Detection and antibiotic sensitivity pattern of avian pathogenic *Escherichia coli* strains among rural chickens in the arid region of north-eastern Nigeria

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Abstract

Aim: To know the prevalence of avian pathogenic *Escherichia coli* (APEC) strains among adult apparently healthy rural chickens slaughtered in Maiduguri, north-eastern Nigeria.

Materials and Methods: Cloacal swabs were examined by Gram staining, biochemical tests such as indole, methyl red, Voges-Proskauer and citrate (IMVC) tests and serotype by standard slide agglutination test with antisera against somatic antigen using six monospecific "O" antisera to *E. coli* belonging to the avian pathogenic *E. coli* group namely O1, O2, O26, O78, O86 and O141. The sensitivity of the isolated APEC strains to 10 antibiotics of human and veterinary use was also determined.

Results: Out of a total of 510 samples examined, 356 (69.8%) were positive for *E. coli*. Of this number 20 (5.6%) samples were positive for O1, 20 (5.6%) for O2, 0 (0%) for O26, 25 (7.0%) for O78, 25 (7.0%) for O86 and 24 (6.7%) for O141 serotypes. The remaining 242 (68.0%) *E. coli* isolates were non typable with the 6 sera of avian pathogenic *E. coli* strains used for the study. The sensitivity profile of the isolates showed complete resistance of all the isolates against ampicillin, tetracycline, nalidixic acid and cefuroxime, while on the other hand all the isolates showed very high susceptibility to oxofloxacin followed by ciprofloxacin and gentamycin. The result of this study suggests that multiple-antimicrobial-resistant APEC isolates are present in rural chickens in Maiduguri, north-eastern Nigeria. In addition to animal health problems created by the resistant strains, there may also be potential danger posed to human health because these strains could easily infect humans through the food chain.

Conclusion: The result of this study suggests that multiple-antimicrobial-resistant APEC isolates are present in rural chickens in Maiduguri, north-eastern Nigeria. Consequently, introduction of surveillance programs to monitor antimicrobial resistance of pathogenic bacteria is strongly recommended in Nigeria.

Key words: Antibiotic resistance, *E. coli*, Nigeria, Rural chickens

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Introduction

Escherichia coli is the most important agent causing secondary bacterial infection in poultry and sometimes may constitute a primary pathogen in disease conditions [1] such as *Coli septicaemia*, coligranuloma or Hjarre's disease and omphalitis [2]. While the majority of *E. coli* strains colonise the avian gastro-intestinal tract and other mucosal surfaces as commensals, certain strains are capable of causing disease and are therefore designated as avian pathogenic *E. coli* (APEC). The pathogenicity is associated to the organism's ability to produce entero- and invasive-toxins, cytotoxic necrotizing factor (CNF), attaching and effacing (eae) genes, siderophores, plasmids, integrons and haemolysins [3]. Colibacillosis is a disease that has been found to be the most frequently reported poultry disease [4] responsible for significant economic losses in aviculture in many parts of the world [5,6]. The increase in both the incidence and severity of colibacillosis indicated that it is likely to continue and become an even greater problem in the poultry industry [7,8,9]. A major tool in reducing both the incidence and mortality from avian colibacillosis is the use of antimicrobial therapy [10]. Though many isolates of *E. coli* are sensitive to several antibiotics, there are a growing number of strains that are becoming resistant to common antibiotics especially

those that are widely used [7,10]. Frequent report of respiratory, diarrhoea, and joint problems caused by the organism following treatment with antibiotics signifies drug resistant strains of this organism in Nigeria [11]. Resistance to two or more classes of antibiotics is now common in both veterinary [6] and human [12] medicine. High incidence of tetracycline and erythromycin resistance by *Escherichia coli* from poultry infections has been reported [13]. These organisms produce a large reservoir of antibiotic resistance in tissues of their hosts probably transfer genes concerned to other organisms [14].

Classical method of characterising avian pathogenic *E. coli* strains is the somatic antigen serologic testing [15]. Surveillance studies of avian pathogenic *E. coli* strains have repeatedly identified a small number of E. coli serogroups such as O1, O2 and O78 as the etiologic agents of a large proportion of colibacillosis infections in poultry [16,17]. These studies of the somatic antigens of avian pathogenic *E. coli* have also shown that many strains are untypable similar genetic diversity within [18] and across [19] serotypes. As APEC share not only identical serotypes with human pathogens but also specific virulence factors and their zoonotic potential which are under consideration [5]. The spread and the contribution of rural poultry to the antigenic diversity of *E. coli* strains have not been ascertained in this part of the country.

The present study was undertaken to examine the prevalence of avian pathogenic *E. coli* strains among rural chickens slaughtered in Maiduguri and to determine the antimicrobial sensitivity pattern of the isolates against some antibiotics of human and veterinary use in Maiduguri.

Materials and Methods

Sample Collection and Processing: Approval of the University of Maiduguri Committee on Animal Care and Use was obtained before the onset of sampling. Cloacal swabs were collected using sterile cotton swabs from 510 live apparently healthy rural chickens brought to local slaughter stalls for dressing in Maiduguri, north-eastern Nigeria from December 2006 to March 2007.

Samples collected were transported on ice packs within one hour of collection to the research and diagnostic laboratory of the Department of Veterinary Medicine, University of Maiduguri for processing.

Isolation and Identification of *E.coli*: Specimens were directly inoculated on MacConkey agar and eosin methylene blue agar and incubated at 37 ^{O}C for 24 hrs for cultural identification and morphological characteristics. Colonies that appeared as button-like pink colouration (lactose fermenter) on MacConkey agar and as greenish metallic sheen on eosin methylene blue agar were presumed to be *E. coli* cultures. The presumptive *E. coli* isolates were further subjected to Gram staining and some standard biochemical tests which included indole, methyl red, voges-proskauer and citrate tests (IMVC) [20].

Serotyping of *E.coli* Isolates: Serotyping of the *E. coli* isolates were done by standard slide agglutination tests with antisera against somatic antigen [21]. Six monospecific "O" antisera to *E. coli* belonging to APEC group were used for the serotyping which included O1, O2, O26, O78, O86 and O141 obtained from Veterinary Laboratories Agency, Weybridge, U.K.

Antimicrobial Sensitivity Testing: Disk diffusion method [22] was used to determine susceptibility of the isolated APEC to some antibiotics of human and veterinary significance on Mueller Hinton agar. Ten antimicrobial discs were used at the following concentrations: ciprofloxacin (10µg), streptomycin (30µg), trimethoprim + sulphametoxazole (30 µg), ampicillin (30 µg), oxfloxacin (10 µg), gentamycin (10 µg), augmentin (30 µg), nalidixic acid (30 µg), cefuroxime (30 µg) and Oxytetracycline (30 µg). The selection of antibiotic disk concentrations was done as recommended by the manufacturers (Oxoid, UK). Zones of complete inhibition were measured to the nearest millimetre with a rule placed at the back of the Petri dish and diameters around each disc were interpreted and reported as susceptible (S), intermediate (I) or resistance (R) on the basis of National Committee for Clinical Laboratory Standards (NCCLS) [22].

Results

E. coli was found in 356 (69.8%) of 510 samples examined. Of this number 20 (5.6%) samples each were positive for O1 and O2, 0 (0%) for O26, 25 (7.0%) for O78 and O86 and 24 (6.7%) for O141 sero types. The remaining 242 (68.0%) *E. coli* isolates were non typable with the 6 sera of avian pathogenic *E. coli* strains used for the study (Figure 1).

Table-1 shows the antibiotic sensitivity profile of APEC isolates from rural chickens in Maiduguri. Complete resistance (100%) of all the isolates was observed against ampicillin, tetracycline, nalidixic acid and cefuroxime while on the other hand, all the isolates showed excellent sensitivity to oxofloxacin followed by ciprofloxacin and gentamycin. Susceptibility of O141 strains was very high (95.8%) to

Table-1: Antibiotic sensitivity pattern of avian pathogenic *E. coli* isolated from rural chickens in Maiduguri, North-eastern Nigeria

Type of Antibiotic	Level of antibiotic sensitivity (%) of *E. coli* isolates														
	O1			O2			O78			O86			O141		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ciprofloxacin	10	80	10	90	10	0	100	0	0	100	0	0	100	0	0
Streptomycin	0	0	100	0	0	100	0	4	96	0	8	92	0	8.3	91.7
Trimethoprim + Sulphametaxazole	0	0	100	0	30	70	0	0	100	0	4	96	0	4.2	95.8
Ampicillin	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Oxfloxacin	90	10	0	100	0	0	96	4	0	100	0	0	100	0	0
Gentamycin	0	70	30	0	85	15	92	8	0	88	12	0	83.3	16.7	0
Augmentin	0	0	100	0	0	100	0	0	100	0	0	100	95.8	4.2	0
Nalidixic acid	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Cefuroxime	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Tetracycline	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100

S = Susceptible; I = intermediate; R = resistant to the respective antibiotic agents according to NCCLS (1) recommendations.

augmentin and moderate susceptibility was recorded in 4.2% of the isolates while none was resistance to the antibiotics. Intermediate susceptibility to streptomycin was presented by strains O78 (4%), O86 (8%) and O141 (8.3%) while all other strains were resistant. Susceptibility to trimethoprim + sulphametaxazole occurred only in strains O2 (30%), O86 (4%) and O141 (4.2%) while all other isolates were resistant.

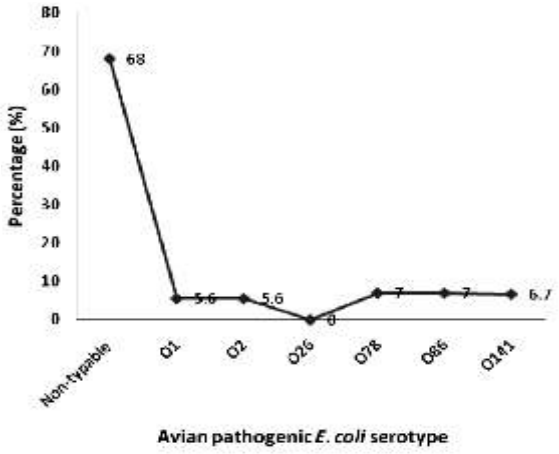

Figure 1: Serotyping of *E. coli* isolated from rural chickens in Maiduguri, North-eastern Nigeria

Discussion

In the present study, very low percentage of APEC serotypes were found from the *E. coli* isolated 20 (5.6%) each for O1 and O2, 25 (7.0%) each for O78 and O86 and 24 (6.7%) for O141 sero types. However no APEC O26 strain was isolated from rural chickens. These findings agree with the earlier reports that there is an apparent paucity of pathogenic *E. coli* in poultry environment [6, 16]. Inability to type 68% of the isolates appears to agree with the earlier reports which stated that many strains cannot be typed [6, 16]. Previous study has shown that O1 is among the most prevalent strain that causes colibacillosis [11, 23, 24] in rural poultry. While serogroup O2 has been reported in a study conducted on sick birds in Zaria by Raji et al. [2], the finding did not agree with the finding in this study. The occurrence of the *E. coli* serogroups O1 and O2 in Nigeria as demonstrated by this study agrees with previous studies [24, 25]. Although serogroup O26 was not isolated in live and apparently healthy chickens in this study, it has being reported to be isolated in cow milk and water bodies [26] and in chickens [2] in Nigeria. The strains isolated in chickens were similar to the strain isolated in cattle feces [26] where it may be a source of *E. coli* contamination to the chickens when they feed on cattle feces [11].

The APEC strains isolated from the chickens in this study clearly demonstrated high level of resistance to the antimicrobial agents tested. Similar report in previous studies showed resistance of clinical isolates of *E. coli* from chickens to tetracycline, chloramphenicol, ampicillin and amino glycosides [24, 27, 28]. However, moderate sensitivity to ciprofloxacin observed in this study is not in conformity with earlier reports of 79% and 90% resistance to ciprofloxacin by *E. coli* [24, 27, 29]. This observed difference in the susceptibility to ciprofloxacin and oxofloxacin by the APEC isolates could be due to fact that the antibiotics are relatively new to the poultry industry in Nigeria

and their use is limited. A similar trend has been reported by Blanco *et al*. [7] in Spain, Son and Gulam [30] in Malaysia, and Van Den Bogaard and Stobberingh [31] in Netherlands. The indiscriminate use of tetracycline by the poultry farmers and its consequent poor activity could be responsible for the 100% resistance of APEC isolates to the agent in this study. This finding has been reported earlier in Iran by Salehi and Bonab [32]. In Nigeria, gentamicin is marketed as an injectable solution and is not commonly used for mass medication in poultry. This could be the reason for the high susceptibility of APEC isolates to the drug in the present study. The findings in this study confirms the significant increase in the incidence of antimicrobial resistance in *E. coli* in Nigeria which is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases. Rural poultry in Nigeria are commonly on free range, with chickens allowed to scavenge for food and water with minimal health care (medication and vaccination). The resistance of APEC isolates from this group of chickens is of great concern and may be as a result of contamination of the environment by resistant strains from other poultry species or commercial chickens.

At slaughter, resistant strains from the gut readily soil poultry carcass and as a result, poultry meat could be contaminated with multi-resistant *E. coli* [33]. Moreover, eggs may become contaminated during laying [34] hence resistant faecal *E. coli* from poultry can infect humans directly and/or indirectly via food. It was shown that antibiotic-resistant *E. coli* could be transferred from poultry to food-handler's hands during food preparation and, finally, to the foodstuff [36]. The transmission of enteric bacteria to consumers via this route has been established, and prevention of food poisoning is the basis for food hygiene and public health regulations in many countries [36].

Conclusion

The result of this study has shown that multiple-antimicrobial-resistant APEC isolates are present in rural chickens in Maiduguri, Nigeria. In addition to animal health problems that could be created by such resistant strains, there may be potential danger posed to human health because these strains could easily infect humans through food chain. Consequently introduction of surveillance programs to monitor emergence of antimicrobial resistant strains among pathogenic bacteria is strongly recommended in Nigeria.

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Competing interests

The authors declare that they have no competing interests.

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