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; Schaufer 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; Hordijk 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; Tuenera 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...
a resistant enterobacteria in companion sampling. The samples were resistant (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; Blak 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; Schaufler et al., 2015; Ljungquist et al., 2016). As commensal organisms in the intestinal tract of animals, ESBL-resistant enterobacteria harboured by dogs may be discharged into the environment, thereby serving as disseminators of genes encoding β-lactam resistance (Costa et al., 2008; Pfiffer 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 (Caratoli 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 fecal 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; Gibson 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; Caratoli et al., 2005; Poiret 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; Inwezeru 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.

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.
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), Dieriks 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. (2015) 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 (Carattoli et al., 2005; Murphy et al., 2010; Schaufler et al., 2015; Nilsson, 2015; Tuereña 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 (Carattoli 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/other animals in France.

The 40.7% E. coli isolation prevalence in this study is higher than 32, 24.5, 20, 19 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 (Poiré 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 (Hordijk 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 (Hordijk 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 clavulante 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 ESBL-producing Enterobacteriaceae should be ‘as found’ (Lerclerc 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 faecal 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). Tuarena 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 penicillioic 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 (Ljunghquist 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 cephalosporinas (Ben Sallem et al., 2012; Tamang et al., 2012; Timofte et al., 2016). Amp C cephalosporinas 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; Tuergena 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; Tuergena 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; Tuergena et al., 2016). Other possible mechanisms by which the isolate 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; Tuergena 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 (Tuergena 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 genra. 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; Tuermen *et al*., 2016). Moreover, because dogs are allowed to stray and scavenge in Nigeria, the sampled dogs could also have acquired bacteria which harboured 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 deposits 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. Feblner *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 *Klebsiella* 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).

The 54% 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 (Caratoli et al., 2005), and Portugal (Costa et al., 2004). In Switzerland (Huber et al., 2013) and UK (Timothe 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 *Klebsiella* 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 Timothe 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 Caratoli 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/trimethoprim resistance among 16 ESBL-producing *Klebsiella* 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 Caratoli 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 harbourited 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.

References


