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Determination of microbiological quality of vegetables irrigated with Kano Abattoir wastewater

Abubakar, M.A[1,*], Olayemi, A[1] and Babatunde, K[1,2]

[1]Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, PMB 3011, Kano, Nigeria
[2]Department of Microbiology, Faculty of Life Science, Obafemi Awolowo University, Osun, Osun state, Nigeria
[3]Department of Zoology, Faculty of Science, University of Lagos, Akoka, Nigeria.

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⌧*Corresponding author:
Musbahu A Abubakar
Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, PMB 3011, Kano, Nigeria
Email: julaibib011@gmail.com

Abstract

Microbiological contamination of vegetables occurs directly along the food chain resulting in a serious Health problem and consequently leading to death during the acute phase of the microbial infection. A total of 15 different samples were randomly purchased and analyzed for the presence of enteropathogenic organisms. Enumeration of aerobic mesophilic bacteria, fungi and coliform counts were carried out using standard procedures which is the use of most probable numbers (MPN). Isolation and identification of some pathogens was also carried out using standard procedures. The mean count of all the three (3) different samples (cucumber, tomato and lettuce) were exceeded the maximum acceptable limit (10^5cfu/g/ml) set by the Food and Agricultural Organizations (FAO). The highest mean count for both bacteria and fungi in all the 15 samples; cucumber with $1.52x10^5$ cfu/g of bacteria and $1.01x10^5$ cfu/g of fungi, tomato with $1.34x10^5$ cfu/g of bacteria and $1.06x10^5$ cfu/g of fungal, whereas, lettuce with $1.18x10^5$ and $1.29x10^5$ of bacteria and fungi respectively. Out of 15 different samples examined the occurrence of enteropathogenic organisms in all the sample were found to be 65% of cucumber while tomato had 60% followed by lettuce with 60%. This shows that cucumber samples are more contaminated due to the higher percentage of occurrence the enteropathogenic organisms in it. The results indicated that the vegetable samples examined in this study did not meet bacteriological quality standards. The implications of the results on human and environmental health are discussed.

1. INTRODUCTION

Vegetables serve as a major part of our food supply and harbors a number of pathogenic microorganisms, which may be dispersed over the plants or appear as micro colonies embedded in the plant tissues. Common vegetables used include cucumber, pepper, tomatoes, onions, red onions, carrots, lettuce, spring onions and radishes and other ingredients such as olives (Osamwonyi et al., 2013). Vegetables have been associated with the outbreaks of food borne disease in many countries (Rahman et al., 2012). Mainly microorganisms and/or their toxins can cause food borne illnesses. Cultivation of vegetables may largely account for such pathogenic contamination. Manures used to promote the growth of crops and vegetables contain a large number of pathogenic microorganisms including *Salmonella*, *Escherichia coli* O157:H7, *Bacillus anthracis*, *Mycobacterium* spp., *Brucella* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Klebsiella* spp., and *M. paratuberculosis*. (Rahman et al., 2012). Therefore, the organic fertilizers applied in the fields pose a great risk towards public health.

The aim of this work is to determine the microbiological quality of vegetables irrigated with Kano Abattoir wastewater. Which was achieved through the followings objectives:

1. To determine the microbiological quality of vegetables irrigated with Kano Abattoir wastewater.
2. To determine the aerobic mesophilic bacterial and fungal count of the vegetables.
3. To enumerate the coliform of the vegetables.
4. To detect the specific pathogenic organisms such as (*Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*) in vegetables.

2. MATERIALS AND METHODS

2.1. Source and Samples Collections

A total of 15 samples were collected from Kano Abattoir wastewater farmland located at Kwakwachi, Nomansland, Fagge local government Kano State. Each sample were properly identified and labeled, placed separately in a sterile plastic bag and transported to the laboratory in an icebox within 2-4 hours of collection (Falomir et al., 2010).

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2.2. Sample Preparation

Samples were prepared according to the method of FAO (1979).

2.3. Enumeration of aerobic mesophilic bacterial count in vegetable sample

1 mL of homogenate was serially diluted in five test tubes containing 9 mL of peptone water. Then to each concentration of 1 mL of each sample was inoculated into a petri dish and pour plate method was done by pouring 15 mL of Nutrient agar on the inoculums. The media was swirled and allowed to solidify. Then the petri dishes were incubated upside at 37 ^{o}C for 24-48 hrs. The plate were counted using Gollenkam colony counter for the accurate result.

2.4. Enumeration of coliforms in vegetable sample

1 mL of sample homogenate was serially diluted in five test tubes containing 9 mL of peptone water to give a dilution of $1x10^1$ up to $1x10^5$ respectively, which was followed by presumptive and confirmatory test.

2.5 Presumptive test of coliforms

Three test tubes each containing 10 mL of MacConkey broth with inverted Durham test tube were each inoculated with 1 mL of the vegetable homogenate to give a dilution of $1x10^1$. From this dilution, 1 mL each was transferred to another two test tubes of MacConkey broth to make a dilution of $1x10^2$. The same procedure was followed to give a dilution of $1x10^3$. All test tubes were incubated at 37 °C for 24 hrs. The number of total coliforms is determined by counting the number of tubes giving positive reaction that showed gas and acid production after 24 hrs. The negative tubes were further re-incubated for 24 hrs. Positive tubes were recorded. The same procedure was done on the water samples.

2.6 Confirmatory test for coliforms

A loopful of coliforms from each gas positive tube from the presumptive test was inoculated into a separate tube containing 10 mg Brilliant Green Lactose Bile Broth (BLGB). The test tubes were incubated at 37 °C for 48 hrs. The formation of gas confirmed the presence of coliform bacteria. The number of positive tubes was recorded. The number of tubes giving a positive reaction was compared to a most probable number (MPN) index table and the number of bacteria present in it was recorded.

2.7 Isolation of *E. coli*

These tests were carried out in accordance with the procedure described by Adrews, et al., 2001. A loopful from each positive tube of the presumptive test was transferred to a separate tube containing BGLB and re-incubated at 37 ^{o}C for 48 hrs. Gas and acid production were recorded. Gas positive tubes were streaked on Eosin Methylene Blue agar (EMB) medium and incubated at 37 ^{o}C for 24 hrs. Golden cream colonies with green metallic sheen confirmed the presence of *E. coli*. (Chesbrough, 2004).

2.8 Isolation of *Salmonella* and *Shigella*

Sample from the test tube that produced gas was streaked using a loopful wire on the Salmonella Shigella agar (SSA) agar and incubated for 24 hrs. Presence of colorless, transparent, with a black center if H_2S is produced indicated the presence of *Salmonella*. Whereas, presence of clear, colorless, transparent colonies indicate *Shigella* (Cheesbrough, 2004).

2.9 Isolation of *S. aureus*

Inoculum from test tube that produced gas was streaked using a loopful wire on the mannitol salt agar (MSA) and incubated at 37 ^{o}C for 24hrs. *S. aureus* ferments mannitol and turns the medium yellow. (Cheesbrough, 2004).

2.10 Gram staining

These tests were done according to Chessbrough, 2005. The Gram staining is by far the most widely used procedure for staining bacteria and separating it into two major groups: Gram positive and Gram negative. Thin film of specimen was spread over a clean grease free slide and allow to air dry. It was then fix by passing it over a Bunsen flame thrice. The film was flood with crystal violet and left for 60 seconds. The slides were washed off and flooded the stain with lugol's iodine and (mordant) and left for 60 seconds. The iodine was then wash-off and the slide was decolorized with acetone (decolorizer) for a second. The slide was wash with safranin (counter stain) for 60 seconds and air-dried. The slide was observed at x100 magnification. A purple color signifies Gram positive, whereas red signifies Gram negative.

2.11 Biochemical Tests

The biochemical tests; indole, vogas proskaeur, citrate, catalase, coagulase, triple sugar iron (TSI) were carried out according to the method described by Cheesbrough, 2005.

3. RESULT AND DISCUSSION

A total of 5 cucumbers, 5 tomatoes and 5 lettuces samples were collected from the Kano Abattoir wastewater farmland located at Kwakwachi, Nomansland, Fagge local government Kano State, Nigeria. Based on this research study, it has been observed that, total viable and coliform counts of the all the 3 samples were very high (Table 1). This revealed that, the cucumber had the highest mean bacterial counts of $1.52x10^5$ cfu/g and fungal counts of $1.10x10^5$ cfu/g, whereas, the tomato had the lowest mean of bacterial and fungal counts of $1.34x10^5$ cfu/g and $1.06x10^5$ cfu/g, and lettuce had mean bacterial and fungal

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counts of $1.18x10^5$ cfu/g and $1.29x10^5$ cfu/g respectively compared with the initial sample. According to the Food and Agricultural Organizations (FAO, 1979), the standard limit for aerobic mesophilic bacterial as well as fungal count should be less than 10^5 cfu/g/ml. The highest bacterial and fungal counts in this research observed may be attributed to the water used during irrigation, unhygienic environments, vehicle exhaust, temperature, improper vegetable storage etc. Exposure of the vegetable to the air, or dust at the point of sale is likely to increase the count of bacteria as well as that of fungi as virtually most of the organisms are carried in aerosol by dust and in air (FDA, 2009).

Table 1: Bacterial, fungal and coliform counts examined in cucumber (A), tomato (B) and lettuce (C). Note: AMBC, aerobic mesophilic bacterial counts; AFC, aerobic fungal counts; CC, coliform counts; cfu/g, colony forming unit per gram.

Sample number	AMBC (cfu/g)	AFC (cfu/g)	CC/mL
A1	1.53×10^5	4.1×10^4	43
A2	1.43×10^5	1.3×10^5	36
A3	1.87×10^5	1.32×10^5	150
A4	1.4×10^5	8.9×10^4	290
A5	1.36×10^5	1.6×10^5	9
Mean	**1.52×10^5**	**1.10×10^5**	
B1	1.6×10^5	1.2×10^5	11
B2	1.9×10^4	1.83×10^5	11
B3	1.52×10^5	7.7×10^5	460
B4	1.5×10^5	1.4×10^5	36
B5	1.9×10^5	1.2×10^5	11
Mean	**1.34×10^5**	**1.06×10^5**	
C1	1.96×10^5	1.7×10^5	240
C2	1.6×10^5	1.8×10^5	43
C3	1.3×10^5	1.5×10^5	460
C4	1.2×10^5	1.54×10^5	16
C5	1.27×10^5	1.3×10^5	11
Mean	**1.18×10^5**	**1.29×10^5**	

The presence of *E. coli*, *S. aureus*, *Salmonella* spp. and *Shigella* in the whole 15 samples. It is discovered that the cucumber sample had the total percentage of 65%, lettuce sample had 60%, whereas tomato sample 60%. The occurrence of *Salmonella* in the vegetables might be attributed to the water used during irrigation of such food as *Salmonella* has been reported to be transmitted via water and *Salmonella* carrier are food handlers (Issa-Zacharia, A, et al., 2010). In this study it was found that the percentages of all the samples that failed to comply with standard is 65%, 60% and 60% respectively (Table 2) and is very much higher than those found in UK 5.1%. The isolates were identified by biochemical tests (Table 3).

Table 2: Percentage frequency of occurrence of the isolates from the cucumber, tomato and lettuce samples.

Sample (total no.)	Number of sample (frequency of occurrence)				Total
	E. coli	*S. aureus*	*Salmonella*	*Shigella*	
Cucumber (5)	3(60%)	4(80%)	2(40%)	4(80%)	65%
Tomato (5)	4(80%)	4(80%)	2(40%)	2(40%)	60%
Lettuce (5)	2(40%)	3(60%)	4(80%)	3(60%)	60%
Total (15)	9(60%)	11(55%)	8(53.3%)	9(60%)	73%

The prevalence of enteropathogenic bacteria such as *E. coli*, *S. aureus, Salmonella* and *Shigella* on the vegetables examined render them as vehicle for food borne infections. *E. coli* when consumed in food are liable to cause peristomstis, mastitis, septicemia and gastrointestinal infections. *Salmonella* is the causative agent of typhoid fever, an enteric fever that can be fatal (Nester et al., 2004). *Shigella* if consumed in food is able to cause abrupt onset diarrhea tenesmus, cramps and lethargy (Prescott, 2013). *S. aureus* when ingested in food is liable of causing abrupt onset, intense vomiting for up to 24 hrs which can be fatal (Prescott, 2013).

4. CONCLUSION

In conclusion, the aerobic mesophilic bacterial, fungal and coliform counts were discovered. In addition, other forms of specific pathogens were detected during the research such as *L. monocytogenes* etc. However, the results obtained in this research revealed that, the microbial counts obtained in cucumber sample were found to be higher than that of tomato and lettuce samples. The occurrence of enteropathogenic bacteria such as; *E. coli*, *Salmonella* and *Shigella* and potentially pathogenic microbes like *S. aureus*. This described the quality of vegetables being irrigated at Kwakwaci is not adequate for human consumption and the vegetables are highly contaminated and contained potentially pathogenic microbes which are very serious to the health when ingested. Some recommendations:

i. Promotion of good hygienic practice among all farmers.
ii. Safety regulators should also be implemented in order to prevent spread of contamination through the water
iii. Further study to determine the role of food borne diseases and microbes in transferring antibiotic resistance in Kano State is highly recommended.

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Table 3: The biochemical characteristic results of isolates from cucumber (A), tomato (B) and lettuce (C) samples. Note: H_2S, hydrogen sulphide; +, positive; -, negative; Y, yellow (acid reaction); R, red (alkaline reaction); ND, no development.

Sample	Growth Media			Gram staining	Biochemical Test										Inference
	EMB	SSA	MSA		Methyl Red	Vogas proskaeur	Indole	Citrate	Catalase	Coagulase	TSI				
											Slant	Butt	H_2S	Glucose	
A	+	+	+	+/-	+	-	+	-	+	+	Y	R	-	+	*E. coli, Shigella, S. aureus*
A	+	+	-	-	+	-	+	-	+	+	Y	R	-	-	*E. coli, Shigella*
A	-	+	+	+/-	-	-	-	+	+	+	R	R	+	+	*S. aureus, Salmonella*
A	+	+	-	-/+	+	-	+	+	-	-	R	Y	-	+	*E. coli, Shigella, S. aureus*
A	-	-	+	-	-	-	-	-	+	+	Y	R	+	+	*Shigella, S. aureus, Salmonella*
B	+	+	-	+/-	+	-	+	+	-	-	Y	R	+	+	*E. coli, S. aureus*
B	+	+	+	+/-	-	-	-	-	+	+	Y	R	-	+	*E. coli, Salmonella*
B	+	-	+	+/-	+	-	+	-	+	+	Y	Y	-	+	*E. coli, Shigella, S. aureus*
B	-	+	+	-/+	-	-	-	+	+	+	ND	R	-	+	*Shigella, S. aureus*
B	+	-	+	+/-	+	-	+	-	+	+	Y	R	+	+	*S. aureus, Salmonella*
C	+	+	+	+/-	+	-	+	-	-	-	Y	Y	-	+	*E. coli, Shigella, S. aureus*
C	+	+	-	-	+	-	+	+	-	-	Y	R	-	+	*E. coli, Shigella*
C	+	+	+	+/-	+	-	+	+	+	+	Y	R	+	+	*Shigella, S. aureus, Salmonella*
C	+	+	+	+/-	+	-	+	-	-	+	Y	Y	+	+	*E. coli, S. aureus, Salmonella*
C	+	+	+	-/+	+	-	+	+		-	Y	R	+	+	*Shigella, S. aureus, Salmonella*

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