Emerging antibiotic resistant enteric bacterial flora among food animals in Abeokuta, Nigeria

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Abstract

*Bacterial resistance to antibiotic in food animals is an emerging public health concern as a result of increasing multi-resistant bacteria found in Abeokuta. Therefore, occurrence rate and resistant profile of the emerging enteric pathogens were determined in food animals in Abeokuta, Nigeria. Fecal and rectal samples of 82 were randomly collected from food animals such as sheep (30), chicken (37) and pigs (15) in three major farms located in Abeokuta, Nigeria. Enteric bacteria isolates obtained were cultured and identified according to standard protocol. Antibiotic susceptibility testing was performed by disc diffusion method and standard micro-tube dilution assay and resistance profile was carried out. Among the food animals surveyed for resistant enteric pathogens, 46% and 36% were chicken and sheep respectively while 18% were pigs. Complete resistance of 100% was shown by all the enteric bacteria obtained to metronidazole, tetracycline and penicillin while highest rate of *Proteus mirabilis* (33.5%) and *Escherichia coli* (20.1%) were multi-resistant strains. Uncontrolled use of antimicrobial agents in food animals is shown to be associated with increasing emergence of antimicrobial resistance in food-borne pathogens which could enhance treatment failure and increase morbidity or death among the populace.*

Keywords: multi-resistant, bacteria, antibiotics, food, animals, pathogens

Introduction

Bacterial antibiotic resistance found among food animals is an emerging public health concern as a result of gross mis-use of antimicrobial agents used as prophylaxis, growth enhancement and for the treatment of infectious diseases (Martínez and Baquero, 2002). Most enteric flora are medically important as infectious agents and usually exhibit antibiotic resistances with severe virulence characteristics (Paterson, 2002; Rupp and Fey, 2003). Antibiotics are extensively used in human and veterinary medicine with or without regulation mostly among small scale farmers, and have potentially led to continuous emergence of multidrug resistant bacteria.

Among the class of antimicrobial agents

used for growth promotion in Nigeria. Tetracyclines, penicillins, macrolides, lincomycin (an analog of clindamycin) and fluoroquinolones are the most commonly used antimicrobial agents in animals for treatment and prevention of diseases. The human health consequence of the agricultural use of antimicrobial agents for growth enhancement in poultry, piggery and other food animals is gradually becoming unavoidable danger to public health (Besser *et al*; 2000). Evaluating the quantity of antimicrobial agents and how they are used in food animals in the Nigeria is important issue to be considered. Administration of antimicrobial agents affects both targeted pathogenic organisms as well as non-target commensals. Thus frequent antimicrobial use creates a pool of

resistant commensal bacteria that contribute to the general increase and dissemination of bacterial resistance worldwide, and can be a source of resistance genes for pathogens (Andreumont, 2003).

Previous studies have shown that animal drugs are grossly misused in Nigeria and are readily available without prescription (Aibinu *et al*; 2007). This creates a pool of resistant organisms both commensals and pathogenic. The use of antimicrobials in veterinary practice greatly influences the prevalence of resistance in animal bacteria and poses a great risk for the emergence of antibiotic resistance in human pathogens as well (Besser *et al*; 2000). Despite the potential threat of this problem, inadequate attention was paid to effort to effectively reduce prolific use of antibiotic majorly for livestock. Many enteric bacteria are now being used to estimate the stemming resistance problem among animal populations because of their high prevalence in the Gram negative bacteria in feces of apparently healthy animals and their ability to harbor several resistance determinants (Aarestrup *et al*; 2008).

Increasing antimicrobial resistance in the commensal bacteria in animals results largely from the selective pressure of antimicrobial agent use and reflects the genetic elements that may transfer to pathogens (Hummel *et al*, 1986, Murray, 1992). Most resistant bacteria have mobile genetic elements, such as R-plasmids and transposons that carry resistance genes. As the number of resistant commensal bacteria increases, the plasmid population becomes larger and may allow more frequent transfer of resistance to pathogenic bacteria such as *Salmonella* and *Shigella*. *Escherichia coli* is predominant species in the normal aerobic fecal flora in many animals with great propensity to transfer genes coding

for resistance to other species, including pathogenic bacteria (Tauxe *et al*; 1989, Murray, 1992). Recent studies have shown an emerging resistance in *E. coli* to third-generation cephalosporins. *Enterococcus faecium* is known as resistant commensal bacteria to quinupristin/dalfopristin which is used as a growth promoter in food animals such as chicken. It has high propensity of transferring resistant gene from animal-to-human (CDFA, 1999). This emerging strain is now found to be resistant to at least ampicillin, chloramphenicol, streptomycin, sulphamethoxazole, tetracycline, amoxicillin/clavulanate, cephalothin, cefoxitin and ceftiofur (Tauxe *et al*; 1989).

Therefore, this study investigates the patterns of enteric bacterial resistance in food animals to common antibiotics, using standard resistance profiling as indicator for detecting and prevention of antimicrobial resistance.

Materials and Methods

Sample collection

Fifty nine (59) fecal and 23 rectal samples were randomly collected from various animal farm locations in Abeokuta, capital of Ogun State. Abeokuta town is located West to Benin Republic, South to Lagos and Atlantic ocean, East to Ondo State and North to Oyo State in South Western Nigeria covering land area of about 16,980.55sq km (NBS, 2007). Abeokuta lies within 7.15°N and 3.35°E in moderately hot tropical climatic zone with mean temperature of 30°C, harmattan of 24°C and annual average rainfall of 1000mm (Shittu *et al*; 2010). Eighty two fecal and rectal samples were collected from major food animals; sheep (30), chicken (37) and pigs (15). Fresh fecal and rectal samples were collected in sterile plastic containers and transported in cold pack to the laboratory

for analysis.

Isolation and identification

Each sample collected was pre-enriched in tryptic soya broth for 24 hours and inoculated directly onto Mac-Conkey agar and Salmonella-Shigella agar (Oxoid, UK) and incubated aerobically at 37°C over night. Pure colony was sub-cultured onto Mac-Conkey and Salmonella-Shigella agar and incubated aerobically at 37°C over night. Biochemical identification was carried out with the use of Microbact 24E test (Oxoid, UK).

Antibiotic susceptibility testing:

Pure isolates were tested for their susceptibility to antibiotics such as gentamycin (10 µg), ampicillin (10 µg), sulphamethaxazole (25/5 µg), penicillin (10 µg), cefotaxime (30 µg), nalidixic acid (30 µg), amoxicillin/clavulanic (5/20 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), nitrofurantoin (30 µg), metronisazole (30 µg) and tetracycline (30 µg) (Oxoid, UK). Antimicrobial resistant profile of the isolates was carried out by the disk diffusion method. The inoculums of 0.5 McFarland turbidity was spread uniformly onto Nutrient agar and antimicrobial disks

were then applied to the surface of the plate. After incubation at 37°C for 18 to 24 h, cultured plates were examined for growth and their respective inhibition zones were interpreted according to CLSI guideline (CLSI, 2011).

Data Analysis

Descriptive statistics was used to analyze the data obtained in this study which were presented in percentages

Results and Discussion

In recent past, there is growing concern on the increasing spread of multi-resistant enteric bacteria isolates from food animal which in turn affect large number of the populace causing alarming rate of mortality (Okeke *et al.*; 2007). Several studies have reported intimate interaction between animals and humans in terms of spread of resistant pathogenic bacteria (Gray *et al.*, 2004; Bester and Essack, 2010; Zhang *et al.*, 2010; Duru *et al.*, 2013). Among the food animals surveyed for resistant enteric bacteria 45% and 37% were chicken and sheep respectively while only 18% were pig (Fig. 1). Chicken is the most common food

Figure 1; Distribution of the common food animals surveyed in this study within Abeokuta

animal which many residents consumed fried, grilled or boiled but numerous multi-resistant commensals are being transferred to the consumers due to poor cooking, unhygienic mode of preparation and sometimes unhygienic raw consumption of the animal (Chinwe *et al.*, 2014).

Different studies have shown that animal drugs are grossly misused in Nigeria and are readily purchased without prescription and poorly administered to the animals by the farmers (Iruka *et al.*; 2007). From the study in Table 1, complete resistance of 100% was shown by all the enteric bacteria obtained to metronidazole, tetracycline and penicillin. Only 80.0% and 66.7% were resistant to Ampicillin and sulphamethaxazole respectively. High rate of resistance to metronidazole and tetracycline is indicated as a result of unregulated use of antibiotics in most food animals. This tetracycline resistance is similar to Blake *et al.*; (2003), who reported dominance of *tetA* resistance gene *tetB* resistance gene in *E. coli* strains from

treated and untreated pigs. This is also similar to reports of increasing Enterobacteriaceae from chickens fed with tetracycline-treated feed that developed resistance within 36 to 48 hours and extended resistance to ampicillin, carbenicillin and sulphonamides (Randall *et al.*, 2004). Unguided use of antimicrobials in food animals influences the rapid development of bacteria resistance and has greatly encouraged emerging pool of resistant bacteria commensals and pathogenic isolates (Aarestrup *et al.*; 2008).

Constant use of antimicrobial agents such as commonly used ones has caused increase in pool of resistant commensal bacteria such as *Escherichia coli* and *Proteus mirabilis* that could be a source of dissemination of resistance genes to other bacteria pathogens (Aarestrup *et al.*, 2001). It was observed (Figure 2) that highest rate of multi-resistant strain found among the food animals were *Proteus mirabilis* (33.5%) and *Escherichia coli* (20.1%). This further

Table 1; Antimicrobial susceptibility pattern of enteric bacteria isolates obtained from different animals in Abeokuta, Nigeria.

Antibiotics	Total number of unrepeatd enteric isolates = 15			
	Susceptible		Resistant	
	N	%	n	%
Gentamycin	14	93.3	01	6.7
Ampicillin	03	20.0	12	80.0
Sulphamethaxazole	05	33.3	10	66.7
Penicillin	00	0.0	15	100.0
Cefotaxime	12	80.0	03	20.0
Nalidixic acid	07	46.7	08	53.3
Amoxycilli/clavulanic acid	07	46.7	08	53.3
Ciprofloxacin	13	86.7	02	13.3
Norfloxacin	08	53.3	07	46.7
Nitrofurantoin	13	86.7	02	13.3
Metronisazole	00	0.0	15	100.0
Tetracycline	00	0.0	15	100.0

suggest frequent contamination of meat and other animal products with enteropathogenic or multi-resistant enteric bacteria spreading through food chain to human and has become food safety concern to the public. Heavy contamination of ready-to-eat animal carcass due to the unhygienic slaughtering, processing and storage in many abattoir and slabs, is another identified source of infection to humans. (Adak *et al.*, 2002, Aibinu *et al.*, 2007). As a result of high level of food animal products contamination, use of antibiotic as growth promoter and prophylaxis, there is unprecedented increase in emergence of antibiotic-resistant bacteria, while inappropriate use of these antibiotics drugs greatly accelerates antibiotic resistance development.

Moreover, the unnecessary use of antibiotic in food animal unnecessarily to healthy farm animals to promote growth and compensate for unsanitary conditions in animal agriculture is gradually posing

danger to human health (Mellon *et al.*, 2001). This has enhanced spread of this new generation of infections which are resistant to antibiotic with serious consequences for public health causing high economic loss of treatment and high morbidity. The elderly and children are those at risk due to their low immune systems and they could be more vulnerable to many infections due to antimicrobial resistant bacteria.

Conclusion

This uncontrolled use of antimicrobial agents in food animals has shown to be associated with increasing emergence of antimicrobial resistance in food-borne pathogens and subsequent multidrug-resistant bacterial infections in humans. These resistant enteric bacteria pathogens could enhance treatment failure and increase morbidity or death. Therefore, working antibiotic regulation should be enforced and inappropriate use of antimicrobial agents in food animals must be discouraged.

Figure 2, Percentage resistance rate of multi-resistant enteric bacteria found in food animals surveyed.

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