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COMPARATIVE EFFECTS OF WASHING SOLUTION AND THE SURVIVAL OF *STAPHYLOCOCCUS AUREUS* ON TOMATOES

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ABSTRACT
Various types of fruits and vegetables are often eaten raw or consumed after minimum processing in which this has been connected to an increase in outbreaks of fresh produce associated with food borne diseases in the recent times. In order to determine the micro-flora, survival of Staphylococcus aureus on ripe and unripe tomatoes and to also asses the efficiency of some washing solutions, 100 tomatoes, both ripe and unripe were randomly bought from different selling points in Sagamu Markets, Ogun State Nigeria. Surface swabs of the tomatoes were cultured on both nutrient broth, and selenite F broth. The sample from nutrient broth was then sub cultured on Blood Agar, Mannitol Salt Agar, and MacConkey Agar, (MSA), Potato Dextrose Agar (SDA), while the one on Selenite F Broth was cultured on Salmonella Shigella Agar (SSA). Density of Staphylococcus aureus ATCC 25923 after pre-inoculation on ripe and unripe tomatoes, and after the application of washing solutions on tomatoes, was determined by surface spread count. The prevalence of any of the isolated micro-flora could not be significantly linked with either ripe or unripe tomatoes (0>0.05).Mean residual counts (log CFU/g) of bacteria was significantly higher in ripe tomatoes (3.52 ±2.40) than unripe tomatoes (2.30 ±1.96) after pre inoculation with staphylococcus aureus (t =2.75, P< 0.05). Among the washing solution, hypochlorite was observed to be twice more efficient than hydrogen peroxide and thrice efficient than saline solution. In conclusion, Staphylococcal colonization of tomatoes skin is significantly reduced in unripe tomatoes. And also, hypochlorite solutions are better than hydrogen peroxide and saline in Staphylococcal decontamination from tomatoes.

Key words: washing solution, *staphylococcus aureus,* tomatoes

INTRODUCTION
Fresh fruit and vegetables have gained increasing recognition as major components of a healthy and balanced diet owing to their health benefits against a range of illnesses such as cancers and cardiovascular diseases (ACMSF, 2005). Increasing health awareness has led to more consumption of fruits and vegetables in recent years However, they are often consumed minimally processed or raw and thus are increasingly being recognized as important disease vehicles (Tournas, 2005). Fruits and vegetables carry microbial flora while passing from the farm to the table. The produce is exposed to potential microbial contamination at every step including cultivation, harvesting, transporting, packaging, storage and selling to the final consumers (FDA, 2009).

Foodborne illnesses are associated with significant morbidity and mortality worldwide (Scallan *et al.,* 2011). Globally, an estimated 2 million people died from diarrheal diseases in 2005; approximately 70% of diarrheal diseases are foodborne. It is also estimated that up to 30% of the population suffer from foodborne illnesses each year in some industrialized countries (WHO, 2011), out of which about 2.2 million people get sick annually from eating contaminated leafy vegetables. That represents about 23% of the 9.6 million cases of food-borne illness each year. Previous studies have shown that produce-containing foods were the food source for approximately half of norovirus outbreaks with an identified simple food vehicle during 2001–2008 (Hall *et al.,* 2012).

Foodborne illnesses are a major threat to the health of people in Nigeria. The Federal Ministry of Health reported 90,000 cases of food poisoning in 2007, while in 1997 Local Government Health Systems profile for Nigeria reported leading causes of deaths in different geo-political zones to be foodborne associated illnesses which accounted for 25% of mortality followed by malaria (21%) and accidents (19%) **(FAO/WHO, 2002,)**. In Nigeria, a large proportion of ready-to-eat fruits and vegetables are sold on the streets, they are sometimes arranged on the trays and hawked, displayed on tables by the road sides and placed in a transparent bowls in ambient tropical conditions. The consumption of these foods is common in major urban and semi- urban Nigerian settings. Fruits vended on the streets is particularly patronised by all categories of people; both the rich and the poor and both elites and the uneducated. In that it is accessible, cheap, convenient and also assure food security for low income urban population and livelihood for a significant proportion of the populace (Ifenkwe , 2012).

Microbiological quality of street vended foods in some parts of Nigeria revealed high bacterial counts of 2.0x106 to 8.2x108 log10 CFU g−1 in a local market and a University cafeteria (Oranusi *et al.,* 2011). Another study in Eastern part of Nigeria, Calabar, reported a total bacterial count range $9.3x10^7$ cfu/ml- $1.8x10^7$cfu/ml from vegetables sold in Nigerian markets. In a recent study from Ondo State, raw tomatoes were screened and average microbial load 10 to 21 x 10^5cfu/mL was reported (Ajayi, 2013). Fresh-market tomatoes are a popular commodity in homes and food service around the world. The inherent risks of contamination by foodborne pathogens present a challenge to the produce industry and regulators. Since fresh-market tomatoes are intended to be consumed fresh, there is no "kill-step" in the processing that would eliminate pathogens in the event that tomatoes become contaminated (Maitland *et al.,* 2011. In order to reduce public health hazard posed by consumption of raw vegetables, pathogens colonizing farm produce, including tomatoes must be reduced in or on the product till they get to the consumers. However, regulatory agencies subjecting farm produce and food products to sanitary treatments does not appear to be given a deserving significant attention in Nigeria. Applications of disinfectant in food industry is an important tool in killing microorganisms and ensuring food quality for food preservation, shelf-life extension, equipment sterilization, elimination of undesirable flavor produced by bacteria during storage and food shipping have been adopted and practised in details in developed countries (GÃÃmez-LÃÃpez, 2007). In view of recent increase in foodborne disease attributed to vegetables that are consumed raw, much more attention is now being paid to the safety of vegetable and fruit products by including various stages of processing in its handling (Chao-Chin Chung 2011). Although, efficacy of the chemical treatments may be limited geographically, as a result of variation in prevailing standard of hygiene and wild streams of microbes. We therefore embarked on this study in order to compare the survival of *Staphylococcus aureus* on ripe and unripe tomatoes, and to also assess the efficacy of washing solutions.

METHODOLOGY

Sterile polythene bags were used to collect and transport the purchased samples to the Laboratory. 100 tomatoes, both ripe and unripe were randomly bought from different sellers in Sagamu markets and kept in sterile polythene bags containing ice packs and were brought to the laboratory. A sterile forceps was used to pick the samples and transferred into sterile distilled water was poured into these containers by gentle shaking. Serial dilution of the washings was done in which 1ml of each washing was added into a test-tube containing 9ml of sterile distilled water. Serial tenfold dilution was carried out from 10^{-1} to 10^{-10}. This was followed by pour plate technique in which 1ml aliquot of each dilution from 10^{-2}, 10^{-5} and 10^{-7} were cultured into blood agar, MacConkey Agar, Manittol Salt Agar (MSA). Selenite F'Broth , Salmonella Shigella Agar (SSA) (for bacteria) and Sabouraud Dextrose Agar (SDA) (for fungi).

Dilents were evenly spread on the plate with the aid of a sterile spreader in order for the microorganisms to produce discreete colonies during growth for easy enumeration. Distinct microbial growth after incubation at 37^0C for 24hrs were counted and recorded. All isolates producing characteristic growth on different agar were identified by standard Microbiological procedures (Cheesbrough Monica, 2000). Also for assessing the antimicrobial affects of ripe and unripe tomato skins, the efficacy of washing solutions both the ripe and unripe tomatoes. The samples were controlled by confirming them by culture to be free of *Staphylococcus aureus* contaminants. Both ripe and unripe tomatoes were inoculated by sub-merging them in standard suspensions of Staphylococcus aureus (ATCC 25923), equivalent to 1x 10^8 organism/ml. They were air dried and after 6hours, the tomatoes skin were swabbed and platted by surface spread for residual Staphylococcus density. Ripe tomatoes that have been inoculated in the same manner; were air-dried .They were later washed with different concentrations of hypochlorite, 3% hydrogen peroxide and brine solutions. After 2 minutes of air drying, the tomatoes skins were swabbed, and suspension made in glucose broth. They were then plated by surface spread method to determine the residual Staphylococcal density for each treatment.

RESULT

Effect of tomato ripeness on microbial isolates was assessed in Table 1. No significant difference was observed between microbial isolation in ripe and unripe tomatoes ($P>0.05$). However, in Table 2, the residual *Staphylococcal* density was significantly lower in unripe tomatoes, when compared with ripe ones. ($t=2.75$, $P<0.05$). After treatment of ripe tomatoes with different concentrations of washing solutions, in Table 3 significant difference was observed in Staphylococcal density under each solution ($P<0.05$), hypochlorite, hydrogen peroxide and Sodium chloride solutions recording the least effective concentrations of 100, 200 and 300ppm respectively.

Table 1. COMPARISON OF MICRO-FLORA OF RIPE AND UNRIPE TOMATOES

		Occurrence of bacteria					
Bacteria	**N**	**Ripe**		**Unripe**		χ^2	**P- Value**
		n	**(%)**	**n**	**(%)**		
Klebsiella spp	50	38	(76.0)	38	(76.0)	0.0001	> 0.05
Staph aureus	50	22	(44.0)	22	(50.0)	2.56	> 0.05
Bacillus spp	50	28	(56.0)	30	(60.0)	1.05	> 0.05
Escherichia coli	50	0	(0)	1	(2.0)	0.01	> 0.05
Staph.epidermidis	50	10	(20.0)	9	(18.0)	0.07	> 0.05
Pseudomonas spp	50	11	(22.0)	8	(16.0)	0.59	> 0.05
Proteus spp	50	27	(54.0)	26	(52.0)	0.04	> 0.05
Malassezia spp	50	50	(100.0)	50	(100.0)	0.10	> 0.05
Aspergillus spp	50	50	(100.0)	50	(100.00	0.10	> 0.05

N = Number of tomatoes examined

Table2: Survival of Staphylococcus aureus on ripe and unripe tomatoes

Ripe Tomatoes			Unripe Tomatoes		
Code	**Bacterial counts CFU/g ($x 10^6$)**	**Mass (g)**	**Code**	**Bacterial counts CFU/g ($x10^6$)**	**Mass (g)**
TR_1	0.25	24.50	TU_1	2.00	24.00
TR2	4.00	28.90	TU2	1.80	22.30
TR3	0.95	30.10	TU3	3.40	20.40
TR_4	0.90	21.40	TU_4	0.70	31.20
TR_5	4.20	22.30	TU_5	4.60	22.00
TR_6	1.40	24.40	TU_6	1.00	24.30
TR_7	1.50	23.00	TU_7	0.65	25.60
TR_8	1.20	31.00	TU_8	0.70	28.60
TR_9	1.50	25.60	TU_9	0.85	30.20
TR_{10}	9.80	27.80	TU_{10}	5.20	27.40
TR_{11}	4.60	25.40	TU_{11}	2.10	30.20
TR_{12}	7.60	27.60	TU_{12}	1.50	26.50
TR_{13}	4.90	20.70	TU_{13}	2.10	30.20
TR_{14}	3.70	22.30	TU_{14}	0.70	29.50
TR_{15}	3.10	21.10	TU_{15}	0.40	30.10
TR_{16}	2.10	26.30	TU_{16}	1.80	26.20
TR_{17}	1.50	21.10	TU_{17}	1.00	28.80
TR_{18}	0.76	22.20	TU_{18}	1.80	27.60
TR_{19}	4.60	21.10	TU_{19}	4.60	30.50
TR_{20}	1.40	22.20	TU_{20}	0.10	28.80
TR_{21}	3.70	27.40	TU_{21}	3.10	29.20
TR_{22}	3.20	23.10	TU_{22}	1.80	30.10
TR_{23}	5.20	22.80	TU_{23}	0.20	28.60
TR_{24}	1.20	29.40	TU_{24}	0.30	29.20
TR_{25}	4.20	21.70	TU_{25}	4.40	30.20
TR_{26}	7.60	28.50	TU_{26}	0.18	25.50

TR_{27}	3.70	26.50	TU_{27}	0.20	28.50
TR_{28}	3.10	30.10	TU_{28}	0.38	29.50
TR_{29}	9.80	28.50	TU_{29}	9.80	30.20
TR_{30}	4.60	26.50	TU_{30}	2.20	28.60
TR_{31}	1.40	24.60	TU_{31}	1.20	29.50
TR_{32}	1.80	30.10	TU_{32}	0.64	28.60
TR_{33}	1.80	28.50	TU_{33}	0.82	30.20
TR_{34}	4.80	29.40	TU_{34}	2.40	29.60
TR_{35}	0.20	25.20	TU_{35}	0.20	28.60
TR_{36}	2.20	26.40	TU_{36}	2.20	30.10
TR_{37}	0.90	28.90	TU_{37}	0.72	29.40
TR_{38}	4.20	29.40	TU_{38}	4.40	25.50
TR_{39}	9.80	30.20	TU_{39}	5.20	28.20
TR_{40}	7.60	28.40	TU_{40}	2.20	30.20
TR_{41}	3.20	27.80	TU_{41}	3.20	28.60
TR_{42}	3.80	30.30	TU_{42}	2.20	26.20
TR_{43}	4.60	28.50	TU_{43}	2.40	25.80
TR_{44}	0.65	30.40	TU_{44}	0.65	30.40
TR_{45}	4.20	28.80	TU_{45}	4.60	30.20
TR_{46}	0.72	30.20	TU_{46}	4.60	28.60
TR_{47}	4.80	28.60	TU_{47}	4.20	25.60
TR_{48}	4.00	27.50	TU_{48}	4.00	28.20
TR_{49}	4.60	28.30	TU_{49}	4.60	29.40
TR_{50}	4.90	30.20	TU_{50}	4.90	28.60

Type of tomatoes (Log CFU/g ml)	**Bacterial count Mean ± SD**	**t**	**P – value**
Ripe n = (50)	3.52 ± 2.47		
Unripe n = (50)	2.30 ± 1.96	2.75	< 0.05

Table 3: Efficacy of different concentrations of washing solutions on *Staphylococcus aureus* on tomatoes

Washing Solution (ppm)	**Concentration of washing solution mean ± SD**	**Bacterial count log CFU/ 1ml**	**F conc.**	**P-value**	**LSD for least efficient**
Hypochlorite	50	7.30 ± 0.70	7.26	< 0.05	100ppm
100	5.27 ± 0.46				
200	5.30 ± 0.76				
300	5.00 ± 0.50				
400	5.00 ± 0.05				
500	5.00 ± 0.50				
Hydrogen Peroxide	50 7.98 ± 0.25	64.59	< 0.05	200ppm	
	100	7.81 ± 0.37			
	200	6.42 ± 0.26			
	300	5.30 ± 0.7			
	400	5.30 ± 0.46			

	500	5.30 ± 0.36			
Saline solution	50	7.90± 0.36	138.05	< 0.05	300ppm
100	7.84± 0.16				
200	7.12± 0.37				
300	6.24± 0.23				
400	6.22± 0.24				
	500	6.20± 0.20			

DISCUSSION

Scientific data has shown that there has been an increase in the number of outbreaks of food-borne disease with consumption of fresh produce (Beuchat, 1997). However, the surface flora on fresh produce may reflect the environmental flora where the products are grown. As a consequence of this, one may expect a low occurrence of potentially pathogenic bacteria such as *Bacillus* cereus and *Listetria monocytogenes* that are commonly isolated from soil and environment. Contamination of the products may take place at all stages pre- and post - harvests and processing (De Roever, 1999). Possible sources of contamination are soil, faeces, manure of human or animal. Microorganisms have been shown to enter farm produce through various pathways available based on the structural integrity of the produce. Microbes strategically invade fruits and vegetables through damage to the natural structure which may include punctures, wounds, cuts and splits; and these injuries can occur during maturation or during harvesting and processing (Zhuang *et al.*, 1995). Several studies have shown that human pathogens can survive and grow in tomatoes and tomato products (Zhuang et al., 1995; Zhuang and Beuchat, 1996;). In the Nigerian postharvest system, packaging of tomatoes in cane baskets, lined with grasses, portends a potential problem of massive proliferation of inocula. In a Market basket survey carried out by Samish *et al* (1963) bacterial contaminants were frequently tomatoes and other products such as cucumbers peas and beans and less often in melons and bananas. Besides steaming down the occurrences of microbial contamination in foods, the incidence of human pathogens in Fruits and Vegetables is another uphill task in food safety. The situation even becomes mores ethical when dealing with tomatoes, which are often eaten raw. To create a public health hazard, pathogens colonizing farm produce, including tomatoes must be able to survive in or on the product till they get to the consumers and regulatory agencies have relentlessly subjected farm produce and food products to sanitary treatments. As a part of a Good Agricultural Practice (GAP) programme, Hernandez-Brenes in 2002, suggests that the background information about the land being used for agricultural production should be maintained. This will also help identify situations that could increase the risk for fresh produce contamination. Important information about the history of the land could include whether the land was previously used for animal feeding, for domestic animal production, as a garbage or toxic waste disposal site, or for mining, oil or gas exploration activities.

In this study, when the microflora of *Staphylococcus aureus* on both ripe and unripe Tomatoes was enumerated and later washed with different washing solutions using different concentration. There was no significant difference between microbial isolation in ripe and unripe tomatoes ($P>0.05$). But, in Table 2, the residual *Staphylococcal* density was significantly lower in unripe tomatoes, when compared with ripe ones. ($t=2.75$, $P<0.05$). After treatment of ripe tomatoes solutions, in Table 3 significant difference was observed in *Staphylococcal* density under each solution ($P<0.05$), hypochlorite, hydrogen peroxide and Sodium chloride solutions recording the least effective concentrations of 100, 200 and 300ppm respectively. Although, a study indicated that surface treatments are ineffective in reducing microbial populations that have been internalized into produce (Pao and Davis, 1999). However, Zhuang and Beuchat (1996) demonstrated that a 15 percent solution of

trisodium phosphate will completely inactivate Salmonella on the surface of tomatoes but will only result in a 2-log reduction (starting concentration = 5.5 logs) of internal populations. Pao and Davis (1999) found that immersing inoculated oranges in hot water or various chemical solutions (200 ppm chlorine, 100 ppm chlorine dioxide, 200 ppm acid anionic sanitizer, 80 ppm peroxyacetic acid, or 2% trisodium phosphate) was effective at reducing generic E. coli populations by 1.8 – 3.1 log cycles on surface areas except for the stem scar. Tested treatments reduced stem scar populations by about 1.0 log.

In this study, it was observed that there was no significant association between microflora of ripe and unripe tomatoes ($P>0.05$). The mean bacteria count of ripe tomatoes 3.52±2.4, was significantly higher than 2.30±1.96 of unripe tomatoes Also, it was found that hypochlorite solution is twice more efficient than hydrogen peroxide solution and thrice efficient than saline solution. Therefore adoption of use of Good Agricultural Practices (GAPs) during growing, harvesting, sorting, packaging, and storage operations for fresh fruits and vegetables is the key to preventing these pathogen contaminations. Ofor *et al.,* 2009. Much more proper washing and storage at safe temperature before consumption should be encouraged among consumers.

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