Original Research Article

Occurrence of Salmonella in wastewater irrigated farms in Meknes City, Morocco

Abstract

The use of polluted water for irrigation is prominent in urban and peri-urban farms in Meknes (Morocco). However, this water may contain many pathogens that cause serious infections. Consumption of contaminated vegetables could be a significant public health problem. Salmonella is a bacteria responsible for multiple body infections, including gastroenteritis, fevers, and more severe symptoms. This study aims to determine the occurrence of Salmonella contamination in urban and periurban farms of Meknes. A total of seventy samples (17 Water, 11 soil, and 42 vegetables) were collected and analyzed using polymerase chain reaction (PCR). The results showed that irrigation water was the most contaminated with Salmonella, followed by soil samples (45.5%) and vegetables (16.6%). This study shows a high prevalence of Salmonella in irrigation water, which can be a source of contamination of crops. It raises public health concerns as farmers and consumers are at risk of infection.

Key-words: Salmonella, irrigation water, polymerase chain reaction, Meknes, Morocco

Introduction

Salmonella is an enteric bacterium that colonizes the gastrointestinal tract. It is responsible for several types of human infections. It causes many clinical manifestations, such as enteric fever, gastroenteritis. Salmonella infections are the most common food-borne illness worldwide. It is transmitted via the fecal-oral route. The most common pathway of infection in humans is the consumption of contaminated food or water.

Worldwide, there is a significant increase in the incidence of foodborne illness associated with the consumption of contaminated fresh produce (Salleh et al et al., 2003; Sivapalasingam et al., 2004). Salmonella is considered the primary pathogen responsible for foodborne outbreaks related to contaminated fruits and vegetables (Liu et al., 2018). Contamination of fresh produce is often linked to potential contamination in the agricultural environment prior to harvest, including soil, composted manure, irrigation water (Tauxe et al., 1997; Islam et al., 2004). Irrigation is an important factor in agricultural production (Liu et al., 2018). The presence of salmonella in irrigation water is considered one of the main sources of crop contamination (Solomon et al., 2002; Islam et al., 2004; Steele and Odumeru, 2004).

In Meknes (Morocco), surface water is the major source of crop irrigation for urban and peri-urban farms, despite the fact that it is notoriously prone to contamination. Moreover, farmers are using untreated municipal wastewater to overcome water shortages. Therefore, irrigation water could be a route for pathogen transmission in agricultural produce, and a cause of transmission of foodborne diseases (Steele and Odumeru, 2004; Allende and Monaghan, 2015). The consumption of contaminated irrigated produce may pose a danger to human health**.** Identifying the specific source of contamination helps to develop strategies and prevent future outbreaks (Liming and Bhagwat, 2004). Improved diagnostic and detection systems have helped with surveillance of foodborne diseases (Ailes et al., 2008). The use of conventional culture-based methods is inexpensive, however it can lack sensitivity (Liu et al., 2019), as well as the possibility of false negative results because of the viable but non-culturable state of existence (Law et al., 2015). The Polymerase Chain Reaction (PCR) has proven to be one of the reliable and highly sensitive microbiological methods for the detection and identification of pathogenic bacteria (Malorny et al., 2003). PCR tests have been developed for the detection of pathogens in water, food and other environmental samples (Imtiaz et al, 2013). In this study, we conducted a PCR approach to assess the presence of Salmonella spp. in irrigation water, irrigated vegetables and soil.

Materials and method

1. Description of the Study area

The study area concerns urban and peri-urban farms located at the three streams that cross the city of Meknes, North East of Morocco (**Figure 1**), the choice of these farms was made because this area is well known for the use of polluted water for irrigation (Dugué et al., 2015). The use of polluted water can be direct through the extraction of sewage/collector water, or indirectly through the use of stream water polluted by discharges.

Figure 1: Map of satellite view of the study area (city of Meknes)

The reuse of agricultural wastewater in urban and peri-urban areas of Meknes is vital for socio-economic dynamics, as farmers have no alternative. The livelihood of farmers is essentially based on the agricultural production that it associates with the activities. Agricultural income accounts for 85% of the total income of the small farm (Dugué et al., 2015). It also contains nutrients that improve crop development (FAO, 2019). Horticultural crops are the most produced in these areas, sold to local markets in the city, but one can also find arboriculture that supplements the income of some farmers. In some areas, there are small forage and livestock plots (Dugué et al., 2015). A cross-sectional study was carried out

on samples of vegetables, irrigation water and soil from different urban and peri-urban farms in Meknes. The study was conducted from December 2021 to March 2022.

2. Sampling and analysis

The sampling was carried out randomly at various urban and peri-urban farms of the city of Meknes. Samples include irrigation water (surface water (10 samples) and wastewater (7 samples)); crop soil samples (11 samples) were taken from pits 1.2 m and 1 m deep; and irrigated vegetables (42 samples). Three types of vegetables were chosen for this study lettuce (*Lactuca sativa*), beet (*Beta vulgaris*) and radish (*Raphanus sativus*). The cultivated soil samples were taken from the same location in the field as the vegetable samples. All samples were collected under aseptic conditions transported on ice to the laboratory and analyzed within 24 hours.

The salmonella isolation and identification was carried out according to ISO 6579 standard (Da Silva et al., 2018). The pre-enrichment was done in buffered peptone water. The enrichment was done in Rappaport Vassiliadis Soya broth and Muller-Kauffmann Tetrathionate Novobiocin broth. Selective differential plating was done with xylose lysine desoxycholate (XLD) agar. The identification was made using bacterial identification using the API 20E.

The molecular confirmation was performed as described by Burkhard Malorny and Reiner Helmuth (Sachse and Frey, 2003). The primers used are the one that amplify a 284 bp fragment of the invA gene encoding the origin of Salmonella DNA replication. The primer sequences were: forward (5'GTG AAA TTA TCG CCA CGT TCG GGC AA3')**;** reverse (5'TCA TCG CAC CGT CAA AGG AAC C3') designed by Rahn *et* al. (2019).

DNA amplification and detection was done after putting PCR tubes in a thermocycler and perform the following thermal cycle program: An initial denaturation of 1 min at 95°C followed by 38 denaturation cycles at 95°C for 30 s, annealing at 64°C for 30 s, then a final extension for 4 min at 72°C, and a final temperature at 4°C. The 10 µL of each PCR product was electrophoresed on a 1.6% agarose gel (1.6 g/100 ml 1X TBE buffer) colored in an Ethidium bromide solution (0.5 µg ml-1) for 10 min, then visualized under ultraviolet light.

3. Statistical analysis

The data collected were processed and analyzed using Excel. The chi-square analysis ($\chi 2$) was used to determine the association between the salmonella contamination rate and all types of samples, and to determine the link between the type of water used for irrigation and the irrigated produce.

Results

A total of 70 samples of irrigation water, soil and vegetables were collected from urban and peri-urban farms in the city of Meknes (Morocco) and examined for Salmonella (**Table 1**). This study showed that irrigation water had the highest contamination rate of *Salmonella spp* (70.6%), followed by irrigated soil 45.5%, and while vegetables were the least contaminated (16.67%). It was also noted that between the two types of irrigation water used, wastewater samples were more contaminated than stream water.

Table 1. Frequency of distribution of the contamination of salmonella in all the study samples

Samples	Number and percentage of positive samples	
Irrigation Water n = 17	12	70.6%
Stream water n= 10	6	60.0 %
Wastewater n = 7	6	85.71%
Soil n=11	6	45.5%
Vegetables n= 42	7	16.67%

Among vegetables samples, lettuce was the most frequently contaminated (27.4%), followed by radish (14.28%). All beet samples collected were negative for salmonella (**Table 2**).

Table 2. Salmonella contamination rate on wastewater-irrigated vegetables

Vegetables samples	Number of samples	Number and percentage of positive samples
Lettuce	14	5 (35.71%)
Beets	14	ND
Radish	14	2 (14,28%)

Table 3. Occurrence of *salmonella spp* in soil and vegetables samples irrigated with different types of irrigation water

	Number and percentage of positive samples	
	Wastewater	**Stream water**
Soil n = 11	4/5 (80.0%)	3/6 (50.0%)
Lettuce n= 14	4/7 (57.14%)	1/7 (14.28%)
Radish n= 14	1/7 (14.28%)	1/7(14.28%)
Beets n=14	ND	ND

A comparison was made of the frequency of contamination of vegetables and soil irrigated by wastewater and stream water (**Table 3**). The soil irrigated by wastewater has been the most contaminated 80.0 %. As for vegetables, wastewater irrigated lettuce was more contaminated

than the one irrigated with stream water, however the contamination rate of radish was the same for wastewater and stream water.

Discussion

The use of polluted water for irrigation may be a vector for the transmission of *salmonella spp* **(**Steele et al., 2004**).** Several studies have shown that irrigation water has been the source of salmonella contamination in vegetables (Ruiz et al., 1987; Melloul and Hassani, 1999; Melloul et al., 2001; Ndiaye et al., 2011; Henao-Herreño et al., 2017). As a study conducted in Senegal in 2011 by Ndiaye et al. (2011), revealed the serotype of Salmonella isolated from irrigation water were the same found in lettuce. In Morocco, a case-control study carried out in 1999 in Marrakech by Melloul and Hassani. (1999), showed a high prevalence of salmonella in groups exposed to wastewater irrigation. Another study in 2001 by Melloul et al. (2001) revealed that serogroups of Salmonella B and C were the most isolated in irrigation waters, and the most found in cultivated vegetables.

In Meknes area, the use of polluted surface water and effluent water is wild spread. This makes vegetables produced likely to be a route for transmission of pathogens and food-borne illnesses. Our study found high salmonella contamination of the irrigation water, no significant difference between wastewater and steam water (X^2= 1.02; p=0.30), hence this water in the study area does not meet Moroccan standards. This can put farmers and their families at risk of infection as they are the most affected group due to the duration and intensity of their contact with wastewater (OMS, 2006).

The prevalence of salmonella in soil is 45.5%, there is no significant difference between the ones irrigated by the waters of the streams and the ones irrigated by the waters of effluents (X^2= 1.31; p=0.25). Soil contamination with *Salmonella spp* may increase the contamination during vegetable harvesting, as the study by Guo et al. (2001)**,** has reported that Salmonella may attach or penetrate to tomatoes grown in soil contaminated with Salmonella and survive for a long time.

The Salmonella contamination of vegetables produced on urban and peri-urban farms in Meknes was valued at 24.0%. However, there is no significant difference between vegetables irrigated with wastewater and the ones irrigated with stream water (X^2= 0.77; p=0.37).

The prevalence of salmonella varies according to the type of vegetables. Several factors affect the frequency of contamination of vegetables, including the surface properties of products and their water retention capacity (Shuval et al., 1997; Abaidoo et al., 2010). In our

study, it showed that the prevalence of salmonella differs significantly between the different types of vegetables (X^2= 6.25; p= 0.03). Lettuce samples were the most frequently contaminated followed by radish. This can be explained by the fact that lettuce has the largest surface area exposed to irrigation water (Samuel et al., 2013). Lettuce and radish pose the greatest risk since they are generally consumed raw. Consumption of these contaminated vegetables could pose a health risk to consumers of these products.

Conclusion

The study showed that wastewater in irrigation could be a risk of contamination of vegetables for farm workers and consumers. It is necessary to adopt the necessary actions to limit the risks associated with the contamination of vegetables by adopting good hygiene practices that involve crop restriction, proper irrigation techniques, and washing of produce, primarily if intended for consumption by an immunocompromised person.

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