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ANTIBIOTIC RESISTANCE AND EXTENDED SPECTRUM BETA-LACTAMASES AMONG *PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA COLI* ISOLATES RECOVERED FROM CATTLE AND CHICKEN IN NIGERIA.

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Abstract: The objective of this study was to determine the antibiotic resistance patterns and the production of extended spectrum beta lactamases by *Pseudomonas aeruginosa* and *Escherichia coli* isolates obtained from cattle and chickens in Nigeria. Out of 150 animals samples analyzed, 76 bacterial isolates were observed. Twenty three were *Pseudomonas aeruginosa* while 53 were *Escherichia coli*. However, 15 (65.2 %), 32 (60.4 %) and 8 (34.8 %), 21 (39.6 %) were *Pseudomonas aeruginosa* and *Escherichia coli* isolates from cattle and chicken respectively. The antibiotic susceptibility studies showed an alarming level of resistance to all the tested antibiotics, reflecting multi-drug resistance strains. The isolates of *Pseudomonas aeruginosa* and *Escherichia coli* that were highly resistant to cephalosporin antibiotics were subjected to ESBL detection using double disc synergy test (DDST). A total of 11 (23.4 %) and 7 (24.1 %) isolates were identified to be ESBL-producers from cattle and chicken respectively. Thus, 4 (8.5 %), 7 (14.9 %) and 2 (6.9 %), 5 (17.2 %) were *Pseudomonas aeruginosa* and *Escherichia coli* isolates from cattle and chicken respectively. This study has reviewed and demonstrated the presence of multiple antibiotic resistant and distribution of ESBL-producing *Pseudomonas aeruginosa* and *Escherichia coli* from animal origin. Thus, preventive measures like a continuous surveillance of the antibiotics used as growth promoter in farm animals, strict use of antibiotics on farm animals, establishment of veterinary antimicrobial resistance, monitoring program worldwide and a strict implementation of infection control practices can go a long way in containing the menace of drug resistance in our environment.

Keywords: Antibiotics Resistance, ESBLs, Animals, *P. aeruginosa, E. coli.*

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INTRODUCTION

Infections with Extended Spectrum Beta-Lactamase (ESBL) producing *Enterobacteriaceae* are found increasingly in humans (Livermore, 2012). Beta-lactam antibiotics are widely used in the treatment of infectious diseases caused by microorganisms in the hospitals and agricultural settings. The indiscriminate use of these drugs has resulted to rapid increase in antibiotics resistance among bacterial organisms which produces enzymes (beta-lactamases) that has ability to degrade or hydrolyse the active site of these antibiotics and thus render them almost inefficacious (Ejikeugwu *et al.,* 2015). These lead to production of extended-spectrum cephalosporins. Shortly, after a variety of extended-spectrum cephalosporins were introduced in the 1980s, bacterial strains producing extended-spectrum β-lactamase (ESBLs) such as TEM or SHV-derived ESBLs emerged in Europe, USA and Germany (Chaudhary and Aggarwal, 2010). Production of an extended-spectrum β-lactamase (ESBL) has been the major resistance mechanism, among microorganisms from the family of *Enterobacteriaceae* and *Pseudomonad*, notably in *Escherichia coli* (Canton *et al*., 2003). ESBLs arise by mutation in the active site of the earlier beta-lactamases as aforementioned, and this allow the bacterial pathogen harbouring them to confer a broader range of activity by allowing attack on oxyiminio cephalosporins such as ceftazidime and cefotaxime (Weldhagen *et al*., 2003).

Pseudomonas aeruginosa is a Gram negative, non-enteric bacillus that is widely distributed in the environment such as soil, water and sometime colonizes animal and human. It thrives well in moist environment and causes a variety of infections such as wound and burn in human and animals (Jawetz *et al*., 2007). *Escherichia coli* belong to the family of Gram negative bacteria called *Enterobacteriaceae,* certain strain such as enteropathogenic and enterotoxigenic were responsible for infant diarrhoea and gastroenteritis which is important in public health. *Escherichia coli* has been implicated as one of the major causes of urinary tract infections in women and men worldwide (Ugbo *et al*., 2015; Cheesbrough, 2010). Antibiotics overuse in humans and animals, hospital cross-infection, the food chain, trade and human migration seem to have contributed to the high dissemination of ESBLs outside hospitals, although the role of these factors is variable. Reports of infection or colonization with ESBL-producing Gram negative bacteria have focused mainly on hospitalized patients or nursing home resident. However, ESBL-producing Gram negative bacteria have also been described in animal and patient in the community, with and without chronic condition (Mirelis *et al*., 2003). *Escherichia coli* producing CTX M-2 beta-lactamase in animal (cattle) has been reported in Japan (Yutake *et al*., 2004); extended spectrum beta-lactamases producing *E. coli* was isolated from dogs and cats in Rome, Italy (Alessandra *et al*., 2004). *Escherichia coli* strains resistant to broad spectrum cephalosporins have been isolated from calves and dogs (Warren *et al*., 2001). Chau and Obegbunam, (2007) reported the presence of high resistant ESBL-producing *E. coli* from chickens in Enugu State,

Nigeria. This study was therefore designed to investigate the presence of multiple antibiotic resistant and distribution of ESBL-producing *Pseudomonas aeruginosa* and *Escherichia coli* from animal origin.

MATERIALS AND METHODS

SAMPLE COLLECTION: A total of 150 animal samples; including cattle (n =75), chicken (n =75) were randomly collected from meat sellers in Abakaliki abattoir environment using sterile knife and sterile lick proof containers. The samples were collected from different parts of the animals' gizzard, muscle, intestine, droplets; and transported to Applied Microbiology Laboratory for analysis.

SAMPLE PREPARATION: Five grams (5 g) of collected meat samples were weighed and transferred to sterile flasks containing 20 ml of phosphate buffer saline (PBS). Samples were homogenized using a meat grinder under aseptic conditions and was stored for further analysis.

MICROBIOLOGICAL ANALYSIS: The diluted meat samples in PBS were inoculated onto Nutrient agar, MacConkey agar and Cetrimide agar, Eosine methylene blue (EMB) for the isolation of Gram negative organisms, incubated aerobically at 37^0C for 18 to 24 hrs. *Pseudomonas aeruginosa* and *Escherichia coli* isolates were identified biochemically using conventional identification techniques including oxidase test, Indole test, Gram staining and colonial morphology such as pigmentation (Cheesbrough, 2010).

ANTIBIOTICS SUSCEPTIBILITY STUDIES

Susceptibility patterns of the isolated organisms were tested against a wide range of antibiotics which includes Ciprofloxacin (5 µg), Tetracycline (30 µg), Nitrofurantoin (100 µg), Amoxicillin (30 µg), Erythromycin (10 µg), Gentamicin (10 µg), Cephalexin (30 µg), Chloramphenicol (10 µg), Amplicillin (25 µg) Ceftazidime (30 µg), Cefotaxime (30 µg), Cotrimoxazole (25 µg) Ceftriaxone (30 µg) and Cefpodoxime (10 µg) (Oxoid, UK) using Kirby-Bauer disc diffusion method (Ugbo *et al*., 2015). The results were interpreted according to the guidelines of Clinical Laboratory Standard Institute (CLSI, 2006).

Screening and detection of ESBL production.

Pseudomonas aeruginosa and *Escherichia coli* isolates that were resistant to any of the third generation cephalosporins including cefotaxime and ceftazidime according to Clinical Laboratory Standard Institute (CLSI) were suspected to be potential ESBL producer and further tested for ESBL production phenotypically (CLSI, 2006). ESBL production in the *Pseudomonas aeruginosa* and *Escherichia coli* isolates were detected phenotypically using the double disc synergy test (DDST) method (Ejikeugwu *et al.,* 2015). An overnight culture of the test bacteria, adjusted to 0.5

McFarland turbidity standards was aseptically inoculated on the surface of Mueller-Hinton agar plates using sterile swab sticks. Amoxicillin/clavulanic acid disc (30 µg) was aseptically placed in the centre of a Petri dish. Cefotaxime (30 µg) and Ceftazidime (30 µg) single antibiotic discs were placed adjacent to the central disc at a distance of 15mm. The plates were incubated at 37 ^{0}C for 18-24 hrs. An increase of ≥5 mm in the inhibition zone diameter for either of the cephalosporins (ceftazidime and cefotaxime) tested in combination with amoxycillin-clavulanic acid versus its zone when tested alone confirms ESBL production phenotypically.

Results

Table 1, shows the distribution of bacteria isolates in various animal, which includes cattle and chicken with percentage distribution of 15 (65.2 %) *P. aeruginosa*, 32 (60.4 %) *E. coli* from cattle and 8 (34.8 %) *P. aeruginosa*, 21 (39.6 %) *E. coli* from chicken. Frequency distribution of bacteria isolates from cattle samples is demonstrated in Table 2. A total of 14 *P. aeruginosa* and 32 *E.coli* with percentage distribution of 5 (35.7 %), 3 (21.3 %), 7 (50.0 %) *P. aeruginosa* and17 (53.1 %) 6 (18.8 %) 9 (28.1 %) were isolated from intestine, muscle and droplet respectively. Table 3 shows frequency distribution of bacteria isolates from chicken samples in percentage, (8); 1 (12.5 %), 0 (0.0 %), 2 (25.0 %), 5 (62.5 %) *P. aeruginosa* and (21); 8 (38.1 %), 2 (9.5 %), 4 (19.1 %), 7 (33.3 %) *E. coli* identified from intestine, gizzard, muscle and droplet respectively. Table 4 demonstrated antibiogram of *Pseudomonas aeruginosa* and *Escherichia coli* isolates in percentage. The antibiotic susceptibility studies showed an alarming level of resistance to all the tested antibiotics reflecting multi-drug resistance strains. However, occurrence of ESBL production from cattle isolates was represented in Table 5. Out of 47 bacteria isolates tested, 11 (23.4 %) isolates were discovered to be ESBL producers, 4 (8.5 %) *P. aeruginosa* and 7 (14.9 %) *E. coli.* Table 6 shows the occurrence of ESBL production from chicken isolates. A total of 29 isolates were tested, 7 (24.1 %) were identified ESBL positive, 2 (6.9 %) *P. aeruginosa* 5 (17.2 %) *E.coli.*

Table 1: Distribution of bacteria isolates in various animal source.

Sample site	No of Samples Examined	Bacteria Isolates (%)	
Source	**in Percentage**	***P. aeruginosa***	***E. coli***
Cattle	75 (50.0)	15 (65.2)	32 (60.4)
Chicken	75 (50.0)	8 (34.8)	21 (39.6)
Total	**150 (100.0)**	**23 (100.0)**	**53 (100.0)**

Table 2: Frequency distribution of bacteria isolates from cattle samples.

Sample Site	No of Samples Examined	Bacteria Isolates (%);	
		P. aeruginosa	*E. coli*
Intestine	25 (33.3)	5 (35.7)	17 (53.1)
Muscle	25 (33.3)	3 (21.3)	6 (18.8)
Droplet	25 (33.3)	7 (50.0)	9 (28.1)
Total	**75 (100.0)**	**14 (100.0)**	**32 (100.0)**

Table 3: Frequency distribution of bacteria isolates from chicken samples.

Sample Site	No of Samples Examined	Bacteria Isolates (%);	
		P. aeruginosa	*E. coli*
Intestine	20 (26.7)	1 (12.5)	8 (38.1)
Gizzard	20 (26.7)	(0.0)	2 (9.5)
Muscle	20 (26.7)	2 (25.0)	4 (19.1)
Droplet	15 (20.0)	5 (62.5)	7 (33.3)
Total	**75 (100.0)**	**8 (100.0)**	**21 (100.0)**

Table 4: Antibiogram of *Pseudomonas aeruginosa* and *Escherichia coli* isolates in percentage.

Antibiotics (µg)	*P. aeruginosa*				*E. coli*	
	S (%)	R (%)			S (%)	R (%)
Cephalexin (30)	10 (43.5)	13 (56.5)	7 (30.4)	16	17 (32.1)	36 (67.9)
Cotrimoxazole (50)	(69.6)				19 (35.8)	34 (64.2)
Ciprofloxacin (5)	12 (52.2)	11 (47.8)	6 (26.1)	17	34 (64.2)	19 (35.8)
Tetracycline (50)	(73.9)				29 (54.7)	24 (45.3)

Nitrofurantoin (100)	4 (17.4)	19 (82.6)	10 (43.5)	13 (56.5)	13 (24.5)	40 (75.5)	
Amoxicillin (30)					15 (28.3)	38 (71.7)	
Erythromycin (10)	12 (52.2)	11 (47.8)	11 (47.8)	12 (52.2)	38 (71.7)	15 (28.3)	
Gentamicin (10)					35 (66.0)	18 (40.0)	
Chloramphenicol (10)	5 (21.7)	18 (78.3)	7 (30.4)	16 (69.6)	37 (69.8)	16 (30.2)	
Amplicillin (25)					12 (22.6)	41 (77.4)	
Ceftazidime (30)	9 (39.1)	14 (60.7)	11 (47.8)	12 (52.2)	22 (41.5)	31 (58.5)	19 (35.8)
Ceftriaxone (30)					34 (64.2)		
Cefotaxime (30)	7 (30.4)	16 (69.6)		8 (34.8)	20 (37.7)	33 (62.3)	
Cefpodoxime (10)	15 (65.2)				29 (54.7)	24 (45.3)	

Key: S- Susceptible; R- Resistant; (%)- Percentage

Table 5: Occurrence of ESBL production from cattle isolates.

Organisms	No of Isolates n(%)	ESBL positive n(%)	ESBL negative n(%)
P. aeruginosa	15 (31.9)	4 (8.5)	11 (23.4)
E. coli	32 (68.1)	7 (14.9)	25 (53.2)
Total	**47 (100.0)**	**11 (23.4)**	**36 (76.6)**

Table 6: Occurrence of ESBL production from chicken isolates.

Organisms	No of Isolates n(%)	ESBL positive n(%)	ESBL negative n(%)
P. aeruginosa	8 (27.6)	2 (6.9)	6 (20.7)
E. coli	21 (72.4)	5 (17.2)	16 (55.2)
Total	**29 (100.0)**	**7 (24.1)**	**22 (75.9)**

DISCUSSION

Antimicrobial drugs have been proved remarkably effective for the control of bacterial infections. However, indiscriminate use of antibiotics in formulating animal feeds and treatment of both

human and animal infections has contributed to multiple antibiotic resistances in bacterial population within our environment. In this study, presence of multiple antibiotics resistant and distribution of ESBL-producing *Pseudomonas aeruginosa* and *Escherichia coli* from animal origin was investigated. Out of 150 animals samples analyzed, 76 bacterial isolates were observed, of which 23 *Pseudomonas aeruginosa* and 53 *Escherichia coli* were isolated. However, 15 (65.2 %), 32 (60.4 %) and 8 (34.8 %), 21 (39.6 %) were *Pseudomonas aeruginosa* and *Escherichia coli* isolates from cattle and chicken respectively (Table 1) and is in line with the work of Busani *et al.,* 2006 were they observed the presence of the same organisms in chicken, beef, and mutton as one of the major cause of zoonotic infection.

Antimicrobial over use in human and animals, hospital cross infection, the food chain, trade and human migration seem to have contributed to the recent dissemination of multiple antibiotics resistance and ESBLs outside hospitals (Mirelis *et al.,* 2003). In our study, the susceptibility results of the bacteria isolates from both cattle and chicken sources showed that *Pseudomonas aeruginosa* and *Escherichia coli* are highly resistance to all the antibiotics tested. This could be as a result of the intrinsic nature of Gram-negative bacteria cell wall. Our findings corroborate the studies done by Dupont and Steele in 1987 which showed that the emergence of antibacterial resistance in animals can follow a general pattern; illustrated by the use of aminoglycosidic growth promoter nourseothricin which was used on farm animals in the former East Germany in the 1980s. Natural resistance of bacteria species to certain antibiotics and possible transfer of antibiotic resistance among species and the use of sub-therapeutic dose of antibiotics in animal feed to improve productivity, could also lead to multiple antibiotics resistance among bacteria. The infections which are caused by multidrug-resistant gram negative bacilli that produce extended spectrum beta- lactamase enzymes have been reported with an increasing frequency in humans (Livermore, 2013) and animals such as chicken, cattle and dogs (Dierikx *et al.,* 2013; Chado *et al.,* 2008 and Hidetake *et al.,* 2004). The numerous β- lactamases are encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposones. In the present study, we observed the presence of ESBL-producing *Pseudomonas aeruginosa* and *Escherichia coli* in both cattle and chickens. A total of 11 (23.4 %) and 7 (24.1 %) isolates were identified to be ESBL-producers from cattle and chicken respectively. However, 4 (8.5 %), 7 (14.9 %) and 2 (6.9 %), 5 (17.2 %) were *Pseudomonas aeruginosa* and *Escherichia coli* isolates from cattle and chicken respectively (Tables 5 and 6). We observed that person-to-animal contact and acquisition from a common source, probably related to food such as chicken and cattle meat products contaminated with these antibiotic resistance bacteria; clearly contribute to ESBL dissemination within families and entire society. The present study is in agreement with that of Chado *et al.* 2008 report on the presence of ESBL producing Enterobacteriaceae isolated from chicken and swine in Portugal and also in concord with the work of Chah and Oboegbunam,

2007 that discovered ESBL production among ampicillin resistant *E. coli* strains from chicken in Enugu State, Nigeria.

The detection of ESBL-producing *Pseudomonas aeruginosa* in our study is in agreement with the work of Aibinu *et al*. (2007). Our findings also akin with the finding of Liebana *et al.,* 2007 were they observed the presence of ESBL mediated resistance organisms in longitudinal farm. In 2010, Sweden reported the presence of *E. coli* carrying bla_{CMY-2} positive strains in imported GPS chickens at their arrival in Sweden (SVARM 2010). Yutaka *et al*., 2004 reported the presence of *E. coli* producing CTX-M-2 bata-lactamase in cattle. The presence of multiple antibiotic resistant and ESBL-producing *Pseudomonas aeruginosa* and *Escherichia coli* from animal origin was observed in this research. This finding could be as a result of wide usage of antimicrobial agent's indiscriminately which includes cephalosporins in animal feed formulation as growth promoter and treatment of infections. Vertical transmission, horizontal transmission as well as recirculation of these isolates at farms and hatcheries may play a role. Thus, preventive measures like a continuous surveillance of the antibiotics used as growth promoter in farm animals, strict use of antibiotics on farm animals, establishment of veterinary antimicrobial resistance monitoring program worldwide and a strict implementation of infection control practices can go a long way in containing the menace of drug resistance in our environment.

REFERENCES

1. Aibinu I., Nwanneka, T and Odugbemi, T. (2007). Occurrence of ESBL and MBL in clinical isolates of *Pseudomonas aeruginosa* from Lagos, Nigeria. *Journal of American Sciences,* 3(4): 81-85.

2. Alessandra, C., Sarah, L., Alessia, F., Gessica, C., Paola, D. M and Battisti, A. (2004). Extended - spectrum beta-lactamases in *Escherichia coli* isolated from Dogs and Cats in Rome, Italy, from 2001-2003. *Antimicrob. agents Chemother.,* 49:833-835.

3. Busani, L., Scavia, G., Luzzi, I and Caprioli, A. (2006). Laboratory surveillance for prevention and control of foodborne zoonosis. *Ann. 1st Super Sanita*., 44: 401-404.

4. Canton R, Coque TM and Baquero F. (2003): Multi-resistant Gram-negative bacilli: from epidemics to endemics. *Curr. Opin. Infect. Dis*., 16**:**315-325.

5. Chado, E., Coque, T. M., Canton, R., Sousa, J. C and Peixe, L. (2008). Antibiotic resistance integrons and Extended spectrum beta-lactamases among Enterobacteriaceae isolates recovered from chicken and swine in Portugal. *Antimicrob Chemother*., 62: 296-302.

6. Chau, K. F and Oboegbunam, S. I. (2007). Extended – spectrum beta lactamase production among ampicillin resistant *Escherichia coli* strains from chicken in Enugu State, Nigeria. *Brazilian J. Microbiol.,* 38:1-4.

7. Cheesbrough, M. (2010).*District Laboratory Practice in Tropical Countries,* Part two, 2nd edn. Cambridge University Press, UK. Pp 143-180.

8. Clinical Laboratory Standards Institute (CLSI) (2006). Performance standards for antimicrobial susceptibility testing.Proceedings of the 16th International Supplement (M100-S16). Wayne, Pa, USA.

9. Dierikx, C., van der Goot, J., Fabri, T., van Essen-Zandbergen, A., and Smith H. (2013) Extended spectrum beta-lactamase- and AmpC-beta-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. *J Antimicrob Chemother.,* 68: 60–67.

10. Dupont, H. L and Steele, J. H (1987). Use of antimicrobial agent in animal feeds. Implications for human health. *Rev. Infect. Dis*., 9: 447-460.

11. Ejikeugwu, C., Iroha, I., Orji, J., Ugwu, M., Okonkwo, E., Ikegbunam, M., Ugbo, E., Moses., I and Nwakaeze, E. (2015). Antibiogram of ESBL- producing *Pseudomonas aeruginosa* isolates of nosocomial origin. *Euro J of Pharm and Med Res.,* 2(4): 92-99.

12. Hidetake, E., Morioka, A and Ishihara, K. (2004). Antimicrobial susceptibility of bacteria isolated from cattle, swine and poultry. *Report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program.* 53: 266-270.

13. Jawetz, Melnick and Adelberg's (2007). Medical Microbiology, Twenty fourth editions. Pp. 161-267.

14. Liebana, E., Batchelor, M and Hopkin, K. (2007). Longitudinal farm study of extended-spectrum bata-lactamase mediated resistance. *J. Clin Microbial.,* 44: 1630-1634.

15. Livermore DM (2012) Current epidemiology and growing resistance of gram-negative pathogens. *Korean J Intern Med.,* 27: 128–142.

16. SVARM 2010. (2011) Swedish Veterinary Antimicrobial Resistance Monitoring. The National Veterinary Institute (SVA), Uppsala, Sweden.

17. Weldhagen, G. F., Poirel, L and Nordmann, P. (2003). Ambler Class A extended-spectrum bata-lactamase in *Pseudomonas aeruginosa;* novel developments and clinical impact. *Antimicrobial Agent Chemother.,* 47: 2385-2392.

18. Ugbo, E., Ezaka, E., Orji, J.,Moses, I., Agumah, N., Nwachi, C., Ogene, L.,Okata-Nwali, D and Ngwu, J. (2015). Mulitidrug resistance profile of clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* of clinical origin. *World Journal of Pharmacy and Pharmaceutical Sciences,* 4 (9): 23-35.

19. Yutaka, S., Naohiro, S., Yohei, D and Yoshichika, A. (2004). *Escherichia coli* producing CTX-2 beta-lactamase in cattle, Japan. *Emerg. Infect. Dis.,* 10: 1-6.