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Original article

Antibiotic resistance of isolates of *Escherichia coli* from chicken in Sokoto Metropolis, Nigeria

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ABSTRACT

A total of 200 faecal samples were collected from the cloaca of chicken (layers) from four different poultry farms within Sokoto metropolis. In this study, 91 lactose fermenters isolates from Mac Conkey agar (presumed to be *E. coli*) were isolated out of which 38 isolates were confirmed as E. coli based on their biochemical characteristics. The isolates were then examined for resistance and sensitivity to different antimicrobials of veterinary and human significance. Antibiotic activities against the isolates were determined by Disc Diffusion test (Kirby and Bauer, 1996). Various antibiotic resistances were observed in all the isolates. There were maximum resistance to Amoxycilline and Cotrimoxazole (100%), followed by Augmentin (90%), Tetracycline (80%) and Nalidixic acid (70%).

High sensitivity to Ofloxacin (95%), Gentamicin (90%) and Nitrofurantoin were noticed. Therefore, based on the findings of this research, it is advised that antibiotics with high sensitivity against the isolates of *E. coli* from this study (Ofloxacin, Gentamicin and Nitrofurantoin, respectively) should be used in the treatment of infections caused by E. coli and also at an appropriate dosage.

1. Introduction

Escherichia coli is one of the most important agent causing secondary bacterial infection in poultry and may also be a primary pathogen (Gross, 1994). Colibacillosis is the most frequently reported disease in surveys of poultry diseases or condemnations at processing (Saif, 2003). In the past few years, both the incidence and severity of colibacillosis have increased rapidly, and current trends indicate that it is likely to continue and become even a greater problem in the poultry industry (Altekruse et al., 2002). Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed et al., 1993). *E. coli* may be sensitive to many antibiotics. However, isolates of E. coli from poultry are frequently resistant to one or more antibiotics, especially if they have been widely used in poultry industry over a long period (e.g. tetracyclines) (Allan et al, 1993; Blanco et al, 1997). Antibiotics once effective at controlling *E. coli* infections are now ineffective due to the bacterium's acquired resistance to these compounds. Resistance to two or more classes of antibiotics is now common place in both veterinary (Gonzalez and Blanco, 1989) and human (Dennesen et al., 1998) medicine.

Concern has been expressed about possible harmful effects on humans through the use of drugs. These include increased microbial drug resistance, drug residues in food, allergic reactions and sensitization to antimicrobials and drug toxicity. Concern about antibiotic resistance and its transmission to human pathogens is important because these resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora.

Escherichia coli as one of the most important agent causing secondary bacterial infection in poultry as well as a primary pathogen has developed resistance to many antibiotics used in the poultry industry hence making treatment of infections caused by *E. coli* difficult.

The aims and objectives of the study are therefore: To isolate *Escherichia coli* from cloacal swab of chickens within Sokoto metropolis. To check the isolates for resistance to antimicrobial agents commonly used in the treatment of infections caused by *E. coli* To give advice on the preferred antibiotics to use in the treatment of infections caused by *E. coli*.

Some antibiotics that were once effective in the treatment of *E. coli* infections are now discovered to be ineffective. This is attributed to the frequency of use of such antibiotics. The result of this research work is expected to come out with antibiotics that would be effective in the treatment of such infections based on antimicrobial resistance and sensitivity to such antibiotics.

2. Materials and methods

2.1. Area of study

The study was carried out within Sokoto Metropolis in Sokoto state, Nigeria. Geographically, the state is situated on latitude 120 15N and 050E, and is 308m above the sea level. Sokoto state occupies an area of short grass savannah vegetation in the South and thorn shrub in the North. It shares boundaries with Zamfara state to the East, Niger republic to the North and Kebbi state to the West and South West. A generally arid region that gradually merges into the desert across the border in Niger republic, it has limited rainfall from Mid-May to Mid-September and is subjected to sahara's harmattan (dry, dust – laden wind) from November to March (Sokoto, 2001).

The state ranks second in the nation livestock population with an estimated number of 3 million cattle, 3.85 million sheep, 4 million goats, 0.8 million camels, 2million chickens and 1 million poultry (Kware, 2008). These animal species are one of the major sources of proteins to the inhabitants of the state and over 75% of them are reared or raised in traditional free range system living on close association with human settlements (Kware, 2008).

2.2. Sample collection and handling

A total of 200 samples were collected. The samples (cloacal swab) were taken from four different poultry farms within Sokoto metropolis. The name and location of the farms are: Adamu farms from Sama road area, Sokoto, Lumana farms in more area, Sokoto, Mukabs farms in Lowcost area and Sulaymawa Farms in Gidan Igwai area in Sokoto.

Cloacal swabs were collected from birds within the four poultry farms. The swabs were then transferred into buffered peptone water and transported in an ice-cold container to the Veterinary Microbiology laboratory. After

collection of the samples (swab stick in the tubes containing peptone water), they were then transferred into the incubator and kept inside for 24hours at 37°C for growth of the microorganisms.

2.3. Sample processing

A loop-full of the peptone water culture was streaked on Mac Conkey agar and incubated at 37 °c overnight (24 hours). The plates were then examined for the presence of pinkish and colourless colonies; suggestive of lactose fermenters and non-lactose fermenters, respectively. The pinkish colonies were then gram stained and observed under a light microscope for cellular characteristics (Zhao, et al., 2001).

2.4. Biochemical characterisation

Biochemical tests comprising of Indole test, Methyl red test, Voges proskauer test, Citrate test and Triple Sugar Iron test were conducted on each suspected isolate based on procedures described by Quinn, et al (2002).

2.5. Disc diffusion method for antibiotic sensitivity tests

Disc diffusion method based on methodology described by Kirby and Bauer (1966) was used to examine the bacterial resistance or susceptibility. One or two discrete colonies of each tested isolates were suspended in a test containing 4mls of physiological saline solution. Sterile inoculation cotton was then used to take the adjusted inocula suspension and spread evenly on nutrient agar to get a uniform inoculum. It was then allowed to partially dry up. Antibiotic sensitivity discs were then applied to the surfaces of the inoculated plates. Each disc was gently placed on the agar ensuring complete contact with the surface. The plates were then incubated at 37°C for 24 hours. The plates were then examined and the diameters of the zones of inhibition to the nearest whole number were measured. The zone diameter for each antimicrobial agent was translated into resistant and sensitive categories according to the interpretation of Kirby and Bauer (1966).

3. Results

The tables below show the results of E. coli isolated from a total number of 200 examined samples from cloacal swab of chicken. A total of 91 (45.5%) of the total samples expressed lactose fermentation on Mac Conkey agar (presumed to be *E. coli*).

Table 1
Total number of lactose fermenters isolated.

Farm	Number tested	Lactose fermenters	Percentage (%)
A	50	22	24
B	50	27	54
C	50	19	38
D	50	23	46
Total	200	91	45.5%

Out of the 91 (45.5%) presumed E. coli isolates from the cloacal swab of chicken, 38 (19%) could be identified as *E. coli*. This is based on their biochemical reactions.

3.1. Antibiotic sensitivity test on E. coli isolates:

A total number of 20 isolates were tested for antibiotic sensitivity and resistance. Of the total 20 isolates tested, 1 (5%) was resistant to Ofloxacin, 2 (10%) were resistant to Gentamicin, 14 (70%) were resistant to Nalidixic acid, 18 (90%) were resistant to Augmentin, 6 (30%) were resistant to Nitrofurantoin, 20 (100%) were resistant to Cotrimoxazole, 16 (80%) were resistant to Tetracycline and 20 (100%) were resistant to Amoxicilline. The results of the antibiotic sensitivity tests are summarized in tables 3 and 4:

Table 2
Result of the biochemically identified *E.coli.*

Farm	Number tested	Number of samples with biochemically identifiedE.coli
A	50	12 (24%)
B	50	8 (16%)
C	50	8 (16%)
D	50	10 (20%)
Total	200	38 (19%)

19% of the total samples examined could be identified as *E. coli*

Table 3
E. coli isolates resistant to the antibiotics.

Antibiotics (Dosage µg)	*E. coli*
Augumentin (30 µg)	18 (90%)
Ofloxacin (5 µg)	1 (5%)
Gentamicin (20 µg)	2 (10%)
Nalidixic acid (30 µg)	14 (70%)
Nitrofurantoin (200 µg)	6 (30%)
Cotrimoxazole (25 µg)	20 (100%)
Tetracycline (25 µg)	16 (80%)
Amoxicillin (30 µg)	20 (100%)

Table 4
E. coli isolates sensitive to the antibiotics.

Antibiotics (Dosage µg)	*E. coli*
Augumentin (30 µg)	2 (10%)
Ofloxacin (5 µg)	19 (95%)
Gentamicin (20 µg)	18 (90%)
Nalidixic acid (30 µg)	6 (30%)
Nitrofurantoin (200 µg)	14 (70%)
Cotrimoxazole (25 µg)	0 (0%)
Tetracycline (25 µg)	4 (20%)
Amoxicillin (30 µg)	0 (0%)

4. Discussion

This research was conducted to determine the antibiotic resistance of isolates of E. coli from cloacal swab of chicken.

In this study, a total of 200 samples were examined out of which 91 of the samples (45.5%) expressed lactose fermentation on Mac Conkey agar (presumed to be *E. coli*). However, 38 of the isolates (19%) were confirmed as E. coli based on their biochemical characteristics.

The rate of E. coli isolates were low when compared to the reports of Sharada et al, (2009) who isolated 65 E. coli from a total number of 85 samples. The variation is attributed to the fact that samples were taken from pathognomonic lesions of colibacillosis at post mortem examination.

Some workers have reported much lower incidence of E. coli isolated from faecal sample (Yadav and Malik, 1971 and Panneerselvam et al., 1988). This is in line with the findings of this research that was conducted from the cloacal swab of chicken. Isolates from lesions typical of colibacillosis could be highly virulent compared to those from cloacal swab of apparently healthy birds (Ghosh, 1988; Krishanmohan and Koteeswaran, 1994).

The sensitivity and resistance patterns of these isolates for various antibiotics are shown in tables 3 and 4. In this study, multiple drug resistance was observed in one of the isolates (5%). It was observed that none of the

antibiotics used were found to be 100 percent effective. This antibiotic resistance was similar to the findings of previous studies (Guerra et al, 2003; Saenz et al., 2003). E. coli isolates showed variable percentages of sensitivity and resistance to the different antibiotics. High levels of resistance were against Amoxicillin (100%), Cotrimoxazole (100%), Augmentin (90%), Tetracycline (80%) and Nalidixic acid (70%). The results obtained in this finding were in variance with the findings of other workers (Ojeniyi, 1989). This indicates that antibiotic resistance pattern varies with different isolates, time and development of multiple drug resistance among different isolates (Gross, 1994). Transmission of resistance plasmids of *E. coli* from poultry to human have also been reported (Mansouri and Shareifi, 2002).

5. Conclusion

Findings from this research clearly demonstrate antimicrobial resistant isolates are commonly present among chickens. Resistance to existing antimicrobials is widespread and is of concern to poultry veterinarians. It has been observed that there is a significant increase in the resistance of *E. coli* isolates from chicken to various antibiotics. This significant increase in the incidence of resistance against antibiotics in the E. coli isolates is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of diseases, resistance transfer among different bacteria and possible cross resistance between antibiotics used in the poultry industry. Therefore, based on the findings of this research, it is advised that antibiotics with high sensitivity against the isolates of E. coli from this study (Ofloxacin, Gentamicin and Nitrofurantoin, respectively) should be used in the treatment of infections caused by *E. coli* and also at an appropriate dosage.

Based on the findings of this research work, the following recommendations were made: The introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria must be vigorously pursued. Emphasis should be given for judicious selection of antibiotics preferably after antibiotic sensitivity testing. There should be a judicious use of such antibiotics at an optimum dose for sufficient duration to ensure effective treatment and control of various diseases caused by *E. coli* in poultry.

References

Allan, B.J., van den Hurk, J.V., Potter, A.A., 1993. Characterization of *Escherichia coli* isolated from cases of avian colibacillosis. Can. J. Vet. Res. 57, 146—151.

Altekruse S.F, Elvinger, F., Lee, K.Y., Tollefson, L.K., Pierson, E.N., Eifert, J., Sriranganathan, N., 2002. Antimicrobial resistance of *Escherichia coli* strains from a turkey operation. J. AM. Vet. Med. Assoc. 221, 411-416.

Bauer, A.W., Kirby, W.M.M., Sheris, J.C., Truck, M., 1996. Antibiotic susceptibility testing by a standardized single disc method. Am. J. clin. pathol. 145, 225- 230.

Blanco, J.E., Blanco, M., Mora, A., Blanco, J., 1997. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. J. Clin. Microbiol 35, 2184-2185.

Blanco, J.E., Blanco, M., Mora, A., Jansen, W.H., Garcia, V., Vazquez, M.L., Blanco, J., 1998. Serotypes of *Escherichia coli* isolated from septicaemic chickens in Galicia (northwest Spain). Vet. Microbiol. 61, 229-2.

Dennesen, P.J., Bonten, M.J., Weinstein, R.A., 1998. Multiresistant bacteria as a hospital epidemic problem. Ann. Med. 30, 176-185.

Freed, M., Clarke, J.P., Bowersock, T.L., Van Alstine, W.G., Balog, J.M., Hester, P.Y., 1993. Effect of spectinomycin on *Escherichia coli* infection in a day old duckling. Avian. dis. 37, 763- 766.

Ghosh, S.S., 1988. *E. coli* serotypes of poultry in Nagaland. Indian. J. Anim. Res. 22, 35- 38.

Guerra, B., Junker, E., Schroeter, A., Malorny, B., Lehmann, S., Helmuth, R., 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. J . antimicrob. Chemother. 52, 489- 492.

Gross, W.B., 1994. Diseases due to *Escherichia coli* in poultry. In C.L. Gyles (ed.). *Escherichia coli* in Domestic Animals and Humans. CAB Int'l: Wallingford, UK, 237—260.

http://www.tradeinvestnigeria.com/featurearticles/517912.htm

Krishnamohan, R.Y., Kooteswaran, A., 1994. Studies on experimental *Escherichia coli* infection in Japanese quail. Indian. Vet. J. 71, 959-963.

Mansouri, S., Shareifi, S., 2002. Antimicrobial resistance pattern of *Escherichia coli* causing urinary tract infections and that of human fecal flora in the South East of Iran. Microb. Drug. Resis. 8, 123 – 128.

Ojeniyi, A.A., 1989. Comparative bacterial drug resistance in modern battery and free – range poultry in a tropical environment. Vet. Record. 117, 11- 2.

Paneerselvam, S., Purushothaman, V., Dorairajan, N., Venugupal, K., 1998. Serotypes of *Escherichia coli* from pathological conditions in poultry and their antibiogram. Ind. J. Poult. Sci. 23(1), 47 -50.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C., 2002. Veterinary Microbiology and Microbial disease. Blackwell publishers. 106- 123.

Saenz, Y., Zarazaga, M., Brinas, L., Ruiz, L.F., Torres, C., 2003. Mutation in gyr A and Part C genes in nalidixic acid resistant *Escherichia coli* strains form food products, humans and animals. J. Antimicrobe. Chemothes. 51, 1001 – 1005.

Saif, Y.M., (2003). Disease of poultry 11th edition Lowa state Press, A blackwell publishing company. 631 – 652.

Sharada, R., Ruban, S.W., Thiyageeswaran, M., 2009. Antibiotic resistance pattern of *Escherichia coli* isolated from poultry in Bangalore. Internet. J. Microbiol. 7(1).

Sokoto, B.A., 2001. The study of local geography for Nigerian secondary schools. 1st edition, Ministry of Education, Sokoto.

Yadav, M.P., Malik, B.S., 1971. Isolation and Serotyping of *Escherichia coli* from chicken and their eggs in India. Indian. Vet. J. 48, 879 – 883.

Zhao, C., Beilei, G.J, sudler, R., Emily, Y., Zhao, S., David, G.W., Meng, J., 2001. Prevalence of Campylobacter spp, *Escherichia coli* and Salmonella serovars in retail chicken, turkey, pork and beef from the greater Washington D.C area. Area. Appl. Environ. Microbial. 67(12), 5431- 5436.