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Research Paper

Cockroaches (*Periplaneta americana*) as possible reservoirs of extended spectrum beta-lactamase (ESBL)-producing pathogenic bacteria

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Abstract

Cockroaches are excellent vectors of microbes since they carry pathogenic microorganisms and transmit same onto sterile surfaces, food, cloths/beddings and equipment/instruments in the hospital and non-hospital environments. Extended spectrum beta-lactamases (ESBLs) are beta-lactamases capable of hydrolyzing many beta-lactam antibiotics including third-generation cephalosporins; and bacteria that produce them occur in both the community and hospital settings. In this study, a total of 517 adult cockroaches comprising 137 cockroaches from hospital environment and 380 cockroaches from residential homes were bacteriologically analyzed for the isolation and identification of pathogenic bacteria expressing ESBLs. Antibiogram was carried out using the Kirby-Bauer disk diffusion method and ESBL production was detected in the isolated bacteria using the double disk synergy test (DDST) method. A total of 101 isolates of *Escherichia coli,* 25 isolates of *Salmonella* species and 5 isolates of *Shigella* species was recovered from the cockroaches emanating from both the hospital and residential homes. The antibiogram result showed that the isolated *E. coli* isolates, *Salmonella* species and *Shigella* species showed varied levels of multidrug resistance to the tested antibiotics especially to the 3rd generation cephalosporins such as ceftazidime, cefoxitin, cefotaxime and ceftriaxone. ESBL expression was detected in 16 isolates of *E. coli* and 4 isolates of *Salmonella* species. None of the isolated *Shigella* species expressed ESBL. Cockroaches may play an important role in the spread of multidrug resistant bacteria pathogens within the hospital and non-hospital environments. Thus, it is important to take cockroaches infestations seriously and be on the lookout for bacteria that harbour drug resistant genes such as those that spur ESBL production.

Keywords: ESBLs, Cockroaches, Multidrug Resistance, Bacterial Infection, Gram-negative bacteria, Nigeria

Introduction

Periplaneta americana known colloquially as cockroaches live in filthy environments in households, food homes and in hospitals where they feed on a vast variety of wastes including feaces, sputum, garbage, human and pet foods[1]. Consequently, they can passively transmit pathogenic microbes including *Salmonella*, *Campylobacter*, *Shigella*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to humans when they feed on leftover food[2]. Cockroaches are among the medically important pests in urban environments and they have been associated with an outbreak of dysentery and other infectious diseases[2,3]. Cockroaches harbour pathogenic microorganisms – which they transmit to non-contaminated environments and/or instruments in either the hospital or non-hospital environments. The use of antibiotics used for the propagation of farm animals and aquaculture has resulted in the selection of pathogenic bacteria resistant to some available drugs.

Pathogenic bacteria may become resistant to the action of antimicrobial agents through several mechanisms including the accumulation of resistant genes and via mutation, but the undue exposure of microbes to antibiotics contributes a great deal to the development and spread of resistant bacteria in both the community and hospital environment[4,5]. Extended spectrum beta-lactamases (ESBLs) are diverse and complex enzymes whose genes are encoded on bacterial plasmids and chromosomes, and they are characterized by their ability to compromise the efficacy of 3rd- generation cephalosporins, penicillins and monobactams but not cephamycins and carbapenems[6]. ESBL-producing bacteria are generally susceptible to β-lactamase inhibitors such as clavulanic acid[7], and their susceptibility to clavulanic acid serves as a basis for their phenotypic detection in the laboratory[8]. Organisms producing ESBLs also carry genes that confer resistance to non-beta-lactams including the quinolones, aminoglycosides, tetracyclines and sulfonamides. These multidrug resistance enzymes are frequently found in members of the *Enterobacteriaceae* including *Escherichia coli* and *Klebsiella pneumoniae* as well as in non-enteric bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [4,7,9]. The selection pressure that drive the evolution of ESBLs has always been associated with the intense use of oxyimino-beta-lactams, widespread use of broad spectrum antibiotics, prolonged hospitalization, indwelling devices and severe underlying diseases[5,10].

However, these organisms can also be transmitted to humans via fomites and other non-human hosts such as cockroaches, and in such circumstance they cause infections[11]. Since arthropods like cockroaches could serve as vehicles through which food supply, fomites and medical devices can be contaminated with pathogens with a concomitant risk of human infections in the hospital and community settings, it is therefore important to take cockroach infestations seriously and be on the lookout for bacteria that harbour drug resistant genes such as those that spur ESBL production. This present study presumptively evaluated cockroaches from both hospital and non-hospital environments as potential sources of ESBL-producing bacteria.

Materials and Methods

Sample collection and processing: A total of 517 *P. americana* adult cockroaches comprising 137 cockroaches from hospital environment and 380 cockroaches from residential homes were collected using the off-target insecticide spray technique. The cockroaches were picked with gloved hands, placed in sterile and labeled universal containers and transported to the Microbiology Laboratory Unit of Ebonyi State University, Abakaliki, Nigeria for analysis. The external body parts and alimentary duct of the cockroaches was used for this study. Each of the samples was aseptically placed in test tubes containing 70 % ethanol for 5 minutes to decontaminate their external body surface and then allowed to air dry. The cockroaches were washed in 5 ml of sterile normal saline in a tube to remove ethanol residues. And their external body parts was removed using sterile forceps which was sterilized after each cut. The alimentary tract of the cockroaches was dissected under a dissecting microscope to dislodge its content. Both the gut content and external body parts of the cockroaches was kept in 5 ml sterile normal saline and allowed for about 10-20 mins to produce a homogenate sample which was used for bacteriological analysis.

Culture and identification of bacteria: An aliquot of 1 ml of the homogenate suspension in the normal saline tube (containing the samples) were aseptically inoculated into 10 ml of Selenite-F broth (Oxoid, UK), and incubated overnight for at 37^{o}C. A loopful of each of the broth culture was inoculated into Deoxycholate Citrate Agar, DCA (Oxoid, UK), MacConckey Agar, MCA (Oxoid, UK) and eosin methylene blue, (EMB) agar (Oxoid, UK). All inoculated culture media were incubated at 37^{o}C for 18-24 hours. Suspected colonies of bacteria growing on the culture media was aseptically cultured on freshly prepared DCA, MCA, and EMB agar plates and incubated at18- 37^{o}C for 24 hours. Bacteria colonies on culture media were identified using standard microbiological techniques[3,12].

Antibiogram: Antimicrobial susceptibility testing was determined by the Kirby-Bauer disk diffusion technique as per the Clinical Laboratory Standard Institute (CLSI) criteria; and as was described previously[13,14]. Single antibiotic disks comprising cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), cefoxitin (30 µg), imipenem (30 µg), ciprofloxacin (5 µg), sulphamethoxazole /trimethoprim (25 µg), chloramphenicol (30 µg), gentamicin (10 µg) and amoxicillin / clavulanic acid (20/10 µg) [Oxoid, UK] was used for susceptibility testing. Briefly, each of the test bacteria (adjusted to 0.5 McFarland turbidity standards) was aseptically swabbed on Mueller-Hinton (MH) agar (Oxoid, UK) plates, and each of the antibiotic disks was placed on the MH agar plates (already swabbed with the test organism) and incubated at 37^{o}C for 18-24 hours. Susceptibility

testing was carried out in triplicate for each of the organism; and the mean inhibition zone diameter (IZD) was taken as the final IZD. The IZD produced was measured, recorded and interpreted as per the CLSI criteria[13].

Screening and confirmatory test for detection of ESBL positive isolates: To screen for the production of ESBLs, the susceptibility of the test bacteria to any of the 3rd generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime) was evaluated. Test bacteria that showed reduced susceptibility to any of the 3rd generation cephalosporins were confirmed phenotypically by double disk synergy test (DDST) for ESBL production[8,15]. Test organisms (adjusted to 0.5 McFarland turbidity standards) were aseptically swabbed on MH agar plates; and antibiotic disks of amoxycillin-clavulanic acid (20/10µg) was placed at the center of the MH agar plate. Supplementary disk of cefotaxime (30µg) and ceftazidime (30µg) were each placed at a distance of 15 mm adjacent to the central disc. The MH agar plates were incubated at 37°C for 18-24 hours. ESBL production was inferred phenotypically when the zones of inhibition of the cephalosporins (cefotaxime 30µg and ceftazidime 30µg) were expanded by the amoxycillin-clavulanic acid disk (20/10µg). A ≥ 5 mm increase in the inhibition zone diameter for either of the cephalosporins tested in combination with amoxycillin-clavulanic acid versus its zone when tested alone confirms ESBL production phenotypically[8,15].

Results and Discussion

Table 1 shows the distribution of the pathogenic bacteria isolated from the cockroaches recovered from both hospital and non-hospital environment. The pathogenic bacteria isolated from the various parts of the cockroaches include *Escherichia coli, Shigella* species and *Salmonella* (Table 1). *E. coli* was the most isolated bacteria from the cockroaches. However, a total of 21 isolates of *Salmonella* species and 5 isolates of *Shigella* species were bacteriologically isolated from the various body parts of the cockroaches analyzed in this study. The antimicrobial susceptibility profile of the *E. coli* isolated from cockroaches in both hospital and residential homes is shown in Figure 1 and 2 respectively. The *E. coli* from hospital was resistant to ceftriaxone (70.8%), ceftazidime (66.6%), cefotaxime (75.0%), cefepime (66.7%), aztreonam (75.0%), cefoxitin (66.7%) and imipenem (62.5%). All *E. coli* isolates from hospital was resistant to sulphamethoxazole-trimethoprim (Figure 1).

Table 1: Distribution of bacterial pathogens isolated

Isolate	Hospitals		Residential Homes		Total
	External body	Alimentary tract	External body	Alimentary tract	
Escherichia coli	7	17	13	64	101
Salmonella species	0	4	1	16	21
Shigella species	0	2	0	3	5
Total	7	23	14	83	127

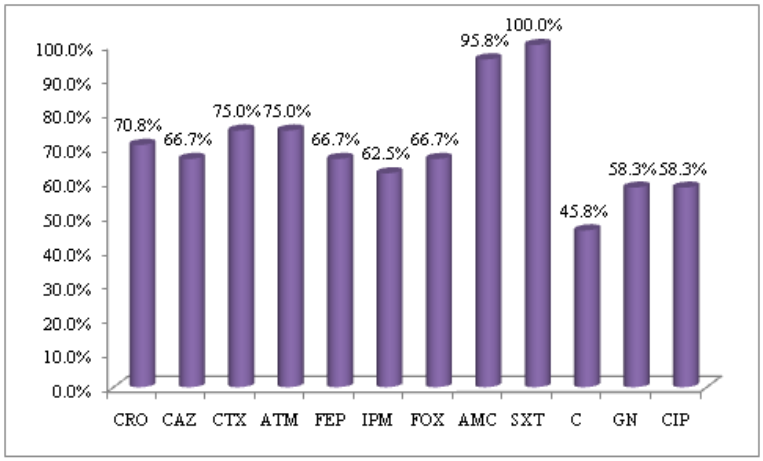

Figure 1: Percentage resistance of *Escherichia coli* isolated from cockroaches in hospital environment

Key: CRO=ceftriaxone, CAZ=ceftazidime, CTX=cefotaxime, ATM=aztreonam, FEP=cefepime, IPM=imipenem, FOX=cefoxitin, AMC=amoxicillin/clavulanic acid, SXT=sulphamethoxazole-trimethoprim, C=chloramphenicol, CN=gentamicin, CIP=ciprofloxacin.

The *E. coli* isolated from cockroaches from residential homes also showed varied levels of resistance to the tested antibiotics. However, they showed high resistance to ceftriaxone, ceftazidime, cefotaxime, imipenem, gentamicin, and amoxicillin-clavulanic acid at the rates of 63.6 %, 71.4 %, 76.6 %, 68.8 %, 62.3 %, 94.8 % respectively (Figure 2). Figures 3 and 4 shows the percentage resistance of *Salmonella* species isolated from hospital and residential homes. The *Salmonella* species isolates from hospital were found to be highly resistant to the 3rd generation cephalosporins including ceftriaxone, ceftazidime, cefepime, and cefotaxime, cefoxitin (Figure 3). The *Salmonella* species isolates from residential homes were also resistant to cefotaxime, cefoxitin, amoxicillin/clavulanic acid, gentamicin, ceftazidime, imipenem, and aztreonam (Figure 4).

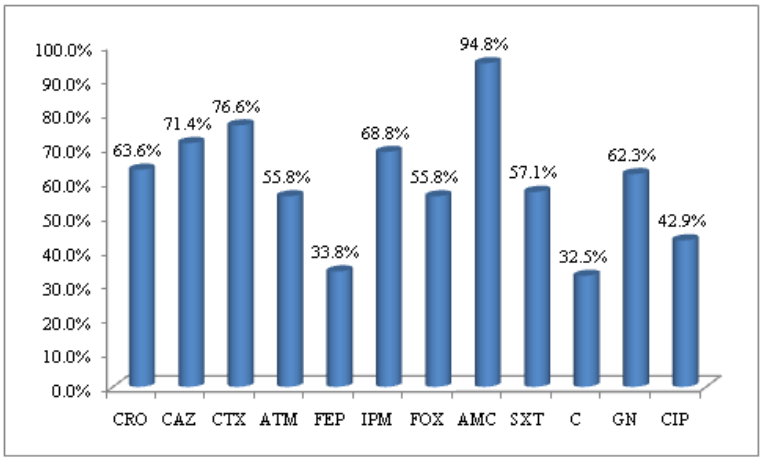

Figure 2: Percentage Resistance of *Escherichia coli* isolated from cockroaches in residential homes

Key: CRO=ceftriaxone, CAZ=ceftazidime, CTX=cefotaxime, ATM=aztreonam, FEP=cefepime, IPM=imipenem, FOX=cefoxitin, AMC=amoxicillin/clavulanic acid, SXT=sulphamethoxazole-trimethoprim, C=chloramphenicol, CN=gentamicin, CIP=ciprofloxacin

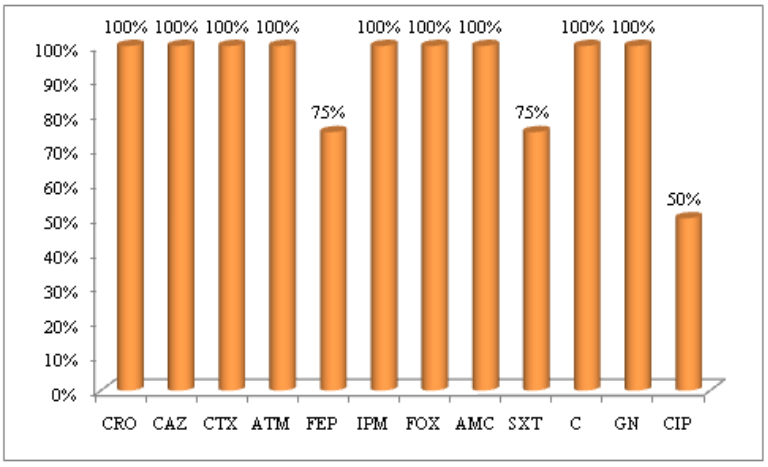

Figure 3: Percentage resistance of *Salmonella* species isolated from cockroaches in hospitals

Key: CRO=ceftriaxone, CAZ=ceftazidime, CTX=cefotaxime, ATM=aztreonam, FEP=cefepime, IPM=imipenem, FOX=cefoxitin, AMC=amoxicillin/clavulanic acid, SXT=sulphamethoxazole-trimethoprim, C=chloramphenicol, CN=gentamicin, CIP=ciprofloxacin

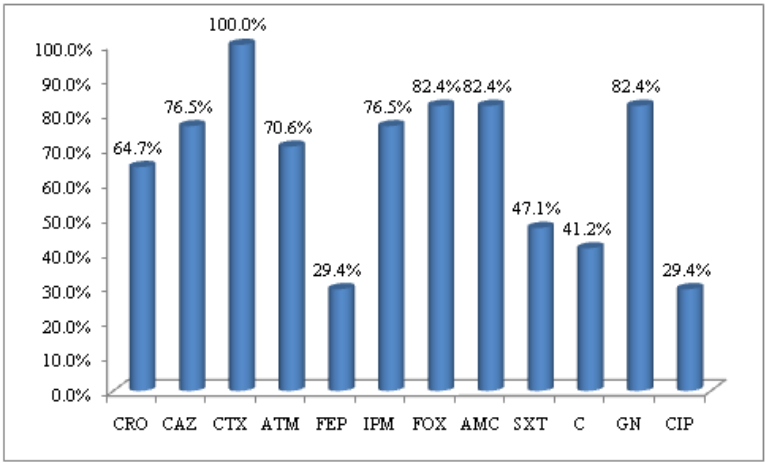

Figure 4: Percentage resistance of *Salmonella* species isolated from cockroaches in residential homes

Key: CRO=ceftriaxone, CAZ=ceftazidime, CTX=cefotaxime, ATM=aztreonam, FEP=cefepime, IPM=imipenem, FOX=cefoxitin, AMC=amoxicillin/clavulanic acid, SXT=sulphamethoxazole-trimethoprim, C=chloramphenicol, CN=gentamicin, CIP=ciprofloxacin

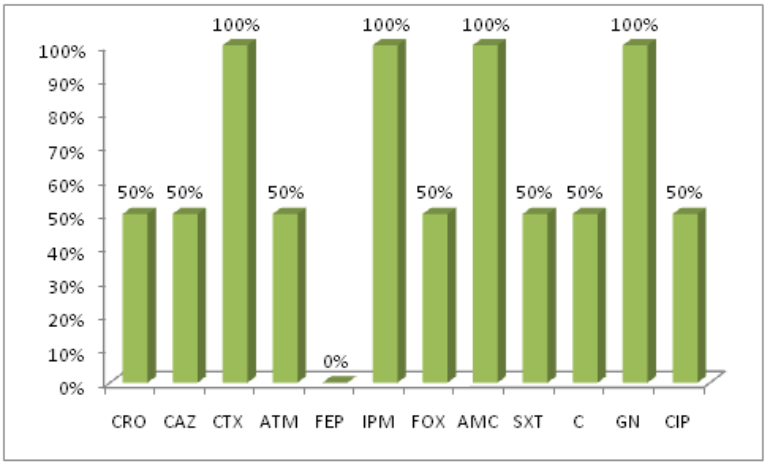

Figure 5: Percentage resistance of *Shigella* species isolated from cockroaches in hospitals

Key: CRO=ceftriaxone, CAZ=ceftazidime, CTX=cefotaxime, ATM=aztreonam, FEP=cefepime, IPM=imipenem, FOX=cefoxitin, AMC=amoxicillin/clavulanic acid, SXT=sulphamethoxazole-trimethoprim, C=chloramphenicol, CN=gentamicin, CIP=ciprofloxacin

Figure 5 and 6 shows the percentage resistance profile of the *Shigella* species isolated from hospital and residential homes. It was observed that the *Shigella* species isolated from hospital was highly resistant to cefotaxime, imipenem, cefoxitin, gentamicin, ceftazidime and amoxicillin/clavulanic acid. All the *Shigella* species isolates from cockroaches emanating from the hospital was completely susceptible to cefepime (Figure 5). All the *Shigella* species isolates from cockroaches emanating from residential homes was found to be resistant to cefotaxime (100%), imipenem (100%), cefoxitin (100%) and gentamicin (100%) (Figure 6). The *Shigella* species isolates from residential homes was also found to be resistant to ceftazidime (66.7%) and amoxicillin-clavulanic acid (66.7%). However, they were all susceptible to cefepime (Figure 6).

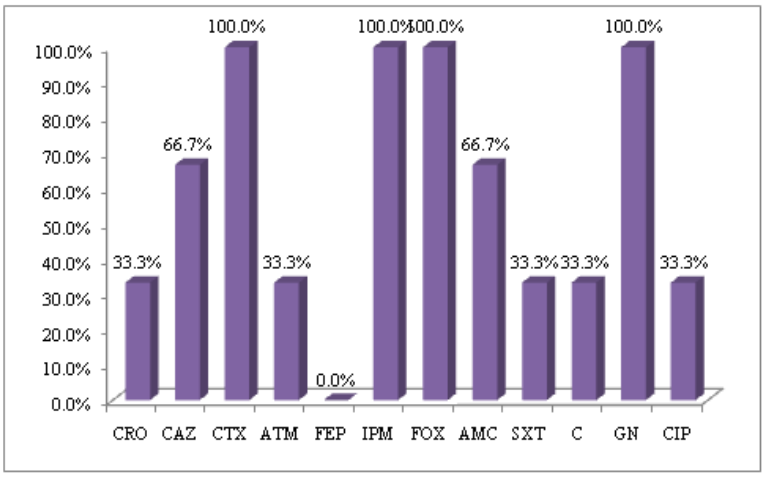

Figure 6: Percentage resistance of *Shigella* species isolated from cockroaches in residential homes

Key: CRO=ceftriaxone, CAZ=ceftazidime, CTX=cefotaxime, ATM=aztreonam, FEP=cefepime, IPM=imipenem, FOX=cefoxitin, AMC=amoxicillin/clavulanic acid, SXT=sulphamethoxazole-trimethoprim, C=chloramphenicol, CN=gentamicin, CIP=ciprofloxacin.

Table 2 shows the occurrence of ESBL-producing bacteria isolated from various body parts of cockroaches recovered from both hospital environment and residential homes. Overall, ESBL expression was phenotypically detected in 16 isolates of *E. coli* and 4 isolates of *Salmonella* species. None of the isolated *Shigella* species expressed ESBL by the double disk synergy test (DDST) method used in this study (Table 2).

Table 2: Frequency of ESBL-producing bacteria

Isolate	Hospitals		Residential Homes		Total
	External Body	Alimentary tract	External Body	Alimentary tract	
Escherichia coli	0	7	2	7	16
Salmonella species	0	1	0	3	4
Shigella species	0	0	0	0	0

The increasing prevalence of antibiotic resistant bacteria is of global concern as they are known to make the treatment of bacterial related infections difficult. Antibiotic resistant bacteria may also lead to increase in the length of hospitalization of a patient, severity of illness and the overall cost of treatment. According to the Scottish Center for Infection and Environmental Health (SCIEH), the emergence and spread of ESBL-producing bacteria which initially looked benign has become one of the major resistance problems that now bedevil our health sector around the world, putting the available antibiotics for treatment of bacterial related infections into risk[16]. In this present study, the antibiogram and occurrence of pathogenic bacteria from cockroaches as possible reservoirs of ESBL-producing bacteria was phenotypically evaluated.

The cockroaches from both the hospital environment and residential homes were found to be carrying potentially pathogenic bacteria including *Escherichia coli, Salmonella* species and *Shigella* species. Overall, a total of 101 isolates of *E. coli,* 21 isolates of *Salmonella* species and 5 isolates of *Shigella* species were bacteriologically isolated from the various body parts of the cockroaches analyzed in this study. The isolation rate of pathogenic bacteria including *E. coli, Salmonella* species and *Shigella* species from this study is similar to the work of Czajka *et al*.[17] and Al-Marjani[18] – who reported similar incidence of pathogenic bacteria isolated from cockroaches that infested hospital environments. According to Kutrup[19], cockroach infestation is common in hospital, and they are usually implicated in a number of hospital and community acquired infection – since they could act as vehicles for the transmission of pathogenic bacteria. In Ghana, the frequency of pathogenic bacteria recovered from cockroach infestation in both hospital environment and residential homes has been reported, and

these reports are similar to ours in which pathogenic bacteria was recovered from cockroaches that infested both hospital and residential homes.

The *E. coli* isolates from cockroaches that emanated from hospital and residential homes were resistant to more than 50 % of the tested antibiotics including the 3rd generation cephalosporins, flouroquinolones, aminoglycosides and monobactam used in this study. However, highest resistance of the *E. coli* isolates from cockroaches recovered from hospital environment was observed in amoxicillin-clavulanic acid (95.8 %) and sulphamethoxazole-trimethoprim (100 %). *E. coli* from residential homes was also resistant to amoxicillin-clavulanic acid (94.8 %) and sulphamethoxazole-trimethoprim (57.1 %). The results of the antibiograms of the *E. coli* isolated from cockroaches recovered from both hospital and residential homes is similar to earlier reports in Brazil, Iraq and Algeria – where pathogenic bacteria isolated from cockroaches were reported to be highly resistant to some commonly used antibiotics including cephalosporins, flouroquinolones and aminoglycosides[18,20,21]. The *Salmonella* species and *Shigella* species isolated from cockroaches that emanated from hospital environment was found to be resistant to over 50 % of the tested antibiotics used in this study. Similar levels of resistance was also observed with the *Shigella* species and *Salmonella* species that were isolated from cockroaches that emanated from residential homes in this study. However, the *Shigella* species isolated from cockroaches from both hospital and residential homes were found to be susceptible to cefepime, a 4th generation cephalosporin. This result is in line with a similar study carried out in Uyo, South-South Nigeria, where Akinjogunla *et al*.[22] reported the occurrence of multidrug resistant bacteria isolated from cockroaches.

The DDST result for the phenotypic confirmation of ESBL production in the isolated pathogenic bacteria from cockroaches emanating from hospital and residential homes was positive for 16 of 101 *E. coli* isolated from both hospital and residential homes and 4 out of 21 *Salmonella* species isolated from both hospital and residential homes. The *Shigella* species isolated from both hospital and residential homes was not positive for ESBL expression by the DDST technique used in this study. The production of ESBL by pathogenic bacteria isolated from cockroaches as reported in this study is similar to previous studies conducted by Abbas *et al*.[2] and Al-Marjani[18]. In Iraq, Al-Marjani *et al*.[18] reported the production of ESBLs by *Enterobacteriaceae*, a result which is similar to ours. Also in Algeria, the production of ESBLs by bacteria isolated from cockroaches (as obtainable in this study) has also been reported[21]. Conclusively**, t**his study has shown that cockroaches carry pathogenic bacteria that are multidrug resistant in nature, and ESBL-producing in nature. The role of cockroaches as a potential reservoir of multidrug-resistant bacteria including those that express ESBLs as demonstrated in our study and with their presence in both hospital and non-hospital environments calls for concerted efforts to bring their infestation of the environment under control.

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