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Antibiotic Resistance Profiles of Salmonella and Escherichia coli Isolates Obtained from Garden Eggs Sold in Abakaliki Metropolis: Insights into Susceptibility Patterns

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ABSTRACT

This investigation aimed to evaluate the antibiotic susceptibility profiles of Salmonella species and Escherichia coli isolated from 100 garden egg samples obtained from diverse vendors in Abakaliki. Samples were transported to the Department of Applied Microbiology Laboratory, Ebonyi State University, Abakaliki, for analysis. Organisms were isolated and identified using standard microbiological techniques, while their antibiotic susceptibility was assessed through the disc diffusion method. Results indicated a prevalence of 60% for Salmonella species and 80% for E. coli among the analyzed samples. The antibiotic susceptibility testing unveiled alarming resistance patterns: Salmonella species displayed complete resistance (100%) to meropenem, perfloxacin, colistin, aztreonam, neomycin, gentamycin, ciprofloxacin, chloramphenicol, and ofloxacin, with only 16.67% susceptibility to streptomycin. Similarly, E. coli isolates exhibited 87.5% susceptibility solely to ofloxacin and 12.5% to chloramphenicol, while showcasing absolute resistance (100%) to other antibiotics tested. This study underscores the role of garden egg sales and consumption in facilitating the widespread dissemination of multi-drug resistant bacterial pathogens within the region. It emphasizes the urgent need for stringent adherence to hygienic practices among garden egg and other fruit vendors as a crucial preventive measure to curb this escalating threat.

Keywords: Garden eggs, Salmonella species, E. coli, antibiotic resistance.

INTRODUCTION

Garden egg (*Solanum melongena*) also called African eggplant is one of the fruits widely consumed both in the rural and urban areas in Nigeria [1-4]. Foods enriched with fresh fruits and vegetables, including garden egg, are vital to human health due to their nutritional values and ability to reduce risk of illnesses [5-6]. Fruits and vegetables are rich source of vitamins and minerals required by the human body for nourishment, growth and metabolism [7-8]. Fruits and vegetables are products of agriculture which are readily obtained from different markets across the country. Most are eaten raw with little or no processing while some are processed by slicing [9-10]. Fruit

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vending is very common probably due to modern lifestyle, industrialization, economic downturn and lack of time to prepare proper meal [11-13]. Thus, there is little information on the incidence of food borne diseases as related to street vending fruits [14-16]. Unrelated factors such as resident micro-flora in the soil, nonresident micro-flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers have resulted in differences in microbial profiles of various fruits and vegetables [17-19]. For instance, in developing countries such as Nigeria, continued use of untreated waste-water for irrigation farming and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contaminations [20-25]. In spite of the health and nutritional values of fruits and vegetables, association of human infections with the consumption of fresh or minimally processed ones have increased in recent years [26-28]. According to [29-32] enteric pathogens such as *Escherichia coli* and *Salmonella* are among the greatest concerns during food-related outbreaks. Several cases of typhoid fever outbreak have been associated with eating contaminated vegetables grown in or fertilized with contaminated soil or sewage [33-34]. Bacteria such as *Salmonella* species, *Shigella* species, *Listeria monocytogens*, *Campylobacter* species, *Aeromonas* species, *Escherichia coli*, S*taphylococcus aureus*, and *Pseudomonas* species have also been associated with contaminated sliced fruits [35-36]. Increase in the occurrence of antimicrobial resistance bacteria in humans, animals and the environment is a major concern in both human and veterinary medicine [9] and Infectious Diseases Report has noted that drug resistant organisms are prevalent worldwide [11]. Antibiotic resistance contaminants contaminate vegetables and fruits through several sources. For instance, manure used in enhancing soil fertility harbours bacteria that can be carrying antibiotic resistant genes, which can be transferred horizontally to soil bacteria [20]. This in turn, can contaminate fruits and vegetables when grown. This study therefore examined the presence and prevalence of *E. coli* and *Salmonella* species and antimicrobial in garden eggs sold within Abakaliki metropolis and their antibiotic susceptibility profiles were investigated.

Plate 1: Photos of two varieties of garden eggs commonly consumed in Abakaliki

Sample Collection

The garden egg samples were purchased from different fruit selling points in Abakaliki International Market, accurately labeled, and transported in sterile nylon bags and were transported to the Department of Applied Microbiology Laboratory of Ebonyi State, University, Abakaliki for analysis.

Isolation, Characterization and Identification of Isolates

Each sample collected was aseptically swabbed with sterile swab stick moistened with sterile water. Each swab stick used on the sample was inoculated into a sterile test tube containing 5ml of nutrient broth. The tubes were incubated at 37°C for 18-24 hours. After incubation, the broth were agitated for proper mixing and a loopful of each was inoculated on sterile *Salmonella-shigella* agar (SS-agar), macConkey agar and eosin methylene blue agar plates by streaking method. The plates were incubated at 37°C for 18-24 hours. After the incubation, suspected colonies of *Salmonella* species on SS-agar and *E. coli* on macConkey and eosine methylene blue agar were subcultured on freshly prepared SS-agar, macConkey and eosine methylene blue agar to get the isolates in pure forms. The pure culture of each isolate was asceptically transferred into nutrient agar slants was the slants were incubated at 37°C for 18-24 hours. After incubation, the tubes were stored in the refrigerator for further identification.

Gram Staining Reaction

This was carried out using a clean grease free slide. Colonies of the test organisms were aseptically used to make thin smears on the glass slides using a sterile wire loop. . The smears were allowed to air dry. The smears were heat-fixed by passing it three time through the bunsen burner flame. There were first covered with crystal violet

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and allowed to stand for 30-60 sec. After that, the stains were washed with slowly running tap water. The slides were then covered again with lugols' iodine, allowed to stand again for 30-60 seconds and were washed off with slowly running tap water. The smears were rapidly decolorized with 70% alcohol until the color of the crstal violet stoped coming out. There were washed off immediately with clean slowly running tap water. The stained slides were counter stained with safranin for 30sec and washed off with clean tap water. The under surface was blotted and the slides were air-dried. . A drop of immersion oil was dropped on the stained slide and examined under X40 and X100 objective lens.

Biochemical Test

Conventional biochemical tests such as catalase test, oxidase test, indole, methyl red, Voges–Proskauer test, citrate utilization, and coagulase and sugar fermentation tests were carried out on the bacterial isolates for further identification [3].

Antibiotic Susceptibility Testing

The susceptibility and resistance profile of the isolates to commonly used antibiotics was determined using Kirby-Bauer disk-diffusion technique. Young cultures (24 hours old) of the test organisms were standardized using Mac Farland equivalents and were inoculated on the surface of aseptically prepared Mueller-Hinton agar plates using sterile swab sticks. Commercially available antibiotics discs including: gentamycin (CN) 5µg, ciprofloxacin (CIP) 30µg, ofloxacin (OFX) 10µg, chloramphenicol (C) 15 µg, colistin (CL) 5µg, nitrofurantoin (F) 30µg, chloramphenicol (C) (10µg), nitrofurantoin (N) 10 µg, sulphamethoxazole trimethprim (SXT) 25 µg and azithromycin (AZ) 10µg, (Oxoid, UK), were aseptically placed onto the surfaces of the inoculated plates using a sterile forceps. The plates were subsequently allowed to stand for 10-15 min to enable the inoculated organisms to pre-diffuse. The plates were incubated at 37 °C for 18-24 hrs and their clear zones of inhibition were measured to the nearest millimeters (mm) using a meter ruler. The interpretation of the measurement as sensitive or resistant were made according to the Clinical Laboratory Standard Institute interpretation guideline for antibiotics sensitivity [5].

RESULTS

Frequency of Occurrence of *Salmonella species* and *E. coli* in the Garden Eggs sold in Abakaliki metropolis

The results showed that a total of 140 organisms comprising of 60(42.86%) *Salmonella* species and 80(57.14%) *E. coli* were obtained from the 100 garden eggs analyzed.

Table 1: Frequency of isolation of *Salmonella* species and *E. coli* from Garden eggs sold within Abakaliki metropolis

Number of Samples analyzed	Number and % of *Salmonella* spp obtained	Number and % of *E. coli* obtained	Total number of isolates obtained
100	60(42.86)	80(57.14)	140

Antibiotic Susceptibility Pattern of *Salmonella* species and *E. coli* isolated from Garden Eggs sold in Abakaliki metropolis

The antibiotic susceptibility pattern of the organisms isolated from the garden egg samples obtained in Abakaliki metropolis showed that the *Salmonella* isolates were 100 % resistant to all the antibiotics evaluated (meropenem, perfloxacin, colistin, aztreonam, neomycin, gentamycin, ciprofloxacin, chloramphenicol and ofloxacin), except Streptomycin to which it recorded 83.3% resistance (Table 2). Similarly, the *E. coli* isolates recorded 100 % resistant to all the antibiotics except 2 (chloramphenicol and ofloxacin) to which it recorded 87.5% and 12.5%, respectively (Table 3).

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Table 2: Antibiogram of *Salmonella* species Isolated from Garden Egg Sold within Abakaliki Metropolis.

S/N	Antibiotics (µg)	Number tested	No. and % Susceptible	No. and % Resistance
1	Meropenem (20)	60	0(0.0)	60(100)
2	Perfloxacin (30)	60	0(0.0)	60(100)
3	Colistin (5)	60	0(0.0)	60(100)
4	Aztreonam (15)	60	0(0.0)	60(100)
5	Streptomycin (10)	60	10(16.67)	50(83.33)
6	Neomycin (10)	60	0(0.0)	60(100)
7	Gentamycin (15)	60	0(0.0)	60(100)
8	Ciprofloxacin (30)	60	0(0.0)	60(100)
9	Chloramphenicol (15)	60	0(0.0)	60(100)
10	Ofloxacin (20)	60	0(0.0)	60(100)

Table 3: Antibiogram of *E. coli* Isolated from Garden Eggs Sold within Abakaliki Metropolis

S/N	Antibiotics (µg)	Number tested	No. and % Susceptible	No. and % Resistance
1	Meropenem (20)	80	0(0.0)	80(100)
2	Perfloxacin (30)	80	0(0.0)	80(100)
3	Colistin (5)	80	0(0.0)	80(100)
4	Aztreonam (15)	80	0(0.0)	80(100)
5	Streptomycin (10)	80	0(0.0)	80(100)
6	Neomycin (10)	80	0(0.0)	80(100)
7	Gentamycin (15)	80	0(0.0)	80(100)
8	Ciprofloxacin (30)	80	0(0.0)	80(100)
9	Chloramphenicol (15)	80	10(12.5)	70(87.5)
10	Ofloxacin (20)	80	70(12.5)	10(12.5)

DISCUSSION

Much importance has been attached to the consumption of fruits and vegetables; which has perhaps led to an increase in microbial infections associated with fresh fruits and vegetables consumption [12]. Several occurrences of severe bacterial infections have often been linked to the members of the Enterobacteriaceae [13]. In this study, *E. coli* and *Salmonella* species were isolated from garden eggs sold in Abakaliki metropolis in Ebonyi State of Nigeria. The occurrence of these enteropathogens in fresh garden eggs which are eaten raw is of public health concern. Outbreak of diseases involving *Salmonella* spp. have been reported globally [13]. In particular, the presence of *E. coli* in ready-to-eat foods like garden egg is an indication of faecal contamination of such foods [14]. The presence of these organisms therefore indicates poor hygienic handling of the fruits in the study area. There are several handling steps that may expose the fruits to contamination by *E. coli* and *Salmonella* species – cultivation, harvesting, transporting, packaging, storage and selling to the final consumers [16]. The sources of faecal contamination to fruits and vegetables might be mostly from the irrigation water contaminated with faecal materials from abattoirs, pit latrine and human wastes [18]. Other sources of contamination could be the soil, improperly composted manure, wastewater, wild and domestic animal wastes human handling, harvesting equipment, transport vehicles, improper packaging and cross contamination [20,23]. The study revealed higher

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occurrence of *E. coli* (57.14%) than *Salmonella* species (42.86%) in the fruits. This agreed with earlier reports by [9] who reported higher prevalence of *E. coli* (30.23%) than *Salmonella* species (11.63%) in garden egg. It is also in accordance with the work of [5] who reported that *Escherichia coli* as one of the most dominant microorganisms in fresh fruits and vegetables. The susceptibility and resistance profile of the *Salmonella* species and *E. coli* obtained in this study showed that only 16.67% of *Salmonella* species were susceptible to only one (i.e. streptomycin) out of ten antibiotics tested. The isolates were totally (100%) resistant to all other antibiotics used. Similarly, the *E. coli* were significantly (87.5%) susceptible to only one of the ten antibiotics (ofloxacin). They were 100% resistant to every other antibiotic used except chloramphenicol to which they were only 12.5% susceptible. This shows that the organisms are multi-drug resistant. The occurrence of multidrug resistant enteropathogens in this widely consumed ready-to-eat fruit is a serious public health issue. [6] also reported a high occurrence (42.1%) of multidrug resistant Enterobacteriaceae in ready-to-eat fruits and vegetables. The multidrug resistant organisms may have been introduced during the application of animal manure in farms as antibiotics used to treat human infections are now routinely used to treat infections in animals [10]. This study has implicated ready-to-eat fruits and vegetables in the spread of multidrug resistant pathogens.

CONCLUSION

The report of high occurrence and level of multidrug resistance of *Salmonella* and *E. coli* isolated from garden eggs in the study area is of serious concern that needs urgent attention. It indicates that antibiotic resistance of bacteria are no longer limited to the clinical settings but also in foods especially fruits and vegetables. It also revealed the ability of raw garden eggs in spreading antibiotics resistance organisms to humans and the environment at large. Also, consumption of such garden eggs could potentially transfer antibiotics resistance genes to gut microorganisms causing serious infections, hence, making treatment options for such infections difficult. According to [8], high concentration of sodium chloride solution or chlorinated water remove most pathogens on the surfaces of fresh produce. It is therefore recommended that the outer surfaces of fresh fruits and vegetables should be properly washed with high concentration of sodium chloride or chlorinated water before consumption to help minimize the risks of food borne infections.

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