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Antibiotics Susceptibility Pattern of Bacteria Isolated from Ready-To-Eat Vegetables Sold in Mohammadu Gumi Market, Kaduna, Nigeria

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Freshly consumed vegetables are considered to have more nutritional value than cooked ones. However, they are a potential source of foodborne illnesses due to possible microbial contamination, this poses safety threat.
Objectives: This study was carried out to determine the bacteriological quality of ready-to-eat vegetables sold in Mohammadu Gumi market, Kaduna and to determine the antibiotics resistance pattern of the bacteria isolates.
Methods: A total of 40 samples of cabbage were collected. Gram staining, growth on selective media and biochemical tests were carried out to identify the isolates. Antimicrobial susceptibility testing was done using the Kirby-Bauer agar disc diffusion method. Methicillin resistant *Staphylococcus aureus* isolates were detected with the use of cefoxitin disc agar diffusion test.
Results: A total of 46 bacteria isolates were obtained with a total colony count range from 4 – 9 x 10^6CFU/ml. The following bacteria were isolated: *Staphyloccocus aureus* (41.3%), *Escherichia coli* (28.3%), *Salmonella* spp (19.6%), *Pseudomonas aeruginosa* (8.7%) and *Serratia* spp (2.2%). The result of antimicrobial susceptibility test showed that the isolates were highly susceptible to ofloxacin: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia* spp (100%), *E. coli* (92.3%) and *Salmonella* spp (87.5%). The isolates were all resistant to penicillin and ampicillin while 89.5% of the *Staphylococcus aureus* isolates were phenotypically methicillin resistant *Staphylococcus aureus.*
Conclusion: This study showed that the vegetables (cabbage) were highly contaminated with antibiotic resistant bacteria, this can be a source of infection to the consumers and a potential means of transmitting multidrug resistant bacteria strains in the community.
Keywords: Antibiotics; Cabbage; Multidrug resistance; Susceptibility

INTRODUCTION

Cabbage (*Brassica oleracea,* family Brassicaceae*)*, is an example of ready to eat vegetable commonly available in the market or hawked by traders. Cabbage has a round shape and is composed of superimposed leaf layers. It is a member of the food family traditionally known as cruciferous vegetables and is very closely related to kale, broccoli, collards and Brussels sprouts.

Cabbage has high nutritional value. It has been found to be very helpful in cancer prevention due to its antioxidant, anti-inflammatory richness, and richness in glucosinolates. Polyphenols rank at the top of the list of phytonutrient antioxidants in cabbage (Liu, 2013; Slavin and Lloyd, 2012). The following anti-inflammatory compounds are found in cabbage: artemetin, betanidin, butein, equol, hydroxyflavone, kaempferol, luteolin, malvidin, naringenin, pelargonodin, purpurogalin, quercitol, and tetrahydrochalcone. Cabbage is an excellent source of vitamin C and a very good source of manganese. It is highly consumed and generally acceptable because of its low economical cost. It is highly rich in low calorie fibers which aids in improving food digestion.

Due to increase in the awareness of the benefits of fresh vegetables, a lot of people now consume vegetables freshly. For the prevention of chronic diseases like heart disease, cancer, diabetes and obesity; also for the prevention and alleviation of several micronutrient deficiencies, the World Health Organization (WHO) recommended the intake of a minimum of 400 g of fruits and vegetables per day (Duedu *et al.*, 2014; Wegayehu *et al.*, 2013). A lot of vegetables are consumed raw including cabbage to retain the natural taste and heat labile nutrients. But these raw vegetables have been shown to harbor pathogenic bacteria, this is of a serious concern (Tambekar and Mundhada, 2006; Cardamone *et al.*, 2015; Nwanko *et al.*, 2015). As much as 70% of diarrheal diseases in developing countries are believed to be of food borne origin (Kusumaningrum *et al.*, 2003, Kang *et al.*, 1998). The risk of disease transmission is much higher among fruits and vegetables consumed raw and/or unwashed (Nazemi *et al.*, 2012; Ebrahimzadeh *et al.*, 2013; Izadi *et al.*, 2006; Said, 2012)

Cabbage can become contaminated with pathogenic micro-organisms whilst growing in fields, or during harvesting, postharvest handling, processing and distribution or even at home (Eraky *et al.*, 2014; Rahman *et al.*, 2014; Pagadala *et al.*, 2015; Maffei *et al.*, 2016). Vegetables cultivated by irrigation can be contaminated by the use of insufficiently treated waste water for irrigation (Mahvi and Kia, 2006). Contamination of soil with animal wastes and increased application of improperly composted manures to soil can also be a source of contamination (Beuchat, 2002).

Only a few studies on bacterial contamination of ready to eat cabbage in North-Western Nigeria has been published; especially the susceptibility of such bacteria to antibiotics. This study was therefore aimed at isolating bacterial contaminants from ready to eat cabbage sold at Muhammadu Gumi market in Kaduna and to determine the antibiotics susceptibility of such bacteria.

METHODOLOGY

Study area

The samples were collected from Mohammadu Gumi market, along Ahmadu Bello Way by Ibrahim Taiwo road, Kaduna. The market is located in the northern part of Kaduna Metropolis and is one of the biggest in Kaduna State, Nigeria. It is situated within latitude 10^0 36' 33.548" N and 7^0 25' 46.2144" E in the North-West region of Nigeria.

Sample collection

A total of 40 samples of ready to eat cabbages were purchased from different vendors at Mohammadu Gumi Market, Kaduna State, Nigeria. Ten samples were obtained from four different sites. The samples were placed in separate sterile conical flasks with ice packs and transported to the Pharmaceutical microbiology laboratory of Kaduna State University, Kaduna for analysis.

Enumeration of aerobic bacteria

For each of the samples, 25 grams was weighed and washed in 100 ml of sterile distilled water using a vortex mixer to ensure homogeneity. This was then used to carry out serial dilutions. Serial dilutions of samples were made up to 10^{-5} dilution in distilled water. This was done by dispensing 1ml of each sample into a test tube containing 9ml of distilled water, this will make the content 10ml and give a dilution of 10^{-1}. The process was repeated until a 10^{-5} dilution was obtained. About 20 ml of nutrient agar was poured into appropriately labelled petri dishes. Thereafter, 0.1 ml was aseptically collected from each dilution of 10^{-5}, and cultured on sterile nutrient agar using spread plate techniques, before incubation at 37°C for 24 hours. Total aerobic bacteria counts were calculated for the colonies examined on nutrient agar. All the colony counts were expressed as CFU/ml.

Isolation and Identification of bacterial isolates

The following selective media: MacConkey agar, Mannitol salt agar, and Cetrimide agar were used to detect the growth of *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. Gram staining of the pure culture was performed according to the method described by Nester (2007). The following biochemical tests were carried out according to Cheesbrough (2005) to identify the isolates: indole, catalase, citrate utilization, methyl red Voges-Proskauer Test, Triple Sugar Iron Agar (TSI) test and oxidase test.

Antibiotic susceptibility test

The susceptibility of the bacterial isolates to different antibiotics was determined using the Kirby- Bauer disc diffusion technique as described by Clinical Laboratory Standard Institute (CLSI, 2013). The following 9 antibiotic discs (Oxoid, Basingstoke) namely, ampicillin (10 µg), gentamicin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg), erythromycin (15 µg), Trimethoprim-sulfamethoxazole (co-trimoxazole) (25 µg), cefoxitin (30 µg), ofloxacin (10 µg), and penicillin G (10 µg),

were used. Bacterial isolates that were resistant to three or more categories of antibiotics were classified as multidrug resistant.

Phenotypic detection of methicillin-resistant *Staphylococcus aureus* isolates

Methicillin resistant *Staphylococcus aureus* isolates were detected with the use of cefoxitin disc agar diffusion test. CLSI, (2013) has recommended cefoxitin due to its ability to enhance induction of altered penicillin binding protein (PBP2a). A 0.5 McFarland standard suspension of the isolate was made and a lawn culture was done on Muller Hinton agar plate. Cefoxitin 30 µg disc was placed and incubated at 37 ºC for 18 hours and diameter zone of inhibition was measured in reflected light. An inhibition zone diameter of ≤21 mm was reported as methicillin resistant.

Evaluation for Multidrug Resistance (MDR) and Multiple Antibiotics Resistant Index (MARI)

Isolates that were resistant to three or more classes of antibiotics and had MARI ≥ 0.3 were categorized as multidrug resistant. The MARI for each isolate was determined by dividing the number of antibiotics to which each test isolate was resistant by the total number of antibiotics tested (Krumperman, 1983).

RESULTS AND DISCUSSION

Aerobic bacteria count

The results obtained showed that the total aerobic bacteria count from the cabbage sampled ranged from 4 x10^6 – 9 x 10^6 CFU/ml. The total viable bacteria count observed in this study was high, similar value was reported by Oranusi and Olorunfemi (2011) while a higher value was obtained by Adeleye *et al.,* (2019) and Abdulahi and Abdulkareem, (2010). The high level of contamination of these ready-to-eat cabbage poses a great concern because they are most times consumed raw. The source of contamination may be through handling by the sellers; even though the vegetable is usually packaged in a bucket, the frequent opening and closing of the bucket in an attempt to fetch out for various customers exposes the vegetables to the atmospheric contaminants. Also the repeated use of the same spoon for serving the vegetable to different buyers can also serve as a source of contamination.

Bacteria Isolated

The following bacteria were isolated *S. aureus* (19), *E. coli* (13), *Salmonella* spp *Pseudomonas aeruginosa* (4) and *Serratia* spp (1), making a total of 46 isolates with 19 isolates being Gram positive and 27 Gram negative. *S. aureus* had the highest prevalence of 41.3% while *Serratia* spp had the least prevalence (Figure 1). Akoechere *et al.,* (2018) in a study on bacterial and parasitic contaminants of salad vegetables sold in markets in Fako Division, Cameroun also recorded a high prevalence of *S. aureus* (35.4%) and the least prevalence in *Serratia* spp (8.5%). The isolation of *E. coli* from these samples was an indication of faecal contamination, sometimes poultry dung is used as manure during cultivation, this can be a source contamination, likewise the source of water used for irrigation (Eraky *et al.,* 2014; Rahman *et al*., 2014; Pagadala *et al.*, 2015; Maffei *et al*. 2016). In a research by Weldezgina and Abelta, (2016) on bacteriological contaminants of some fresh vegetables irrigated with Awetu River in Jimma town, South Western Ethiopia, similar microorganisms were isolated from the vegetables and the irrigation water. Consumption of any of these organisms poses a serious health hazard. Previous reports where *E. coli* and *Salmonella* spp were isolated from cabbage include the following: Eni *et al.*, (2010) reported *E. coli* (4.2%), *Salmonella* spp (12.5%) from Sango Ota, Nigeria; Mritunjay and Kumar, (2017) and Alemu *et al.*, (2018) reported *E. coli* (16.7%) and (31.4%) respectively from Dhambad city, India and Arba Minch town, Southern Ethiopia.

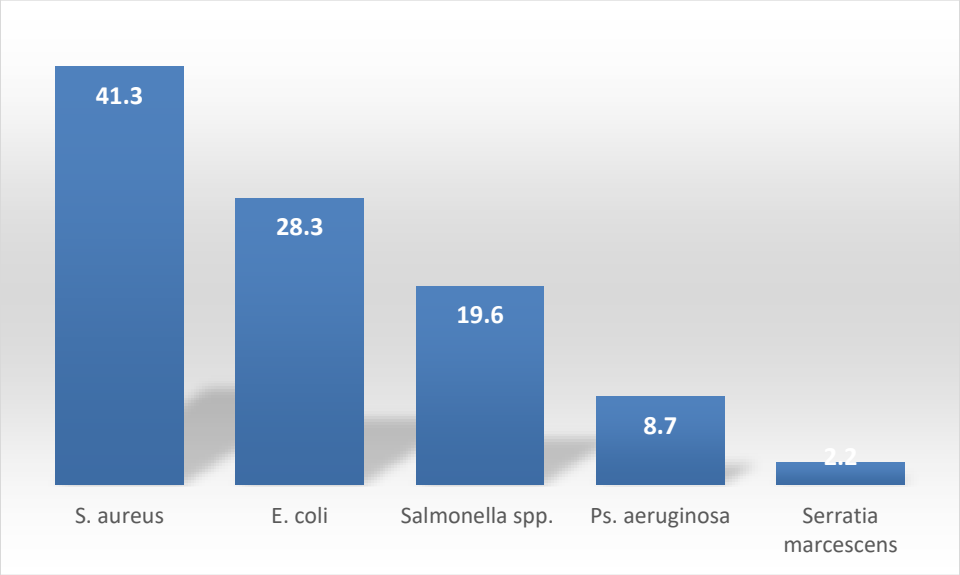

Figure1: Percentage occurrence of bacterial isolates from cabbage

Antibiotic Susceptibility

Both the Gram positive and Gram-negative organisms demonstrated a high level of resistance to erythromycin (100%), ampicillin (100%) and penicillin (100%) (Tables 1 and Figure 2). Akoechere *et al.*, (2018) also reported a high level of resistance of bacterial isolates from cabbage to erythromycin (75.6%). Both the Gram positive and Gram-negative organisms were highly susceptible to ofloxacin, 100% and 92.3% respectively. Considering the overall resistant pattern of the isolates to antibiotics, it was observed that the Gram-negative isolates were more resistant than the Gram-positive isolates. The nature of the outer membrane of the Gram-negative envelope distinguishes them from Gram positive bacteria, it is the main reason for their resistance to a wide range of antibiotics including beta lactams, quinolones and other antibiotics (Miller, 2016; Datta and Gupta, 2019).

Table 1: Antibiotic Susceptibility of Gram-negative and Gram-positive isolates

Antibiotics	Gram negative isolates (n=27)		Gram positive isolates (n=19)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ofloxacin	92.3	7.7	100.0	0
Gentamicin	23.1	76.9	26.3	73.7
Penicillin	0	100.0	0	100.0
Erythromycin	0	100.0	0	100.0
Ampicillin	0	100.0	0	100.0
Cefoxitin	0	100.0	10.5	89.5
Chloramphenicol	11.5	88.5	0	100.0
Tetracycline	3.8	96.2	0	100.0
Cotrimoxazole	15.4	84.6	5.3	94.7

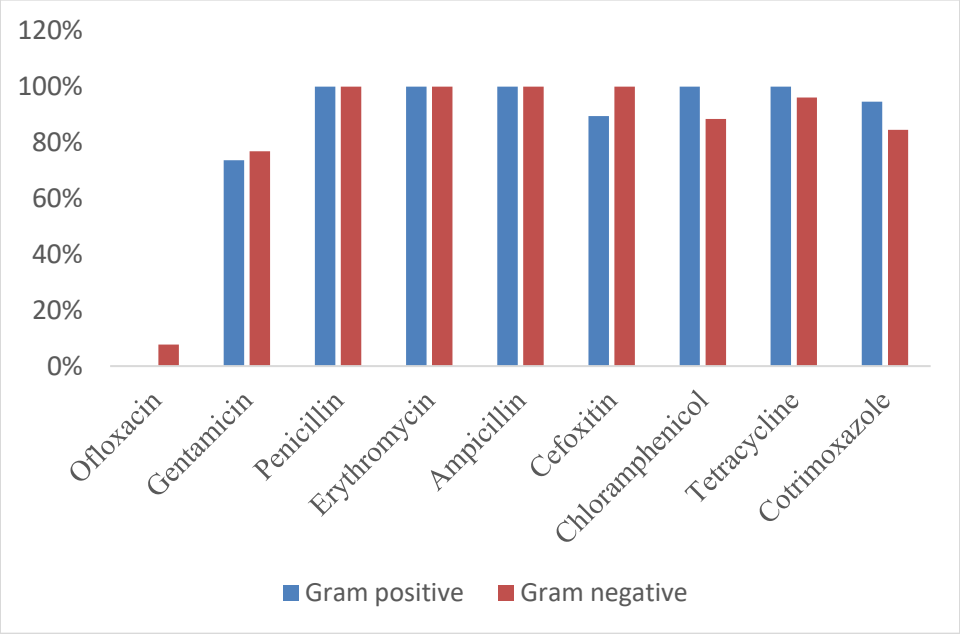

Figure 2: Resistance Pattern of Gram negative and Gram-positive isolates

Table 2: Multiple Antibiotic Resistance Index (MARI) of bacterial isolates from cabbage samples

MARI	Number of organisms	Percentage %
0.1	0	0
0.2	1	2.2
0.3	2	4.3
0.4	4	8.7
0.5	6	13.0
0.6	2	4.3
0.7	4	8.7
0.8	8	17.4
0.9	18	39.1
1.0	1	2.2
	46	100

It is alarming to discover that 97.8% of the isolates in this study had MARI ≥ 0.3 (Table 2) and were multidrug resistant; this implies that they were all resistant to antibiotics of three or more categories. Irrational antibiotic use might be common in this environment. The consumption of these vegetables harbouring multidrug resistant strains will lead to further spread of multidrug resistant bacterial strains which can lead to treatment failure and/or antibiotic resistance when such individuals contract infections. It is therefore imperative that there should be strong advocacy against misuse and abuse of antibiotics in our society. The results of the detection of MRSA showed 89.5% phenotypic MRSA isolates, this is of public health concern because MRSA isolates are always resistant to antibiotics and are responsible for increased length of hospital stay, increased hospital costs and high hospital mortality rate (Zhen *et al.*, 2020)

CONCLUSION

The ready-to-eat cabbage sampled were highly contaminated, the bacteria isolated include *S. aureus*, *E. coli*, *Salmonella* spp. *Pseudomonas aeruginosa* and *Serratia* spp. A high percentage of the isolates were multidrug resistant. Ofloxacin, an example of a quinolone was the most active antibiotic in this study. Since cabbage is usually consumed raw it is recommended that the government and relevant

agencies should give regular training on the need for proper hygiene by handlers, sellers and consumers of ready-to-eat vegetables. Implications of consuming contaminated vegetables should also be made clear.

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