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Prevalence and Antibiogram of Generic Enterococci in Ready-to-Slaughter Beef Cattle

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Abstract

Rectal swabs were collected from 95, systematic randomly selected, apparently healthy beef cattle, in order to isolate generic enterococci in Nsukka Southeast, Nigeria, and thus to determine the antibacterial resistance profile of the isolates. Isolation of enterococci was done using Slanetz-Bartley enterococci selective medium. 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. From 95 swabs, 93 (97.89%) were positive for enterococci. Of the 93 isolates, 10 (10.75%) were haemolytic *Enterococcus* species, while 83 (89.25%) were non-haemolytic *Enterococcus* species. Out of 75 isolates, all (100%) were resistant to cefoxitin, 66 (88%) were resistant to ampicillin, 71 (94%) to amoxicillin/clavulanic acid, 68(90.7%) to ceftriaxone, 42 (56%) to streptomycin, 74 (98.67%) to gentamicin, 16 (21.3%) to tetracycline, 5 (6.7%) to vancomycin, 62 (82.7%) to sulphamethoxazole/trimethoprim, and 1 (1.3%) to chloramphenicol and ciprofloxacin. None of the isolate was resistant to imipenem. The enterococcal isolates exhibited 22 resistance patterns. Out of 75 isolates, 1 (1.3%) isolate was resistant to 1 class of antibacterial agents, 9 (12%) were resistant to 2 classes, and 65 (86.7%) to 3 or more classes. This study has shown that cattle slaughtered in Nsukka Southeast Nigeria are potential reservoirs and disseminators of multidrug-resistant enterococci.

Keywords: antibiogram, enterococci, multidrug resistant, bovine
Abbreviations: Ampicillin-AMP, Amoxicillin/clavulanic acid-AMC, Streptomycin-STP, Gentamicin-GEN, Cefoxitin-CEF, Ceftriaxone-CTR, Sulfamthoxazole/trimethoprim-SXT

Introduction

Currently, one of the greatest challenges/threats facing mankind is antimicrobial resistance as it constitutes health crisis (WHO, 2014). Although antibacterial resistance is a natural phenomenon for microbial adaptation, acquired resistance evolved following inappropriate use of antimicrobials in human and veterinary medicine (Tenover, 2006; Laxminarayan *et al*., 2013). Developing countries, particularly the African countries, including Nigeria, are important regions for emergence of antimicrobial-resistant organisms. This is due to lack or lax control of antimicrobial use in these countries (Laxminarayan *et al*., 2013; Martins *et al*., 2015). It has been observed that many antimicrobial-resistant commensal/indicator organisms enter the food chain undetected, because these organisms, especially the non-clinical isolates, are often neglected (WHO, 2014; Morrison and Rubin, 2015). The one health oriented approach for antimicrobial resistance surveillance involved monitoring the spread of resistance among non-clinical isolates of commensal organisms in food-producing animals (WHO, 2014). Enterococci are ubiquitous Gram-positive catalase-negative organisms, part of normal commensal flora in the gastrointestinal tract of humans and animals (Tremblay *et al*., 2011; Werner *et al*., 2013; Beukers *et al*., 2015). They are also commonly found in the soil, water and other environments (Silva *et al*., 2012; Klibi *et al*., 2014). Reports showed considerable diversity in enterococcal strains isolated from food-producing animals, both major (*E. faecalis* and *E. faecium*) and minor species (such as *E. mundtii*, *E. gallinarum*, *E. durans*, *E. casseliflavus*, *E. hirae* etc.) have been reported (Nam *et al*., 2010; Wener *et al*., 2013; Li *et al*., 2014, Iweriebor *et al*., 2015). For long time, the potential of enterococci as pathogens and reservoirs of antimicrobial resistance genes (ARGs) was neglected or underestimated (Sood *et al*., 2008); they were considered non-pathogenic commensals, the reason for their widespread use as probiotics and food preservatives (Krocko *et al*., 2007; Werner *et al*., 2013). By the time their pathogenic potential and capacity to habour ARGs became evident, interest aroused, and all enterococcal species were found to be opportunistic pathogens and to habour variety of ARGs (Nam *et al*., 2010; Werner *et al*., 2013).

Contamination of animal carcasses with antimicrobial-resistant enterococci is often unavoidable due to the ubiquitous nature of the organisms and their ability to adapt to varying environmental conditions (Huys *et al*., 2004; Krocko *et al*., 2011;

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Tremblay *et al*., 2011). This situation is worse in developing nations, including Nigeria, because unhygienic measures are employed during animal slaughter (Ugwu *et al*., 2015). Meat contamination with antimicrobial-resistant enterococci constitutes adverse impact on the food chain, and poses threat to public health following direct and indirect contact with and consumption of contaminated meat and associated meat products (Donabedian *et al*., 2003; McGowan *et al*., 2006; Hammerum *et al*., 2010; Werner *et al*., 2013). Evidences support zoonotic transmission of antimicrobial-resistant enterococci (Stobberingh *et al*., 1999; Donabedian *et al*., 2003; Nam *et al*., 2010; Werner *et al*., 2013). Significantly, enterococci are among the leading causes of nosocomial and community-acquired infections worldwide (Kuhn *et al*., 2000; Manolopoulou *et al*., 2003; Jackson *et al*., 2010; Werner *et al*., 2013; Beukers *et al*., 2015), a capacity attributed to their intrinsic resistance to many classes of antimicrobial agents (Li *et al*., 2014), and worsened by acquisition of multiple resistance genes (Hayes *et al*., 2004; McGowan *et al*., 2006; Nam *et al*., 2010). Most enterococcal isolates from humans and food-producing animals are multidrug resistant strains (Ruzauskas *et al*., 2009). This underlines the widely reported compromise and complication in antimicrobial therapy often observed in treating enterococcal-associated infections (Vergis *et al*., 2001; Hershberger *et al*., 2005; Li *et al*., 2014; Kilonzo-Nthenge *et al*., 2015).

There are calls for increased monitoring of the extent of antimicrobial resistance in enterococci harboured by food-producing animals (Jackson *et al*., 2010; Ristori *et al*., 2012; WHO, 2014). *Enterococcus* species of human, veterinary, and food origin have been used as indicators of occurrence and transfer of antimicrobial resistance (Bager *et al*., 1998; Kuhn *et al*., 2000; Nam *et al*., 2010). They provide accurate information on previous exposure to antimicrobial agents (Nam *et al*., 2010). Isolation of antimicrobial-resistant enterococci from foods of animal origin raised questions regarding the occurrence of antimicrobial-resistant enterococci in food-producing animals (Borgen *et al*., 2001; Hayes *et al*., 2003; Šustáčková *et al*., 2004; McGowan *et al*., 2006; Krocko *et al*., 2007; Koulman *et al*., 2009; Krocko *et al*., 2011). There is increasing numbers of reports on isolation of antimicrobial-resistant enterococci from food-producing animals in the last decennium (Anderson *et al*., 2008). Determination of antibiogram of enterococcal isolates from food-producing animals is essential for monitoring the spread of resistance in food-borne bacteria (Jackson *et al*., 2010; Ugwu *et al*., 2015b). This helps in evaluation of trends and identification of mitigation strategies including empirical treatment of infections associated with the organisms (Ugwu *et al*., 2015b). Surveillance studies to screen food-producing animals as potential reservoirs and disseminators of antimicrobial-resistant enterococci have been conducted in America (Hershberger *et al*., 2005; Anderson *et al*., 2008), and some countries in Asia (Stobberingh *et al*., 1999; Seo *et al*., 2005; Shin *et al*., 2006; Han *et al*., 2011; Li *et al*., 2014), Europe (Aarestrup *et al*., 2000; 2002; Kaszanyitzky *et al*., 2007; Kempf *et al*., 2008; Brtkova *et al*., 2010; Kasimoglu-Dogru *et al*., 2010; Trembley *et al*., 2011) and Africa (Klibi *et al*., 2014; Iweribor *et al*., 2015) Unfortunately, there is paucity of information on occurrence of antimicrobial-resistant enterococci in food-producing animals in Nigeria. Three reports (David, 2014; Amaechi and Nwankwo, 2015; Amaechi 2015) on antimicrobial-resistant enterococci in food-producing animals in Nigeria, exist in available literature. Moreover, no study has been conducted to screen beef cattle slaughtered in Southeast, Nigeria, whereas cattle is the main source of animal protein for the Nigeria populace. Thus, there is need to screen beef cattle slaughtered in Southeast, Nigeria to determine if they are reservoirs of antimicrobial-resistant enterococci. The objectives of this study, therefore, were to isolate enterococci from ready-to-slaughter beef cattle in Nsukka Southeast, Nigeria and determine the antimicrobial resistance profile of the isolates.

Materials and Methods

Sampling

Cattle meant for slaughter at Nsukka abattoir between April and June 2015 were sampled. Ninety five beef cattle consisting 10% of total slaughter within the period of the study were selected using a 1 in 5 systematic random sampling technique. Prior to slaughter, rectal swab was collected from each of the cattle using sterile swab stick. The samples were transported aseptically and processed within 1 hour of collection in the Veterinary Microbiology Laboratory, Department of Veterinary Pathology and Microbiology, University of Nigeria.

Isolation of enterococci

The swabs were aseptically inoculated into brain heart infusion broth supplemented with 6.5% sodium chloride and incubated at 37 °C for 48 hours aerobically. A loopful of the cultures was sub-cultured onto Slanetz-Bartley agar and incubated at 37 °C for 48 hours aerobically, for selective isolation of enterococci. Pinkish, reddish or maroon-coloured tiny colonies were taken as presumptive enterococci. Purification of the isolates was done by sub-culturing on nutrient agar plates and incubated at 37 °C for 48 hours. Pure cultures of the isolates were then inoculated onto nutrient agar slants, incubated at 37 °C for 48 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, bile aesculin, growth at 45 °C and haemolytic test following standard methods.

Determination of antibiogram of enterococcal isolates from beef cattle

Antibacterial resistance/susceptibility of 75 enterococcal 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 plates using sterile swab stick. Twelve antibacterial agents (Oxoid) belonging to 7 classes were used and they included: ciprofloxacin (5 µg), ampicillin (10 µg), ceftriaxone (30 µg), cefoxitin (30 µg), amoxicillin/clavulanic acid (30 µg), imipenem (10 µg), sulphamethoxazole/trimethoprim (25 µg), chloramphenicol (30 µg), gentamicin (30 µg), streptomycin (10 µg), vancomycin (5 µg) and tetracycline (30 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.

Results

Prevalence of generic enterococcal isolates from beef cattle

Out of 95 rectal swabs cultured, 93 (97.89%) yielded positive growth (Fig. 1). Of the 93 isolates, 10 (10.75%) were haemolytic *Enterococcus* species while 83 (89.25%) were non-haemolytic *Enterococcus* species.

Antibiogram of enterococcal isolates from cattle

Out of 75 isolates, all (100%) were resistant to cefoxitin, 66 (88%) were resistant to ampicillin, 71 (94%) to amoxicillin/clavulanic acid, 68 (90.7%) to ceftriaxone, 42 (56%) to streptomycin, 74 (98.67%) to gentamicin, 16 (21.3%) to tetracycline, 5 (6.7%) to vancomycin, 62 (82.7%) to sulphamethoxazole/trimethoprim, and 1 (1.3%) to chloramphenicol and ciprofloxacin. None of the isolate was resistant to imipenem (Fig. 2). The enterococcal isolates exhibited 22 resistance patterns with AMP-AMC-STP-GEN-CEF-CTR-SXT being the most dominant pattern (Table 1). Out of 75 isolates, 1 (1.3%) isolate was resistant to 1 class of antibacterial agents, 9 (12%) were resistant to 2 classes, and 65 (86.7%) to 3 or more classes (Fig. 3).

Discussion

The enterococcal isolation prevalence of 97.89% recorded in this study, suggested that Slanetz and Bartley's agar could be a highly sensitive medium for selective isolation of enterococci from rectal swabs obtained from food-producing animals (Klibi *et al*., 2014). It also suggested that almost all the beef cattle slaughtered in the study area harboured enterococci in their gastrointestinal tract. Isolation of 83 (89.25%) non-haemolytic enterococcal strains against 10 (10.75%) haemolytic strains in this study, suggested that non-haemolytic species of *Enterococcus* may be the dominant strains associated with beef cattle slaughtered in the study area. This finding may be attributed to the health status of the animals. The haemolytic isolates in this study may belong to *E. faecium*, *E. faecalis*, *E. hirae* and *E. casseliflavus* which have been reported to colonize gastrointestinal tract of cattle (Anderson *et al*., 2008; Jackson *et al*., 2010; Werner *et al*., 2013; Beukers *et al*., 2015), while the non-haemolytic isolates may belong to non-haemolytic enterococcal species such as *E. gallinarum* and *E. mundtii* also reported to be endogenous in cattle (Nam *et al*., 2010; Klibi *et al*., 2014). Therefore, enterococcal isolates in this study may be part of endogeneous flora or pathogens in the sampled animals (McGowan *et al*., 2006; Nam *et al*., 2010).

The 97.89% enterococcal prevalence in this study is higher when compared with 91, 79, 73 and 61.4% enterococcal isolation prevalence reported in faecal samples of 96 food animals in Tunisia (Klibi *et al*., 2014), 305 poultry products in The Netherlands (Van Den Braak *et al*., 1998), 22 and 57 beef samples in USA (McGowan *et al*., 2006) and Poland (Rozanska *et al*., 2015), respectively. It is also higher when compared with enterococcal isolation prevalence reported in 232 Tibetan pigs (Li *et al*., 2014), 275 minced beef samples in Germany (Klein *et al*., 1998), 362 environmental streptococci isolated from cases of bovine mastitis in USA (Rossito *et al*., 2002), 636 raw meat samples in Italy (Pesavento *et al*., 2014), 112 food of animal origin in Poland (Chajęcka-Wierzchowska *et al*., 2012) and 343 retail chicken meat in USA (Kilonzo-Nthenge *et al*., 2015), respectively. But it is however lower when compared with 100% enterococcal isolation prevalence reported in faecal samples of 35 and 39 healthy cattle in USA (Anderson *et al*., 2008) and Tunisia (Klibi *et al*., 2014), 262 samples of ground beef in USA (Hayes *et al*., 2003) and 260 samples of food of animal origin in Czech Republic (Krocko *et al*., 2011), respectively. The differences in enterococcal separation prevalence in these studies, reflects variation in rate of enterococcal colonization, meat contamination, number of samples processed, health status of sampled animals, and the method of enterococcal isolation in the various studies.

The moderate (21.3%) tetracycline resistance in the present study suggested selection to the drug. This resistance may be due to acquisition of tetracycline resistance genes following use selection pressure (Pesavento *et al*., 2014). Tetracycline is commonly used in treating bacterial infections of food-producing animals in Nigeria because of its broad spectrum effect (Ugwu *et al*., 2015a). The 21.3% resistance to tetracycline in this study is lower when compared with 24.5, 45, 42.9, 35.3 and 80% tetracycline resistance reported among faecal enterococcal isolates from dairy cattle in USA (Jackson *et al*., 2010), beef cattle in Lithuania (Ruzauskas *et al*., 2009), healthy beef and dairy cattle in USA (Anderson *et al*., 2008), food of animal origin in Italy (Pesavento *et al*., 2014) and poultry products in Czech Republic (Kolar *et al*., 2002), respectively. McGowan *et al*. (2006), Krocko *et al*. (2007) and Duckova *et al*. (2014), Liu *et al*. (2013), Cetinakaya *et al*. (2013), and Rozanska *et al*. (2015) reported 24.3, 56 and 44.4, 92.5, 12 and 74.3% tetracycline resistance among enterococcal isolates from food of animal origin in USA, Slovakia, China, Turkey and Poland, respectively. Nam *et al*. (2010) and Li *et al*. (2014) reported 69.5 and 64.3% tetracycline resistance among enterococcal isolates from milk of mastitic dairy cattle in Korea, and pigs in Tibet, China, respectively. These results are also lower than that of the present study. However, the 21.3% tetracycline resistance in the present study is higher than 14.9 and 20.1% tetracycline resistance reported among enterococcal isolates from food animals in Tunisia (Klibi *et al*., 2014) and minced meat in Germany (Klein *et al*., 1998). The varying tetracycline resistance rates may be related to the differences in the use of the drug in food animal production in the study areas.

The low rate (1.3%) of ciprofloxacin resistance in this study suggested low selection against the drug. This finding suggested that the isolates may have acquired genes encoding for fluoroquinolones resistance at a low proportion (Werner, 2012). The low fluoroquinolones resistance in this study may be related to the fact that this class of antibacterial agents is not commonly used in food-producing mammals (probably because of their

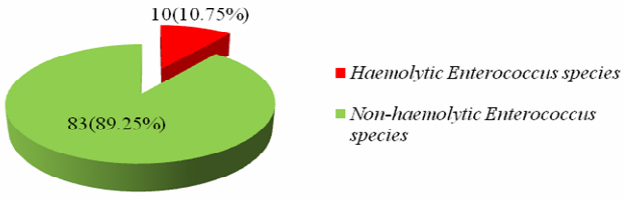

Fig. 1. Prevalence of generic enterococci in healthy beef cattle

high cost) in the Nigeria (Eze *et al*., 2013; Ugwu *et al*., 2015b). The 1.3% ciprofloxacin resistance in this study is lower when compared with 9.5, 8, and 24.1% ciprofloxacin resistance reported among faecal enterococcal isolates from cattle in USA (Anderson *et al*., 2008) and Lithuania (Ruzauskas *et al*., 2009), and food animals in Tunisia (Klibi *et al*., 2014) respectively. Hershberger *et al*. (2005) reported ciprofloxacin resistance of 55 and 45% among faecal enterococcal isolates form beef and dairy cattle in USA, respectively. Klein *et al*. (1998), McGowan *et al*. (2006), Li *et al*. (2014), Pesavento *et al*. (2014) and Cetinakaya *et al*. (2013) reported 2.7, 4.3, 3.6, 7.69 and 33.3% ciprofloxacin resistance among enterococcal isolates from minced meat in Germany, meat samples in USA, pigs in Tibet China, food of animal origin in Italy and Turkey, respectively. Aarestrup *et al*. (2002) reported ciprofloxacin resistance which is higher than that observed in this study among enterococci isolated from pigs in Denmark and Sweden. These results are also higher than that of the current study. Variation in ciprofloxacin resistance in these studies may be due to differences in the use of the drug in food-producing animals in the study areas (Hershberger *et al*., 2005).

Similarly, low rate (1.3%) of chloramphenicol resistance in this study suggested that the isolates exerted low selection against the drug. This may be related to the fact that chloramphenicol has long been banned and is no longer used in food animal production in the study area. The 1.3% chloramphenicol resistance in the present study is similar to 1% chloramphenicol resistance reported among enterococcal isolates from minced meat in Germany (Klein *et al*., 1998). But it is lower than 8, 30, 6.41 and 28.6% chloramphenicol resistance reported among enterococcal isolates from cattle in Lithuania (Ruzauskas *et al*., 2009), food animals in China (Liu *et al*., 2013), food of animal origin in Italy (Pesavento *et al*., 2014), and retail beef in Poland (Rozanska *et al*., 2015), respectively. Higher chloramphenicol resistance than that of this study, was also reported by Aarestrup *et al*. (2002) among enterococcal isolates from Danish and Swedish pigs.

Enterococcal resistance to aminoglycoside, particularly to gentamicin and streptomycin, is critical because of their use (in combination with ampicillin) in empirical treatment of enterococcal infections (Klein *et al*., 1998; Arias *et al*., 2010; Kristich *et al*., 2011; Werner, 2012). The CLSI recommended the use of 120 µg and 300 µg gentamicin and streptomycin discs, respectively, for assessing high level resistance to these drugs (CLSI, 2012). In the hereby experiment, low level resistance of the isolates to the drugs was assessed using 10 µg discs for both gentamicin and streptomycin. Low level resistance to these drugs by enterococci suggests possible high level resistance to them (CLSI, 2012). In the current study, high rate (98.7%) of resistance to gentamicin (98.7%) and streptomycin (56%) suggested that the isolates exerted high selection against aminoglycoside. The result also showed that the isolates exerted higher selection to gentamicin than to streptomycin. This finding suggested that the isolates may have acquired genes encoding for resistance to aminoglycoside (Thal *et al*., 2000; Werner, 2012). While the present study determined low level aminoglycoside resistance (LLAR), there is a high probability that the isolates would also exhibit high level aminoglycoside resistance (HLAR); enterococcal strains exhibiting HLAR are multidrug-resistant (Rozanska *et al*., 2015). Nevertheless, the aminoglycoside resistance observed in this study could also be inherent in the isolates, since enteroccoci have been reported to exhibit moderate intrinsic resistance especially to low level aminoglycoside (Barbosa *et al*., 2009; Kristich *et al*., 2014). This finding of high rate of LLAR in this study is a cause for concern, because, an aminoglycoside resistance gene confers co-resistance to similar drugs (Febler *et al*., 2011). The 98.7% gentamicin resistance observed in this study is higher when compared with 25 and 1.59%; 16.7, 30, 2.2, 1 and 6.5% gentamicin resistance reported among enterococcal isolates from food of animal origin in Slovakia (Krocko *et al*., 2007; Duckova *et al*., 2014) and Italy (Pesavento *et al*., 2014); food animal in China (Liu *et al*., 2013), farm animals in Southwest, Nigeria

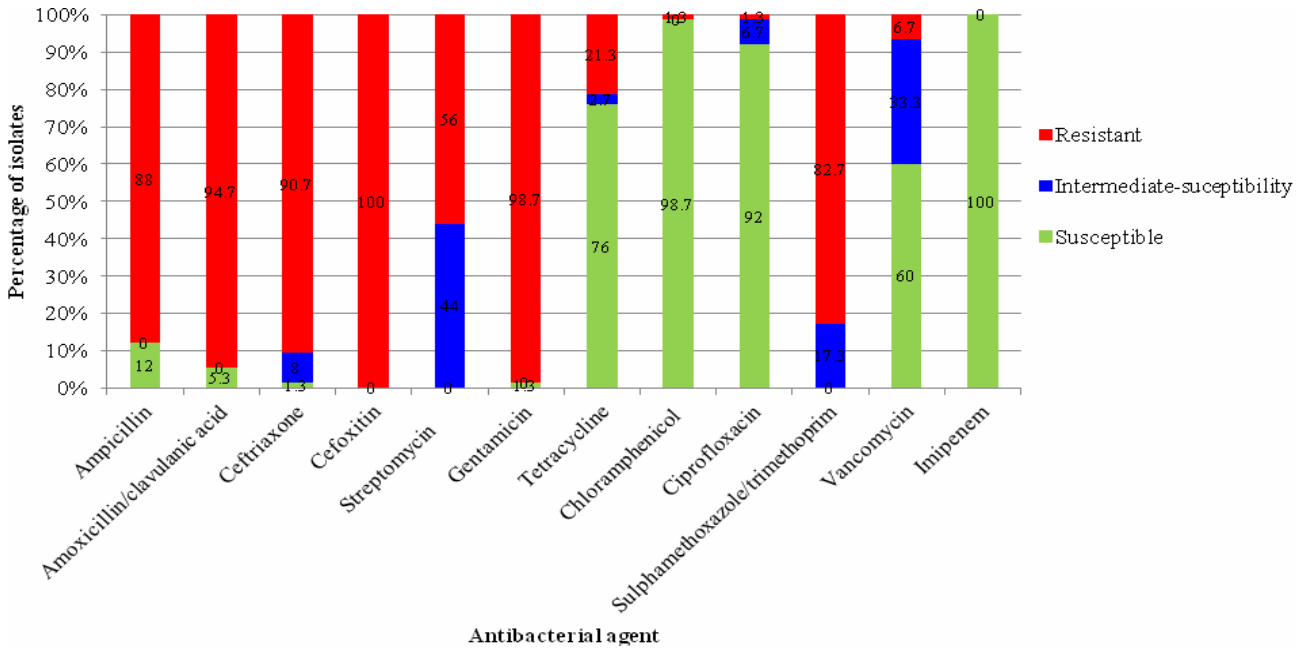

Fig. 2. Antibiogram of 75 enterococcal isolates from healthy beef cattle

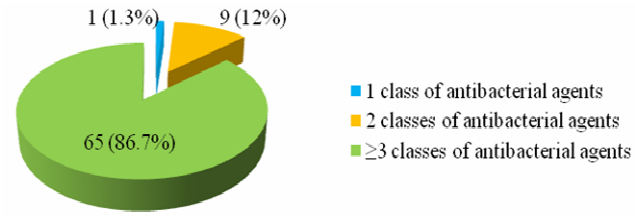

Fig. 3. Number of antibacterial classes to which 75 enterococcal isolates from beef cattle were resistant

(David, 2014), pigs in Denmark (Aarestrup *et al*., 2002), and meat samples in USA (McGowan *et al*., 2006), respectively. In this study, the 56% streptomycin resistance is higher than 14.6 and 40% streptomycin resistance among enterococcal isolates from meat and cattle reported in USA (McGowan *et al*., 2006) and Lithuania (Ruzauskas *et al*., 2009), respectively. Klein *et al*. (1998), Liu *et al*. (2013), Klibi *et al*. (2014) and Rozanska *et al*. (2015) reported 1, 50.3, 5.7 and 15% streptomycin resistance among enterococcal isolates from minced meat in Germany, food of animal origin in China, food animals in Tunisia, retail beef in Poland, and pigs in Sweden, respectively. These findings including that of Aarestrup *et al*. (2002) among enterococcal isolates from Danish pigs are also lower than streptomycin resistance recorded in the present study. Differences in application of these drugs in treatment of infections in the study areas may account for the variation in their resistance. In Nigeria, streptomycin is often combined with penicillin to exert broad-spectrum effect in treatment of food-producing animals (Ugwu *et al*., 2015b). The high LLAR observed in this study may be a cause for concern because of compromise (if aminoglycosides resistance genes are acquired) that would occur during treatment of enteroccocal infection in humans and animals in the study area (Donabedian *et al*., 2003).

The CLSI also recommended the use of 6 µg/mL vancomycin supplemented agar or broth for detection of vancomycin-resistant enterococci (VRE) (CLSI, 2012). Low level vancomycin resistance, a presumptive test for vancomycin resistance, was determined in the hereby experiment using 5 µg vancomycin disc. Demonstrable vancomycin resistance (6.7%) in this study suggested selection against the drug. This suggested that the isolates possibly acquired and haboured vancomycin resistance genes (VRGs). Although the isolates were not confirmed to be VRE, this finding is critical because vancomycin is regarded as last-line of defense against many bacterial pathogens, especially ampicillin/aminoglycoside-resistant enterococci (McDonald *et al*., 1997; Kolar *et al*., 2002; Li *et al*., 2014). The 6.7% vancomycin resistance in this study is higher than 3.53 and 2.9% vancomycin resistance among enterococcal isolates from food of animal origin and retail beef reported in Italy (Pesavento *et al*., 2014) and Poland (Rozanska *et al*., 2015), respectively. But it is lower when compared with 8, 17 and 7.49% vancomycin resistance among enterococcal isolates from cattle, food of animal origin and pigs reported in Lithuania (Ruzauska *et al*., 2009), Slovakia (Duckova *et al*., 2014) and Denmark (Aarestrup *et al*., 2002), respectively. Variation in resistance rates to aminoglycosides and glycopeptide (vancomycin) in these studies may be due to differences in usage of the drugs in the study areas and/or concentration (whether low or high level) of the drugs used in the various studies.

The high rate (82.7%) of sulphamethoxazole/trimethoprim resistance in this study suggested high selection against the drug. This high potentiated sulfonamide resistance may be due to extensive use of the drug (because of

Table 1. Resistance patterns exhibited by 75 enterococcal isolates from beef cattle

Keys: AMP-Ampicillin, AMC-Amoxicillin/clavulanic acid, STP-Streptomycin, GEN-Gentamicin, CEF-Cefoxitin, CTR-Ceftriaxone, CIP-Ciprofloxacin, TET-Tetracycline, CHL-Chloramphenicol, VAN-Vancomycin, IMP-Imipenem, SXT-Sulphamethoxazole/trimethoprim

S/N	Resistance pattern	Frequency	Percentage
1.	GEN-CEF-CTR-SXT	1	1.33
2.	AMP-GEN-CEF-CTR	1	1.33
3.	AMP-AMC-GEN-CEF-SXT	1	1.33
4.	AMP-AMC-STP-GEN-CEF-CTR-TET-CHL-SXT	1	1.33
5.	GEN-CEF-SXT	1	1.33
6.	AMC-GEN-CEF-CTR-CIP-TET-SXT	1	1.33
7.	AMP-AMC-GEN-CEF-CTR-VAN-SXT	1	1.33
8.	AMP-GEN-CEF-SXT	1	1.33
9.	AMP-AMC-GEN-CEF-CTR-TET	1	1.33
10.	AMP-AMC-CEF-CTR	1	1.33
11.	AMP-AMC-STP-GEN-CEF-CTR	2	2.7
12.	AMC-S-GEN-CEF-CTR-SXT	2	2.7
13.	AMP-AMC-STP-GEN-CEF-SXT	2	2.7
14.	AMP-AMC-STP-GEN-CEF-CTR-TET-VAN	2	2.7
15.	AMC-GEN-CEF-SXT	2	2.7
16.	AMC-GEN-CEF-CTR-TET-SXT	2	2.7
17.	AMP-AMC-STP-GEN-CEF-CTR-VAN-SXT	2	2.7
18.	AMP-AMC-GEN-CEF-CTR-TET-SXT	3	4
19.	AMP-AMC-GEN-CEF-CTR	6	8
20.	AMP-AMC-STP-GEN-CEF-CTR-TET-SXT	6	8
21.	AMP-AMC-GEN-CEF-CTR-SXT	11	14.7
22.	AMP-AMC-STP-GEN-CEF-CTR-SXT	25	33.3
	Total	75	100

its broad-spectrum effect) in food animal production in Nigeria. Acquisition of genes encoding resistance to potentiated sulfonamide may be the cause of this high sulphamethoxazole/trimethoprim resistance observed in this study (Werner, 2012).

The 82.7% sulphamethoxazole/trimethoprim resistance in this study is lower than 100% sulphamethoxazole/trimethoprim resistance among enterococcal isolates from food animals reported in Tunisia (Klibi *et al*., 2014).

Resistance of enterococci to β-lactams, particularly ampicillin, is important because they are critical in treatment of enterococcal infections (Klein *et al*., 1998, Kolar *et al*., 2002; Kristich *et al*., 2014; Arias *et al*., 2010). High rate (88%) of ampicillin resistance in this study suggested high selection against the drug. The high ampicillin resistance also suggested that the isolates may have produced β-lactamases (major mechanism of β-lactam resistance) which hydrolysed the β-lactam ring of the drug to penicilloic acid (Livermore and Brown, 2001; Werner, 2012; Urmunova, 2015). The isolates may also have exerted resistance to the ampicillin by changing their cell wall permeability, expressing active efflux pump, gene mutation and/or altered penicillin binding protein (PBP) receptors, a well-known intrinsic (natural) β-lactam resistance mechanism in enterococci (Livermore and Brown, 2001; Kak and Chow, 2002; Kristich *et al*., 2014; Li *et al*., 2014; Urmunova, 2015). The high ampicillin resistance may be a result of selective pressure due to inappropriate use of β-lactams (probably because they are cheap) in Nigeria (Chah and Nweze, 2001; Ugwu *et al*., 2015b). High rate (94%) of resistance to amoxicillin/clavulanic acid (a β-lactam-β-lactamase inhibitor) in this study suggested that the isolates produced AmpC β-lactamases (cephalosporinases), the major mediators of resistance to β-lactam-β-lactamase inhibitors (Ben Sallem *et al*., 2012). Other mechanisms of resistance to β-lactam-β-lactamase inhibitors that the isolates might have exhibited include: hyper-production of class A β-lactamases (i.e. TEM-1 or SHV-1), production of class D plasmid-mediated enzyme, chromosomal or plasmidic class C β-lactamase, and modification of outer membrane permeability (Chaïbi *et al*., 1999; Dwarz and Bonomo, 2010). The 88% ampicillin resistance in this study is higher when compared with 1.2, 4.2, 9.52, 2.24 and 16.7% ampicillin resistance recorded among enterococcal isolates from Tibetan pigs (Li *et al*., 2014), food animals in China (Liu *et al*., 2013), and food of animal origin in Slovakia (Duckova *et al*., 2014), Italy (Pesavento *et al*., 2014) and Turkey (Cetinakaya *et al*., 2013), respectively. The result also contrasts Klibi *et al*. (2014) and Klein *et al*. (1998) who did not detect ampicillin resistance among enterococcal isolates from food animals in Tunisia and minced meat in Germany, respectively.

The high β-lactam resistance rates among isolates (which are non clinical strains) in this study, is not in agreement with Lopes *et al*. (2005) and Pesavento *et al*. (2014) who stated that resistance of enterococci to β lactam antibiotics seems to be associated with clinical strains. The finding of higher ampicillin resistance in this study is worrisome, because, the use of extended-spectrum β-lactams (ESBL) would further be resorted for use in treating enterococcal infections in food-producing animals in the study area. However, the 94% amoxicillin/clavulanic acid resistance in the current study is higher when compared with 0.32% amoxicillin/clavulanic acid resistance among enterococcal isolates from food of animal origin reported in Italy (Pesavento *et al*., 2014).

High rate of resistance to ceftriaxone (90.7%) and cefoxitin (100%) in this study suggested production of extended-spectrum β-lactamases (ESBLs) (Geser *et al*., 2012). Ceftriaxone and cefoxitin are oxyimino-cephalosporins (extended-spectrum-β-lactams [ESBL]) whose resistance is mediated by ESBLs (Carattoli, 2008; Geser *et al*., 2012). Confirmation of ESBLs production is done using ceftriaxone plus clavulanic acid disc synergy test (CLSI, 2012). Single discs of these drugs were used in the hereby experiment. Thus, the finding of high ESBL-resistance in this study, suggested that the isolates acquired genes encoding for ESBLs in high proportion. This may be a result of inappropriate use of ESBL in human and veterinary medicine in Nigeria. Possible sources of the ESBLs-encoding genes were organisms which contaminated ingested feed, vegetation, drinking water and/or cattle environment (Silva *et al*., 2012; Klibi *et al*., 2014). It is also possible that because of resistance to β-lactams (e.g. ampicillin and amoxicillin/clavulanic acid), ESBL were used in treating previous infection(s) in the sampled animals. The finding of high ESBL-resistance in this study is a cause for real concern because ESBL-resistant enterococci could rapidly spread ESBL-encoding genes to bacteria flora in human consumers of the beef, owing to location of ESBLs genes on highly promiscuous mobile genetic elements (Ewers *et al*., 2012). Moreover, it is known that ESBL-resistant organisms exhibit co-resistance to other classes of antibacterial agents including aminoglycosides, fluoroquinolones, tetracyclines, phenicols, and potentiated sulfonamides (Gniadowski, 2001; Coque *et al*., 2008; Geser *et al*., 2012). Thus, resistance to drugs belonging to these classes of antibacterial agents in this study may also be mediated by ESBLs which the enterococcal isolates might have produced. Therefore, the presence of ESBL-resistant (multidrug-resistant) enterococci in the sampled animals is worrisome because it adversely impacts the food chain and poses a huge threat to the health of consumers of beef and associated products from these cattle. Moreover, the overall consequence of high ESBL-resistance rate observed in this study is, if unchecked, the use of carbapenem may be resorted for use in production of food-producing animals in the study area. The 100% resistance to ceftriaxone in this study is higher when compared with 35.4% ceftriaxone resistance among enterococcal isolates from minced meat reported in Germany (Klein *et al*., 1998).

Interestingly, none of the isolates to 3 or more antibacterial agents tested in this study exhibited demonstrable resistance to carbapenem (imipinem), the last resort drug for the treatment of multidrug-resistant bacteria (Abraham *et al*., 2014). This finding suggested that carbapenem have not been abused in food animal production in Nigeria. Howbeit, this status (non-carbapenem resistance in enterococcal isolates from cattle) should be maintained; because, the emergence of carbapenem-resistant enterococcal strains in food-producing animals, would definitely result in rapid dissemination of these organisms to the public due to unhygienic animal slaughter practices in Nigeria. Consequently, untreatable and highly fatal infections would emerge since carbapenem-resistant organisms are superbugs resistant to all known therapeutic agents

(Johnson and Woodford, 2012; Abraham *et al*., 2014).

In this study, resistance of 65 (86.7%) isolates coupled with 22 (100%) multidrug resistance patterns, further suggested that high numbers of enterococci colonizing cattle slaughtered in the study area are multidrug resistant strains (Tenover, 2006; Rozanska *et al*., 2015). This finding corroborates previous reports that most enterococcal isolates from food-producing animals exhibit multidrug resistance (Ruzauskas *et al*., 2009). This multidrug resistance exhibited by the isolates could be due to acquisition of multidrug resistance genes (such as ESBLs, fluoroquinolones and vancomycin resistance genes) and also to intrinsic resistance to some antibacterial agents (Paulsen *et al*., 2003; Fischer and Philips, 2009; Kristich *et al*., 2014). The 86.7% multidrug resistance in this study is lower than 100% multidrug resistance among enterococcal isolates from food reported animals in Canada (Trembley *et al*., 2011). But it is higher than 18.5, 52 and 52.6% multidrug resistance among enterococcal isolates reported in Tunisia (Klibi *et al*., 2014), Poland (Rozanska *et al*., 2015) and Korea (Nam *et al*., 2010), respectively.

Conclusions

This study has shown that a sizeable percentage (97.89%) of cattle slaughtered in Nigeria was positive of multidrug-resistant enteroccoci and prove that cattle are potential reservoirs and disseminators of multidrug-resistant enterococci. These organisms are reservoirs of multiple resistance genes and transfer them to bacteria flora of humans when consumed together with meat and meat products. Therefore, their presence in slaughtered cattle portends huge adverse impact on the food chain and poses serious health threat to the consumers of the beef and associated products from the cattle. However, further studies to deduce high level vancomycin and aminoglycosides resistance, and the antimicrobial resistance genes harboured by the isolates is recommended.

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