

Multidrug resistant *Escherichia coli* isolates of poultry origin in Abeokuta, South Western Nigeria

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OGUNLEYE, A. O., M. A. OYEKUNLE, A. O. SONIBARE: Multidrug resistant *Escherichia coli* isolates of poultry origin in Abeokuta, South Western Nigeria. Vet. arhiv 78, 501-509, 2008.

ABSTRACT

Thirty nine *Escherichia coli* (*E. coli*) isolated from septicemic clinical cases between October 2005 and March 2006 from eleven poultry farms in Abeokuta, South West Nigeria were tested for their in vitro antimicrobial drug sensitivity pattern. The *E. coli* were isolated from two hundred and fifty samples comprising of intestine, kidneys, lungs, hearts, ovary, spleen and colo-rectum from diseased chickens (mostly commercial layers) submitted for post-mortem examination. Eight of the poultry farms studied disclosed that they had used between 3 to 7 different antimicrobial agents either for treatment, prophylaxis or as growth promoters within the studied period. The result indicated that the 39 *E. coli* isolates showed nineteen different multidrug-resistant patterns to Nitrofurantoin (100 µg), Cefuroxime (20 µg), Norfloxacin (10 µg), Cotrimoxazole (50 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Chloramphenicol (10 µg), Ampicillin (10 µg, 25 µg), Ofloxacin (5 µg), Penicillin G (5 i.u), Amoxylin (20 µg), and Cloxacilin (5 µg, 10 µg) discs that were tested.

Key words: *Escherichia coli*, multidrug resistant, poultry

Introduction

E. coli is one of the most common causes of infection by gram negative bacteria (DIEKEMA et al., 1999). Although drug resistance in gram negative bacteria is not uncommon, its occurrence among isolates of *E. coli* was considered unusual (MUDER et al., 1997; VROMEN et al., 1999). Furthermore, there are more data on the prevalence of antimicrobial resistance among gram positive organism especially methicilin resistant

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Staphylococcus aureus and vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium* than there are for gram negative bacteria (FEINGOLD et al., 1994; BONILLA et al., 1997). Nevertheless, there are reports of resistance of *E. coli* to antibiotics with associated treatment failure (TALAN et al., 2004; BLONDAEU, 2004). Included in the list of affected antimicrobials are penicillin, cephalosporin, sulpha drugs (FLUTT et al., 2000; SAHM et al., 2001) and fluoroquinolones (GOETTSCHE et al., 2000).

Fluoroquinolone resistant *E. coli* strains often show resistance to other drugs such as ampicillin, tetracycline, chloramphenicol, trimethoprim, sulphamethoxazole and Gentamycin (GARAU et al., 1999; KOMP et al., 2003). And there has been a significant increase in fluoroquinolones resistant *E. coli* in many countries over the last few decades (GARAU et al., 1999; VAN BELKUM et al., 2001; VIROY et al., 2005).

There is growing concern over the transmission of resistant bacteria through the food chain and to that effect, the W.H.O. has recognised that antibiotic use in animals affects antibiotic resistance in human (ANONYMOUS, 2000). In the current study we studied the antimicrobial resistant pattern of *E. coli* isolated from eleven poultry farms, eight of which were known to have a history of multiple antimicrobial usage for both prophylaxis and therapeutic purposes in Ogun State, Nigeria.

Materials and methods

Sample collection. The study was carried out between October 2005 and March 2006 in eleven poultry farms comprising one breeding farm and ten commercial laying hen farms. Eight of the farms had a history of previous usage of various antibiotics, either for therapy or prophylaxis in Abeokuta, Ogun State. Carcasses from birds that had died of suspected septicaemic clinical cases were collected and aseptic specimens of liver, intestine, kidneys, lungs, heart, ovary, spleen and colorectum, totalling two hundred and fifty samples, were obtained from the series over the stated period. The specimens were immediately transported to the laboratory for bacteriological investigation.

Bacteriological analysis. The samples were streaked onto 5% sheep blood agar and MacConkey agar and incubated aerobically at 37 °C for 24 - 48 hours. All isolates that fermented lactose within 24 - 48 hours on MacConkey agar were further identified morphologically and biochemically according to standard methods (BARROW and FELTHAM, 1993). The *in vitro* antimicrobial sensitivity for the *E. coli* isolates was carried out by the agar - disc diffusion test (MATSEN and BARRY, 1974) using discs of Nitrofurantoin (NIT) 100 µg, Cefuroxime (CEF), 20 µg, Norfloxacin (NOR), 10 µg, Cotrimoxazole (COR), 50 µg, Nalidixic acid (NAL), 30 µg, Chloramphenicol (CHL) 10 µg, Ampicillin (AMP) 25 µg, Ofloxacin (OFL), 5 µg, Penicillin G (PEN) 5 unit, Amoxylin (AMX), 20 µg, Cloxacillin (CXC), 5 µg, Cefuroxime (CEF), 30 µg, Ampicillin (AMP) 10 µg, using oxoid iso-sensitest agar.

Results

From the two hundred and fifty samples that were examined, a total of thirty nine *E. coli* isolates were obtained. Each of the thirty nine isolates was resistant to between 5 and 12 antimicrobial agents. These isolates were from various organs: liver 26/39 (67%), lungs 4/39 (10%), kidneys 1/39 (2.5%), ovary 1/39 (2.5%), colorectum 2/39 (5%), and intestine 4/39 (10%). Table 1 shows the pattern of antimicrobial resistance exhibited by the *E. coli* isolates. Table 2 shows the history of antibiotic usage in the poultry farms used for the study and Table 3 shows the relationship between the antimicrobial resistance pattern obtained with the antimicrobials previously used in the respective farms.

Table 1. Antimicrobial disc resistant pattern exhibited by the *Escherichia coli* isolated from different farms

	Multidrug resistant pattern	Frequency	Farm sources
1	R-CIP-NOR-OFL-NIT-TET-PEN-AMX-CXC-CHL-CEF-AMP-GEN	3	1,2
2	R-CIP-NOR-NIT-TET-CHL-CEF-AMP-COR-GEN-NAL	3	1,2
3	R-CIP-NOR-NIT-TET-CHL-CEF-AMP-GEN-OFL-AMX	11	1,11
4	R-CXC-NIT-PEN-NOR-CHL-GEN	1	3
5	R-TET-NOR-NIT-COR-AMP-CHL-NAL	3	4,5
6	R-NOR-NIT-TET-CEF-COR-AMP-GEN-NAL	3	10
7	R-PEN-NIT-CEF-COR-CHL	1	1
8	R-NOR-NIT-TET-PEN-CXC-CEF-AMP-GEN-COR-NAL	1	1
9	R-CIP-NOR-NIT-TET-PEN-CXC-CEF-COR-AMP-NAL	1	1
10	R-CXC-NIT-PEN-CEF-AMP	1	1
11	R-NIT-CEF-TET-AMP-NAL	1	6
12	R-CXC-NIT-PEN-TET-CEF-AMP	1	6
13	R-NIT-CEF-TET-NOR-COR-CHL-AMP-NAL	1	5
14	R-NOR-COR-CEF-TET-AMP-NAL	1	9
15	R-NOR-COR-CEF-NIT-AMP-GEN-PEN-NAL	1	9
16	R-NOR-COR-CEF-AMP-GEN-TET-NAL	1	9
17	R-CIP-NIT-COR-CEF-TET-AMP-CHL-GEN-NAL	2	7
18	R-CIP-CEF-NOR-TET-AMP-CHL-GEN-OFL	2	7
19	R-CIP-CEF-NOR-TET-AMP-GEN-OFL	1	7
	Total	39	11

R- Resistant, NIT- Nitrofurantoin, CEF- Cefuroxime, NOR- Norfloxacin, COR- Cotrimoxazole, GEN- Gentamycin, TET- Tetracycline, CIP- Ciprofloxacin, NAL- Nalidixic acid, CHL- Chloramphenicol, AMP- Ampicillin, OFL- Ofloxacin, PEN- Penicillin, AMX- Amoxylin, CXC- Cloxacillin

Table 2. History of previous antimicrobial drug usage in the farms

S/N	Farm studied	Previous antimicrobial drug
1	1	Enrofloxacin, Tetracycline (four time), Gentamycin injectable (twice), Streptomycin injectable (twice)
2	2	Enrofloxacin, Furatadone, Gentamycin, Norfloxacin
3	3	Enrofloxacin (Twice), Gentamycin (Twice), Tetracycline (Three times), Furatadone (Twice), Streptomycin
4	4	Tetracycline only
5	5	Oxytetracycline, Tylosine, Enrofloxacin, Gentamycin injectable (280 mg) Furatadone
6	6	Not available
7	7	Not available
8	8	Oxytetracycline, 20%, Norfloxacin, Gentamycin, Streptomycin injectable
9	9	Gentamycin injectable (three times), Tetracycline, Enrofloxacin (three times), Norfloxacin, Furatadone (twice), Furazolidone (three times) injectable
10	10	Gentamycin (three time) injectable, Enrofloxacin (twice), Oxytetracycline 20% injectable
11	11	Not available

Table 3. Comparison of antimicrobial resistance pattern with the antimicrobial agents previously used in the respective farms

S/N	Multidrug resistant pattern	Frequency	Farm source	Related drug previously used
1	R-CIP-NOR-OFL-NIT-TET-PEN-AMX-CXC-CHL-CEF-AMP-GEN	3	1, 2	D-CIP-GEN-TET-NOR
2	R-CIP-NOR-NIT-TET-CHL-CEF-AMP-COR-GEN-NAL	3	1,2	D-CIP-GEN-TET-NOR
3	R-CIP-NOR-NIT-TET-CHL-CEF-AMP-GEN-OFL-AMX	11	1, 11	D-CIP-GEN-TET-NOR
4	R-CXC-NIT-PEN-NOR-CHL-GEN	1	3	D-CIP-GEN-TET-STEP
5	R-TET-NOR-NIT-COR-AMP-CHL-NAL	3	4, 5	D-CIP-GEN-TET-NOR
6	R-NOR-NIT-TET-CEF-COR-AMP-GEN-NAL	3	10	D-CIP-GEN-TET-NOR
7	R-PEN-NIT-CEF-COR-CHL	1	1	D-CIP-GEN-TET-NOR
8	R-NOR-NIT-TET-PEN-CXC-CEF-AMP-GEN-COR-NAL	1	1	D-CIP-GEN-TET-NOR

Table 3. Comparison of antimicrobial resistance pattern with the antimicrobial agents previously used in the respective farms (continued)

S/N	Multidrug resistant pattern	Frequency	Farm source	Related drug previously used
9	R-CIP-NOR-NIT-TET-PEN-CXC-CEF-COR-AMP-NAL	1	1	D-CIP-GEN-TET-NOR
10	R-CXC-NIT-PEN-CEF-AMP	1	1	D-CIP-GEN-TET-NOR
11	R-NIT-CEF-TET-AMP-NAL	1	6	Not available
12	R-CXC-NIT-PEN-TET-CEF-AMP	1	6	Not available
13	R-NIT-CEF-TET-NOR-COR-CHL-AMP-NAL	1	5	D-CIP-GEN-TET-NOR
14	R-NOR-COR-CEF-TET-AMP-NAL	1	9	D-CIP-GEN-TET-NOR
15	R-NOR-COR-CEF-NIT-AMP-GEN-PEN-NAL	1	9	D-CIP-GEN-TET-NOR
16	R-NOR-COR-CEF-AMP-GEN-TET-NAL	1	9	D-CIP-GEN-TET-NOR
17	R-CIP-NIT-COR-CEF-TET-AMP-CHL-GEN-NAL	2	7	Not available
18	R-CIP-CEF-NOR-TET-AMP-CHL-GEN-OFL	2	7	Not available
19	R-CIP-CEF-NOR-TET-AMP-GEN-OFL	1	7	Not available
	Total	39	11	

D - Drug previously used

Discussion

Antimicrobial resistance has grown to become a global problem (GUNNER et al., 2004). Antibiotic usage, that is, inappropriate use in terms of over use and misuse both in humans, veterinary use and agriculture is considered the most important factor promoting the emergence, selection and dissemination of antibiotic resistant micro organisms in both veterinary and human medicine (NEU, 1992; WITTE, 1998; GUNNER et al., 2004). Large proportions of antibiotics (50%) of the total global consumption are administered to food producing animals for prophylaxis treatment and growth promotion purposes, but 80% of such total administration is unnecessary (HARRISON and LEDERBERG, 1998).

The practice of indiscriminate use of antibiotics in food producing animals has undesirable consequences on human health because of the presence of drug residue in foods (which may be the parent compound or compounds derived from the parent drugs or both, including metabolites and residue bound to macromolecules on the one hand and the selection of resistant bacteria in animals on the other), thus jeopardizing

the effectiveness of the treatment of bacterial, fungal and parasitic infections worldwide (WEBER, 1979; COSGROVE and CARMEH, 2003).

This study presents a good example of typical use of antimicrobial agents by most poultry farmers in Nigeria and the possible consequences of such practices. Table 2 shows the disclosed history of antimicrobial usage in the farms studied (a very difficult task to accomplish because of reluctance on the part of the farmers to release the information). Seven of the eight farms with disclosed history used between three to seven different antimicrobial agents at different times, either for prophylaxis, treatment or growth promoting purposes. One farm (farm four) disclosed a history of tetracycline usage only. Three farms did not disclose antibiotic usage at all. However, the detailed dose/dosages of administration of the antibiotics and the duration of the therapy were not disclosed. The inability to access the antimicrobial usage record on demand is a reflection of bad management practice, because it makes it difficult to consider the justification for the use of such antimicrobial agents.

However, all the farms with a history of antimicrobial usage used at least one of quinolones (enrofloxacin, ciprofloxacin, norfloxacin). And, in ten out of the eleven farms, nineteen different types of multidrug resistant patterns were observed from the thirty nine *E. coli* that were isolated, as shown in Table 1. The resistant pattern was such that the *E. coli* was resistant to ciprofloxacin, norfloxacin, nalidixic acid (quinolones), penicillin, amoxycillin, cloxacillin (penicillin group of drugs), cefuroxime (2nd generation cephalosporin), gentamycin (Broad spectrum aminoglycoside), nitrofurantoin (nitrofurans), tetracycline, chloramphenicol, and cotrimoxazole (potentiated sulphonamide). The observation of resistance to the quinolones agrees with the earlier findings of *E. coli* to quinolones in many countries (GARAU et al., 1999; VIROY et al., 2005). The resistance to the penicillin group of drugs, tetracycline chloramphenicol, gentamycin may have been precipitated by the resistance to the quinolones (GARAU et al., 1999; KOMP et al., 2003). The consequences of these resistance patterns would be grave in terms of treatment of diseases in poultry and the possibility of transfer of drug resistance to humans.

Although the mechanism of spread of antibiotics resistance from food animals to humans, remains controversial, colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers and there is evidence that animals are a reservoir for *E. coli* found in humans (LINTON et al., 1977).

It is also known that resistant faecal *E. coli* from poultry can infect humans both directly and through food, by colonizing the human intestinal tract and contributing resistant genes to humans endogenous flora (VAN DEN BOGAARD et al., 2001). In Nigeria, ADETOSOYE (1980) reported multidrug resistant *E. coli* from humans and animals; one strain contained resistant factors for tetracycline, and two other strains contained resistant factors for ampicillin, chloramphenicol and compounds of sulphonamide.

OJENIYI (1989) also described direct transmission of *E. coli* resistant to streptomycin, sulphonamide and tetracycline from poultry to poultry attendants. Elsewhere, chickens have also been described as a source of antibiotic resistance in humans (SINGH et al., 1992; AMARA et al., 1995; AL GHAMBI, 1999).

The findings from this work support the need for a critical review of the usage of antimicrobial agents in livestock in Nigeria and the importance of taking concrete steps in terms of policy to curtail the indiscriminate use of antimicrobial agents in a bid to prevent the possible adverse consequences in animal production, as well as in humans. There is an urgent need to formulate a policy and put the necessary plan in place to execute a policy targeted at the promotion of rational use of antimicrobial agents, as an important element in antimicrobial resistant containment.

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Received: 5 March 2007

Accepted: 24 November 2008

OGUNLEYE, A. O., M. A. OYEKUNLE, A. O. SONIBARE: Multipla rezistencija izolata bakterije *Escherichia coli* podrijetlom iz peradi iz Abeokute u jugozapadnoj Nigeriji. *Vet. arhiv* 78, 501-509, 2008.

SAŽETAK

Ukupno je 39 izolata bakterije *Escherichia coli* bilo izdvojeno iz peradi sa septikemijom od listopada 2005. do ožujka 2006. s 11 farmi u Abeokuti u jugozapadnoj Nigeriji. Svi su izolati bili pretraženi na osjetljivost prema antimikrobnim tvarima. *E. coli* bila je izdvojena iz 250 uzoraka tkiva različitih organa: crijeva, bubrega, pluća, srca, jajnika, slezene i izlaznoga crijeva (rektuma) oboljelih pilića dostavljenih na postmortalnu pretragu. Na osam od 11 pretraženih farmi bilo je rabljeno od tri do sedam različitih antimikrobnih tvari za liječenje, profilaksu ili pak kao promotori rasta. Ustanovljeno je da je 39 izolata *E. coli* pokazivalo 19 različitih načina višestruke otpornosti prema nitrofurantoinu (100 µg), cefuroksimu (20 µg), norfloksacinu (10 µg) kotrimoksazolu (50 µg), ciprofloksacinu (5 µg), nalidiksičnoj kiselini (30 µg), kloramfenikolu (10 µg), ampicilinu (10 µg, 25 µg), ofloksacinu (5 µg), penicilinu G (5 i.j.), amoksicilinu (20 µg) i kloksacilinu (5 µg).

Ključne riječi: *Escherichia coli*, mnogostruka otpornost, perad
