



## INCIDENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACTERIA ISOLATED FROM APRONS OF MEAT VENDORS IN AWKA, ANAMBRA NIGERIA

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Article Received on  
28 Jan 2016,

Revised on 19 Feb 2016,  
Accepted on 10 Mar 2016

DOI: 10.20959/wjpps20164-6114

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### ABSTRACT

Food handlers with poor personal hygiene and inadequate knowledge working in Abattoirs could be potential sources of infections of many public health bacteria. This study evaluated the frequency and susceptibility profile of three Gram negative bacterial isolates from aprons of meat vendors an Abattoir in Awka, South-eastern Nigeria. Randomly collected fresh swabs (100) from aprons of meat vendors were analyzed on different bacteriological media. Colonies were identified by basic identification techniques; and susceptibility profile was evaluated on Muller-Hinton agar using agar diffusion method. Of the 100 swab samples collected, the total Gram negative bacteria isolated from the aprons was 43, the predominant was *Escherichia coli* (44.2%), followed by *Shigella* (39.5%) and *Salmonella* spp (16.3%). The isolates were resistant to almost all the tested agents except levofloxacin and the isolates recorded 100% resistance to the beta-lactams (ampicillin, ceftriaxone and cefuroxime). This study confirmed

the presence of multidrug resistant *Escherichia coli*, *Shigella* and *Salmonella* on the apron of meat vendors in abattoirs in Awka. These public health important bacteria from food handlers may pose significant risk on the consumers. It is therefore essential for our government to implement food handlers training on food safety, conduct periodic medical checkup and regular monitoring of personal hygiene.

**KEYWORDS:** Antibiogram, bacteria, resistance, food safety, aprons, meat vendors, Awka.

## INTRODUCTION

Aprons are worn by the meat vendors to maintain hygiene and protect clothes from spills, wear and tear. However, because of their frequent contact during animal slaughter, transfer and sales of meat, as well as poor personal hygiene/sanitization, the aprons may be potent fomites for the spread of many bacteria.<sup>[1,2]</sup> Poor personal hygiene amongst meat handlers and the inadequate handling of meat products in abattoirs are possible sources of acquiring microbial pathogens that cause food borne infections.<sup>[3]</sup> Meat handlers who harbor bacteria may contaminate meat with their fingers during processing, and finally to infection of consumers. It is on record that cross-contamination is a crucial factor in the outbreaks food borne diseases/infections.<sup>[4-5]</sup> Food borne diseases are widespread and are of great public health concerns.<sup>[3,6]</sup> Microbes that are inherent microflora in animals' tissues, air, and environment are possibly contaminants of meat surfaces. Contamination could be due to unhygienic slaughtering, handling as well as processing of the meat products.<sup>[7]</sup> Surfaces contaminated with microbes run the potential risk of transmission of the pathogens to food during processing. Thus Food borne diseases/infections due to contact with hands and surfaces depends greatly on the level of contamination.<sup>[2]</sup>

Studies have demonstrated that meat handlers harbor well-recognized food-borne pathogens such as *Salmonella* spp. and *Escherichia coli* asymptotically.<sup>[8-9]</sup> Therefore meat vendors with poor personal hygiene and inadequate knowledge working in abattoirs could be potential sources of infections of many enteropathogenic bacteria.<sup>[2]</sup> This study is therefore aimed at assessing the incidence of *Salmonella*, *Shigellia* and *E.coli* as public health important bacteria and their antibiotic susceptibility pattern on the aprons of meat vendors in an abattoir in Awka, Anambra Nigeria. The significance of this study lies on the fact the meat vendors may transfer resistant strains from apron to raw meat and finally to the consumers.

## METHODS

### 2.2.1 Sample collection

One hundred (100) samples were collected an abattoir from the aprons of meat sellers/vendors using sterile swab sticks. The collected samples were processed within 2h after collection at the laboratory section of Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria.

### 2.2.2. Sample culturing and purification

The swab sticks containing the samples were swirled into the sterile nutrient broth and incubated at 37°C for 24 hours. Following the incubation, the bacterial isolates were purified by growing/inoculating them in different bacteriological media: MacKonkey agar (*E.coli*) and salmonella –shigella agar (SS Agar). The characteristic bacteria observed on these media were aseptically isolated and subjected to microscopical and appropriate biochemical tests for proper identification.

### 2.2.3 Identification and confirmation of Isolates

The various isolates obtained were characterized by subjecting them to morphological (colonial morphology), and biochemical tests.<sup>[10]</sup> The isolates were then Gram stained and the following biochemical tests were carried out to further identify the organisms to species level: Catalase test, Coagulase test, citrate, urea, Indole test and Oxidase test.

### 2.2.4 Antimicrobial screening/sensitivity studies

Antimicrobial susceptibility pattern of the bacterial isolates were evaluated using the Kirby-Bauer disk diffusion assay following the guidelines of Clinical Laboratory Standard Institute (CLSI) 2005<sup>[11]</sup> as described by Akbar and Anal 2013.<sup>[6]</sup> Antibiotic disc (Oxoid, UK) containing the following antibiotics was used: Ceftriaxone 30µg, Levofloxacin 5µg, Gentamycin 30µg, Azithromycin 15µg, Cefuroxime 30µg and Ampicillin (10µg), Each antibiotic disc was placed over the lawn and incubated at 37°C for 24hours. Then the clear zone around each antibiotic disc was measured in millimeter and recorded.

## RESULT

The aprons worn by the meat vendors were contaminated by various bacteria, many of which had been identified as food borne pathogens. Total Gram negative bacteria recorded from the aprons is 43 bacterial isolates. Of the 43 that were positively identified to be contaminated the predominant was *Escherichia coli* (44.2%), followed by *Shigella* (39.5%) and *Salmonella* spp (16.3%) as shown in figure 1.

Figure 2 shows the antibiogram of the *Salmonella* isolates to some antibiotics. It is observed that all the isolates were resistant to almost all the tested agents except levofloxacin. The isolates recorded 100% resistance to beta-lactams (*Ampicillin*, *ceftriaxone* and *cefuroxime*).

The sensitivity of the isolates of *E.coli* to the agents is shown in figure 3. Similar to the antibiogram of the *Salmonella spp*, the *E.coli* were equally very resistant to the antibacterial agents except Levofloxacin and gentamicin that recorded 100% and 47.4% sensitivity. *Shigella spp* were also subjected to antibiotic sensitivity study. Their susceptibility profile is shown in the figure 3. Similarly the isolates were sensitive to levofloxacin (88.2%) and gentamicin (47.1%) but recorded high resistance to ceftriaxone (70.6%) and Azithromycin (82.4%).

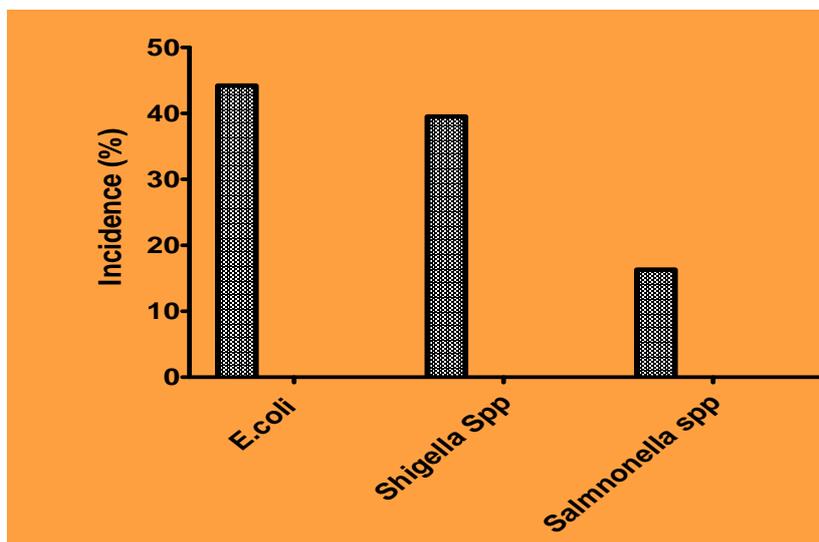


Figure 1: Incidence of Gram negative bacterial isolates from Apron of meat vendors.

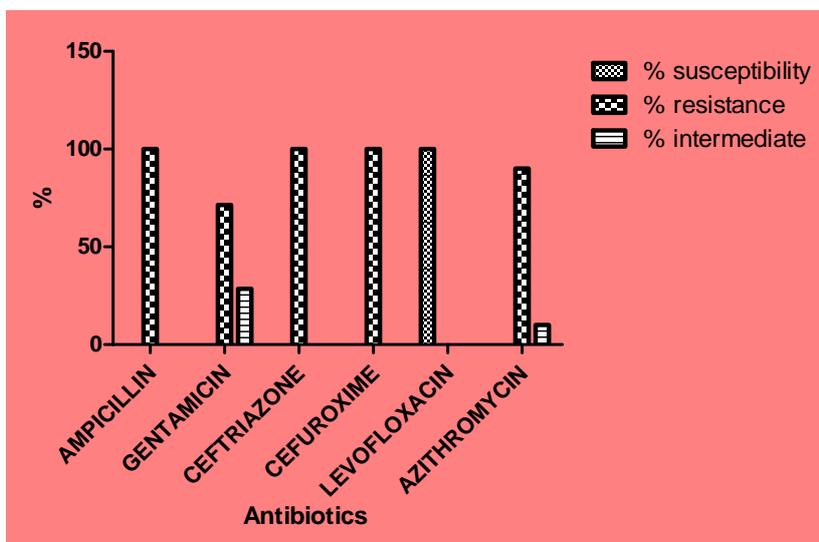
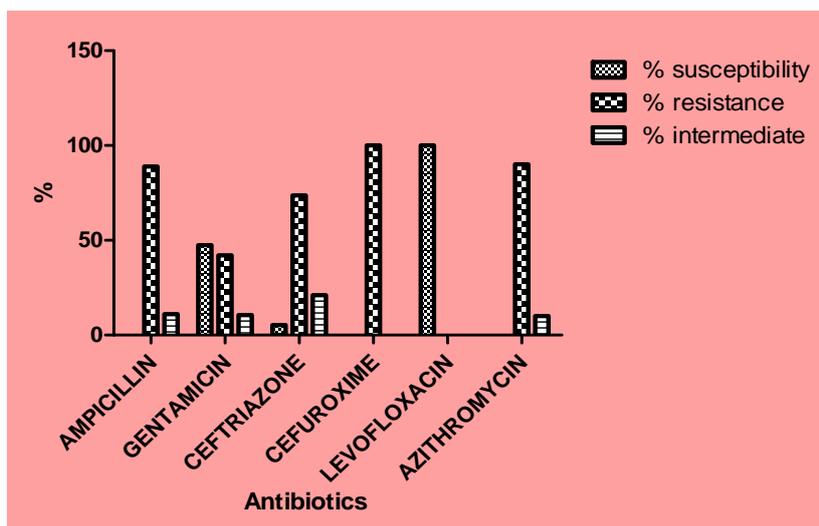
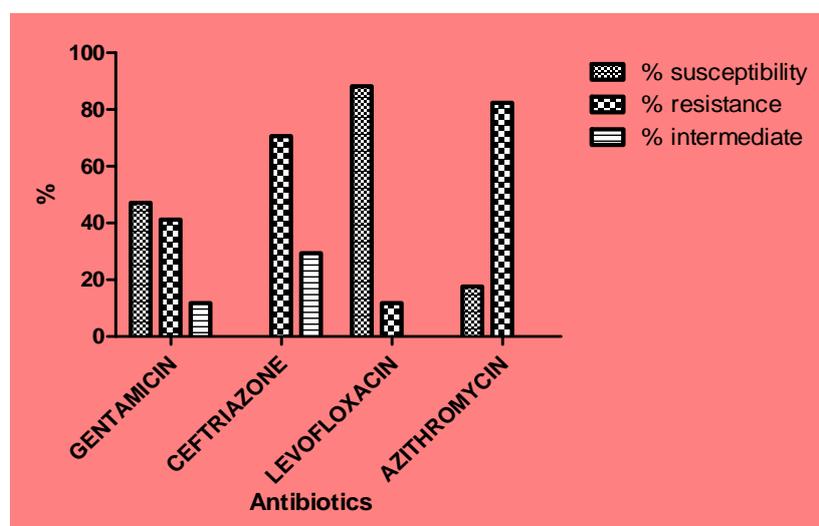


Figure 2: Antibiogram of salmonella spp.



**Figure 3: Antibigram of E.Coli spp.**



**Figure 4: Antibigram of Shigella spp.**

## DISCUSSION

It has been established that contaminated food is the main source of transmission for many pathogenic bacteria. It is the major cause of enteric diseases in developing countries. Several studies have demonstrated that meat handlers harbour microorganisms especially bacteria asymptotically.<sup>[8-9]</sup> Meat handlers with poor personal hygiene and inadequate knowledge working in establishments like abattoir could be potential sources of infections of many enteropathogenic bacteria.<sup>[3]</sup> Likewise, meat handlers who harbour pathogenic bacteria may contaminate foods with their fingers/aprons during food processing, and finally to infection of consumers.<sup>[7]</sup>

In this study, aprons swab culture from meat vendors were investigated for the presence of food borne Gram negative bacteria. The result showed positive for public health important bacteria such as *Salmonella* spp, shigella and *E.coli*. Our finding is similar to the report of a similar study by Eruteya *et al.* 2012<sup>[2]</sup> in Portharcourt, South-south Nigeria. They recorded high level of *E.coli* and *Salmonella* among other bacterial spp. from aprons of meat vendors. Presence of these public health important bacteria from food vendors may pose significant risk on the consumers. *S. Typhi* is one of the principal causes of food and water borne gastroenteritis in human. The WHO had estimated that 16 million new cases of typhoid fever are recorded annually worldwide. Salmonellosis and shigellosis remain a major public health problem across the globe.<sup>[1,13-14]</sup> Similarly the Centers for Disease Control and Prevention (CDC) estimates that 48 million cases of food-borne illnesses occur in the United States annually. Many of these illnesses are caused by *Salmonella* spp. and *E. coli*. The situation is worsened by the production of toxins by *E. Coli*.<sup>[9,15-16]</sup> The high isolation rate of *Shigella* (39.5%) as compared to salmonella (16.3%) among the bacterial isolates points to the fact that the sanitary practices among the meat vendors are not satisfactory. This is because *Shigella* organisms do not have any natural reservoirs in animals and can only spread from person to person.<sup>[12]</sup> In addition, *Salmonella* species which are flora of intestinal tract of animals and humans, expectedly would have recorded a higher carriage among food handlers. The antibiotic sensitivity profiles of the bacterial isolates are presented in figures 2-4. It is alarming that all the bacterial isolates were so resistant to commonly used agents. However, it must be mentioned that all the isolates had strains that were sensitive to levofloxacin and moderately sensitive to gentamicin. Thus levofloxacin was the most active of all the evaluated antibiotics. Our finding is in line with a study conducted by Sultana *et al.* .2014.<sup>[17]</sup> They reported a similar 0% resistance of food borne isolates to levofloxacin. Almost all the isolates of *Salmonella* spp and *E.coli* recorded 100 % resistance to beta-lactams (ampicillin, ceftriaxone and cefuroxime) and other commonly used agents. Similar resistance profiles of *Salmonella* spp to commonly used agents have also been reported.<sup>[3,12]</sup> This resistance to the beta-lactams may be due to enzymatic activity. Extended-spectrum  $\beta$ -lactamases (ESBLs) have been found in the members of Enterobacteriaceae. Gelinski *et al*<sup>[18]</sup> recently reported that there is increasing number of enterobacterial strains producing ESBLs. Extended spectrum  $\beta$ -lactamases (ESBLs) enzymes have been identified in large numbers from different regions and are significantly detected in various *E. coli* strains. Mo *et al.* 2014<sup>[19]</sup> stated that even in the absence of selective pressure from antimicrobial agents cephalosporin resistance has developed in *Escherichia coli*. The incidence of ESBL-producing

environmental Salmonella isolates is on the increase.<sup>[18,20]</sup> The multi-antibiotic resistance profile of the isolated bacteria is suggestive that they can overcome the likely sanitary norms practiced by the vendors and might originate from the vendors body as frequent antibiotic use might favor development of resistance in bacteria Eruteya et al, 2012.<sup>[2]</sup>

## CONCLUSION

Isolation of multidrug resistant public health important bacteria E.coli, Salmonella, Shigella from apron of meat vendors poses significant risk on the consumers. There is thus, urgent need for applying proper hygienic practices among the food vendors as we equally recommend the use disposable aprons by the meat vendors as these will restrict transfer and the spread of food borne pathogens.

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