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https://dx.doi.org/10.4314/nvj.v43i4.5 **ORIGINAL ARTICLE**

PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCTION IN CLINICAL AND NON - CLINICAL ISOLATES OF KLEBSIELLA SPECIES OF ANIMAL ORIGINS IN ABEOKUTA, NIGERIA.

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SUMMARY

Production of extended-spectrum β-lactamases (ESBLs) confer resistance to β-lactam antibiotics. This study investigated the presence of ESBL-producing *Klebsiella spp* in clinical and non-clinical samples from different animal species in Abeokuta, Nigeria. The species of *Klebsiella* were determined by biochemical characterization (Oxoid Microbact GNB 24E®), while phenotypic ESBL-production was confirmed by using the cefpodoxime and cefpodoxime/clavulanic acid combination disc kit. Ninety-five *Klebsiella* isolates were investigated in this study. Fifty-five (57.9%) of the isolates were obtained from the faeces of apparently healthy animals while 40 (42.1%) were from clinical samples. The *Klebsiella* isolates were identified as follows: *K. oxytoca* (34.7%), *K. pneumoniae* (26.3%), *K. ozaenae* (18.9%), *K. terrigena* (13.7%), *K. rhinoscleromatis* (5.3%), *K. planticola* (1.1%). Eight (8.4%) out of the 95 isolates of *Klebsiella* spp were identified as ESBL-producers. These included four isolates of *Klebsiella pneumoniae*, three of *Klebsiella oxytoca* and one of *Klebsiella ozaenae.* Five out of the eight ESBL-producing isolates were from clinical samples while three isolates were from the faeces of apparently healthy animals. The ESBL-producing isolates were 100% resistant to ampicillin, cefotaxime, ceftazidime, cefpodoxime, streptpmycin, tetracycline, compound sulfonamide, trimethoprim/sulphomethoxazole, and nalidixic acid. Apparently healthy carriers and sick animals can serve as sources of transmission of ESBL-producing *Klebsiella* spp to other animals and humans.

Key words: Apparently healthy animals, clinical samples, Extended Spectrum Beta-Lactamases (ESBLs), *Klebsiella species*, Nigeria.

INTRODUCTION

Klebsiella species are increasingly been implicated in urinary and respiratory tract infections (Podschun and Ullmann, 1998). Most infections occur in immunocompromised subjects especially under hospital settings (Podschun and Ullmann, 1998). Although *Klebsiella* are principally opportunistic pathogens, they may cause very severe disease such as septicaemia, pneumonia, soft tissue infections and urinary tract infections (Podschun and Ullmann, 1998). The involvement of multi antibiotic resistant *Klebsiella species* identified as ESBL-producers in hospital outbreaks of infections both in humans and animals is on the increase (Podschun and Ullmann, 1998). In such outbreaks, therapeutic options for treating clinical cases are limited. Until recently, *K. pneumoniae* and *K. oxytoca* have been considered to be the only pathogenic *Klebsiella species* (Podschun and Ullmann, 1998). However, the newer species such as *K. terrigena* and *K. planticola*, formerly regarded as "environmental" *Klebsiella species*, have been demonstrated to occur in human clinical specimens (Podschun and Ullmann, 1998). *Klebsiella planticola*, in particular, has been isolated with astonishing frequency from human infectious processes (Podschun and Ullmann, 1998). Many veterinarians and physicians ordinarily do not regard *Klebsiella species* as important pathogens. The organism is rarely considered as primary aetiology of diseases. The *Klebsiellae* isolated from clinical samples are often regarded as contaminants or as secondary invaders and as such undeserving of serious attention (Alenka *et al.,* 2007). Moreover, clinicians and pathologist do not usually consider the possibility of the involvement of *Klebsiella species* as a suspect in disease condition. Therefore, many clinicians and pathologists fail to request for *Klebsiella* isolation when samples from sick animals or from carcasses are being submitted to diagnostic laboratories for microbiological investigations. *Klebsiella species* are therefore hardly recognized as important causes of clinical conditions in animals. Hence, isolation of *Klebsiella species* in clinical samples may be accidental findings (Dorina *et al.,* 2014). This may be responsible for failure of most diagnostic Microbiology laboratories to fully identify isolates of *Klebsiellae to* species level. In most cases, isolates are identified based on colonial and cultural characteristics alone without further biochemical characterisation to determine the particular species involved (Dorina *et al.*, 2014). This has resulted in under reporting of the involvement of *Klebsiella species in* clinical conditions. Many public and private diagnostic laboratories are poorly funded and clients may be unwilling to pay extra fees for detailed bacterial characterization required for species identification. Lack of resources for the provision of necessary reagents for proper in-depth characterisation hampers correct identification of bacterial isolates to species level (Dorina *et al*., 2014). Notwithstanding that some veterinarians may fail to suspect *Klebsiella* in disease conditions, the organism has all the same been recognised as important primary or opportunistic pathogen causing severe extra intestinal infections in humans and in many animals' species (Dorina *et al*., 2014). There is gross underestimation of the involvement of *Klebsiella* in disease conditions because of poor documentation, inadequate disease reporting and exclusion of the organism by clinicians and pathologists from tentative and differential diagnosis (Dorina *et al*., 2014). *Klebsiellae* have been implicated in various disease conditions in

humans and in animals (Roberts *et al*., 2000). In particular, *Klebsiella species* are known to cause mastitis and bacteraemia in cattle (Marcos *et al*., 2007; Dorina *et al.*, 2014), severe infection of the lower respiratory tract (Kaushik and Kalra, 1983), septicaemia and urinary tract infection in dogs (Ling and Ruby, 1983), cervicitis and metritis in mare (Kikuchi *et al*., 1987) as well as pneumonia and septicaemia in foals (Kreig and Holt, 1984; Roberts *et al*., 2000). *Klebsiella species* is very prominent among bacterial aetiologies of nosocomial infections in veterinary hospital settings (Glickman, 1981; Alenka *et al.,* 2007). Antimicrobials are used in animals for different reasons, antimicrobials are used for the treatment and control of animal diseases (chemotherapy), for the prevention of diseases (prophylaxis) and to enhance growth and performance (growth promotion) (Carattoli, (2009). Beceiro *et al*., 2013). In food animals, antimicrobials are applied on herd basis in which case both sick and in-contact animals within a flock are treated together (metaphylaxis) (Beceiro *et al*., 2013). The situation is different in companion animals where animals are treated on individual basis. The two most important demerits of antimicrobial usage in animals are the risk of development and dissemination of resistant bacterial strains as well as the presence of antimicrobial residues in animal source-foods (Ewers *et al*., 2014). The association of ESBL-genes with mobile genetic elements including plasmids, transposons, insertion sequences and integrons enhance the intra- and inter- species transfer of ESBL-production trait among bacteria (Stokes and Gillings, 2011; Ojo *et al*; 2016). This has facilitated the global spread of ESBL trait across diverse geographical and climatic regions. ESBL-genes are easily transferable among the enteric bacterial microflora in human and animal guts (Stokes and Gillings, 2011).

These can be distributed through faecal contamination of water, food and the environment (Stokes and Gillings, 2011). The involvement of ESBL-producing *Klebsiella species* in animal disease conditions and their reservoirs in apparently healthy animals in Abeokuta, Nigeria has not been previously investigated. Only very limited information is available on the occurrence of ESBL-producing *Klebsiellae* of animal origin in Nigeria. This study identified phenotypic ESBL producers among clinical and non-clinical isolates of *Klebsiella* species from different animal sources in Abeokuta, Nigeria. ESBL-producing *Klebsiella* isolates were tested for susceptibility to selected antimicrobial agents.

MATERIALS AND METHODS

Study design

In this study, culture collection of *Klebsiella species* isolates preserved on nutrient agar slopes at the Veterinary Microbiology laboratory of the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria was investigated for species determination, detection of ESBL-producing strains and antimicrobial susceptibility of the identified ESBL-producing strains. The culture collection included isolated obtained from clinical and non-clinical samples from different animal species (cattle, sheep, goats, pigs, horses, dogs, cats, chickens, guinea fowl) over a five-year period (January 2012 to December 2016) (Table 1).

Table 1: Clinical and non-clinical sources of *Klebsiellae* in different species of animals in Abeokuta, Nigeria

Animal sources	Sample sources							Total (%)
	Non-clinical	Clinical						
	Faeces	Faeces	Urogenital tract	Milk	Lung	Blood	Liver	
Cattle	9	0	-	5	3	0	0	17 (17.9)
Sheep	6	2	-	0	2	0	0	10 (10.5)
Goats	8	0	-	2	0	0	0	10 (10.5)
Pigs	7	1	3	0	0	0	0	11 (11.6)
Horses	2	0	-	0	0	0	0	2 (2.2)
Dogs	6	3	5	0	1	1	2	18 (18.9)
Cats	6	0	-	0	0	0	0	6 (6.3)
Chicken	9	5	-	0	4	0	1	19 (20.0)
Guinea fowls	2	0	-	0	0	0	0	2 (2.1)
Total (%)	55 (57.9)	11 (11.6)	8 (8.4)	7 (7.4)	10 (10.5)	1 (1.1)	3 (3.2)	95 (100)

Only isolates recoverable from nutrient agar slopes were included in the study. Some of the preserved isolates could not be recovered from agar slopes.

Sample Sources of *Klebsiella species*

The *Klebsiella spp* isolates investigated in this study were obtained from different samples sources including faeces, milk from mastitis, tissues (lungs and liver samples collected at post mortem

examination), blood and swabs of urogenital tracts (Table 1).

Recovery of Isolates from Nutrient Agar Slopes

Suspected isolates of *Klebsiella species* preserved on nutrient agar slopes in half-ounce bottles were removed from the refrigerator allowed to equilibrate to room temperature. Five to eight millilitres of freshly prepared sterile tryptic soy broth (TSB, Oxoid Basingstoke, UK) was dispensed directly into each of the nutrient agar slopes. This was incubated at 37 ^{o}C for 18 to 24 hours. A loopful of the bacterial broth culture was taken from the half-ounce bottles containing the agar slope with TSB. This was inoculated onto MacConkey agar plates and incubated at 37 ^{o}C for 18 to 24 hours. The plates were examined for colonies that resemble those of *Klebsiella species*.

Biochemical characterization and Identification of Klebsiella *species*

The colonial characteristics of the organisms were recorded. Suspected *Klebsiella* isolates were tested for oxidase and catalase production. The isolates were further identified using biochemical tests kit (Oxoid Microbact GNB 24E®) for substrate utilization as indicated by colour change. The test was carried out as described by the manufacturer and reaction read accordingly using a colour chart provided with the kit. After reading the colour change reaction, the results were interpreted using computer software package (Oxoid Microbact® 2000 version 2.03) designed for the test kit.

Selective isolation of ampicillin- and cefotaxime-resistant *Klebsiella species*

A loopful of the TSB culture was inoculated onto MacConkey agar supplemented with ampicillin (100mg/L; Amp_{100}). Inoculated plates were incubated at 37^{0}C for 18 to 24 hours and examined for bacterial growth. Colonies of *Klebsiella* species on MacConkey+Amp_{100} were selected for further screening on MacConkey agar containing cefotaxime supplement (1mg/L; CTX_1). Isolates that grew on both selective agar media (that is, resistance to ampicillin and cefotaxime) were suspected as ESBL-producers.

Phenotypic confirmation of ESBL-producing isolates:

Isolates recovered following the selective culture on Mac-Amp100 and Mac CTX, were tested for phenotypic ESBL-production using the cefpodoxime combination disc kit (Oxoid DD0029) test. Briefly, isolates were inoculated onto Nutrient agar (NA) supplemented with cefotaxime (1mg/L). Bacterial colonies on NA-CTX were emulsified in normal saline and adjusted to 0.5 McFarland standard and used as inoculum. A sterile cotton swab was dipped into the bacterial suspension and inoculated onto the entire surface of Mueller Hinton agar (MHA) plate by a 'back and forth' swabbing in a three-dimensional orientation. Two discs, one containing cefpodoxime (10 µg) and the other containing both cefpodoxime + clavulanic acid (10 µg /L) were then placed firmly on the inoculated MHA. This was incubated at 35 ^{o}C for 18 hours. *Escherichia coli* ATCC 25922 was used for positive control. After incubation, the diameter of zones of inhibition around the cefpodoxime and cefpodoxime + clavulanic acid combination discs were measured. The value of the inhibition zone around the cefpodoxime disc was compared to the value of the inhibition zone

around cefpodoxime + clavulanic acid combination disc. Isolates that showed a difference of ≥5mm between the inhibition zones of cefpodoxime alone and cefpodoxime + clavulanic acid combination discs were confirmed as phenotypic ESBL-producers.

Antimicrobial Susceptibility Testing

Isolates were tested for susceptibility to cefotaxime (FOT, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime/clavulanic acid (FOT/CLA), ceftazidime/clavulanic acid (CAZ/CLA), cefoxitin (FOX, 30 µg), compound sulphonamide (SMZ, 300 µg), streptomycin (STR, 10 µg), trimethoprim (TMP, 5 µg), tetracycline (TET, 30 µg), chloramphenicol (CHL, 10 µg), ampicillin (AMP, 10 µg), enrofloxacin (ENRO, 5 µg), gentamicin (GEN, 10 µg), nalidixic acid (NAL, 30 µg), ertapenem (ETP, 10 µg), and sulphamethoxazole/trimethoprim (SXT, 25 µg) by the disk diffusion method. A bacterial inoculum in normal saline was prepared and adjusted to 0.5 McFarland standards. This was inoculated onto the entire surface of Mueller Hinton agar in a Petri dish. Thereafter, the antibiotic discs (Oxoid) containing the antibiotic to be tested were carefully and aseptically applied to the surface of the agar. The plates were incubated at 35 ^{o}C. After 18 hours of incubation, the diameter of the zones of inhibition of bacterial growth around each of the antibiotic disc was measured. The results were interpreted according to the recommendation of the Clinical Laboratory Standards Institute (CLSI, 2015). In every occasion, *Escherichia coli* ATCC25922 was used as positive control.

Statistical analysis

Data were expressed in absolute values and in percentages and compared by Chi-square test at $p<0.05$ probability level using Statistical Software Package for Social Sciences (SPSS, version 16, 2007).

RESULTS

Bacterial isolates

Ninety-five suspected *Klebsiella* isolates were investigated in this study. Fifty-five (57.9%) of the isolates were obtained from the faeces of apparently healthy animals while the remaining 40 (42.1%) were from clinical samples such as diarrhoeic faeces, urogenital discharges milk from mastitis, blood, lung and liver tissues of various animal species (Table 1).

Identification of *Klebsiella species*

The *Klebsiella* identified in this study belong to six species, including ; *K. oxytoca* (34.7%), *K. pneumoniae* (26.3%), *K. ozaenae* (18.9%), *K. terrigena* (13.7%), *K. rhinoscleromatis* (5.3%), *K. planticola* (1.1%) (Table 2).

Table 2: *Klebsiella* species identified in clinical and non-clinical samples from animals in Abeokuta, Nigeria

Klebsiella species	Cattle	Sheep	Goats	Pigs	Horses	Dogs	Cats	Chickens	Guinea fowls	Total (%)
K. pneumoniae	5	1	0	1	1	8	4	5	0	25 (26.3)
K. rhinoschleromatis	0	0	1	1	0	1	0	2	0	5 (5.3)
K. oxytoca	7	3	5	5	0	3	2	7	1	33 (34.7)
K. ozaenea	2	4	2	3	0	3	0	3	1	18 (18.9)
K. terrigena	3	2	2	1	0	3	0	2	0	13 (13.7)
K. planticola	0	0	0	0	1	0	0	0	0	1 (1.1)
Total (%)	17 (17.9)	10 (10.5)	10 (10.5)	11 (11.6)	2 (2.2)	18 (18.9)	6 (6.3)	19 (20.0)	2 (2.1)	95 (100)

The rate of detection of *K. oxytoca* and *K. pneumoniae* was significantly higher ($p<0.05$) than for the other species. *Klebsiella. pneumoniae* was detected more in clinical samples. (Table 3).

Table 3: Sample sources of *Klebsiella* species isolated from animals in Abeokuta, Nigeria.

Klebsiella species	Non-clinical samples	Clinical samples						Total (%)
	Faeces	Faeces	Urogenital tract	Milk	Lung	Blood	Liver	
K. pneumoniae	2	3	4	5	8	1	2	25 (26.3)
K. rhinoschleromatis	5	0	0	0	0	0	0	5 (5.3)
K. oxytoca	22	6	2	1	1	0	1	33 (34.7)
K. ozaenea	12	2	2	1	1	0	0	18 (18.9)
K. terrigena	13	0	0	0	0	0	0	13 (13.7)
K. planticola	1	0	0	0	0	0	0	1 (1.1)
Total (%)	55 (57.9)	11 (11.6)	8 (8.4)	7 (7.4)	10 (10.5)	1 (1.1)	3 (3.2)	95 (100.0)

Eight (8.4%) out of the 95 isolates of *Klebsiella* spp were identified as ESBL-producers. These included four isolates of *Klebsiella pneumoniae*, three of *Klebsiella oxytoca* and one of *Klebsiella ozaenae*. Five out of the eight ESBL-producing isolates were from clinical samples including diarrhoeic faeces (2 isolates), mastitic milk (1 isolate), lung tissue (1 isolate) and discharge from urogenital tract (1 isolate). The remaining 3 isolates were from the faeces of apparently healthy animals (Table 4).

Table 4: Extended spectrum β-lactamase producing *Klebsiella species* isolated from animals in Abeokuta.

S/N	ESBL-producing *Klebsiella* species	Animal Source	Sample source	Antimicrobial resistance profile	Year of isolation
1	*K. pneumoniae*	Dog	Urinary tract infection	AMP-CEF-CTX-CAZ-STR-TET-CIP-CHL-SUL-SXT-TMP-NAL-GEN	2014
2	*K. pneumoniae*	Dog	Diarrhoeic faeces	AMP-CEF-CTX-CAZ-STR-TET-SUL-SXT-TMP-NAL	2014
3	*K. oxytoca*	Goat	Faeces	AMP-CEF-CTX-CAZ-STR-TET-CIP-CHL-SUL-SXT-TMP-NAL-GEN	2016
4	*K. oxytoca*	Goat	Faeces	AMP-CEF-CTX-CAZ-STR-TET-CIP-CHL-SUL-SXT-TMP-NAL-GEN	2016
5	*K. pneumoniae*	Cattle	Milk (mastitis)	AMP-CEF-CTX-CAZ-STR-TET-CIP-CHL-SUL-SXT-TMP-NAL-GEN	2013
6	*K. oxytoca*	Pigs	Faeces	AMP-CEF-CTX-CAZ-STR-TET-CHL-SUL-SXT-TMP-NAL	2014
7	*K. pneumoniae*	Chicken	Lung tissue	AMP-CEF-CTX-CAZ-STR-TET-CHL-SUL-SXT-TMP-NAL	2013
8	*K. ozaenea*	Cat	Faeces (Diarrhoeic)	AMP-CEF-CTX-CAZ-STR-TET-CHL-SUL-SXT-TMP-NAL-GEN	2013

Key: cefotaxime –FOT, ceftazidime – CAZ, cefoxitin –FOX, gentamicin –GEN, ertapenem --ETP nalidixic acid --NAL, compound sulfonamide –SMZ, streptomycin –STR, trimethoprim --TMP tetracycline –TET, chloramphenicol -- CHL, ampicillin --AMP, enrofloxacin--ENRO cefotaxime/clavulanic acid --FOT/CLA, sulphamethoxazole/trimethoprim—SXT, ceftazidime/clavulanic acid -- CAZ/CLA.

Antimicrobial susceptibility profile of ESBL-producing *Klebsiella* species.

The ESBL-producing isolates were all (100%) resistant to ampicillin, cefotaxime, ceftazidime, cefpodoxime, streptpmycin, tetracycline, compound sulfonamide, trimethoprim, sulphomethoxazole, and nalidixic acid. They showed varying degrees of resistance to ciprofloxacin (50%), chloramphenicol (87.5%) and gentamicin (62.5%) but were all susceptible to ertapenem. Four multiantibiotic resistance pattern were detected (Table 4).

DISCUSSION

In this study, *Klebsiella* spp were recovered from clinical and non-clinical samples of different animal species. This study suggested that *Klebsiella* may exist as commensals in animals without manifestation of disease in the host but can also be involved in clinical conditions of considerable severity. This is similar to the findings of other authors where *Klebsiella* was isolated from apparently healthy animals as well as from cases of clinical infections (Kilonzo-Nthenge *et al*., 2008; Fielding *et al.,* 2012; Ojo *et al.*, 2012). *Klebsiella* species in apparently healthy host can assume pathogenic roles following immunosuppression due to the presence of a stressor such as change in weather conditions, poor management, concurrent primary pathogen, age extremes, poor nutrition or debility (Podschun and Ullmann, 1998; Ghanem *et al*., 2015). It is therefore important to prevent the establishment of *Klebsiella* species as possible aetiology of disease, by avoiding stressful conditions through proper nutrition, good hygiene, good animal husbandry, and prompt treatment of primary infections (Ghanem *et al*., 2015). *Klebsiella* species were isolated from diverse sources and across different animal species. This showed that the organism could cause infections in different body systems (Glickman, 1981; Podschun and Ullmann, 1998). *Klebsiella* was isolated from clinical samples including diarrhoeic faeces, milk from cases of mastitis in cows, lung tissues, blood, urogenital discharges and the liver. *Klebsiella* species is known to cause both intestinal and extraintestinal diseases especially pneumonia and septicaemia (Ghanem *et al*., 2015). The isolation of *Klebsiella* from blood as seen in this study is suggestive of the septicaemia. The isolation of *Klebsiella* from different animal species including cattle, sheep, goat, dogs, cats and poultry showed that *Klebsiella* spp could cause diseases in varieties of animals. The organism was isolated from cases of gastroenteritis, mastitis, urinary tract infections, pneumonia and septicaemia. This is similar to the observation of previous authors where *Klebsiellae* were isolated from different clinical condition of different animal species across the globe (Gameel *et al.,* 1991; Roberts *et al.,* 2000; Munoz *et al*., 2006; Alenka *et al*., 2007; Ribeiro *et al.*, 2008, Ewers *et al.*, 2014). *Klebsiella* spp is part of the normal microflora of the gut of humans and animals (Podschun and Ullmann, 1998). The organism can be shed in the faeces of apparently healthy host and be transmitted to cause disease in susceptible hosts of different species of animals (Ewers *et al.*, 2014). Faecal shedding is results in environmental contamination and widespread dissemination of the organism within the community (Alenka *et al*., 2007). *Klebsiella* spp have emerged as important aetiology of nosocomial infection (Zaki *et al*., 1980; Podschun and Ullmann, 1998). Therefore, it is important to note that animal patients can serve as the foci of nosocomial transmission to other patients, clients,

veterinarians and workers within the hospital setting. In this study, six different species of *Klebsiella* were identified in clinical and non-clinical samples from animals. These included *K. pneumoniae, K. oxytoca, K. ozaenae, K. terrigena, K. rhinoscleromatis,* and *K. planticola. Klebsiella planticola* was the most frequently encountered species. *Klebsiella oxytoca* and *K. pneumoniae* were isolated from both clinical and non-clinical samples, but *K. pneumoniae* was more common in clinical samples. *K. ozaenae* was also identified in clinical samples. In previous studies, *K. pneumoniae* was also isolated more frequently from clinical conditions of humans and animals (Podschun and Ullmann, 1998; Ewers *et al.*, 2014). However, other species of *Klebsiella* have also been associated with clinical conditions in humans and animals either as primary pathogen or as opportunistic invader (Gameel *et al.,* 1991; Roberts *et al.,* 2000; Munoz *et al*., 2006; Alenka *et al*., 2007; Ribeiro *et al.,* 2008; Ewers *et al.*, 2014). Extended-spectrum β-lactamase producing *Klebsiella* spp were detected in both clinical and non-clinical samples of animal origin. This confirmed the involvement of ESBL-producing *Klebsiella spp* in disease conditions of animals as previously reported by other authors Ewers *et al.*, 2014. It also showed that apparently healthy animals could harbour ESBL-producing *Klebsiella* spp in their gut and serve as reservoir for environmental contamination and dissemination to other animals and humans. The detection of ESBL-producing *Klebsiella* species has implication in the treatment of clinical infections caused by *Klebsiella* species and is of public health significance. Diseases caused by ESBL-producing *Klebsiella* species could be refractory to antimicrobial therapy. This could lead to protracted illness, increase cost of veterinary care, increased case fatality, increased risk of nosocomial transmission and widespread dissemination in the environment. Humans can also contract infection through direct and indirect contact with animals or contaminated environment and through consumption of contaminated animal-source foods. The ESBL-producing *Klebsiella* species detected in this study were not only resistant to the β-lactam antibiotics but also to antimicrobials from other classes. It is generally recognised that most ESBL-producing bacterial strains showed multiantibiotic resistance traits as displayed by those encountered in this study (Ewers *et al.*, 2014; Bush and Bradford, 2016). The multidrug resistance property of ESBL-producing bacteria has been associated with co-location of resistance genes of other antimicrobials on the same mobile genetic elements as ESBL genes. Multiantibiotic resistant can also be due to the presence of other resistance mechanisms such as efflux pumps and alter outer membrane proteins (porins) in addition to the production of ESBLs (Pitout and laupland, 2008; Beceiro *et al*., 2013; Ojo *et al.*,2016). The increasing reports of multidrug resistant among bacterial isolates of animal origin could be due to non-judicious use of antimicrobials in food and companion animals as a result of unrestricted access and overdependence on antimicrobial (Hao *et al,* 2014; Ojo *et al.*, 2016). Poor disease diagnosis and exclusion of microbiological investigations including isolation and antimicrobial susceptibility testing before application of antimicrobial agents play a major role in antimicrobial misuse by veterinarians. In Nigeria, unauthorized persons indiscriminately use antimicrobials in farm and in companion animals. Despite the existence of good legislation guiding the distribution of antimicrobial, these drugs are sold over-the-

counter without prescription. This has led to misuse and over-use which can hasten the process of resistance development and accelerate wide spread dissemination of resistant traits.

CONCLUSION

Multiantibiotic resistant ESBL-producing *Klebsiella spp* were detected in clinical and non-clinical samples from different animal species in Abeokuta, Nigeria. Apparently healthy carriers and sick animals can serve as sources of transmission of ESBL-producing *Klebsiella spp* to other animals and humans either in hospital settings or within the community.

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