Occurrence and antibiotic resistance of Staphylococcus species isolated from Moringa leaf salad 'Kwadon Zogale' sold in Wukari metropolis, North-Eastern Nigeria.

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Abstract

Moringa leaf salad is an affordable meal in parts of Taraba state and can also be a source of infectious agents due to its vulnerability to contamination. *Staphylococcus* species is one of the major contaminants of this salad due to mode of preparation and serving with a bear hand and can also be expose to various infectious agents. Therefore, this research was carried out to determine the occurrence and antibiotic resistance of *Staphylococcus* species. A total number of twenty samples were collected for this research. Four samples were collected from each of the five vendors. The total bacterial count in the food ranged from 1.0×105 to 8.8×105. *Staphylococcus* species was highly prevalence especially *Staphylococcus aureus* (66.7%) and other *Staphylococcus* species (33.3%). Antibiotic sensitivity pattern of *Staphylococcus aureus* from salad sample shows that It was sensitive to CN=12(100), AMX=10(83.3), NB=4(33.7), S=9(75), resistance to CH=1(8.3), CP=5(41.7) S=1(8.3) and intermediate to CH=11(91.7), CPX=7(58.3), E=12(100), LEV=12(100), APX=12(100), RD=12(100), AMX=2(16.7), NB=2(16.7), S=2(16.7). The multiple antibiotic indices ranged from 0.2–0.3. The high incidence of bacterial contamination of ready to eat Moringa leaf salads reported in this study may be accounted for lack of basic sanitation requirements for processing the product that requires no pre-heating before consumption.

Keywords: Moringa meal, Infectious agents, Antibiotics, Resistance.

Introduction

Moringa Leaf (Kwadon Zogale) can be used to prepare vegetable salad, which is a special delicacy in Northern Nigeria [1]. The traditional vegetable salad made from Moringa leaves is sometimes prepared with raw spices such as onions, pepper, fresh tomatoes, seasoning and sometimes grounded groundnut cake locally referred to as kulikuli is added (Cookpad.com). Zogale is not a main dish; it is usually eaten as an accompaniment or alone as a snack antioxidant and they contain properties that are anti-inflammatory, anti-diabetic, cholesterol lowering and cardio-protective according to Marcus [2]. This means that anybody with any kind of health issues can eat this super-food.

The traditional way of preparing Moringa leaf salad (Kwadon zogale) involves cooking and mixing with spices and other ingredients using bare hands; and it is a known fact that *Staphylococcus* species lives on the skin as normal flora, which can then be transferred to the salad during preparation and may cause harm to humans sometimes through the release of toxins as described by Tambekar, et al. [3]. Sometimes the vegetable salad can be contaminated through exposure to aerosols and dusts in the market or on the streets [4].

Therefore, the occurrence of *Staphylococcus* species is inevitable in the production of the locally made Moringa Leaf Salad (Kwadon Zogale). Antibiotic resistance of *Staphylococcus* species has become the major public health burdens in the globe especially *Staphylococcus aureus* as reported by Yugueros, et al. [5]. This has been a very serious challenge in the treatment of infections that are cause by these pathogens [6]. In many countries clinical strains are quite often multi-resistant, which significantly reduces the therapeutic options for treatment of staphylococcal infections. The resistance mechanism against methicillin involves the acquisition of the mecA gene, which is a determinant of a unique penicillin binding protein, (PBP)2a, that has reduced affinity for β-lactames, including cephalosporins [7]. It is important to carry out this work because vegetables salads have been found to be sources of pathogens [8]. Sometimes the local Moringa leaf salad is usually produced and served unhygienic and the screening for antibiotic susceptibility of pathogens is crucial because of the indiscriminate use of antibiotics in treatment of diseases among human and animal populations [9].

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Material and Methods

Study area

This study was carried out in the new and old markets of Wukari in Wukari Local Government Area of Taraba State. Wukari town is the headquarters of Wukari Local Government Area in Taraba State, Nigeria and is situated at latitude 7.85◦ North, longitude 9.78◦ East and 152 meters' elevation above the sea level in the northern part of Nigeria.

Sample Collection

A total of twenty (20) samples of Moringa leaf salad (kwadon zogale) were collected in a sterile universal container from different vendors in New and Old Wukari markets. The samples were collected aseptically in a sterile container. They were labelled and immediately transported to the research laboratory of the Department of Microbiology, Federal University Wukari for the microbial analysis.

Total bacteria count

The total bacteria were counted on plates after the serial dilution of the samples. Twenty-five gram of the salad sample was placed in sterile 225 ml buffer peptone water and was thoroughly mixed, from which 1 ml was transferred to a test tube containing 9 ml of sterile buffer peptone water as diluent. The serial dilution was repeated in other sets of nine tubes containing buffer peptone water and was diluted to 10-8. From the last two dilutions 0.1 ml was dispensed to two separate petri dishes. Prepared and cooled molten nutrient and mannitol salt agar were poured gently and swirled, allowed to solidified and were incubated at 37oC for 24hrs. After which the bacteria colony were counted using colony county and average was taken [10].

Isolation of Staphylococcus species

Aseptically, 1 millimeter of the diluted sample was taken using a sterile pipette and then it was pour using a pour plate method into a prepared Mannitol salt aga and allowed to solidify before incubation. The culture plate was incubated at a temperature of 37ºC for 24hrs. The incubated culture plate was morphologically observed for their colour, form, elevation, size, margin, texture [10].

Identification of Staphylococcus species

After incubation, the colonies of the different culture media were examined and recorded based on the shape, colour, border, texture, and general appearance of individual bacterial colonies on each plate and biochemical characteristics with reference to Bargeys systematic Bacteriology manuals [10]. The following is used to identify bacteria:

Biochemical test: The biochemical tests considered for this research were Gram reactions, catalase, coagulase, urease and citrate tests were and were carried out for the identification of *Staphylococcus* species according to [10] and the isolates were confirm using Microgen *Staphylococcus* ID kits.

Standardization of inoculum: Dilution from each of the suspension of the test isolates was prepared by picking a 24hrs colony of the isolates using sterile wire loop into sterile test tube containing sterile normal saline to form turbidity that match with 0.5 scale of McFarland's standard (1.5 × 108 cells/ml) as described by Coyle [11].

Antibiotic susceptibility test: *Staphylococci* isolates was tested for their sensitivity to antibiotics by means of disc diffusion method [12]. The antibiotic susceptibility pattern was determined using Kirby-Bauer-NCCLS modified single disc diffusion technique [10]. The standardized inocula were inoculated by streaking on prepared Mueller-Hinton agar using sterile swab stick; the antibiotic disc was placed aseptically on the inoculated medium with the help of sterile forceps and incubated at 37°C for 24hrs. The zones of inhibition cleared by each of the antibiotics against the test organisms were measured and the results were interpreted using the guideline from CSLI [13] and all the results were recorded appropriately. Gram positive (+ve) multiple susceptibility antibiotic discs from Optun Laboratory Nigeria LTD containing CPX-Ciprofloxacin (10µg), NB-Norfloxacin (10µg), CN-Gentamycin (10µg), AML-Amoxil (20µg), S-Streptomycin (30µg), RD-Rifampicin (20µg), E-Erythromycin (30µg), CH-Chloramphenicol (30µg), APX-Ampiclox (20µg), and LEV-Levofloxacin (20µg) were used.

Multiple antibiotics resistance (MAR) index: Multiple antibiotics resistance was determined for each of the selected bacterial isolate using the formula MAR=X/Y where X is the number of antibiotics to whish the test isolates displayed resistance to and Y is the total number of antibiotics to which the test organism has been evaluated for sensitivity [14].

Multiple Antibiotic Resistance (MAR) Index

MAR index=Number of antibiotics isolate is resistant to/Total number of antibiotics tested

MAR 1/10 Therefore, MAR=0.1

Results and Discussion

The total bacteria count on Moringa leaf salad 'kwadon zogole' ranged from 1.0 x 105 to 8.8 x 105 as presented in Table 1 and the presumptive identification and characterization of bacteria isolate based on their morphological characteristics microscopy gram stain and biochemical test as presented in Table 2 shows that present of *Staphylococcus* species in the food sample. The frequency of occurrence of *Staphylococcus* species isolates on the salad samples. The result showed that *Staphylococcus aureus* was the most frequent (66.7%) followed by *Staphylococcus* species (33.3%) as presented in Figure 1.

Antibiotic susceptibility patterns of *Staphylococcus aureus* from the salad sample. Susceptibility of each isolate to a panel of ten (10) antimicrobial substances was assessed by disk diffusion on Mueller-Hinton agar Plates. Isolates of S. aureus shows sensitive to CN=12(100), AMX=10 (83.3), NB= 4(33.7), S= 9(75), Resistance CH= 1(8.3), CPX=5(41.7), NB=6(50), S=1(8.3), intermediate CH=11(91.7), CPX=7(58.3), E=12(100), LEV=12(100), APX=12(100), RD=12(100), AMX=2(16.7), NB=2(16.7), S=2(16.7) (Tables 3 and Table 4).

Table 1: *Microbiological count (CFU) of Kwadon zogale obtains from the five locations.*

S/N	Sample Site	Total Bacteria Count (TBC)	*Staphylococcus* Count (SC)
1	**First Site**		
	1	Too Numerous	4.0 x 105
	2	4.8 x 105	No Growth
	3	4.4 x 105	No Growth
	4	4.0 x 105	No Growth
2	**Second Site**		
	1	3.6 x 105	1.2x 105
	2	Too Numerous	No Growth
	3	4.8 x 105	4.0 x 105
	4	8.0 x 105	No Growth
3	**Third Site**		
	1	1.0 x 105	4.0 x 105
	2	Too Numerous	4.0 x 105
	3	Too Numerous	1.6 x 105
	4	Too Numerous	4.0 x 105
4	**Fourth Site**		
	1	1.0 x 105	No Growth
	2	6.0 x 105	No Growth
	3	Too Numerous	4.0 x 105
	4	No Growth	No Growth
5	**Fifth Site**		
	1	6.0x 105	8.0 x 105
	2	8.0 x 105	No Growth
	3	8.8 x 105	4.0 x 105
	4	1.12 x 105	8.0 x 105

Table 2: *Biochemical characteristics of presumptive Staphylococcus species isolated from Moringa leaf salad 'kwadon zogole.*

		Microscopy		Biochemical Test				
S/N	**Colony Characteristics**	**Morphology**	**Gram Stain**	**Catalase**	**Coagulase**	**Citrate**	**Urease**	**Bacteria Isolate**
1	Smooth, creamy, glistening, flat with entired edge.	Cocci	+in cluster	+	+	+	+	Staphylococcus species
2	Rough, slightly yellowish,Dull with undulate.	Rod	+in chain	+	_	+	_	Bacillus species
+=Positive, -=Negative, spp=species, Coagula=Coagulase								

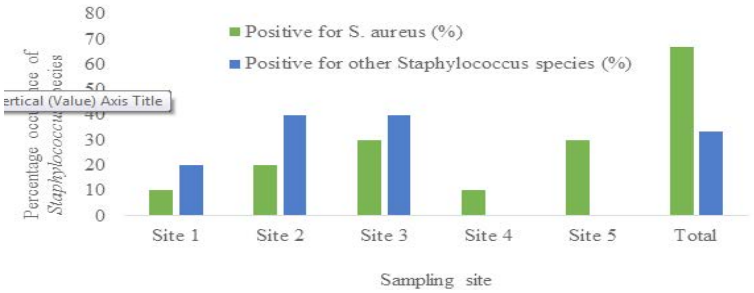

Figure 1*. Percentage of occurrence of Staphylococcus species in the food samples.*

Table 3: *Antibiotic susceptibility test of Staphylococcus aureus isolated from the food sample.*

S/N	Antibiotics	S (%)	I (%)	R (%)
1	CH	0(0.0)	11(91.7)	1(8.3)
2	CPX	0(0.0)	7(58.3)	5(41.7)
3	E	0(0.0)	12(100)	0(0.0)
4	LEV	0(0.0)	12(100)	0(0.0)
5	CN	12(100)	0(0.0)	0(0.0)
6	APX	0(0.0)	12(100)	0(0.0)
7	RD	0(0.0)	12(100)	0(0.0)
8	AML	10(83.3)	2(16.7)	0(0.0)
9	NB	4(33.7)	2(16.7)	6(50)
10	S	9(75`)	2(16.7)	1(8.3)
CPX=Ciproflox, NB=Norfloxacin, CN=Gentamycin, AML=Amoxil, S=Streptomycin, RD=Rifampicin, E=Erythromycin, CH=Chloramphenicol, APX=Ampiclox, Lev=Levofloxacin, S=Sensitivity, I=Intermediate, R=Resistance.				

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Table 4: *Antibiotic resistance patterns of Staphylococcus species isolated from the food samples.*

S/N	Pattern	Frequency (NO of isolates)
1	CPX	4
2	NB	3
3	CH, NB	1
4	CPX, NB	1
5	CPX, S, NB	1
CPX=Ciproflox, NB=Norfloxacin, CH=Chloramphenicol, S=Streptomycin, NO=Number.		

Table 5: *Multiple antibiotic resistances (MAR) index of Staphylococcus species isolated from Moringa leaf salad.*

Number of multi-resistant isolates	Antibiotic combination	MAR index
1	2	0.2
1	2	0.2
1	3	0.3

Table 5 shows Antibiotic resistance pattern of *Staphylococcus aureus* from salad sample. Four (4) isolates were resistant to CPX, three (3) resistant to NB, one isolate was resistant to CH, NB and the other isolate was resistant to CPX, NB. One of the isolates was resistant to CPX, S and NB. The multiple antibiotic resistances (MAR) index of *Staphylococcus* species isolated from the salad sample ranged from 0.2–0.3.5.

The high incidence of bacterial contamination of Moringa leaf salad 'kwadon zogole' observed in this study demonstrates that salads are frequently colonized by *Staphylococcus* species which is of great risk to human health. The percentage of occurrence of *Staphylococcus* species in the salad samples from the (5) five site in Wukari, shows that *Staphylococcus* species is highly prevalence. This could be due to fact that the food that has been handled unsafely, such as preparation and use of contaminated utensil [3]. *Staphylococcus* species lives on the skin as normal flora, which can then be transferred to the Moringa leaf salad during preparation and can cause harm to the human system according to Tambekar, et al. [3].

The high incidence of bacterial contamination of ready to eat Moringa leaf salads reported in this study may be accounted for lack of basic sanitation requirements for processing products that requires no pre-heating before consumption. Another reason may be using a low quality of water during washing and pre-disinfection of the fresh vegetables and fruits during salads production [15].

Staphylococcus aureus bacterium caused staphylococcal infections in humans, according to Mayo Clinic, [16]. Although these bacteria are usually harmless and are commonly found in the surface of the skin. Mayo Clinic reported that they can prevail over the body's natural protection, once the skin is damaged or injured to produce infection. The infections caused by *Staphylococcus aureus* range from superficial skin infection to life threatening ones. Skin infections are the most common effects of *Staphylococcus aureus*. These infections can start as a simple crusting of the skin known as impetigo. Other skin infections caused by *Staphylococcus aureus* are folliculitis, an inflammation of a hair follicle, furuncle a small abscesses affecting the skin and sub cutaneous tissues, and carbuncle a collection of furuncle [17].

People eating contaminated Moringa salad develop symptoms within one to six hours. *Staphylococcus aureus* caused stomach and skin diseases in humans. Toxic shock syndrome caused by *Staphylococcus* is life threatening, Toxic shock syndrome include redness of the skin, fever and low blood pressure, it usually involves organs and systems, such as gastrointestinal, muscular, renal, hematologic and nervous systems.

The antibiotic resistance pattern demonstrated by the bacterial isolates in this study revealed that *Staphylococcus* species was resistance to CPX, NB, CH, and S. Multiple antibiotic resistant *Staphylococcus* species isolated from the vegetable salad can be a threat to the health of the consumer. Many isolates of *Staphylococcus* species have evolved resistance to both synthetic and traditional antimicrobial chemotherapy and their prevalence outside the hospital is of potential epidemiological threat [18].

The antibiotic resistance pattern obtained in this study is a serious challenge to public health because of the higher demanding for salads in different homes, societies and functions. In recent years, the number of documented outbreaks of human infections associated with the consumption of raw vegetables has greatly increased. The resistant ability of the organisms can be transferred from one organism into another, through the antibiotic resistance plasmids [19]. The MAR index of 0.2 indicates that the S. aureus most been isolated from environment where the antibiotic resistant to all frequently use [20].

Conclusion

In conclusion, the high bacterial load ranged from the lowest to the highest 1.0x105 to 8.8x105 respectively. The percentage of occurrence of *Staphylococcus aureus* and *Staphylococcus* species was 66.7% and 33.3% respectively. The antibiotics susceptibility test of *Staphylococcus aureus* isolated from the salad sample shows that Erythromycin, Levofloxacin, Gentamycin, Ampiclox, Refampicin, Ampiclox was not resistant to the isolates from the salad sample, isolates was senstive to Gentamycin, Ampiclox, Norfloxacin, Streptomycin and other isolate was intermediate to Chloramphenicol, Ciproflox, Erythromycin, Levofloxacin, Ampiclox, Rifampicin, Amoxil, Norfloxacin, and Streptomycin.

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The multiple antibiotic resistances (MAR) index of *Staphylococcus* species isolate ranged from 0.2-0.3. The MAR index of 0.3 showed that the isolate might have been originated from the environment were the antibiotics are frequently used. The high incidence of bacterial contamination of Moringa leaf salads reported in this study may be accounted for lack of basic sanitation requirements for processing products that requires only pre-heating of the salad vegetable before consumption and the addition of raw ingredients. Another reason may be using a low quality of water during washing and pre-disinfection of the fresh vegetables during salads preparation.

There is therefore, the need for regulatory bodies to ensure that microbiological standards are established and a good hygiene practiced by food processor and marketers for the handling and distribution of salad vegetables. There is need for awareness on qualities of antibiotics sold for treatment of infection cause as results of consumption of contaminated vegetable salad. This will help reduce the antibiotic resistance pattern of bacteria in the environment.

Conflict of Interest

All authors do not have any possible conflicts of interest.

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