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Original Article

Prevalence and Antibiogram of Generic Extended-Spectrum β-Lactam-Resistant Enterobacteria in Healthy Dogs

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Abstract

This study was conducted to isolate generic extended-spectrum β-lactam-resistant enterobacteria from household dogs in Nigeria, and to determine the antibacterial resistance profile of the isolates. Rectal swabs were collected from 100, randomly selected, apparently healthy household dogs. Isolation of ESBL-resistant enterobacteria was done using Mac Conkey agar supplemented with 4 µg/ml of ceftazidime. Phenotypic characterization of the isolates to generic level was done following standard biochemical methods. Phenotypic resistance of the isolates to antibacterial agents was determined using the disc diffusion method. Out of 27 ESBL-resistant enterobacterial isolates, 40.7% were *Escherichia coli*, 37% were *Klebsiella* species, 18.5% were *Salmonella* species, while 3.7% was *Proteus* species. Resistance of the *E. coli* isolates was 81.8% to ampicillin, 27.2% to streptomycin, 54.5% to ciprofloxacin and tetracycline, 45.4% to enrofloxacin, 90.9% to sulphamethoxazole/trimethoprim, 9.1% to amoxicillin/clavulanic acid and 0% resistant to gentamicin. Resistance of the *Klebsiella* isolates was 80% to ampicillin, 20% to streptomycin and amoxicillin/clavulanic acid, 30% to ciprofloxacin and enrofloxacin, 60% to tetracycline, 90% to sulphamethoxazole/trimethoprim and 10% to gentamicin. Resistance of the *Salmonella* isolates was 100% to sulphamethoxazole/trimethoprim, 80% to gentamicin and ampicillin, 60% to streptomycin and tetracycline, 20% to amoxicillin/clavulanic acid, and 0% to ciprofloxacin and enrofloxacin. The *Proteus* isolate was resistant to streptomycin and gentamycin. All the isolates were resistant to ceftazidime and cefotaxime. Resistance of the isolates to more than 3 classes of antibacterial agents tested was 81.8% for *E. coli*, 70% for *Klebsiella* and 100% for *Salmonella*, respectively. This study has shown that household dogs in Nigeria, are colonized by ESBL-resistant *Enterobacteriaceae* and are potential reservoirs and disseminators of these organisms.

Keywords: antibiogram, β-lactam-resistant, *Enterobacteriaceae*, extended-spectrum, companion animals, canine

Introduction

Antimicrobial resistance is a serious threat to the future of antimicrobial chemotherapy, and negatively impacts the health of human and animals (WHO, 2014; Morison and Rubin, 2015). The one health oriented approach in curbing antimicrobial resistance involved assessment of the impact of animals in close association with humans (Guerra *et al*., 2014; Schaufler *et al*., 2015). Companion animals such as dogs represent potential sources of spread of ESBL-resistant organisms owing to the extensive use of antimicrobials as those used in humans, especially β-lactams, in veterinary practices dealing with small animals, and their close contact with humans (Guardabassi *et al*., 2004; Ma *et al*., 2009; Ewers *et al*., 2012; Hordjik *et al*., 2013; Tamang *et al*., 2012). Extended-spectrum β-lactams (i.e. 3rd – [e.g. ceftazidime, cefpodoxime, etc and 4th-generation cephalosporins) are critical drugs in medicine, used for the treatment of bacterial infections (especially multidrug-resistant infections) in humans and companion animals (WHO, 2011; Ewers *et al*., 2012; Hordijk *et al*., 2013; Tuerena *et al*., 2016). Inappropriate use of extended-spectrum β-lactams especially in developing nations (due to lack of strict policy regarding the use of antibacterial agents) has led to selection pressure and development of resistance to these drugs by enterobacteria (Ugwu *et al*., 2015). Resistance to extended-spectrum β-lactams in enterobacteria is mediated mainly by extended-spectrum β-lactamases (ESBLs) (Tamang *et al*., 2012; Ljungquist *et al*., 2016). ESBL-resistant enterobacteria are multidrug-resistant and exhibit resistance to all β-lactams and many classes of antibacterials including

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aminoglycosides, fluoroquinolones, phenicols, lincosamides, potentiated sulfonamides and tetracycline (Coque *et al*., 2008; Dierikx *et al*., 2012; Schaufler *et al*., 2015). Therapeutic options for treating infections associated with ESBL-resistant organisms are often limited due to phenotypic multidrug resistance (Dierikx *et al*., 2012; Schaufler *et al*., 2015). It is well known that ESBL-resistant enterobacteria are common cause of community and hospital-associated infections in humans and companion animals (Sun *et al*., 2010; Rubin and Pitout, 2014). These infections are often fatal due to limited therapeutic options, resorting to the use of carbapenems is usually the treatment option (Dierikx *et al*., 2012; Blaak *et al*., 2014). This has resulted in increasing numbers of isolation of carbapenem-resistant organisms (superbugs) from humans and animals, including companion animals, worldwide (Shaheen *et al*., 2013; Guerra *et al*., 2014; Yousfi *et al*., 2015). Determination of phenotypic antibacterial resistance profile of ESBL-resistant enterobacteria is useful for empirical treatment of infections associated with such organisms (Ugwu *et al*., 2015; Schaufler *et al*., 2015).

Direct physical contact between dogs and humans, especially dog owners/caretakers, children and veterinarians, has been identified as a putative risk for the spread of ESBL-resistant enterobacteria (Weiler *et al*., 2011; Meyer *et al*., 2012; Schaufller *et al*., 2015; Ljungquist *et al*., 2016). As commensal organisms in the intestinal tract of animals, ESBL-resistant enterobacteria haboured by dogs may be discharged into the environment, thereby serving as disseminators of genes encoding β-lactam resistance (Costa *et al*., 2008; Pfeiffer *et al*., 2010; Tuerena *et al*., 2016). Beta-lactam resistance genes present in the discharged enterobacteria could be acquired by horizontal transfer to pathogenic human bacteria, thereby complicating infections and compromising antibacterial therapy in carriers (Caratolli *et al*., 2005; Ugwu *et al*., 2015; Ljungquist *et al*., 2016).

Reports on the occurrence of probable exchange of ESBL-resistant organisms between humans and animals raised questions on the role of companion animals as reservoirs of these organisms (Weiler *et al*., 2011; Schmeidel *et al*., 2014). There have been calls for assessment of companion animals as potential reservoirs and disseminators of ESBL-resistant organisms (Ewers *et al*., 2011; Weiler *et al*., 2011; Cozma *et al*., 2015a). Since the first report on isolation of ESBL-resistant *E. coli* in faecal sample of a laboratory dog in Japan (Matsumoto *et al*., 1998), improvements have been made on isolation of ESBL-resistant enterobacteria from clinical samples of dogs worldwide (Feria *et al*., 2002; Lim *et al*., 2009; Gibbson *et al*., 2010; O'Keefe *et al*., 2010; Timofte *et al*., 2011; Shaheen *et al*., 2011; Huber *et al*., 2013; Dierikx *et al*., 2012; Weiler *et al*., 2014; Rzewuska *et al*., 2015). Evidences support zoonotic transmission of ESBL-resistant enterobacteria from companion animals to humans and *vice versa* (Weiler *et al*., 2011; Meyer *et al*., 2012; Rubin and Pitout, 2014; Carvalho *et al*., 2016; Ljungquist *et al*., 2016).

Surveillance studies to assess dogs as potential reservoirs and disseminators of ESBL-resistant bacteria have been conducted in several countries in Europe (Costa *et al*., 2004; Caratolli *et al*., 2005; Poirel *et al*., 2013; Gandolfi-Decristophoris *et al*., 2013; Hordijk *et al*., 2013; Johard *et al*., 2015; Cozma *et al*., 2015a, Cozma *et al*., 2015b; Baede *et al*., 2015; Ljungquist *et al*., 2016), South America (Moreno *et al*., 2008; Rocha-Gracia *et al*., 2015; Oleivera *et al*., 2016; Carvalho *et al*., 2016), Asia (Sun *et al*., 2010; So *et al*., 2012; Tamang *et al*., 2014) and Australia (Sidjabat *et al*., 2007). No single report regarding the presence of ESBL-resistant enterobacteria in companion animals in Nigeria, exist in the literature whereas most Nigerian households keep dogs as pets, guard and/or hunting dogs. Reports in the literature which assessed animals as reservoirs of ESBL-resistant organisms in Nigeria, were those from food animals (Chah and Oboegbulem, 2007; Eze *et al*., 2013; Duru *et al*., 2013; Inwezerua *et al*., 2014; Olowe *et al*., 2015; Ugwu *et al*., 2015; Torres *et al*., 2015). This may paint a picture that only food animals are reservoirs of ESBL-resistant organisms in Nigeria. This is a matter of concern because companion animals in Nigeria may harbour these multidrug-resistant organisms and continually serve as source of dissemination of ESBL-encoding genes to the gut microbiota of animals and humans. Therefore, there is need to screen companion animals in Nigeria as potential reservoirs of ESBL-resistant organisms. This will help in evaluation of trends, identification of mitigation strategies and empirical treatment of infections associated with these organisms (Ugwu *et al*., 2015). The objective of this study, therefore, was to isolate ESBL-resistant enterobacteria from household dogs in Nsukka, Southeast, Nigeria, and to determine the antibacterial resistance profile of the isolates.

Materials and Methods

Sampling

This cross-sectional study was conducted between January and July, 2016. Households with dogs in Nsukka Southeast, Nigeria, were identified purposively, using snowballing technique. Rectal swabs were collected using sterile swab sticks from 100 (one dog per household), randomly selected apparently healthy dogs of varied breeds, sexes and ages (puppies and adults). Distinguishing body marks of each dog was appropriately noted and each house was visited once to avoid the possibility of re-sampling. The samples were transported aseptically in ice packs and processed within 2 hours of collection in the Veterinary Microbiology Laboratory, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka.

Isolation and generic identification of ESBL-resistant enterobacteria from dogs

The swabs were cultured on Mac Conkey agar supplemented with 4 µg/ml of ceftazidime and incubated at 37 °C for 24 hours aerobically. The morphology of different colonial types were described and recorded appropriately. Purification of the isolates was done by sub-culturing on plain Mac Conkey agar and incubated at 37 °C for 24 hours. Pure cultures of the isolates were then inoculated onto nutrient agar slants, incubated at 37 °C for 24 hours and stored in refrigerator at 4 °C as stock cultures until needed for further analysis. Phenotypic characterization of the isolates to generic level was done by subjecting them to various tests such as Gram staining, catalase, urease, oxidase, indole, methyl red, triple sugar iron agar and citrate test, and sub-culturing on eosin methylene blue agar following standard methods.

Determination of the antibiogram of the ESBL-resistant enterobacterial isolates from dogs

Antibacterial resistance/susceptibility of the ESBL-resistant isolates was determined by the disc diffusion method (CLSI 2012). The isolates were sub-cultured on nutrient agar, incubated at 37 °C for 24 hours. Then, colonies of each of the isolate were adjusted to 0.5 McFarland's turbidity standard (equivalent to 1 x 10^8 colony forming unit/ml) in sterile nutrient broth. The standardized broth cultures were incubated for 10 minutes at 37°C and then inoculated onto sterile Mueller-Hinton agar using sterile swab sticks. Ten antibacterial agents (Oxoid®) belonging to 6 antibacterial classes were used and they included: ceftazidime (30 µg), cefotaxime (30 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), tetracycline (30 µg), streptomycin (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg) and sulphamethoxazole/trimethoprim (25 µg). The discs were placed strategically on the inoculated Mueller-Hinton agar plates. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition around each disc was measured with a meter rule. Each test was performed in triplicate and the mean inhibitory zone diameter (IZD) calculated to the nearest whole millimeter (mm) for each isolate and each antibacterial agent. The IZD was interpreted as susceptible, intermediate or resistant according to the Clinical and Laboratory Standards Institute (CLSI) (2014) criteria for aerobic isolates.

Results

Prevalence of generic ESBL-resistant enterobacterial isolates from dogs

Out of the total of 100 rectal swab samples processed for isolation of ESBL-resistant enterobacteria, 27 gave positive culture. Of the 27 ESBL-resistant isolates, 11 were *Escherichia coli*, 10 were *Klebsiella* species, 5 were *Salmonella* species, while 1 was *Proteus* species (Fig. 1).

Antibiogram of generic ESBL-resistant enterobacterial isolates from dogs

Out of the 11 ESBL-resistant *E. coli* isolates, all were resistant to ceftazidime and cefotaxime, 81.8% to ampicillin, 27.2% to streptomycin, 54.5% to ciprofloxacin and tetracycline, 45.4% to enrofloxacin, 90.9% to sulphamethoxazole / trimethoprim, and 9.1% to amoxicillin / clavulanic acid (Fig. 2). None of the *E. coli* isolates was resistant to gentamicin.

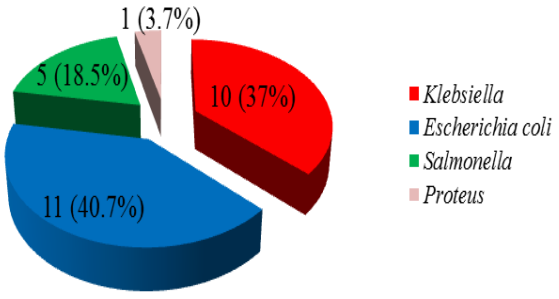

Fig. 1. Prevalence of extended-spectrum β-lactam-resistant enterobacterial isolates from dogs

Out of the 10 ESBL-resistant *Klebsiella* isolates, all (100%) were resistant to ceftazidime and cefotaxime, 80% to ampicillin, 20% to streptomycin and amoxicillin/clavulanic acid, 30% to ciprofloxacin and enrofloxacin, 60% to tetracycline, 90% to sulphamethoxazole / trimethoprim and 10% to gentamicin (Fig. 3).

Out of the 5 ESBL-resistant *Salmonella* isolates, all (100%) were resistant to ceftazidime, cefotaxime and sulphamethoxazole / trimethoprim, 80% to gentamicin and ampicillin, 60% to streptomycin and tetracycline, and 20% to amoxicillin / clavulanic acid (Fig. 4). None of the isolates was resistant to ciprofloxacin and enrofloxacin.

The ESBL-resistant *Proteus* isolate was resistant to ceftazidime, cefotaxime, streptomycin and gentamicin.

Out of the 11 *E. coli* isolates, 1 was resistant to one and two classes of the antibacterial agents tested while 9 were resistant to 3 or more classes (Fig. 5). Of the 10 *Klebsiella* isolates, 1 was resistant to one class of antibacterial agents tested, 2 were resistant to two classes while 7 were resistant to 3 or more antibacterial classes tested. All the *Salmonella* isolates were resistant to 3 or more classes of the antibacterial agents tested. The *Proteus* isolate exhibited resistance to two classes of antibacterial agents tested.

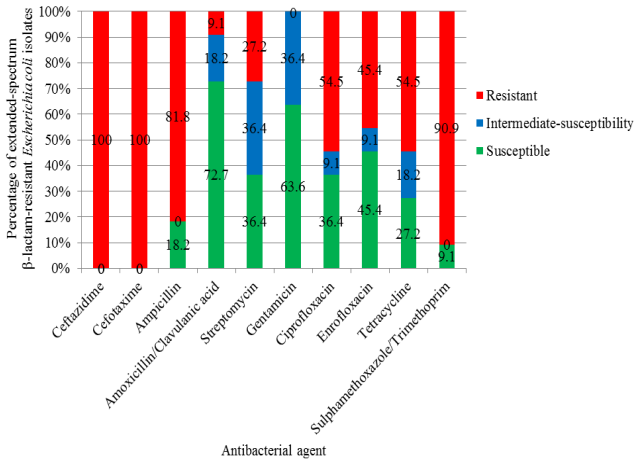

Fig. 2. Antibiogram of 11 generic extended-spectrum β-lactam-resistant *Escherichia coli* from dogs

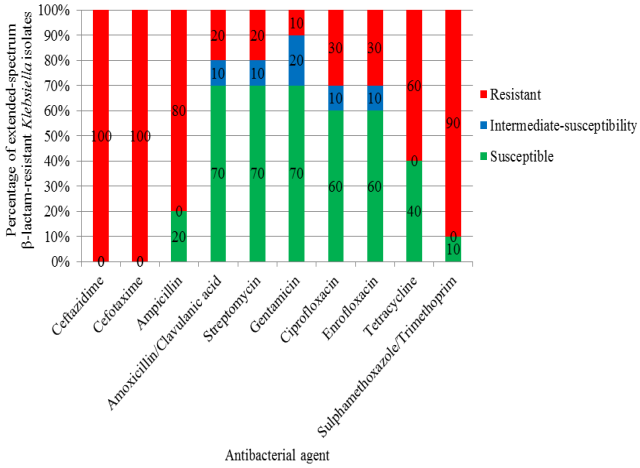

Fig. 3. Antibiogram of 10 generic extended-spectrum β-lactam-resistant *Klebsiella* isolates from dogs

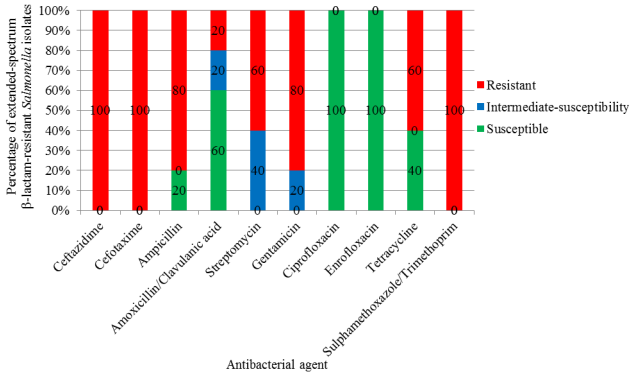

Fig. 4. Antibiogram of 5 generic extended-spectrum β-lactam-resistant *Salmonella* isolates from dogs

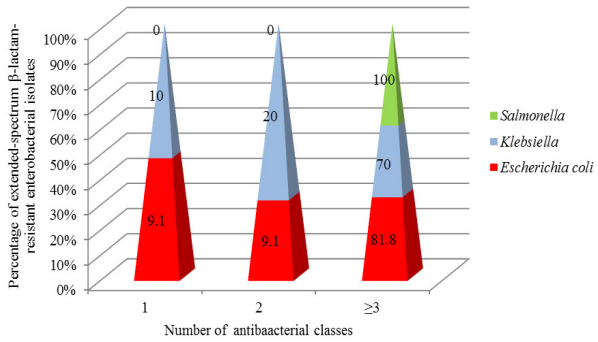

Fig. 5. Resistance of extended-spectrum β-lactam-resistant enterobacterial isolates to antibacterial classes

Discussion

Isolation of ESBL-resistant enterobacteria from 27 dogs (27%) among 100 apparently healthy dogs in this study, suggested a high prevalence of ESBL-resistant enterobacteria colonization of household dogs in Nsukka. This finding calls for concern because these dogs potentially serve as reservoir and disseminators (by faecal shedding) of these organisms into the environment thereby posing health threat to individuals that get in contact with them (Moreno *et al*., 2008; Sun *et al*., 2010; Meyer *et al*., 2012; Belas *et al*., 2014; Torkan *et al*., 2015; Carvalho *et al*., 2016). Environmental contamination by the isolates could result in their entry into the food chain (Woerther *et al*., 2013; Belas *et al*., 2014; Schaufler *et al*., 2015), thereby magnifying the health threat posed to humans and animals. Dog owners, children and veterinarians, could acquire these organisms following contact with faeces from the dogs or formites contaminated by the organisms (Sun *et al*., 2010; Ewers *et al*., 2012; Belas *et al*., 2014; Ljungquist *et al*., 2016; Torkan *et al*., 2016). Moreover, contact with companion (pet) animals have been linked with colonization of intercontinental travelers by ESBL-resistant enterobacteria; therefore, these organisms could spread from Nigeria to other parts of the globe (Ewers *et al*., 2010; van der Bij and Pitout, 2012; Belas *et al*., 2014; Woerther *et al*., 2013; Ljungquist *et al*., 2016).

The 27% ESBL-resistant enterobacteria isolation prevalence recorded in this study, is higher than 17 and 12% ESBL-producing enterobacteria isolation prevalence in rectal swabs of 174 and 202 healthy dogs/cats reported in Switzerland, respectively (Gandolfi-Decristophoris *et al*., 2013). It is also higher than 9, 28.9, 2 and 3.6% ESBL-resistant/producing enterobacterial isolation prevalence in 22 household dogs in Sweden, 58 faecal samples of healthy dogs, among clinical enterobacterial isolates from 65 dogs/cats/horses and 608 dogs/cats reported by Ljungquist *et al*. (2016), Cozma *et al*. (2015a), Dierikx *et al*. (2012) and Bogaerts *et al*. (2015) in Sweden, Romania, The Netherlands and Europe, respectively. The result is however lower than 62.1 and 30% ESBL-producing enterobacterial isolation prevalence in faecal samples of 29 healthy dogs/cats and among 110 dogs/cats/others reported by Cozma *et al*. (2015b) and Poirel *et al*. (2013) in Romania and France, respectively. A longitudinal study conducted in the Netherlands, reported 45-63% ESBL-producing enterobacterial isolation prevalence in faecal samples of 38 dogs (Baede *et al*., 2015); this result is also higher than the ESBL-resistant enterobacteria prevalence (27%) recorded in the present study. The variation in enterobacteria isolation prevalence reported in these studies may be due to disparity in the method of isolation/processing, media/concentration of extended-spectrum cephalosporin used for the isolation and type of sample (whether clinical or non-clinical) used in the various studies, and usage of extended-spectrum β-lactams in the various study areas (Damborg *et al*., 2015). ESBL-resistant *Enterobacteriaceae* have been isolated elsewhere using the same concentration of extended-spectrum cephalosporin as in this study (Schaufler *et al*., 2015)

Isolation of four enterobacteria genera (*E. coli*, *Klebsiella*, *Salmonella* and *Proteus*) in this study, suggested acquisition / transfer of genes encoding ESBLs by/among the enterobacterial isolates within the gut of the sampled dogs. Possible sources of isolates in this study include contaminated food (including pet foods and meat/meat products) and drinking water, dogs' environment (walking and living environment), veterinary hospital environment on previous visitation, owners/handlers, and/or formites contaminated by the ESBL-resistant organisms which the dogs had contact with during straying/scavenging (Caratolli *et al*., 2005; Murphy *et al*., 2010; Schaufler *et al*., 2015; Nilsson, 2015; Tuerena *et al*., 2016; Ljungquist *et al*., 2016). It is also possible that the isolates acquired genes encoding ESBLs from other organisms (commensals or transient pathogens) in the sampled dogs following previous exposure to extended-spectrum β-lactam used in treating the animals (Caratolli *et al*., 2005; Teurena *et al*., 2016; Ljungquist *et al*., 2016).

In this study, 40.7% *E. coli* isolation prevalence compared against *Klebsiella* (37%), *Salmonella* (18.5%) and *Proteus* (3.7%), suggested that *E. coli* may be the dominant ESBL-resistant enterobacteria genus colonizing dogs in Nsukka. The result also suggested that the *E. coli* isolates may have acquired ESBLs-encoding genes more than the other genera. It has been reported that *E. coli* acquires ESBLs more than any other enterobacterial genera in companion animals (SVARM, 2011). The highest *E. coli* isolation prevalence (40.7%) recorded in this study differs from the result of a similar study conducted in Enugu State, Nigeria, in which *Klebsiella* was the dominant (67.4% isolation prevalence) enterobacteria genus isolated from healthy pigs (Ugwu *et al*., 2015). The result of this study, also contrasted Poirel *et al*. (2013) who reported *Klebsiella*, with

51.5% prevalence, as the dominant genus among 33 ESBL-producing enterobacterial isolates from dogs/cats/others in France.

The 40.7% *E. coli* isolation prevalence in this study is higher than 17, 3.2, 24.5, 20, 1.9 and 3% ESBL-resistant/producing *E. coli* isolation prevalence reported in faecal samples of 53 healthy dogs in Mexico (Rocha-Gracia *et al*., 2015), oral swabs of 31 healthy dogs in Brazil (Oliveira *et al*., 2016), faecal samples of 109 healthy dogs in China (Sun *et al*., 2010), faecal/urine samples of companion animals in Chile (Moreno *et al*., 2008), 209 dog faecal deposits in Denmark (Damborg *et al*., 2015) and among 944 clinical *E. coli* isolates from companion animals in America (Shaheen *et al*., 2011), respectively. It is also higher than 16, 7, 6.6 and 2.6% ESBL-resistant/producing *E. coli* isolation prevalence in 100 extra-clinical faecal samples of dogs in Germany (Schaufler *et al*., 2015), among 445 clinical *E. coli* isolates from companion animals in United Kingdom (UK) (Timofte *et al*., 2016), faecal samples of 75 healthy dogs/cats (Costa *et al*., 2004) and 39 healthy dogs (Costa *et al*., 2008) reported in Portugal, respectively. Pomba *et al*. (2009), Tamang *et al*. (2012), Rzewuska *et al*. (2015), Caratolli *et al*. (2005), O'Keefe *et al*. (2010), Huber *et al*. (2013) and Feira *et al*. (2002) reported 1.6, 2.9, 28.2, 7, 5, 7.5 and 1.4% ESBL-producing/resistant *E. coli* isolation prevalence among 61, 730, 628, 298, 257, 107 and 72 *E. coli* isolates from urine/urinary tract of sick dogs in Portugal, dogs/cats in Poland, intestinal *E. coli* isolates from stray dogs in South Korea, sick/healthy dogs/cats/rat in Italy, urine/urinary tract of sick dogs/cats in America and Switzerland, and healthy dogs in Portugal, respectively. These results are also lower than 40.7% *E. coli* isolation prevalence recorded in the current study. In Brazil, Carvalho *et al*. (2016) reported 28.5% ESBL-resistant / producing *E. coli* isolation prevalence among 42 faecal *E. coli* isolates from household dogs, a finding that is also lower than the result (40%) of the current study. An Iranian study also reported lower ESBL-producing *E. coli* isolation prevalence in faecal samples of healthy cats (Akhtardanesh *et al*., 2015). The *E. coli* isolation prevalence in the present study, is however, lower than ESBL-producing *E. coli* isolation prevalence recorded among 20 faecal ESBL-producing enterobacterial isolates from healthy dogs/cats in Romania (Cozma *et al*., 2015b), 63 faecal *E. coli* isolates from hospitalized dogs in South Korea (So *et al*., 2012), 33 ESBL-producing enterobacterial isolates from companion animals in France (Poirel *et al*., 2013), 15 *E. coli* isolates from urine samples of 138 sick cats in Italy (Nebia *et al*., 2014) and in faecal samples of 20 each of healthy and diarrhoeic dogs in the Netherlands (Hordjik *et al*., 2013), respectively. It is also lower than 62% ESBL-resistant *E. coli* isolation prevalence in faecal samples from 13 sick dogs reported by Damborg *et al*. (2011) in Denmark. Murphy *et al*. (2009) reported 0% ESBL-resistant *E. coli* isolation prevalence among 188 dogs in America, a finding that contrasted the result of the present study.

The 37 % *Klebsiella* isolation prevalence in this study is higher than 15% ESBL-producing *Klebsiella* isolation prevalence among 20 faecal ESBL-producing enterobacterial isolates from healthy dogs/cats reported by Cozma *et al*. (2015b) in Romania. The least isolation prevalence of *Proteus* (3.7%) in this study may be attributed to the fact that this organism is mostly isolated from urinary tract of infected dogs than faecal samples of healthy animals (Feria *et al*., 2012). The variation in isolation prevalence of ESBL-resistant enterobacteria genera reported in these studies, may be related to the differences in sample size, type of sample processed (whether clinical or non-clinical), rate of contamination of dog's food/drinking water or environment by ESBL-resistant enterobacteria and usage of extended-spectrum cephalosporins in the various study areas (Hordjik *et al*., 2013; Baede *et al*., 2015).

It is recommended that phenotypic ESBLs production be detected using extended-spectrum β-lactam (such as ceftazidime, cefotaxime or cefpodoxime) with/without clavulanate in double disc synergy test (DDST) (CLSI, 2012). Recently, it was recommended that when using the new interpretative breakpoints (for EUCAST and CLSI), routine ESBL testing is no longer necessary and reporting of susceptibility results to penicillins and cephalosporins for ESBLs-producing *Enterobacteriaceae* should be 'as found' (Lerclerq *et al*., 2013; Timofte *et al*., 2016; CLSI, 2014). In the hereby experiment, resistance of the isolates to the drugs were tested using single discs. The fact that all the isolates (100%) in this study exhibited resistance to extended-spectrum β-lactams (ceftazidime and cefotaxime) tested, further suggested they produced ESBLs. The high rate of extended-spectrum β-lactam resistance in this study may be as a result of frequent use of extended-spectrum β-lactams in small animal medicine in Nigeria. Moreover, extended-spectrum β-lactams are also frequently used in Nigerian hospitals, and high prevalence of ESBL-resistant organisms in food animals, is a good indication that the drugs are used in these animals in the country (Torres *et al*., 2015; Ugwu *et al*., 2015). Thus, many potential sources of ESBLs genes existed, acquisition of the genes by the isolates might have been facilitated by poor personal and public hygiene in Nigeria (Woerther *et al*., 2013; Schaufler *et al*., 2013). The high extended-spectrum β-lactam resistance in this study, calls for real concern, because, if the selective pressure resulting from indiscriminate use of these critical drugs is not curtailed (by implementing antibacterial stewardship programs), the use of carbapenems would be resorted for treating multidrug-resistant infections in companion animals in Nigeria. Consequently, this would result in spread of superbugs (associated with untreatable and highly fatal infections) in companion animals and humans in Nigeria. The 100% cefotaxime resistance in this study is similar to 100% cefotaxime resistance reported among 32 clinical ESBL-resistant *E. coli* isolates from companion animals in UK (Timofte *et al*., 2016); 4 faceal ESBL-producing *E. coli* isolates from healthy dogs in Portugal (Costa *et al*., 2004), 8 uropathogenic ESBL-producing *E. coli* isolates from dogs/cats in Switzerland (Huber *et al*., 2013) and 21 ESBL-resistant *E. coli* isolates from sick/healthy pets in Italy (Caratolli *et al*., 2005), respectively. The 100% ceftazidime resistance in this study is also similar to 100% ceftazidime resistance reported by Timofte *et al*. (2016). Tuerena *et al*. (2016) reported 27.2% extended-spectrum cephalosporins resistance among *E. coli* isolates from companion animals/environment/hospital in Liverpool, United Kingdom. This finding is lower than the extended-spectrum cephalosporins resistance recorded in this experiment.

High ampicillin resistance observed in the hereby experiment, further suggested production of ESBLs by the isolates. Ampicillin is a β-lactam which is rendered ineffective by ESBLs (Ugwu *et al*., 2015). The high ampicillin resistance in this study could also be as a result of production of β-lactamases (a major mechanism of β-lactam resistance) which hydrolysed the β-lactam ring of the drug to penicilloic acid, change in cell wall permeability, expression of active efflux pump, gene mutation and/or altered penicillin binding protein (PBP) receptors in the isolates (Urmunova , 2015). The rate of ampicillin resistance (80-81.8%) among isolates in this study is lower than that (100%) observed among isolates of the same genera in healthy pigs in Enugu State, Nigeria (Ugwu *et al*., 2015).

The 81.8% *E. coli* ampicillin resistance in this study is lower than 100% ampicillin resistance among 21 ESBL-resistant *E. coli* isolates from sick/healthy pets, 8 uropathogenic ESBL-producing *E. coli* isolates from dogs/cats, 32 ESBL-resistant clinical *E. coli* isolates, and 14 ESBL-resistant *E. coli* isolates from extra-clinical faecal samples of dogs reported by Caratolli *et al*. (2005), Huber *et al*. (2013), Timofte *et al*. (2016) and Schaufler *et al*. (2015) in Italy, Switzerland, UK and Germany, respectively. The variation in ampicillin resistance recorded in these studies could be related to differences in usage of ampicillin in the various study areas (Ugwu *et al*., 2015). Ampicillin is not known to be a drug of choice in small animal medicine in Nigeria, but it is frequently used in humans (alone or in combination with cloxacillin) and food-producing animals (Chah and Nweze, 2001; Ugwu *et al*., 2015). Thus, these factors could also have contributed to high ampicillin resistance observed in this study (Ljungquist *et al*., 2016).

The *Proteus* isolate in this study was susceptible to ampicillin, suggesting lack of selection against the drug. This finding suggested that, although the isolate exhibited phenotypic extended-spectrum β-lactam resistance, it may not harbour genes encoding ESBLs (Caratolli *et al*., 2005; Beceiro *et al*., 2011). It also suggested that the extended-spectrum β-lactam resistance exhibited by the *Proteus* isolate occurred by other mechanisms such as expression of efflux pump, plasmid-mediated Amp C β-lactamases or plasmid-mediated metalo-β-lactamases (Batchelor *et al*., 2005; Damborg *et al*., 2011; Harada *et al*., 2014; Urmunova, 2015).

Demonstrable amoxicillin/clavulanic acid resistance among *E. coli* (9.1%), *Klebsiella* and *Salmonella* (20%) isolates in this study, suggested selection against the drug. This also suggested that *Klebsiella* and *Salmonella* isolates exerted more selection to the drug than the *E. coli* isolates. However, variation in number of isolates in each genus tested against amoxicillin/clavulanic acid in this study might be responsible for the discrepancies observed in the resistance rates. Nevertheless, the result suggested production of other β-lactamases such as AmpC cephalosporinases (Ben Sallem *et al*., 2012; Tamang *et al*., 2012; Timofte *et al*., 2016). Amp C cephalosporinases mediate resistance to 3rd-generation β-lactams/cephalosporins and are not inhibited by clavulanic acid and other β-lactamase-inhibitors such as sulbactam, avibactam and tazobactam (Ben Sallem *et al*., 2012; Tamang *et al*., 2012; Tuerena *et al*., 2016) while ESBLs are inhibited by clavulanic acid and other β-lactamase-inhibitors (Ben Sallem *et al*., 2012). Nonetheless, amoxicillin / clavulanic acid resistance observed in this experiment may be as a result of selection following the use of the drug in the animals (Seiffert *et al*., 2013; Tuerena *et al*., 2016). Potentiated amoxicillin is widely used for treating bacterial infections associated with β-lactamases in humans and small animals worldwide, including in Nigeria (Radford *et al*, 2011; Tuerena *et al*., 2016). Other possible mechanisms by which the isolates exerted selection to amoxicillin/clavulanic acid in this study include hyper-production of class A β-lactamases (TEM-1 or SHV-1), production of class D plasmid-mediated enzyme, chromosomal or plasmidic class C β-lactamase, ampicillinase C and/or modification of outer membrane permeability (Dwarz and Bonomo, 2010). The finding of moderate to high amoxicillin/clavulanic acid resistance in this study is a cause for some concern because the use of potentiated amoxicillin has been related to the development of ESBL resistance (especially AmpC-mediated) in enterobacterial isolates from humans and companion animals (Aldeyab *et al*., 2012; Johard *et al*., 2015; Tuerena *et al*., 2016). Again, organisms exhibiting resistance to potentiated amoxicillin are known to be multidrug resistant, thus posing huge health threat to the dogs and individuals (humans and animals) in contact with them (Tamang *et al*., 2012). However, the ESBLs produced by the isolates might also have mediated resistance to the amoxicillin component of the drug (Tuerena *et al*., 2016). Notably, isolates from healthy pigs in Enugu State, Nigeria, belonging to the genera reported in the present study, showed 100% amoxicillin/clavulanic acid resistance (Ugwu *et al*., 2015). Therefore, it is also possible that potentiated amoxicillin resistance observed in this study emanated from the food chain. The *Proteus* isolate in this study, was susceptible to amoxicillin/clavulanic acid, this suggested absence of selection against the drug.

The 9.1% *E. coli* amoxicillin/clavulanic acid resistance in this study, is higher when compared with 4 and 0% amoxicillin/clavulanic acid resistance among ESBL-resistant/producing *E. coli* isolates from dog faecal deposits and healthy/sick dogs/cats/rat reported by Caratolli *et al*. (2005) and Damborg *et al*. (2015) in Italy and Denmark, respectively. But it is lower than 57.1 and 71.4% amoxicillin/clavulanic acid resistance among ESBL-resistant *E. coli* isolates from companion animals reported by Caratolli *et al*. (2005) and Schaufler *et al*. (2015) in Italy and Germany respectively. It is also lower than 100% amoxicillin/clavulanic acid resistance among ESBL-resistant *E. coli* isolates from companion animals in Switzerland (Huber *et al*., 2013) and UK (Timofte *et al*., 2016).

In this study, higher rate of ciprofloxacin (54.5%) and enrofloxacin (45.4%) resistance among the *E. coli* isolates as against the *Klebsiella* and *Salmonella* isolates with 30 and 0% resistance to both drugs, respectively, suggested higher selection to these fluoroquinolones by the *E. coli* isolates than the other genera. The result also suggested that the *E. coli* isolates exerted selection against ciprofloxacin more than enrofloxacin. This moderate to high fluoroquinolones resistance may be due to acquisition of genes encoding fluoroquinolones resistance following use-selection pressure. This finding of moderate to high fluoroquinolones resistance in this study, calls for real concern, because these drugs are not known to be commonly used in small animal medicine in Nigeria. However, these drugs are frequently used in humans and food-producing animals in

Nigeria (Ugwu *et al*., 2015); thus, it is possible the fluoroquinolone resistance observed in this study, emanated from the food chain and/or environment (Schaufler *et al*., 2015; Ljungquist *et al*., 2016; Tuerena *et al*., 2016). Moreover, because dogs are allowed to stray and scavenge in Nigeria, the sampled dogs could also have acquired bacteria which haboured fluoroquinolone determinants (served as source of transfer of these genes to the isolates) from various sources. However, the ESBLs produced by the isolates could also have mediated resistance to fluoroquinolones in the isolates (Coque *et al*., 2008; Huber *et al*., 2013). Cross resistance of ESBL-resistant *Enterobacteriaceae* to fluoroquinolones have been reported (Jacoby, 2005; Frank *et al*., 2011; Schaufler *et al*., 2015).

Enterobacterial isolates in the same genera, obtained from healthy pigs in Enugu State, showed 12.9-50% ciprofloxacin resistance (Ugwu *et al*., 2015). The 54.5% *E. coli* ciprofloxacin resistance in this study is higher when compared with 26, 50 and 0% ciprofloxacin resistance reported by Pomba *et al*. (2009), Tamang *et al*. (2014) and Poirel *et al*. (2013) among 61 clinical *E. coli* isolates from dogs/cats in Portugal, 6 and 16 ESBL-producing *E. coli* isolates from stray dogs in South Korea and companion/domestic animals in France, respectively. But it is lower when compared with 100% ciprofloxacin resistance reported by So *et al*. (2012) and Huber *et al*. (2013) among 24 faecal ESBL-resistant *E. coli* isolates from hospitalized dogs in South Korea and 8 uropathogenic ESBL-producing *E. coli* isolates from dogs/cats in Switzerland, respectively. Timofte *et al*. (2016) recorded 65% ciprofloxacin resistance among clinical ESBL-resistant *E. coli* isolates from dogs in UK; this result is also higher than the findings (54.5%) of the present study. The 45.4% *E. coli* enrofloxacin resistance in this study is higher than 35.7 and 20% enrofloxacin resistance among ESBL-resistant/producing *E. coli* isolates from extra-clinical faecal samples of dogs and dog faecal deposists reported by Schaufler *et al*. (2015) and Damborg *et al*. (2015) in Germany and Denmark, respectively. It is lower than 92 and 100% enrofloxacin resistance among ESBL-producing *E. coli* isolates reported by Shaheen *et al*. (2011) and Huber *et al*. (2013) in America and Switzerland, respectively. The *Proteus* isolate in this study was susceptible to the fluoroquinolones (ciprofloxacin and enrofloxacin) tested.

In this study, resistance to aminoglycosides was observed among isolates in all the four genera. The rate of aminoglycoside resistance was higher among the *Salmonella* isolates (80% and 60% gentamicin and streptomycin resistance, respectively) than the *E. coli* and *Klebsiella* isolates with lower resistance to the drugs. This finding suggested that the *Salmonella* isolates exerted more selection to the aminoglycosides than the other genera. *Salmonella* have been reported to be highly resistant to aminoglycosides (CLSI, 2012; CLSI 2014). In fact, the CLSI recommended that *Salmonella* isolate which exhibited susceptibility to aminoglycoside, should be reported as resistant because these drugs may not be clinically effective against the isolate (CLSI, 2014). The *Proteus* isolate exhibited resistance to both gentamicin and streptomycin. The aminoglycosides resistance noted in this study, may be as a result of acquisition of genes encoding aminoglycoside resistance following use-selection pressure (Ugwu *et al*., 2015). Gentamicin and streptomycin (often combined with penicillin to exert a broad-spectrum effect) are frequently used for treating bacterial infections in humans and animals (especially in small animals) in Nigeria. It is also possible that the isolates acquired an aminoglycoside gene, which encoded resistance to the drugs (gentamicin and streptomycin) tested in this study. Febler *et al*. (2011) reported that a gene can encode resistance for many aminoglycosides. However, the ESBLs produced by the isolates could also have mediated the aminoglycoside resistance (Coque *et al*., 2008; Huber *et al*., 2013). Morosini *et al*. (2006) reported cross-resistance of ESBL-resistant enterobacteria to aminoglycosides. The aminoglycoside resistance in this study calls for serious concern, because there is a high correlation between gentamicin resistance and multiresistance profiles in nosocomial strains including ESBL-resistant enterobacteria (Meirelles-Pereira *et al*., 2002; Carvalho *et al*., 2016).

The 0% *E. coli* gentamicin resistance in this study is similar to 0% gentamicin resistance among 8 faecal ESBL-resistant *E. coli* isolates from dogs reported by Damborg *et al*. (2011) in Denmark. It is lower than 64.3, 66.7, 33.3, 100 and 20% gentamicin resistance observed among ESBL-resistant / producing *E. coli* isolates from extra-clinical faecal samples from dogs in Germany (Schaufler *et al*., 2015), stray dogs in South Korea (Tamang *et al*., 2012), sick/healthy dogs/cats/rat in Italy (Caratolli *et al*., 2005), and sick cats/dogs in Switzerland (Huber *et al*., 2013), respectively. The 10% gentamicin resistance among *Klebsiella* isolates in this study is lower when compared with 100% gentamicin resistance among 16 ESBL-producing *Klesbsiella* isolates from rectal/urine samples of companion/domestic animals reported by Poirel *et al*. (2013) in France. The 27.2% *E. coli* streptomycin resistance in this study is lower than 50, 66.7 and 75% streptomycin resistance reported by Tamang *et al*. (2012), Caratolli *et al*. (2005) and Costa *et al*. (2004) in South Korea, Italy and Portugal, respectively. The variation in aminoglycosides resistance observed in these studies may be due to differences in their usage in the study areas and/or the number of isolates tested in the various studies. The variations may also be related to differences in the rate of production of ESBLs by the isolates as well as the rate of acquisition of aminoglycoside determinants (Coque *et al*., 2008; Huber *et al*., 2013). ESBLs have been reported to mediate resistance to aminoglycosides (Huber *et al*., 2013).

High (54.5% for *E. coli* and 60% for *Klebsiella* and *Salmonella*) tetracycline resistance observed among the isolates in this study, suggested selection against the drug. The tetracycline resistance may be due to acquisition of genes encoding tetracycline resistance as a result of use-selection pressure. In Nigeria, 5% (short-acting) tetracycline is frequently used (due to its broad-spectrum effect) in small animal clinical practice while the long-acting (20%) tetracycline and other forms of the drug, are extensively used in humans and food-producing animals (Ugwu *et al*., 2015). Thus, selective pressure for tetracycline resistance might have emanated from various sources. ESBLs have also been reported to mediate tetracycline resistance in enterobacteria (Huber *et al*., 2013). The 54% *E. coli* tetracycline resistance in this study is higher than 12.5% tetracycline resistance among 8 ESBL-resistant *E. coli* isolates from dogs reported by (Damborg *et al*., 2011) in Denmark. But, it is lower than 75, 95.2, 75 and 100% tetracycline

resistance observed among ESBL-producing *E. coli* isolates from companion animals in South Korea (Tamang *et al*., 2012), Italy (Caratolli *et al*., 2005), and Portugal (Costa *et al*., 2004). In Switzerland (Huber *et al*., 2013) and UK (Timofte *et al*., 2016), 100% tetracycline resistance was observed among ESBL-resistant/producing *E. coli* isolates from companion animals; this result is also higher than that of the current work. The 60% *Klebsiella* tetracycline resistance observed in this study is lower when compared with 100% tetracycline resistance among ESBL-producing *Klesbsiella* isolates from rectal/urine samples of companion/domestic animals reported in France (Poirel *et al*., 2013).

In this study, tetracycline resistance was not observed in the *Proteus* isolate; thus, suggesting it did not exert selection against the drug. This finding suggested that the isolate may not be *P. mirabilis* which is intrinsically resistant to tetracycline (Magiorakos *et al*., 2011; Dierikx *et al*., 2012)

The 100% sulphamethoxazole/trimethoprim resistance among *Salmonella* isolates in this study, suggested complete selection to potentiated sulphonamide in this genus. It also suggested that *Salmonella* isolates exerted selection to the drug more than the *E. coli* and *Klebsiella* isolates with 90.9 and 90% sulphamethoxazole/trimethoprim resistance, respectively. The high potentiated sulphonamide resistance among the isolates may be due to selection following acquisition of genes encoding potentiated sulphonamide resistance (Torkan *et al*., 2015). Sulphamethoxazole/trimethoprim is frequently used in humans and animals (mostly in canine clinical practice) in Nigeria (Ugwu *et al*., 2015). The 90.9% *E. coli* sulphamethoxazole/trimethoprim resistance in this study is higher than 59, 60 and 58.3% sulphamethoxazole / trimethoprim resistance reported by Timofte *et al*. (2016), Damborg *et al*. (2015) and Tamang *et al*. (2012) in UK, Denmark and South Korea, respectively. It is lower than 95.2, 100 and 85.7% sulphamethoxazole trimethoprim resistance among ESBL-resistant/producing *E. coli* isolates from companion animals, and extra-clinical faecal samples of dogs reported by Caratolli *et al*. (2005), Huber *et al*. (2013) and Schaufler *et al*. (2015) in Italy, Switzerland and Germany, respectively. The 90% sulphamethoxazole / trimethoprim among *Klebsiella* isolates in this study is lower when compared with 100% sulphamethoxazole/triemthoprim resistance among 16 ESBL-producing *Klesbsiella* isolates from faecal/urine samples of companion/domestic animals reported by Poirel *et al*. (2013) in France. Variations in potentiated sulphonamide resistance in these studies may be related to differences in method used for antimicrobial sensitivity and drug concentration in the various studies, and usage of potentiated sulphonamide in the various study areas. In the present study, selection against potentiated sulphonamide was not observed in *Proteus* isolate.

Resistance to three or more classes of antibacterial agent implies multidrug resistance (Tenover 2006; Nam *et al*., 2010; Rzewuska *et al*., 2015). In this study, resistance of all the *Salmonella* isolates to three or more of the antibacterial agents suggested that isolates in the genus exhibited higher multidrug resistance when compared with the multidrug resistance of the *E. coli* (81.8%) and *Klebsiella* (70%) isolates. The 81.8% *E. coli* multidrug resistance in this study is lower than 93.9 and 100% multidrug resistance among ESBL-resistant/producing *E. coli* isolates from extra-clinical faecal samples of dogs and healthy pets reported by Schaufler *et al*. (2015) and Caratolli *et al*. (2005) in Germany and Italy, respectively. The result is however higher than 31.3 and 66.7% multidrug resistance among 112 ESBL-resistant *E. coli* isolates from companion/domestic animals and 12 ESBL-producing *E. coli* isolates from stray dogs reported by Filipovic *et al*. (2007) and Tamang *et al*. (2012) in Montenegro and South Korea, respectively. The *Proteus* isolate in this study exhibited resistance to two classes (β-lactam and aminoglycoside) of antibacterial agents tested. Therefore, it may not be regarded as a multidrug-resistant strain. The high multidrug resistance exhibited among enterobacterial isolates in this study, poses huge health threat to individuals (humans and animals) that have direct physical (dog owners, dog caretakers/handlers, children and veterinarians) and/or indirect (general public via contact with the primary contacts, environment and the food chain) contact with the dogs (Torkan *et al*., 2015; Srisanga *et al*., 2016). These individuals could acquire these multidrug resistant organisms. Consequently, there could be compromise in antibacterial therapy in colonized and/or infected individuals (Ugwu *et al*., 2015).

Conclusions

This study has shown that ESBL-resistant enterobacteria are harboured by a sizeable percentage (27%) of household dogs in Nsukka, Southeastern Nigeria. *E. coli* is the most prevalent genus of ESBL-resistant enterobacteria colonizing household dogs within the study area. Thus, household dogs in the study area are potential reservoirs and disseminators of ESBL-resistant enterobacteria and genes encoding ESBLs production. This has tremendous impact on the ecology of antibacterial resistance and potentially it can impact negatively on the food chain. Therefore, attention should be paid on the use of extended-spectrum β-lactams in companion animals in Nigeria. However, further studies to determine the types of ESBLs genes harboured by the isolates are recommended.

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