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Isolation, identification and characterisation of bacteria from ripe tomatoes grown under different agronomic conditions

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ABSTRACT

Background: Tomatoes are exposed to potential microbial contamination. The objective of the study was to determine the presence of bacteria in and on tomato fruits grown under different agronomic conditions in Lusaka. **Methods:** Bacteriological survey of 27 samples of fresh tomatoes collected from different sources was carried out and analysed. Analysis was done by culturing the tomatoes on different medias: MacConkey, chocolate, blood and shingella and salmonella agar. Following isolation of pure colonies, biochemical tests were done, SIM, catalase, oxidase, TSI, simmons citrate agar and the urease test. **Results:** Nine bacteria were identified: Escherichia coli, citrobacter, enterobacter, Vibrio spp, Shigella, Salmonella, Klebsiella and Streptococcus aureus. E. Coli and Staphylococcus aureus were found to be the dominant species. Results indicated that the microbial load in samples from fields near the sewer was higher than in samples from far away from the sewer system. **Conclusion**: Tomatoes grown near the sewer streams are contaminated with bacteria from entrobactriaceae family.

Key words: Bacteria, Contaminants, Pathogens, Tomato

Introduction

The vulnerability of the tomato to contamination by mostly salmonella and other major human pathogens like vibrio cholera and Escherichia coli bacteria that are common cause of foodborne illness has remained a mystery. If the illness is not detected early and controlled, it can cause death. According to [1], vegetables and fruits have been associated with outbreaks of foodborne diseases in many countries. Organisms involved include bacteria, viruses and parasites. According Olsen et al [2], water can be a carrier of many microorganisms including pathogenic strains of *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, *Shigella*spp., *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclosporacayetanensis*, *Toxi-plasmagondii*, and the Norwalk and hepatitis A viruses.

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Even small amounts of contamination with some of these organisms can result in food borne illness

Food borne illness is most often associated with gastrointestinal symptoms, including diarrhoea, nausea, and/or vomiting. Food borne illness may be bacterial, viral, or parasitic in nature. Although most ailments have acute symptoms that resolve within a week, complications including severe dehydration, bacteraemia, renal and hepatic impairment, neurologic symptoms, miscarriage, and surgical complications can occur [3].The frequency of microorganisms such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter* and *Cyclospora* are subject to wide variation from study to study. The prevalence of *Campylobacter* is mostly at low levels, whereas the prevalence of *Salmonella* is higher. *E. coli* O157:H7 and *Listeria monocytogenes* were in general found in a higher frequency compared to *Salmonella.*[4,5,6,3,7]. Hedberg et al [3] concluded that the presence of pathogenic microorganisms on raw tomatoes vary considerably. According to Samish et al [17], three species of *salmonella* are recognized; *Salmonella typhi*, *S. Choleraesuis* and *S. enteriditis*, which have more

than 1400 antigenically distinct serotypes. The possibility of uptake of bacteria by roots of hydroponically grown tomato plants was investigated and found that bacteria can exist as endophytes in tomato plants grown under conditions that simulate commonly used agronomic practices. It was conclude that the point of entry of bacteria into plants includes stomata, germinating radicles, and areas of emergence of lateral roots. However, the major points of entry appear to be wounds that naturally occur as a result of growth through root hairs, at the root emergence, and at epidermal conjunctions. [8,2,9,10], characterized a three-phase process in which roots of hydroponically grown tomato plants become infected. The inner tissues of sound produce are generally considered to be sterile as stated by [11]. However, endophytic bacteria are known to reside in a wide range. Sanitation contributes to produce safety [12,13,14,15].

Foodborne diseases are widespread and growing public health problems both in developed and developing countries like Zambia. Salmonellosis is the major problem in most countries though in Zambia not much work has been done to show and publicise information on this disease. Cholera and salmonellosis are major public health hazard in Zambia and tomatoes are among the important vehicles of transmission of these diseases. The objective of the study was to isolate, identify and characterised bacteria from ripe tomatoes grown under different agronomic conditions.

Methodology

Sample collection

A total sample of nine tomatoes were collected from each of three different sources, namely, ZAF Lusaka (far from the sewer system), Kaunda Square and Garden Compound (both near the sewer system). From each location three tomatoes were collected from a farmer who used chemical fertilizer, three from a farmer who used sludge as mature and the other three from a farmer who used sewer water/Tap water. Samples were triplicate for each sampling site to bring the total number to 27. The figures from each sampling site where tomatoes were collected are shown as figure 1 to 9.

Fig 1: Tomatoes fields at the ZAF area, Kaunda Square, Garden Compound

Sample preparation

Nine tomatoes (from different sources) were immersed in 70% alcohol for 15 minutes for surface disinfection and then air dried for15 minutes. A sterile surgical blade was used to cut the tomato in two equal parts. For tomatoes were an outer part was used, sterile swabs were used to swab the outer body of the tomatoes and then pated directly on four different media.

Sample inoculation and culture of bacteria

Four different culture media (MacConkey, blood agar, chocolate agar and *shigella salmonella* Agar) were used. The media were prepared according to the manufacturers recommendations. The media were reconstituted in distilled water and then sterilized at 121^{o}C for 15 minutes. The swabs were inoculated directly using streaking method for each tomato on all the four media. For the fruit pulp, (inner part of the tomato), both spreading and streaking methods were used. The inoculated plates were then incubated at 37^{o}C for 24 hours.

Identification of bacteria

Discrete colonies were transferred from the petri dish to another new petri dish of nutrient agar and then streaked to isolate pure colonies. Plates were then incubated at 37^{o}C for 24 hours. At this step the pure culture of the microorganisms were obtained. The gram stain technique was then used to study the staining properties of the bacterial colony and the bacteria were identified using biochemical tests.

Results and data analysis

Results

Isolated bacteria

Various bacteria species were observed in the tomato fruits, although variations were observed from one sampling site to another. At ZAF area no bacteria were isolated from the fruit pulp and only two (table 1) types of bacteria were isolated from the outer part of the tomato.

Table 1: Bacteria isolated from tomatoes from ZAF

TYPES OF MANURE	FRUIT PULP	OUTER PART
Chemical fertilizer	No bacterial growth(isolate)	*Escherichia coli* *Staphylococcus aureus*
Tap water	No bacterial growth(isolate)	*Escherichia coli* *Staphylococcus aureus*
Chicken manure	No bacterial growth(isolate)	*Escherichia coli* *Staphylococcus aureus*

Samples collected from Garden compound, Showed bacteria presence in the fruit pulp and outer part as shown in Table 2.

Table 2: Bacteria isolated from tomatoes from garden compound

TYPES OF MANURE	FRUIT PULP	OUTER PART
Chemical fertilizer	No growth	*Escherichia coli* *Staphylococcus aureus*
Sewer water	*Escherichia coli* *Shigella. sp* *Salmonella. sp* *Streptococcus. sp* *Staphylococcus aureus*	*Escherichia coli* *Staphylococcus aureus*
Sludge	*Escherichia coli* *Shigella sp* *Salmonella sp* *Streptococcus sp* *Staphylococcus aureus*	*Escherichia coli* *Staphylococcus aureus*

Due to the treatment of chemical fertilizer, bacteria were found only on the outer part of the tomato and these bacteria could be from the air or soil. The sludge or manure and sewer water used are always contaminated with bacteria from human feaces thus increasing the chances of bacteria transportation into the tomato pulp. Specimen from Kaunda Square had bacterial contamination in both outside and inside as shown in table 3.

Table 3: Bacteria isolated from samples from Kaunda Square

TYPE OF MANURE	FRUIT PULP	OUTER PART
Chemical fertilizer	*Escherichia coli*	*Escherichia coli*
Sewer water	*Shigella* *Salmonella* *Streptococcus* *Escherichia coli* *Staphylococcus aureus* *Klebsiela* *Vibrio spp* *Enterobacteria*	*Escherichia coli* *Staphylococcus aureus*

	Citrobacter	
Cattle manure	*Shigella* *Salmonella* *Klebsiella* *Escherichia coli*	*Escherichia coli* *Staphylococcus aureus*

The sewer water had the largest number of bacteria. And some bacteria like *Vibrio* were not found in tomatoes grown with cattle manure. This showed that some bacteria were mostly found in water contaminated with animal feces. Fertilizer is usually treated, thus if the treatment is effective 99% of bacteria are killed and since the fertilizer is dry, it does not support bacterial growth. Bacteria found are presented in table 4.

Table 4: Morphological identification

NAME OF BACTERIA	COLONY COLOR	SHAPE	GRAM STAIN
Staphylococcus	Yellowish colonies on blood agar	Cocci	Gram positive
Streptococcus	Yellowish-greenish colonies on blood agar	Cocci	Gram positive
Enterobacter	Large mucoid colonies with pink centres on MacConkey	Rods Lactose fermenter	Gram negative
Citrobacter	Large spreading light pink/yellow colonies on MacConkey.	Rods Lactose fermenter	Gram negative
Vibrio spp	Tiny slightly pinkish colonies on MacConkey.	Rods	Gram negative
Shigella	Creamy like colonies on blood agar. Colorless on MacConkey	Rods Non lactose fermenter	Gram negative
Salmonella	Tiny black colonies on XLD and tiny colonies on *Shigella-salmonella agar*	Rods Non lactose fermenter	Gram negative
Klebsiella	Mucoid pinkish colony on MacConkey	Rods Lactose fermenter	Gram negative
Escherichia coli	Pink mucoid colonies on MacConkey.	Rods Lactose fermenter	Gram negative

Analysis of the bacteria that was cultured on the various media indicated both gram positive and negative bacteria from the sampled tomatoes. Of the bacteria observed, some of them were rod shaped while other were cocci shape. In addition the morphological appearance of the colonies was noted as part of the isolate characterization

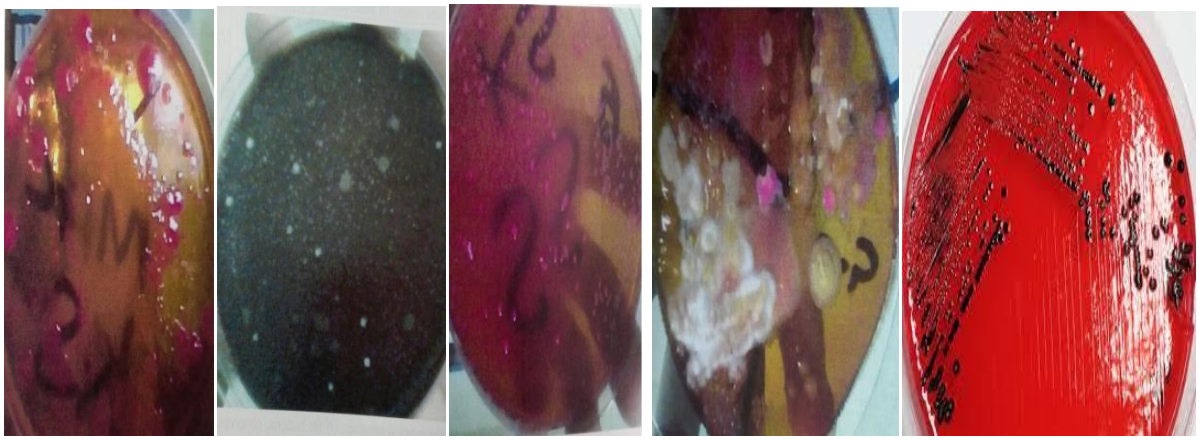

Fig 2: inoculates on MaCconkey, Chocolate, *shigella-salmonella*,blood and XLD agar

Table 5: Colony identified per sampling site

SAMPLING SITE	COLONIES IDENTIFIED
ZAF	5 /50
Kaunda Square	15 / 1000
Garden compound	10 / 1000
Total	30

In total 30 colonies out of numerous colonies observed were identified. The identification was done by first growing pure colonies as shown below which were later subjected to gram stain and lastly biochemical tests.

Fig 3: Pure colonies inoculated on blood agar, nutrient agar and MacConkey agar

Table 7: Gram stain identification

SAMPLING SITE	GRAM –VE BACTERIA		GRAM +VE BACTERIA
ZAF	*Escherichia coli*		*Staphylococcus aureus*
Kaunda Square	*Shigella* *Escherichia coli* *Vibrio spp* *citrobacter*	*Salmonella* *Klebsiella* *Enterobacter*	*Staphylococcus aureus* *Streptococcus spp*
Garden compound	*Shigella* *Escherichia coli*	*Salmonella*	*Staphylococcus aureus* *Streptococcus spp*

Of the total number of species, a greater percentage of bacteria observed were from Kaunda square. ZAF had the least and this can be explained as being due to the fact that ZAF is located far from contaminated water, whereas Kaunda square and garden compounds are near the sewerage treatment ponds and use contaminated water for irrigation.

Biochemical identification

Of the 30 isolates cultured and subjected to biochemical analysis, 16 were identified as shown in the table 8. A total of 14 isolates plus the numerous colonies that grew could not be identified due to lack of resources.

A combination of morphological characteristics and biochemical reactions were used to identify the bacteria based on standard bacteriological manuals.

Table 8: Organisms isolated from tomatoes

Site	Bacteria isolated	Total number of identified bacteria	Total number of unidentified bacteria	Number and characteristics of fungus observed
Zaf	**Outer part** • *Escherichia coli* • *Staphylococcus aureus* **Fruit pulp** No bacteria isolated	**2**	**3**	**3 -white -pinkish -black**
Kaunda square	**Outer part** • *Escherichia coli* **Fruit pulp** • *Shigella* • *Salmonella* • *Streptococcus* • *Escherichia coli* • *Staphylococcus aureus* • *Klebsiella* • *Vibrio spp* • *Enterobacteria* • *Citrobacter*	**9**	**6**	**-white -black**
Garden compound	**Outer part** • *Escherichia coli* **Fruit pulp** • *Shigella* • *Salmonella* • *Streptococcus* • *Escherichia coli* • *Staphylococcus aureus*	5	**5**	-white 3 -black -yellowish

Data analysis

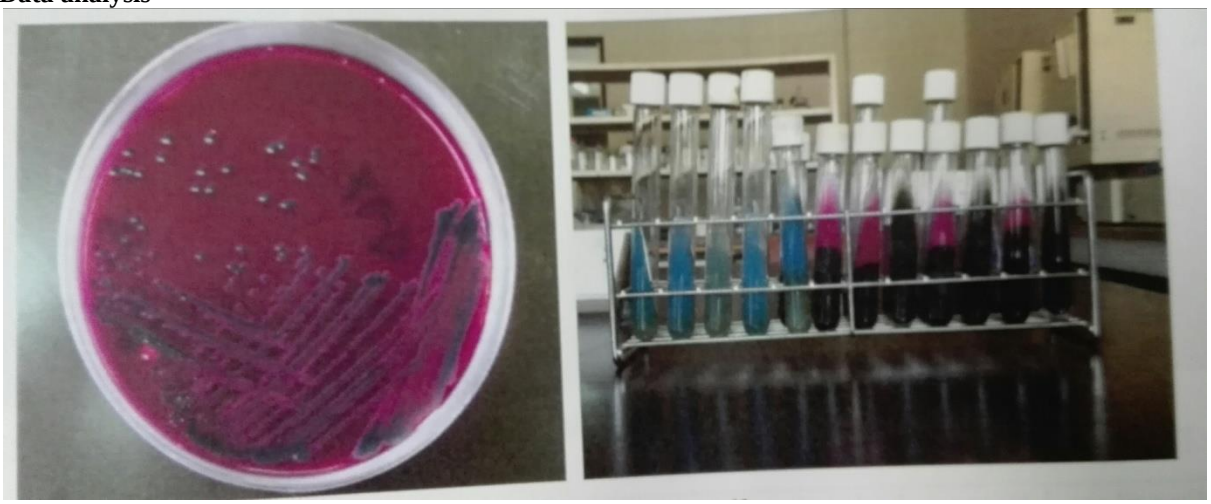

Fig 4: Isolation and Biochemical analysis of *Salmonella*

The colonies that grew on XLD were biochemically analysed and the figure 10 shows color change. Observations made on the biochemical tests (TSI and Citrate) showed that *Salmonella* strains produces hydrogen sulphate differently in that some strains changes the whole test tube to black while other strains changes the slant to pink and buttom to black as shown on the figure 10.

Fig 5: shows the analysis of *E. Coli* using SIM

Table 9: identification of gram negative fermentative enterobacteriacea

Isolate No.	SIM			TSI			Citrate	Catalase	Oxidase	Urease	Probable bacteria
	H_2S	I	M	S	B	G/H_2S					
01	+ve	-ve	+	K	A	-/+	+ve	-ve	-ve	-ve	*Salmonella*
02	-ve	+	+	A	A	+/-	-ve	-ve	-ve	-ve	*E.coli*
03	-ve	-ve	-ve	A	A	+/-	+ve	-ve	-ve	+ve	*Klebsiella*
04	-ve	-ve	+	K	A	+/-_	+ve	-ve	-ve	-ve	*Citrobacter*
05	-ve	-ve	+	A	A	+/-	+ve	-ve	-ve	+ve	*Enterobacter*
06	-ve	-ve	+	A	A	-/-	-ve	-ve	+ve	-ve	*Vibrio spp*
07	-ve	-ve	-ve	K	A	-/-	-ve	-ve	-ve	-ve	*Shigella*

KEY

A= Acid
K= Alkaline
-/-ve= Negative
+/+ve= Positive
S= Slant of the tube
TSI= Triple sugar iron slants
M= Motility
H_2S= Hydrogen sulphide production
I= Indole
G= Gas production
B= Bottom of the test tube
SIM= Sulphur, Indole Motility media

From table 9, the probable microorganisms were known. This was achieved through the biochemical tests (color change), as shown Figure 12.

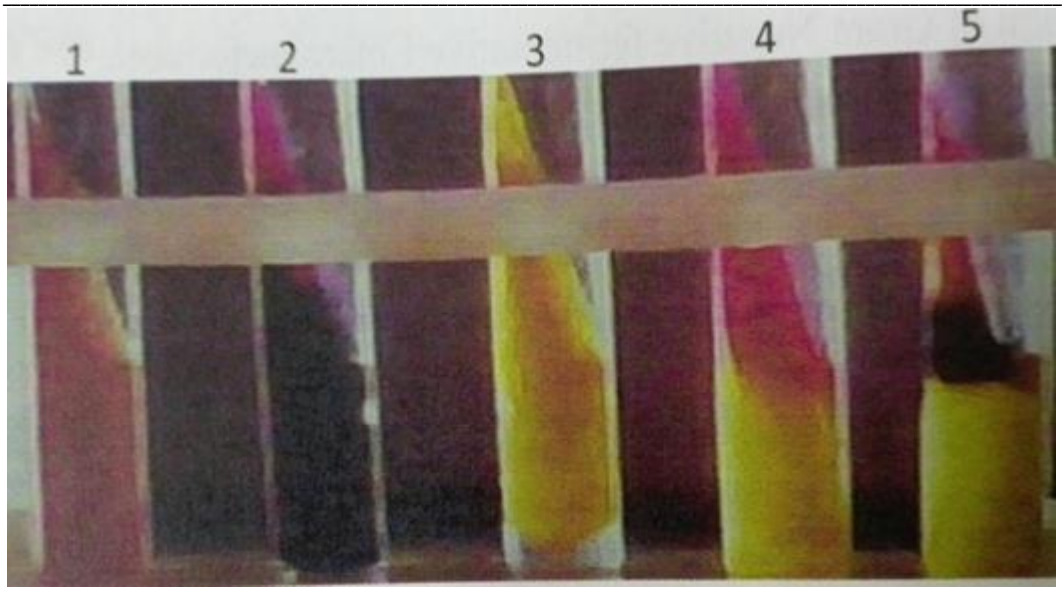

Fig 6: TSI (triple sugar iron slants)

Test tube 1: control
Test tube 2: *Salmonella* strain 1
Test tube 3: *E. coli* strain
Test tube 4: *Shigella strain*
Test tube 5: *Salmonella* strain 2

Figure 12 shows colour changes due to presence of different strains of bacteria after 24 hours incubation on TSI media. Strains of *Salmonella* produce hydrogen sulphate thus turning the color from red to black (butt) and pink (slant). *E. Coli* is a lactose fermenter, turning the colour to yellow and produces gas which demonstrated by cracks and space at the bottom of the test tube. *Shigella* is differentiated from *E.coli* in that it turns the colour to yellow (butt) and pink (slant). While some strains of *Salmonella* turns the colour to yellow (butt), pink (slant) and black on the middle.

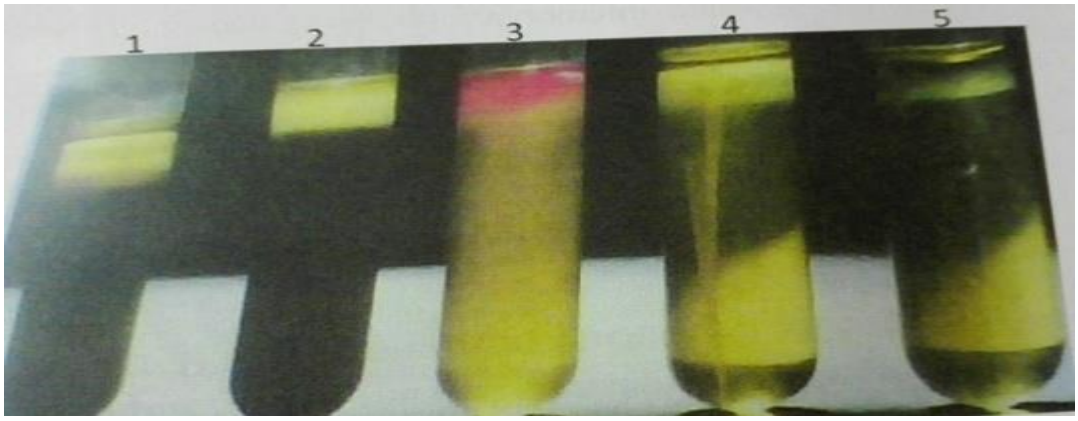

Fig 7: Sulphur, Indole Motility media

Table 10: SIM Analysis

Test tube No.	H2S	Indole	Motility
1	+ve	-ve	-ve
2	+ve	-ve	-ve
3	-ve	+ve	-ve
4	+ve	-ve	+ve
5	+ve	-ve	-ve

Identification of gram positive bacteria

A number of bacterial isolates were isolated on various media. Two types of colonies were identified as colony one(1) and colony 2. Colony 1 was a pure white colony with a morphology of a cocci and was gram positive. On identification with catalase test, there were no bubbles and was identified as *streptococcus* species of bacteria. Colony 2 was identified as *staphylococcus* spp as there was gas production on catalase reaction test.

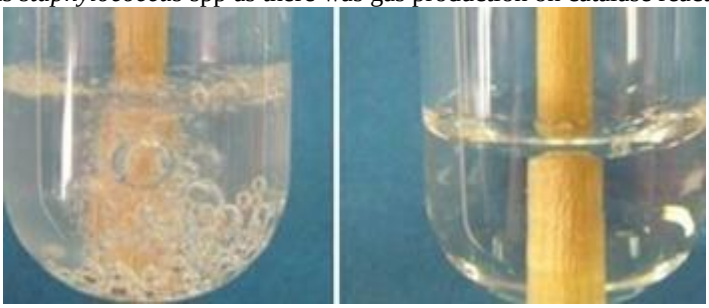

Fig 8: Catalase test

- First one : Bubbles production
- Second one: No bubbles production

Colony 2 was further grown on MSA, the media turned yellow from red and yellow colonies grew on the media. From this observation, the probable bacterium was *staphylococcus aureus.*

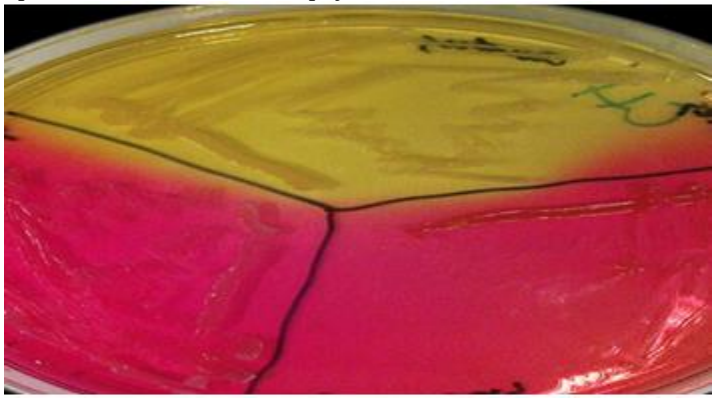

Fig 9: *Staphylococcus aureus* on MANITAL SALT AGAR

Discussion

Tomatoes from ZAF had bacteria only on the outer part of the sample, where *E. Coli* and *S. Aureus* were identified. The inner part of the sample was found to be sterile as no bacteria was isolated. The findings were attributed to soil and air contamination. Tap water is used for irrigation at this site, and since the water is treated and not contaminated, it washes away a number of microorganisms from the outer surface of the tomatoes thus reducing the number of microorganisms from soli and air to fruit.Samples from Kaunda square had the largest number of public health significance bacteria (*Shigella*, *Salmonella*, *Streptococcus*,

Escherichia coli, Staphylococcus aureus, Klebsiela, Vibrio spp, Enterobacteria, Citrobacter). This was due to the usage of sewer water which is heavily contaminated with dangerous microorganism for irrigation of vegetable crop, tomatoes gardens are near the ponds. Bacteria from the ponds can easily be absorbed into the gardens with tomatoes. Also during watering, sewer water increases the number or load of microorganisms on the tomato and soil, instead of reducing the number of bacteria from the soil and air on the outer part of the tomato. As the number of bacteria increase, synergism is achieved and through this, bacteria can find ways of getting into the tomato were conditions are favourable. The entry of bacteria into tomato is achieved by producing certain enzymes that facilitate their quick entry. The soil and water around the roots of the tomato plants is heavily loaded with numerous different types of microorganisms. It has been observed by microbiological scientist that as the plant absorbs water (sewer water) from the soil, microorganisms are also absorbed together with water (the enzymes they (bacteria) produce facilitate their absorption) and these are microorganisms that reflect in the tomato fruit (tomato pulp).Tomato samples collected from Garden compound had bacteria both in the pulp and outer part of the sample. Bacteria that were successfully identified included *Shigella, Salmonella, Streptococcus, Escherichia coli, Staphylococcus aureus.* In this area, untreated sewer water is used for irrigation direct on vegetable and tomato plants. Less number of bacteria was successfully identified even though the area was highly contaminated. This is because, if the area is more contaminated (too numerous bacteria) bacteria starts to compete for the little nutrients on the media leading to growth suppression of the bacteria which cannot stand the environment thus resulting in growth bacteria which are labile to environmental insults. Some bacteria produce antibiotics which inhibits the growth of other microorganism and these bacteria requires special media for growth which was not available.

A stem depression, which remains after the tomato stem has been pull off, is very often populated by bacteria more than the underlying fruit pulp [16]. This fact suggests that bacteria may penetrate into the inner pulp from this area. Bacteria belonging to the same families as those found on leaves of the surface of the tomato itself could be found in the inner pulp of the plant. Thus it could well be envisaged that some bacteria find a suitable medium for survival and multiplication in the stem depression, and penetrate from there into the growing fruit thus contaminating the fruit. Such theory also finds support from the findings made by Samish [17].

The first finding indicates that *Serratia* suspensions, which had been applied upon the sepals of growing tomatoes, were later recovered in the stem depression and later in the tomato pulp. Secondly, the central core of the tomato indicated higher bacterial concentration than the peripheral tissues (except the stem depression).Representative of the two bacterial families most often found within the tomatoes belong to the normal epiphytal flora of these plants. They presumably progress into the fruit tissue more readily than other numbers of this flora because of their comparatively smaller size and motility [17]. These bacterial isolated may affect the nutritive value of the tomato because they utilize the tomato`s nutritive value and minerals to multiply and in the process degrade the nutritive value of the tomato fruit.The use of animal manure leads to a potential risk of transmitting bacteria, but the key to using it as a fertilizer, is knowing how to use it safely and correctly. Chicken manure can also contain pathogen like *Escherichia coli* and *Salmonella.* Under normal circumstances the manure undergoes decomposition when temperatures are maintained at between 54^{o}C and 66^{o}C for a number of days. Thus treatment of this nature reduces the number of microorganisms in the manure, reducing the chances of contaminating fruits and vegetables which utilize chicken manure.

The bacteria isolated from the study were identified as belonging to the family of enterobacteriaceae. Enterobacteriaceace family is divided into two groups of lactose fermenters (*E. Coli, Klebsiella* and *Enterobacter),* and non lactose fermenting group *(Shegella, and Salmonella).* A large number of bacteria from this family were found because the contamination is always associated with animal manure. These bacteria are found in the digestive tract of animals and as animals pass their waste these bacteria are discharged to the environment. These bacteria become pathogenic to human once in the environment.

All the identified bacteria are of public healthy significance to human beings. In animals these bacteria are needed as part of the normal flora. They only become deadly as the number increases because they start working together and as this process continue (synergism) they start to exchange their genetic materials through conjugation, transformation and transduction. Some bacteria such as *E. Coli,* in this kind of arrangement end up producing *Shegella* like toxin that can easily cause diseases.

As earlier mentioned, the microorganisms documented are of public healthy significance. Of these, *Salmonella* is the most deadly because it can cause diseases if few cells (15 to 20) are ingested. This is totally dependable on the age of the patient, and the health status of the

host (strength of the immune system). Depending on which strain is ingested, this bacteria can cause diseases within 6 to 48 hours. *Vibrio spp, Shigella, Klebsiella* and *E.coli* can be very pathogenic as well.

All the bacteria identified in this research have been identified by other researchers in other countries as indicated in the literature review. And as other researchers indicated, the considerable fluctuations in bacterial content of the tomatoes obtained from different fields could be due to a number of factors encountered during the research, such as climate influences, and agrotechnical practices.

While every effort must be made to prevent contamination of tomatoes during production, transportation, processing and handling, much improvement is still needed in some part of the world especially Africa-Zambia if hygienic production of tomatoes is to be ensured.

Unless measures are taken to decontaminate them, their safety may not be assured. This study has provided an overview of the hazards associated with tomatoes eaten raw.

Conclusion and recommendation

Conclusion

Bacteria from tomatoes grown under different agronomical conditions were culture and isolated. The isolates which were characterized as being rod shape and cocci shape were identified using different biochemical tests. From this study it can be concluded that:

1. Tomatoes grown near the sewer streams were contaminated with bacteria from entrobactriaceae family, of public health significance, which include; *E. Coli, Salmenella, Shigella, Klebssiella, S. Aureus streptococcus Citrobacter, Enterbacter* and *Vibrio.*
2. Fresh tomatoes from the local markets could be harbouring many microbial contaminants and pathogens.

Recommendations

Application of the principles from the following recommendations may result in reduced risk of illness associated with consumption of raw tomatoes in salads and many other undercooked foods.

1. Further basic and applied research is needed in order to better understand modes of contamination of raw tomatoes before harvesting and during post-harvest handling and when subjected to various agronomic practices. Furthermore, determination of conditions that influence bacterial attachment, growth and survival on tomatoes may be significant.
2. Control of pathogenic microorganisms should involve multidisciplinary teams with a wide range of technical, sociological, educational and administrative skills through health education programmes especially to small scale farmers.
3. Hygienic principles should be applied during production.
4. Epidemiological studies should be carried out to address the role of raw tomatoes as vehicles of microorganisms capable of causing diseases.
5. There should be continual training of tomato growers and handlers at all levels in order to control microbiological hazards that may be influenced by current and changing cultural agronomic processing, distribution and preparation practices.
6. Professional and domestic food grower and consumers should be better educated about the principles of personal hygiene and decontamination of raw tomatoes grown under bad agronomic conditions.

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