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Serovars and biochemical characterization of *Escherichia coli* isolated from colibacillosis cases and dead-in-shell embryos in poultry in Zaria-Nigeria

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ABSTRACT

This study was designed to determine the isolation rate, serovars and biochemical profiles of *E. coli* from cases of colibacillosis and dead-in-shell embryos in Zaria-Northern Nigeria. The isolation rate of *E. coli* from hatcheries studied were 4.67% and 7.50% from farms of Simtu Agricultural Company and National Animal Production Research Institute (NAPRI) Shika Zaria, Nigeria respectively. Twenty *E. coli* isolates from clinical cases of colibacillosis were also used for this study. The Simtu farm *E. coli* isolates showed 97.5% motility, while isolates from both NAPRI and clinical colibacillosis cases were 100% motile. The results of carbohydrate fermentation are variable without specific character, except for *E. coli* isolates from clinical cases of colibaccillosis that showed 100% fermentation especially for lactose, ducitol, rhamnose and xylose. The major serovars recorded from clinical cases of colibacillosis were serovars O8:K50 and O9:K30. Serovars from the dead-in-shell embryos were O78:K80, O8:K50, O9:K30, and O26:K60. Untypable isolates made up the greatest percentage of serogroup of *E. coli* studied. The antibiotic susceptibility testing indicated that many of the isolates were resistant to more than one antibiotic. Ciprofloxacin was the antibiotic to which majority of isolates were sensitive (85% of the clinical cases and 100% of both the Simtu and the NAPRI farms' isolates). It is concluded that other methods for controlling *E. coli* should be evaluated, so that the emergence of resistant isolates be limited and the cost involved in prophylactic and therapeutic treatment programs be reduced.

Key words: *Escherichia coli,* serovars, biochemical profiles, dead-in-shell embryos

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Introduction

Colibacillosis is one of the principal causes of mortality and morbidity in chickens and turkeys resulting in significant economic losses to poultry industry. *Escherichia coli* causes different vars of disease syndromes in poultry, including: acute colisepticaemia, sub-acute fibrinopurulent synositis, yolk sac infection, cellulitis, swollen head syndrome and coligranuloma (GOMIS et al., 2001; ALLAN et al., 1993). The most common form of colibacillosis is characterized as an initial respiratory infection (air sacculitis) in 3 to 12-week-old broiler chickens and turkeys, which is frequently followed by generalised septicaemia, perihepatitis, and pericariditis (BOPP et al., 2005). Infection is generally enhanced or initiated by predisposing factors, such as mycoplasmas or viral infection, and the environment (GOMIS et al., 2001; BOPP et al., 2005).

E. coli isolates pathogenic for poultry commonly belong to certain serogroups, particularly the serogroups O78, O1, and O2, and to some extent O15 and O55 (GROSS, 1994; CHART et al., 2000). The virulence attributes such as: aerobactin iron sequestration (DOZOIS et al., 1992), capsules (e.g. K1), lipopolysaccharide, cytotoxins such as α-haemolysin (NGELEKA et al., 1996) resistance to the bactericidal effect of serum (DHOMOULIN et al., 1990) and fimbrial (pili) adhesion (ARNÉ et al., 2000; JEFFREY et al., 2000) are associated with pathogenic *E. coli* that cause colisepticaemia. Reduced hatchability is one of the major problems in the hatchery industry, which has adversely affected the rapidly expanding poultry production in Nigeria. This has been related to nutritional deficiencies and infertility in breeders, faulty incubation, embryonic malpositions and bacterial infections of embryo (WOOLEY et al., 2000; AKINYEMI and OJEH, 1982; FALADE, 1977). Bacterial infection of the embryo is a major cause of reduced hatchability, early chick mortality and poor performance (WOOLEY et al., 2000; KABILIKA and SHARMA, 1997). In Nigeria, AKINYEMI and OJEH (1982) and FALADE (1977) isolated some bacteria species including *E. coli* from infected chicken embryo in Ibadan, Oyo State. Although numerous studies have been conducted on the prevalence and isolation of *E. coli* in poultry in Nigeria, there is scarcity of information on biochemical profiles, serological data, antibiotic susceptibility of this important agent of mortality and morbidity in poultry. The present study was undertaken to investigate biochemical and serological properties of *E. coli* isolated from cases of colibacillosis and dead-in-shell embryos in Zaria, Nigeria, and their susceptibility to antimicrobial agents.

Materials and methods

Sample collection and isolation of E. coli from dead-in-shell embryos. A total of 600 unhatched and unpipped eggs were selected from five batches of a hatch from the private hatchery, Simtu Agricultural Industrial Limited, Zaria, as well as from a government owned hatchery unit in NAPRI of Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Selected eggs for hatching were candled on the 6th day of incubation to eliminate infertile eggs. The eggs were again candled on the 18th day of incubation. The embryonated eggs that died between the 6th and 21st day of incubation were used for this study. All samples were macroscopically examined. Eggs with cracks and those embryos that pipped the shell but failed to hatch were discarded to minimize the incidence of external contamination. The surface of each egg was disinfected by cleaning with disinfectant, chlorohexidine (Salvon^{R}) solution, and dried with ethyl alcohol for 15 minutes. Flamed wire loop was used to take about 0.2 mL of the yolk contents which was streaked on MacConkey agar for primary isolation. This was then incubated at 37 ^{o}C for 24 h under aerobic conditions.

Sample collection and isolation of E. coli from clinical cases of colibacillosis. Birds that died from cases of colibacillosis and those sacrificed for confirmatory diagnosis from flock outbreak from clients that submitted birds to the Avian Unit of the Veterinary Teaching Hospital, ABU, Zaria, were used for this study. A sterile cotton swab was used for sampling the tissues and organs with lesions (liver, gallbladder, spleen, air sac, pericardium and heart) from clinical cases of suspected colibacillosis.

Identification of E. coli. Bacteria were identified on the basis of their cultural characteristics, morphological and physiological properties. For example, on MacConkey agar, colonies appeared as button-like, of pinkish colouration (lactose fermenter), while on eosin methylene blue agar the colonies appeared as greenish metallic sheen. Following identification, the colonies were sub-cultured on nutrient agar slant for storage at 4 ^{o}C in the refrigerator for further studies and characterization. The colonies were then subjected to biochemical tests as described by BOPP et al. (2005).

Biochemical characterization. E. coli isolates were subjected to standard biochemical tests, including catalase, indole, motility, hydrogen sulfide, carbohydrates fermentations, phenylalanine deaminase, bile esculin hydrolysis, methyl red, Voges Proskauer, citrate, urease and gelatine liquefaction, previously described in detail by GOMIS et al. (2001).

Fermentation of carbohydrates by E. coli isolates. The *E. coli* isolates were characterized by their ability to utilize the following sugars: maltose, lactose, sucrose, dulcitol, adonitol, salicin, raffinose, dextrin, xylose, rhamnose and mannitol. The indicator used for sugar fermentation (Bromothymol blue broth) base was prepared by dissolving peptone (10 g), sodium chloride (5 g) and bromothymol blue (0.018 g) in 1 litre of distilled water. Each carbohydrate solution was prepared by dissolving 1% of the corresponding sugar in the broth base medium, and only salicin was prepared at 0.5%. Each *E. coli* isolate was inoculated into prepared sugar medium and incubated at 37 °C for 24 h. The test was recorded as positive when the medium turned from bluish colour to yellow, while for negative reaction the medium remained blue (GROSS, 1994; BOPP et al., 2005).

Serotyping assay method. Serotyping of the isolates was carried out using slide agglutination tests with antisera against somatic antigen groups according to standard methods described by ORSKOV et al. (1977).

Antimicrobial susceptibility tests. Fifty-two *E. coli* isolates comprising twelve from clinical cases and twenty from dead-in-shell embryos, were subjected to antimicrobial *in vitro* susceptibility testing. The *E. coli* isolates were tested against 10 and 9 antimicrobial agents for clinical cases of colibacillosis and dead-in-shell embryo, respectively. The selection of antibiotic disk concentrations and interpretation of the zone size were done as recommended by the manufacturers (Oxoid, UK) and the National Committee for Clinical Laboratory Standards (NCCLS) (1990). The following antibiotics concentration per disk were used: ciprofloxacin (5 μg), sulphamethazole-trimethroprim (25 μg), streptomycin (10 μg), penicillin G (10 unit), tetracycline (30 μg), chloramphenicol (30 μg), ceftrixazone (30 μg), cephalothin (30 μg), ampicillin (10 μg), amoxicillin (25 μg).

Results

In this study, *E. coli* was isolated in 28 (4.7%) and 45 (7.5%) dead-in-shell embryos from Simtu and NARPI hatcheries, respectively. Twenty *E. coli* isolates were randomly selected from clinical colibaccillosis positive cultures of poultry samples submitted to Microbiology laboratory of Department of Veterinary Pathology and Microbiology, Zaria, Nigeria. The Simtu farm *E. coli* isolates showed 96.43% motility, while isolates from both NAPRI and clinical colibacillosis cases were 100% motile. The results also showed that 46.4% of the Simtu isolates, 24.4% of the NAPRI and 25% of the clinical colibacillosis cases hydrolysed bile aesculin.

All the isolates obtained from clinical colibacillosis, in birds from Simtu and NAPRI hatchery farms fermented lactose on MacConkey, and showed greenish metallic sheen on eosin methylene blue agar. The results of carbohydrate fermentation were variable without specific character, except for *E. coli* isolates from clinical cases of colibaccillosis that showed 100% fermentation especially for lactose, ducitol, rhamnose and xylose (Table 1). The *E. coli* isolates from dead-in-shell embryos from Simtu farm showed 100% fermentation for xylose, ducitol and lactose. The NAPRI isolates showed 100% fermentation rate for lactose and 97.8% fermentation rate for each of the following sugars: xylose, rhamnose and ducitol (Table 1). Many of the isolates exhibited resistance to more than one antibiotic. Ciprofloxacin was the antibiotic to which high numbers of isolates were sensitive, with 85% of the clinical cases and 100% of both the Simtu and the NAPRI farms isolates. Antibiotic resistance to penicillin and cephalothin was 100% (Table 2). The 93 *E. coli* isolates derived from clinical cases of colibacillosis and dead-in-shell embryos were serotyped. Twenty-two of the 93 isolates were assigned to O serogroups.

Table 1. Carbohydrates fermentation of various *E. coli* isolates from colibacillosis cases and dead-in-shell embryos in poultry (%).

Carbohydrates	Clinical cases of colibacillosis	Dead-in-shell embryos from Simtu farm	Dead-in-shell embryos from NAPRI farm
Xylose	100	100%	97.8
Mannitol	95	92.5	93.3
Raffinose	95	85.7	80.0
Sorbitol	75	75.0	91.1
Adonitol	10	32.1	17.8
Rhamnose	100	96.4	97.8
Lactose	100	100	100
Sucrose	65	75.0	82.2
Maltose	95	92.9	68.9
Dextrin	85	75.0	46.7
Ducitol	100	100	97.8
Salicin	65	60.7	77.8

Table 2. *In vitro* antibiotic susceptibility of *Escherichia coli* isolated from dead-in-shell embryos and colibacillosis (%)

	Resistance		
	Dead-in-shell isolates		
Antibiotic disc potency (μg)	Clinical colibacillosis cases	Simtu farm isolates	NAPRI isolates
Tetracycline 30 μg	60	19	81
Streptomycin 10 μg	90	75	81
Chloramphenicol 30 μg	70	NT	NT
Ampicillin 10 μg	80	88	31
Cephalothin 30 μg	100	100	100
Penicillin 10 unit	100	100	100
Amoxicillin 25 μg	65	94	38
Ciprofloxacin 5 μg	5	0	0
Ceftriazone 30 μg	75	63	25
Sulfamethoxazole-Trimethroprin 25 μg	70	50	75

NT = Not tested

Table 3. Various Serovars of *E. coli* isolates from colibacillosis cases and dead-in-shell embryos in poultry

Serovars	Clinical cases of colibacillosis	Dead-in-shell embryos Simtu farm	Dead-in-shell embryos NAPRI farm	Total
O8:K50	2	2	1	5
O9:K30	2	-	-	2
O86:K62	1	-	-	1
O9:K9	-	-	2	2
O9:K28	-	-	1	1
O9:K34	-	-	3	3
O99:K	-	-	1	1
O8:K	-	-	1	1
O4:K3	-	1	-	1
O26:K60	-	-	1	1
O112:K68	-	-	1	1
O137:K79	-	-	1	1
O13:K11	-	1	-	1
O78:K80 O8:K41	-	1	-	1
Rough	14	20	32	66
Not included for typing	1	3	1	5
Total	20	28	45	93

Five isolates were not analysed, while the remaining 66 isolates analysed were found to be non-typeable rough isolates. The 22 typeable isolates of *E. coli* were distributed among the O serogroups as follows: 5, 3, 2 and 2 isolates for O8:K50, O9:K34, O9:K30 and O9K:9; respectively, and one isolate for each of the serogroups O86:K62, O9:K28, O99:K, O8:K, O4:K3, O26:K60, O112:K68, O137:K79, O13:K11, O78:K80 and O8:K41 (Table 3).

Discussion

The isolation rate of *E. coli* from dead-in-shell embryos from Simtu farm and NAPRI were 4.7% and 7.5%, respectively. The findings were in agreement with those of KABILIKA and SHARMA (1997), GROSHEVA (1971), ORAJAKA and MOHAN (1986), who

also isolated *E. coli* predominantly from dead-in-shell embryos, although the percentage of *E. coli* isolates varied between different authors. The variation in the percentage of *E. coli* isolates may be partly related to the prophylactic and therapeutic use of certain antibiotics, vaccination against respiratory viruses, and improved hatchery sanitation. The biochemical profiles of *E. coli* isolated from cases of clinical colibacillosis and dead-in-shell embryos were similar to those previously reported (GOMIS et al., 2001; BOPP et al., 2005).

Eighty-five of these isolates were serologically typed in detail in South Africa. The findings obtained in this study disagreed with the reports of CLOUD et al. (1985) and ORAJAKA and MOHAN (1986), which recorded a high incidence of serovars O1, O2 and O78 in cases of colibacillosis and dead-in-shell embryos. In this study, serovars O8, O9 and O78 were most frequently isolated. FALADE (1977), in Oyo State, Nigeria, serotyped *E. coli* isolates from yolk sac of dead chicken embryos, and the serogroups found in his study were O141 and O139, although they are not among the known serogroups normally associated with pathogenic lesions in poultry. None of the serogroups have been isolated in the present investigation.

The serogroup isolated in this study is O86. This serogroup is known to be highly pathogenic for 3-5 day-old chicks (BURKHANOVA, 1980). Besides this, O86 and O26 groups isolated in this investigation are among the enteropathogenic *E. coli* known to be associated with infant haemorrhagic colitis and bloody diarrhoea (CRAVIOTO et al., 1979). This is suggestive of the possible zoonotic effect of some of the *E. coli* serogroups associated with dead-in-shell embryos. The O8 serogroup has also been associated with hatchery losses and early chick mortality in India (VENUGOPALAN et al., 1974; ARUNACHALAN et al., 1974), while HINTON and LINTON (1982) had reported the association of colibacillosis to the presence of O8 and O9 in South Africa.

The present study also found rough untypeable isolates of *E. coli* (66 of the 88 isolates analysed). This finding is in agreement with CLOUD et al. (1985) who reported 63.5% untypeable isolates of *E. coli* from yolk sac disease. However, ORAJAKA and MOHAN (1986) found only 26% untypeable bacteria isolates from dead-in-shell embryos. Very little information is available on the association of rough untypeable *E. coli* isolates with embryonic mortality. However, ROSENBERGER et al. (1985) reported that O2 serovars and untypeable *E. coli* of avian origin are among virulent avian *E. coli* in colibacillosis. This observation was not confirmed in the present study.

The results obtained from this study suggest that multiple antibiotic resistance is widely spread among the local isolates of *E. coli* isolated from poultry. These observations agree with the report by BLANCO et al. (1997) and CLOUD et al. (1985) that attributed the development of drug resistance to frequent usage of drugs in veterinary practices at sub-optimal concentrations. It is also very significant to note that almost all the *E. coli* isolates

showed very high resistance to streptomycin, tetracycline and ampicillin. This gives rise to a serious concern because these drugs are still considered the most recommendable for the treatment of colibacillosis in both animal and man. There is, therefore, an urgent need to reverse this notion in the light of present study with regards to the sensitivity pattern of each particular isolates of *E. coli.*

In the present study, most isolates were highly sensitive to ciprofloxacin and ceftriazone, but many of the other antibiotics that are used extensively in poultry industry were less effective (CLOUD et al., 1985; OJENIYI, 1989). It would be correct to change the drug prescription to these fluorated piperazinyl-substituted quinoline derivates in the light of the information obtained in the present study. Ciprofloxacin and other fluorated piperazinyl- substituted quinoline began to be used by the poultry farmers in Zaria only recently. Many of the other antibiotics, such as the sulpha compounds, tetracycline and cephalothin, that have been used extensively in the poultry industry, are less effective. Although Ceftrixazone is rarely used, the high incidence of resistance to this compound can be associated with a transferable plasmid also carrying resistance to the tetracyclines (OJENIYI, 1989).

It is also disturbing to note that chloramphenicol which is the drug of choice for the treatment of colibacillosis and other enteric pathogens, showed high resistance with regard to *E. coli* from clinical cases of colibacillosis in this environment. Generally speaking, the majority of the isolates were highly resistant to the common, less costly antibiotics used in poultry industry. Certainly other methods for controlling *E. coli* should be evaluated, so that the emergence of resistant isolates be limited and the cost involved in prophylactic and therapeutic treatment programs be reduced.

In conclusion, this study documents colibacillosis and dead-in-shell embryo in northern Nigeria. There was no difference observed in the serovars distribution in the colibacillosis and dead-in-shell embryos. Serovar O8:K50 was more frequently encountered in the study followed by O9:K34 then by O9:K9 among others. Serovars O1 and O2 were not found in this present study. The majority of *E. coli* isolates were rough so they could not be typed because only the smooth isolates can be typed easily. The serological, biochemical and antibiotic sensitivity characterization of *E. coli* isolates associated with recently diagnosed avian colibacillosis and dead-in-shell embryos should be used in developing new methods for control of these diseases.

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RAJI, M., J. ADEKEYE, J. KWAGA, J. BALE, M. HENTON: Serovarovi i biokemijske osobine izolata bakterije *Escherichia coli* izdvojenih kod kolibaciloze i iz zadušaka kokoši u Zariji, Nigerija. Vet. arhiv 77, 495-505, 2007.

SAŽETAK

Istraživanje je provedeno radi određivanja stope izdvajanja, serotipizacije i određivanja biokemijskih osobina izolata *E. coli* kod kolibaciloze peradi i zadušaka u Zariji u sjevernoj Nigeriji. Stopa izdvajanja *E. coli* iz promatranih valionica bila je 4,67% na 7,50% farmi Poljoprivrednoga dobra Simtu i Nacionalnog instituta za proizvodnju i istraživanje životinja, Shika Zaria, Nigerija. Dvadeset izolata *E. coli* izdvojenih iz peradi oboljele od kolibaciloze bilo je također upotrijebljeno u istraživanju. 97,5% izolata *E. coli* s farme Simtu bilo je pokretljivo, dok su izolati Nacionalnog instituta i oni izdvojeni kod kliničke kolibaciloze bili 100% pokretljivi. Rezultati fermentacije ugljikohidrata bili su varijabilni, osim u izolata kod kliničke kolibaciloze u kojih je dokazana fermentacija laktoze, dulcitola, ramnoze i ksiloze. Glavni serovarovi ustanovljeni kod kliničke kolibaciloze bili su O8:K50 i O9:K30. Iz zadušaka su bili izdvojeni serovarovi O78:K80, O8:K50, O9:K30 i O26:K60. Izolati se najvećim dijelom nisu mogli tipizirati unutar pretraživanih seroloških skupina. Mnogi izolati bili su otporni na više antibiotika. Većina sojeva (85% izdvojenih kod kliničke kolibaciloze te 100% s farme Simtu i Nacionalnoga instituta) bila je osjetljiva prema ciprofloksacinu. Zaključuje se da treba vrednovati druge metode za kontrolu *E. coli* tako da bi se pojava otpornih sojeva mogla ograničiti te smanjiti troškovi profilakse i terapije.

Ključne riječi: *Escherichia coli,* serovarovi, biokemijske osobine, zadušci