



INCIDENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCERS IN CONTACT SURFACES OF MEAT AND MEAT PRODUCTS VENDORS IN KANO METROPOLIS

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ABSTRACT

Extended Spectrum Beta Lactamases (ESBLs) are enzymes produced by certain types of bacteria which break down the active ingredients in many of the common antibiotics making them inactive or ineffective. There are at least two hundred (200) different types of ESBL enzymes documented. The first infection of ESBL was reported in Greece in 1960s. Infections with Extended Spectrum Beta Lactamases (ESBLs) producing organisms *are of* great clinical significance because of their association with refractile diseases in humans. It is therefore epidemiologically important to public health to continue survey for the incidence of ESBLs from hands, knives and tables of meat and meat products vendors in Kano metropolitan. A total of 96 swab samples of hands, knives and tables (32 of each) of fresh meat, *Balangu, Kilishi* and *Tsire* vendors at different retailing points in Kano metropolis were randomly collected using sterile swab sticks. Direct culture method using Eosin Methylene Blue Agar was employed for the detection of enteric bacteria. Isolates were identified using biochemical tests. Clinical Laboratory Standard Institute Break Point procedure was used to confirm antimicrobial susceptibilities. From the 96 swab samples analyzed, the following organisms were isolated *Enterobacter aerogenes* 5(14.29%), *E-coli* 17(48.57%), *Klebsiella pneumoniae* 8(22.86%), *Proteus mirabilis* 3(08.67%), *Salmonella species* 2(05.71%). Also, 12(34.29%) of the organisms were isolated from hands with the following percentages: *Enterobacter aerogenes* 2(16.67%), *E-coli* 4(33.33%), *Klebsiella pneumoniae* 4(33.33%), *Proteus mirabilis* 1(08.33%) and *Salmonella species* 1(08.33%). 11(37.14%) organisms were detected from knives and reads as follows: *Enterobacter aerogenes* 2(18.18%) *E-coli* 6(54.54%), *Klebsiella pneumoniae* 2(18.18%), *Salmonella species* 1(09.09%). 12(34.29%) organisms were isolated from the tables which include *Enterobacter aerogenes* 1(08.33%), *E-coli* 7(58.33%), *Klebsiella pneumoniae* 2(16.67%) and *Proteus mirabilis* 2(16.67%). Twelve 12(34.29%) of the enteric bacteria isolates were confirmed ESBLs positive. Statistical analysis shows no significant difference in the incidence of ESBLs among the different samples. It is recommended that hands and tables should be sanitized and the meat be well protected with nylon leather cloth to prevent meat contamination with enterobacteria.

Key words: Fresh Meat, Meat products, ESBLs, Sensitivity, Antimicrobials.

INTRODUCTION

Microorganisms are in association with our foods because of their ecological predisposition and nutritional requirement for their growth and development. More than 400 foodborne illnesses were reported by the Centre for Disease Control and Prevention (2011). The illnesses are caused either by the microorganisms themselves or by the toxins they produced (Assefa *et al.*, 2015). Bacteria are one of the etiological agents of about two thirds of foodborne diseases in both humans and animals with great clinical significance contributing to the high morbidity and mortality rate (Andes and Craig, 2012).

Extended-spectrum-beta-lactamases (ESBLs) are plasmid-mediated beta-lactamase of predominantly Bush class A, so far described only in gram negative bacilli (Emery and Weymouth, 1997). Extended-Spectrum-Beta-Lactamases are capable of efficiently hydrolyzing penicillin, narrow spectrum cephalosporin (cefotaxime, ceftazidime) and monobactams aztreonam). Beta-Lactamase, inhibitors (clavulanic acid, sulbactam and tazobactam) generally inhibit ESBLs-producing strain (Naumoski and Paizkill, 1996).

The overall incidence of ESBL among patient in Kano is 37.1%. *Escherichia coli* recorded a incidence of 37.8% followed by *Klebsiella pneumoniae* with 36.4%. with 4.8% in Saudi Arabia an incidence rate of 12% in Younde, Cameroun, 15.8% and Riyadh, Saudi Arabia. (Yusuf *et al.*, 2011)

Meat and meat products can be contaminated through contacts with utensils and equipments such as spoons, grinders, sausage stuffers, pots and casings (Henok *et al.*, 2015). However, the main source of contaminating bacteria is the exterior of the animal with great proportion coming from its natural surface flora, water, soil, feed and manure, as well as the intestinal contents (Ndahi *et al.*, 2013).. Knives, cloths, air and hands are secondary sources of bacteria to the meat (Abubakar *et al.*, 2014). These problems can pre-dispose the meat products sold in these areas to contamination by members of the Enterobacteriaceae family which are associated with poor sanitary practices (Shamsuddeen *et al.*, 2017). Enteric microbes that are believed to be capable of being transmitted by food handlers include *E. coli*, *Salmonella spp.*, *Shigella spp.*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium butulinum*, *Campylobacter sp.* *Yersinia*, *Citrobacter*, and *enterobacter* species (Biswas *et al.*, 2011). Pathogens originating from raw animal products such as *Klebsiella* and *proteus* can contaminate hands from where they could be transferred to foods, equipment and other food workers (Assefa *et al.*, 2015). Accordingly, the meat and related products are being incriminated as sources through which drug resistance bacteria could be transmitted to humans or other animals and with epidemiological consequences.

Antibiotics such as cephalosporins carbapenems, penicillins and monobactams are reported to have lethal effect on bacteria by inhibiting bacterial cell wall biosynthesis, targeting the penicillin-binding proteins

(PBPs). The beta-lactam ring binds to these different PBPs, rendering them useless to perform their role in cell wall synthesis. This then leads to death of the bacterial cell due to osmotic instability or autolysis (Deepti and Deepthi, 2010). Beta-lactam antibiotics are the most effective and commonly used agents in the treatment of infectious diseases (Öztürk *et al.*, 2015). They are mainly semi-synthetic compounds, originating from fungi and bacteria found in the environment constituting of about 60% of the worldwide antibiotic usage (Andes and Craig, 2012 ; Öztürk *et al.*, 2015). Some beta-lactams have a very narrow antimicrobial spectrum, while others have a very broad spectrum and targets both Gram-positive and Gram-negative bacteria (Deepti and Deepthi, 2010).

However, few in the bacterial population termed as Extended Spectrum Beta Lactamase producers (ESBLs) are resistant against β -lactam antibiotics (Maria, 2012). Resistance against beta-lactams is primarily mediated by a structural change of the penicillin PBPs or by bacterial production of enzymes. Other mechanisms are decreased permeability or active transportation via efflux pumps (Deng *et al.*; 2013). More than 1,000 different β -lactamase enzymes have been reported in various species of bacteria with wide variations in their chemical structure and catalytic activities (Tidwell, 2008). When bacterial populations have these resistant subgroups, treatment with β -lactam can result in widespread and more potent resistant strain (İsmail and Haydar, 2016).

Extended Spectrum Beta Lactamases have been reported to be disseminated through foods, vegetables and water by many workers (Ananias and Roland, 2017). It is therefore the intention of the current work to monitor meat being processed by butchers for the presence of ESBLs Producers from hands, knives and tables of meat and meat products vendors in Kano Metropolis, Kano, State, Nigeria. This is with the view to addressing Hazard Analyses and Critical Control Point (HACCP) areas requiring better attention in order to promote public health and food hygiene habit in the society.

MATERIALS AND METHODS

Field Survey for the Study Area and Population

The survey was conducted in Kano Metropolitan, Kano State, Nigeria with a population of 13 million people (NPC, 2017) (11.7574° N, 8.6601° E). Kano is one of the commercial centers of livestock in West Africa (Maigari and Dabo, 2018). According to Dossa *et al* (2015), livestock keeping is the most popular Urban Agriculture (UA) practitioner households in Kano State, and that household involved in livestock keeping accounted for about 90% of UA. Meat and meat products is significant portion of the diet of a large population in Kano. These products are commonly sold as ready to eat products within Kano Metropolis.

Sample Collection and Processing

Thirty-two (32) Swab samples (each) of hand, knives and tables of Fresh meat, *Balangu*, *Kilishi* and *Tsire* vendors were

randomly collected using sterile swab sticks containing 1ml of peptone water from different selling centers within the Metropolis.

The samples were thoroughly shaken and 1ml pipetted into test tubes containing 9mls of peptone water (10^{-1}). The test tubes were further diluted to 10^{-5} as described by Food and Agricultural Organization of the United Nations (FAO, 1979).

Enumeration of Coliform Bacteria

The Most Probable Number (MPN) method was used for the enumeration of coliform bacteria as described by Atlas (1997). Each of the three tubes containing 9ml of Lactose broth with an inverted Durham tube was autoclaved to expel air and sterilized. Each tube was inoculated with 1ml of the swab sample to give a dilution of 10^{-1} . From these dilution 1ml aliquots each was transferred to another set of 3 tubes of lactose broth (LB) to make a dilution of 10^{-2} . The same procedure was followed to give a dilution of 10^{-3} . All the tubes were incubated at 37°C for 24 hours. Tubes that showed gas and acid production after 24 hours were recorded as positive for the presence of Coliforms. Negative tubes were further re-incubated for 24 hours after which positive tubes were recorded. Number of gas positive tubes was compared with the most probable number table to obtain the estimated number of Coliforms per gram of sample (MPN/g) at 95% P-value.

Confirmatory Test for Coliforms

Confirmatory test for coliform was carried out as described by Shamsuddeen (2015). A loopful from each gas positive tube from the presumptive test was inoculated into a separate tube containing Eosine Methylene Blue agar plate (EMB) and the tubes were incubated at $37\pm 1^{\circ}\text{C}$ for 24hours. Formation of bluish black colony with green metallic sheen and reddish colonies confirms the presence of coliform bacteria. The numbers of positive tubes were recorded and the most probable number of the Coliforms was determined from an MPN index table. Positive colonies from the confirmatory test were further sub cultured into tubes of lactose broth at 37°C for 24 hours for gas production. This is completed test for coliform. Colonies were then preserved on nutrient agar slant at 0°C for further analysis.

Identification of Isolates

Gram staining was carried out to identify the morphological features of the isolates. Biochemical tests such as motility, IMVIC Tests, Urease production, Oxidase, Triple Sugar Iron Agar Test (TSI) were conducted according to standard procedure for the identification of bacterial colonies as described by Cheesbrough, (2005). The enteric bacteria were further confirmed using API 20E identification kit.

Screening of Isolates for ESBLs

Inoculums Standardization

The isolates were cultured on prepared Brain Heart Infusion (BHI) Agar (Biotech,

England) plates and incubated at 37°C for 24 hours so as to obtain confluent growth for sensitivity test. Few colonies of isolates from BHI plates were dispensed in sterile normal saline to match the 0.5 McFarland standards for sensitivity tests.

Antibiotic Sensitivity Testing

The antibiotics susceptibility test of the isolates was performed on Mueller–Hinton agar by Kirby-Bauer disk diffusion technique and results interpreted according to criteria recommended by the CLSI, (2011).

Clinical Laboratory Standard Institute (CLSI) Break point for ESBLs Screening

One to 5 colonies was picked from a purity plate, with a sterile wire loop and emulsified into 5 ml of sterile saline in a test tube. The saline was stirred with the loop to uniformly mix the colonies in the saline. The turbidity of the saline was adjusted to match the standard McFarland Standard No. 0.5, Biomerieux. Then antibiotic discs ceftaxidime (CAZ)(30µg) Oxoid, England and Ceftriaxone (CXN) (30 µg) Oxoid, England were independently and gently placed on the agar surface and the plates incubated at 37 °C aerobically for 18-24 hours. The diameters of zones of inhibition around each disc, was measured using a ruler, and compared against the zone diameter interpretative standards recommended by the Clinical Laboratory Standard Institute (2011). Zone diameter of inhibition equal to or less than 22mm for Ceftaxidime and less than or equal to 25mm for Ceftriaxone discs were interpreted as positive for the presence

of an Extended Spectrum Beta Lactamases (ESBLs).

Double Disc Synergy Test (DDST) For Extended Spectrum Beta Lactamases Confirmation

The confirmatory test for the presence of ESBLs was performed using the procedure employed by (Yusha'u *et al.* 2010 ; Shamsuddeen *et al.* 2017). A sterile swab stick was used for inoculation on to the surface of prepared Mueller-Hinton Agar (Biotech, England). Augmentin (Oxoid England) disc was gently placed at the center of the inoculated agar plate and a disc of Ceftriaxone (CXN) (30ug) (Oxoid England) 20mm apart (center to center). Another disc of ceftazidime (CAZ) (30ug) was also placed on the plate 20mm (center to center) from the Augmentin disc. Synergistic effect of both the Ceftriaxone disc and the Ceftazidime disc towards the Augmentin disc were observed. Clear extension of the edge of either or both Ceftriaxone and ceftazidime inhibition zone towards the Augmentin disc confirms the presence of ESBLs.

Statistical Analysis

Chi square test of independence was used to analyze the categorical data for the analysis of meat contact surfaces using Maxstat software version 3.0.

RESULTS AND DISCUSSIONS

Ninety-six (96) swab samples of Hands, Knives and Tables of meat and meat product vendors were analyzed for the presence of ESBLs from which thirty-five (35) enteric

bacterial species were isolated, thus, re-establishing the occurrence of bacteria in meat and meat products. This is in conformity with the findings of Henok *et al.* (2015) in a similar study conducted on meat and meat products. Out of the 35 enteric bacteria isolated, *E. coli* had the highest percentage of Incidence with 17(48.57%), followed by *Klebsiella pneumoniae* 8(22.86%) with *Salmonella* species having the least count 2(05.71%). The high frequency of *E. coli* indicates that the sources are contaminated. The presence of *E. coli* on hands, knives or tables of the vendors is an indication of faecal cross contamination probably at one stage of preparation or from the processing materials used (Shamsuddeen and Dahiru, 2018). The microbiological quality of meat and meat products is strongly influenced by the conditions of hygiene existing during their production and handling. The natural source of *E. coli* is the human intestine and although most strains are harmless, some serotypes O157:H7 can cause serious illness (Assefa *et al.*, 2015). *E. coli* is not commonly found on hands, but its presence gives a better indication of recent fecal contamination with enteric pathogens probably during processing (Moshood *et al.*, 2012). In a similar study conducted by Ananias and Roland (2017) on roasted beef samples, it is noted that having functional hand washing facilities is critical to the control of *E. coli*. Cross-contamination may occur as a result of handling meat or meat products with unwashed hands which consequently leads to the introduction of microbes on the products.

The transmission of pathogenic bacteria from the environment and the unhygienic processing utensils to the hands, knives or tables of the vendors then finally to the foods is facilitated by imperfect hand washing (Ananias and Roland, 2017). The use of dirty items and for mites by the vendors, inadequate personal hygiene and the use of unsafe processing water might contribute to the presence of *Klebsiella* and other coliform on the hand of the vendors (Bukar *et al.*, 2010). Usually all processing of meat and meat products including retailing of the finished products are carried out on wooden tables, this may give room to cross contamination and might be the reason for higher coliform counts when compared with bacterial counts on knives. When these organisms are transferred from hands to the food could cause mild to severe infections such as diarrhea, typhoid and cholera (CDC, 2011). The most obvious sources of faecal coliform is the intestine and the skin surfaces of the animal, then followed by knives, tables and other processing utensils which can directly get on to the hands of vendors through cross contamination. These faecal coliform can be transferred to the products if not properly processed. The high occurrence of Gram-negative bacteria is an indication of poor sanitary conditions of processing and mishandling (Iheagwara and Okonkwo, 2016).

The 35 enterobacterial isolates were subjected for ESBLs screening using CLSI Break point procedure, and 12(34.29%) were found to be ESBLs positive. Bacterial isolates from the hands and tables of the vendors were found to be having the highest

percentage of the ESBLs organisms 12(34.29%) each, when compared with those detected from Knives 11(31.42%). Among the ESBLs organisms, *E-coli* was the most prevalent 8(66.665), followed by *Klebsiella* 2(16.67%) and *Enterobacter* 2(16.67%).

ESBLs confer resistance to most beta-lactam antibiotics, including 3rd- and 4th-generation cephalosporins, which led to increased prevalence of enterobacteriaceae (Alejandro, 2013). Studies conducted by Sarah *et al.* (2014) have demonstrated similar pathogenic *E. coli* and *Klebsiella* strains on contact surfaces of meat products. Heat treatment and hand hygiene prevent transmission, but hand could be re contaminated by touching used items during preparation (Sarah *et al.*, 2014). Direct contact with human or animal carriers and the environment are great sources of *E-coli* especially to our foods and most of these transmissions are via hands of the working personnel (Hetty *et al.*, 2013).

Higher ESBLs counts 7(58.33%) were detected from the hands of vendors while

4(33.33%) were detected from tables and 1(8.33%) from knives. Hand involvement in the production and retailing activities coupled with its participation in daily activities may contribute to its high bacterial counts. In a cross-sectional study carried out in Malaysia by Aliyu *et al.* (2016) indicates that majority of the meat vendors are using the same water placed in a container to wash their hands and utensils throughout the retailing period, thus the contaminated water continued to contaminate subsequent meat, contact surfaces and processing environment (Table 1). Most of the local producers in Nigeria use wooden tables for meat and meat products preparations processing and retailing, this may lead to cross contamination under unsanitary conditions. Being pathogenic, these microorganisms may cause outbreak of diseases when consumed. Poor hygienic practices may as well lead to the transfer of plasmids from the environment to the non ESBLs producing isolates hence acquiring plasmids for ESBLs production (Shamsuddeen and Dahiru, 2018).

Table 1: Distribution of ESBLs Organisms Isolated from swab samples of Hands, Knives and Tables of Meat and Meat Product Vendors in Kano Metropolis

Isolated organisms	Hands			Knives			Tables			Total Organisms Isolated
	N	E	NE	N	E	NE	N	E	NE	
<i>Enterobacter aerogenes</i>	2	1	1	2	0	2	1	1	0	5
<i>Eschericia coli</i>	4	3	1	6	1	5	7	2	3	17
<i>Klebsiella pneumoniae</i>	4	1	3	2	0	2	2	1	1	8
<i>Proteus mirabilis</i>	1	0	1	0	0	0	2	0	2	3
<i>Salmonella specie</i>	1	0	1	1	0	1	0	0	0	2
Total	12	7	5	11	1	10	12	4	8	35

Key: N= Number Isolated E = ESBLs , NE = Non- ESBLs

CONCLUSION

Results of the study indicated that the hands, Knives and tables of meat and meat product vendors were contaminated with pathogens such as *E. coli* spp and *Salmonella*. The presence of these pathogenic microbes on contact surfaces when transferred to the meat products could pose a serious public health hazard to those consuming them. *E. coli* contamination was higher on all contact surfaces followed by *Klebsiella pneumoniae*. Some level of contamination with ESBLs organisms was also observed in all contact surfaces. Hands of the meat vendors had the highest count (7) followed by tables (4), then knives (1). The detection of these organisms on meat and meat products contact surfaces signify danger that could be associated with poor personal hygiene, inadequate knowledge and practice on Hazard Analysis and Critical Control Points (HACCP) principles during the preparation, processing, packaging and retailing of the products in the study area, a challenge to the public health policy makers.

FUTURE PERSPECTIVE

These findings emphasize hand hygiene not only after handling raw meat but also after contact with knives and tables used for preparation. There is need for educating and monitoring the producers of meat and meat products during preparation, processing and marketing. Hygiene practices, hazard analysis and critical control points, microbiological risk evaluation, and quality management should be inculcated to the processors. The consumers also need to be

educated on the ill effects of eating contaminated food products. Maintenance of good personal hygiene, prompt sanitation of all meat contact surfaces and environmental sanitation is as well essential to minimize the risk of direct and cross-contamination of the meat and meat products. This will ensure meat quality and public health protection.

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