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Original article

Antibiotic resistance: Detection of extended-spectrum beta-lactamase in Enterobacteriaceae from garden eggs

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ABSTRACT

Background: The emphasis on sustainable good health through the consumption of a healthy diet has necessitated the consumption of fresh vegetables, which could harbour the presence of members of the Enterobacteriaceae, antibiotic resistance and extended-spectrum beta-lactamase (ESBL). As a result, this study investigated the presence of antibiotics resistance (AR) and ESBL in Enterobacteriaceae isolated from garden eggs. **Methodology:** One hundred (100) garden egg samples were randomly purchased from 10 different vendors into sterile bags. Samples were serially diluted and cultured on MacConkey agar for the isolation of Enterobacteriaceae, then characterised and identified. Antibiotic susceptibility test was carried out on isolates following the Kirby-Bauer disc diffusion method. Double disc synergy test (DDST) was used to detect ESBL production. **Result:** Forty-three isolates were identified to belong to the family Enterobacteriaceae with *Klebsiella* spp. being the most dominant specie (51.16%), *Escherichia coli* (30.23%), *Salmonella* (11.23%) and *Enterobacter aerogenes* (6.98%). Of all the isolates, (65.12%) were multi-drug resistant (MDR). The isolates showed highest frequency of resistance to erythromycin (90.7%), gentamicin (34.9%), sulfamethoxazole/trimethoprim (32.6%), ofloxacin (30.2%), ciprofloxacin (25.6%), imipenem (14%), ceftriaxone (11.6%) and nalidixic acid (0.0%). For ESBL production, 23(53.49%) were positive. The ESBL positive isolates (n=23) were *Klebsiella* spp. 14(60.87%) and *Escherichia coli* 9 (39.13%). No ESBL production was detected in *Salmonella* spp. and *Enterobacter aerogenes* isolates. **Conclusion:** This study detected the presence of AR and ESBL in Enterobacteriaceae from garden eggs. Consumption of garden eggs contaminated with these bacteria pose a potential problem of infection and spread of resistance in the environment through food.

Introduction

Fresh vegetables being sources of minerals, vitamins and fibre are often eaten raw and form an important part of a healthy diet for humans as a result of the health benefits they provide [1,2]. One of such vegetables widely consumed amongst the rural and urban population in Nigeria is the garden egg (*Solanum melongena*) also known as African eggplant [3,4]. It is a warm-season crop often cultivated in the North central region of Nigeria and is consumed raw as it supplies the body with fibre, vitamins and minerals [5]. In recent years, there has been growing emphasis on the consumption of fruits

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and vegetables; which has possibly led to an increase in microbial infections associated with fruits and vegetables consumption [6]. This is not surprising as fresh vegetables could act transmitters of antibiotic-resistant bacteria to humans [7]. In many cities in Nigeria, fresh vegetables, especially ready-to-eat vegetables are often displayed on road sides, purchased and consumed without to washing, making them microbiologically unsafe for consumption.

Extended-spectrum beta lactamases (ESBLs) are a rapidly evolving group of beta-lactamases which share the ability to hydrolyse third-generation cephalosporins and aztreonam but are inhibited by clavulanic acids. They are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that make treatment options for infections difficult [8]. Extended-spectrum beta lactamases producing bacteria is no longer confined to the health care system but represents a growing challenge of food safety. Extended-spectrum beta lactamases production is a very important mechanism of antibacterial resistance in Enterobacteriaceae [9]. The application of manure to soil and irrigation of vegetables with untreated wastewater during vegetables production could be a potential source of ESBL-producing bacteria in fresh vegetables [10]. Previous studies have reported ESBL-producing bacteria in vegetables, although with varying results of the presence and absence of ESBL-producing bacteria [7,11-13]. However, paucity of information on ESBL-producing bacteria in garden eggs exists. The sustainable development goal on the demand for good health makes it imperative to investigate the presence of ESBL-producing Enterobacteriaceae from garden eggs. Hence, this study focused on detecting antibiotic resistance and the presence of extended-spectrum beta-lactamase in Enterobacteriaceae isolated from garden eggs sold within Lokoja metropolis, Kogi State, North-Central, Nigeria.

Methods

Sample collection

The study was carried out in Lokoja, Kogi State, North-Central Nigeria. One hundred (100) garden eggs were randomly purchased from 10 vendors within the state capital metropolis into separate sterile bags. Samples were stored under ice and transported to the Laboratory of the Department of Biological Sciences, Federal University Lokoja, Kogi State for microbiological analyses.

Isolation and characterisation of bacterial isolates

Approximately 50g of garden eggs sample from each vendor was placed in 450mL of sterile Peptone water (Oxoid Ltd., Basingstoke, Hampshire, UK) and shaken vigorously following aseptic techniques. Ten-fold serial dilution was made with sterile peptone water and 1mL of each homogenate was plated following the pour plate technique on MacConkey agar (Oxoid Ltd., Basingstoke, Hampshire, UK) plates, incubated at 35^0C for 24 hours.

Bacterial isolates obtained from agar plates incubated for 24 hours after serial dilution were sub-cultured repeatedly to obtain pure cultures. The cultures were stored in glycerol broth for further identification by cultural characteristics, morphology and biochemical tests [14] following the Bergey's Manual of Determinative Bacteriology [15]. API 20E test kit (bioMerieux, Hazelwood, MO, US) was also used for the confirmation of the biochemical identification of bacterial isolates.

Antibiotic susceptibility testing

The isolates were sub-cultured onto MacConkey agar to ensure that the isolates were discrete and pure. The antibiotic susceptibility testing was performed by the Kirby-Bauer disc diffusion method as modified by the Clinical and Laboratory Standards Institute [16]. Suspension of each bacteria colony was made by emulsifying a loop-full of the confluent growth of the pure culture of the isolates in 5 millilitres (mL) of sterile distilled water and standardized to 0.5 McFarland Standard. This standard contains approximately 10^7 CFU/mL. A sterile cotton wool swab was inserted into each test-tube containing the standardized inoculums suspension. The swab was rotated several times with firm pressure on the inside wall of the test-tube to remove excess fluid and then used to inoculate the surface of a dry Mueller Hinton agar (MHA) plate.

Eight antibiotic discs were used: ofloxacin (OFX) (5µg), gentamicin (CN) (10µg), ceftriaxone (CRO) (30µg), erythromycin (E) (15µg), sulfamethoxazole /trimethoprim (SXT) (25µg), imipenem (IMP) (10µg), ciprofloxacin (CIP) (5µg), nalidixic Acid (NA) (30µg). All plates were incubated at 37^0C for 24 hours. The diameters of zones of inhibition were measured to the nearest millimeter using a metre rule. Control strain *K. pneumoniae* ATCC 700603

was used in the testing to validate the results of the antibiotic discs.

Phenotypic detection of ESBLs

The double disc synergy test (DDST) as described by [17] was used for the detection of ESBLs. The test was performed on Mueller-Hinton agar (Oxoid Ltd., Hampshire, England) inoculated with a bacterial suspension of 0.5 Mc Farland's turbidity. Synergy was determined between a 20/10µg amoxicillin-clavulanate (Oxoid Ltd., Hampshire, England), 30µg ceftazidime and ceftriaxone (Oxoid Ltd., Hampshire, England) at a distance of 15mm apart from the edge of the amoxicillin/clavulanate disc. The cefoxitin (Oxoid Ltd., Hampshire, England) was placed in any available space on the plate. Extension of the edge of the inhibition zone by >5mm towards the disc of amoxicillin/clavulanate, together with susceptibility to cefoxitin was interpreted as positive for ESBL production [16].

Statistical analysis

The diameters of the zones of inhibition (mm) of the organism to the antibiotics tested were interpreted as signifying susceptibility, intermediate or resistant according to the approved [16] susceptibility zone diameter interpretative standard. Descriptive statistics was carried out using the R v4.1.3(R Studio).

Results

Isolation and characterisation of bacterial isolates

Forty-three bacteria belonging to the family Enterobacteriaceae were isolated from the garden eggs. *Klebsiella* spp. was most dominant with an occurrence of 51.16% (n=22) while *Enterobacter* spp. was least dominant with an occurrence of 6.98% (n=3). The occurrence of the isolates is shown in **table (1).**

Antibiotics susceptibility testing

Enterobacteriaceae isolates displayed their most frequent resistance (90.7%) against erythromycin (E). From the *Klebsiella* spp. isolates, the highest resistance (95.46%) was shown to erythromycin (E). *Klebsiella* spp. displayed no resistance to NA and imipenem (i) antibiotics. *Esherichia coli* isolates displayed their highest resistance (92.31%) to erythromycin (E). Multidrug resistance was observed in 65.11% (n=28) of all isolates and the occurrence of the isolates is depicted in **table (2)**. **Figure 1** shows the susceptibility pattern of bacteria isolates to the test antibiotics.

Phenotypic detection of ESBLs

Fifty-three percent (53%) of Enterobacteriaceae isolates were positive for the production of ESBLs. ESBL production was detected in 32.6% of *Klebsiella* spp. isolates while ESBL was detected in 20.9% of *Escherichia coli* isolates. *Salmonella* spp. and *Enterobacter* spp. isolates showed no production of ESBLs. The distribution of ESBL production in Enterobacteriaceae isolates is shown in **figure (2).**

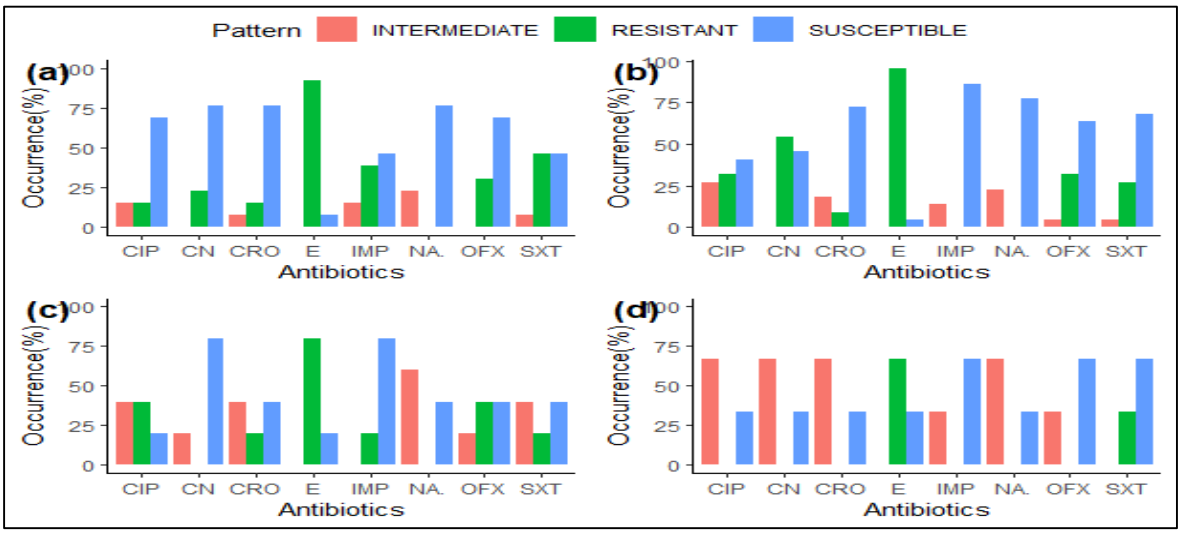
Table 1. Occurrence of bacterial isolates in garden egg samples.

S/NO	Isolates	Frequency (n)	% n
1	*Klebsiella* species	22	51.16
2	*Escherichia coli*	13	30.23
3	*Salmonella* species	5	11.63
4	*Enterobacter aerogenes*	3	6.98
	Total	**43**	**100**

Table 2. Occurrence of multidrug resistance against antimicrobial classes.

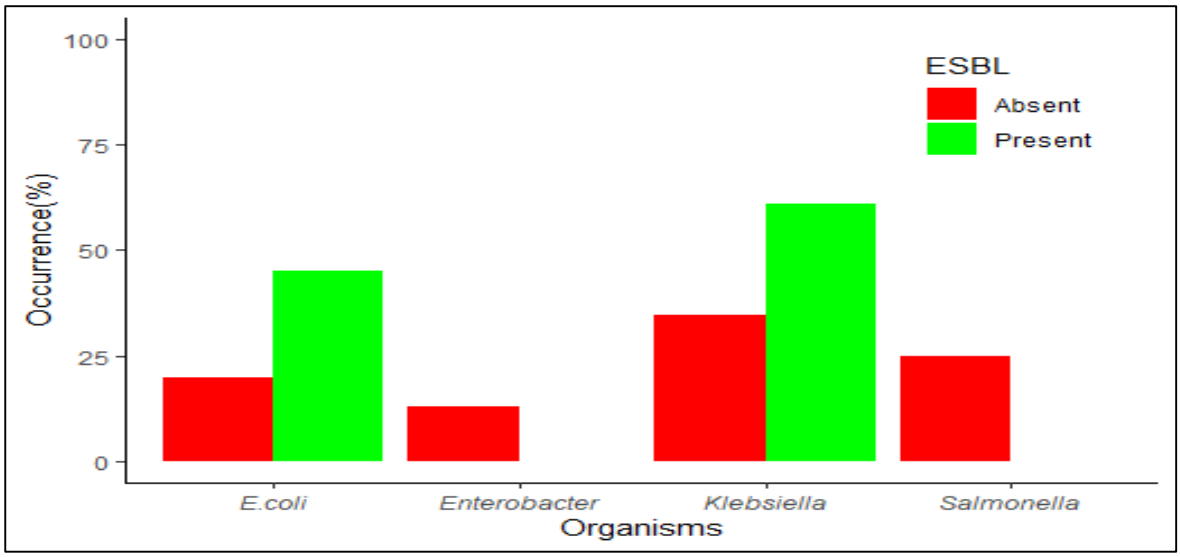
S/NO	Isolates	R0	R3	n (%) MDR
1	*Klebsiella* species	6	16	16 (72.7)
2	*Escherichia coli*	3	10	10(76.9)
3	*Salmonella* species	3	2	2(33.3)
4	*Enterobacter aerogenes*	3	0	0(0.0)
	Total	**15**	**28**	**28(65.12)**

Figure 1. Antibiotic susceptibility pattern of Enterobacteriaceae isolates.



(a) *Klebsiella* spp. (b) *Escherichia coli* (c) *Salmonella* spp. (d) *Enterobacter aerogenes*
CIP=Ciprofloxacin; CN=Gentamicin; CRO=Ceftriaxone; E=Erythromycin ; IMP=Imipenem; NA= nalidixic acid; OFX=Ofloxacin; SXT=Sulfamethoxazole/Trimethoprim.

Figure 2. Distribution of ESBL production in Enterobacteriaceae isolates.



Discussion

Numerous outbreaks of severe bacterial infections are often linked to the members of the Enterobacteriaceae. This study demonstrates the presence of Enterobacteriaceae in garden eggs which is in consonance with previous reports [1,18,19]. The dominance of *Klebsiella* spp. and *E. coli* in the Enterobacteriaceae isolated from the samples, indicate faecal contaminations from sources such as irrigation, application of manure by farmers and unhygienic handling by sellers. This finding is in line with the works of [1], who encountered *Escherichia coli* and *Klebsiella* spp. as the most dominant isolates in fresh fruits and vegetables originating from different countries of the world. The high occurrence of *Klebsiella* spp. among the Enterobacteriaceae isolates was divergent from the work of [19] who studied the prevalence of Enterobacteriaceae on fresh produce and food safety practices. Unlike our findings, their study reported *E. coli* as being the most dominant isolate. Furthermore, the occurrence of *Klebsiella* spp. reported in our study exceeds the reports of [20] that reviewed the contamination of fresh produce with antibiotic-resistant bacteria and reported a lower occurrence of *Klebsiella* spp. One possible factor that could be responsible for the higher occurrence of *Klebsiella* spp in our study is improper waste disposal into water bodies, which end up being used as irrigation or wash water for garden eggs and other fresh vegetables. This calls for caution in waste disposal as this could negatively affect spread of bacteria from water to humans. In addition, this is an indication of poor hygienic practices in the study area as the presence of *Klebsiella* spp. is associated with severe infections in humans [21,22]. The occurrence of *Salmonella* spp. was relatively low, however, its presence in fresh garden egg could have a negative impact on public health as garden eggs are often consumed raw and outbreaks involving *Salmonella* spp. have been reported globally [23,24]. *Enterobacter aerogenes* had an occurrence which is similar to work of [25] who reported 6.4% for the occurrence of *Enterobacter aerogenes.*

The susceptibility of the Enterobacteriaceae isolates to different classes of antibiotics reveals the presence of Multi drug resistant (MDR) Enterobacteriaceae in garden eggs. The increased exposure of bacteria to antibiotics in the environment could be responsible for increased MDR bacteria in garden eggs. **Bitew and Tsige** [26] also reported a high occurrence of multidrug resistant Enterobacteriaceae of 42.1%. The frequent resistance of Enterobacteriaceae against erythromycin as reported in our study is possibly from application of manure in farms as antibiotics are routinely used to treat infections in animals. Preliminary studies carried out on vegetable farms in our study area shows the wide use of manure to grow vegetables. Similarly, [25] in an experiment, reported a high frequency of resistance to erythromycin in retail poultry and beef. In addition, this study shows NA was highly effective against all Enterobacteriaceae isolates with no resistance which is in contrast with the findings of [27] who reported 72% resistance of isolates to NA. This difference is probably as a result of the sources of the samples and location. In like manner, Imipenem (IPM) was highly effective against *Klebsiella* spp. with *Klebsiella* spp. showing no resistance against imipenem (IPM). Carbapenems (Imipenem) are widely known to be an effective option for the treatment of infections caused by ESBL-producing Enterobacteriaceae [28,29].

The presence of ESBL producing Enterobacteriaceae in garden eggs in the study area confirms the earlier reports of fresh vegetables as carriers of resistance genes [7,11,30]. In contrast with the findings of [31], *Klebsiella* spp. accounted for a higher proportion of ESBL producing Enterobacteriaceae isolates than *E. coli.* which is an indicator of a growing ESBL producing Enterobacteriaceae not just in the clinical community, but in food and the environment.

Conclusion

The occurrence of ESBL producing bacteria in food especially fresh vegetables is a continuous threat to food safety and sustainable good health. This study detected the presence of antibiotics resistance and extended-spectrum beta-lactamase in Enterobacteriaceae isolated from garden eggs sold within Lokoja metropolis, North-Central Nigeria. The high frequency of occurrence of antibiotic resistance in *Klebsiella* spp. *and E. coli* isolated from garden eggs is of public health importance as it is an indication that antibiotic resistance in these bacteria are no longer restricted to the clinical environment but also in food especially ready-to-eat vegetable. It also demonstrates the potential ability of garden eggs in spreading antibiotics resistance to human and the environment at large. Consumption of such garden eggs could potentially transfer antibiotics

resistance genes and extended-spectrum beta lactamase genes to gut microorganisms causing serious infections, hence, making treatment options for such infections difficult. Regular antibiotic surveillance and hygienic practices are required to mitigate the spread of resistance in food and in the environment.

Disclosure of potential conflicts of interest

The authors report that there were no conflicts of interest.

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