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

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Abstract

This study was conducted to isolate generic extended-spectrum β-lactam (ESBL)-resistant enterobacteria from pigs reared in Enugu State Southeast, Nigeria and determine the antibacterial resistance profile of the isolates. Rectal swabs were collected from 190, randomly selected, apparently healthy pigs. Isolation of ESBL-resistant enterobacteria was done using Mac Conkey agar supplemented with 2 µg/ml of cefotaxime. 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 46 ESBL-resistant enterobacterial isolates, 4 (8.7%) were *Escherichia coli*, 11 (23.9%) were *Salmonella* species, while 31 (67.4%) were *Klebsiella* species. Resistance of the *Salmonella* isolates was 45.5% to ciprofloxacin, 36.4% to ofloxacin and levofloxacin, 9.1% to norfloxacin, amikacin and gentamicin, 27.3% to streptomycin, 72.7% to chloramphenicol and 90.9% to tetracycline. Resistance of the *Klebsiella* isolates was 93.5% to ampicillin, 12.9% to ciprofloxacin, 19.4% to ofloxacin and levofloxacin, 9.7% to norfloxacin and streptomycin, 64.5% to chloramphenicol and 38.7% to tetracycline. Resistance of the *E. coli* isolates was 100% to gentamicin, 75% to ampicillin and streptomycin, 50% to ciprofloxacin, norfloxacin, chloramphenicol and tetracycline, and 25% to ofloxacin, levofloxacin and amikacin. All the isolates were resistant to ceftriaxone, cefotaxime, ceftazidime, cefepime, cefpodoxime, amoxicillin/clavulanic acid and aztreonam. Resistance of the isolates to more than 3 classes of antibacterial agents tested was 54.8% for *Klebsiella*, 90.9% for *Salmonella* and 100% for *E. coli*, respectively. This study has shown that pigs reared in Enugu State Southeast, Nigeria, are colonized by ESBL-resistant *Enterobactericeae* and are potential reservoirs and disseminators of these organisms.

Keywords: antibiogram, β-lactam-resistant, Enterobacteriaceae, extended-spectrum, porcine

Introduction

Antimicrobial resistance has recently been identified as one of the greatest threat to human health and developing countries, including Nigeria, are worst hit by this crisis (WHO, 2014). Extensive use of antibacterial agents in food-producing animals has been rated a major cause of this crisis (Geser *et al*., 2012). The situation is worsened by indiscriminate use of antibacterial agents, especially β-lactams, in sub-therapeutic/therapeutic doses for growth promotion, prophylaxis and treatment of bacterial diseases in food-producing animals (Waters *et al*., 2011). Developing countries, including Nigeria, are major culprits due to the lack of strict policy regarding the use of antibacterial agents in humans and food-producing animals (Wall, 2014). This has led to increased resistance to most of the antibacterial agents (especially the β-lactams) (CLSI, 2012). Enterobacteriaceae constitute normal bacterial flora in intestinal tract of food-producing animals and humans (Ben Sallem *et al*., 2012). Indiscriminate use of β-lactams (particularly 3rd- [e.g. cefotaxime, ceftriaxone, cefpodoxime, ceftazidime] and 4th- [e.g. cefepime] generation cephalosporins) has led to selection pressure and development of resistance to these drugs by enterobacteria (Geser *et al*., 2011). These 3rd and 4th-generation cephalosporins are extended-spectrum β-lactams (ESBL) whose resistance in enterobacteria is mediated by extended-spectrum beta-lactamases (ESBLs) (Garcia-Graells *et al*., 2010; Geser *et al*., 2011).

Extended spectrum β-lactam (ESBL)-resistant enterobacteria also exhibit resistance to many other classes of antibacterial agents including aminoglycosides, fluoroquinolones, phenicols and potentiated sulfonamides (Gniadikowski, 2001; Carattoli, 2008; Geser *et al*., 2012). Thus, ESBL-resistant enterobacterial isolates are multidrug

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resistant (Li *et al*., 2014). This multidrug resistance accounts for the limited therapeutic options available for treating infections associated with ESBL-resistant enterobacteria (Blaak *et al*., 2014). ESBL-resistant enterobacteria are the most prevalent causes of nosocomial and community-acquired infections globally (Li *et al*., 2014). These infections are often fatal due to limited therapeutic options, while the treatment option is using the last resort drug carbapenems (Canton *et al*., 2012; Blaak *et al*., 2014). This has also resulted in the recent increasing numbers of enterobacterial resistance to the carbapenems (Zurflu *et al*., 2013; Blaak *et al*., 2014). Therefore, determination of antibacterial resistance profile of ESBL-resistant enterobacteria is useful for empirical treatment of infections associated with such organisms.

As commensal organisms in the intestinal tract, ESBL-resistant enterobacteria harboured by food-producing animals could be discharged into the environment, thereby serving as disseminators of genes encoding for β-lactam resistance. 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 (Garcia-Graells *et al*., 2010). Presence of ESBL-resistant enterobacteria in faecal samples of food-producing animals represents a risk for carcass contamination at slaughter and subsequent potential for contamination of retail meat products (Geser *et al*., 2011). This contamination risk is particularly important in developing countries, such as Nigeria, where inadequate hygienic practices are employed during meat processing. Consumption of contaminated meat results in colonization of humans with ESBL-resistant bacteria, jeopardizing subsequent antibacterial therapies in carriers (Geser *et al*., 2011).

Isolation of ESBL-resistant enterobacteria in meat and meat products raised questions about the presence of ESBL-resistant organisms in food-producing animals (Jensen *et al*., 2006; Jouini *et al*., 2007; Hammad *et al*., 2008; Doi *et al*., 2010). There have been improvements in isolation of ESBL-resistant enterobacteria in food-producing animals worldwide (Geser *et al*., 2011). Zoonotic transmission of ESBL-producing enterobacteria has been reported (Ewers *et al*., 2012; Schmeidel *et al*., 2014). With the current global target of "one world, one health", efforts are being intensified to detect sources of antibacterial resistance (especially β-lactam resistance) in order to curb its spread in both humans and animals (Wall, 2014). Surveillance studies to assess food-producing animals as potential reservoirs and disseminators of ESBL-resistant bacteria have been conducted in several countries in Europe (Aestrup *et al*., 2006; Meunier *et al*., 2006; Cloeckaert *et al*., 2007; Girlich *et al*., 2007; Smet *et al*., 2008; Wu *et al*., 2008; Cortes *et al*., 2010; Goncalves *et al*., 2010; Geser *et al*., 2011; Geser *et al*. 2012; Hammerrum *et al*., 2014), Asia (Duan *et al*., 2006; Tian *et al*., 2009; Yuan *et al*., 2009; Li *et al*., 2014; Wang *et al*., 2014), Africa (Jouini *et al*., 2007; Ben Sallem *et al*., 2012), North America (Wittum *et al*., 2010) and South America (Fernandes *et al*., 2009). Unfortunately, there is paucity of information on ESBL-resistant bacteria in food-producing animals in Nigeria. In available literature, there are four reports (Chah and Oboegbulem, 2007; Eze *et al*., 2013; Duru *et al*. 2013 and Inwerezua *et al*., 2014) on surveillance of food-producing animals as reservoirs of ESBL-resistant enterobacteria. Chah and Oboegbulem (2007), Eze *et al*. (2013) and Duru *et al*. (2013) detected 9.4% among 172 ampicillin-resistant *E. coli*, 25.5% and 22.2% among 141 *E. coli* isolates from chicken in Enugu, Ebonyi and Imo States in Southeast, Nigeria, respectively, whereas Inwerezua *et al*. (2014) detected no ESBL-resistant isolate in healthy cattle in Oyo State Southwestern, Nigeria. No study has been conducted to assess pigs reared and slaughtered in Enugu State Southeast, Nigeria, as potential reservoirs of ESBL-resistant organisms, whereas pigs constitute a major source of animal protein (because it is relatively cheaper than beef, chicken, chevron etc.) for the populace of Enugu State, Nigeria. These pigs may harbor ESBL-resistant enterobacteria which are consumed together with the pork meat by the population, hence increasing the dissemination of antimicrobial resistance genes. Therefore, there is need to assess pigs reared and slaughtered in Nigeria as reservoirs of ESBL-resistant organisms. More importantly, determination of antibiogram of isolates from food-producing animals is essential for monitoring the spread of resistance in food-borne bacteria. This would help in evaluation of trends and identification of mitigation strategies. The objective of this study, therefore, was to isolate ESBL-resistant enterobacteria from pigs reared in Enugu State, 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 June 2013. Out of the 17 Local Government Areas (LGAs) in Enugu State, six were selected purposively for the study: Igbo-Eze North, Nsukka, Udenu, Udi, Eziagu and Enugu North. In these LGAs, 16 pig farms were visited. Rectal swabs were collected using sterile swab sticks from 190 randomly selected pigs reared in the farms. Each Local Government Area 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 ESBLs-resistant enterobacteria from pigs

The swabs were cultured on Mac Conkey agar supplemented with 2 μg/ml of cefotaxime 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 following standard methods.

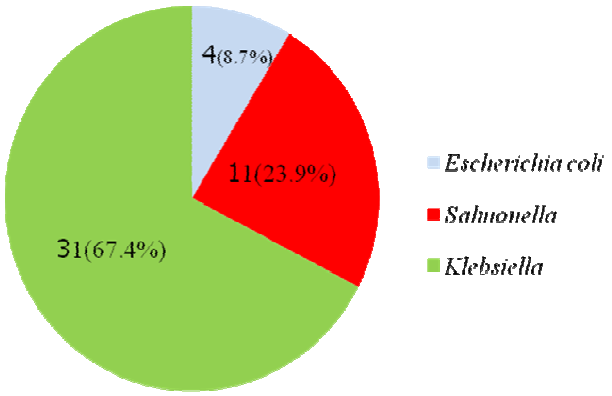

Fig. 1. Prelevance of extended-spectrum ß-lactam-resistant enterobacterial isolates from pigs

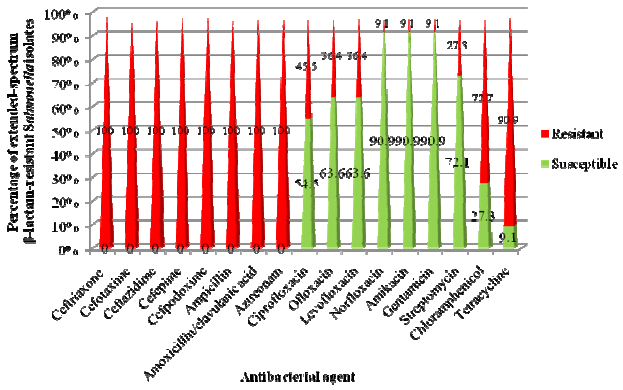

Fig. 2. Antibiogram of extended-spectrum ß-lactam-resistant *Salmonella* isolates from pigs

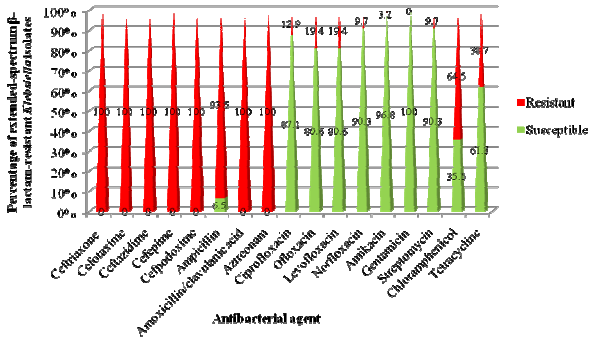

Fig. 3. Antibiogram of extended-spectrum ß-lactam-resistant *Klebsiella* isolates from pigs

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

Antibacterial 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

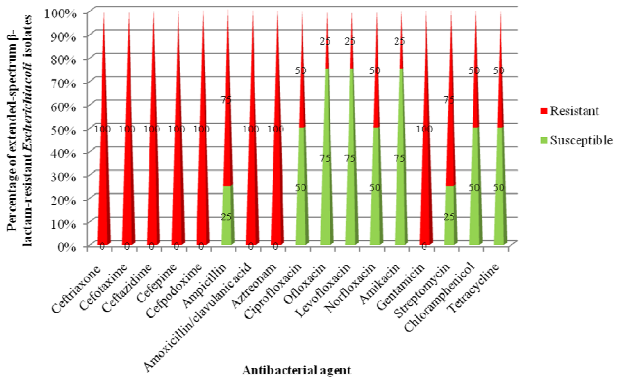

Fig. 4. Antibiogram of extended-spectrum ß-lactam-resistant *Escherichia coli* isolates from pigs

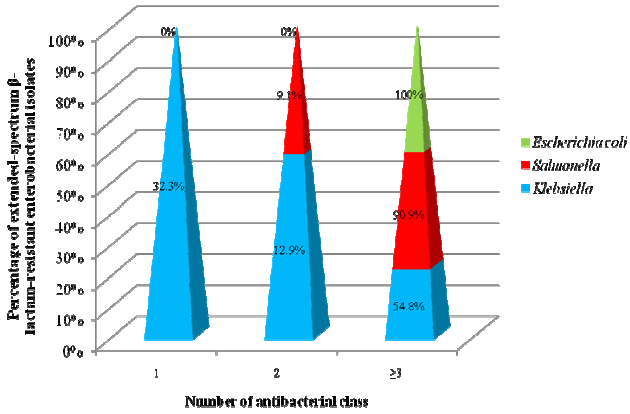

Fig. 5. Resistance of extended-spectrum ß-lactam-resistant enterobacterial isolates to antibacterial classes

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 plates using sterile swab stick. Seventeen antibacterial agents (Oxoid®) belonging to 5 antibacterial classes were used and they included: cefepime (30 µg), cefotaxime (30 µg), cefpodoxime (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), norfloxacin (10 µg), ofloxacin (5 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), streptomycin (10 µg), tetracycline (30 µg), gentamicin (10 µg), amikacin (30 µg), chloramphenicol (30 µg), amoxicillin/clavulanic acid (20/10 µg), aztreonam (30 µg) and ampicillin (10 µg). The discs were placed strategically on the inoculated Mueller-Hinton agar plate. 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 millimeters 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) (2012) criteria for aerobic isolates. For the purpose of the study, isolates with intermediate-susceptibility were classified as resistant.

Results

Prevalence of generic ESBL-resistant enterobacterial isolates from pigs

Out of the total of 190 rectal swab samples processed for isolation of ESBL-resistant enterobacteria, 46 (24.2%) gave positive culture. Of the 46 ESBL-resistant isolates, 4 (8.7%) were *E. coli*, 11 (23.9%) were *Salmonella* species, while 31 (67.4%) were *Klebsiella* species (Fig. 1).

Out of the 11 ESBL-resistant *Salmonella* isolates, all (100%) were resistant to ceftriaxone, cefotaxime, ceftazidime, cefepime, cefpodoxime, ampicillin, amoxicillin/clavulanic acid and aztreonam, 5 (45.5%) to ciprofloxacin, 4 (36.4%) to ofloxacin and levofloxacin, 1 (9.1%) to norfloxacin, amikacin and gentamicin, 3 (27.3%) to streptomycin, 8 (72.7%) to chloramphenicol and 10 (90.9%) to tetracycline (Fig. 2).

Out of the 31 ESBL-resistant *Klebsiella* isolates, all (100%) were resistant to ceftriaxone, cefotaxime, ceftazidime, cefepime, cefpodoxime, amoxicillin/clavulanic acid and aztreonam, 29 (93.5%) to ampicillin, 4 (12.9%) to ciprofloxacin, 6 (19.4%) to ofloxacin and levofloxacin, 3 (9.7%) to norfloxacin and streptomycin, 20 (64.5%) to chloramphenicol and 12 (38.7%) to tetracycline (Fig. 3). None (0%) of the *Klebsiella* isolates was resistant to gentamicin.

Out of the 4 ESBL-resistant *E. coli* isolates, all (100%) were resistant to ceftriaxone, cefotaxime, ceftazidime, cefepime, cefpodoxime, amoxicillin/clavulanic acid, aztreonam and gentamicin, 3 (75%) to ampicillin and streptomycin, 2 (50%) to ciprofloxacin, norfloxacin, chloramphenicol and tetracycline and 1 (25%) to ofloxacin, levofloxacin and amikacin (Fig. 4).

Out of the 31 *Klebsiella* isolates, 10 (32.3%) were resistant to one class of the antibacterial agents tested, 4 (12.9%) to two classes, while 17 (54.8%) were resistant to three or more of the antibacterial agents tested (Fig. 5). Of the 11 *Salmonella* isolates, 1 (9.1%) was resistant to two antibacterial classes tested, while 10 (90.9%) were resistant to three or more classes. All (100%) the *E. coli* isolates were resistant to three or more classes of the antibacterial agents tested.

Discussion

In this study, the prevalence and antibiogram of ESBL-resistant enterobacterial isolates from pigs reared in Enugu State, Nigeria, was determined. The fact that 46 (24.2%) enterobacteria isolates were obtained from the cultured rectal swab samples using Mac Conkey agar supplemented with 2 µg/ml of cefotaxime, suggested that the isolates were ESBL-resistant enterobacteria. Cefotaxime is a 3rd-generation cephalosporin (extended-spectrum β-lactam) and its resistance is mediated by ESBLs (Geser *et al*., 2012). Therefore, the growth of the isolates on the extended-spectrum β-lactam-supplemented medium suggested that they might have produced ESBLs. ESBL-resistant enterobacteria isolation prevalence of 24.2% recorded in this study is higher than 8.4% prevalence of ESBL-producing *Enterobacteriaceae* reported by Reist *et al*. (2013) in calves slaughtered in Switzerland. Isolation of three genera (*Klebsiella*, *E. coli* and *Salmonella*) of ESBL-resistant enterobacteria in this study, suggested that there could have been acquisition/transfer of genes encoding for ESBLs production by/among the enterobacterial organisms (Schmeidel *et al*., 2014). The 67.4% *Klebsiella* isolation prevalence suggested that the genus may have the capacity of acquiring genes encoding for ESBLs more than *Salmonella* and *E. coli* with isolation prevalence of 23.9% and 8.7%, respectively. Among ESBL-resistant enterobacteria, *Klebsiella* acquired the greatest variety of genes encoding for ESBLs production and it produced the greatest variety of ESBLs (Paterson *et al*., 2003; Nobrega *et al*., 2013). Studies conducted elsewhere in Africa also reported isolation of ESBL-resistant *Klebsiella* more frequently than any other genus in *Enterobacteriaceae* (Blomberg *et al*., 2005; Gangoue-Pieboji *et al*., 2005). The 67.4% isolation prevalence of ESBL-resistant *Klebsiella* in the present study is higher than 28.6% isolation prevalence of ESBL-producing *Klebsiella* reported by Guandong and Avci (2013) in food of animal origin in Turkey.

Salmonella was isolated in this study at the prevalence of 23.9%. Isolation of ESBL-resistant *Salmonella* from food-producing animals have been reported in Brazil (Fernandes *et al*., 2009), Germany (Rodriguez *et al*., 2009) and from a slaughterhouse and poultry farm in Korea (Bae *et al*., 2013) and Spain (Silva *et al*., 2013), respectively.

In the current study, isolation prevalence of ESBL-resistant *E. coli* was 8.7%. This result is lower than 9.4% isolation prevalence of ESBLs-producing *E. coli* reported by Chah and Oboegbulem (2007) among 171 ampicillin-resistant *E. coli* isolates from chicken in Nigeria. Smet *et al*. (2008), Geser *et al*. (2011), Garcia-Graells *et al*. (2010) and Wasinski *et al*. (2013) reported 60, 92.3, 42 and 11.7% isolation prevalence of ESBLs-producing *E. coli* in poultry carcasses in Belgium, broilers in Belgium, pigs and cattle in Switzerland, and meat samples in Poland, respectively. In the Netherlands, Overdevest *et al*. (2011) and Leverstein-van Hall *et al*. (2011) reported 80 and 94% prevalence of ESBLs-producing *E. coli* in chicken meat and raw meat, respectively. Escudero *et al*. (2010), Schmid *et al*. (2013), Adenaike *et al*. (2013), Ben Sallem *et al*. (2012) and Guandong and Avci (2013) reported 72, 21.4, 45, 13.8 and 44.4% prevalence of ESBL-producing *E. coli* from pigs in Spain, beef cattle in Germany, roasted beef in Nigeria, food-producing animals in Tunisia and food of animal origin in Turkey, respectively. The isolation prevalence of ESBL-resistant *E. coli* (8.7%) in this study is lower when compared with those of the previous studies. The variation in isolation prevalence of ESBL-resistant enterobacteria recorded in these studies could be related to differences in the use of β-lactams in human and veterinary medicine in the various study areas. The findings of this study therefore suggest sizeable prevalence of ESBL-resistant enterobacteria in pigs reared in Enugu State, Nigeria. This portends huge adverse health impact on the food chain in the study area.

In the disc synergy/combination test for phenotypic detection of ESBLs production, ceftazidime and cefotaxime plus clavulanic acid (clavulanate) discs were used (Warren *et al*., 2001; CLSI, 2012). Resistance of the isolates to the drugs was tested in the hereby study (with single discs) to further confirm that they produced ESBLs. The fact that all

tested isolates were resistant to the extended-spectrum β-lactams tested (e.g. cefepime, cefpodoxime, ceftriaxone, ceftazidime and cefotaxime) further suggested that they produced extended-spectrum β-lactamases (ESBLs). ESBLs are hydrolytic enzymes that breakdown the β-lactam ring of β-lactams, extended-spectrum β-lactams (3rd and 4th-generation cephalosporins/β-lactams) and monobactams (Garcia-Graells *et al*., 2010). The 100% resistance to ESBL in this study is similar to the findings of Duru *et al*. (2013) among ESBLs-producing *E. coli* isolates from poultry in Imo State, Nigeria. The 100% cefotaxime resistance observed in this study is higher than 84.6% cefotaxime resistance reported by Schmid *et al*. (2013) among ESBLs-producing *E. coli* isolates from beef cattle in Germany. High rates (100%) of resistance to ampicillin and aztreonam among all the genera of enterobacterial isolates were observed in the hereby experiment, further suggesting the production of ESBLs by the isolates. These drugs (ampicillin – β-lactam and aztreonam – monobactam) are β-lactams which are rendered ineffective by ESBLs (Garcia-Graells *et al*., 2010). Ampicillin resistance rate (100%) recorded in this study is similar to the findings of Guandong and Avci (2013), who reported 100% ampicillin resistance among ESBLs-producing *Klebsiella* and *E. coli* isolates from food of animal origin in Turkey. The recorded 100% ampicillin resistance in this study is higher than 75% ampicillin resistance reported among ESBLs-producing *E. coli* isolates from roasted beef in Nigeria (Adenaike *et al*., 2013). Rate of resistance (100%) to aztreonam by all the genera in the present study is higher when compared with 65.4 and 28.9% aztreonam resistance reported by Schmid *et al*. (2013) and Guandong and Avci (2013) among ESBL-resistant *E. coli* isolates from beef cattle in Germany and food of animal origin in Turkey, respectively. It is also higher than 42.9% aztreonam resistance reported by Guandong and Avci (2013) among ESBLs-producing *Klebsiella* isolates in Turkey.

High rate (100%) of amoxicillin/clavulanic acid by all the genera in this study suggestsed that the isolates might have produced other β-lactamases such as AmpC cephalosporinases. AmpC cephalosporinases mediate resistance to 3rd-generation β-lactams/cephalosporins, but are not inhibited by clavulanic acid and other β-lactamase inhibitors (such as sulbactam and tazobactam) (Ben Sallem *et al*., 2012). Conversely, the ESBLs are inhibited by clavulanic acid and other β-lactamase inhibitors (Garcia-Graells *et al*., 2010; Ben Sallem *et al*., 2012). Amoxicillin/clavulanic acid resistance rate (100%) in this study is higher when compared with 53.8, 6.7 and 90% amoxicillin/clavulanic acid resistance reported by Schmid *et al*. (2013), Guandong and Avci (2013) and Duru *et al*. (2013) among ESBL-producing *E. coli* isolates from cattle in Germany, food of animal origin in Turkey and poultry in Nigeria, respectively. It is also higher than 3.1% amoxicillin/clavulanic acid resistance among ESBLs-producing *Klebsiella* isolates reported by Guandong and Avci (2013). The high rate (100%) of resistance to all the tested β-lactams demonstrated by all the genera in this study, may have resulted due to the frequent use of β-lactams in preparation of animal drugs and treatment of bacterial infections in animals within the study area (Chah and Nweze, 2001). Recently, the use of extended-spectrum β-lactams in humans and animals has tremendously increased in Nigeria. This could have resulted in the high resistance to all the β-lactams tested in this study. The *Salmonella* isolates exhibited the highest rate of resistance to the β-lactams, which further suggested that isolates in the genus may have been exposed more to the drugs than their counterparts in the other genera obtained in this study. The high rate of resistance to ESBL in this study calls for real concern, as these drugs (ESBL) are the only available drugs for treatment of resistant bacteria. If the resistance continues to spread at the rate observed in this study, carbapenems may be resorted for use in production of food animals, while this situation could result in development of superbugs.

In this study, resistance rates of the *Salmonella* isolates to ciprofloxacin (45.5%), ofloxacin and levofloxacin (36.4%), and norfloxacin (9.1%), suggested that isolates in the genus have not developed complete resistance to fluoroquinolones. This may also explain similar low resistance rate of the *Klebsiella* isolates to fluoroquinolones. The low fluoroquinolone resistance among the *Salmonella* and *Klebsiella* isolates may be due to infrequent use of these drugs in swine medicine within the study area. Nevertheless, the low fluoroquinolone resistance may not be unconnected to the ESBLs which the isolates might have produced (Morosini *et al*., 2006). Cross-resistance to fluoroquinolone by ESBL-resistant *Salmonella* isolates has been reported (Pasquali *et al*., 2005). High resistance (50%) of the *E. coli* isolates to ciprofloxacin and norfloxacin in this study suggested that they exerted higher selection against the drugs than to the other tested fluorquinolones with lower rates of resistance (25% for ofloxacin and levofloxacin). Geser *et al*. (2011), Schmid *et al*. (2013), and Guandong and Avci (2013) reported 42.9, 15.4 and 31.1% ciprofloxacin resistance among ESBLs-producing *E. coli* isolates from pigs and cattle in Switzerland, beef cattle in Germany and food of animal origin in Turkey, respectively. The rates of ciprofloxacin resistance in these previous studies are lower when compared with the ciprofloxacin resistance (50%) among *E. coli* isolates in the present study. However, the result is lower than 78% ciprofloxacin resistance reported by Eze *et al*. (2013) among ESBLs-producing *E. coli* isolates from chicken in Nigeria. The 25% ofloxacin resistance rate in this study is lower when compared with 68.8 and 69.4% reported by Li *et al*. (2014) and Eze *et al*. (2013) among ESBLs-producing *E. coli* isolates from chicken in China and Nigeria, respectively. The 12.9% ciprofloxacin resistance shown by *Klebsiella* isolates in this study contrasts 23.8% ciprofloxacin resistance exhibited by ESBLs-producing *Klebsiella* isolates from food of animal origin in Turkey (Guandong and Avci, 2013). Differences in fluoroquinolones resistance in this study may suggest variation in usage of the drugs in production of food animals in the study areas. Eze *et al*. (2013) noted that the use of fluoroquinolones in food-producing animals is not common in Nigeria, so this may account for the relative lower fluoroquinolone resistance rates observed in this study. Nevertheless, cross resistance of ESBLs-resistant *Enterobacteriaceae* to fluoroquinolones have severally been reported (Jacoby, 2005; Frank *et al*., 2011).

Low rates of resistance to amikacin and gentamicin (9.1%), and streptomycin (27.3%) by the *Salmonella* isolates suggested that they exerted low pressure for selection against the aminoglycosides. Similar low resistance rates were exhibited by the *Klebsiella* isolates against streptomycin (9.7%), amikacin (3.2%) and gentamicin (0%). The high resistance rates (100% for gentamicin, 75% for streptomycin and amikacin) among the *E. coli* isolates suggested that they exerted higher selection pressure against the aminoglycosides, more than their counterparts in the other genera. The high streptomycin resistance may be related to the fact that streptomycin is frequently used in combination with penicillin to exert a broad spectrum action for treatment of bacterial infections of pigs in the study area. The 75% streptomycin resistance in this study is similar to 75% streptomycin resistance recorded among ESBLs-producing *E. coli* isolates from pigs and cattle in Switzerland (Geser *et al*., 2012). It is higher when compared with 60% streptomycin resistance reported by Reich *et al*. (2010) among ESBLs-producing *E. coli* isolates from chicken carcasses in Germany. The 100% gentamicin resistance among the *E. coli* isolates in this study is higher when compared with 42.9, 15.4, 6.7 and 34.5% gentamicin resistance among ESBLs-producing *E. coli* isolates reported by Geser *et al*. (2011) in Switzerland, Schmid *et al*. (2013) in Germany, Guandong and Avci (2013) in Turkey and Li *et al*. (2014) in China, respectively. Rate of amikacin resistance (75%) among the *E. coli* isolates in this study, contrast with 8.4% amikacin resistance reported by Li *et al*. (2014) among ESBLs-producing *E. coli* isolates from chicken in China. Low amikacin resistance among the *Salmonella* (9.1%) and *Klebsiella* (3.2%) isolates suggested that these genera exerted low pressure for selection against the drug. In Turkey, an amikacin resistance rate of 4.4% was reported by Guandong and Avci (2013). These results are higher than the amikacin resistance among *Klebsiella* isolates in this study, but it is lower when compared with 9.1% amikacin resistance among the *Salmonella* isolates obtained in the present study. The aminoglycoside resistance observed in this study is probably due to production of ESBLs by the isolates (Carattoli, 2008). Cross-resistance of ESBL-resistant enterobacteria to aminoglycosides has also been reported (Morosini *et al*., 2006).

The high rate (72.7%) of chloramphenicol resistance by the *Salmonella* isolates suggested that the genus have acquired resistance against the drug more than the *E. coli* (50%) and *Klebsiella* isolates (64.5%). Chloramphenicol resistance rates recorded in this study is higher than the findings of Reich *et al*. (2010) and Geser *et al*. (2011) who reported 38 and 47.6% chloramphenicol resistance among ESBLs-producing *E. coli* isolates in Switzerland and Germany, respectively. High rate (90.9%) of tetracycline resistance among the *Salmonella* isolates suggested that they exerted high selection pressure for resistance against the drug more than the *Klebsiella* (38.7%) and *E. coli* (50%) isolates. This demonstrable resistance to tetracycline by the enterobacterial isolates may have resulted due to frequent use of the drug in treatment of bacterial diseases in pigs within the study area. Tetracycline is a broad-spectrum antibacterial agent often used for prophylaxis and treatment of infections in food-producing animals. Geser *et al*. (2011), Adenaike *et al*. (2013), Guandong and Avci (2013) and Reich *et al*. (2010) reported 71.4, 65, 77.8 and more than 50% tetracycline resistance among ESBLs-producing *E. coli* isolates from pigs in Switzerland, cattle in Nigeria, food of animal origin in Turkey and chicken carcasses in Germany, respectively. The tetracycline resistance rates reported in the previous studies is higher when compared with the recorded 50% tetracycline resistance among the *E. coli* isolates in the present study. Guandong and Avci (2013) reported 69.8% tetracycline resistance among ESBL-producing *Klebsiella* isolates in Turkey. This result is higher when compared with 38.7% tetracycline resistance exhibited by *Klebsiella* isolates in this study. ESBLs-resistant enterobacterial isolates have been reported to exhibit co-resistance to tetracycline and phenicols (Morosini *et al*., 2006).

Multidrug resistance implies resistance to three or more classes of antibacterial agent (Tenover *et al*., 2006). Resistance of all the *E. coli* 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 *Salmonella* (90.9%) and *Klebsiella* (54.8%) isolates. Adenaike *et al*. (2013) reported 89% multidrug resistance among ESBL-resistant *E. coli* isolates from roasted beef in Northern Nigeria. Studies showed that ESBLs-producing enterobacteria are multidrug resistant (Guandong and Avci, 2013; Blaak *et al*., 2014). Therefore, the multidrug resistance observed in this study may be as a result of ESBLs that the isolates might have produced (Blaak *et al*., 2014). The high multidrug resistance exhibited among enterobacterial isolates in this study, portends adverse impact on the food chain in the study area. This is because consumers of pork meat and associated processed foods from these pigs could acquire these multidrug resistant organisms. Consequently, there could be compromise in antibacterial therapy in colonized and/or infected individuals (Garcia-Graells *et al*., 2010).

Conclusions

This study has shown that ESBL-resistant enterobacteria are harboured by a sizeable percentage (24.2%) of pigs reared in Enugu State, Southeastern Nigeria. *Klebsiella* is the most prevalent genus of ESBL-resistant enterobacteria colonizing pigs reared within the study area. Thus, pigs reared in the study area are potential reservoirs and disseminators of ESBL-resistant enterobacteria and genes encoding for ESBLs production. This has tremendous impact on the food chain and ecology of antibacterial resistance. However, further studies to elucidate the types of ESBLs genes harbored by the isolates are recommended.

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