The International Journal Of Engineering And Science (IJES)
||Volume||2 ||Issue|| 12||Pages|| 01-06||2013||
ISSN(e): 2319 – 1813 ISSN(p): 2319 – 1805

Incidence of Extended Spectrum β-lactamase Producing Bacteria and Multidrug Resistance Strains from Processed Meat 'Suya' Sold in a University Community

[1,]Adenaike, O., [2,]Olonitola, O.S., [3,]Ameh, J.B. and [4,]Whong, C.M.Z.
[1,2,3,4]*Department of Microbiology, Ahmadu Bello University, Zaria. Nigeria*

----------**ABSTRACT**----------
Extended spectrum β-lactamases (ESBLs) producing Enterobacteriaceae notably E.coli that are also multidrug resistance (MDR) have emerged worldwide as a frequent cause of infection in hospitals and in the community. A total of forty (40) isolates identified as E.coli were obtained from 150 samples of processed meat 'suya' sold in a University community. The isolates were screened for ESBL production and confirmed using double disk synergy test (DDST). Resistance to cefpodoxime or ceftriaxone indicated potential ESBLs production while multidrug resistance (MDR) was taken as resistance to four or more antibiotics. Eighteen (45%) of the isolates were found to be ESBLs producers while multidrug resistance was exhibited by 22 (55%) of the isolates. Sixteen (16) out of the MDR strains were ESBLs producers. Pearson's correlation coefficient showed significant correlation ($p<0.05$) between ESBLs production and multidrug resistance in E.coli isolated in this study. Four (10%) organisms were susceptible to all the antibiotics and constitute the population with multiple antibiotic resistance (MAR) index of 0.0. Result obtained showed 80% of the isolates had MAR index of 0.2 and above. This indicates that there were no strict rules governing antibiotic prescriptions and usage in the environment where this study was conducted.

KEY WORDS: ESBLs, 'suya', DDST, MDR, MAR index.

Date of Submission:23 September 2013 Date of Acceptance: 25 December 13

I. INTRODUCTION

Processed meat products are defined as those in which the properties of fresh meat must have been modified by the use of one or more procedure such as grinding, addition of seasoning agents, alteration of colour or heat treatment[1]. Processed meat products in Nigeria comprise 'Tsire' or 'Suya', 'Kilishi' and 'Balangwu'.'Suya' is a popular Nigerian traditional processed ready-to-eat roasted meat product. It is served or sold in public places, along streets, in club houses, restaurants, picnics and homes. It is prepared from boneless meat of animals such as mutton, beef or goat. The meat is trimmed from associated connective tissues, nerves and vessels. The meat is artfully sliced into very thin continuous sheets which are then cut into pieces. The pieces of meat are staked on sticks, spiced with groundnut powder/flour, salt, vegetable oil and flavourings such as monosodium glutamate or others. The sticks are then arranged round the heat place for the meat to roast and the duration of roasting depends on such factors as fire intensity and size of meat. The traditional roasting of 'suya' is usually done by wood smoke[2]. The prepared 'suya', when being sold are usually packaged in leftover newspapers and sometimes in cellophane or nylon bags. Most of the stages of 'suya' preparation, materials used, packaging, the handlers and the surrounding environment can serve as source of contaminants to the meat product[3].

The Enterobactericeae are major pathogens in animals as well as humans[4]. Many strains of *E.coli* are harmless and are found naturally in the gut of humans and animals. Traditionally, its presence in foods has been an indication of faecal contamination of food or water. However, particular strains are pathogenic; traveller's diarrhoea and haemolytic uraemic syndrome (HUS) are caused by some *E.coli* strains. The pathogenic types are said to be rare, but in the last few years, there have been several food borne outbreaks from certain strains of *E.coli* [5].

Great attention has been paid to bacterial resistance to antibiotics in both human and animal populations for adverse impacts on morbidity and mortality from diseases caused by resistant bacteria, economic cost of therapy and real risks of the spread of resistant strains among animals and humans[6]. Emerging antibiotic resistant *E.coli* strains have been increasingly reported in many developing countries and is causing serious public health concerns. *E. coli* has been recognized as a reservoir for resistant genes and can occupy multiple niches in human and animal hosts[7]. Acquired resistance to β-lactam antibiotics is mainly mediated by extended spectrum β-lactamases (ESBLs)[8]. The emergence of *E.coli* that produces extended spectrum β-lactamases (ESBLs) and are multidrug resistant (MDR) poses antibiotic management problems[9].

ESBLs were first described in 1980s and have been detected in *Klebsiella* spp. and later in *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens* and other Gram negative bacilli. ESBLs are enzymes conferring broad resistance to penicillins, aztreonam and cephalosporins (with the exception of cephamycins). These enzymes are able to hydrolyze 3rd and 4th generation cephalosporins and monobactams. They are an increasing important cause of transferable multidrug resistance in Gram negative bacteria throughout the world[10]. In fact, one of the important features of ESBL producing strains is resistant to multiple clinically important antibiotics[11].

More has been focused on hospitals as the primary reservoir and place of transmission of many antimicrobial-resistant organisms, there is need to shift interest to the role of non-hospital community, such as foods as a significant reservoir of resistant pathogens[12]. This study is therefore aimed at assessing the presence of ESBLs production and multidrug resistance among *E. coli* isolated from the popular, traditionally processed ready-to-eat Nigerian meat product, 'suya'.

II. MATERIALS AND METHODS

ISOLATION AND CHARACTERIZATION

A hundred and fifty (150) samples of 'suya' were purchased from three selling points in Samaru campus of Ahmadu Bello University, Zaria, Nigeria. Twenty five grams of each sample was homogenized using a disinfected blender and enriched in 225 ml buffered peptone water before incubating at 37 °C for 18 hours. A loopful of the homogenate was plated on EMB agar plates, and incubated at 37°C for 18-24 hours. Colonies with green metallic sheen were picked as presumptive *E.coli*. The presumptive isolates were subjected to routine IMVIC tests (Indole, Methyl red, Voges Proskaur and citrate utilization tests) among other tests[13]. Isolates giving atypical responses for any of the above named tests were examined further using Microgen^{TM}Gram negative Identification A system. The data obtained by the Microgen GN-ID A microwell strip was designed to generate a 4 digit octal code which was used to interpret the result from the Microgen Identification System Software[14].

Antimicrobial susceptibility test

For susceptibility test, *E. coli* strains were tested for their susceptibility to nine antimicrobial agents using the disk diffusion method on Mueller Hinton agar. The inocula were standardize by adjusting the turbidity of the culture to match that of 0.5 MacFarland standard from which 0.1ml of the cultures were plated on Mueller Hinton agar plates , and spread evenly using a sterile spreader. The following antibiotic discs; Ampicillin 10µg, cephalothin 30µg, Cefpodoxime 10µg, Ceftriaxone 30µg, Ciprofloxacin 5µg, trimethoprim-sulfamethoxazole 25µg, tetracycline 30µg, amikacin 30µg and amoxicillin/clavulanic acid 25µg (Oxoid Ltd., Basingstoke, Hampshire, England) were placed gently and allowed to stand for 5minutes to diffuse[15]. Results obtained from this test were used in the screening for ESBL production. Organisms with diameters of zones of inhibition of <22mm and <25mm for cefpodoxime and ceftriaxone respectively were regarded as potential ESBLs producers. Phenotypic confirmatory test was performed using amoxicillin-clavulanic acid (augmentin). Enhanced diameter of zone of inhibition with any of these agents in presence of amoxicillin-clavulanic acid confirms ESBLs production. Multidrug resistance (MDR) was taken as resistance to four or more of the antibiotics tested[16].

II. RESULTS

Characterization of the isolates as identified by Microgen Gram negative identification kit is shown in Table 1. Only the utilization of ornithine was found to vary among the different *E. coli* strains. Fig. 1 shows the percentage resistance of the isolates to the antimicrobial agents tested. Highest resistance was found in cephalothin, followed by ampicillin with 85% and 75% resistance respectively. No *E.coli* strain was resistant to ciprofloxacin and amikacin.

Table 1: Identification of *E.coli* using Microgen GN A Identification kit

Code	Lys	Orn	H_2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Octal Code	Identi-fication
Sye1	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E.coli*
Sye2	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E.coli*
Sye3	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E.coli*
Sye4	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E.coli*
Sye5	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E.coli*

Key**:** Lys-Lysine; Orn-Ornithine; H_2S-Hydrogen sulphide; Glu-Glucose; Man-Mannitol; Xyl-Xylose; ONPG-Ortho-nitrophenol- galactosidase; Ind-Indole; Ure-Urease; VP-Voges Proskauer; Cit-Citrate; TDA-Tryptophan deaminase acid
Sye- *E.coli* isolated from 'suya'
+ Positive;
- Negative.

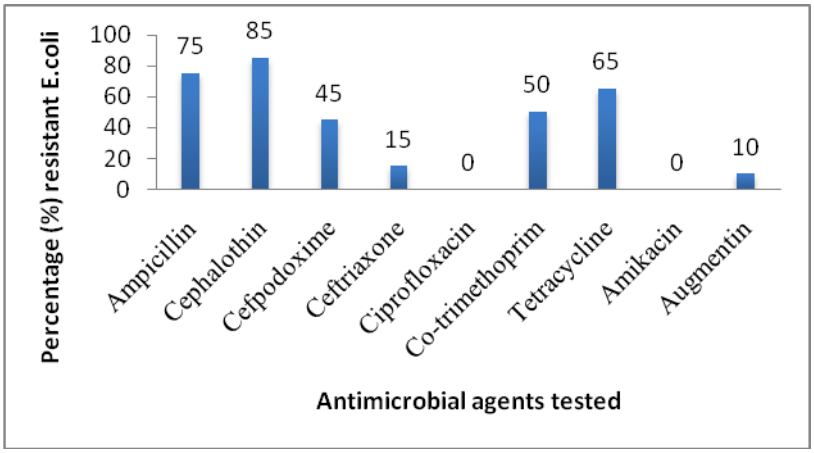

Fig 1: Percentage resistant *E.coli* against the antimicrobial agents tested

Out of forty isolates of *E.coli* screened for ESBL expression, 18(45%) were positive and comfirmed for ESBL production (Fig. 2). Table 2 shows the resistance phenotypes of the organisms. *E.coli* with multiple antibiotic resistance combinations of 4, 5, 6, and 7 were regarded as the multidrug resistance strains. Twenty two of the entire isolates (55%) exhibited multidrug resistance. All the multidrug strains were resistant to AMP, KF and TE. Highest frequency was found in the antibiotic resistance combinations of 4 and 5 with 8(20%) and 10 (25%) of the organisms respectively. MDR *E.coli* strains in this category also had the highest frequency of phenotypic patterns of AMP, KF, SXT, TE and AMP, KF, CPD, SXT, TE. Sixteen (16) MDR-*E.coli* strains were ESBLs producers i.e. 73% of the multidrug resistance strains while 16 out of the 18 ESBLs producers exhibited multidrug resistance (i.e. 89% of the ESBLs producers). Pearson correlation showed significant correlation ($P< 0.05$) between ESBLs production and multidrug resistance in *E. coli* isolated from 'suya'.

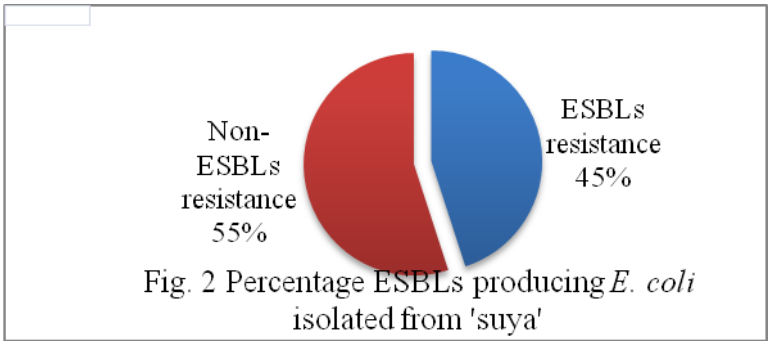

Fig. 2 Percentage ESBLs producing *E. coli* isolated from 'suya'

Table 2: Resistance phenotypes of *E. coli* isolated from 'suya'

Single antibiotic resistance		**Multiple antibiotic resistance**		
Number of isolates (%) in the Category	Resistance Phenotype	Number of antibiotic combinations	Number of isolates (%) with the pattern	Resistance Phenotype
4(10)	KF	2	2 (5)	SXT, TE
			2 (5)	AMP, KF
		3	2 (5)	AMP, KF, CPD
			2 (5)	AMP, KF, SXT
			2 (5)	AMP, KF, TE
		*4	6 (15)	AMP, KF, SXT, TE
			2 (5)	AMP, KF, CPD, TE
		*5	4 (10)	AMP, KF, CPD, CRO, TE
			6 (15)	AMP, KF, CPD, SXT, TE
		*6	2 (5)	AMP, KF, CRO, SXT, TE, AMC
		*7	2 (5)	AMP, KF, CPD, CRO, SXT, TE, AMC

Key: AMP-Ampicillin; KF- Cephalothin; CPD- Cefpodoxime; CRO-Ceftriaxone; CIP- Ciprofloxacin; SXT-Sulphamethoxazole-trimethoprim (Co-trimethoprim); TE-Tetracycline; AK-Amikacin; AMC- Amoxycillin-clavulanic acid (Augmentin); *Multidrug resistance

Fig.3 indicates percentage multidrug resistance strains, that is, those with resistant antibiotic combinations of 4 and above. The non-multidrug resistant *E.coli* strains are those with resistant antibiotic combinations of less than four while the susceptible ones are the *E.coli* strains sensitive to all the antibiotics. Four (10%) organisms were susceptible to all the antibiotics and constitute the population with multiple antibiotic resistance index of 0.0 (Fig. 4). A high antibiotic resistance was observed in these organisms which had 80% isolates with multiple antibiotic resistance (MAR) index of 0.2 and above.

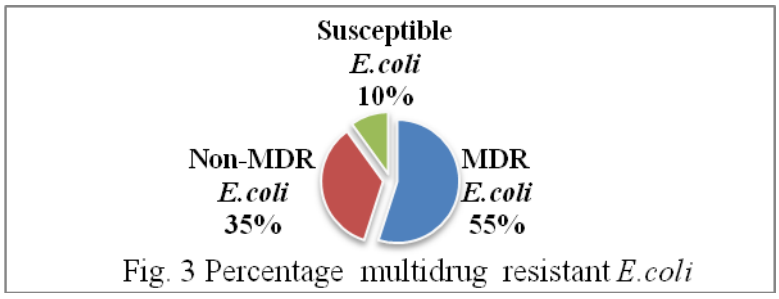

Fig. 3 Percentage multidrug resistant *E.coli*

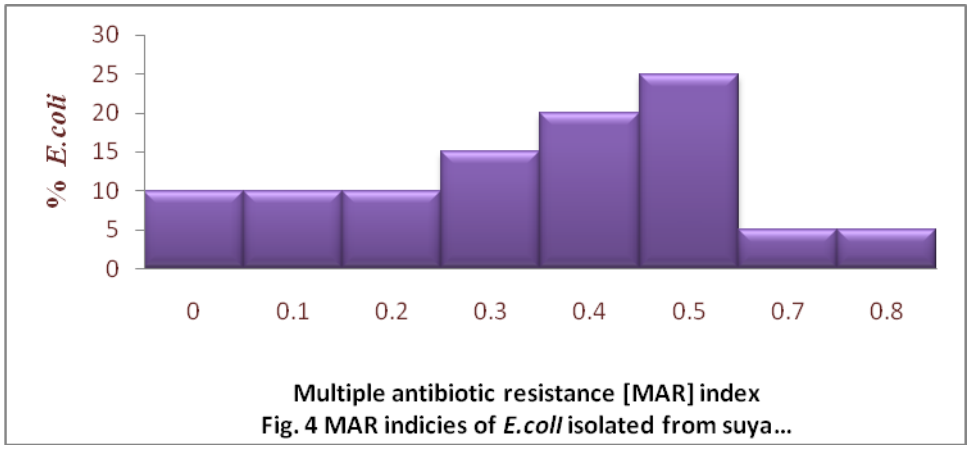

Fig. 4 MAR indicies of *E.coli* isolated from suya…

III. DISCUSSION AND CONCLUSION

The incidence of *E.coli* in 'suya' may have occurred as a post processing contaminant due to the fact that 'suya' is prepared and sold in the open along the streets. Since meat offers a rich medium for bacterial growth, the presence of *E.coli* is inevitable[17]. The dissemination of *E.coli* in food production units may equally occur via faecal cross-contamination between groups of animals (or individuals), and the contamination of food derived from animals may occur during processing in the abattoir[18]. Antimicrobial-resistant bacteria, including *Escherichia coli* are frequently isolated from the commensal gut flora of food animals[19]. Antimicrobial resistant bacteria have been identified along production path of 'suya'. It has been confirmed that on-farm and slaughter cattle are important sources of antimicrobial resistant *E.coli* transmissible to humans through beef[20]. This could be as a result of large amount of antimicrobial drugs used in food production. The use of antibiotics in treatments or as food supplements for farm animals leaves behind drug-resistant microbes in milk, eggs and meat that could encourage the development of resistant traits and transfer to other bacteria, making consumers more vulnerable to the resistant varieties[21].

The study showed 100% susceptibility to ciprofloxacin and amikacin. Several studies have established that susceptibility to ciprofloxacin or other fluoroquinolones are quite high among ESBL producing *Enterobacteriaceae*[22]. The problems of Multi-drug resistance (MDR) have been the driving force for the development of newer quinolones[23]. This agrees with earlier studies which stated that treating infection caused by ESBL with cephalosporins often does not yield good therapeutic result and suggested that fluoroquinolones and aminoglycosides could be alternative choices[24].Multiple antibiotic resistance (MAR) index is a measure of the extent of antimicrobial agent resistance for the isolates in the group studied. It is calculated as $^{a}/_{b}$ where "a" represents the number of antibiotics to which the isolates was resistant and "b" represents the total number of antibiotics to which the isolate was exposed[25]. MAR index gives an indirect suggestion of the probable source of the organism[26]. MAR index values greater than 0.2 indicates that the isolates were recovered from samples originating from high-risk sources[25]. Most probably, there are no strict rules concerning antibiotic prescriptions and usage in such areas.

All the multidrug resistance strains had 100% resistance to ampicillin, cephalothin and tetracycline. Ampicillin is a commonly used broad-spectrum aminopenicillin, which inhibits the final stage of bacterial cellwall synthesis and ultimately leads to cell lysis. Its usefulness is however, limited by its susceptibility to β-lactamase hydrolysis produced by the organism (Jain *et al*., 2011). Up to 90% of ampicillin resistance in *E.coli* is due to the production of TEM-1 β-lactamase[9]. This enzyme is able to hydrolyze penicillins and early cephalosporins such as cephalothin and cephaloridine. TEM-type β-lactamases are most often found in *E. coli* and *K. pneumonia*, also in other species of Gram-negative bacteria with increasing frequency[28]. Tetracycline resistance is already emerging in clinical isolates in our community[22]. Tetracyclines belong to a family of broad-spectrum antibiotics that include tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline, and a number of other semisynthetic derivatives. These antibiotics inhibit protein synthesis in Gram-positive and Gram-negative bacteria. Since their introduction in late 1950s, they have been widely used in clinical and veterinary medicine, as well as for prophylaxis and growth promotion in food animals. Resistance of this class of antibiotics is widespread because of the possible misuse and overuse of these drugs, limiting their utility in treating infections[29].In conclusion, this study revealed the presence of extended spectrum and multi-drug resistance *E.coli* strains in the widely acceptable and popularly consumed ready-to-eat processed meat 'suya'. Measures should be taken to increase the hygienic condition in the preparation of 'suya'. Public enlightment campaign teams should be set up to educate people on the measures which antibiotic should be taken and the effect of adding antibiotics to animal feed as growth promoters should be looked into.

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