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Antibiogram Profiles of *Escherichia coli*, *Salmonella* and *Listeria* Species Isolated Along the Processing Line of Sale of Frozen Poultry Foods

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ABSTRACT

In this study, thirteen bacteria strains isolated along the processing line of sale of frozen poultry foods in retail outlets of Bodija market, Ibadan, Nigeria, were subjected to antibiogram assay using the Bauer-Kirby disc diffusion method. *E. coli* and *Salmonella* sp. displayed almost similar resistant patterns (50%) and were totally resistant to tetracycline, augmentin, cotrimoxazole and amoxicillin. The *E. coli* strains were at best moderately sensitive to ofloxacin while *Salmonella* sp. were moderately sensitive to gentamicin and ofloxacin. The resistance level for the *Listeria* spp. was 62% while it was 70% with the pathogenic isolate. All the *Listeria* sp. were completely resistant (0.00±0.00 mm) to six of the antibiotics used: cephalexin, cotrimoxazole, ampicillin, clindamycin and augmentin. The best reaction seen was a weakly sensitive level demonstrated by weighing scale *Listeria* spp. with ciprofloxacin (13.00±0.42 mm). Since these bacteria species were sensitive to the quinolones, so their use is being encouraged.

Key words: Antibiogram assay, resistant specie, frozen chicken, bacteria species, antibiotics

INTRODUCTION

Pathogens such as *E. coli*, *Salmonella* and *Listeria* sp. are causes of foodborne illnesses and death in humans. Cross-contamination of foods by these organisms can occur coupled with the spread of genes resistant to commonly used antibiotics along various points in the processing line of sale of poultry products. This is of both serious public health and economic concerns. The symptoms of foodborne illnesses, resulting from the consumption of pathogen contaminated foods, can range from mild to more severe indications such as diarrhea, fever, nausea, vomiting, abdominal cramps, dehydration, meningitis, endocarditis, kidney failure and septicemia (Darwin and Miller, 1999). Commonly used antibiotics in both human and animals in Nigeria include tetracycline, gentamicin, ampicillin, augmentin and amoxicillin.

Frozen poultry foods have emerged to be an alternative source of protein to the teaming Nigerian population owing to their relatively cheaper prices as compared to other meat and protein sources such as goat, sheep, pig and cattle. Most enterprises have thus joined in the import, sale and distribution of frozen poultry foods which have led to a consequential increase in the number of retail frozen poultry foods operators in most vicinities in the country. Reduction or elimination of foodborne microorganisms should be one of the most important concerns for the food industry. Food handlers play a major role in ensuring food safety throughout the chain of producing, processing, storage and preparation, sale and distribution (Apata, 2009).

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 (Apata, 2009). This increase in the use of antibiotics has played a significant role in the emergence of antibiotic resistance bacteria (Ashraf and Shah, 2011). Increasing episodes of multi-drug resistant pathogens can result in failure of antibiotic therapy in both animals and human and this also facilitate the transmission of antibiotic resistance between and among bacteria strains and species. Because of the growing global concerns that antibiotic resistance bacteria can be transmitted from animals to humans, there is an increase in public and government interest in phasing out inappropriate antibiotic use in animal husbandry (Apata, 2009). Improvement in the hygienic practice of handling raw animal products and adequate heat treatment to eliminate antibiotic resistant bacteria may play a role in preventing the spread. More attention should be focused on increasing antibiotic surveillance capacity to cope with the spread of emerging resistances and on alternative approach to sub-therapeutic antibiotics in poultry that can positively influence poultry health and produce safe edible products (Apata, 2009).

Antibiogram studies have been carried out on pathogenic organisms present in poultry feeds in Nigeria (Ezekiel *et al.*, 2011) with none reported from bacteria isolates along the processing line of sale of frozen poultry foods which this study intends to address. This study therefore isolated *Escherichia coli*, *Salmonella* and *Listeria* sp. along the processing line of sale of frozen chicken and assessed the antibiotic sensitivity profile of these isolates.

MATERIALS AND METHODS

Isolation and characterization of strains: Thirteen bacterial strains (7 Gram negative and 6 Gram positive) comprising 2 *E. coli* strains (*E. coli* from nylon covering (ECN) and *E. coli* O157:H7 (ECH7P) from weighing scale), 5 *Salmonella* strains (2 meat and table *Salmonella* isolates (SSM1, SSM2, SST1, SST2) and a *Salmonella* Enteritidis (SEP) isolated from weighing scale) and 6 *Listeria* strains (1 knife, 2 meat, 1 Table, 1 weighing scale *Listeria* sp. (LSK2, LSM1, LSM2, LST1, LSW) and a *Listeria monocytogenes* (LMP) isolated from carton) were isolated along the processing line of sale of frozen poultry meat according to Barrow and Feltham (1993). The direct slide agglutination technique was utilized for serology. The *E. coli*, *Salmonella* and *Listeria* isolates were subjected to agglutination tests with specific *E. coli* O157:H7, *Salmonella* O (poly A to S) and O, factor 9.

Antibiotic sensitivity test: The Bauer-Kirby disc diffusion method of Bauer *et al.* (1966) was used to test the sensitivity of the isolates. According to the manufacturer's specification, nutrient agar (LAB M, Lancashire, UK) was prepared and dispensed in 26 petri dishes. Then 1 mL \times 10⁸ CFU of a 24 h broth culture (HiMedia Labs, Pvt. Ltd., Mumbai, India) of the 13 test cultures were inoculated into each of the solidified agar plates in replicates and gently spread. Plates were allowed to dry after which the antibiotic sensitivity discs (Gram negative for *E. coli* and *Salmonella*, Gram positive for *Listeria*) were placed onto the petri dishes followed by incubation of the preparation at 37°C for 24 h. Zone of inhibition around each antibiotic indicated sensitivity of the organism present in the culture to that antibiotic. The gram negative antibiotic sensitivity disc contained TET: Tetracycline, AUG: Augmentin, OFL: Ofloxacin, GEN: Gentamicin, NAL: Nalidixic Acid, NIT: Nitrofurantoin, COT: Cotrimoxazole and AMX: Amoxicillin (Abtek Biological Ltd., England).

The gram positive disc comprises E: Erythromycin, OF: Ofloxacin, CIP: Ciprofloxacin, CD: Clindamycin, CX: Cephalexin, CO: Cotrimoxazole, AP: Ampicillin and FX: Ceftriaxone. (PolyTes Med. Labs, Nigeria).

Data analysis: Microsoft excel, 2007 was used for data analysis. The results were presented in mean zones of inhibition \pm Standard deviation (mm) from the mean and sensitivity range was assigned as S: Sensitive, MS: Moderately sensitive, WS: Weakly sensitive and R: Resistant according to Adetunji and Adegoke (2008).

RESULTS

Antibiotics sensitivity test was carried out on the strains used for serology. The mean zones of inhibition \pm standard deviations (mm) for the two *E. coli* strains are presented in Table 1 with both strains displaying similar pattern of susceptibility to various antibiotics. The level of resistance and sensitivity displayed by the organisms was 50% (Table 3). Both strains (ECN and ECH7P) were totally resistant to tetracycline, augmentin, cotrimoxazole and amoxicillin. ECH7P was weakly sensitive to ofloxacin, gentamicin, nalidixic acid and nitrofurantoin with zones of inhibition ranging from 11-12 mm. However, ECN was moderately sensitive to ofloxacin (19.0 \pm 1.84 mm) though weakly sensitive to nalidixic acid (11.0 \pm 0.14 mm) and was weakly resistant to gentamicin (9.0 \pm 0.14) and nitrofurantoin (8.0 \pm 0.28) (Table 1). 53.13% resistance was observed for the *Salmonella* spp. (Table 3) which was slightly higher than obtained with *Salmonella* Enteritidis (SEP) (50%). All *Salmonella* strains were completely resistance to tetracycline, augmentin, cotrimoxazole and amoxicillin (0.0 \pm 0.0 mm). The *Salmonella* sp. were at best moderately sensitive to gentamicin and ofloxacin. Though *Salmonella* Enteritidis (SEP) was sensitive to ofloxacin (21.5 \pm 0.07 mm), it was moderately and weakly sensitive to nalidixic acid (17.0 \pm 0.14 mm) and gentamicin (10.5 \pm 0.07 mm), respectively (Table 1). Also, it was moderately resistant to nitrofurantoin (8.5 \pm 0.07 mm) (Table 1).

The resistance level for the *Listeria* spp. was 62% (Table 3) while a very high resistance (70%) was obtained with the pathogenic isolate (LMP). All the *Listeria* sp. were completely resistant (0.0 \pm 0.0 mm) to six of the antibiotics used: cephalixin, cotrimoxazole, ampicillin, clindamycin, augmentin (Table 2). None of the strains was Sensitive (S) or Moderately Sensitive (MS) to all antibiotics. The best reaction seen was a weakly sensitive level demonstrated by LSW with ciprofloxacin (13.0 \pm 0.42 mm). The *Listeria monocytogenes* (LMP) strain showed total resistance to seven of the antibiotics used.

Table 1: Mean zone of inhibition (mm) formed by two strains of *E. coli* and using gram -ve antibiotics sensitivity discs

Gram-ve discs							
TET	AUG	OFL	GEN	NAL	NIT	COT	AMX
2.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R	19.0 \pm 1.84 ^{MS}	9.0 \pm 0.14 ^R	11.0 \pm 0.14 ^{WS}	8.0 \pm 0.28 ^R	0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R
1.5 \pm 0.7 ^R	0.0 \pm 0.0 ^R	11.0 \pm 0.71 ^{WS}	11.0 \pm 0.71 ^{WS}	12.0 \pm 0.28 ^{WS}	11.0 \pm 0.14 ^{WS}	0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R
0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R	10.0 \pm 0.85 ^R	9.5 \pm 0.07 ^R	13.5 \pm 0.07 ^{WS}	15.5 \pm 0.07 ^{MS}	0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R
0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R	11.5 \pm 0.07 ^{WS}	7.5 \pm 0.07 ^R	11.5 \pm 0.07 ^{WS}	11.5 \pm 0.07 ^{WS}	0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R
0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R	2.0 \pm 0.00 ^R	6.5 \pm 0.07 ^R	13.5 \pm 0.07 ^{WS}	9.5 \pm 0.07 ^R	0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R
0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R	11.0 \pm 0.14 ^{WS}	6.5 \pm 0.21 ^R	13.0 \pm 0.14 ^{WS}	9.5 \pm 0.07 ^R	0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R
0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R	21.5 \pm 0.07 ^S	10.5 \pm 0.07 ^{WS}	17.0 \pm 0.14 ^{MS}	8.5 \pm 0.07 ^R	0.0 \pm 0.0 ^R	0.0 \pm 0.0 ^R

Values are Mean \pm SD (mm), TET: Tetracycline, AUG: Augmentin, OFL: Ofloxacin, GEN: Gentamicin, NAL: Nalidixic Acid, NIT: Nitrofurantoin, COT: Cotrimoxazole and AMX: Amoxicillin. R: Resistant, MR: Moderately resistant, WS: Weakly sensitive and MS: Moderately sensitive, S: Sensitive

Table 2: Mean zone of inhibition (mm) formed by *Listeria* spp. using gram +ve antibiotics sensitivity discs

Gram +ve discs										
Strains	-----									
n = 2	E	CIP	GEN	CX	COT	FX	AP	CD	AUG	OF
LMP	5.00±0.40 ^R	12.00±0.28 ^{WS}	9.00±0.42 ^R	0.00±0.00 ^R	0.00±0.00 ^R	1.0±0.10 ^R	0.00±0.00 ^R	3.00±0.14 ^R	2.00±0.00 ^R	7.00±0.14 ^R
LSK2	9.00±0.14 ^R	10.00±0.28 ^R	8.00±0.85 ^R	0.00±0.00 ^R	0.00±0.00 ^R	3.0±0.10 ^R	0.00±0.00 ^R	0.00±0.00 ^R	0.00±0.00 ^R	8.00±0.28 ^R
LSM1	5.00±0.10 ^R	13.00±0.42 ^{WS}	11.00±0.14 ^{WS}	0.00±0.00 ^R	0.00±0.00 ^R	2.0±0.00 ^R	0.00±0.00 ^R	0.00±0.00 ^R	1.00±0.10 ^R	8.00±0.28 ^R
LSM2	8.00±0.00 ^R	11.00±0.42 ^{WS}	6.00±0.57 ^R	0.00±0.00 ^R	0.00±0.00 ^R	3.0±0.10 ^R	0.00±0.00 ^R	0.00±0.00 ^R	0.00±0.00 ^R	6.0±0.00 ^R
LST1	10.00±0.28 ^R	10.00±0.00 ^R	6.00±0.28 ^R	0.00±0.00 ^R	0.00±0.00 ^R	4.0±0.00 ^R	0.00±0.00 ^R	0.00±0.00 ^R	4.00±0.00 ^R	8.00±0.00 ^R
LSW	7.00±0.42 ^R	13.00±0.42 ^{WS}	10.00±0.28 ^R	0.00±0.00 ^R	0.00±0.00 ^R	2.0±0.00 ^R	0.00±0.00 ^R	0.00±0.00 ^R	0.00±0.00 ^R	10.0±0.28 ^{MR}

Values are Mean±SD (mm), E: Erythromycin, OF: Ofloxacin, CIP: Ciprofloxacin, CD: Clindamycin, GEN: Gentamicin, CX: Cephalixin, COT: Cotrimoxazole, AP: Ampicillin, AUG: Augmentin, FX: Ceftriaxone. R: Resistant, MR: Moderately resistant, WS: Weakly sensitive, MS: Moderately sensitive

Table 3: Resistance patterns of the various bacteria isolates

Bacteria isolate	No. of isolates	No. of antibiotics	Resistance antibiotics
<i>E. coli</i> spp.	1	8	TET, AUG, COT, AMX
<i>E. coli</i> O157:H7	1	8	TET, AUG, COT, AMX
<i>Salmonella</i> spp.	4	8	TET, AUG, COT, AMX
<i>Salmonella enteritidis</i>	1	8	TET, AUG, COT, AMX
<i>Listeria</i> spp.	5	10	CX, COT, AP, CD, AUG
<i>Listeria monocytogenes</i>	1	10	E, CD, CX, COT, AP, AUG, FX

TET: Tetracycline, AUG: Augmentin, COT: Cotrimoxazole, E: Erythromycin, CD: Clindamycin, CX: Cephalixin, AP: Ampicillin, AMX: Amoxicillin and FX: Ceftriaxone

DISCUSSION

Despite the successful use of antibiotics in veterinary and human practice for therapeutic treatment, disease prophylaxis and as growth promoter, the reduced efficacy of the drugs noticed overtime is worrisome. Due to the multiplicity of antibiotics, infections caused by drug-resistant bacteria did not represent a medical problem until the early 1980s. However, evolution of bacteria towards resistance has been considerably accelerated by the selective pressure exerted by over-prescription of drugs in clinical settings and their heavy use as growth promoters for farm animals (Charpentier and Courvalin, 1999). Sharada *et al.* (2010) stated that antibiotics pattern varies with different isolates, time and development of multiple drug resistance among different bacteria isolates.

The 50% resistant level were comparable with Adetunji and Isola (2011) who recorded 40 and 60% resistance level in *E. coli* ECN and *E. coli* O157:H7, respectively though they were abattoir isolates. Al-Ghamdi *et al.* (1999), Barka and Kihal (2010) and Sharada *et al.* (2010) reported resistant *E. coli* isolates from chicken showing extremely high resistance to tetracycline and cotrimoxazole, similar reports were made by Olatoye (2010) in beef isolates. Antibiogram profiles that showed that *E. coli* spp. were resistant to one or multiple antibiotics such as reported by previous researchers Elmali *et al.* (2005), Aibinu *et al.* (2007), Olatoye (2010) and Adetunji and Isola (2011). Ofloxacin should be a drug of choice in the treatment of *E. coli* infections as noted from this study. Comparably, Sharada *et al.* (2010) discovered poultry *E. coli* isolates that were highly sensitive to ciprofloxacin and enrofloxacin (83%), pefloxacin (76.92%) and norfloxacin (75.39%). Beef isolates of *E. coli* spp. were also sensitive to ofloxacin (Adetunji and Isola, 2011).

However, Aibinu *et al.* (2007) recorded high resistance of poultry and human isolates of *E. coli* O157:H7 strains to the quinolones including ofloxacin. In poultry feeds, both *E. coli* and *Salmonella* sp. were highly sensitive (>70%) to the fluoroquinolones (Ezekiel *et al.*, 2011).

Similar resistance pattern (as obtained for the *E. coli* strains) was demonstrated by the *Salmonella* spp. in this study. This is possibly because both bacteria species belong to the same family (Enterobacteriaceae). The *Salmonella* strains were resistant to multiple antibiotics but sensitive to gentamicin and ofloxacin as comparable to findings in some other studies (Jones *et al.*, 2002; Cardoso *et al.*, 2006; Yah and Eghafona, 2007; Enabulele *et al.*, 2010; Soomro *et al.*, 2010; Adetunji and Isola, 2011). Ciprofloxacin and ofloxacin are fluoroquinolone antimicrobial that is increasingly and successfully used for the treatment of septicaemic salmonellosis in humans (Soomro *et al.*, 2010). Ciprofloxacin has been recorded as a very sensitive drug against clinical isolates of *Salmonella* isolates in Adamawa state, Nigeria by Doughari *et al.* (2007). In contrary, in a study carried out by Moon (2011), most *Salmonella* sp. tested were resistant to ciprofloxacin and ofloxacin but sensitive to norfloxacin.

The levels of antibiotic resistance displayed by *Listeria* strains were higher than other two species above. The 70% resistance is very alarming and this calls for a serious effort in the prevention of *Listeria* contamination in foods. This resistance level was contrary to the low plane reported by Ruiz-Bolivar *et al.* (2011). High resistance levels were also observed by Charpentier *et al.* (1995) and Issa *et al.* (2011), they reported 100% resistance to ampicillin in akin to that observed in this study however, it is at per with the findings of Ruiz-Bolivar *et al.* (2011). Persistent bacteria infections prevalent in Nigeria can possibly be due to the continuous use of drugs like ampicillin and augmentin that were completely resistant to the *Listeria* sp. tested in this study. The total resistance to cephalixin is expected as *Listeria* sp. has been reported to be naturally and intrinsically resistant to cephalosporins (Yucel *et al.*, 2005).

Though we would encourage that the use of the quinolones in Nigeria be emphasized, the general resistant pattern noticed in this study from samples isolated from the processing line of sale of frozen retail poultry is suggestive of an age-long problem associated with the use of antibiotics of veterinary use. For instance, tetracycline is the most commonly available drug for use as growth promoter and routine chemoprophylaxis among livestock in Nigeria (Olatoye, 2010) and has been and still been prescribed in most hospitals in Nigeria since the decades making it very easy for local pathogenic bacteria strains to develop resistance to it. Also, a reflection of this is also seen in the treatment of human diseases associated with foodborne illnesses which subsequently lead to prolonged illnesses, drug wastages, serious economic losses and threat to public health. In various livestock farms and homes, improper drug administration (over-dosage and under-dosage) are seen while the production of fake drugs owing to lax monitoring activities of regulatory agencies promotes the emergence of multi-drug resistant pathogens. This further underscores the need for adequate protection of consumers from zoonotic pathogens. Molecular studies are important to determine the gene encoding for the high and prevalent resistance to antimicrobials and plasmid transfer demonstrated by these local strains since transmission of resistance plasmids of pathogenic poultry organisms to human have been reported (Mansouri and Shareifi, 2002).

CONCLUSION

The findings generated from this research therefore provide the evidence of the existence of multi-resistant strains of pathogenic and zoonotic organisms and the emergence of super-bacteria

in ready to eat frozen poultry products, thus representing a potential threat to human health. There is a need to do further studies on inert factors that aid bacteria to develop resistance to antibiotics and proffer a solution to inhibit this process.

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