Original Research Article

ANTIBIOTIC SENSITIVITY PROFILES OF *SALMONELLA TYPHIMURIUM* AND *E. COLI O157: H7* ISOLATES FROM READY TO EAT CHICKEN MEAT.

ABSTRACT

BACKGROUND: The global increase in the use of antibiotics in poultry and livestock production has significantly resulted in the emergence of antibiotic resistance bacteria. There is a growing global concern of the effect of antibiotic resistance on both animals and humans. The aim of this study was to determine the antibiotic resistance profile of *Salmonellae typhimurium* and *E coli 0157:H7* in ready to eat chicken meat.
METHOD: A set of 500pieces of chicken parts were purchased from sampled eateries in Ibadan. Standard microbiological techniques were used to isolate *Salmonella* and *E. coli* biochemically and serologically. All confirmed isolates were subjected to in vitro antibiotic susceptibility testing against 8 antibiotics of different classes of Gram negative antimicrobial-impregnated multidisks (ABTEK)
RESULT: The resistant pattern revealed E coli 0157:h7 and Salmonella isolates resistant to 5 drugs namely; Ceftazidime, Cefuroxime, Augmentin Nitrofurantoin and Ampicillin. Highest resistance of E coli isolates to Augumentin and Ampicillin both having a frequency of 11 (84.6%) and least resistance to Gentamicin, Ciprofloxacin and Ofloxacin 0 (0. 00%).Three antibiotics that showed 100% sensitivity by all the isolates were Gentamicin, Ciproflozacin and Ofloxacin.
CONCLUSION: The study revealed that Isolated *E. coli* and *Salmonella* strains from ready to eat chicken parts were resistant to five antibiotics; Ceftazidime, Cefuroxime, Augmentin Nitrofurantoin and Ampicillin and very susceptible to three antibiotics namely; Gentamicin, Ciproflozacin and Ofloxacin.

Keywords: *E. coli, Salmonella*, Antibiotics, Antibiotic-resistance, ready-to-eat chicken

INTRODUCTION

The worldwide increase in the use of antibiotics in poultry and livestock production industry to treat and prevent infectious bacterial diseases and as growth promoters at sub-therapeutic levels in feeds has led to bacterial resistance to antibiotics during the past years [1]. This increase in the use of antibiotics has played a significant role in the emergence of antibiotic resistance bacteria [2]. Increasing episodes of multi-drug resistant pathogens can result in failure of antibiotic therapy in both animals and human and this also facilitates the transmission of antibiotic resistance between and among bacteria strains and species [1].
Food of animal origin represents the major route of human exposure to foodborne pathogens with antimicrobial resistance [3]. Wide usage of antibiotics in the diet of domestic animals has made drug resistant bacteria which could be transferred to human beings [4]. There is an increase in public and government interest in phasing out inappropriate antibiotic use in animal husbandry [1]. In recent years, the problem of resistant strains to multiple drugs (MDR) is increasing and most studies in Iran and other countries have shown high resistance of *Salmonella* strains to several antibiotics [5-7].
Similar findings on multiple drug resistance of *E. coli* strains have been reported from Nigeria and other parts of the world [8-13]. The prevalence of antimicrobial resistance among food-borne pathogens increased during recent decades [8,14]. The frequent and unnecessary use of antimicrobial agents for farming and therapeutic purpose in animals and human are contributing to create resistant strains. Drug resistant bacteria are harder to treat with the common antibiotics [15].
The aim of this study was to identify and establish the antibiotic sensitivity profiles of the *Salmonella typhimurium* and *E. coli O157: H7* isolates from ready to eat chicken meat.

MATERIALS AND METHOD

A total of 500 ready-to-eat chicken meats were purchased from 25 eateries in Ibadan to prepare 1000 samples. The eateries were grouped into standard, semi standard and substandard based on the general outlook, perceived level of hygiene standard and the quality of food and service offered by the

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eateries. A combined method of the FDA/AOAC BAM Salmonella Isolation Procedure, [16] and some modifications were used to isolate E coli and Salmonella.
The obtained data was analyzed using descriptive statistics such as frequency tables and charts. Stastistical Package for Social Sciences (SSPS) version 21.0 (SPSS Inc. Chicago, III, USA) was used for Inferential analysis. Level of correlation between the eatery type and isolation of either of the organisms (*E. coli* and *Salmonella*) was determined and a $p \leq 0.05$ were considered significant.

Antibiotic Susceptibility Testing:

Susceptibility tests was performed using the Kirby-Bauer method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) using method as described by Hudzicki which describes the Kirby-Bauer Disk Diffusion Test method on Mueller Hinton Agar, some modifications were however made [17]. All confirmed isolates were subjected to in vitro antibiotic susceptibility testing against 8 antibiotics of different classes Gram negative antimicrobial-impregnated multidisks (ABTEK) comprising Ceftazidime (30µg), Cefuroxime (30 µg), Gentamicin(10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Augumentin (30µg), Nitrofurantoin (300µg), Ampicillin (10µg) and was carried out. All was according to current recommendation of NCCLS. A summary of the method is as follows;
One to several colonies was suspended in 0.85% saline to produce an even visible turbidity of 0.5mcfarland standard, the turbidity was adjusted by adding more bacteria or saline & compare to 0.5mcfarland standard. The inoculum was used within 15mins of preparation, the application of disk was done within 15mins of inoculating plate and incubation was within 15mins from application of disk. The zone diameter was determined by measuring the radius of the zone, from the center of the antibiotic disk to a point on the circumference of the zone where a distinct edge is present. This measurement was multiplied by 2 to determine the diameter of the zone of inhibition.
Interpretation and reporting of result was done using the CLSI susceptibility and interpretative breakpoints [18]. For each drug, it was indicated whether the zone sizes were susceptible (S), intermediate (I) or resistant (R).

RESULT

Out of 1000 samples tested, 27 biochemically confirmed isolates were subjected to antimicrobial testing against 8 different antimicrobial agents. *E coli 0157:H7* isolates showed highest resistance to both Augmentin and Ampicillin with a frequency of 11(84.6%) and least resistance to Gentamicin, Ciprofloxacin and Ofloxacin 0(0. 00%). Two antibiotics that showed 100% sensitivity by all the isolates were Gentamicin and Ciprofloxacin (Table 3).
The antimicrobial resistant pattern showed that 38.5% of the isolates had the highest resistant profile to 5 antibiotics namely Ceftazidime, Cefuroxime, Augmentin, Nitrofurantoin and Ampicillin (Table 4).
In the antibiogram results all *Salmonella* isolates (100%) were sensitive to ciprofloxacin which is a member of the fluoroquinolones and was the most effective antibiotic followed by Gentamicin (92.3%). Almost all isolates (84.6%) showed considerable resistance to cefuroxime, Augmentin and Ampicillin (Table 6). The antimicrobial resistant pattern of *Salmonella* isolates showed that 5(38.5%) of the isolates had the highest resistant pattern and were resistant to 5 antibiotics namely; Ceftazidime, Cefuroxime, Augmentin Nitrofurantoin and Ampicillin (Table 7).

Table 1. Standard values and performance breakpoints

S/NO	ANTIMICROBIAL AGENT	CODE	CONC PER DISC (µg)	SENSITIVE	INTERMEDIATE	RESISTANCE
	Ceftazidime	CAZ	30	≥21	18-20	≤17
	Cefuroxime	CRX	30	≥18	15-17	≤14
	Ciprofloxacin	CPR	5	≥21	16-20	≤15

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Gentamicin	GEN	10	≥15	13-14	≤12
Ofloxacin	OFL	5	≥16	13-15	≤12
Augmentin	AUG	30	≥18	14-17	≤13
Nitrofurantoin	NIT	300	≥17	15-16	≤14
Ampicillin	AMP	10	≥17	14-16	≤13

NCLS Manual 2012 [19]

Table 2. Antimicrobial resistant profile of *E. coli 0157:H7* with abtek (Cm-12-8nr100) rapid lab

S/No	**Presumptive isolate Sensitive values**	**CAZ (≥21)**	**CRX (≥18)**	**GEN (≥15)**	**CPR (≥21)**	**OFL (≥16)**	**AUG (≥18)**	**NIT (≥17)**	**AMP (≥17)**
1	E13	24	21	23	28	34	-	24	-
2	E20	23	22	32	36	36	-	18	-
3	E42	27	28	19	32	15	20	17	15
4	E48	30	22	22	36	34	22	26	21
5	E118	9	-	18	36	34	-	24	-
6	E121	12	-	18	30	34	-	26	-
7	E138	13	11	18	32	28	-	24	-
8	E276	-	-	20	38	34	-	25	-
9	E284	-	-	38	36	30	-	14	-
10	E409	-	-	20	26	22	-	-	-
11	E459	-	-	24	26	28	-	11	-
12	E487	-	-	24	34	26	-	12	-
13	E500	-	-	20	32	28	-	10	-

E (E coli), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ciproflozacin (CPR), Oflaxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ampicillin (AMP).

Table 3. Antibiogram result of *E coli 0157:H7* isolates

S/NO	Types of Antibiotics	Disc Potency	Number of Isolate,T=13		
			Resistant {n(%)}	Sensitive {n(%)}	Intermediate :{n(%)}
1	Ceftazidime(CAZ)	30 µ	9(69.23)	4(30.77)	-(0.00)
2	Cefuroxime(CRX)	30 µ	9(69.23)	4(30.77)	-(0.00)
3	Gentamicin(GEN)	10µ	-(0.00)	13(100.00)	-(0.00)
4	Ciprofloxacin(CPR)	5µ	-(0.00)	13(100.00)	-(0.00)
5	Ofloxacin (OFL)	5µ	-(0.00)	12(92.31)	1(7.69)
6	Augmentin (AUG)	30µ	11(84.6)	2(15.38)	-(0.00)
7	Nitrofurantoin(NIT)	300µ	5(38.46)	8(61.54)	-(0.00)
8	Ampicillin (AMP)	10µ	11(84.62)	1(7.69)	1(7.69)

Table 4. Antimicrobial Resistance Pattern (Phenotype) *E coli 0157:H7* Isolates

Number	Antimicrobial Resistant Pattern(ARP)	ARP Frequency (%)
2	AUG, AMP (2)	2 (15.4)
4	CAZ, CRX, AUG, AMP (4)	4 (30.8)
5	CAZ, CRX, AUG, NIT, AMP (5)	5 (38.5)

Number: number of antibiotics the isolate is resistant to, ARP Frequency (%) = the number of *E coli* isolates that have the resistant pattern of the presented antibiotics.

Table 5. Antimicrobial sensitivity profile for *Salmonella Typhimurium* isolates

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S/No	Presumptive isolate	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
	Sensitivity values	≥21	≥18	≥15	≥21	≥16	≥18	≥17	≥17
1	S3	23	-	22	30	30	20	16	16
2	S6	20	-	22	34	26	-	-	-
3	S20	24	24	24	36	36	-	25	-
4	S97	25	24	22	42	37	18	28	14
5	S157	-	-	18	30	28	-	10	-
6	S227	-	-	17	28	28	-	21	-
7	S229	-	-	22	24	20	-	25	-
8	S235	-	-	20	24	19	-	23	-
9	S313	-	-	18	34	24	-	-	-
10	S426	-	-	22	34	26	-	-	-
11	S436	-	-	20	40	28	-	-	-
12	S447	-	-	22	30	28	-	10	-
13	S492	-	-	22	40	26	-	-	-

S (Salmonella), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ciproflozacin (CPR), Oflaxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ampicillin (AMP).

Table 6. Antibiogram results of *S typhimurium* isolates

S/NO	Type of Antibiotics	Disc Potency	Number of isolates, T=13		
			Resistant {n (%)}	Sensitive {n (%)}	Intermediate {n (%)}
1	Ceftazidime (CAZ)	30µg	9 (69.23)	3 (23.08)	1 (7.69)
2	Cefuroxime (CRX)	30 µg	11 (84.62)	2 (15.38)	0 (0.00)
3	Gentamicin (GEN)	10µg	0 (0.00)	12 (92.31)	1 (7.69)
4	Ciprofloxacin (CPR)	5µg	0 (0.00)	13 (100.00)	0 (0.00)
5	Ofloxacin (OFL)	5µg	0 (0.00)	11 (84.62)	2 (15.38)
6	Augmentin (AUG)	30µg	11 (84.62)	2 (15.38)	0 (0.00)
7	Nitrofurantoin (NIT)	300µg	7 (53.85)	5 (38.46)	1 (7.69)
8	Ampicillin (AMP)	10µg	11 (84.62)	0 (0.00)	2 (15.38)

Table 7. Antimicrobial resistance pattern (phenotype) for *S. typhimurium*

Number	Antimicrobial Resistant Pattern (ARP)	ARP Frequency(%)
1	CRX (1)	1 (7.79)
2	AUG, AMP (1)	1 (7.79)
4	CRX, AUG, NIT, AMP (1) CAZ, CRX, AUG, AMP (3)	4 (30.8)
5	CAZ, CRX, AUG, NIT, AMP (5)	5 (38.5)

Number: number of antibiotics the isolate is resistant to; ARP Frequency (%) = the number of Salmonella isolates that are resistant to the presented antibiotics.

This study showed that, most of the *Escherichia coli* and Salmonella isolates had multi-drug resistance and majority of the antibiotics were found inactive against them. Out of the thirteen isolated

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as E coli, nine showed multidrug resistance to at least four antibiotics. Four isolates had multidrug resistance to four antibiotics while six isolates had multidrug resistance to five antibiotics namely; Ceftazidime, Cefuroxime, Augmentin Nitrofurantoin and Ampicillin. Thirteen *Salmonella* isolates had multidrug resistance to at least four antibiotics used in the study. Four of the isolates showed resistance to four antibiotics while another six isolates were resistant to five antibiotics.
In both *Salmonella* and *E coli* isolates Ciprofloxacin and gentamicin were found to be very active. For *Salmonella* isolates, 13 (100) were sensitive to ciprofloxacin and twelve (92.31) isolates were sensitive to gentamicin. All the *E coli* isolates were (100) sensitive to both ciprofloxacin and gentamicin irrespective of the eatery type.

DISCUSSION

Antimicrobial susceptibility of *Salmonella* strains is useful for epidemiological purposes [20]. In this study, based on the previous literature, resistance to 3 different classes of antibiotics was defined as a multi-drug resistance [21]. Some bacterial strains are now resistant to three or more classes of antimicrobial substances – the currently accepted definition of multi-drug resistance (MDR). CDC reported that while multi-drug resistance declined in *Salmonella* isolates in recent years, this was due to a reduction in numbers of *S. Typhimurium* isolated [22].
Previous reports had indicated that the amount of multidrug resistance ranges between 5-60% [20]. The reasons behind the increasing resistance of species that caused food borne diseases were due to the uncontrolled use of antibiotics on farm animals that resulted in destruction of sensitive bacteria and selection of resistant strains to multiple antibiotics. Through food, these strains could directly infect humans or transfer resistance genes to human endogen [23].
The use of antimicrobial agents in animal production has been identified as an important factor which select for antimicrobial resistant bacterial strains [24]. This might be due to an inevitable genetic response to the strong selective pressure imposed by antimicrobial agents which played a vital role in the evolution of antibiotic resistance among bacteria. These bacteria then pass the plasmid containing resistance gene among other bacterial cells and species [25]. Globally, antimicrobial resistant bacteria resident in the gut of carrier animals contribute significantly to environmental contamination and spread of antimicrobial resistant bacterial strains, hence the need to continuously monitor antimicrobial resistance in zoonotic and commensal bacteria of animal origin for the protection of public health [26-28]. The results of this study indicated highest resistance to cefuroxime, augumentin and ampicillin by *Salmonella* isolates and resistance to augmentin and ampicillin by *E. coli* isolates. The most obvious reason that could be responsible for the high frequency of antibiotic resistance was due to the uncontrolled use of antibiotics for various purposes. Previous study observed a similar resistant profile amon [29]. In this research, the two isolates were most sensitive to ciprofloxacin, 100% sensitivity was observed. Similar study observed that some of the isolates were sensitive to Ofloxacin (100%), Gentamicin (100%), Nalidixic Acid (98%) and Nitrofurantoin (99%) [30]. It was reported previously that *E. coli* isolates were sensitive to Levofloxacin (80%) and Ciprofloxacin (80%); a few number of isolates were sensitive to Azithromycin (30%) and Nalidixic acid (30%) and resistant to Tetracycline (80%), Ampicillin (90%), Erythromycin (90%), Amoxicillin (90%) and Metronidazole (100%) [31].
Fluoroquinolones were introduced in the 1980s and are fluorinated derivatives of quinolones. In ciprofloxacin, the ethyl group of norfloxacin was replaced by a cyclopropyl group, increasing the spectrum of action to include Gram-positive bacteria and not compromising its activity against Gram-negative bacteria [32]. Fluoroquinolones, as all quinolones, are bactericidal antimicrobials that inhibit the catalytic activity of bacterial DNA gyrase (Topoisomerase II) and Topoisomerase IV, which are essential for the replication and transcription of bacterial DNA [32]. International organizations, such as the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE), as well as regulating authorities, had expressed their concern with the development of resistance in microorganisms that were pathogenic both for humans and animals, including zoonotic agents, such as *Campylobacter spp.* and *Salmonella spp.*, particularly to some antimicrobial classes, including fluoroquinolones [33]. Fluoroquinolones are intensively used in poultry production and have allowed better treatment of several diseases; however, their prudent, ethical, and professional use is essential [34]. The inherent risks of the inadequate use of antimicrobials in poultry production could include the introduction of bacterial resistance, environmental contamination, and the accumulation of residues in poultry products [34]. Consumer union in the US had concluded that overuse of antibiotics in food animals was increasing greatly. Human beings were at risk due to potential presence of the superbug in meat

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and poultry and its general migration into the environment. Numerous health organizations including World Health Organization, America Medical Association, American Public Health Association, just to mention but a few agreed and had called for a significant reduction in use of antibiotics for food animals, [35]. FDA has banned the use of ciprofloxacin in 2005 after reported cases of ciprofloxacin-resistant campylobacter in nearly 20% sampled chicken breast.

Antibiotics are often used for therapy of infected humans and animals as well as for prophylaxis and growth promotion of food producing animals. Many findings suggest that inadequate selection and abuse of antimicrobials may lead to resistance in various bacteria and make the treatment of bacterial infections more difficult [36]. Antimicrobial resistance in *E. coli* were reported worldwide. Treatment for *E. coli* infection has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents [37].

To deal with multi-drug resistant organisms, it is usually recommended that potentially synergistic antimicrobial combinations be used [38]. The issue of antibiotic resistance is very complex. In many parts of the world, poultry producers have proactively and voluntarily taken steps toward finding alternative ways to control disease while reducing antibiotic use. Two classes of antibiotics, that FDA deemed critically important to human medicine, especially for treating foodborne illness in human beings namely flouroquinolones and cephalosporins, were phased-out of chicken production for a number of years. The FDA's proposed Veterinary Feed Directive (VFD) ensures that all antibiotics are administered to food producing animals under the supervision and prescription of licensed veterinarians [39].

Some commonly used antibiotics had become ineffective in treating infections from resistant bacteria resulting in choosing alternatives that could cause complications and more severe side-effects. It is for these reasons that fluoroquinolones have been banned in poultry production in the US [40]. These drugs were also prohibited in chicken farms in Australia, Finland and Denmark. The World Health Organisation (WHO) had issued warning about the risks for 18 years. There is a full ban of antibiotic use as a growth promoter in the European Union since 2006.

Care must be taken to reduce usage of antibiotics, with a particular focus on those antibiotics considered to be of critical importance for human medicine like the flouroquinolones and cephalosporins [40].

Other considerations include, timely clinical inspections to identify and treat sick animals before disease spreads to others; assessment of animal based welfare parameters to maintain a hygienic and healthy living environment; use of laboratory tests to detect animals at risk of developing disease [22].

CONCLUSION

This study confirmed that most of the isolated Escherichia. coli and Salmonella strains were multi-drug resistant. However, both isolated Salmonella and E coli strains were susceptible to Ciprofloxacin and gentamicin.

Recommendation

Base on the outcome of this study, the followings are hereby recommended:

- Careful restrictions of antibiotics use in animals most especially those that are prescribed for human infections
- Consideration for new food safety regulations and surveillance programs with a priority on molecular subtyping of zoonotic foodborne pathogens.
- Establishment and enforcement of guidelines for antibiotic distribution in the country by the Federal Ministry of Health (FMOH) and the National Agency for Food and Drug Administration and Control (NAFDAC) respectively.
- Need to do more research on a possible and sustainable alternative to antibiotics from readily available sources.
- All poultry farms should be duly registered and representative of the regulatory body within the locality should examine samples from poultry farms to ensure the birds are antibiotic free before being sold or slaughtered.
- Finally, a one health approach should be deeply considered

CONSENT: The study was explained to the Manager of the eateries who permitted the commencement of the study.

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ETHICAL APPROVAL: The ethical approval for this study was obtained from UI/UCH, Research and Ethics Committee, Institute of Medical Research and Advanced Training (IMRAT). Ibadan.

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