanica* is capable of ingesting *Salmonella* contaminated food and excreting viable bacteria in its faeces. When compared with other results, this is probably the longest *Salmonella* excretion time, and we assume that the cockroaches could have continued to excrete the pathogen for an extended period of time if they had lived longer. Since our *Salmonella* test strains were recovered only after enrichment, the number of cells shed by the infected cockroaches must have been very low. Our *S. aureus* test strain could also survive in cockroach gut for two weeks. However, the increasing difficulty to isolate *Salmonella* and *S. aureus* from the faecal pellets with time indicated that the pathogens were not multiplying in the gut of cockroach. The observed survival and shedding rate of our test strains in cockroach gut may not hold true for other strains because the phenomena seem to be associated with bacterial strain, species of cockroaches and antagonism effects of endogenous gut bacteria as observed in other studies (Klowden and Greenberg 1976, Dillon and Dillon 2004)). This may also explain the inability of our *Shigella* test strain to survive in cockroach gut even for a day.

The pathogens considered in this study were reported to be isolated in Ethiopia from various kinds of raw foods (Tegegne and Ashenafi 1998, Molla et al. 1999, Zewde 1999, Geyid et al. 1991) and ready-to-eat cooked foods (Wolde-Tensay and Tesfaye 1992, Erku and Ashenafi 1998, Muleta and Ashenafi 2001). High levels of drug resistance were observed in *Salmonella* and *Shigella,* isolated from diarrhoeal patients in various parts of Ethiopia (Awole et al. 2002, Mache 2001, Assefa et al. 1997). Use of antibiotics for empirical treatment of bacterial food-borne infections in humans and indiscriminate and continuous use of sub-therapeutic doses of antibiotics in animals in Ethiopia are possible factors for the dissemination of drug resistant pathogens in the environment. This study has demonstrated that cockroaches can contribute to the dissemination and spread of food-borne pathogens and multiple drug resistance in human environments for an extended period of time. The observations made in this study indicate the need to use molecular epidemiology to categorically establish the link between isolates from cockroaches and those from humans.

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