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https://dx.doi.org/10.4314/nvj.v39i2.4 **ORIGINAL ARTICLE**

Detection of some resistance genes in *Salmonella* isolated from Poultry farms in Abia and Imo States, Southeastern Nigeria

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SUMMARY
Sixteen *Salmonella* Gallinarum and 24 *Salmonella* Pullorum strains isolated from chickens were screened for resistance to 11 antibacterial agents using the disc diffusion method. Five of the *Salmonella* Gallinarum and five of *S.* Pullorum strains resistant to streptomycin, gentamicin, tetracycline and sulfamethoxazole/trimethoprim were screened for presence of *strA/strB, aac (3)-II, aac (3)-1V, tetA and tetB* and *sul 1 (dfr/A)* and *sul 3 (dfr/G)* resistance genes. A singleplex PCR with resistance gene specific primers was used to ascertain the presence of the target resistance gene. All the *Salmonella* isolates studied were resistant to ampicillin while 95% were resistant to tetracycline and streptomycin. None of the isolates were resistant to ofloxacin and ciprofloxacin. Three of the sulphamethaxazole/trimethoprim-resistant isolates haboured *dfrA* and *dfrG* genes while one of the gentamicin-resistant isolates was positive for *aac (3)-II* genes. None out of the 10 streptomycin and tetracycline resistant isolates harbored any *strA/strB, tetA* and *tetB* genes. In addition, none of the 10 gentamicin resistant isolates harbored *aac (3)-IV* genes. The high resistance rates recorded in this study may be attributed to indiscriminate use of antibacterial agents.

Key words: *Salmonella***,** antimicrobials, resistance genes, Polymerase chain reaction, Southeastern Nigeria

INTRODUCTION
The Genus *Salmonella* comprises of Gram-negative, non-sporing rods (2-4 x 0.5um) that do not have capsules and are in the Family *Enterobacteriaceae* (OIE Manual, 2006), some of which are pathogenic. Members of this Genus, except *S.* Pullorum and *S.* Gallinarum, are motile and have long flagellae (OIE Manual, 2006). They grow readily on ordinary media and most agars, forming large, thick, grayish white, domed shaped colonies on deoxycholate citrate media. All ferment glucose but not lactose, all reduce nitrates to nitrites and all can survive for several months away from the host (Hossain *et al.,* 2006).

Salmonellae are found in the intestinal tract of humans and many animals, including domestic animals, such as chickens and cattle. Chickens are natural host for both *S.* Pullorum and *S.* Gallinarium (Snoeyenbos, 1991). *Salmonella* is a well-known genus because members of the genus have the

ability to cause disease. More than 2,500 serotypes of *Salmonella* have been identified, only about 10% of these have been isolated from poultry (Gast, 1997). Incidences of occurrence of *Salmonella* have been traced to only few forms, mostly *S* Pullorum, *S* Typhimurium and *S.* Enteritidis (Breslow 2002). Salmonellosis is the name of a group of infectious diseases caused by *Salmonella* serotypes and include fowl typhoid, typhoid fever, paratyphoid fever, and food poisoning (OIE Manual, 2006). These infections can be reduced through proper hygiene and personal and social responsibility.

Microbial resistance is the loss of sensitivity by a microorganism to an antimicrobial to which it was originally susceptible. This resistance can be acquired by mutations in chromosomal DNA or by the acquisition of extra- chromosomal genomic material by means of plasmids and transposons (Vazquez *et al*., 2005). The growing resistance of pathogenic bacteria to antimicrobials has raised the concern that the widespread use of antimicrobials in animals' production may promote the development of resistant bacteria or resistance genes that can be transferred to bacteria that cause disease in humans (Wegener *et al.,* 1997). A Major public health problem has been the emergence and spread of antimicrobial resistance in bacteria populations. There is a significant increase in the frequency of isolation of bacteria that were once sensitive to routine drugs, but are now resistant to nearly all drugs in the market (Nogueira *et al.,* 1999).

The emergence and persistence of antibiotic resistance in *Salmonella spp*. continue to pose serious risks to human and animal health (Joseph *et al*., 2008).

Resistance to antimicrobial drugs was first reported in the studies published in 1907 by Paul Ehrlich, who recorded the emergence of trypanosomes resistant to rosaniline chemotherapy.

The emergence of bacterial resistance was also recorded after sulfonamide and penicillin started to be used in veterinary and human medicine in the 1940s (Quinn *et al.,* 2001) There is a growing concern as to the proper use of antibacterials. One negative aspect of the use of antimicrobial is the selection of multi- resistant microorganism, limiting the therapeutic possibilities, and increasing not only the lethality rates, but also treatment costs (Nogueira *et al.,* 2005). The presence of plasmids of high molecular weight (50 to 100kb) has been demonstrated, where genes encoding for toxins are found, as well as genes that confer multi-resistance to antimicrobials (Vazquez *et al.,* 2002). Previous study has shown that antimicrobial use in animal production systems may lead to the increase of drug- resistance among animal pathogen (Heider *et al.,* 2009; Mann *et at.,* 2011; Morley *et al.,* 2011).

This study was conducted to determine the antibacterial resistance profile and resistance genes in *Salmonella* Gallinarum and *S.* Pullorum from chickens in Abia and Imo States, Nigeria**.**

MATERIALS AND METHODS

Bacterial isolates

Stocked cultures of *Salmonella* Gallinarum (16 strains) and *Salmonella* Pullorum (24 strains) were used for the study. The isolates were obtained from chickens in Abia and Imo States (Nwiyi *et al.,* 2016).

Antimicrobial resistance profile

Antimicrobial resistance profile of the Salmonella isolates was determined by the disc diffusion method of Kirby Bauer (1966) as described by the Clinical Laboratory Standard Institute (CLSI, 2014). Each *Salmonella* isolate was enriched in nutrient broth for 15 minutes at 37^{o}C before swabbing on to the surface of dried plates of Mueller-Hinton agar (MHA). A total of 11 antimicrobial agents were used for the study. The antibiotics used was obtained from Oxoid, (United Kingdom) and their concentration were as follows: Ampicillin (30µg), tetracycline (25µg), gentamicin (10µg), perfloxacin (10µg), oxfloxacin

(30µg), sulfamethoxazole/trimethoprim (30µg), streptomycin (10µg), ciprofloxacin (5µg), levofloxacin (30µg), nalidixic acid (30µg) and ceftriaxone (10µg). The antibiotic disks were placed on the agar surface, sufficiently separated from each other so as to avoid overlapping of inhibition zones. Each plate carried a maximum of six discs and each test was performed in duplicate. After 30seconds of pre-diffusion, the plates were incubated at 37^{o}C for 24 hours after which the diameter of inhibition zones were measured with a metre rule and the inhibition diameter for each isolate and each antimicrobial agent was calculated to the nearest whole number. For each antimicrobial agent, the isolates were recorded as sensitive or resistant according to the interpretation guidelines of CLSI (2014).

Detection of antimicrobial resistance genes in the *Salmonella* isolates

Ten strains (five *Salmonella* Gallinarum and five *Salmonella* Pullorum) resistant to gentamicin, sulfamethoxazole-trimethoprim, streptomycin and tetracycline were selected and screened for presence of genes conferring resistance to streptomycin (*strA/B*), gentamicin [*aac (3)*-ii and *aac(3)*-iv,] tetracycline (*tetA* and *tetB*) and sulfamethoxazole/trimethoprim [*sul* 1 (*dfr/A*) and *sul* 3 (*dfr/G*)]. Each test isolate was grown on MacConkey agar and genomic DNA was extracted from the colonies using the boiling method and in accordance with the protocol of Danifor Biotechnology (2012). The target resistance genes were detected using singleplex polymerase chain reaction (PCR). The cycling conditions and primer sequence were as described by Ma *et al.* (2007). The PCR was performed in 30µl volumes containing 3µl of buffer (100mmol/L Tris-HCl (pH 9), 1.5mmol/L $MgCl_2$, 500mmol/L KCl, 0.1% gelatin), 100mmol/L concentration each of dATP, dGTP, dGTP and dCTP, 10pmol of each primer, and 0.9U of Taq DNA polymerase (Inqaaba Biotechnical Industries Ltd, South- Africa), with 2.0µL of template DNA. The primer sequence (Table 1) and cycling conditions used in this study are: Initial denaturation step at 95^{O}C for 5min, followed by annealing at 35^{o}C for 30 sec and a final elongation at 72^{O}C for 2min. The amplified genes were electrophoresed in 1.5% agarose gel and a 100-bp DNA ladder was used as a size maker. After staining with ethidium bromide, the gel was visualized and photographed under transilluminator ultraviolet (UV) light with gel documentation (MB) Fermenters USA.

TABLE I: Primer set for resistance genes detection

Resistance es	Primer sequence
strA/strB-F	ATGGTGGACCCTAAAACTCT
strA/strB-R	CATCTAGGATCGAGACAAAG
aac(3)-11- F	ACGCGGAAGGCAATACGGA
aac(3)-11-R	TAACCTGAAGGCTCGCAAGA
aac(3)-IV- F	TGCTGGTCCACAGCTCCTTC
aac(3)-IV-R	CGGATGCAGGAAGATCAA
tetA-F	GCTACATCCTGCTTGCCTTC
tetA-R	CATAGATCGCCGTAAGAGG
tetB- F	TTGGTTAGGGGCAAGTTTTG
t*etB*-R	GTAATGGGCCAATAACACCG
sul1(dfr/A)- F	CGGCGTGGCTACCTGAACG
sul1(dfr/A)-R	GCCGATCGCGTGAAGTTCCG
sul3(dfr/G)-F	CAACGGAAGTGGGCGTTGTGGA

RESULT AND DISCUSSION

The resistance profile of *Salmonella* isolates to 11 antimicrobial agents is presented in Table II. *Salmonella* Pullorum isolates were resistant to ampicillin, streptomycin and tetracycline while 91.7, 58.3 and 50.0% were resistant to nalidixic acid, sulfurmethaxazole/trimethoprim and perfloxacin, respectively while *Salmonella* Gallinarum isolates were all resistant to ampicillin and gentamicin, while resistance to streptomycin, tetracycline and nalidixic acid was demonstrated by 87.5, 87.5

sul3(dfr/G)-R	GCTGCACCAATTCGCTGAACG

F=Forward, R=Reverse

and 75.0%, respectively, of the isolates. In this study, all the *Salmonella* isolates were

TABLE 2: Antibacterial resistance profile of *Salmonella* Pullorum (n=24) and S*almonella* Gallinarum (n=16) isolated from chickens in Abia and Imo states

Antibacterial agent	*S. Pullorum* (n=24)	*S. Gallinarum* (n=16)	Total (n=40)
	No resistant (%)	No resistant (%)	No resistant (%)
Ampicillin	24 (100)	16 (100)	40 (100)
Tetracycline	24 (100)	14 (87.5)	38 (95)
Streptomycin	24 (100)	14 (87.5)	38 (95)
Nalidixic acid	22 (91.7)	12 (75)	34 (85)
Sulfamethoxazole/trimethoprim	14 (58.3)	12 (75)	26 (65)
Gentamicin	12 (50)	16 (100)	28 (70)
Perfloxacin	12 (50)	4 (25)	16 (40)
Ofloxacin	0 (0)	0 (0)	0 (0)
Ciprofloxacin	0 (0)	0 (0)	0 (0)
Levofloxacin	0 (0)	4 (25)	4 (10)
Ceftriaxone	0 (0)	2 (12.5)	2 (5)

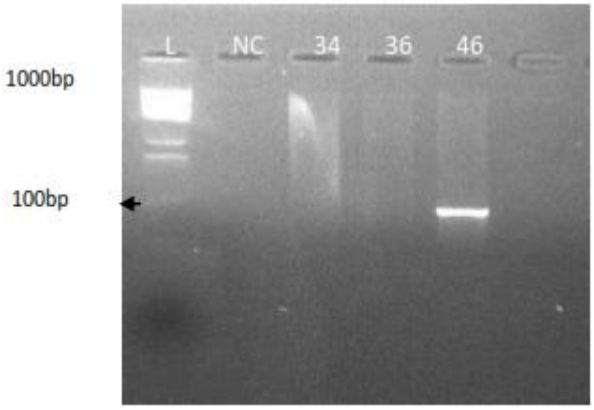

Figure 1: Polymerase chain reaction results for *Salmonella* isolates analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1kb DNA ladder (molecular marker). Sample 46 is positive for *aac* (3)-II resistance gene with band at 100bp while samples 34 and 36 are negative for *aa (3)-II* resistance gene. NC is a no DNA template control

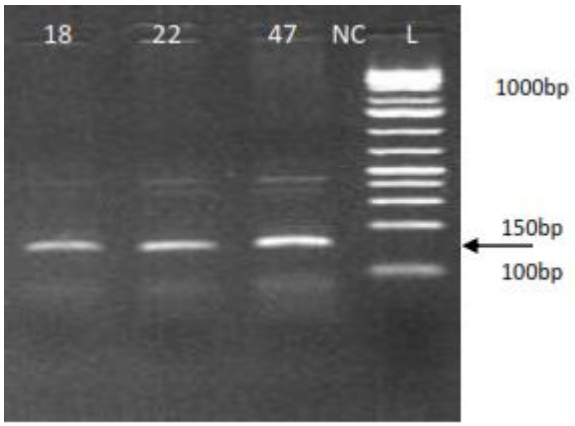

Figure 2: Polymerase chain reaction results for *Salmonella* isolates analyzed with 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1kb DNA ladder (molecular marker). Samples 18, 22 and 47 are positive for *Sul-1-(dfr/A)* resistance gene with bands at 150bp. NC is a no DNA template control

evaluated for resistance against five classes of antimicrobial agents namely: the aminoglycosides, quinolones, cephalosporins, sulphonamides and tetracycline. Resistance to antibiotics seen among *Salmonella* strains has become a global problem (Baggesen *et al.*, 2000). Lee *et al.* (2003) in his study reported *Salmonella* resistance to many antibacterial agents in many countries including Nigeria. The *Salmonella* strains in this study, demonstrated high resistance rates to the

commonly available antimicrobial agents (ampicillin, tetracycline, streptomycin, and nalidixic acid). The resistance to commonly available antimicrobials may be a reflection of their indiscriminate use in poultry used in veterinary practice (Smith *et al.*, 2011). This wide and indiscriminate use may also explain the emergence of resistance to this class of antimicrobial agents. ofloxacin and ciprofloxacine are not used in veterinary practice in the study area. This may explain the reason why the *Salmonella* strains were not resistant to these antimicrobial agents. Sulfamethaxazole/ trimethoprim and gentamicin have shown evidence of resistance due to poor response of *Salmonella* to these drugs on usage by farmers Ojo *et al.* (2012).

Ampicillin resistance was observed in all the isolates and this is in agreement with the findings of Deekshit *et al.* (2012) who reported 100% resistance to ampicillin. The frequent use of ampicillin for treatment by most poultry farmers as well as the low dose applied may be responsible for the high resistance recorded in this study and this agrees with the observation by Suresh *et al.* (2006).

Resistance of *Salmonella* to streptomycin in this current study was 100% and this is in agreement with Sultana *et al.* (1992), but in disagreement with Cardoso *et al.* (2006) and Carraminana *et al.* (2004). Cardoso *et al.* (2006) in his study on *Salmonella* in Pakistan reported that *Salmonella* were resistant to streptomycin at 64.2%, while fluoroquinolones (levofloxacin and ciprofloxacin) are now widely prescribed and used in veterinary practice (Smith et al., 2011). The high resistance rate to streptomycin observed in the present study may be due to indiscriminate use of streptomycin which results in emergence of resistance and this is in agreement with Wannaprasat *et al.* (2011).

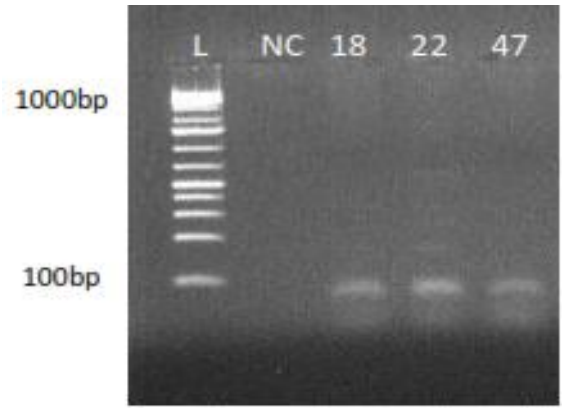

Figure 3: Polymerase chain reaction results for *Salmonella* isolates analyzed with 1.5% agarose gel electrophoresis stained with

Tetracyclines are commonly used for treatment of animal disease before antibiotic susceptibility test is determined. It is the most commonly used antibiotic in Nigeria and many other third world countries (Baggesen *et al.* 2000). This may explain the reason for the high resistance this drug recorded in this study. In this study, the high level of resistance to tetracycline recorded is in agreement with the findings of Nde and Logue (2007), who reported similar levels of resistance (76.3%) to tetracycline in *Salmonella* isolated from broiler in Midwestern, United States. Tetracycline has been one of the most commonly used growth promoters and as a result, resistance to tetracycline should be expected (Jones and Ricke, 2003).

In this study, the frequency of resistance to sulfamethaxazole/trimethoprim (75%) and gentamicin 50% in the *Salmonella* isolates is similar to the findings of Fashae (2010) and Molbalk *et al.* (2002). The high level of resistance to these drugs in this study and from isolates from food animal reaffirms the importance of the need to strengthening the collaboration between veterinary and public health sectors on the appropriate methods of detection and reporting of zoonotic food borne pathogens (Adesiji and Fagbami, 2006).

The low rate of *Salmonella* resistance to ofloxacin, ciprofloxacin and ceftriaxone in

ethidium bromide. L is 100bp-1kb DNA ladder (molecular marker). Samples 18, 22 and 47 are positive for *Sul-3-(dfr/G)* resistance gene with bands at 100bp. NC is a no DNA template control

Salmonella strains in poultry infections in the study area is ciprofloxacin, followed by oxfloxacin, levofloxacin, ceftriazone and perfloxacin.

The *strA/strB* genes were not detected in any of the streptomycin resistant *Salmonella* strains. Similarly, the ten tetracycline resistant strains were negative for *tetA* and *tetB* genes. One of the gentamicin resistant *Salmonella* strains (*S*. Pullorum) haboured *aac (3)-II* gene (Figure 1) while none was positive for *aac (3)-IV* gene. Three of the 10 sulfamethoxazole/trimethoprim resistant strains harboured both *sul-1*(*dfr/A*) (with amplificon size of 150bp, Figure 2) and *sul 3 (dfr/G)* gene (with amplicon size of 100bp, Figure 3). Two of these strains were *Salmonella* Gallinarum.

In this study strains of *S.* Gallinarum and *S*. Pullorum resistant to streptomycin, gentamicin, tetracycline and sulfamethoxazole/trimethrim were investigated for the presence of some genes that code for resistance to these agents. Streptomycin and tetracycline resistant strains did not haboured the *strA/B* nor *tetA* and *tetB* genes and this finding is in contrast to those of Chiou and Jones (1995), who reported that all the streptomycin resistance isolate examined contained both s*trA* and s*trB* genes. Similarly, Pezella *et al*. (2004) reported that 84% of streptomycin resistance isolate contain *strA* and *strB* genes. The resistance of *Salmonella* to streptomycin resistant genes may be due to efflux and this suggests that resistance may be chromosomally mediated. The findings in this study is in disagreement with Nde and Logue (2007), who reported that *tetA* genes was detected in more than half of the *Salmonella* isolates examined in North Dakota, USA. Similarly, Deekshit *et al.* (2012) reported that *tetA* and *tetB* resistant genes occur in Italy. The implication of this finding is that resistance may be chromosomal associated. One gentamicin resistant gene *aac (3)-II* was detected in the study out of 10 *Salmonella* isolates tested, while non was resistant to gene *aac (3) -IV* and his finding disagrees with those of Maynard *et al*. (2003), who found that 10 *Salmonella* isolates tested positive for gentamicin resistant gene *aac (3)-II* while 8 tested positive for *aac (3)-IV* gene. This suggests that the resistance is both plasmid and chromosomally mediated. Three *Salmonella* isolates tested carried the sulfamethaxazole/trimethoprim resistant genes *sul-1-dfr/A* and *sul-3-dfr/G*, and this finding is in agreement with Deekshit *et al*. (2012), who in his work reported that all *Salmonella* isolates carried resistant genes for *sul-1-dfr/A* and *sul-3-dfr/G*. The possible reason may be due to modification or replacement of antimicrobial target; the resistance here may be plasmid mediated. From the study, the detection of resistant genes observed in *aac (3)-II, sul-1-dfr/A* and *sul-3-dfr/G* as indicated by the bands suggests that resistivity was plasmid mediated this study is similar to the observation of Cardoso *et al.* (2006). Therefore, indiscriminate use should be avoided. The drug of choice for the treatment of

CONCLUSION

The following resistance genes was detected: *aac (3)-11*, *sul-1-(dfr/A)* and *sul-3-(dfr/G).* This may be the reason for reoccurring antimicrobial resistance observed in treatment of *Salmonella* in poultry farms in the study area.

REFERENCES

ADESIJI, Y. O. and FAGBAMI A.H. (2006): Epidemology of bacterial zoonois in Nigeria. Nigeria journal of health and biomedical science, 5: 20 – 25.

BAGGESEN, D. L., SANDRANG, D and AARESTRUP, F.M. (2000): Characterization of Salmonella enterica Serovar Typhimimnurum DY 104 Isolated from Danmark and Comparison with Isolates from Europe and the United States. Journal of clinical microbiology, 13: 1581 – 1586.

BRESLOW, L. (2002). Encyclopedia of public health. New York: Macmillan reference

CARDOSO, M.O., RIBEIRO, A.R., SANTOS, L.R., PILOTTO, F., MORAES, H.L.S SALLE, C.T.P., ROCHA,S. L.S and NASCIMENTO. V.P (2006): Antibiotic resistance in *Salmonella Enteritidis* isolated from broiler carcasses. Brazil journal of microbiology, 37:368-371.

CARRAMINANA, J. J., ROTA, C., AUGUSTIN, I and HERRERA, A. (2004): High prevalence of multiple resistance to antibiotics in *Salmonella* serovars isolated from a poultry slaughterhouse in Spain. Veterinary microbiology, 104:133-139.

CHIOU, C.S. and JONES, A. L. (1995): Expression and Identification of the StA – StB gene pair from streptomycin – resistant Erwinia amylovora. Gene, 592 (1): 47 – 51.

Clinical and Laboratory Standard Institute (2014). A model for laboratory services. GP 26-A4. lab medicine. 1: (46) 26

Danifor Biotechnology (2012). www.omicsonline.org/biotechnology 2012

DEEKSHIT, V. K., KUMAR, B.K., RAI, P., SRIKUMAR, S. and KARUNASAGAR, I. (2012): Detection of class 1 integrons genes in salmonella Weltevreden and silent antibiotic resistance genes in some seafood-associated non-typhoidal isolates in south- west coast of India. Journal of applied microbiology, 112:1113-1122

FASHAE, K., OGUNSOLA, F., AARESTRUP, F.M. and HENDIKSEN, R.S. (2010): Antimicrobial susceptibility and serovars of salmonella from chickens and human in Ibadan, Nigeria. Journal of infectious diseases, 4:484-494.

GAST, R. K. (1997): Paratyphoid infections. In: diseases of poultry. Calnek, B. W., Barnes, H. J. Beard, C. W., McSoughald, l. R. and Saif, Y. M., (eds). 10th ed. Iowa state university, AMES. IA. Pp 97-121.

HEIDER, L.C., FUNK, J.A., HOET, A.E., MEIRING, R.W., GEBREYES, W.A and WITTUM, T.E (2009): Identification of *Escherichia coli* and *Salmonella* enteric organisms with reduced susceptibility to ceftriaxone from fecal samples of cows in dairy herds. American journal of veterinary resources, 70: 389-393

HOSSAIN, M.S., CHOWHURY, E.H., ISLAM, M.M., HAIDER, M.G and HOSSAIN, M.M (2006): Avian *Salmonella* infection isolation and identification of organisms and histopathological study. Banglidesh Journal of veterinary medicine, 4: 7-12

JONES, F.T. and RICKE, S. C. (2003): Observations on the history of the development of antimicrobials and their use in poultry feeds. Poultry science, 82:613-617.

JESOPH, S.N., SAPKOTA, A.R., CULLEN, P., WAGNER, D., HULET, M., HAYES, J., SAHU, S., GADWAL, L.E and CARR, B.H (2008). Reduced resistance to antibiotics among Salmonella spp. Recovered from U.S organic poultry farms. America Society for Microbiology Conference 2008 Proceeding, 1752 N Street, N. W.

Washington, D.C world wide web; www.asm.org

Kirby Bauer, W (1966). Antimicrobial susceptibility testing by the Kirby Bauer disc diffusion method. American Journal of Biological Technology, 1:50-55

LEE, Y.J., KIM, S.K., KWON, K.Y and TAK, R.B (2003): Biochemical characteristics and antimicrobial susceptibility of *Salmonella* Gallinarum isolated in Korea. Journal veterinary science, 4: 161-166.

MA, M., WANG, H.I., YONG, Y.Y., ZHANG, D and LIU, S (2007): Detection of antimicrobial resistance genes of pathogenic *Salmonella* from swine with DNA microarray, Journal veterinary diagnosis investigation, 19: 161-167.

MANN, S., SILER, D., JORDAN, D and WARNICK, L.D (2011): Antimicrobial susceptibility of fecal *Escherichia coli* isolates in dairy cows following systemic treatment with ceftiofur or penicillin. Foodborne pathogenic diseases, 8; 861-867

MAYNARD, C., FAIRBROTHER, J.M., BEKAL, S., SANSCHAGRIN, R.C., LEVESQUE, R and BROUSEAU, A (2003): Antimicrobial resistance genes in enterotoxigenic Escherichia Cole: O149 :K91 isolates obtained over a 23years period from pigs. Antimicrobial agent chemotherapy, 47:3214-3221.

MOLBAK, K. P., GERNER, S and WEGERNER, H.C (2002): Increasing quinolone resistance in *Salmonella* enterica serotype *Enteritidis*. Emerging infectious disease, 8:514-515

MORLEY, P. S., DARGATZ, D.R., HYATT, G.A., DEWELL, J.G., PATTERSON, B.A and BURGESS, A (2011): Effects of restricted antimicrobial exposure on antimicrobial resistance in fecal *Escherichia coli* from feedlot cattle. Foodborne Pathogenic disease, 8: 87-98

NDE, W.C and LOGUE, C.M. (2007): Characterization of antimicrobial susceptibility and virulence genes of *Salmonella* serovars collected at a commercial turkey processing plant. Journal of applied microbiology, 104:215-223.

Nogueira, M.S., Nascimento, A.M and Chartone-Souza, E (1999). A cao de produtos naturalis na inibicao do crescimento bacteria e do fluxo genico e na origem de mutantes resistance. Genetics and Molecular Biology, 22: 431

NOGUEIRA, L.A., GESTEIRA, T.C.V and MAFEZOLI, J (2005): Oxytetracycline residues in cultivated marine shrimp (*Listopenaeus Vannamen*). Aquaculture, 254: 748-757.

NWIYI, P.O., CHAH, K.F and SHOYINKA, S.V.O (2016): Molecular detection of *Salmonella* isolated from poultry farms in Abia and Imo States Southeast Nigeria. International Journal of Current Microbiology and Applied Sciences, 5(7):961-968.

OIE, MANUAL (2006): Fowl typhoid and pullorum disease. In: Terrestrial menual. Office international des Epizooties (OlE), Paris, france.pp.538-548.

OJO, O.E., OGUNYINKA, D.G., AGBAJE, M., OKUBOYE, J.O., OLUGBENGA, O. K and OYEKUNLE, M.A (2012): Antibiogram of enterobactericeae isolated from free range chickens in Abeokuta, Nigeria. Veterinary Archives, 82:577-589.

PEZZELLA, C., RICCI, A., DIGIANNATALE, E., LUZZI, I. and CARATTOLI, A. (2004): Tetracycline and streptomycin

resistance genes, transposons, and plasmids in *Salmonella enteric* isolates from animals in Italy. Antimicrobial agents and chemotherapy, 48:903-908.

QUINN, P.J., CARTER, M.E., MARKEY, B.K. and CARTER, G.R. (2001): Clinical Veterinary Microbiology. Mosby-year Book Europe Ltd., London, Pp: 120-121.

SMITH, S. I., FOWORA, M.A., GOODLUCK, H.A., NWAOKORIE, F.O., ABOABA, O.O. and OPERE, B. (2011): Molecular typing of *Salmonella spp* isolated from food handlers and animals in Nigeria. International journal of molecular epidemiology and genetics, 2: 73-77

SNOEYENBOS, G. H. (1991): Pullorum disease. In: calnek, B, W., BARNES, H. J, BEARD, C. W., REID, W. M. YODER, H. W., editors. Diseases of poultry. Ames, iowa; state university press; Pp. 73-86.

Sultana, K., Bushra, A. M and Nafisa, I (1992). Evaluation of antibiotic resistance in clinical isolates of Salmonella typhi from Islamabad. Pakistan Journal of Zoology, 27: 185-187

SURESH,T., HETHA, A.A., SREENIVASAN, M.D., SANGEETHA, N. and LASHMANAPERUMALSAMY, P. (2006): Prevalence and antimicrobial resistance of *Salmonella* Enteritidis and other *Salmonellas* in the eggs and egg-storing trays from retails markets of Coimbatore, South India. Food microbiology, 23:294-299.

VAZQUEZ, N. J., LOPEZ V. Y., SUAREZ, G. F., ESLAVA, C. and VERDUGO, R. A.(2002): Caracterizacion y clonacion de los genes que expresan una enterotoxina l. t en Salmonella gallinarum. Anales del 27^0 congreso centroamericano y del caribe de avicultura; 2002; La habana.

VAZQUEZ, N. J.CORDOBA, B. C., LOPEZ, N. Y. and MANCERA, M. A. (2005): identification del gene da la integrasa tipo 1 y perfil da Resistencia antimicrobiana em salmonella enteritidis. Cuajimalpa (cited 2005 Nov 24). Available from: www,vet-uy.com/articulos/artic_micro/001/micro001.htm.

WANNAPRASAT, W., PADUNGTOD, P. and CHUANCHUEN, R. (2011): Class 1 integrons and virulence genes in *salmonella* enterica isolates from pork and humans. International journal of antimicrobial agents, 37:457-461.

WEGENER, H. C., BAGER, F. and AARESTRUP, F. M. (1997): Vigilancia da Resistencia aos antimicrobianos no homem, nos produtos alimentares e no gado na Dinamarca. Euro surveillance 3(2):17-19.