

**COCKROACH-ASSOCIATED FOOD-BORNE BACTERIAL
PATHOGENS FROM SOME HOSPITALS AND FOOD
HANDLING ESTABLISHMENT IN ADDIS ABABA**

By Erdaw Tachbele Betre

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Table of Content	page
Acknowledgment -----	I
Table of content-----	III
List of Tables -----	IV
Abstract -----	V
1. INTRODUCTION -----	1
2.LITRATURE REVIEW-----	5
2.1 Identification and Classification of Cockroaches-----	5
2.2 Life cycle, Distribution and Ecology of Cockroaches-----	6
2.3 Medical Importance of Cockroaches -----	8
2.4 Common Food-borne Bacterial Pathogens -----	17
2.4.1 <i>Salmonella spp</i> -----	17
2.4.2 <i>Shigella spp.</i> -----	19
2.4.3 <i>Escherichia coli O157: H7</i> -----	21
2.4.4 <i>Staphylococcus aureus</i> _ -----	25
2.4.5 <i>Bacillus cereus</i> -----	26
3.OBJECTIVES OF THE STUDY -----	29
3.1 General Objective -----	29
3.2 Specific Objectives -----	29

4. MATERIALS AND METHODS	30
4.1. Sampling areas	30
4.2. Collection, Processing and Identification of Cockroaches	30
4.3. Isolation of Bacteria	31
4.4. Identification of Bacteria	33
4.5. Survival and Excretion of Bacteria from Experimentally Infected Cockroaches	35
4.6. <i>In vitro</i> Drug Susceptibility Test.	37
5. RESULTS	39
5.1 Identification of Cockroaches;	39
5.2. Isolation and identification of <i>Salmonella</i> , <i>Shigella</i> , <i>E. coli</i> O157, <i>S. aureus</i> , and <i>B. cereus</i>	39
5.3. Survival and excretion of bacteria in experimentally infected <i>Blattella germanica</i>	42
5.4 <i>In vitro</i> Drug Susceptibility Test	45
6.DISCUSSION	53
7.CONCLUSION AND RECOMMENDATION	62
8.REFERENCES	64

List of Tables

Page

Table 1. Type and number of bacteria isolated from gut and external body surfaces of cockroaches collected from hospitals and restaurants----	40
Table 2. Survival and excretion of <i>Salmonella B</i> by <i>B. germanica</i> following ingestion of 10 ⁶ CFU/g contaminated food-----	43
Table 3. Survival and excretion of <i>S. aureus</i> by <i>B. germanica</i> following ingestion of 10 ⁶ CFU/g contaminated food-----	45
Table 4. Antibiotic susceptibility pattern of 12 <i>Salmonella</i> Isolates-----	47
Table 5. Antibiotic susceptibility pattern of 2 <i>Shigella</i> isolates-----	48
Table 6. Antibiotic susceptibility pattern of 2 <i>E. coli</i> O157 isolates-----	49
Table 7. Antibiotic susceptibility pattern of 17 <i>S. aureus</i> isolates-----	50
Table 8. Antibiotic susceptibility pattern of 25 <i>B. cereus</i> isolates-----	51
Table 9. Multi-drug resistant strains of Bacterial Isolates-----	52

Abstract

A study was carried out to determine the role of cockroaches as reservoirs and vectors of food-borne bacterial pathogens in some hospitals and food handling establishments in Addis Ababa. A total of 1600 adult cockroaches were captured aseptically from eight study sites between December 2002 and June 2003, and all were identified as *Blattella germanica*. Ten cockroaches were pulled as one sample from each of the eight study areas and killed with chloroform. Using selective media, their external surface wash and internal (gut) homogenates, after adequate decontamination of the external surface, were culturally examined for the presence of *Salmonella*, *Shigella*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Bacillus cereus*. We have also initiated challenge studies to evaluate survival and excretion of *Salmonella B*, *Shigella B*, and *Staphylococcus aureus* in *B. germanica* following ingestion of 10^6 CFU/g of contaminated food. In the process of isolation and identification of the test pathogens, 12 *Salmonella* spp., 2 *Shigella B*, 2 *E. coli* O157, 17 *S. aureus* and 25 *B. cereus* isolates were made. Furthermore, most of the isolates were resistant to two or more antimicrobial drugs in a susceptibility test. In the challenge experiment, cultural examination of fecal pellets showed that *Salmonella* and *S. aureus* could be excreted for 35 and 14 days post infection, respectively. However, culture examination of fecal pellets of *Shigella B* infected cockroaches failed to yield the bacterium for 30 days post infection. These results indicate that cockroaches (*B. germanica*) are the possible reservoirs and vectors of multi-drug resistant food-borne pathogens in hospitals and food-catering areas that may be responsible for nosocomial and community acquired infections. Hence, there is a need to implement effective cockroach controlling

programs focusing on hygiene. Continuous surveillance and rational use of antimicrobial drugs are also required in order to minimize the emergence and spread of drug resistant pathogenic bacteria by cockroaches. Further work is essential to establish the natural transmission of human food-borne diseases by cockroaches.

INTRODUCTION

Ethiopia is a country where nearly 16 people die of infectious diseases for every 1000 people each year (WHO, 1996), and a lot more suffer from varieties of illnesses. Infectious diseases are the major causes of mortality and morbidity, out of which food-borne diseases (FBD) take the major part. The great numbers of FBD are avoidable under appropriate hygienic practices.

The World Health Organization defines food-borne diseases as " diseases of infectious or toxic nature caused by, or thought to be caused by the consumption of food or water (Loir *et al.*, 2003)." More than 250 FBDs have been described with various symptoms depending on the etiological agents, though diarrhea and vomiting are the most common (Loir *et al.*, 2003). Among FBDs, there are many different disease-causing pathogens that can contaminate foods, while food poisoning is caused by microbial toxins, or other harmful substances that are present in food.

In most countries, bacteria are the leading cause of FBD and appear to be causative agents of two-thirds of the recorded FBD outbreaks (Loir *et al.*, 2003). Bacteria causing food borne infections have a pathogenesis centered on their ability to penetrate, survive and multiply in host cells. The pathogenesis of bacteria causing food poisoning depends on their capacity to produce toxins after ingestion (in the digestive tract) or before (toxins preformed in foodstuff).

Miscellaneous bacteria (including Gram positive and Gram negative ones) produce toxins that cause food poisoning, resulting in symptoms ranging from gastrointestinal

disorders to paralysis and death. Some food-borne diseases are well recognized, but are considered re-emerging because they have recently become more common. For example, outbreaks of salmonellosis have been reported for decades, but within the past 25 years the disease has increased in incidence in many continents (WHO/OMS, 2002).

Other food-borne pathogens like *E. coli* O157 are considered emerging because they are new microorganisms or because the role of food in their transmission has been recognized only recently. In 1996, an outbreak of *E. coli* O157:H7 in Japan affected over 6,300 school children and resulted in 2 deaths (WHO/OMS, 2002).

New food-borne disease threats occur for a number of reasons. According to WHO/OMS (2002) increase in international travel and trade (globalization), microbial adaptation, and changes in the food production system as well as human demographics and behavior are the major factors contributing to the emergence of FBD that pose a considerable threat to human health and the economy of individuals, families and nations.

Food consumers in Addis Ababa and elsewhere in Ethiopia 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 in Ethiopia could be dissemination of the pathogens to foods and/or utensils of catering centers due to small animals such as cockroaches that live closely with humans in urban

environments. In fact, various investigations around the world revealed that cockroaches living close to human dwellings are important carriers of etiologic agents from all group of pathogens: viral, bacterial, protozoan and helminthes (Burgess and Chetwyn, 1981; Ramirez, 1989; Agbodaze and Owusu, 1989; Fotedar *et al.*, 1991a; Cloarec *et al.*, 1992; Pai *et al.*, 2003a; Pai *et al.*, 2003b).

Cockroaches have been in existence for about 360 million years (Cochram, 1982). During the intervening time, the cockroaches have undergone very little structural change; nevertheless, there is a growing population of at least 4000 named species, which range in length from 5 mm to 90 mm (Cochram, 1982). Although one is apt to think of cockroaches primarily as pests, far less than 1% (not more than 50 species) of cockroaches actually cause concern to humans as domestic pests. Among them, *Blattella germanica*, *Blatta orientalis*, *Periplaneta americana* and *P. australasiae* can infest any place provided suitable conditions such as shelter, water, food and warmth are available (Burgess, 1984). The important factor that makes cockroaches medically important pests is their living and feeding habits. They are obnoxious insects and indiscriminate feeders. They consume every type of food material of animal and plant origin and, by inhabiting diverse unhygienic sites, such as cesspits, garbage bins, groceries, hospitals etc., they disseminate the pathogenic microbes en route (Burgess and Chetwyn, 1981; Cloarec *et al.*, 1992; Pai *et al.*, 2003 B).

The concern about cockroaches' potential health problem in Ethiopia is that people might undermine the vector role of these pests. In view of the facts that the number of immunocompromised people and drug resistant pathogenic strains are increasing

simultaneously, and the role of cockroaches as mechanical vectors and/or reservoir hosts to opportunists and real pathogens is unknown in Ethiopia, it is important to start investigation into this problem.

In this work, isolation and identification of *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* O157:H7 from cockroaches collected from 4 hospitals (Black Lion, Yekatit 12, Zewkitu Memorial and Menilik II) and 4 restaurants around Kazanchis and Aware were undertaken. In addition, survival rate in experimentally infected cockroaches and drug susceptibility test for the isolated pathogens were done.

2. LITERATURE REVIEW

2.1. Identification and Classification of Cockroaches

Typically, cockroaches are large, oval and dorso-ventrally flattened insects. The pest species range in length from 10 to 50 mm. The head bears a pair of compound eyes, long filamentous antennae with numerous annulations and ventral chewing mouthparts for omnivorous feeding. The thorax has three distinct segments, of which the first is evidenced dorsally by a large pronotum which is sometimes so well developed as to partially hide the head when viewed from above. Each thoracic segment has a pair of strong legs covered in stout setae and terminating in paired claws; pad-like pulvilli are present on the last tarsal segment in some species. Two pairs of wings are present in the adults of most cockroaches, each with a network of veins. The wings are thickened to a leathery texture and are closed scissors-like over the metathorax and abdomen. The abdomen is clearly segmented and its only appendages are paired cerci at the posterior end (Burgess, 1993).

Cockroaches are grouped in the order *Dictyoptera* and suborder *Blattaria* which have four families among which the three, *Blaberidae*, *Blattellidae*, and *Blattidae* contain species of some medical importance (Burgess, 1993). Representative species include *Rhyparobia maderae*, *Blattella germanica*, and *Blatta orientalis* respectively.

2.2. Life cycle, Distribution and Ecology of Cockroaches

Cockroaches are exopterygote insects in which the life cycle is of the so-called indirect or incomplete type. Once matured, and about a week after mating, the female cockroach produces eggs, which she deposits in a protective covering, referred to as the ootheca or egg case (Cochram, 1982). At the peak of her reproductive capacity, the female cockroach may produce up to two egg cases per week and a total of 10-84 egg cases (14-40 eggs per case) in her lifetime (Baumholtz *et al.*, 1997). The female may carry the case internally or externally, depending on the species (Cochram, 1982). In most cases, the female cockroach tries to hide or bury her ootheca, but will attach it to almost anything if a hiding place cannot be found (Baumholtz *et al.*, 1997). After the ootheca is deposited, the female is believed to leave it alone. By the time they hatch from out of the eggs, the nymphs resemble the adults, although they are smaller and have undeveloped wings and genitalia. After hatching, the nymph's transformation to adults progress through a series of 6-16 molts (Cochram, 1982). Once fully matured, the adult cockroach may live from days to years, depending on the species as well as environmental conditions and especially the availability of water (Baumholtz *et al.*, 1997).

Mature cockroaches will breed all year long if the conditions are favorable. Consequently, the cockroach poses a more alarming threat to humans as potential vector when compared with other vectors such as flies, which are dormant in winter (Baumholtz *et al.*, 1997).

Moisture is essential to the cockroach's well being. If adult cockroaches are placed in a dry atmosphere, they will die within 2-4 weeks, while nymphs or eggs would deteriorate even more rapidly, and love darkness (Anonymous 1958, cited in Baumholtz *et al.*, 1997). In addition to moisture, cockroaches need food. Experiments with *B. germanica* and *P. americana* have shown that adult cockroaches can survive for 3-6 weeks without food, while newly hatched cockroaches will usually die with 8-9 days (Baumholtz *et al.*, 1997). There seems to be no dietary restrictions for the cockroaches, as they eat almost anything.

Currently, approximately 16 species of cockroaches represent a potential threat to human health and well-being (Cochram, 1982). *B. germanica*, *P. americana* and *B. orientalis* are three of the most important cockroach species with regard to their importance to man. Because of its distribution, *B. germanica* is the most important cockroach pest on a worldwide basis (Cochram, 1982). It is also considered to be world's most common domiciliary pest, and is also found in hospitals (Fotedar *et al.*, 1993). Although *B. germanica* is found all over the world, it is believed that this cockroach originated in northeast Africa (Cochram, 1982).

Adult *B. germanica* generally measure 10-15 mm in length with a light yellowish-brown color in males and a slightly darker color in females. Both nymphs and adults have two longitudinal, black, parallel bands separated by a lighter stripe on the pronotum. Males and females mature at the same time and mate within the first 7-10 days of adult life. Males will mate repeatedly while females usually mate only once. After mating, the

incubation period of the ootheca is 2-4 weeks with 37-44 eggs per ootheca and a 90% hatch rate. Females will produce 4-8 egg cases per life span, which usually lasts about 100 days. Although seemingly adaptive to most conditions, this species prefers places with easy access to warmth, moisture, and food (Bauholtz *et al.*, 1997).

Periplaneta americana is distributed throughout the temperate, and sub-tropical regions of the world. It is larger than *B. germanica*, 35-40mm in length. It has a shining red to chocolate brown color. Males and females are about the same size and adults live a year or longer (Cochram, 1982). The mated female can produce a capsule once a week until she produces 21-59 egg cases in her lifespan, with an average of 16 eggs per case (Bauholtz *et al.*, 1997). Although *P. americana* prefers 28°C, it is still active from 21 to 33 °C.

Blatta orientalis is distributed in temperate zones of the world. This cockroach is intermediate size, measuring 20-27mm in length, and reddish brown to black in color. Adults live for 35 to 180 days. After mating, an ootheca is produced in 8-10 days. Females produce an average of 8 oothecas, with 16 eggs each. It prefers a cool environment (Bauholtz *et al.*, 1997).

2.3. Medical importance of cockroaches

Studies have shown that cockroaches can be directly linked to asthma by producing allergenic particles from their feces and shed exoskeleton. In one study, homogenized *B. germanica* was administered via a nebulizer to test for bronchial irritation. All

subjects with positive skin test to the cockroach extract had bronchial irritation, but subjects with a negative skin test had no bronchial reaction (Bernton *et al.*, 1972). Twarog *et al.* (1979) have identified three main cockroach extract allergens: Cr-I, Cr-II, and Cr-III. In a study of bronchial asthma, 40% of patients in China were allergic to German cockroaches (Tsai and Chen, 1999, cited in Fathpour, 2003). Furthermore, Pomes *et al.* (2002) demonstrated that sensitization to cockroach allergens is associated with the development of asthma.

Aside from the reports on allergies, there is also strong evidence that cockroaches are involved in spreading bacteria, fungi, protozoa and helminthes that have public health significance (Pai *et al.*, 2003a). For the last 50 years, a number of studies were conducted to isolate and identify microorganisms of medical importance from cockroaches to ascertain their vector potential in the transmission of diseases. Most of the evidences on the vector potential of cockroaches come from one of two models of study: either (i) collecting cockroaches from a specific location and isolate pathogens, or (ii) experimental infection of cockroaches with pathogenic organisms and recovery of the pathogens. In both models, the results were determined by testing for the external and/or internal presence of the pathogenic organisms for possible connection between the positive recovery of a pathogenic organism and the cockroach's potential to act as a vector for human disease.

Under experimental condition, cockroaches have been shown to acquire, maintain, and excrete a number of viruses such as *poliomyelitis* viruses and *Hepatitis B*. However, the

natural transmission of viral diseases by the insects remains to be established (Cochram, 1982).

Cockroaches are also known to harbor naturally at least two important human fungal pathogens, namely *Aspergillus fumigatus* and *A. niger*, although there are indications that there are many more (Baumholtz *et al*, 1997).

Some protozoa have been either found or inoculated into cockroaches. In this line of investigation, *Entamoeba histolytica* / *E. dispar* cysts were found on the cuticle and/or in the gut of *P. americana* and *B. germanica*, both in the field survey and after experimental infections of the cockroaches with the cyst. These findings indicate that cockroaches harbor the *E. histolytica* / *E. dispar* cysts and play a role as potential mechanical disseminators (Pai *et al.*, 2003 a). The authors have also reported that cockroaches act as vectors of *coccidian* parasites such as *Toxoplasma gondii*.

Cockroaches have been shown to harbor a large number of helminthes such as *Ancylostoma duodenale*, *Ascaris lumricoides*, *Enterobius vermicularis*, *Trichuris trichria* (Burgess, 1993), *Suifunema caudelli*, *Galebia aegyptiaca*, and *Hammerschmidtella desingi* (Mimioglu and Sahin, 1976).

Bacteria are recognized as other organisms, that cockroaches can acquire and excrete. In this regard, more than 100 species of bacteria have been isolated from or passed through cockroaches (Cuden and Markovetz, 1987). It has also been shown that cockroaches harbor and transmit bacteria, both in nature and experimental conditions.

About 40 species of pathogenic bacteria, including at least 25 from the *Enterobacteriaceae* group, that cause gastroenteritis in many, and 20 other species of bacteria have been introduced into cockroaches in the hopes of finding a connection between cockroaches and human diseases (Ramirez, 1989).

In a study towards isolating and identifying pathogenic bacteria from cockroaches, Devi and Murray (1991) collected 221 cockroaches from hospitals, houses, animal sheds, grocery stores, and restaurants in various parts of the South East Coastal Regions of India and studied the presence of *Salmonella* in the insect. In the study, 4.1% of the cockroaches were positive for nine different strains of drug resistant *Salmonella* that were commonly isolated from humans and animals in this locality. According to the authors, isolation of *Salmonella* from cockroaches collected from the livestock premises and human dwellings suggested that they might act as significant reservoirs of *Salmonella* in nature. In addition, recovery of strains from the cockroaches that were common to humans and animals in the area suggested a transmission role for the cockroaches (Devi and Murray, 1991).

A study was conducted to isolate and identify microorganisms medically important from cockroaches and determine their vector role in the epidemiology of nosocomial infections. In this line of research, Fotedar *et al.* (1991a) isolated and compared the type and number of medically important bacteria from cockroaches collected from hospital and residential area. In the study, 158 out of 159 (99.4%) hospital cockroaches (test) and 113 out of 120 (94.2%) residential area cockroaches (control) were found carrying medically important microorganisms. Significantly, higher numbers of test cockroaches were carrying a higher bacterial load with multiple drug resistant strains as compared to

control cockroaches, suggesting their involvement in the transmission of drug resistant bacteria. Furthermore, various fungi and parasitic cysts of medical importance were also isolated from test and control cockroaches, signifying that cockroaches act as potential vectors of medically important bacteria, parasites and fungi (Fotedar *et al.*, 1991a) in nosocomial infection.

In another study conducted by Foedar *et al.* (1991b), the possibility that hospital cockroaches may act as vectors of drug resistant *Klebsiella spp.* was investigated in one of the Indian Hospitals. In the study, occurrence of *Klebsiella spp.* in wounded patients was compared with the incidence of the pathogens in the cockroaches of the same hospital. Accordingly, similar strains of *Klebsiella spp.* were isolated from 28.3% hospital cockroaches and 28.1% of wounded patients. Drug resistant patterns of *Klebsiella spp.* isolated from the hospital patients and the cockroaches also appeared in roughly equal percentage (96.3% and 85.9%, respectively). These results suggest that hospital cockroaches may act as vectors of drug resistant *Klebsiella spp.* and may contribute to the epidemiology of nosocomial infection (Foedar *et al.*, 1991b).

A study was also carried out to determine the vector role of hospital infesting cockroaches in an outbreak of nosocomial infection due to extended spectrum beta-lactamase-producing *K. pneumonia* in a neonatal unit in South Africa. The organisms isolated from the cockroaches were indistinguishable by pulsed-gel electrophoresis from those colonizing infants, suggesting that cockroaches are possible vectors of pathogenic bacteria in the hospital environment (Cotton *et al.*, 2000). About 56% prevalence of enterobacteria and an 18% prevalence of coagulase negative

staphylococci were found in cockroaches captured in a Brazilian hospital, out of which the most frequent enterobacteria were *K. pneumonia*, *Enterobacter aerogenes*, *Serratia marcescens*, *Hafnia alvei*, *Enterobacter gergovitae*, *Enterobacter cloacae*, and *Serratia spp.*, ranging from 17% to 60% (Prado *et al.*, 2002). Both enterobacteria and coagulase negative staphylococci showed significance resistance to antimicrobials, including oxacillin (Prado *et al.*, 2002).

Oothuman *et al.* (1989), have also isolated *Shigella boydii*, *S. dysenteriae*, *Salmonella typhimurium*, *K. oxytoca*, *K. oazaena* and *Serratia marcescens* from almost all 104 cockroaches collected from hospital kitchens in Malaysia. From 157 cockroaches collected from hospitals 56 species of bacteria were isolated, and 14 of them were pathogenic to man (Rivault *et al.*, 1993.).

A recent study conducted revealed that 70% of cockroaches collected from hospitals in Iran were found to be positive for *Salmonella spp.* and some of the isolates were resistant to antimicrobial drugs (Fathpour *et al.*, 2003), suggesting that cockroaches act as natural reservoirs of drug resistant *Salmonella*. The authors also showed that the inoculation of 10^6 CFU of *Salmonella* in to cockroaches via their food could infect the uncontaminated cockroaches. These contaminated cockroaches transfer infection to other colony members, and *Salmonella* was stable in cockroaches for more than 10 months.

Agbadaze and Owusu (1989) collected 208 cockroaches from kitchens in Acra and isolated *Salmonella* (6/208), *Shigella dysentriae*(1/208) and other *coliforms* (64/208). In

an experiment conducted by Umannabuike and Irokamulo (1986), 4 isolates of *Compylobacter jejuni* were found from a total of 690 cockroaches (*P. americana* and *B. orientalis*) collected from kitchens and poultry houses in Vom, Nigeria. All that points to the fact that these insects could play an important role in the transmission of these pathogenic organisms.

A qualitative study done in Bangladesh on *P. americana* revealed the presence of 31 species of bacteria belonging to 16 genera (Paul *et al.*, 1992), and most of them were *Salmonella*, *Shigella*, *S. aureus*, *B. cereus*, and *E. coli*. According to the authors, the highest prevalence of bacterial flora both in number and types occurred in stomach followed by intestine and the external body.

To assess their biological vector potential, cockroaches were fed with *Pseudomonas aeruginosa* in incremental doses of 10^2 , 10^5 , or 10^7 bacteria. In the cockroaches fed 10^5 and 10^7 doses, the *Pseudomonas* multiplied in the gut of the cockroach and there were detectable traces in the feces for up to 114 days. Because there is the possibility of the organism multiplying in the gut, the authors contended that cockroach could play a significant role in the epidemiology of *P. aeruginosa* infection (Fotedar *et al.*, 1993).

Blatta orientalis excreted viable tubercle bacilli after ingestion of heat-fixed sputum smears (Allen, 1987). However, there is no information on the dissemination of *Mycobacterium* through hospital cockroaches (Fotedar *et al.*, 1991a). Very recently, Fischer *et al.* (2003) demonstrated that orally infected nymphs of *B. germanica* could harbor and shed viable and virulent *Mycobacterium*. Pai *et al.* (2003b) have isolated 6

species of non-tuberculous mycobacteria from hospital cockroaches compared to none from household cockroaches suggesting the possible role of the insects in the epidemiology of the nosocomial infections due to non-tuberculous mycobacteria. The non-tuberculous mycobacteria have been recognized as important etiologic agents in hospital acquired infection (Phillips and Von Reyn, 2001).

Helicobacter pylori was successfully cultured from excreta of cockroaches fed with *H. Pylori* for 1 day after removal of the challenge (Imamura *et al.*, 2003), even though *H. pylori* culturability from contaminated materials is usually elusive (Hulten *et al.*, 1996). In contrast, *H. pylori* was not recovered from the external surfaces of the cockroaches. These results suggest that cockroaches are potential vector for *H. pylori* infection and that the transmission route is likely via their excretion of *H. Pylori* (Imamura, *et al.*, 2003).

A laboratory study showed that the pathogenic serotype O119 *E. coli* taken up by *B. orientalis* continued to be passed out in the feces for 20 days (Burgess *et al.*, 1973).

There are many diseases caused by pathogenic bacteria that have been found naturally in or on to cockroaches. The diseases are both general and specific infections such as bubonic plague (*Yersinia pestis*), dysentery (*Shigella alkalenscens*), diarrhea (*Shigella para dysenteriae*), urinary tract infection (*Pseudomonas aeruginosa*), abscesses (*S. aureus*), food poisonings (*Clostridium perfringens*, *Escherchia coli*, *Streptococcus faecalis*, *P. aeruginosa*), gastroenteritis (*Salmonella schotmuelleri* *S. bredentyphosa*),

leprosy (*Mycobacterium leprae*), and nocardiosis (*Actinomyces spp.*) (Baumholtz *et al.*, 1997).

The disease caused by the pathogenic bacteria experimentally introduced either in to or on to cockroaches include Asiatic cholera, pneumonia, diphtheria (*Cornybacterium diphtheria*), gladders (*Pseudomonas malle*), anthrax (*Bacillus anthracis*) black leg (*Clostridium chauvoei*), tetanus (*Clostridium tetani*) and tuberculosis (*Mycobacterium spp*) (Baumholtz *et al.*, 1997).

Most of the common food-borne bacteria are believed to have multiple drug resistance to a number of antimicrobials (Gedebu *et al.*, 1987;Geyid and Lemeneh1991; Fotedar *et al.*, 1991b; Devi and Murray, 1991; Mache *et al.*, 1997A; Mache *et al.*, 1997B, Assefa *et al.*, 1997; Molla *et al.*, 1999; Mengistu *et al.*, 1999; Fathpour *et al.*, 2003). It is well known that these disease-causing bacteria are wide spread in Ethiopia (Dagnew *et al.*, 1995).

The evidence for cockroaches acting as vectors for bacterial disease transmission remains circumstantial. Although there is a great deal of material demonstrating the cockroaches' ability to pick up and later excrete pathogens over the last 50 years, there is not yet proof that the cockroach is a vector for human disease (Davis and Breslin 2003). Without question it is important to continue to understand the cockroach's possible role as a vector and, concomitantly, to learn more effective ways to control or eliminate the cockroach, especially in places such as hospitals and food service areas.

2.4. Common Food borne bacterial pathogens

2.4.1. *Salmonella*

The genus *Salmonella* is Gram-negative bacillus that generally is facultatively anaerobic, non-sporulating, non-capsulate, motile, oxidase negative, aerogenic, non-lactose fermenting, urease-negative, citrate utilizing and KCN-negative in the family of Enterobacteriaceae (Sleigh and Duguid, 1989a).

According to WHO (1988), compared with other Gram-negative rods, *Salmonella* is relatively resistant to various environmental factors. *Salmonella* grows at temperatures between 8 and 45⁰C, at water activities above 0.94 and in a pH range of 4 to 8. *Salmonella* is also able to multiply in an environment with a low level or no oxygen. However, the bacterium is sensitive to heat and will not survive temperatures above 70⁰C, and even more sensitive to gamma and beta rays. *Salmonella* has been shown to be resistant to drying even for years, especially in dry feces, dust, and other dry materials such as food. Furthermore, several months of survival has been reported in 20% salt, especially in commodities with a high protein or fat content such as certain sausages (WHO, 1988).

Salmonella is one of the leading causes of bacterial gastroenteritis (Darwin and Miller, 1999), and it is capable of causing a variety of disease syndromes: enteric fever (typhoid fever), bacteremia, enterocolitis and focal infections. Enterocolitis is, by far, the most common manifestation of disease caused by *Salmonella* but bacteremia and focal

infections can accompany or follow enterocolitis. Enteric fever is caused primarily by *S. typhi* and *S. paratyphi* and occasionally by other serotypes. Although approximately 2,000 serotypes of *Salmonella* have been associated with enterocolitis, *S. typhimurium*, *S. enteritidis* and *S. heidelberg* are incriminated for the majority of the infection (CDC, 1994). The incubation period is typically 6 to 48 hours, and is followed by headache, abdominal pain, diarrhea, and vomiting. The diarrhea can contain blood lymphocyte and mucus. Fever, malaise and muscle aches are quite common symptoms usually resolved within a week but *Salmonella* can be shed in feces for up to 20 weeks by children under 5 Years of age and for 8 weeks by adults (Darwin and Miller 1999).

It is estimated from volunteer studies that 10^5 to 10^{10} bacteria are required to initiate an infection (Glaser and Newman, 1982), but the exact amount needed varies with the strain, what is consumed, and physiological state of the host (Darwin and Miller, 1999). For instance, infective dose is found to be about 50 organisms per gram for *S. napoli* in chocolate and less than 10 organisms per gram for *S. typhimurium* in cheddar cheeses (WHO, 1988). It is generally believed that large inoculum is required to overcome the stomach acidity and to compete with the normal flora of the intestinal tract. The infection dose decreases where *Salmonella* is consumed with foods that traverse the stomach rapidly (i.e. liquids) or with food that neutralizes the stomach acidity (i.e. cheese, milk) (Taux and Pavia, 1998 cited in Darwin and Miller, 1999). Individuals with high gastric pH, such as the elderly, are more susceptible to infection (Darwin and Miller 1999). It has also been shown that pretreatment of mice with streptomycin, which reduces the amount of the normal flora, decreases the dose of *Salmonella* required to infect 50% of

the mice (Bohnhoff *et al.*, 1964). Similar effects of antibiotic treatment have been observed in humans (Ryan *et al.*, 1987).

According to Centers for Disease Controls and Prevention (CDC 1991), the incidence of salmonellosis in the united state has steadily increased since World War II. The reasons for this include an increase in the proportion of the population older than 60 years, changing agricultural and food distribution methods, increased consumption of raw or slightly cooked foods, an increase in the number of immunocompromised or chronically ill people, and deterioration of the public health infrastructure. Transmission of *Salmonella* to humans is usually by consumption of contaminated food but human-to-human transmission and direct animal to human transmission can occur. However, the most common sources of *Salmonella* are beef, poultry, and eggs (Darwin and Miller 1999). Studies conducted around the world have also shown that cockroaches can directly involve in the transmission of *Salmonella* via contaminating human food (Oothuman *et al.*, 1989; Paul *et al.*, 1992; Fathpouer *et al.*, 2003).

2.4.2. *Shigella*:

The genus *Shigella* consists of four serogroups: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). *Shigella* species are Gram negative, facultatively anaerobic, non-sporulating, non-motile rods in the family *Enterobacteriaceae*. They do not decarboxylate lysine or ferment lactose within two days. They utilize glucose and other carbohydrates, producing acid but not gas. Neither citrate nor malonate is used as the sole carbon source for growth, and they are inhibited

by KCN (Sleigh and Duguid 1989 b). *Shigella* causes bloody diarrhea (dysentery) and non-bloody diarrhea. Shigellosis often begins with watery diarrhea accompanied by fever and abdominal cramps but may progress to classic dysentery with scant stools containing blood, mucus, and pus (Farmer III, 1999). All four species of *Shigella* are capable of causing dysentery, but *S. dysenteriae* 1 has been associated with a particularly severe form of illness related to its production of shiga toxin (Farmer III, 1999). In developing countries, the most prevalent serogroups are *S. flexneri* and *S. dysenteriae*, with the latter being the most frequent cause of epidemic dysentery (Farmer III, 1999).

Shigella infections are responsible for an estimated 300,000 illness and 600 deaths per year in the United States, and more than 600,000 deaths per year worldwide (Baer et al. 1999). *Shigella* species are typically transmitted by direct or indirect fecal-oral contact. As a result, shigellosis has been associated with outbreaks in day-care centers, nursing homes, and institutionalized populations (Baer et al., 1999). Shigellosis is an important public health problem, especially in developing countries where hygienic practices are very poor. Annual incidence rates of 8.9 per 100,000 have been reported through active surveillance in food Net in 1996, next to *Campylobacter* (23.5) and *Salmonella* (14.5) (Tauxe et al., 2000).

Shigellosis, although commonly regarded as water-borne, is also a food-borne disease restricted primarily to higher primates, including humans (Andrews and Jacobson, 1998). Food handlers with poor personal hygiene usually spread it among humans. Different *Shigella spp.* have been isolated from cockroaches suggesting the possible

role of the insects in disseminating the bacterium (Oothman, *et al.*, 1989; Paul, *et al.*, 1992; Agbodaze and Owusu, 1989).

2.4.3. Enterohemorrhagic *Escherichia coli* (*E.coli* O157:H7)

Escherichia coli O157:H7 is so-named because it expresses the 157th somatic (O) antigen identified and the 7th flagellar (H) antigen. The recognition of EHEC as distinct class of pathogenic *E. coli* resulted from two outbreaks. The first was the 1983 report by Riley *et al.* (1983), and the second was by Karamoli *et al.* (1983). Riley *et al.* (1983) reported a distinctive gastrointestinal illness characterized by severe cramping, abdominal pain, watery diarrhea followed by glossy bloody diarrhea and little or no fever. This illness designated hemorrhagic colitis (HC), was associated with the ingestion of undercooked hamburger at a fast food restaurant. Stool cultures from these patients were positive for a rarely isolated *E. coli* O157:H7.

The association of sporadic cases of hemolytic uremic syndrome (HUS) with fecal cytotoxin and cytotoxin-producing *E. coli* in stools was observed (Karamoli *et al.*, 1983). HUS is a combination of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure that is preceded typically by bloody diarrhea indistinguishable from HC (Nataro and Kaper, 1998). Thus, two key clinical and microbiological observations, one based on a rare *E. coli* serotype and the other based on production of a specific cytotoxin, led to the recognition of a novel and increasingly important class of enteric pathogens causing intestinal and renal disease. The bacteria grouped under enterohemorrhagic *E. coli* are strains that cause HC and HUS, express shiga-like toxin

(Stx) cause A/E lesion on epithelial cells, and possess a Ca. 60 MDa plasmid such as *E. coli* O157:H7 (Natar and Kaper, 1998).

Shigatoxins produced by *E. coli* O157:H7 are the principal virulence factors responsible for HC and HUS in humans (Galland *et al.*, 2001). *E. coli* O157: H7 is a dominant shiga toxin producing (STEC) stereotype in many parts of the world and historically has been the type most commonly associated with large out breaks (Paton and Paton, 1998).

E.coli O157: H7 or H7⁺ strains are regularly reported in the world literature associated with cases and outbreaks of diarrhea, HC, HUS and related conditions (Bettelhiem, 2001).

According to a worldwide systemic review study conducted by Garge *et al.* (2003) death or end-stage renal disease (ESRD) occurs in about 12 % patients 4 years after diarrhea-associated HUS, and 25 % survivors demonstrate long-term renal sequelae caused by *E.coli* O157 infection.

The salient features of EHEC epidemiology include a reservoir in the intestinal tract of cattle and other animals; transmission by a wide variety of food items, with beef being a major vehicle of infection; and a very low infectious dose (10 to 100 organisms) enabling high rates of attack and person-to-person transmission (Kolling and Matthews, 2001).

CDC estimates the annual disease burden of *E.coli O157: H7* in the United States to be more than 20,000 infections and as many as 250 deaths (Boyle *et al.*, 1995), but the failure of many clinical laboratories to screen for this pathogen greatly complicates any estimate. In some areas, *E. coli O157:H7* is more frequently isolated from routine stool specimens than are *Shigella spp* and it is the second or third most frequently isolated organism after *Campylobacter* and/or *Salmonella spp.* (Nataro and Kaper, 1998), and accounts 50 to 80% of all EHEC infections.

Human infection with *E. coli O157* has been reported from over 30 countries on six continents. Annual incidence rates of 8 per 100,000 population or greater have been reported in regions of Scotland, Canada and the USA (Mead and Griffin, 1998). Infection with *E.coli O157* is more common in the warm summer months in both northern and southern hemispheres (Mead and Griffin, 1998), and shedding by animals also seasonal.

A large outbreak of bloody diarrhea caused by *E.coli O157* infections occurred in Southern Africa in 1992, after an isolation of *E.coli O157* from an elderly man undergoing surgery for lower gastrointestinal bleeding in Johannesburg in 1990(Effler *et al.*, 2001). In addition, investigators in Malawi and Angola reported outbreaks of diarrheal illness caused by *E. coli O157* (Effler *et al.*, 2001). Therefore, outbreak of *E. coli O157* infections could be a potential danger for the developing world including Ethiopia. According to Effler *et al.*,(2001), draught, carriage of *E.coli O157* by cattle, and heavy rains with contamination of surface water appear to be important factors

contributing to the Southern African outbreak. The O-serotype predominantly associated with human infections in Great Britain is *E. coli* O157 and isolations have almost doubled in the last 6 years (Stevens *et al.*, 2002).

Healthy cattle are a major reservoir for human infection with *E.coli* O157 (Griffin and Tauxe, 1991); however, it is not known why the bacterium cannot cause disease in cattle (Effler *et al.*, 2001). Environmental studies have shown that the organism can persist in manure, water troughs, and other places on farms (Mead and Griffin, 1998). In addition to cattle, the organism has been isolated from deer, sheep, goats, horses, dogs, birds and flies (Mead and Griffin, 1998). *E. coli* O157 is transmitted by food and water, directly from one person to another, and occasionally through occupation exposure (Nataro and Kaper, 1998).

Because of their ecological niche, it has been suggested that cockroaches play a role in the transmission of disease by harboring and disseminating pathogenic organisms (Oothuman *et al.*, 1989). Their omnivorous habits of feeding and indiscriminate deposition of fecal material make them ideal agents for the transmission of microorganisms (Fotedar *et al.*, 1993). The alimentary tract of cockroaches serves as a reservoir for multiplication of certain pathogens and thus subsequently excretion in their droppings (Fotedar *et al.*, 1993). As *E.coli* O157 is wide spread in the environment (Mead and Griffin, 1998), there is ample opportunity for the insect to become contaminated with it and in turn to contaminate the human environment.

2.4.4. Staphylococcus aureus

Staphylococcus aureus is a facultative anaerobic Gram positive coccus. It is non-motile and catalase and coagulase positive (Collins *et al.*, 1991). Cells are spherical, single or paired cocci, or form grape-like clusters. The staphylococcal cell wall is resistant to lysosome and sensitive to lysotaphin, which specifically cleaves the petaglycine bridges of *Staphylococcus spp.*(Loir *et al.*, 2003). Some *S. aureus* strains are able to produce staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisonings (Loir *et al.*, 2003).

Staphylococcus aureus is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance (Loir *et al.*, 2003). The bacterium is one of the most common causes of nosocomial and community-acquired infection (Smith *et al.*, 1999). According to Gillet *et al.*, (2002), panton-valentine leukocidin (PVL) producing *S. aureus* strains caused rapidly progressive haemorrhagic, necrotising pneumonia in young immunocompetent people, with high lethality rate. The spectrum of staphylococcal infections ranges from pimples and furuncles to toxic shock syndrome and sepsis, most of which depend on numerous virulence factors. On the other hand, some infections, such as staphylococcal poisoning, rely on one single type of virulence factors, staphylococcal enterotoxins (SEs). The symptoms of staphylococcal poisoning are abdominal cramps, nausea, vomiting, sometimes followed by diarrhea. The onset of symptoms is rapid (from 30 min to 8 hr) and usually spontaneous reduction is observed after 24 h (Loir *et al.*, 2003).

Since *S. aureus* does not form spores, its contamination can be readily avoided by heat treatment of food. Nevertheless, it remains a major cause of FBD because it can contaminate food products during preparation and processing. *S. aureus* is indeed found in nostrils, and on skin and hair of warm-blooded animals. Up to 30 to 50% the human population are carriers. It is able to grow at a wide range of temperatures (7 °C to 48.5 °C with an optimum of 30 to 37 °C), pH (4.2 to 9.3 with an optimum of 7 to 7.5), and sodium chloride concentrations (up to 15% NaCl) (Loir *et al.*, 2003). These characteristics enable *S. aureus* to grow in wide variety of foods. This, plus their ecological niche, can easily explain their incidence in foodstuffs that require manipulation during processing, including fermented food products, such as cheese.

Although warm-blooded animals are believed to be the major reservoir of *Staphylococcus spp.* (Loir *et al.*, 2003), they were also isolated both from the gut and body surface of cockroaches collected from hospitals and residents in different parts of the world in an attempt to establish the vector role of the insect in the transmission of the pathogen (Paul *et al.*, 1992, Oothuman, *et al.*, 1989; Burgess, 1984; LeGuyader *et al.*, 1989; Prado *et al.*, 2002).

2.4.5. *Bacillus cereus*

The genus *Bacillus* comprises the Gram-positive rods that grow aerobically and form heat-resistant spores, and contains two medically important species: *B. anthracis* and *B. cereus*. These species are distinguished from other species because they form a

phospholipase that splits lecithin in egg yolk; their colonies on the egg yolk become surrounded by a circular zone of opacity in 1-5 days at 37 °C (Green,1989). The activity of *B. anthracis* on the egg yolk is much weaker than that of *B. cereus*. In addition, *B. anthracis* is the only non-motile species in the genus (Green, 1989).

Bacillus cereus spores occur in soil, air, vegetables, and in many raw and processed foods. Consumption of foods that contain $>10^6$ *B. cereus* / g may result in food poisoning as a result of enterotoxins produced by most strains of *B. cereus* (Doyle, 2001). Two types of food-poisoning syndromes have been recognized as a result of consumption of *B. cereus* contaminated foods. The first is the diarrheal type, caused by a heat-labile enterotoxic complex, and characterized by abdominal pain with diarrhea 8 to 16 h after ingestion of the contaminated food (Lagan and Turnbull, 1999). The second is the emetic type, caused by heat-stable enterotoxins, and characterized by nausea and vomiting 1 to 5 h after eating offending foods (Lagan and Turnbull, 1999). A survey of ready-to-serve moist foods in USA showed that *B. cereus* spores were detected in 80-100% of noodles, mashed potatoes and rice, in 50-83% of cooked vegetables, and in 25-75% of gravies sampled (Doyle, 2001).

Cooking destroys vegetative bacteria, but spores of food borne bacterial pathogens such as *B. cereus* are very resistant. Heat resistance of spores is decreased by acidity and is increased by low water activity (e.g. High salt concentration) and gradual heating (Doyle, 2001). Enterotoxic activity appears to be distinct from the hemolysin and phospholipase produced by *B. cereus* and can elicit accumulation of fluid in ligated rabbit ileac loops (Williams, 1986).

There are different reports indicating that cockroaches could harbor *B. cereus* (Paul *et al.*, 1992, and Oothman, 1989). As *B. cereus* is ubiquitous in the environment there is an opportunity for cockroaches to become contaminated and in turn contaminate the human environment and foods.

3. OBJECTIVES OF THE STUDY

3.1 GENERAL OBJECTIVE:

To isolate and identify food-borne bacterial pathogens from cockroaches captured from hospitals and restaurants and to determine the vectorial role of the prevalent cockroach species.

3.2. SPECIFIC OBJECTIVES:

1. To identify the prevalent cockroach species in selected hospitals and restaurants from Addis Ababa.
2. To isolate and identify the following important bacterial pathogens from the collected cockroaches: *Salmonella* spp., *Shigella* spp., *S. aureus*, *E. coli* O157:H7 and *B. cereus*;
3. To evaluate the survival and excretion rate of some bacterial isolates in experimentally infected cockroaches ;
4. To assess drug-sensitivity pattern of the isolated strains against selected antibacterial drugs;

4. Materials and Methods

4.1. Sampling Areas

Four Hospitals (Tekur Anbesa, Zewditu Memorial, Yekatit 12, and Minilik II) and four catering centers in Aware and Kazanchis areas in Addis Ababa were considered in this study. In each hospital different wards, staff duty rooms, and food processing centers were used as specific sampling sites. Where as coffee/tea machine, kitchens, food and drink service cabinet were used in the restaurants. Samples of cockroaches were collected from all study sites once a week for twenty weeks (December 2002 – June 2003).

Collection, Processing and Identification of Cockroaches

4. 2.1. Collection of Specimens

Cockroaches were collected using sterilized screw-capped 250 ml jars and sterile hand-gloves (Paul *et al.*, 1992). Each time 10 cockroaches were caught and pulled as one sample from each of the eight sampling areas. Only cockroaches caught whole and alive were considered in the study.

4.2.2. Identification of cockroaches

Identification of cockroaches to species was done based on keys provided by Burgess (1993).

4.2.3. Processing of specimens

The collected cockroaches were brought to the laboratory in pull of ten and killed in a sterile jar using chloroform soaked cotton. Then, the external body surface was washed by vortexing in 5 ml sterile normal saline for two minutes, and the wash taken as external body homogenate sample. After the external body washing, the cockroaches

were soaked in 90% ethanol for 5 minutes to decontaminate the external surfaces and were dried. The cockroaches 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 sterilized with ethanol and flamed between dissections. The excised gut was then homogenized in sterile petridish containing 5 ml of sterile normal saline water. A small portion of the external surface homogenate and the gut emulsion were transferred separately in to appropriate enrichment and growth media for isolation and identification of the target bacteria.

4.3. Isolation of Bacteria

Primary Enrichment

A total of 320 specimens consisting of 160 external body surface and 160 internal (gut) homogenates of the cockroaches were analyzed. For primary enrichment 1 ml of each homogenate was inoculated separately in to 9 ml of buffered peptone water (BPW) (OXOID) and incubated at 37⁰ C for 18-24hrs.

Isolation of *Salmonella* and *Shigella*

A volume 0.1ml of growth from BPW was inoculated in to 10 ml of Rappaport-Vassilidias(RV) broth (OXOID) and incubated at 42⁰ C for 24-48hrs for secondary enrichment. Then, it was streaked on Xylose Lysine Deoxycholate (XLD) agar (OXDID). After 18-24 hrs of incubation at 37⁰ C, *Salmonella* and *Shigella* were distinguished by their characteristic appearance on the XLD Agar: *Salmonella* has pink colonies with or

without black centers or glossy black centers or completely black colonies, where as *Shigella* has red colonies (Collins *et al.*, 1991)) and these were picked for further identification.

Isolation of *Escherichia coli* O157:H7

A loopful of overnight growth from BPW was streaked on Sorbitol MacConkey Agar (SMAC) (OXOID) and incubated at 37 °C for 18 -24 hrs. After 18 -24 hrs incubation, non-sorbitol fermenting presumptive *E. coli* colonies were characterized as colorless or neutral/gray with a smoky center and 1-2 mm in diameter, and these were picked for further biochemical and serological tests.

Isolation of *Staphylococcus aureus*

Growth from BPW was heavily plated on Mannitol Salt Agar (OXOID) and incubated at 37 °C for 48 hrs. Mannitol fermenting (golden colony color) and cluster-forming gram-positive cocci were further characterized using biochemical and enzyme tests.

Isolation of *Bacillus cereus*

After BPW culture was heat treated for 10 minutes at 70°C in a water bath (to kill mesophilic vegetative bacteria), a loopful of growth was streaked on *Bacillus cereus* Selective Medium and incubated at 37°C for 18-24 hrs. Lecithinase positive (pink colonies surrounded by precipitate zone) were picked and further characterized by morphology, gram reaction, and catalase and spore tests.

4.4 Identification of Bacteria

Identification was done using standard biochemical testes described by (Farmer III, 1999).

***Salmonella* Identification: Biochemical and serological Identification**

Growth from a single colony of presumptive *Salmonella* was seeded in to differential tubed media. Triple sugar Iron Agar (TSA), Lysine Iron Agar (LIA), urea Agar, citrate, motility medium, Kovac's reagent, malonate, mannitol and glucose broths were used for biochemical identification of *Salmonella*. The presumptive *Salmonella* isolates were confirmed as Gram negative, motile rods, glucose fermenting, Lysine decarboxylase positive, H₂S positive (TSI & LIA), urease negative, indole negative , citrate positive and malonate negative. Following biochemical tests, *Salmonella* grouping antisera were employed as an aid in the detailed identification of the genus *Salmonella* (BBL, Salmonella Grouping Antisera).

***Shigella* identification: Biochemical and serological Identification**

Colonies of isolated presumptive *Shigella* were inoculated into Triple sugar Iron Agar (TSA), Lysine Iron Agar (LIA), urea Agar, citrate, motility medium, Kovac's reagent, malonate, manitol and glucose broth were used for biochemical identification of *Shigella*. The isolates were identified as Gram-negative rods, negative for H₂S, urease, gas (glucose), motility, lysine decarboxylase, malonate and citrate. Cultures that yield

biochemical reactions typical of *Shigella* were tested with BBL *Shigella* grouping antisera to classify the genus in to serogroups.

***Escherichia coli* O157:H7 Identification: Biochemical and serological Identification**

Non-sorbitol fermenting suspicious *E. coli* isolates were subjected to Triple sugar Iron Agar (TSA), Lysine Iron Agar (LIA), urea Agar, citrate, motility medium, Kovac's reagent, malonate, mannitol and glucose broths as a biochemical confirmation of *E. coli*. The isolates were identified as Gram-negative rods, lactose fermenting, Lysine decarboxylase positive, urease negative, citrate negative, motile, indole positive, H₂S negative and mannitol positive. Dry spot *E.coli* O157 latex agglutination test (OXOID) and Bacto *E.coli* H7 antiserum H7 were employed for confirmatory identification of *E.coli* O157:H7.

***Staphylococcus aureus* identification**

Microscopic examination was done to study cell shape and arrangement of the isolates. Potassium hydroxide (KOH) solution was used for determination of Gram reaction of the isolates. Similarly, 3% hydrogen peroxide (H₂O₂) was used for catalase test for staphylococci isolates. For biochemical characterization Baird-Parker (1966 cited in Collins *et al.*, 1991) modification of Hugh-Liefson's medium was used for oxidative-fermentative (OF) test. Further conformation of *S. aureus* was done using DNAase and coagulase tests (Collins *et al.*, 1991).

4.5. Survival and Excretion of Pathogenic Bacteria from Experimentally Infected cockroaches.

4.5.1. Screening of cockroaches that were free from the test bacterium

The cockroach species used for the challenge experiment was *B. germanica* collected from the same study sites considered in this work. Each cockroach was transferred to a sterile test tube containing sterile food. Fecal pellets were collected for isolation and presumptive identification of the test pathogens to make sure that cockroaches were not previously contaminated with *Salmonella*, *Shigella* or *S. aureus*. Isolation and biochemical identification of the target pathogenic bacteria was done as in 4.3 and 4.4. Each cockroach was checked three times every 2-3 days 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).

4.5.2. Exposing cockroaches to the test pathogen

Salmonella group B, *Shigella* group B, and *S. aureus*, which had been isolated from the cockroaches collected from hospitals and restaurants, were used as test pathogens. A single colony of each test bacterium was added to 10 ml of Tryptic Soy Broth (OXOID), and incubated at 37°C for 36 hrs. The resulting growth was diluted serially in BPW and 10⁶ cells/ml were fed to the insects (Badillo-vargas and Holbrook, 2002) for an hour.

Four uncontaminated and starved cockroaches were transferred aseptically to a 100 ml test tube containing 1g of food (a mixture of milk, wheat powder and sucrose) contaminated with 0.1 ml of 10^6 CFU test bacterium, and allowed to feed on for one hour. Another group of four uncontaminated cockroaches were transferred to a similar test tube but containing 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, hanged over in a sterile 250 ml jar and kept at room temperature for 24 hr. The exposed portion of the mesh was rapped with sterile aluminum foil to prevent contamination from external source. The set up allowed fecal pellets to be collected from the bottom of the jar passed through the mesh with out cockroaches' external body contamination. 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. Then, every 2-3 days, the cockroaches were transferred to new sterile containers containing sterile food. The excreted bacteria in the former jar were identified. The experiments were extended until the excretion of the bacteria in question ceased or, if excretion continued, until all test cockroaches were dead for a maximum period of 40 days.

4.5.3. Recovering of the test pathogens from experimentally infected

Cockroaches

In order to determine whether cockroaches were carrying challenged bacteria (*Salmonella B*, *Shigella B*, or *S. aureus*), their feces were cultured on the respective selective growth medium. The feces were picked over 48 – 72 hr period, using sterile forceps, 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*, a volume of 0.1 ml of overnight broth aliquots was dispensed into RV broth. After 24-48 hr incubation at 42 °C, enriched culture was streaked on XLD agar (Badillo-Vargas and Holbrook, 2002). The feces of control insects not exposed to the test pathogens were set up and analyzed in the same way. Isolation of *Salmonella and Shigella* was also attempted by direct plating on XLD without RV broth enrichment. All confirmatory biochemical and serological tests were done on the isolates as in 4.3 and 4.4.

4.6. In vitro Drug susceptibility Testing

Single disc diffusion susceptibility test, also termed as Kirby-Bauer method, was employed (Bauer *et al.*, 1966) to determine the antimicrobial susceptibility patterns of bacteria isolated from cockroaches collected from the study sites. The isolates were subjected to the following Oxoid drug discs of the indicated concentrations: Ampicillin (30µg), Sulfamethoxazole (25 µg), polymyxin B (30 µg), Carbenicillin (10µg), Cephalothin (30 µg), Chloramphenicol (30 µg), Gentamycin (10 µg), Kanamycin (30

µg), Streptomycin (10 µg), Tetracycline (30 µg), Augmentin (30 µg), Clindamycin (2 µg), Oxacillin (5 µg), Erythromycin (15 µg), Penicillin-G (10 µg), Vancomycin (30 µg), and Bactroban (5 µg).

Briefly 4-5 discrete colonies representative of the pure test organisms were inoculated in to 4-5 ml of Tryptic Soy Broth and adjusted to Mc Farland barium sulfate standard to bring to cell density of approximately $10^7 - 10^8$ CFU /ml .Mc Farland turbidity standard was prepared by mixing 0.1 ml BaCl₂ (1%) with 9.9 ml H₂SO₄ (1%) (Jorgenson *et al.*, 1999). Muller Hinton (MH) (OXOID) plates were prepared and warmed to room temperature for plating. A sterile cotton swab was dipped in to the standardized suspension of TSB and swabbed on to the surface of MH agar plates. After swabbing, the MH agar plates were allowed to dry to 3-5 minutes. Thereafter, the discs of the aforementioned antibiotics were put on to the agar plate using disc dispenser and incubated at 35 °C for 24 hours.

The zone of inhibition diameter of the antibiotic disc was measured by using metal sliding caliper. The measured figures were translated in to descriptive terms as susceptible (S), intermediate (I) or Resistant (R) according to cut-off points given by National Committee for Clinical Laboratory Standard (NCCLS, 1998 cited in Jorgenson *et al.*, 1999). The intermediate strains were included with the sensitive strains for convenience since they were relatively few. For quality control, reference strains *S. aureus* ATCC 6538 for gram positive and *E. coli* ATCC 25922 for gram negative ones were used.

5. Result

5.1 Identification of cockroaches

In this study, 1600 cockroaches were collected from 4 hospitals and 4 restaurants in Addis Ababa from December 2002 to June 2003. All cockroaches were identified as *Blattella germanica* using identification keys of Burgess (1993).

5.2 Isolation and identification of *Salmonella*, *Shigella*, *S. aureus*, *E. coli*

O157:H7* and *B. cereus

A total of 12 *Salmonella* spp. 17 *S. aureus* , 2 *E. coli* O157 , 2 *Shigella* spp and 25 *B. cereus* were isolated and identified from the total of 320 samples (160 gut and 160 external body homogenates) of 10 cockroaches pulled as one sample collected from the study sites considered in this work (Table 1)

Table 1. Type and number of bacteria isolated from gut and external body surfaces of cockroaches collected from hospitals and restaurants

Bacteria isolates	Types of specimen		Cockroach collection sites		Total
	Gut	External Surface	Hospitals	Restaurants	
<i>Salmonella spp.</i>	10	2	11	1	12
<i>S. aureus</i>	10	7	10	7	17
<i>E. coli O157</i>	2	-	2	-	2
<i>Shigella B</i>	1	1	-	2	2
<i>B. cereus</i>	11	14	10	15	25

5.2.1. *Salmonella* isolation and Identification

Using biochemical and serological tests, the isolates were classified as *Salmonella B* (4/12), *Salmonella D* (4/12), and *Salmonella E* (4/12), according to their somatic (O) antigens. From the total of 12 *Salmonella* isolates 10 were isolated from gut homogenates and 2 were from external body surface homogenates of the cockroaches. Based on the study site 11 *Salmonella* isolates were from hospital and 1 from restaurant cockroaches (Table 1).

5.2.2. *Shigella* Isolation and Identification

Suspected *Shigella* colonies from XLD agar plates were identified biochemically and using polyvalent antiserum. Two *Shigella* isolates were identified as *Shigella B* (BBL *Shigella* polyvalent antisera). The isolates were one from the external body surface homogenate and the other from the gut homogenate of the cockroaches each one from hospital and restaurant samples (Table 1).

5.2.3. *Escherichia coli* O157 Isolation and Identification

Thirteen Non-Sorbitol fermenting isolates from SMAC were identified as *E. coli* using biochemical tests. The isolates identified as *E. coli*, were subjected to the Oxoid Dry spot *E. coli* O157 latex agglutination test and H7 antiserum. Two of them were found positive for *E. coli* serogroup O157 but negative for H7 antiserum. Both of them were from the gut homogenates of the cockroaches collected from the hospital study sites (Table 1).

5.2.4. *Staphylococcus aureus* Isolation and Identification

Based on morphological and biochemical characters, 17 *S. aureus* isolates were identified. Of the total isolates, 10 were from external homogenates and 7 were from gut homogenates of the cockroaches. According to the study sites, 10 isolates were from hospital and 7 were from restaurant cockroaches (Table 1)

5.2.5. *Bacillus cereus* Isolation and Identification

Pink Lecithinase positive colonies from *Bacillus cereus* selective medium were picked and characterized as gram-positive rods, catalase and spore positive. A total of 25 *B. cereus* isolates were identified, 10 from hospital and 15 from restaurant specimens; and 11 were isolated from gut and 14 were from body surface homogenates of the cockroaches (Table 1)

5.3. Survival and Excretion of Bacteria in Experimentally Infected

B. germanica

5.3.1. Survival of *Salmonella B* in and its excretion by *B. germanica*

following ingestion of 10^6 CFU/g contaminated food

Every 2-3 days both test and negative control groups of cockroaches' excreta were analyzed for detection of *Salmonella B*. In the challenge group, all excreta samples were positive for salmonella for 35 days, after which all cockroaches were dead. The carcass (gut) of the challenge group was also found positive for *Salmonella B*. All positive samples obtained were from RV enriched cultures streaked on XLD plates. All direct streaks on XLD from an overnight broth failed to yield *Salmonella* throughout the study period except the first day fecal sample (Table 2). All excreta samples from negative control group were found to be negative both with and without RV enrichment throughout the study period.

Table 2. Survival and excretion of *Salmonella B* by *B. germanica* following ingestion of 10⁶ CFU/g contaminated food

Excreta sampling time	Direct streaked on XLD (without RV-enrichment)	RV-enriched (Streaked on XLD)
2 days	++	++++
5 days	-	++++
8 days	-	++++
11 days	-	++++
14 days	-	+++
17 days	-	+++
20 days	-	+++
23 days	-	++
26 days	-	++
29 days	-	+
32 days	-	+
35 days	-	+

Key: ++++ too many; +++ many; ++ few; +vary few; - negative

5.3.2. Survival and excretion of *Shigella B* by *B. germanica* following ingestion of 10⁶ CFU/g contaminated food

Every 2-3 days excreta from both test and negative control cockroaches were analyzed for the presence or absence of *Shigella B*. Culture examination of fecal pellets of *B. germanica* after exposure to *Shigella B* contaminated food failed to yield the bacterium on XLD agar plates both with and without RV enrichment. The experiment was extended for 30 days. By then, all challenged cockroaches were found dead. However, it was possible to recover *Shigella B* on XLD agar plates, after direct and RV enrichment, from the contaminated feed. Finally, the carcass of the cockroaches were examined for the presence of *Shigella B* and found to be negative on XLD plates both after primary and RV enrichment. The negative control group was treated the same way and found negative for the bacterium.

5.3.3. Survival and excretion of *S. aureus B* by *B. germanica* following ingestion of 10⁶ CFU/g contaminated food.

In the challenge group, culture examinations of fecal samples on MSA were positive for *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* (Table3).

Table 3. Survival and excretion of *S. aureus* B by *B. germanica* ingestion of 10⁶ CFU/g contaminated food

Sampling Time	Streaked on MSA	Coagulase Test
2 days	+++	+
5 days	+++	+
8 days	++	+
11 days	++	+
14 days	++	+

Key: +++ many; ++ few; + vary few; - negative

Gut of dead bodies of the test cockroaches was positive for *S. aureus* within a day of their death. All MSA positive colonies were subjected to catalase, OF, and coagulase tests and found to be typical of *S. aureus*.

5.4. In Vitro Drug Susceptibility Test

A total of 12 *Salmonella*, 2 *E. coli* O157, 2 *Shigella*, 17 *S. aureus* and 25 *B. cereus* isolates were tested for different Gram-negative and Gram-positive battery of antibiotics (Tables 4-8).

As revealed in table 4, 8-10 of 12 *Salmonella* isolates were resistant to sulfamethoxazole, chloramphenicol, cephalothin, ampicillin, augmentin, streptomycin,

carbenicillin, and tetracycline. Whereas, almost all of them were susceptible to polymyxin B, gentamycin, and kanamycin. While only two of the *Salmonella* isolates were susceptible to all antibiotics, ten were resistant to 3 or more drugs.

Only two *Shigella B* were isolated, and both of them were susceptible to polymyxin B, cephalothin, gentamycin, and kanamycin. However, the isolates were resistant to ampicillin, sulfamethoxazole, carbenicillin chloramphenicol, streptomycin, tetracycline, and augmentin (Table5). Similarly both *E. coli O157* isolates were susceptible to polymyxin B, carbenicillin, gentamycin, kanamycin, tetracycline, but resistant to ampicillin, sulfamethoxazole, cephalothin, chloramphenicol, streptomycin, and augmentin (Table 6).

Almost half of the 17 *S. aureus* isolates were resistant to cephalothin, chloramphenicol, kanamycin tetracycline, and 14 to 17 of the isolates were resistant to erythromycin, oxacillin, vancomycin, clindamycin, penicillin, augmentin and bactroban. Only gentamycin was found to be effective against all (Table7). There was no difference in the pattern between strains isolated from hospital and restaurant cockroaches.

All *B. cereus* isolates were resistant to cephalothin, penicillin, augmentin, and bactroban, but over 19 of the 25 isolates were susceptible to chloramphenicol, oxacillin, gentamycin, kanamycin, streptomycin, tetracycline, clindamycin,, erythromycin, and vancomycin (Table 8).

Table4. Antibiotic Susceptibility pattern of 12 salmonella Isolates

	A N T I M I C R O B I A L S										
Pattern	AMP	SXT	PB	CA	CF	C	GM	K	S	Te	AMC
Sensitive	2	3	10	4	2	3	12	11	3	2	2
Intermediate	----	---	2	----	----	----	----	----	1	2	----
Resistant	10	9	----	8	10	9	----	1	8	8	10

Key: AMP= Ampicillin

SXT =Sulphamtoazol

PB= polymyxin B

CA =Carbenicillin

CF=Cephalothin

C=Chloramphenicol

GM= Gentamycin

K= Kanamycin

S = Streptomycin

Te= Tetracycline

Amc= Augmentin

Table 5. Antibiotic Susceptibility pattern of 2 *Shigella* isolates

	A N T I M I C R O B I A L S										
Pattern	AMP	SXT	PB	CAR	CF	C	GM	K	S	Te	AMC
Sensitive	----	----	2	---	----	----	1	1	----	----	----
Intermediate	-----	-----	-----	---	2	----	1	1	----	-----	----
Resistant	2	2	----- -	2	----	2	----	-----	2	2	2

Key: AMP= Ampicillin

SXT =Sulphamtoazol

PB= polymyxin B

CA =Carbenicillin

CF=Cephalothin

C=Chloramphenicol

GM= Gentamycin

K= Kanamycin

S = Streptomycin

Te= Tetracycline

Amc= Augmentin

Table 6. Antibiotic Susceptibility pattern of 2 *E. coli* O157 Isolates

Pattern	A N T I M I C R O B I A L S										
	AMP	SXT	PB	CA	CF	C	GM	K	S	Te	AMC
Sensitive	----	--	2	1	--	----	2	2	----	1	--
Intermediate	---	---	----	1	----	----	---	----	--	1	--
Resistant	2	2	---	---	2	2	----	---	2	--	2

Key: AMP= Ampicillin

SXT =Sulphamtoazol

PB= polymyxin B

CA =Carbenicillin

CF=Cephalothin

C=Chloramphenicol

GM= Gentamycin

K= Kanamycin

S = Streptomycin

Te= Tetracycline

Amc= Augmentin

Table 7 Antibiotic Susceptibility pattern of 17 *S. aureus* Isolates

Pattern	A N T I M I C R O B I A L S												
	CF	C	GM	K	S	Te	CC	OX	E	P	V	AMC	MUP
Sensitive	2	7	15	8	7	9	----	1	3	---	1	----	----
Intermediate	7	2	2	1	----	----	1	----	---	---	---	---	---
Resistant	8	8	----	8	10	8	16	16	14	17	16	17	17

Key: CF=Cephalothin

C=Chloramphenicol

GM= Gentamycin

S = Streptomycin

Te= Tetracycline

K= kanamycin

CC= clindamycin

OX= Oxacillin

E =Erythromycin

P= Penicillin

V= Vancomycin

Amc= Augmentin

MUP = mupirocin (Bactroban)

Table 8. Antibiotic Susceptibility pattern of 25 *B. cereus* Isolates

Pattern	A N T I M I C R O B I A L S												
	CF	C	GM	K	S	Te	CC	OX	E	P	V	AMC	MUP
Sensitive	----	24	24	24	23	21	18	---	21	----	20	----	----
Intermediate	---	----	----	----	1	1	1	----	---	----	---	----	---
Resistant	25	1	1	1	1	3	6	25	4	25	5	25	25

Key: CF=Cephalothin

C=Chloramphenicol

GM= Gentamycin

S = Streptomycin

Te= Tetracycline

K=kanamycin

CC=clindamycin

OX= Oxacillin

E =Erythromycin

P= Penicillin

V= Vancomycin

Amc= Augmentin

MUP = mupirocin (Bactroban)

Table 9. Multi-drug resistant strains of Bacterial Isolates

Antibiogram	<i>Salmonella</i> (No & %)	<i>Shigella</i> (No & %)	<i>E.coli</i> O157 (No & %)	<i>S. aureus</i> (No & %)	<i>B. cereus</i> (No & %)
Single drug	--	--	--	--	--
Double drugs	--	--	--	1(6%)	--
Triple drugs	1(8%)	--	--	--	--
Four drugs	1(8%)	--	--	1(6%)	10(40%)
Five drugs	--	--	--	1(6%)	10(40%)
> Six drugs	8(67%)	2(100%)	2(100%)	14(82%)	5(20%)
All drugs	--	--	--	--	---
Sensitive to all drugs	2(17%)	--	--	--	--

NB: Number of drugs tested is 11 for gram negative and 13 for gram positive ones.

8. Discussion

In this study, 1600 cockroaches were collected from hospitals and restaurants from December 2002 to June 2003, in Addis Ababa and all were identified as *B. germanica*. This cockroach 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 unit, table drawers and even the ceilings of the restaurants were found infested with cockroach. *B. germanica* is the most abundant and closely associated with humans on worldwide basis, and was found to carry at least one bacterial pathogen (Badillo-Vargas and Holbrook, 2002). 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 potential role of invertebrate vectors in the spread of several infectious diseases is undenied. It has been established that cockroaches' external body parts like antennae, mouth parts and legs as well as their faces, which retain, in most cases, pathogens without loss in their virulence, are ideally suited for disease dissemination (Paul *et al.*, 1992). However, they do not suffer from any disease (Devi and Murray, 1991).

The hazard of transmission of pathogens by cockroaches results from their nocturnal activity during which they visit various contaminated sites. The insects have the habit of vomiting, re-ingesting it and feeding on their own feces (Badillo-Vargas and Holbrook,

2002). These habits increase their potential to contaminate household foods by spreading bacteria to other members of the population. The common infections associated with cockroaches are bacillary and amoebic dysentery, cholera, enteric fever, food-poisoning, leprosy, tuberculosis, fungal infections, anthrax, pneumonia, septicemia, helminthic infestation, hepatitis, poliomyelitis etc. (Paul *et al.*, 1992).

In this study, isolation and identification of selected food-borne bacterial pathogens was done from the cockroach, *B. germanica*. This work reports for the first time the presence of *Salmonella spp.*, *S. aureus*, *Shigella*, *E. coli O157* and *B. cereus* in *B. germanica* from Ethiopia. The isolation of these pathogens from this cockroach species, captured in hospitals and restaurants, indicates that domestic pests can pose health problem. Except *E. coli O157*, others have been isolated from different species of cockroaches inhabiting different human and animal dwellings, restaurants and hospitals elsewhere outside Ethiopia (Oothman, *et al.*, 1989, Paul *et al.*, 1992, Fathpouer *et al.*, 2003; Agbodaze and Owusu, 1989; Burgess, 1984; LeGuyader *et al.*, 1989; Prado *et al.*, 2002). 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 introduce rare and emerging pathogens in to the community.

It has been reported that strains of EHEC, *E. coli O157:H7* or *H* are currently associated with diarrhea outbreaks, HC and HUS (Bethlehem, 2001). Although the major reservoir of *E. coli O157* is believed to be intestinal tract of cattle (Griffin and Tauxe, 1991), there are reports indicating the presence of the bacteria in flies (Kobayashi *et al.*, 1999). Further more, feeding experiments of *E. coli O157* to

houseflies showed that the ingested bacteria were harbored in the intestine of flies and continued to be excreted at least for 3 days after feeding, suggesting that houseflies are not simple mechanical vectors of EHEC (Kobayashi *et al.*, 1999). Considering the abundance of cockroaches and the very low infective dose of *EHEC O157* (10-100 bacteria), its presence in the *B. germanica* might enable high rates of transmission through foods, and out breaks. Both isolates were recovered from gut of hospital cockroaches. The multi-drug resistant feature further indicates the importance of hospital cockroaches in nosocomial infection.

It has been established that *Salmonella* is one of the most important food-borne pathogens, which cause different types of diseases (Darwin and Miller, 1999). *Salmonella* has been isolated from different species of cockroaches found in hospitals, restaurants, residents, schools, animal shelters etc. through out 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),

In the present study, 10 of the 12 *Salmonella* isolates were from the gut, and the rest 2 were recovered from the external surface of the cockroaches. These results suggest that cockroach intestine acts as major reservoir for bacterial flora. The external body parts of *B. germanica* had lower *Salmonella* load, probably due to dry external body surface. Moreover, 11 of the isolates were from hospital cockroaches and found to be resistant to 3 or more drugs tested, suggesting their possible role as reservoir and vectors of drug resistant *Salmonella* in health facilities and contribution to nosocomial infections. Most of our *Salmonella* isolates (8-10/12) have shown multi-drug resistance

pattern common in *Salmonella typhimurium* DT104, which is known to be resistant to ampicillin, chloramphenicol, streptomycin, sulphaamides, and tetracycline (IFST,1997). The antibiotic resistance probably emerged as a result of widespread use of antibiotics and antimicrobial both prophylactically and therapeutically.

In agreement with our results for *Salmonella*, different workers reported that hospital cockroaches were known to carry drug-resistant *Salmonella* and other pathogenic bacteria (Fotedar *et al.*, 1991b; Rivault *et al.*, 1993; Cotton *et al.*, 2000 Prado *et al.*, 2002; Fathpouer *et al.*, 2003).

Shigellosis is commonly considered as water and food-borne disease primarily found in higher primates including man (Andrews and Jacobson 1998). 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 residents indicating their importance in the dissemination of the bacterium (Oothuman, 1989; Paul, 1992; Agbogaze and Oswusu 1989). Similarly, we isolated two *Shigella* B from *B. germanica* each from gut and external body surface homogenates of restaurant cockroaches. Both isolates were resistant to seven antimicrobials using a standard disc diffusion assay. The results confirmed that *B. germanica* could be potential vector for multi-drug resistant *Shigella* in food establishment areas in Ethiopia too.

Staphylococcus aureus is one of the most common community-acquired and health-care related infections (Smith *et al.*, 1999). Moreover, it remains a major cause of FBD

because it can contaminate food products during preparation and processing. Although *S. aureus* is commonly found in nostrils, on the skin and hair of warm-blooded animals (Loir *et al.*, 2003), the possible role of cockroaches in its dissemination cannot be overlooked. Along with other pathogenic bacteria, *S. aureus* has been isolated from different species of cockroaches collected in hospitals and residents elsewhere (Fotedar *et al.*, 1991a; Paul *et al.*, 1992; Oothuman *et al.*, 1989; Burgess, 1984; LeGuyader *et al.*, 1989; Prado *et al.*, 2002).

In agreement with others result, *S. aureus* was recovered from *B. germanica* collected from hospitals and restaurants. Ten out of seventeen of the isolates were from the gut and the rest seven were from the external body surfaces homogenates of the cockroaches indicating the bacterium can survive both in intestine and body surface of the insects. Almost proportional numbers of *S. aureus* isolates were found from hospital and restaurant cockroaches. These findings suggest a potential role of cockroaches, in the dissemination of *S. aureus* in hospitals and food catering centers. Furthermore, all *S. aureus* isolates were resistant to all antibiotics tested except gentamycin suggesting the potential danger of cockroaches as reservoirs of multi-drug resistant *S.aureus* strains.

Antimicrobial resistance is increasing and *S. aureus* isolates have multiple drug resistance to commonly used antibiotics. In this study, although the number of isolates tested is small, the resistant rates show an increasing trend compared to previous studies in Tekur Anbesa Hospital and elsewhere in Ethiopia (Gedebou, 1982; Gedebou *et al.*, 1987; Lindtjorn *et al.*, 1989; Wolday and Erge, 1997; Mengistu *et al.*, 1999).

The increasing resistant rates of the isolates to most antibiotics may be an indication of the wide spread use of antibiotics. *S. aureus* is the most frequent cause of nosocomial infections caused by Gram-positive bacteria. Although all isolates were susceptible to gentamycin, the detection of high rates of multiple drug resistant isolates including these against vancomycin is cause for concern. Particularly, vancomycin resistant isolates could cause serious problem, because vancomycin is one of the few reserved drugs used for the treatment of serious bacterial infections. Strains of vancomycin resistant *S. aureus* (VRSA) have been reported from Japan, the United States, France, United Kingdom, Germany, Spain, Scotland, Hong Kong and Greece (Tenover *et al.*, 2001). Most of these isolates appear to have developed from pre-existing Methicillin resistant *S. aureus* infection. The emergence of such resistance could produce morbidity and mortality similar to that caused by *S. aureus* infections in the era before antibiotics became available.

Isolation of 25 *B. cereus* strains from hospital and restaurant cockroaches suggests the possible vector role of the insects in contaminating human foods.

In this study, there was no evidence of increasing or decreasing drug resistance pattern of *B. cereus* to common antibiotics tested. Since *B. cereus* produce β -lactamase, it is expected to be resistant to penicillin, oxacillin, cephalothin, and the same is observed. The isolates were also completely resistant to augmentin and bactroban. However, over 19 of the 25 isolates were susceptible to the other 8 antimicrobials tested (Table 8), which is also common drug resistance pattern of the bacterium demonstrated in the earlier studies (Drobniewski, 1993; Lagan and Turnbull, 1999).

Although the mechanical transmission of pathogens has received considerable attention among researchers, there are few attempts done to determine whether cockroaches sustain internal infections after ingesting them for an extended period of time. Burgess *et al.*, (1973) determined that oriental cockroaches could maintain and excrete viable *E. coli* for 20 days after a single oral dose of the bacterium. It was also shown that cockroaches could become infected with *S. typhimurium* and *S. montevideo* simply by feeding on animal excrement containing the bacteria (Jung and Shaffer, 1952, cited in Badillo-Vargas and Holbrook, 2002). Allen (1987) demonstrated that *B. orientalis* fed with heat-fixed tubercle sputum smears were excreting viable tubercle bacilli at least for four weeks. Recently, Fischer *et al.*, (2003) confirmed that orally infected nymph of *B. germanica* could harbor and shed viable and virulent *Mycobacterium*. Badillo-Vargas and Holbrook (2002) showed that 10-15% of nymph of *B. germanica* can sustain *Salmonella* for 21 days after a single dose of 10^4 CFU/ μ l of BPW, and observed that nymphs are more susceptible for *Salmonella* infection than their adults.

In the present study, it has also been tried to evaluate survival and excretion rate of *Salmonella*, *Shigella*, and *S. aureus* isolates in *B. germanica* fed with food contaminated with 10^6 CFU/g of bacteria in a separate experimental setting for each test pathogen. In the case of *Salmonella B*, culture examinations of fecal pellets of cockroaches were positive for the bacterium for 35 days after which all the test cockroaches were dead. Even the gut of the cockroaches was positive after their death. It is evident from this result that *B. germanica* is capable of ingesting *Salmonella* contaminated food and excrete viable bacteria in its feces. As compared with other results, this is probably the

longest *Salmonella* excretion time, and we assume that the cockroaches could continue excreting the bacteria for extended period of time if they lived longer.

Survival and shedding rate of *Salmonella* from the gut of experimentally infected cockroaches seems to be associated with bacterial strain, species of cockroaches and antagonism effects of endogenous gut bacteria. For instance, *S. typhimurium* was shed in droppings of the cockroaches for 7 days after infection and live *Salmonella* were detected in the digestive tract of 11 days after infection, however, *S. typhosa* died in cockroaches within 24h (Fischer *et al.*, 2003). Prior investigations with *P. americana*, *B. germanica* and *B. orientalis* fed approximately 10^9 CFU of *S. oranienburg* indicated that their feces were found to be positive for 10, 12 and 20 days, respectively. Infection of *P. americana* with graded doses of *S. typhimurium* and *S. montevideo* demonstrated persistence for at least 7 days when 10^9 CFU or more were ingested (Klowden and Greenberg, 1976).

In another study, *Salmonella* was recovered for 17 days after feeding 3×10^9 bacteria (King *et al.*, 1959, cited in Klowden and Greenberg, 1976). According to these authors, factors contributing to the elimination of *Salmonella* from the cockroach most likely relate to the physical washout resulting from normal gut motility, as well as microbial antagonism by the indigenous gut flora.

In the challenge study, it was also shown that *B. germanica* shed *S. aureus* for 14 days post infection, by then all the test cockroaches were dead, confirming the reservoir potential of the gut of the cockroach for the bacterium. We were not able to compare

this result for there was no other challenge study done on *B. germanica* with *S. aureus*. However, *Shigella B* was not recovered from the culture examination of fecal pellets of experimentally infected *B. germanica* following for 30 days. This is probably due to the antagonistic effect of the indigenous bacteria in resisting colonization of the gut by non-indigenous species (Dillon and Dillon, 2004).

7. Conclusion and Recommendation

Bacterial food-borne diseases are the major concern in public health programs worldwide. Among the predominant bacteria involved in these diseases, *Salmonella*, *Shigella*, *E. coli* O157:H7, *S. aureus*, and *B. cereus* are the major cause of gastroenteritis resulting from the consumption of contaminated foods. Circumstantial evidences from different researches conducted elsewhere outside Ethiopia indicate the potential vector role of cockroaches in dissemination of pathogenic bacteria and contamination of food and/or utensils in catering centers and hospitals. Isolation of the above-mentioned pathogens from hospital and restaurant cockroaches (*B. germanica*) in this study, confirm that they can act as a potential vectors of medically important bacteria. Persistence of *Salmonella* and *S. aureus* in experimentally fed *B. germanica* for 35 and 14 days post infection, respectively, also show that the insects are the possible reservoir of the bacteria and could play a role in nosocomial and community acquired transmission of infectious diseases. Therefore, control of cockroaches, especially in hospitals and food handling establishments should help to control the reservoir of the bacteria. Although there is a great deal of materials demonstrating that cockroaches could harbor and excrete pathogens, their vector role for human disease remains to be proven. Hence, researchers in this field should continue to investigate some of the following problems to establish the natural disease transmission to humans by the cockroaches. (1) Prevalence and species diversity of cockroaches in Ethiopia; (2) The virulence degree of a bacterium after passing through cockroach's gut; (3)

Multiplication capacity of experimentally infected bacteria in the gut of cockroaches;(4) Normal cockroaches' gut flora analysis and understand the relationship between the insect and its microbes that could provide new strategies for controlling cockroaches using entomopathogens;(5) The negative result of *Shigella* survival in experimentally fed cockroach; and (6) Dominant cockroach species and their vectorial capacity for bacteria, viruses, protozoa and helminthes should be explored in order to have direct proof for incriminating cockroaches in the mechanical transmission of human food-borne diseases. (8) Safe insecticides should be sought from local herbs for controlling domestic cockroaches.

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