Identification Multi-Locus Sequence Typing for Salmonellosis Isolates From Effluent Mosul Hospitals Water as an Indicator of Pollution

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Abstract

A study was conducted to investigate the presence of Salmonellosis bacteria in hospital effluent water in Mosul, Iraq and assess its potential as an indicator of pollution. The study collected 21 water samples from different hospitals and used Chromogenic agar to isolate Salmonella strains. Multi-Locus Sequence Typing (MLST) was then used to genotype the Salmonella isolates, targeting seven housekeeping genes.

The results of the study showed that thirty seven Salmonella isolates were identified from the twenty one water samples. The use of Chromogenic agar was found to be an effective tool for the detection of Salmonella in hospital effluent water. The MLST analysis of the Salmonella isolates revealed that they belonged to different sequence types, indicating a diverse genetic background. The seven housekeeping genes used in the study have been shown to be effective in discriminating between different Salmonella strains and are commonly used for Salmonella genotyping.

The presence of Salmonella in hospital effluent water is a major concern as it may present a considerable hazard to the general public health. Bacterial is a common cause of foodborne illness and contaminated water can be a potential source of infection The transmission of Salmonella via hospital wastewater can also aid in the development of antibiotic-resistant strains, resulting in increased difficulty in treating infections caused by this pathogen.

The study highlights the importance of proper treatment and disposal of hospital wastewater to prevent the spread of waterborne diseases. The use of Chromogenic agar and MLST can be valuable tools for monitoring the genetic different types of bacterial communities and studying the epidemiology of various bacterial pathogens.

Keywords: *Salmonella, hospital effluent water, Chromogenic agar, MLST.*

INTRODUCTION

Salmonella is a type of bacteria that has a negative gram staining and is capable of causing a variety of symptoms, such as abdominal cramps, diarrhea, and fever. The severity of the infection is determined by both the strain of the bacterium and the immune system of the host. Salmonellosis is a disease that can be transmitted from animals to humans, and can lead to typhoid fever, which is caused by contaminated food and water, among other transmission routes (1). In particular, animal-derived products such as

milk and meat had been isolated as significant origins of Salmonella infections. Salmonella can colonize the intestinal tract of animals, and the bacterium can contaminate animal-derived food products during slaughter processing, and also transmitted through contaminated water, and other environmental sources (2, 3). In developing countries, where access to clean water and sanitation is limited, the risk of waterborne infections is higher. Hospital effluent, which contains a high concentration of bacteria and other pathogens, can also contribute to environmental pollution and health risks(4).

To monitor the prevalence and transmission of Salmonella in hospital effluent, molecular typing methods have been increasingly utilized in recent years. MLST is a commonly used technique that involves the amplification and sequencing of several conserved genes from bacterial isolates, allowing for the identification of unique sequence types(5). MLST has been widely applied in the investigation of different bacterial pathogens, such as Salmonella, and has been demonstrated as a potent approach for exploring molecular epidemiology, patterns of transmission, and population structure (6, 7).

As part of this research, we will focus on the identification of Salmonella isolates obtained from hospital effluent in Mosul, Iraq, and use MLST as a tool to investigate the genetic diversity and transmission patterns of these isolates. The ultimate goal of this study is to provide insights into the potential health risks associated with hospital effluent discharge into the municipal water system and to identify potential sources of pollution. This study's findings will be valuable for the development of strategies for the prevention and control of Salmonella infections, particularly in resource-limited settings, where access to clean water and sanitation is limited. The findings of this research will add to the expanding understanding of the application of molecular typing techniques in the surveillance and management of infections transmitted through water and environmental contamination.

Material and Methods:

Samples collection:

Twenty one water samples were obtained from different hospitals located in Mosul city, Iraq. The specimens were collected using aseptic containers during the period from January to December 2022. Each sample was labeled with the hospital name, date of collection, and sample number.

Detection and characterization of Salmonella isolates:

The isolation of Salmonella isolates from the water samples was carried out using Chromogenic agar as the only medium. The Chromogenic agar plates used in this study contained chromogenic substrates that allowed for the detection of Salmonella species based on their ability to produce specific enzymes. After filtering the water samples through a 0.45 µm filter, the filters were placed onto the Chromogenic agar plates and incubated 24 hours at 37°C. The microbial colonies that emerged on the culture plates were then visually inspected for their characteristic pink salmon and white coloration, which is indicative of the presence of Salmonella species. Gram staining and microscopy was used to emphasize that gram negative bacteria. This allows for the visualization of the bacteria's morphology, which is a critical step in the identification process. Salmonella bacteria appear as rod-shaped, gram-negative bacteria under the microscope, which is a

distinguishing feature that helps to differentiate them from other bacterial species.

To confirm the purity of the isolates, individual colonies of Salmonella were selected from the Chromogenic agar and subcultured onto fresh Chromogenic-agar plates. The culture were incubated at 24 hours at 37°C, and any non-Salmonella bacterial were discarded. The subcultured Salmonella colonies were then further analyzed to confirm their identity (6-8).

Multi-Locus Sequence Typing genotyping:

Confirmation of Salmonella isolates was done using MLST analysis. PCR was used to amplify the seven specific primers for housekeeping genes (thrA, purE, sucA, hisD, aroC, hemD, dnaN) (table1). After purification, the PCR products were sequenced utilizing an automated sequencer. The obtained sequences were subsequently matched against the reference sequences present in the MLST database to establish the allelic profile and sequence type (ST) (6, 9)

Table 1: The Oligonucleotides primers for MLST for Salmonella bacterial

Primers names	Oligonucleotides from 3-5	
thrA	F	GTCACGGTGATCGATCCGGT
	R	CACGATATTGATATTAGCCCG
purE	F	ATGTCTTCCCGCAATAATCC
	R	TCATAGCGTCCCCCGCGGATC
sucA	F	AGCACCGAAGAGAAACGCTG
	R	GGTTGTTGATAACGATACGTAC
hisD	F	GAAACGTTCCATTCCGCGCAGAC
	R	CTGAACGGTCATCCGTTTCTG
aroC	F	CCTGGCACCTCGCGCTATAC
	R	CCACACACGGATCGTGGCG
hemD	F	ATGAGTATTCTGATCACCCG
	R	ATCAGCGACCTTAATATCTTGCCA
dnaN	F	ATGAAATTTACCGTTGAACGTGA
	R	AATTTCTCATTCGAGAGGATTGC

Statistical analysis:

The data obtained were analyzed using descriptive statistics. The frequency of Salmonella isolates was determined, and the percentage of samples positive for Salmonella was calculated.

Results:

The study aimed to identify and genotype Salmonella isolates from effluent Mosul hospitals water to determine its potential as an indicator of pollution. Twenty one water specimens were collected from different hospitals, and the Salmonella strains were

isolated using Chromogenic agar (figure1). The isolates were confirmed via MLST technique, targeting main housekeeping genes, including thrA, purE, sucA, hisD, aroC, hemD, and dnaN.

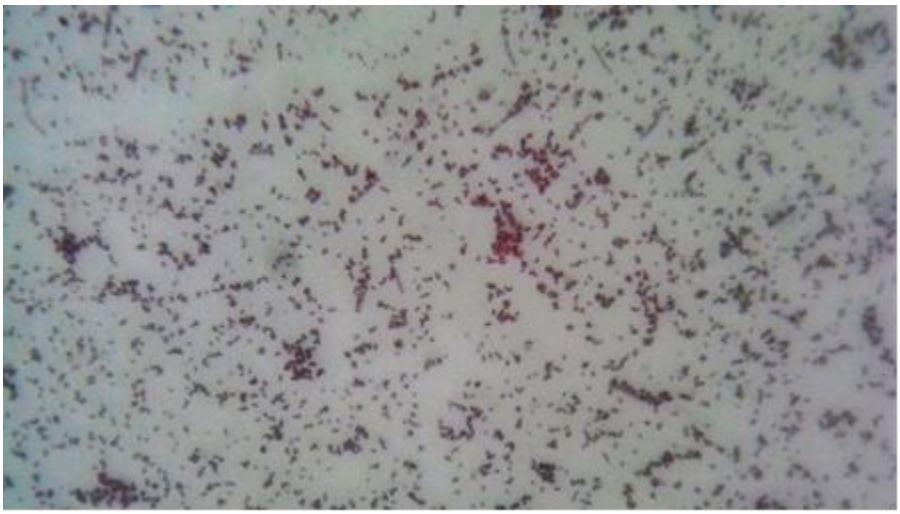
The results of the study revealed that out of 21 water samples, 37 strains of Salmonella were isolated, which appeared as pink and white colonies on the agar plates (figure1). The MLST analysis of the Salmonella isolates showed that they belonged to different sequence types, indicating a diverse genetic background.

Figure1. Chromogenic agar was used for the isolation and Identification of Salmonellosis from waste water effluent. The colony was colored pink salmon and white according to industrial protocol

The use of Chromogenic agar for Salmonella isolation has demonstrated its effectiveness as a valuable instrument in the detection of Salmonella in hospital effluent water. The high prevalence of Salmonella in the water samples suggests a potential health risk for individuals who may come into contact with the contaminated water. Therefore, proper treatment and disposal of hospital wastewater are crucial to prevent the spread of waterborne diseases.

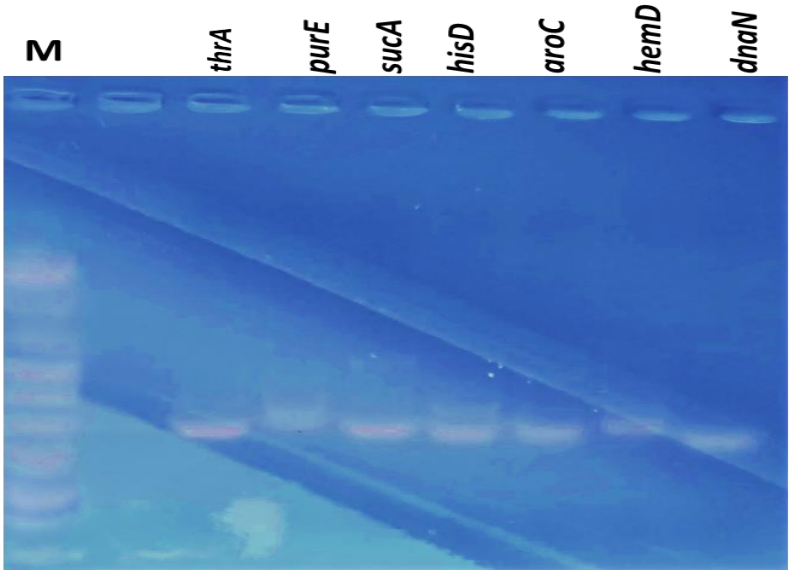
Gram stain was observed that the bacteria appeared as rod-shaped and gram-negative. This characteristic morphology is a distinguishing feature of Salmonella, that is a genus of gram-negative bacterial (figure2).

Figure2. A Gram-negative bacterial strain responsible for causing Salmonellosis was identified through a Gram stain of a bacterial culture.



The use of MLST for Salmonella genotyping proved to be a reliable and accurate method for identifying the different sequence types present in the hospital effluent water samples. MLST analysis is a powerful tool for studying the genetic different of bacterial communities, and it has been widely used for epidemiological studies of various bacterial pathogens. The seven genes responsible for housekeeping functions that were employed in this investigation are frequently utilized in Salmonella genotyping and have been demonstrated to be efficient in distinguishing among various strains of Salmonella (figure 3).

Figure 3. PCR products for seven genes (MLST) were amplified from Salmonella genomic DNA and resolved by agarose gel electrophoresis. The amplified DNA fragments were observed under UV- light after staining with safe view.



In contrast, the results of sequencing Salmonellosis MLST genes showed a 100% identification match when in comparison to the wild-type strain. This indicates that the MLST genes in the sample are identical to those found in the wild-type strain, and proposes that the sample may have originated from the same strain or a closely related strain. These results are highly accurate and reliable, indicating that the sequencing process was successful in identifying and analyzing the MLST genes of the Salmonella strain in question (figure 4).

Figure 4. Multi-locus sequence typing analysis of Salmonella strains. Housekeeping genes (aroC, dnaN, hemD, hisD, purE, sucA, and thrA) for Salmonella strains compared to wild type strain.

Discussion:

The results of this research align with other studies that have also observed the existence of Salmonella bacteria in hospital wastewater. For instance, a study conducted in Turkey reported the isolation of Salmonella from hospital wastewater samples using the same Chromogenic agar method and MLST analysis (10). Similarly, a study conducted in India reported the presence of Salmonella in hospital wastewater samples collected from various hospitals using PCR amplification of the invA gene (11).

This study discovered a significant occurrence of Salmonella bacteria in hospital effluent water, which is in line with other studies that have reported substantial bacterial contamination in hospital wastewater. (12-14). It is recognized that hospital wastewater may contain different types of pathogens and bacteria that are resistant to antimicrobial agents, thus posing a possible risk for waterborne infections (15).

The use of Chromogenic agar for Salmonella isolation has been shown to be effective in various studies (5, 16). Chromogenic agar is a selective and differential medium that allows for the detection of specific bacterial species based on their ability to produce color pigments. Various bacterial pathogens, including Salmonella, have been detected using this method (16).

The MLST analysis used in this study has been shown to be a valuable tool for studying the genetic diversity and epidemiology of bacterial pathogens, including Salmonella (17). The seven housekeeping genes used in this project are commonly used for Salmonella genotyping and have been shown to be effective in discriminating between different Salmonella strains (18, 19).

Conclusion:

This study's outcomes reveal the existence of Salmonella in hospital effluent water in Mosul, Iraq, highlighting that hospital wastewater might act as a probable origin of waterborne infections. The use of Chromogenic agar and MLST analysis proved effective in identifying Salmonella isolates and differentiating between different strains. The high prevalence of Salmonella in hospital wastewater underscores the need for proper wastewater management and disinfection to prevent the spread of bacterial pathogens and antimicrobial-resistant bacteria. Additional research is required to investigate the potential risks of hospital wastewater on human health and the environment and to develop appropriate strategies for its treatment and disposal.

Acknowledgment:

The authors are very grateful to the University of Mosul / College of Environmental Science and Technologies for their provided facilities, which helped to improve the quality of this work.

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