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Full Length Research Paper

Antibiotic resistance of *Escherichia coli, Listeria* and *Salmonella* isolates from retail meat tables in Ibadan municipal abattoir, Nigeria

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Antibiotics sensitivity test was assayed on thirty (30) isolates (10 each for *Escherichia coli, Listeria* and *Salmonella*) from retail meat (beef) tables in Ibadan municipal abattoir, Nigeria. The isolates were tested for sensitivity for eight (*Listeria*) and ten (*Escherichia coli* and *Salmonella*) commonly used antibiotics using Bauer-Kirby disc diffusion assay. Antibiotics sensitivity profile expressed in mean zone of inhibition (mm) ± standard error of mean showed that all the isolates were resistant to three or more antibiotics. All the isolates were resistant to tetracycline. The incidence of antibiotic resistance in virulent strains: *E. coli* O157:H7 (60%) and *Salmonella typhi* (60%) was higher than the non virulent strains: *E. coli* (40%) and *Salmonella* spp, (50%), respectively. The overall incidence of antibiotics resistance in *Listeria* strains was relatively lower (37.5%) than the other pathogens. The high rate of resistance revealed abuse of antibiotic usage in cattle. The public health significance of these findings is that the resistant strains from meat tables may find their way into human population through food chain and occupational exposure.

Key words: Meat table, *Escherichia coli, Listeria, Salmonella*, antibiotics sensitivity, abattoir.

INTRODUCTION

Microbial food safety is an increasing public health concern worldwide. External contamination of meat constitutes a constant problem in most developing countries abattoirs where there are many potential sources of infection (Lawrie, 1979). The microbial surface contamination of carcasses has been repeatedly reported to have a significant effect on the meat shelf life. Moreover, contaminants may also include pathogens which can penetrate into the meat (Elmossalami and Wassef, 1971). Unhygienic abattoir processes spread such diseases as *Salmonellosis*, *Cholera*, *Escherichia coli* food poisoning and listeriosis caused by contamination of meat, a serious public health concern (Neil et al., 2002). Microorganisms owe their resistance to their ancestors' ability to adapt and change. An unfortunate consequence of this process, however, is the development of microbial resistance to clinical antibiotics, food preservatives and disinfection processes. However, cells that already possess some degree of resistance, or acquire it later (through mutation or genetic exchange) may proliferate or survive. Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries including Nigeria (Okeke et al., 2005; Ojo et al., 2009). Many pathogens are developing resistant to most currently used antibiotic and there are increasingly frequent reports of pathogens which are resistant to almost all available antibiotics (levy, 1998).

E. coli is a widespread intestinal commensal organism found in human and animal, resulting from fecal contamination or contamination during animal slaughter. It is often found in soil, water and foods. Shiga toxin-producing *E. coli* (STEC) O157 has emerged as a public health threat following its initial identification as a pathogen in a 1982 outbreak of illness associated with the consumption of undercooked ground beef (Riley et al., 1983).

Listeria spp. have been known to be susceptible to

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many antibiotics that are active against Gram positive bacteria (Hawkins et al., 1984), but more recently, reports of resistance in *Listeria* spp. have been published (Franco et al*.,* 1994; Roberts et al., 1994; Abrahim et al., 1998; Schwaiger et al., 2010). During the 1980s, a number of listeriosis outbreaks were linked with the consumption of contaminated foodstuffs such as coleslaw (Schlech et al., 1983), pasteurized milk (Fleming et al*.,* 1985) and soft cheeses (Piffaretti et al., 1989). Onyemelukwe et al*.* (1983) isolated *Listeria monocytogenes* in Northern Nigeria, from 19 patients with clinical condition characterized by meningitis, meningo-encephalitics, spontaneous peritonitis, septicemia, arthritis, pelvic infection and arthritis.

Antibiotic resistance has increased among *Salmonella* in various areas of the world (Su et al., 2004) and resistance to multiple antibiotics has become a significant trend with *Salmonella thyhimurium* (*S. thyhimurium* D T 104) and several other non thyphoidal serotypes (Weinberger, 2005). *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever). Typhoid fever is a global infection with a fatality rate of 10%. The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa), especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Anita et al., 2002; Doughari, 2005). The World Health Organization (WHO) estimated an annual infectious rate of 21.6 million and approximate death rate of 600 000 with the highest percentage in Africa and Asia. Resistance to a number of antibiotics by *S. typhi* has become a serious problem. Strains of *S. typhi* resistant to chloramphenicol and other recommended antibiotics have been identified in several parts of Latin America, Asia and Africa (Benoit et al., 2003).

It is evident that antibiotic resistance is becoming more and more widely reported in all bacteria pathogens and that the occurrence of antibiotic resistance poses major risks to human health. While many antibiotic-resistant bacteria in foods are currently saprophytic or commensal in habit, their resistance genes can be transferred to other food-borne bacteria, including pathogenic species within the gastrointestinal tract (Perreten et al*.,* 1997). This process may have undesirable clinical implications within human and livestock population having contact with such resistant pathogens. All surfaces in contact with meat are supposed to be kept clean to minimize the risk of bacterial contamination (Butterworth and Heinemann, 2000). Meat processing involving unhygienic floor dressing of carcasses is a common practice in most developing countries including Nigeria, thereby resulting in carcass contamination and isolation of pathogenic microorganisms from meat and slaughtering facilities (Umolu et al*.,* 2006; Ojo et al., 2009). This study determined the antibiotic sensitivity profile of Ibadan municipal abattoir isolates of *E. coli, Listeria* spp. and *Salmonella* spp. from meat tables in other to determine resistant strains of these isolates.

MATERIALS AND METHODS

Thirty (30) bacterial strains comprising of 7 *L. monocytogenes* (SLM 1, 2, 3, 4, 5, 6 and 7), 3 *Listeria* spp. (SLS 1, 2 and 3), 6 *E. coli* O157:H7 (SEH 1, 2, 3, 4, 5 and 6), 4 *E. coli* (SE 1,2,3 and 4), 4 *S. typhi* (SST 1, 2, 3 and 4) and 6 *Salmonella* spp. (SSS1, 2, 3, 4, 5 and 6) were isolated from meat tables according to Barrow and Feltham (1993). Salmonella test cultures were subjected to agglutination tests with *Salmonella* O antiserum factor 9 and *Salmonella* O antiserum (poly A to S) (Difco^{TM}, UK) to identify *S. typhi* strains. The Bauer-Kirby disc diffusion method (Bauer et al., 1966) was used to test the sensitivity of the isolates. According to the manufacturer's specification, 9.2 g of nutrient agar (Fluka, Germany) was dissolved in 500 ml of distilled water followed by sterilization in autoclave at 121°C for 15 min. The 15 ml agar each was then dispensed into thirty (30) Petri dishes. One milliliter 10^8 CFU of a 24 h broth culture of the 30 test cultures were then inoculated into each of the solidified agar plates 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 positive antibiotic disks used include cotrimoxazole, 25 µg; chloramphenicol (10 µg), cloxacilline (5 µg), erythromycin (5 µg), gentamicin (10 µg), augmentin (30 µg); streptomycin (10 µg) (Abtek Biological Ltd, England), while the Gram negative antibiotic disks used include: nitrofuratoin (100 µg); gentamicin (10 µg); ampicillin (10µg); cefuroxime (30 µg); chloramphenicol (10 µg); ofloxacin(5 µg); amoxicillin (30 µg); norfloxacin (10 µg); tetracycline (50 µg); ciprofloxacin (5 µg) (Poly-Tes med; Laboratories, Nigeria). The results were presented in mean zones of inhibition (mm) ± standard error of mean and sensitivity range were assigned as S- sensitive; MS- moderately sensitive; WS- weakly sensitive; R- resistant (Adetunji and Adegoke, 2008).

RESULTS

In this study, *L. monocytogenes* and *Listeria* spp. showed resistant to three out of the eight (38.5%) antibiotics used (Tables 1 and 4). *L. monocytogenes* was highly sensitive to gentamicin and streptomycin. The least sensitive drugs were cloxacillin, tetracycline and chloramphenicol (Table 1). *Listeria* spp. was highly sensitive to gentamicin, erythromycin and streptomycin. The least sensitive drugs were cloxacillin, tetracycline and chloramphenicol (Table 1). The incidence of antibiotic resistance in *E. coli* O157:H7 was high (60%) when compared with the *E. coli* strains (40%) (Tables 2 and 4). *E. coli* O157:H7 was sensitive to cefuroxime but least sensitive to a wide range of antibiotics: Ciprofloxacin, tetracycline, ampicillin, amoxillin, gentamicin and chloramphenicol (Table 2). *E. coli was* sensitive to cefuroxime, ciprofloxacin and gentamicin, norfloxacin, while the least sensitive drugs were tetracycline, amoxillin, gentamicin and chloramphenicol (Table 2). Antibiotics resistance of *S. typhi* (60%) was higher than that of *Salmonella* spp. (50%) (Table 4). *S.*

Table 1. Mean zone of inhibition (mm) of *L. monocytogenes* and *Listeria* spp. to antibiotics.

Antibiotic	Mean zone of inhibition (mm)(n-7) *L. monocytogenes*	Sensitivity (n-7) *L. monocytogenes*	Mean zone of inhibition (mm)(n-3) *Listeria* spp.	Sensitivity (n-3) *Listeria* spp.
Augmentin (AUG)	7.73±0.43	WS	6.67±0.33	MS
Cloxacillin (Cxc),	0.00±0.00	R	0.00±0.00	R
Tetracycline (TETRA)	2.28±0.22	R	1.50±0.86	R
Cotrimoxazole (COT)	9.29±0.38	MS	8.00±0.58	MS
Gentamicin (GEN)	9.93±0.49	S	9.67±1.20	S
Chloramphenicol (CHL)	4.00±0.41	R	1.67±0.96	R
Streptomycin (STR)	11.14±0.51	S	10.67±0.23	S
Erythromycin (ERY)	8.93±0.23	MS	9.83±0.60	S

Values represent mean zone of inhibitions ± standard error of mean. S- Sensitive; MS- moderately sensitive; WS- weakly sensitive; R- resistant.

Table 2. Mean zone of inhibition of *E. coli* 0157:H7 and *E. coli* to antibiotics.

Antibiotic	Mean zone of inhibition (mm)(n-6) *E. coli* 0157:H7	Sensitivity(n-6) *E. coli* 0157:H7	Mean zone of inhibition (mm)(n-4) *E. coli*	Sensitivity (n-4) *E. coli*
Nitrofurantion (N)	4.50±0.43	WS	5.25±0.96	WS
Ciprofloxacin (CIP)	0.00±0.00	R	9.00±2.16	S
Tetracycline (TE)	0.00±0.00	R	4.00±0.40	WS
Norfloxacin (NB)	7.33±0.42	MS	8.00±0.42	S
Amoxillin (AX)	0.00±0.00	R	0.00±0.00	R
Ofloxacin (OF)	7.58±0.37	MS	7.75±0.48	MS
Chloramphenicol	0.00±0.00	R	I.75±0.42	R
Cefuroxime (CF)	10.00±0.00	S	7.25±1.92	S
Ampicillin (AM)	0.00±0.00	R	0.00±0.00	R
Gentamicin (GN)	0.00±0.00	R	9.25±0.48	S

Values represent mean zone of inhibitions ± standard error of mean. S- Sensitive; MS- moderately sensitive; WS- weakly sensitive; R- resistant.

typhi was highly sensitive to amoxillin but was least sensitive to a wide range of antibiotics: Tetracycline, ciprofloxacin, ampicillin, ofloxacin, cefuroxime and norfloxacin (Table 3). *Salmonella* spp. *was* highly sensitive to gentamicin and amoxillin but was resistance to tetracycline, ciprofloxacin, ampicillin, ofloxacin and norfloxacin (Table 3).

DISCUSSION

The overall incidence of antibiotic resistance in *Listeria* strains was still relatively lower (37.5%) than that of E. *coli* O157:H7 (60%), *E. coli* (40%), *S. typhi* (60%) and *Salmonella* spp. (50%). Since the first report of antibiotic resistance of *Listeria* strains (Polyart-Salmeron, 1990), there has been a continuing emergence of *Listeria* strains isolated from food, meat, environment and clinical isolates in cases of listeriosis which are resistant to 2 or more antibiotics (Arpin et al*.,* 1992; Charpentier et al.,1995). The resistance patterns shown by *L. monocytogenes* and *Listeria* spp. strains to tetracycline in this study, agreed with earlier report by Poyart-Salmeron et al. (1990) who deter-mined a new class of tetracycline resistance gene test(s) in *L. monocytogenes* BM4210 and 37 kb plasmid, PIP811 in *L. monocytogenes* strains isolated from a patient with meningo-encephalitis, respectively. The report by Poyart-Salmeron et al. (1990) in which *L. monocytogenes* isolated from a patient with meningo-encephalitis was resistant to erythromycin, streptomycin and chloram-phenicol is similar with this study where abattoir isolates tested were sensitive to gentamicin and streptomycin. Also, findings of an earlier work done by Adetunji and Adegoke (2008) on *L. monocytogenes* isolated from local cheese (wara) differed from this study where they reported augmentin as a highly sensitive drug. This is probably due to abuse of this drug leading to the development of resistance.

The results of this study showed that the incidence of antibiotic resistance in *E. coli* O157:H7 is high (60%) when compared with the non-pathogenic *E. coli* strains (40%). The result agrees with previous report by Olatoye

Table 3. Mean zone of inhibition of *S. typhi* and *Salmonella* spp. to antibiotics.

Antibiotic	Mean zone of inhibition (mm)(n-4)*S. typhi*	Sensitivity (n-4)*S. typhi*	Mean zone of inhibition (mm)(n-6)*Salmonella* spp.	Sensitivity (n-6)*Salmonella* spp.
Nitrofurantion (N)	4.63±0.40	WS	5.33±0.24	WS
Ciprofloxacin (CIP)	1.75±1.02	R	0.00±0.00	R
Tetracycline (TE)	0.00±0.00	R	1.17±0.34	R
Norfloxacin (NB)	0.75±0.36	R	0.00±0.00	R
Amoxillin (AX)	9.50±0.64	S	9.92±0.58	S
Ofloxacin (OF)	0.75±0.38	R	3.50±0.47	R
Chloramphenicol	5.00±0.40	WS	4.58±0.40	WS
Cefuroxime (CF)	2.00±0.61	R	6.75±0.56	MS
Ampicillin (AM)	1.00±0.50	R	0.00±0.00	R
Gentamicin (GN)	6.75±0.48	MS	10.08±0.13	S

Values represent mean zone of inhibitions ± standard error of mean. S- Sensitive; MS- moderately sensitive; WS- weakly sensitive; R- resistant.

Table 4. Resistance patterns of different bacteria isolates.

Bacteria isolate	Number of isolate	Number of antibiotic	Resistance pattern	Percentage (%)
L. monocytogenes	7	8	Ofl, Tetra, Chlor	37.5
Listeria spp.	3	8	Ofl, Tetra, Chlor	37.5
E. coli 0157:H7	6	10	Amo, Amp, Tetra, Chlor	40
E. coli	4	10	Nitro, Cipro, Tetra, Amo, Chlor, Amp, Gen	60
S. typhi	4	10	Cipro, Tetra, Nitro, Ofl, Cerf, Amp.	60
Salmonella spp.	6	10	Cipro, Tetra, Nitro, Oflo, Amp.	50

CIPRO, Ciproflaxin; TETRA, tetracycline; NITRO, norfloxacin; AMO, amoxilin; OFL, oflaxicin; CHLOR, chloramphenicol; CERF, cefuroxime; AMP, ampicillin; GEN, gentamicin.

(2010) where he recorded high resistance of *E. coli* 0157:H7 to 4 or more antibiotics. In this study, *E. coli* 0157:H7 strains were resistance to nitrofuratoin, tetracycline, ampicillin, gentamicin, ciprofloxacin and chloramphenicol. This showed near similarity in resistance recorded by Olatoye (2010) in which *E. coli* 0157:H7 strains isolated in his study from beef in Ibadan metropolis showed a resistance to tetracycline, nitrofuratoin and chloramphenicol. The meat could have been contaminated on the floor or retail tables as shown by this study. Al-Ghamdi et al. (1999) also reported resistant *E. coli* isolates from chicken showing resistance to tetracycline (99.1%), spectomycin (95.7%), TMP + SMX (92.2%), gentamicin (89.7%), ampicillin (88.7%) and chloramphenicol (57.0%), while ceftazidime and nitrofurantoin were highly effective with resistance rates of ≤ 2.6%. However, our study showed nitrofuntoin to be weakly effective.

Antibiotics resistance of *S. typhi* (60%) is higher than that of *Salmonella* spp. (50%). The high level of resistance showed by *S. typhi* to ampicillin and ciprofloxacin is similar to Su et al. (2004) and Chiu et al. (2005) report where resistance were recorded for ampicillin and ciprofloxacin in non typhoid *Salmonella* serotypes and genome sequence of *Salmonella enterica* serovar and *Cholera suis*, respectively. Resistance to a number of antibiotics by *S. typhi* has become a serious problem as shown in the results obtained in this study which records resistance to tetracycline, oxfloxacin, ciprofloxacin, nitrofloxacin, cerufoxime and ampicillin. Similar resistance pattern had been reported by Doughari et al. (2007), although, with resistance to other antibiotics (amoxillin, chloramphenicol and cotrimazole) for clinical isolates in Adamawa state, Nigeria. Ciprofloxacin recorded as most sensitive drug by Doughari et al. (2007) is at par with the findings in this study where it was resistant. This could be due to development of resistance as a result of misuse of this antibiotic in disease conditions.

This study reveals that all the strains tested were resistant to tetracycline and each of the strains showed multiple resistances to 3 or more antibiotics. The multiple resistances observed were to those antimicrobials frequently employed in veterinary practices. The public health

significance of this study is that the resistant strains from meat tables may find their way into human population through food chain, meat consumption and occupational exposure. Proper decontamination of tables before carcass processing is therefore very important. The high rates of resistance found in this study can be explained by the widespread use of antibiotics agents given to cattle as prophylaxis, growth promoters or treat-ment. We recommend more restrictions on the irrational use of antibiotics and public awareness activities should be undertaken to alert the public on the risks of the un-necessary use of antibiotics. There should be extensive educational programmes for abattoir workers, meat butchers and buyers for proper hygiene on meat tables and the need for comprehensive HACCP program. Governments at all levels should put in place a nation-wide surveillance programme to monitor microbial trends and resistance patterns of abattoir isolates in Nigeria.

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