Relative Bacteriological Assessment of Spoilt Fruits and Vegetables Sold in Minna, Niger State.

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Abstract
An investigation was carried to study the different bacterial species present in some spoiled fruits (orange and watermelon) and vegetables (spinach and tomato) sold in a Kasuwan gwari market. Samples were aseptically collected from the Kasuwan gwari market into a sterile universal container and a plastic bag, and were transported to the Microbiology Laboratory of Federal University of Technology Minna, Niger State. Samples were serially diluted and inoculation of these samples on various media was done via the pour plate method. The isolated bacteria were identified via Gram reactions and other biochemical tests. The antibiogram of the bacterial isolates was carried using various antibiotic discs. The highest bacteria count was observed in oranges ($1.56x10^4$), followed by tomatoes ($1.12x10^4$), spinanch ($1.08x10^4$) and then watermelon ($1.04x10^4$). The isolated bacteria were identified as Klebsiellapneumoniae (25%), *Streptococcus pyogenes (16.67%), Salmonella typhi(33.33%) and*Staphylococcus aureus (25%) respectively. The study revealed that the susceptibility of *Klebsiellapneumoniae to Gentamycin, Septrin and Reflacine was 100% while its resistance to Augmentin, Streptomycin, Tarivid and Penicillin was 100%. Streptococcus pyogenes on the other hand, also exhibited 100% susceptibility to Ofloxacin, Erythromycin and Augmentin, and 100% resistance to Cloxicillin, Ceftazidine and Ceftriaxone. SimilarlySalmonellatyphi and Staphylococcusaureus also exhibited 100% susceptibility to Ofloxacin, Erythromycin, Augmentin, Nalidixic acid, Streptomycin, Tarividand Septrin while 100% resistance to Gentamycin, Augmentin, Penicillin, Reflacine, Rifamipicin, Norfloxacin and Levofloxacin in Salmonellatyphi and Staphylococcusaureus was also observed. However due to the high resistant bacteria isolates, associated with the spoiled fruits and vegetables, it is therefore imperative to ensure that the Government enlightens thefarmers who harvest, package and transport various fruits and vegetables for public consumption,on the menace associated with microbial contamination of various fruits and vegetables to ensure that the microbial spoilage especially with resistant bacteria is controlled and curtailed adequately. In addition, the Government should also create adequate awareness on the misuse of antibiotics to ensure that the spread of resistant bacteria isolates in the environment (such as soil or water) is curtailed and controlled adequately.*

Keywords: Fruits, Vegetables, Antibiotics, Microorganisms

Introduction

Fruits and vegetables are edible substances which contain growth factors, such as vitamins and essential minerals that enablehumans live a healthy life (Al Hindi *et al*., 2011).

A fruit is basically the edible part of a matured ovary, of a flowering plant. They are usually eaten raw, and are classified as eitherfleshy or dry fruits. Fleshy fruits are furtherclassified into berry (such as orange, tomato,pineapple, pawpaw, and banana), drupes(such as plume, coconut, almond, cherry) andpomes (such as apple and pear), while the dryfruits, are classified as dehiscent (such as pod, follicle and capsule) andindehiscent fruits (like achene, samara and cashew) (Jay, 2000).

Vegetables are considered as the leafy outgrowth of plants or plants shoot consumed as food by eitherhumans or other animals (Yusuf *et al.,* 2004). Basically they are protective foods used to maintain good health and prevent diseases.

Generally, the succulent nature of fruits and vegetables enables microbes to invade easily. The high content of various sugars, minerals, vitamins and amino acids in both fruits and vegetables enhances the proliferation and survival of various microorganisms (Bhale, 2011), thus bringing about microbial spoilage of these fruits and vegetables.

However, thesusceptibility of various fruits and vegetables to microbial contamination is mainly due to their various chemicalcompositions, pH or moisturecontents whichpredispose them to microbial spoilage. Basically, microbial spoilage is a condition that occurs when microbes such as fungi and bacteria grow and degrade tissues, such as pectins, thus presentingthe whole fruit into a slimy mass. The microbes also degrade starch and sugars, therebyproducing substances that alter the color, texture, flavour and odour of the food (Rawat, 2015).

Most microorganisms associated with the surfaces of either a fruit or vegetable are soil inhabitants (Janisiewicz and Korsten, 2002;Andrews and Harris, 2000).However, microbial contaminationof most fruits and vegetablescould also occur either during their growth season (via direct contact with contaminated manure, sewage, irrigation water, wastewater from livestock operations or wild and domestic animals) or during their harvest, handling, transportation, post-harvest storage, marketing or even after a purchase by a consumer (Erak*yet al.,* 2014; Rahman*et al.,* 2014; Pagadala*et al.,* 2015; Maffei*et al.,* 2016) (as seen in Figure 1)

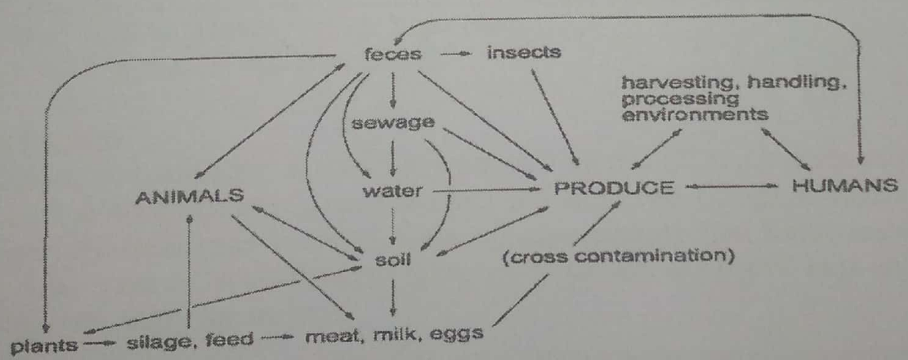

Figure1: Illustration on how farm produce get contaminated with pathogenic microorganism
Source: Maffei *et al.* (2016)

Based on the improper postharvest practices performed by most farmers, spoilage bacteria are introduced on most farm products during their harvest, packaging, storage or transportation. This thereby renders most farm product undesirable for consumption. Based on this, it is therefore necessary that bacteria associated with the spoilage of these farm produce are determined. This is the main focus of this study

Materials and Method

Study Area

Minna is a city, with an estimated population of 304,113. It's the capital of Niger State, one of Nigeria's 36 federal states, and is the headquarters of Chanchaga Local Government Area. The area surveyed wasKasuwangwari market.

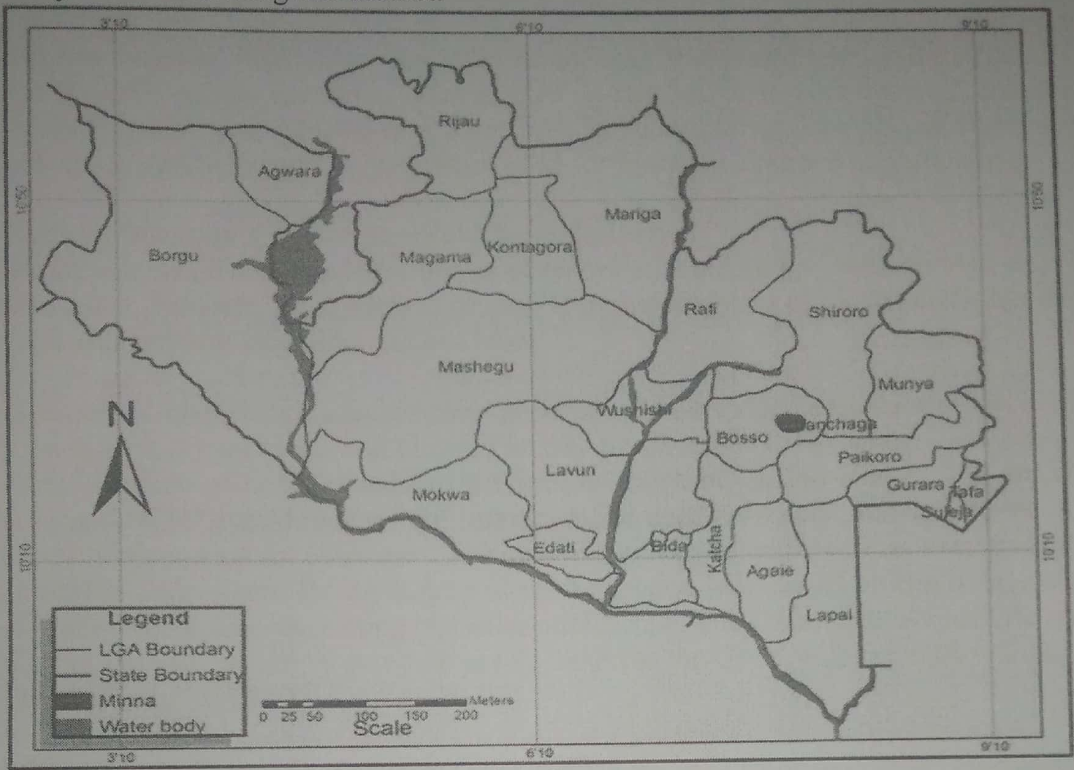

Figure 2: Map of Nigeria showing Minna Niger State
Source: Niger State Bureau of Statistics (2010)

Sampling Collection

A total of 12 samples [consisting of three (3) samples from two (2) spoilt fruits namely Oranges (*Citrus sinensis*) &Watermelon (*Citrulluslanatus*) and from two (2) spoilt vegetables namely Spinach (*Spinaciaoleracea*) and Tomato (*Solanumly copersicum*)] from Kasuwangwarimarket in Minna Niger State were collected in sterile universal containers and plastic bags and transported to microbiology laboratory for analysis.

Preparation of Media

The media used are: Nutrient agar, MacConkey agar and Salmonella Shigella agar. These media were prepared according to the manufacturer's instruction (28g/L, 52.55g/L, and 63g/L) respectively and were sterilized at 121°c for 15 minutes in the autoclave except the Salmonella Shigella agar that was boiled with frequent agitation. After that all were allowed to cool and poured under aseptic condition into the Petri dishes.

Inoculation of samples
The spoilt areas of the fruits and vegetables were washed with sterile water; after which the spoilt areas were cut out and 5g of it was weighed. It was then put into sterile universal bottle containing 10ml of sterile water. The mixture was shaken and then serially diluted into five (5) test tubes to reduce the concentration of microorganisms in the test tube. Nutrient agar plates were inoculated with 1 ml from the serially diluted test tubes of 10^{-2}, using the Pour Plate Technique (to isolatevarious bacteria). The plates were allowed to solidify, inverted and incubated at 37ºC for 24hrs for bacterial colony formation. Thecolonies on Nutrient agar wereobserved, counted and recorded.Each colony was isolated in a pure form by sub culturing into a fresh Nutrient agar, MacConkeyagar and Salmonella Shigellaagar plate for further studies and identification.

Bacterial Identification and Biochemical Characterization
The discrete colonies from these sub cultured plates were identified by Gram staining techniques (Sherman, 2005; Prescott, 2002; Holt *et al*., 1994) and other types of biochemicaltests namely: Indole test, catalase test, coagulase test and so on.

Gram staining: a smear was made on a clean grease-free glass slide, the smear was heat-fixed and then flooded with crystal violet and allowed to stay for 60seconds. The smear was then diluted with Gram's iodine after 60seconds which acts as mordant on the smear. The smear was decolorizing with 95% ethyl alcohol for 1minute. After decolourization, the smear was counter stain with safraninand left for 60seconds. This was finally washed with distilled water and the slide was allowed to drain water. Finally a drop of oil immersion was placed on the slide and it was viewed at x100 (objective lens) using binocular microscope. The Gram positive bacteria retained the colour of the dye, crystal violet (dark purple) whereas the Gram negative retained the colour of the dye, safranin (reddish).

Indole Test: The test is used to determine the ability of the organisms to breakdown amino acids, tryptophan to indole. Peptone water broth rich in tryptone was dispensed in test tubes, autoclaved and inoculated with test organism. It was then incubated at 37 ^{0}C overnight. Few drops of Kovac's reagent were added and shaken. Red colouration which separated out in the alcohol interphase depicted a positive result while a yellow colouration depicted a negative result (Cheesbrough, 2010).

Catalase test: The test bacteria colony was picked and emulsified with 3% hydrogen peroxide on a clean glass slide and bubble formation was observed. If there is bubble formation, it is positive (+ve) and no bubble formation means it is negative (-ve).

Coagulase test: The test is used to differentiate coagulase positive *Staphylococcus aureus* from Coagulase Negative *Staphylococcus* (CONS). The test bacteria was picked and emulsified with a drop of blood serum on the slide and coagulation was observed. If there is coagulation it is positive (+ve) whereas no coagulation means negative (-ve).

Blood haemolysis test: Blood agar was prepared using 95ml sterile nutrient agar which was cooled to 45 ^{0}C and 5.0ml of blood was added and mixed thoroughly. The obtained blood agar was dispensed into sterile petri dishes and then allowed to solidify. The test organism (bacteria) was inoculated in the plate and incubated for 24hours. After 24hours of incubation, greenish and creamy zones surrounding the colonies indicated alpha haemolysis, whitish and creamy zones surrounding the colonies indicated beta haemolysis and scanty whitish zones surrounding the zones indicated gamma haemolysis (Sherman, 2005 and Holt *et al.*, 1994).

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was done using Kirby-Bauer method on Mueller Hinton Agar. 3.8 g of this agar was dispensed into a sterile conical flask. One hundred milliliter (100 mL) of distilled water was poured into the flask and stirred to dissolve the agar. The mixture was autoclaved and then poured into petri dishes. On gelling, a sterile swap stick was used to spread the standardized inoculums from a test tube, onto a Petri dish. After the inoculums have been spread, the positive and the negative antibiotic sensitivity discs were introduced using sterile forceps and then the plates were incubated for 24 hours at 37^0C.

Results and Discussion

Result

Out of all the spoiled fruits and vegetables analyzed, orange had the highest microbial count of $1.56x10^4$while spinach had the least microbial count of $7.8x10^3$

Table 1: Total plate count of 6 fruits and vegetables sampled in Kasuwan gwari market Minna, Niger state.

Sample	Microbial load (cfu/ml) 10^2		
	A	B	C
Orange	$1.04x10^4$	$1.24x10^4$	$1.56x10^4$
Watermelon	$9.2x10^3$	$8.4x10^3$	$1.04x10^4$
Spinach	$8.4x10^3$	$7.8x10^3$	$1.08x10^4$
Tomatoes	$9.6x10^3$	$1.16x10^4$	$1.12x10^4$

Table 2: Biochemical test of the Isolated Bacteria

Isolation Code	Cell Shape	Gram Reaction	Catalase	Coagulase	Haemolysis	Indole	Suspected Organism
KG1	R	-	-	-	-	-	*Salmonella typhi*
KG2	R	-	+	-	-	-	*Klebsiella pneumonia*
KG3	R	-	+	-	-	-	*Klebsiella pneumonia*
KG4	R	-	-	-	-	-	*Salmonella typhi*
KG5	C	+	+	+	-	-	*Staphylococcus aureus*
KG 6	R	-	+	-	-	-	*Klebsiella pneumonia*
KG7	R	-	-	-	-	-	*Salmonella typhi*
KG8	C	+	-	-	β	-	*Streptococcus pyogenes*
KG9	R	-	-	-	-	-	*Salmonella typhi*
KG 10	C	+	-	-	β	-	*Streptococcus pyogenes*

KG 11	C	+	+	+	-	-	*Staphylococcus aureus*
KG 12	C	+	+	+	-	-	*Staphylococcus aureus*

Key: KG= Kasuwangwari, C= Cocci, R= Rod, + = Positive, - = Negative, β = Beta haemolysis.

Twelve (12) bacterial isolates were obtained (as seen in Table 3). *Salmonella typhi* 4(33.33%) had the highest frequency of occurrence followed by *Klebsiella pneumoniae* and *Staphylococcus aureus* which had their frequency of occurrence to be 3(25%) and *Streptococcus pyogenes* 2(16.67%) had the least frequency of occurrence (Table 3).

Table 3: Frequency of Occurrence of Isolated Bacteria from Kasuwan gwari Markets.

Isolates	**Kasuwangwari (f / %)**
Klebsiella pneumoniae	3(25)
Streptococcus pyogenes	2(16.67)
Salmonella typhi	4(33.33)
Staphylococcus aureus	3(25)
Total	**12(100)**

Table 4.1 : Antibiotic Sensitivity of the Gram negativeBacterial Isolated.

Bacteria isolates	**Pattern**	**CN %**	**AU %**	**NA %**	**S %**	**OFX %**	**SXT %**	**PN %**	**PEF %**
Klebsiella pneumoniae	S	3(100)	0(0)	1(33.3)	0(0)	0(0)	3(100)	0(0)	3(100)
	I	0(0)	0(0)	1(33.3)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	0(0)	3(100)	1(33.3)	3(100)	3(100)	0(0)	3(100)	0(0)
Salmonellatyphi	S	0(0)	0(0)	4(100)	4(100)	4(100)	4(100)	0(0)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	4(100)	4(100)	0(0)	0(0)	0(0)	0(0)	4(100)	4(100)

Key: OFX= Tarivid, PEF= Reflacine, AU= Augmentin, CN= Gentamycin, SXT= Septrin,NA= Nalidixic acid,S= Streptomycin and PN= Penicillin.

Table 4.2: Antibiotic Sensitivity of Gram positive Bacterial Isolated.

Bacteria isolates	Pattern	S %	CH %	E %	RD %	NB %	LEV %
Staphylococcusaureus	S	3(100)	3(100)	3(100)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	0(0)	0(0)	0(0)	3(100)	3(100)	3(100)
		OFL %	**ERY %**	**CXC %**	**CAZ %**	**CTR %**	**AUG %**
Streptococcuspyogenes	S	2(100)	2(100)	0(0)	0(0)	0(0)	2(100)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	0(0)	0(0)	2(100)	2(100)	2(100)	0(0)

Key: S= Streptomycin, CH= Chloramphenicol, E= Erythromycin, RD= Rifamipicin, LEV= Levofloxacin, NB=Norfloxacin, CXC= Cloxicillin, ERY= Erythromycin, CAZ= Ceftazidime, AUG= Augmentin, CTR= Ceftriaxone, OFL= Ofloxacin

Discussion

This study revealed that high microbial count was observed in oranges compared to spinach. This could be based on the fact that, due to the presentation of most fruits especially oranges in a ready to consume form, most oranges sold in the market are easily prone to microbial contamination from the sellers due to inadequate use of personal protective wears and inadequate hand hygiene before and after peeling the oranges, thus enhancing rapid transfer of large microbial contaminants on the surfaces of these oranges, and this thereby leads to a high degree of microbial spoilage of these fruits.This finding is in agreement with the studies carried out by Muhammad*et al.*(2017).

This study also revealed that *Salmonellatyphi*4(33.33%) had the highest frequency of occurrence when compared to other bacteria isolated. This could be enhanced by the unhygienic packaging of most fruits and vegetables in the markets, which in turn encourages the transfer of feacal contaminants on the surfaces of these farm products through frequent perching ofinsects such as flies. This result is in line with the studies carried out by (Adebolu, 2001; Olayemi, 2007; Omemu, 2005; Tambekar, 2006; Uzeh, *et al.,* 2009).

*The study revealed that the isolated microbial contaminants were hundred percent (100%) resistant to*Augmentin, Streptomycin, Tarivid, Penicillin, Cloxicillin, Ceftazidine, Ceftriaxone, Gentamycin, Reflacine, Rifamipicin, Norfloxacin and Levofloxacin. This could be based on the fact that most microbial contaminants from various environmental sourcessuch as water, soil, animals or humansassociated with the microbial spoilage of these farm products were illegally exposed to most available antibiotics in the study area. This result conforms to the findings of Brown (2004).

Conclusionand Recommendations

This study revealed thatfour bacteria isolates namely; *Klebsiellapneumoniae,Streptococcus pyogenes, Salmonella typhi,* and *Staphylococcus aureus*wereisolated from the spoiled fruits and vegetables samples. However most of these microbial contaminants on these farm products were hundred percent (100%) resistant to most antibiotics used in this study, hence there is a need for Government to enlighten rural farmers on the need to practiceadequate precautions during preharvest and post-harvest of these farm products to ensure that microbial contaminants, particularly resistant organisms associated with microbial spoilage are curtailed and controlled.

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