Salmonella spp. Implication in Products of Wastewater Irrigation

Amina Abubakari
Department of Laboratory Technology, Faculty of Health Sciences, Kumasi Technical University, Kumasi, Ghana

Abstract

This study was conducted to assess the occurrence of Salmonella spp. in lettuce (Lactuca sativa), manured soil and wastewater. An initial analysis was carried out for indicator organisms such as E. coli and Total coliforms to be able to ascertain their presence prior to assessment of pathogenic microorganism. The study was carried out in Kumasi the second largest city in Ghana. A total of 111 samples were aseptically collected and analysed for Salmonella spp. using standard microbiological laboratory protocols. Out of 111 samples, 6(5.4%), 11(9.9%) and 4(3.6%) showed positive for lettuce, manured soil and irrigation water respectively. When one way ANNOVA was used to analysed the data the mean positive values showed no significant difference among the 3 groups of samples (P=0.17). The study revealed that, wastewater which farmers rely on for unrestricted irrigation all year round, lettuce which is consumed raw and manured soil from irrigation fields are all contaminated with Salmonella spp.

Keywords: Salmonella spp.; Irrigation; Lettuce; Implication; Wastewater

Introduction

Salmonella spp. is one of the pathogenic bacterium which causes diarrhoea, fever, and abdominal cramps 12 to 72 hours after infection usually lasting 4 to 7 days. In some persons, the diarrhoea may be so severe and infection may sometimes spread from the intestines to the blood stream, and then to other body sites which could result in death. In Africa, over 30,000 children are reported to die every year due to the consumption of contaminated food [1]. Fresh fruits and vegetables are increasingly recognized as potential sources of diseases [2,3]. Vegetables that are eaten uncooked are among the products associated with food borne disease outbreaks in the Unites States [4]. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness. Every year, approximately 42,000 cases of Salmonellosis are reported in the United States (CDC). Children are the most likely to get Salmonellosis and the rate of diagnosed infections in children less than five years old is higher than the rate in all other persons. Vegetables can become contaminated by pathogens while growing, during harvest, by post-harvest handling, or distribution. It is estimated that approximately 400 persons die each year with acute Salmonellosis (CDC). In some persons it can also result in pain in their joints, irritation of the eyes, and painful urination which could last for months or years, sometimes leading to chronic arthritis which is difficult to treat. Population growth, coupled with increasingly lack of water supply and sanitation give rise to large volumes of wastewater, which sometimes end up in our water bodies either partially treated or untreated.

Desperate farmers who do not get portable water for irrigation depend on this water source to irrigate their produce. Demand for fresh fruit and vegetables as well as provision of job opportunities and income for farmers and their family members is the driving force for this activity. Although this is good for both consumers and farmers and their families, the health risk associated with it is highly significant since wastewater irrigation has been implicated in most pathogenic disease outbreaks.

Microbiological quality of lettuce on three farms where wastewater is used in Kumasi were studied to determine the CFU levels of Total coliforms, faecal coliforms, Enterococci, E. coli and Salmonella using standard methods. Total coliforms on the lettuce varied from 4.93 × 104 cfu to 6.17 × 104 cfu. Faecal coliforms ranged from 3.48 × 103 cfu to 4.66 × 104 cfu and E. coli 2.98 × 103 cfu to 3.86 × 104 cfu. Salmonella and Enterococci levels ranged from 2.50 × 102 cfu to 2.72 × 102 cfu and 0.68 × 10 cfu to 2.05 × 10 cfu respectively. Studies have extensively looked at this particular area but in bit and pieces, not holistically on all the possible sources of contamination of the raw eaten vegetables by these organism and more so Salmonella which is pathogenic and pose serious health challenge to the public. Studies conducted in Kumasi by Keraita et al. [5] show that the microbiological contaminants in irrigation water sources in most cases exceed the WHO recommendation. According to Amoah et al. [6], poultry manure which most wastewater irrigation vegetable farmers use are sources of potential health risk.

Objective of study

- The broader objective was to assess Salmonella spp. implication in wastewater, lettuce (Lactuca sativa) and manured soils on which vegetables are cultivated
- To achieve this broader objective, initial identification and quantification of Total coliforms and E. coli as indicator organisms in the same samples was carried out

Materials and Methods

Study site and design

The study was conducted in Kumasi, where an early survey was done, mapping out the wastewater irrigation sites. Kumasi is the capital of...
of the Ashanti Region of Ghana where majority of the farmers engage in wastewater irrigation farming. Hand-held GPS was used in capturing the entire wastewater irrigation site within the study area which was subsequently superimposed on the google earth map to obtain clear locations of the study area showing all the farms and irrigation waters (Figure 1). During the initial survey farmers and their families working on the various irrigation farms numbered approximately 250 but one hundred and fifty (150) farmers agreed to be recruited and also allowed samples to be taken from their farms for this study. Kumasi is located between latitude 6°30 and 7°00 N and longitude l°30 and 2°00 W. It has a wet semi-equatorial climate with an annual rainfall of around 1400 mm with two distinct rainy seasons. The mean annual temperature is 25.7°C with a relative humidity ranging from 53%-93%. Kumasi is the second largest city in Ghana and it is about 270 Km north to the national capital, Accra. It has a growth rate of 5.4% with an average household size of 5.1 persons [7]. Majority of the areas in Kumasi were wastewater is used for irrigation lie in the South-Eastern.

Figure 1: Kumasi an insert in Ghana map showing irrigation farms and impoundments for irrigation.

Sampling procedure

Three whole lettuce heads were aseptically picked from each bed with sterile Knives into sterile plastic bags (Stomacher™ lab system from Seward, UK) and sealed. From each bed, the lettuce heads were selected at random from the far ends and also in the middle to represent triplicate samples.

Manured Soil from beds were vegetables were sampled were taken in triplicates with sterile spatula into sterile plastic bags (Stomacher™ lab system) sealed and put into cooling boxes with cooling elements.

Wastewater used for irrigation was sampled into sterile pre-labelled 500 ml bottles.

Samples were transported in cooling boxes containing cooling elements into the Microbiology Research laboratory for analysis within three hours.

Laboratory analysis for Salmonella spp. in irrigation samples

Lettuce were weighed and washed with distilled water (1 L) each. Serial dilutions of $10^{-1} - 10^{-2}$ were made by taking 1mL of the sample added to 9 mL of peptone water in test tubes and vortexed for thorough blending. It was then incubated at 37°C for 24hrs. Tubes that showed growth by the presence of turbidity and gas were selected for another inoculation on selenite broth and incubated at 37°C for 24hrs.

Positive samples were later inoculated onto Salmonella Shigella (SS) agar plates and incubated at 37°C; suspected colonies were purified by sub culturing onto fresh plates. Typical Salmonella colonies which were colourless with black centers were inoculated on Triple Sugar Iron agar (TSI) slants and incubated at 37°C for 24 hrs.

Confirmation was done by using latex agglutination kit for Salmonella (Salmonella polyvalent O group A-Z antiserum agglutination test kit).

One gram (1g) wet weight of manured soil was added to 9 mL of peptone water, incubated at 37°C for 18-24 hrs. Tubes which showed growth were inoculated into 9 mL of selenite broth and incubated again at 37°C for 18-24 hrs.

Positive tubes with turbid appearance were transferred onto Salmonella Shigella (SS) agar plates, incubated of 37°C for 18-24 hrs. Plates showing colonies of colourless with black centers were purified.

First confirmation was done by using TSI slants and then salmonella polyvalent 'O' group A-Z antiserum latex kit.

A serial dilution of $10^{-1} - 10^{-2}$ was prepared by pipetting 1ml of the wastewater sample into 9 mL of peptone water and incubated at 37°C for 18-2 4 hrs. Turbid tubes were considered positive. Subsequent inoculation was done in tubes containing selenite broth and inoculated at 37°C for 18-24 hrs.

Positive tubes were selected and culturing done on Salmonella Shigella (SS) agar and incubated at 37°C for 18-24 hrs.

Salmonella colonies on the plates were identified morphologically as colourless colonies with black centers, isolates were purified and then inoculated by stabbing TSI slants and stored at 37°C for 24 hours and observed for gas production and slant colour for confirmation.

Serological confirmation was also than using salmonella polyvalent 'O' group A-Z antiserum latex kit according to the instructions provided by the manufacturer.

A loop-full of the test organism's pure colony was added to one drop of Salmonella antisera distributed onto a reaction card. An even suspension was obtained by careful emulsification, after which the card was gently whirled.

The test organism was recorded positive for Salmonella spp. if agglutination occurred within 1-2 minutes. Salmonella spp. antiserum was substituted for normal saline as control for another typical reaction.

Laboratory analysis for total coliforms and E. coli in lettuce, manured soil and wastewater

Lettuce were weighed and homogenized with distilled water. 1 millilitre of water samples were pipetted and a dilution of each made up to $10^{-1} - 10^{-2}$ with 9ml of distilled water. The later diluent ($10^{-2}$) was added to a beaker containing 90ml of distilled water mixed with one pack of colisure powder (Quanti-Tray®/2000).

10 g of dry manured soil sample was added to 90 ml of distilled water in a beaker and mixed thoroughly. One sachet of colisure powder was poured into the sample mixture and allowed to mix well by shaking.

The mixture was allowed to stand for some time while shaking it in between to give room for thorough mixture. 1ml of the wastewater was pipetted into 9ml of distilled water and subsequent dilutions were
made from the 10ml to a factor of $10^{-1}$-$10^{-2}$. The later diluent was poured into 90 ml of distilled water. It was then poured into the quantitray and heat sealed. Incubation was done at 35°C for 18-24 hrs.

Readings for total coliforms and *E. Coli* was done by identifying yellow to red magenta wells indicating that, the coliforms have metabolize colisur's nutrient-indicator and when 6-watt, 365 m, UV light in a dark room was passed through the surface of the sample the wells that fluoresce given purple colour were counted as containing *E. coli* colonies per 100 ml of sample.

## Results

A total of one hundred and eleven (111) each of lettuce, manured soil which supports the growth of wastewater irrigated produce and then wastewater used for irrigation (irrigation water) were aseptically collected and analysed. Result (Table 1) indicated *Salmonella* spp. presence in lettuce, manured soil and irrigation water as 6 (5.4%), 11(9.9%) and 4(3.6%), respectively. Manured soil had the highest percentage of *Salmonella* spp. which may be due to the fact that most of the manures are from chicken containing high loads of *Salmonella* according to previous research and are poorly treated.

<table>
<thead>
<tr>
<th>Samples type (n=111)</th>
<th><em>Salmonella</em> spp. positive result</th>
<th>Percentage positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce</td>
<td>6</td>
<td>5.4</td>
</tr>
<tr>
<td>Manured soil</td>
<td>11</td>
<td>9.9</td>
</tr>
<tr>
<td>Irrigation water</td>
<td>4</td>
<td>3.6</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>18.91</td>
</tr>
</tbody>
</table>

Table 1: Number of Samples indicated *Salmonella* spp. positive.

Apart from this study determining the presence of *Salmonella* spp. in the samples, concentrations were also determined for both indicator organisms and *Salmonella* spp. for both the wet and the dry seasons.

Result of this study indicated significant variations ($P<$0.05) in the mean log concentrations of all the microorganisms investigated in the wet and the dry seasons (Table 2).

For *Salmonella* spp., the mean log concentrations for the dry season was found to be within the WHO standard for unrestricted irrigation with the wet season been a little above the WHO standard.

Total coliforms and *E. coli* presented mean log concentrations between 3-4 logs higher than the acceptable standards for unrestricted irrigation in the dry and wet seasons (Table 2).

### Microorganism

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Mean log cfu/100ml ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>2.524 ± 0.984</td>
<td>0.017</td>
</tr>
<tr>
<td>Wet Season</td>
<td>3.692 ± 0.388</td>
<td></td>
</tr>
<tr>
<td>Total coliform</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>7.557 ± 0.201</td>
<td>0</td>
</tr>
<tr>
<td>Wet Season</td>
<td>7.915 ± 0.371</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Concentrations of *Salmonella* spp. in samples expressed in log cfu/100 ml of samples.

## Discussion

Unrestricted agricultural practices with wastewater should be carefully monitored and planned and supported with hygiene protocols. This is to reduce un-necessary associated health risk especially in poor communities due to lack of strict policies on the use of wastewater. The World Health Organization [8] made recommendations for standard thresholds of 103 FC/100ml and ≤ 1 egg/L of faecal coliform, respectively in wastewater used for unrestricted irrigation. Although manured soil application on farms by agricultural farmers provide nutrients to the vegetables, it introduces pathogens which poses health risk to man which requires serious attention [9]. A study in Zaria, Nigeria, showed that water sampled from households with livestock contains *Salmonella* spp. while water from households without livestock had no *Salmonella* spp. [10]. The difference is believed to be the introduction of the pathogens through animal husbandry operations such as the application of their faeces as organic manure without adequate treatment. The presence of *Salmonella* spp. in the products of this current study therefore agrees with the facts that, since most farmers within the study area apply untreated poultry manure it could highly be the source within urban and peri-urban [11,12].

This study presented results with *E. coli* and Total coliforms concentrations in the samples to be high for both seasons with mean levels exceeding the $1 \times 10^3$/100ml recommended by WHO [13] for unrestricted irrigation. There were excesses of 3 to 4 logs in all seasons which could be attributed to the fact that about 90% of wastewater generated even in the capital cities of Ghana are not treated and eventually mixed up with storm drain which most farmers rely on for unrestricted irrigation [14]. The results also confirmed studies by Gupta et al. [15] which reported high concentrations of *E. coli* in vegetables irrigated with wastewater. Amoah et al. [16] also confirmed in their studies that, drains and streams highly contaminated with faecal coliform concentrations above the WHO acceptable level was the primary water source used in irrigation of most vegetables in Accra. On the seasonal effects, the dry samples had lower concentrations of *E. coli* and total coliform concentrations compared to those reported for the wet season which confirms the studies reported by Seidu et al. [17], which indicated in their study that, lettuce from wastewater irrigated farms had a six times chance of *E. coli O157:H7* contamination during the wet season compared to the dry season which is similar to the observation from this current study. This study observed low concentrations of *Salmonella* spp. in all the samples for both seasons, agreeing with the WHO limits of within 3-4 logs [13]. Although they were present in low concentrations, *Salmonella* spp. and *E. coli O157:H7* are reported as having low infectivity dose of 10-100 cells [18] which can result in a very huge health effect or outcomes. If this is the case then, unrestricted irrigation should be devoid of these pathogenic microorganisms and therefore the needed caution. Often reports indicate that fresh produce
is responsible for about 22% of all food borne disease infections [19,20]. Report by Sivapalasingam et al. [4] indicates a strong link between lettuce and pathogenic organisms that can cause food borne illnesses. Their report further indicated that 29% (5 out of 17) of all lettuce-related outbreaks were linked to pathogenic microorganisms and 38% (five out of 13) of all these outbreaks with fresh produce were associated with lettuce. Their observation deferred slightly from results obtained from this study which could be due to the high temperatures (24.7°C-37.2°C) experienced during the study which could inhibit the growth of Salmonella spp.

Consumer’s food safety depends on series of event beginning from the production fields or on-farm, market and finally to consumers for consumption with each of these events been essential. Estimates of contaminated food illnesses in developing countries presents a major challenge since infections are mostly treated at home and often not documented [21]. Studies have indicated that Salmonella spp. infect over 1.6 million humans within United States costing the nation about $15 billion yearly [22]. Although this study also detected Salmonella spp. in wastewater, manured soil and more especially lettuce which is consumed raw, little is known in Ghana about the health cost implications of pathogenic organisms which needs to be given attention. Involvement of Salmonella spp. in these samples agrees with reports from studies indicating the presence of pathogenic microorganisms been generally transmitted directly via contaminated foods, water and crops [23,24]. The role of free-ranging birds and livestock in pathogen recirculation and local transmission has been noted on and around farms [25,26]. This is in line with this current study that has detected microbial pathogens in high levels in the manured soil of irrigation fields. The rather high levels of Total coliforms and E. coli in all the samples agree with a previous study conducted by Amoah et al. [27]. Again it is revealed that about 50-90% of the vegetables consumed in the major cities in West Africa are produced from urban and peri-urban communities, where most of the irrigation water is polluted [28]. Since majority of the salad been prepared are obtained from the sources where this study took samples [29], policy makers should attach serious importance to it and enforce the existing policies on unrestricted irrigation practices. Again an observation from the study showed that farmers mostly used manure from livestock on their farms but these serve as pathogen reservoir and consequently serves as a channel for the transmission of pathogens which also agrees with a similar report by Oliveira et al. [30]. Faecal matter of grazing livestock was also frequently observed close to the irrigation areas. These eventually are washed into the nearby irrigation water during a rain fall event increasing contamination by pathogens greatly as also reported by Karesh et al. [31]. This current study has therefore shown that microbial contamination of vegetables especially those that are consumed raw may occur through contamination from the farm which could result from the quality of soil, and irrigation water [6]. Although poor post-harvest handling of in the market and during food preparation may also contribute to the contamination [26].

Conclusion

This study has revealed that, unrestricted vegetables from Kumasi has high concentrations of E. coli and Total coliforms with their mean levels exceeding $1 \times 10^3/100$ ml which presents an excess of 3-4 logs of E.coli and Total coliforms beyond the World Health Organization recommendations. There are also some levels of pathogenic microorganism such as Salmonella spp. present in the water used for irrigation, manured soil and the vegetables produced under these conditions. These therefore predispose both farmers and consumers to infections due to accidental ingestion of manured soil and wastewater and the consumption of lettuce.

The conclusions from this study coupled with the fact that stake holders in the use of wastewater for irrigation and consumers of the produce lack basic knowledge in the area; more education is needed as well as enforcement of existing policies. More pragmatic and effective low cost irrigation technologies should also be promoted.

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References


