زانکۆی سه لاحه دین- هه ولیر

Salahaddin University-Erbil

Prevalence of Some Pathogenic Bacteria among Food Handlers in Erbil City

Research Project
Submitted to the Department of Biology in partial fulfillment of the requirements for the degree of BSc. in Biology

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Supervisor Certification

I certify that this research project was prepared under my supervision at the Department of Biology, College of Science/ Salahaddin University- Erbil in partial fulfilment of the requirements for the degree of BSc. in **Biology**.

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Dedication

This research is dedicated to:

This Research specialized for those who supported us during four years of studying in order to achieve the meaning of success on the whole of life especially our parents, our friends and all of the others.

(Mohamad &Hawkar)

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First of all, we would like to thank (God almighty) for giving us powerful and a great mind to complete our studying positively. We would like to thank our sweet supervisor (Nishtiman S. Hasan) who without her support and good ideas we couldn't achieve our goals and finally we would like to mention the role of our family especially our parents and all of the kind people's who helped us.

(Mohamad &Hawkar)

Abstract

Food handlers with poor personal hygiene could be potential sources of infection due to pathogenic bacteria. This study was designed to determine the prevalence of some pathogenic bacteria among the food handlers in Erbil city. For this, during September, 2022 to March 2023, 52 samples were collected from urine, fingernail, palm of both hands and nasal swabs of food handlers of different jobs and ages. The samples were cultured on bacteriological culture media and bacterial species were identified following standard procedures, afterward, analyzed for the presence of *Staphylococcus aureus* (*S.aureus*), *E. coli* (*Escherichia coli*), *Salmonella* and *Shigella*. According to the results, 16.3% of the samples were found positive for *S.aureus*, 10.5% positive for *E. coli* and 0.4% positive for *Salmonella* whereas *Shigella* were not found in any samples. *S.aureus* isolates were the most prevalent one amongst the bacterial isolates. Additionally, food handlers showed the highest prevalence of bacteria (34.6%) in their fingernail contents. These findings indicate the need for intensive training/retraining and health education of all food service employees and strengthening the existing screening methods to control the problem of bacterial infestation in food handlers.

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Chapter One: Introduction

Food-borne diseases (FBD) are a major public health concern around the world. The rate of food-borne diseases is estimated at 30% in developed countries, and up to 2 million deaths are reported each year (Abebe *et al.*, 2020). CDC estimates that each year 1 in 6 Americans get sick from contaminated food or beverages and 3,000 die from foodborne illness (CDC, 2023).

Food can be contaminated by physical, chemical, and microbiological agents. The microbial agents responsible for food-borne diseases are bacteria, viruses, parasites, and fungi. However, the main causes of foodborne illness are bacteria, which accounts for 66% of foodborne disease (Mengist *et al.*, 2018, Belhu *et al.*, 2020). Poor sanitation habits and food handling, inadequate food safety programs, lack of knowledge of food handlers, lack of clean water supply, and poverty are reasons for FBDs in developing countries (Eltayeb *et al.*, 2020).

A food handler is anyone who works in eating and drink establishments and handles food or contacts with any utensils or equipment that are probable to be in contact with food, such as chopping boards, bowls, plates, or cutlery (Al Bayari *et al.*, 2023). In addition, the human body surface is always in contact with environmental microorganisms and becomes readily colonized by certain microbial species, gram-negative, gram-positive bacterial and parasitic infections in clinical specimens (Eltayeb *et al.*, 2020). The spread of foodborne diseases through food handlers is a persistent and common global problem. Infected food handlers with poor hygiene practice working in foodservice establishments are potential transmitters and sources of the disease due to pathogenic organisms such as infection with several enteropathogenic bacteria. They can transmit both non-enteric and enteric parasitic and bacterial infections through the food that they handled (Eltayeb *et al.*, 2020). Generally, *Escherichia coli, Bacillus cereus, Salmonella, Hepatitis, Shigella, Staphylococcus aureus, Rotavirus, Brucella* and other enteric bacteria are the most common etiologic agents causing

FBDs which could be harbored and transmitted via food handlers (Awol *et al.*, 2019, Eltayeb *et al.*, 2020).

Food handlers who harbor and excrete microbial agents may contaminate foods by transmission from their faces via their fingers into the food processing chain, and finally to healthy individuals (Mengist *et al.*, 2018). Compared to other parts of the hand, the area beneath the fingernails harbors the most microorganisms and is most difficult to clean(Mengist *et al.*, 2018). Hence, the hand is the major vehicle of the transmission of numerous microbes, including the enteric species, its contamination of plays a key role in the fecal-oral transmission of diseases (Eltayeb *et al.*, 2020).

As mentioned above knowing the prevalence and detection of pathogenic bacteria among food handlers are crucial and could help intervention and prevention of serious food borne diseases. Therefore, the present study aimed to estimate the presence of some pathogenic bacteria among food handlers working in food establishments in Erbil city.

Chapter Two: Materials and Methods

2.1 Materials

2.1.1 Laboratory Equipment's and Apparatus

The instrument and their manufactures used in this study are summarized in table (2.1)

Table 2.1 equipments and instruments, company and origin

No.	Equipment	Company	Country
1	Autoclave	hirayama	Japan
2	Balance	kern	Germany
3	Disposable Petri plat	Afco-Dispo	Jordan
4	Incubator	Memmrt	Germany
5	Light microscope	Olympus	Japan
6	Oven	Memmert	Germany
7	Transport swab	Vircell	Spain

2.1.2 Culture Media:

Ready media which were used listed in table (2.2). Prepared and sterilized according to the manufacturing information on the container.

Table 2.2 Media with their manufacturing company

No.	Culture media	Company/Origin
1	MacConkey agar	Oxoid, UK
2	Eosin Methylene Blue agar	Oxoid, UK
3	Mannitol Salt agar	LabM, UK
4	Triple sugar iron agar	Oxoid, UK.
5	Xylose- Lysine Deoxycholate	Oxoid, England
6	Salmonella Shigella agar	Oxoid, England
7	Brain Heart Infusion agar	Oxoid, England

2.1.3 Chemicals:

The chemical substances that were used in this study are shown in the following table (2.3).

Table 2.3: Chemicals, company, and origin

No.	Chemicals	Company/Origin
1	Crystal violet	Fluka/Switzerland
2	Ethanol	Scharlau/spain
3	Gram stain	Atom scientific/ UK
4	Kovacs reagent	Merck,Germany

2.2 The Methods

2.2.1 Specimen Collection

A total of 52 samples from food handlers' hands (finger nails and palms of both the left and the right hands) as well as from their nose and urine were collected during September to March, 2022-2023 in Erbil city. Samples were directly transported to the laboratory in a cooler box with ice packs (4 to 8 ◦C) for analysis (FDA, 2020, Lubis *et al.*, 2020).

2.2.2 Isolation of bacterial pathogens

The specimens were initially cultured onto Xylose Lysine Deoxycholate (XLD) and Salmonella Shigells agar (SSA) to isolate colonies of Shigella and Salmonella species. Other MacConkey agar (MA) and Mannitol Salt Agar (MSA) plates were inoculated to detect the *E. coli* and *S. aureus* bacteria in the samples respectively. Plates incubated for 24 h at 37 °C (FDA, 2023).

2.2.3 Identification of Bacterial Isolates

2.2.3.1 Microscopic Examination

2.2.3.1.1 Gram Staining Method

Gram stain was the first examining test usually done after bacterial growth for the differentiation between Gram negative and Gram positive according to Talaro (2002).

All the bacteria isolated were examined for gram reaction. A small quantity of bacterial suspension from the 24 hours pre-grown colony incubated at 37°C was spread on a glass slide, and a thin smear was made, the film air-dried and then fixed using the gas flame of benzene burner. The fixed smear stained with Gram stain and examined under microscope oil immersion lens (Tille, 2018).

2.2.3.2 Cultural Identification

Each sample inoculum was streaked (swap) on XLD, SSA, MA and MSA. After overnight incubation of all cultured media, the growth of *Salmonella* and *Shigell*a species were detected by their characteristic appearance on XLD agar (*Salmonella*: red with or without a black center; *Shigella*: red/pink colonies).The individual pink colonies on MA were identified as *E. coli* as well as small circular yellow colonies with yellow zones on MSA were examed as *S. aureus*. Furthermore, the pink colored colonies appeared after incubation at 37ºC for 24 hours on MA were confirmed by streaking on eosin methylene blue agar (EMB)(FDA, 2023).

2.2.3.3 Identification Based on Biochemical Tests

Biochemical identification of suspected colonies was carried out according to the methods recommended by FDA (2023) employing the following tests: Indole production, Citrate utilization, Urease, Catalase, Sugar fermentation and H_2S production tests for gram negative bacteria. Mannitol fermentation, coagulase, DNase and catalase for *S.aureus.*

Chapter Three: Results and Discussion

Food borne disease outbreaks continue to happen, despite the progress achieved in food quality and safety, the restricted research on food handlers and food handling practices in food establishments indicates that food handling problems need to be highlightened. Food handlers act as a vehicle for microorganisms causing a potential risk to the public health(Allam *et al.*, 2016a). Therefore, the current study was conducted to evaluate the occurrence of pathogenic bacteria among apparently health food handlers from different restaurants at Erbil city.

3.1 Identification and characterization of pathogenic bacteria.

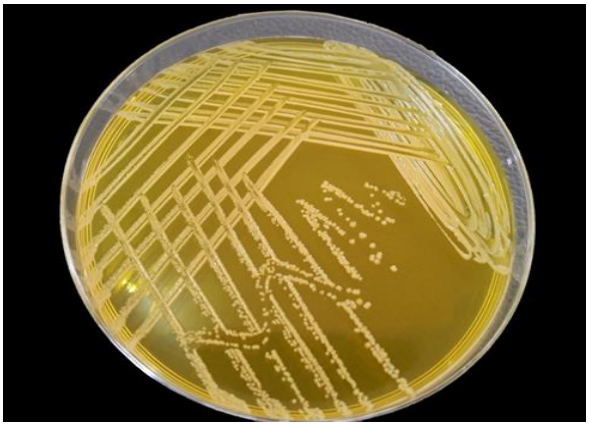
Identification of bacterial isolates include some morphological, cultural and biochemical tests. On the basis of conventional microbiological and biochemical examination out of the total 52 food handler samples taken, 27.49% of the samples were positive for pathogenic bacteria as indicated in table (3.1).

On mannitol salt agar which is a selective medium and one of the most important tests for identification of *S. aureus*, colonies were round, smooth, glistening with golden yellow pigmentation. Likewise, El-Jakee *et al.* (2010) reported the golden yellow pigmentation of *S. aureus* colonies. The golden yellow color of *S. aureus* is due to staphyloxanthin pigment. Those isolates that lack this pigment are white in color (Tille, 2018).

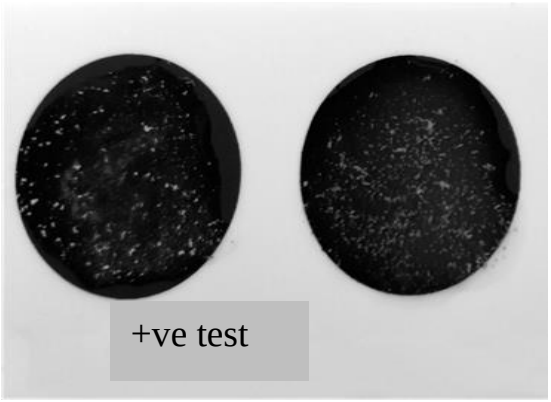
For Enterobacteriacaeae, the following media MacConkey Agar (MCA) and EMB for *E. coli* detection, Purple colonies on MCA and dark centered and flat, with metallic sheen on EMB were identified as *E. coli* as clear in figure (3.4*).* On the other hand, palm, fingernail, nose and urine swabs were inoculated onto the media recommended for *Salmonella* spp. including: Xylose lysine deoxycholate agar (XLD) and Salmonella shigella agar (SS) (Oxoid, UK) medium, *Salmonella* colonies, characterized by producing pink-red colonies with black centres on XLD medium, and white colonies

with black centres on SS medium figure (3.2) exhibit this characteristic. The colonies were confirmed as Gram negative bacteria using Gram staining procedures. These characteristics come in accordance with the corresponding cultural characteristic of isolated bacteria that mentioned byAl-Humam and Mohamed (2022) Also, FDA (2023) mentioned that rapid identification methods.

Regarding microscopical examination and biochemical characteristics, pure colonies were identified according to their gram staining and other microscopically characteristic. *S.aureus* was gram positive grape shaped arrangement whereas *E.coli and Salmonella* were gram negative rods in shape, non-spore forming as shown in figure (3.1), (3.2) and (3.3) respectively. Then bacterial isolates were subjected to the related biochemical tests. Results illustrated in fig (3.2) showed that all *S. aureus* isolates were positive for catalase, slide coagulase, DNase and hemolytic activity. Moreover *Salmonella* isolates gave positive for motility, citrate, H_2S production and catalase while negative for indole production as in figure (3.2). Additionally, table (3.3) illustrates that *E coli* isolates were had given positive test for catalase, indole, and for lactose and glucose fermentation, but negative test for oxidase, citrate and urease.Hasan and Hoshyar (2019) Al-Humam and Mohamed (2022) and FDA (2023) mentioned that such characteristics usually are coming in accordance with those belonging toisolated bacteria.

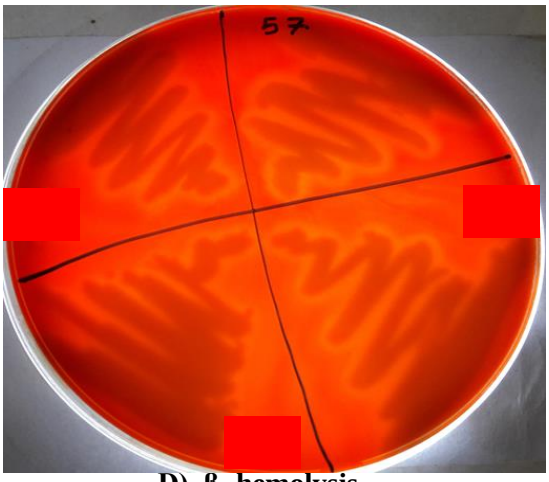


A) Mannitol fermentation

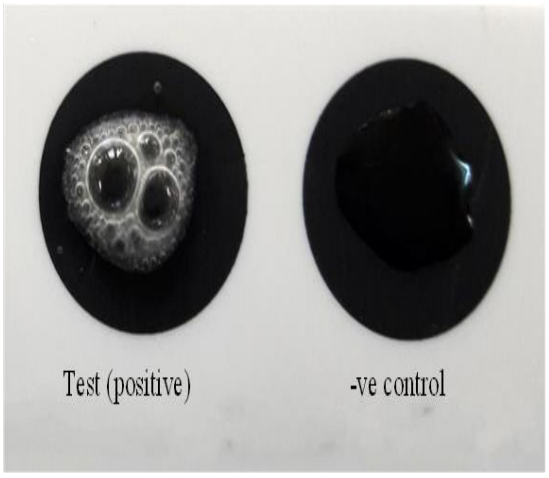


B) Slide coagulase test

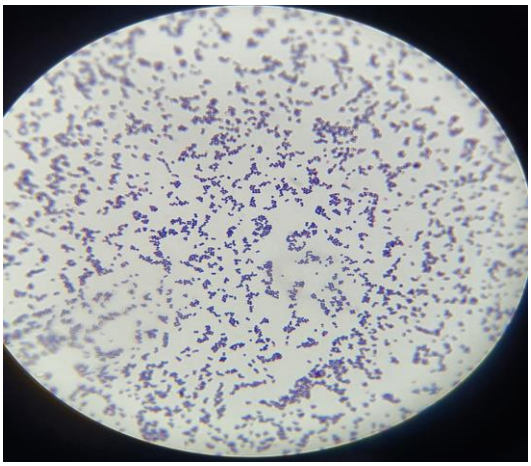
C) DNase production



D) β- hemolysis



E) Catalase test



F) Gram stain

Figure 3.1 Some biochemical, cultural and microscopical characteristics of *S.aureus* isolates

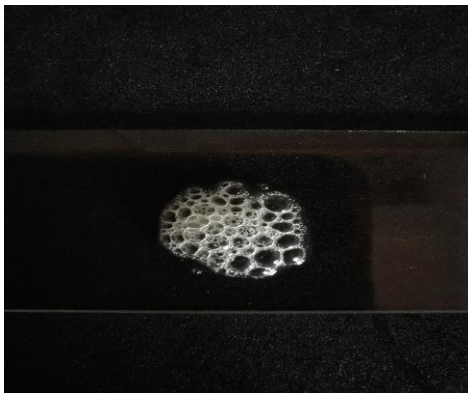
A) SS agar

B)+ve Citrate test

C)-

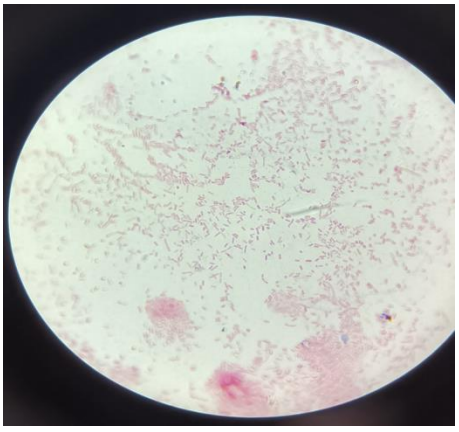
Ve indole test

D) + Ve Motility test



E)

Catalase test

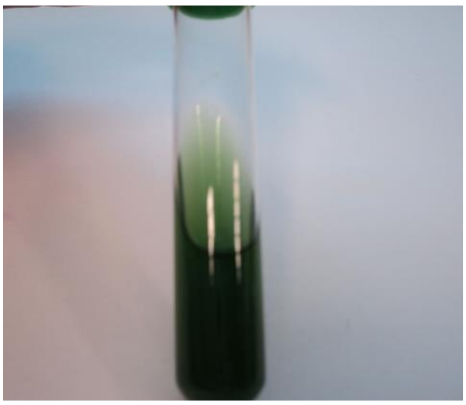


F)

Gram stain

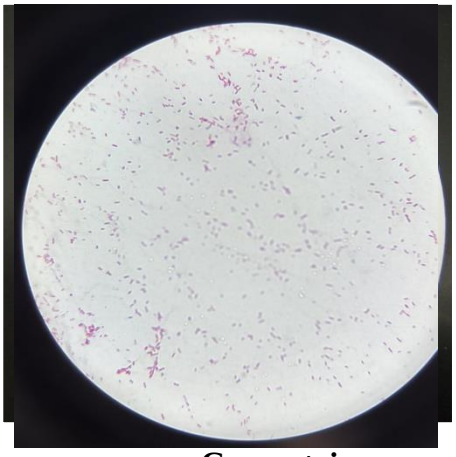
Figure 3.2 Some biochemical, cultural and microscopical characteristics of *Salmonella* isolates.

Indole Test / +ve test



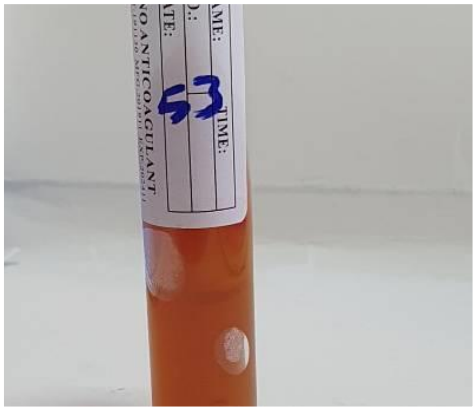
Citrate Test / -ve test

Catalase Test: +ve test



Gram stain

Urease Test : -ve test



Suger fermentation and H_2S production.

Figure 3.3 Some biochemical tests of *E. coli* isolates

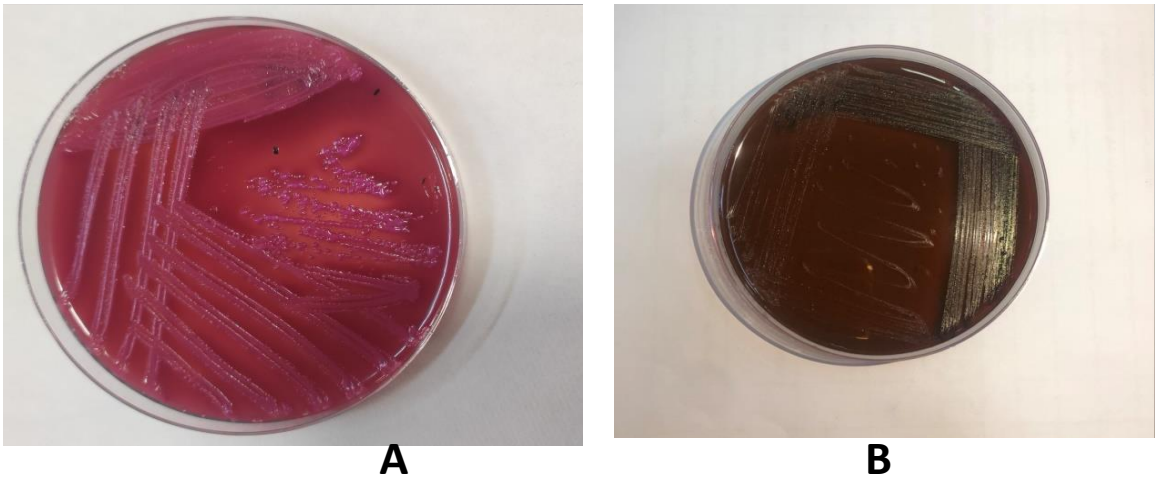


Figure 3.4 Growth of *E. coli* on **A**. MacConkey agar, **B**. Eosin methylene blue

3.2 Prevalence of Isolated Pathogenic Bacteria.

In this survey of 52 food handlers were examined, 34.6% (n = 18) were carrier of some pathogenic bacteria mainly *S.aureus* and *E.coli* in their fingernails, 25% (n = 13) were found to be harboring *S.aureus* and *E.coli* in their palm and nostrils, while 25% (n = 13) of urine samples were positive for *S.aureus, E.coli* and *Salmonella* based on microbiological and biochemical examination as shown in table (3.1).Our results are nearly in agreement with previous studies reported from other parts of the country (Eltayeb *et al.*, 2020).

Table (3.1), also displays that the most prevalent pathogenic bacteria were *S.aureus* (16.3%) followed by *E.coli* (10.5%) and *Salmonella* (0.4%). This was nearly similar to the studies which were reported *S.aureus* as a predominant bacteria (Saeed and Hamid, 2010, Eltayeb *et al.*, 2020). A higher prevalence of *S.aureus* was reported by Nasrolahei *et al.* (2017) from Iran (65.4%) compared to the current study. High prevalence of *S. aureus* in this study was because it is the true pathogenic bacteria included in the resident micro-flora of the skin and 40—50% of healthy peoples carry *S .aureus* in the anterior nostrils of the nose and skin. Food handlers may contaminate food with *S. aureus* (common cause of food poising) if they don't properly wash their

hands after using a toilet, after making contact with their nose, and before touching food (Eltayeb *et al.*, 2020).

E coli is considered one of the enteric pathogens that are believed to be capable of being transmitted by food workers. The second prevalent contaminant isolated from the studied group was *E coli* (10.5%). This prevalence is in accordance with the reported prevalence by Nasrolahei *et al.* (2017) and Eltayeb *et al.* (2020). This result which reflects hand contamination with fecal matter pointed to inadequate and poor hand washing habits among food handlers as well as the different used methods of contaminant detection(Allam *et al.*, 2016b). It should be kept in mind that, although not analyzed in this study, the isolated *E. coli* could comprise strains such as entero-haemorrhagic *E. coli* O111 or even *E. coli* O157:H7.

Salmonella showed a very low frequency of occurrence. Of the 52 samples examined, it was detected in only one sample (Table 3). The low frequency of *Salmonella* recorded in this study is in line with that previously reported by Ndu *et al.* (2022) who found that only 1(0.4%) out of 229 urine samples was positive for the pathogen. However, the small sample size in both studies may account for the low incidence

In the present study no *Shigella* species were recovered from any samples which are similar with the results from Addis Ababa University reported no *Shigella* isolate (Aklilu *et al.*, 2015). However, a study in Jimma University, Jimma, Ethiopia reported 0.8% *Shigella* isolate from food handlers (Gemechu *et al.*, 2022). These differences might be due to the higher standards of education, differences in geographical variation and the type of sample and culture media used(Gemechu *et al.*, 2022).

Three of the bacteria are categorized as the agent of food-borne disease; therefore, we stated all food-handlers who were confident with the pathogen are at risk of becoming a source of transmission.

Table 3.2 Frequency and type of bacteria isolated from food handlers in Erbil city

Variables	Bacterial frequency(%)				Total positive (%)
	S.aureus	***E.coli***	***Shigella***	***Salmonella***	
nail	9(17.3)	9(17.3)	0	0	18(34.6)
palm	8(15.3)	5(9.6)	0	0	13(25)
nose	10(19.2)	3(5.7)	0	0	13(25)
Urine	7(13.4)	5(9.6)	0	1*(1.9)	13(25)
Total	34(16.3)	22(10.5)	0	1(0.4)	57(27.4)

Conclusions and Recommendations

Conclusions

The study indicates clearly the relatively high frequency of occurrence of pathogenic organisms (27.4%) among food handlers in the city of Erbil. Among the pathogenic organisms identified, *S. aureus* were the most prevalent. Our results also indicate that these findings indicate that the spread of food-linked pathogens and diseases is highly likely and that there is an imperative need for protective measures, including increased public awareness about foodborne diseases, regular monitoring of food handlers for pathogenic organisms, and intensive training on primary health care and hygiene.

Recommendations

Future studies should be conducted to reveal the species distributions of the isolates, and possible risk factors associated with pathogens in food handlers should be included in order to plan appropriate interventions.

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