Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.9, 2016

Multidrug Resistance and Multiple Antibiotic Resistance Index of Escherichia coli Strains Isolated from Retailed Smoked Fish

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Abstract
E. coli isolated from retailed smoked fish were subjected to antibiotic sensitivity testing using nine (9) antibiotics belonging to six different mode of action namely β-lactams, fluoroquinolone, aminoglycosides, sulphonamide, tetracycline and β-lactam-β-lactamase. Based on the resistance pattern of the isolates, the Multiple Antibiotic Resistance (MAR) index for each isolate was calculated. The results of the study revealed that 16% of the isolates exhibited multidrug resistance (MDR) and 53% of the isolates had MAR Index of 0.2 and above. Multidrug resistance (MDR) was taken as resistant to four or more antibiotics tested. This is suggestive of the origin of the isolates to be from area of high antibiotic usage.
Keywords: Aminoglycosides, β-lactams, β-lactam-β-lactamase, Fluorouinolones, MAR index

INTRODUCTION

Antimicrobial resistance is currently the greatest challenge worldwide. It decreases the effectiveness of drugs that decrease morbidity and mortality associated with serious and life-threatening infections and thus, compromising human health. Antimicrobials are not only fundamental for the treatment of infections in humans and animals, but are also essential substances in agriculture and animal husbandry, where they are applied at subtherapeutic levels as growth promoters in bird, swine, beef, and fish food **(**Moreira *et al*., 2004).

Food contamination with antimicrobial resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human clinical significance. Since the last decade, the prevalence of antimicrobial resistance among food borne pathogens has increased, possibly as a result of selection pressure created by the use of antimicrobials in animal health (Nyenje *et al*., 2012). *E. coli* is a classic example of enteric bacteria causing gastroenteritis. *E. coli* including other coliforms and bacteria such as *Staphylococcus* spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. Such organisms should not be present on fresh-caught fish. The contamination of food of fish origin with pathogenic *E. coli* probably occurs during handling of fish and during the production process (Novotny *et al*., 2004).

Multidrug resistance (MDR) is a common problem that hurdles chemotherapy. To overcome this problem, it is obligatory to identify the multidrug resistance pattern of an isolate. An attempt was done in this study to isolate a few *E.coli* strains from retailed smoked fish, to determine their MDR pattern and MAR Index by subjecting the isolates to antibiotic study.

METHODS AND MATERIALS

Identification and characterisation of E. coli

A hundred and fifty samples of retailed smoked fish were purchased from sellers in Samaru-Zaria, Nigeria. Identification and characterisation were carried out using conventional methods and confirmed using Microgen Gram negative Identification kit (Adenaike *et al*., 2013).

Antibiotic Susceptibility Testing

Susceptibility of *E.coli* strains to nine antibiotics was determined using the disc- diffusion method as recommended by Clinical Laboratory Institute Standards (CLSI, 2008). The bacterial isolates were grown for 18h on nutrient agar. They were suspended in 2 ml sterile normal saline and turbidity adjusted to match McFarland Opacity Standard No0.5 (equivalent to 1.5 x 10^8 bacterial density). Bacterial suspensions of 0.1 ml were dispensed on the surface of sterile Mueller-Hinton agar plate and spread evenly using a sterile spreader. This was allowed to dry for 5 min and antibiotic discs were dispensed on the surface of the media and incubated aerobically at 37^{o}C for 18 h. The susceptibility patterns of the isolates to the different antibiotics were noted as Sensitive (S) or Resistant (R) as per CLSI standards (CLSI, 2008). Intermediate-resistance was taken as full blown resistance. A standard strain *E.coli* ATCC 25922, obtained from National Institute for Pharmaceutical Research, Idu, Abuja. Nigeria was used as quality control. The following antimicrobial agents (single discs, Oxoid Ltd., Basingstoke, Hamphire, England) were tested: Ampicillin (10µg), Cephalothin (30µg), Cefpodoxime (10µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Trimethoprim/Sulphamethoxazole (25µg), Tetracycline (30µg) Amikacin (30µg) and amoxicillin-clavulanic acid (25µg) [CLIS, 2008].

Identification of Multidrug Resistance (MDR) Strains

The number of antibiotic each bacterium was resistant to in the disc diffusion test was noted for identification of

multidrug resistant strains. Multidrug resistance (MDR) was taken as resistant to four or more antibiotics tested (Ezekiel *et al*., 2011).

Calculation of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistance (MAR) index was calculated as $^{a}/_{b}$ where a represents the number of antibiotics to which the isolates were resistant and 'b' represents the total number of antibiotics to which the isolate was exposed (Apun *et al*., 2008).

RESULTS AND DISCUSSION

The pattern of resistance is shown in Table 1. Ten resistance phenotypes were obtained; two single antibiotic resistance types (KF and AMP resistance) and eight multiple resistance types with varying combinations of 2, 3, 4 and 6 antibiotics.

Table 1: Resistance pattern of *E. coli* isolated from smoked fish (n=38)

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 (11)	KF	**2**	2 (5)	KF, TE
2 (5)	AMP		2 (5)	AMP, KF
			2 (5)	CPD, TE
		3	2 (5)	KF, CPD, CRO
			4 (11)	AMP, KF, TE
			2 (5)	CPD, CRO, TE
		***4**	2 (5)	AMP, KF, SXT, TE
		***6**	4 (11)	AMP, KF, CPD, CRO, SXT, TE

Key: AMP-Ampicillin; KF- Cephalothin; CPD- Cefpodoxime; CRO-Ceftriaxone; CIP- Ciprofloxacin; SXT-Sulphamethoxazole-trimethoprim (Co-trimethoprim); TE-Tetracycline; AK-Amikacin; * Multidrug resistant strains

Six (16%) of the E. coli strains had resistance to four or more antibiotics and are regarded as multidrug resistant strains. Multiple drug resistance has become a common feature of many microorganisms especially the human pathogens. Data obtained in this study however is much lower than those of Subramani and Vignesh, (2012) of which 50% of the isolates tested in their study exhibited multidrug resistant character, suggesting the existence of greater frequency of MDR strains in the study area.

There was no resistance to three antibiotics i.e. ciprofloxacin, amikacin and amoxicillin-clavulanic acid. This agrees with the work of Karczmarczyk et al. (2011), where the MDR E.coli obtained from environmental samples showed 0% resistance to both amikacin and ciprofloxacin, also 3% resistance to amoxicillin-clavullanic acid. High susceptibility levels to the newer, more valuable antimicrobial compounds, such as fluoroquinolones, β-lactam-β-lactamase inhibitor, as well as aminoglycosides could yield good therapeutic results in treating infections caused by MDR E.coli and probably Enterobacteriaceae in general.

Results of several studies have revealed bacterial resistance to ampicillin, cephalothin and tetracycline at times co-trimethoprim [Karczmarczyk et al. (2011), Sharma and Rai, (2012) and Adenaike et al. (2013)]. This is because these antimicrobial agents are inexpensive and affordable by the common Man. The inexpensive drugs in developing countries are widely available without prescription from authorized health institutions and pharmacies, as well as from unauthorized patent medicine shops and other distributors (Sharma and Rai, 2012).

12 (32%) of the *E.coli* were susceptible to all the antibiotics tested and are referred to as the susceptible *E.coli* (Fig. 1).

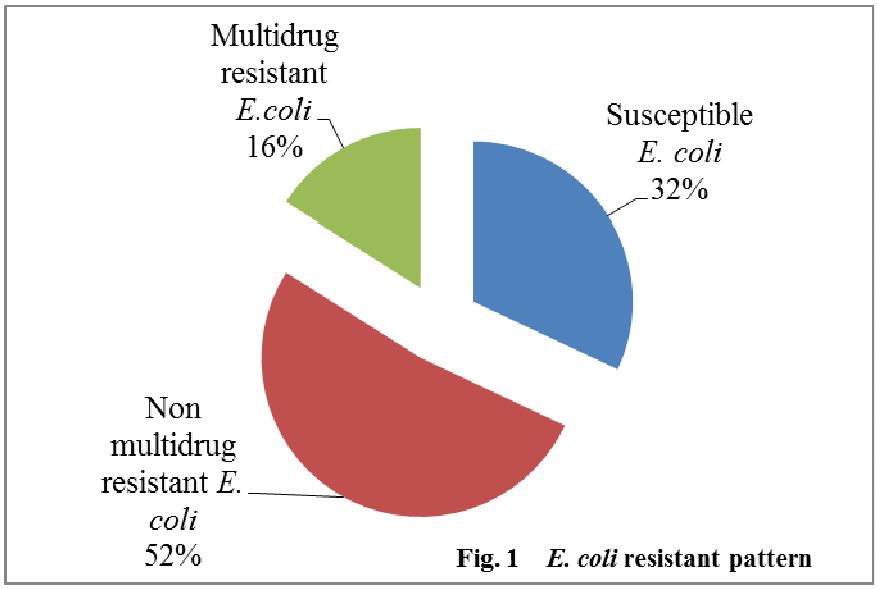

Fig. 1 *E. coli* resistant pattern

These susceptible *E.coli* also constituted the population with MAR index 0.0 (Fig. 2). A larger portion (52%) of the bacteria were regarded as non-multidrug strains. These were resistant to less than four antibiotics. Multiple antibiotic resistance (MAR) index is a measure of the extent of antimicrobial agent resistance for the isolates in the group studied. Multiple antibiotic resistance index is illustrated in Fig. 2.

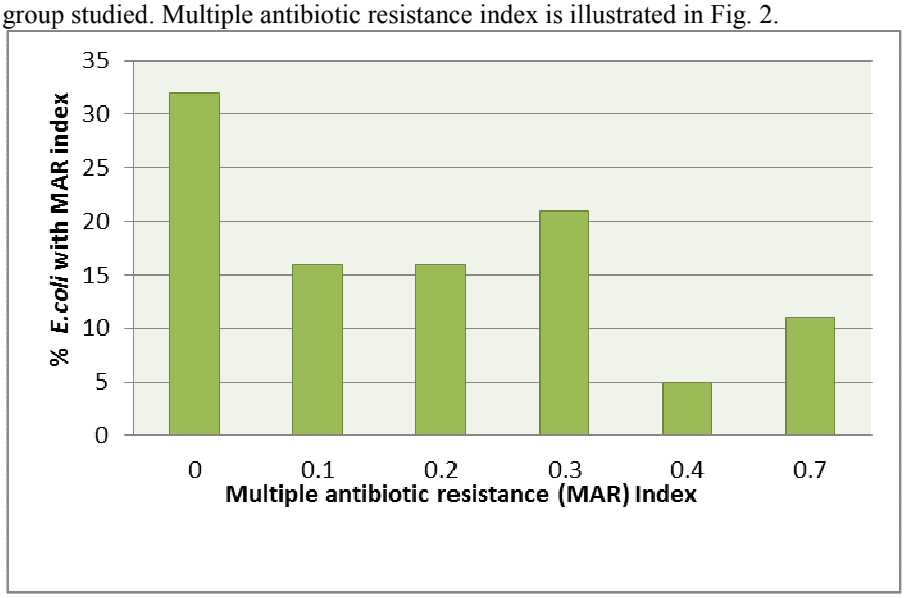

Fig. 2 Multiple antibiotic resistance indices of *E. coli*
(53% of *E. coli* had MAR index of 0.2 and above)

According to Mishra *et al*. (2013), MAR index of 0.2 or higher indicates high risk sources of contamination, MAR index of 0.4 or higher is associated with human faecal source of contamination. Thenmozhi *et al*. (2014), also states that MAR index values > 0.2 indicate existence of isolate from high – risk contaminated source with frequency use of antibiotics while values ≤ 0.2 show bacteria from source with less antibiotics usage. High MAR indices mandate vigilant surveillance and remedial measures. In this study, 53% of *E. coli* having MAR index of 0.2 and above is worrisome. Sensitivity patterns and treatment must be guided by laboratory investigations.

Conclusion

Ingestion of antibiotics is known to provide selective pressure ultimately leading to a higher prevalence of resistant bacteria. Therefore, 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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