

Microbial Science Archives (ISSN: 2583-1666)

An International Research Journal

Journal homepage: www.microbial.sciencearchives.org

<https://doi.org/10.47587/MSA.2023.3308>



Research Article

Diagnostic study of some bacterial pollutants isolated from different food and environmental samples in the city of Mosul, Iraq

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Received: Aug 10, 2023/ Revised: Sept 2, 2023/Accepted: Sept 10, 2023

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Abstract

The research included a study of some bacterial pollutants. 144 samples were collected from different food and environmental sources, including leafy vegetables (celery, cabbage and lettuce), local dairy products (fresh buffalo milk, fresh cow's milk and local cheese), meat products (frozen minced meat, meat (non-frozen minced meat, sausage and chicken) and environmental samples from farm soil and irrigation water taken from the river. The isolates were purified on different culture media, and the total number of aerobic bacteria and the total number of coliform bacteria were calculated, in addition to the isolation and identification of some bacterial genera based on phenotypic and microscopic characteristics and the VITEK2 technique, as 14 genera and different species of bacteria were diagnosed according to the diagnostic keys adopted in the Bergey's Manual of Systematic Bacteriology. The results showed that the highest percentage of the total number of aerobic bacteria in food samples was in local cheese samples, reaching 118.3% the highest value was 209 x10⁵ CFU/ml, while the lowest percentage was in fresh cow's milk, amounting to 37.67%. The highest percentage of the total number of coliform bacteria in food samples was in celery, which amounted to 86.3%, and the lowest percentage was in cabbage, which amounted to 28.1%. From the 14 different genera and species of bacteria, that were isolated and diagnosed, the highest percentage of isolation was 68.05% for *Escherichia coli*. and 34.02% for *Proteus spp.*; then the lowest percentage of the isolates was to *Staphylococcus lentus*, which amounted to 4.86%. Using the VITEK2 technique six different species of bacteria were diagnosed, which were: *Staphylococcus lentus*, *Staphylococcus saprophyticus*, *Kocuria kristinae*, *Enterococcus faecalis*, *Enterococcus gallinarum* and *Enterococcus casseliflavus*.

Keywords: Bacterial contaminants, Meat products, Dairy products, Coliform bacteria, VITEK2

Introduction

Food contamination is one of the most dangerous types of pollution that poses a direct threat to the public health (Garvey, 2019). Food pollution and its problems have increased in recent years and led to many diseases such as food poisoning, which is caused by some microorganisms like bacteria, fungi, parasites, and viruses (Bintsis, 2017). Humans may play a major role in bringing these germs to foodstuffs due to the low level of health awareness and personal hygiene among workers in the field of preparing and making food (Thomas, 2018). Food or waterborne diseases occur when food or water contaminated with certain microbes is ingested and the microbes establish themselves in

the host (human or animal), transmitting intestinal toxins that affect the life of the organism (Todd, 2020). Some foods transmit microorganisms that cause food contamination, such as dairy products, milk, cheese, cooked and uncooked meat products, processed meat (sausage), poultry, vegetables in general, and leafy vegetables in particular (Yu et al., 2015). Food contamination may occur at the beginning of the food chain. Some wild animals, such as flies and rodents, are classified as sources of soil and field contamination, resulting in human infection through direct contact with farms or contamination of poultry fields (Sheng et al., 2022). In fields and farms, microbial contamination results from fertilizers or fodder contaminated due to improper storage or animal feces, which lead to contamination of the soil and water used for

irrigation; Therefore, preventive measures are prescribed as part of established good agricultural practice systems, and pre-harvest contamination control and reduction is a key aspect of food safety (Sarfraz et al., 2017). Domestic and industrial wastewater has a direct impact on river water. When these pollutants reach the river, the water becomes contaminated with different types of organic pollutants that contain harmful microorganisms and when it is used to irrigate farmland, it transfers pollutants to it (Zhang et al., 2020). Pollution is a global problem of concern to environmental professionals and has serious consequences for humans and components of the ecosystem. Due to neglect of environmental problems, many areas have become polluted, such as markets due to failure to use correct storage and display methods, and residential areas due to poor municipal work and the lack of health and environmental awareness among the population (Gourama, 2020). Among the most common foodborne bacterial pathogens are *Salmonella*, *Escherichia coli*, *Shigella*, *Bacillus*, and *Staphylococcus*. The aim of the study to isolate and identify different types of bacterial contaminants from food and environmental samples by different methods.

Materials and Methods

Sampling

The study was conducted in the laboratories of the College of Environmental Science and Technology and the College of Science at the University of Mosul - Iraq, for the period from September 2022 to February 2023. 144 samples were collected from different sources, including four different groups. The first group included leafy vegetables (celery, cabbage, lettuce), the second group included local dairy products (fresh buffalo milk, fresh cow's milk, and local cheese), and the third group included meat products (Frozen minced meat, non-frozen minced meat, sausage, chicken) which were collected from the local markets of the city of Mosul in a healthy and clean manner and placed in sealed polyethylene bags and transported in sealed packages. A package with small ice cubes to protect it from environmental changes until it reaches the laboratory. The fourth group included environmental samples from the soil of a farm in the Rashidieh area and irrigation water from the farms themselves (pumps drawing water directly from the river). The meat has been kept in a freezer at a low temperature -18°C until it is prepared for operation.

Prepare samples for estimation of the total number of bacteria and the total number of coliform bacteria 10 gm of the solid samples, such as frozen and non-frozen meat samples, sausage, chicken, cheese, and leafy vegetables, were weighed (taking into account the addition of calcium chloride to the cheese sample to disintegrate it), and placed in a sterilized electric mixer (blender) with 90 ml of distilled water for a period of time. 2 minutes, at 230 r/min. This was the first 10^1 dilutions, from which the rest of the dilutions were made. Where 1 ml of it is withdrawn into a test tube containing 9 ml of normal saline solution, and this is the second dilution 10^2 . The rest of the dilutions are completed with the fifth dilution 10^5 .

For liquid samples such as milk and water, 1 ml of the sample was taken and added to 9 ml of normal saline to obtain the first dilution 10^{-1} and complete the rest of the dilutions as mentioned above. For soil samples, 1 gm of the sample was taken and 9 ml of distilled water was added to a test tube and it was shaken well for 5-10 minutes to homogenize the solution and disintegrate the soil, by a micropipette 1 ml of the mixture was added to a test tube contained 9 ml of normal saline, this was the first dilution 10^{-1} , and the previous steps were followed for the rest of the dilutions. 0.1 ml of the fourth and fifth dilutions (to make replicates) were cultured on Nutrient agar medium to estimate the total number of aerobic bacteria, and 0.1 ml of the second and third dilutions (to make replicates) were cultured on MacConkey agar medium to estimate the total number of coliform bacteria, the plates were incubated with a temperature 37°C for 24 hours, after which the number of colonies developing on the dishes was counted and multiplied by the reciprocal of the dilution factor and the average number was extracted.

Isolation and purification of bacterial isolates

To isolate different species of bacteria from food and environmental samples, 0.1 ml of the fourth and fifth dilutions (replicates) were cultured on multiple media such as Nutrient agar (N.A) to isolate *Klebsiella spp.*, *Enterococcus spp.* (Jebrail Chicho & Sorchee, 2023). Mannitol salt agar (M.S.A) used to isolate *Staphylococcus spp.*, *Streptococcus spp.* (Hamad & Atiyea, 2021). Salmonella-Shigella agar (S.S) medium used to isolate *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*. (Tagar & Ahmed, 2022). MacConkey agar (M.A) medium used to isolate *Bacillus spp.*, *Proteus spp.* (Zaman et al., 2022). Blood agar medium (B.A) was used to isolate *Kocuria kristinae* (Al-Hasseney, 2022). The Petri dishes were turned over and incubated in the incubator at 37°C for 24 hours, then the growth of the colonies was monitored and the results were recorded. to preserve the isolates for a short period ranging from one to two months, the pure bacterial isolates were cultured in tubes containing Nutrient agar (Slant) and incubated at a temperature of 37°C for 24 hours, then placed at a temperature of 4°C . As for keeping the isolates for a long period, the isolates were inoculated in test tubes containing 5 ml of brain heart infusion broth with 15% sterile glycerol, then the test tubes were incubated at a temperature of 37°C for 24 hours and finally frozen at -20°C (Ikken et al., 2021).

Diagnosis of isolates

Depending on international sources, the isolated bacteria were diagnosed with several tests, including

Phenotypic Test

The cultivation characteristics were studied on different media. The shape, size, and color of the colonies were observed, and they were diagnosed based on the cultural and phenotypic characteristics.

Microscopic test

smears were prepared from pure colonies grown on different media

and stained with the gram stain. Using light microscopy, the shape of the cells and their ability to stain were observed to be diagnosed as whether they were positive or negative.

VITEK2 technique

This technique was used to diagnose bacterial colonies grown on different media, and the technique included 64 biochemical tests to diagnose bacterial isolates (Dos Reis et al., 2022).

Results and Discussion

Food contamination with different types of bacteria is a serious problem because it causes many diseases (Abebe, 2020). Annually, 600 million people around the world are infected with various diseases transmitted through food (WHO, 2020). Food contamination occurs due to environmental, animal or human sources, and pollution of the surrounding environment often occurs due to human activity and animal feces (AkedA, 2015). The results showed that the studied food samples contained different numbers of total aerobic bacteria, the highest percentage of the total number of bacteria was in the samples of local cheese, reaching 118.3%, and the highest value of the total

number of bacteria in it was 209×10^5 CFU/ml, while it was the lowest Its percentage in fresh cow's milk samples amounted to 37.67% as shown in Table 1. The reason may be due to cheese contamination due to primitive manufacturing methods, poor storage, contamination coming from utensils or manpower, or that it is old-fashioned (Heyi et al., 2023).

Although most bacteria may be unsatisfactory, it is known that all kinds of cheese contain It contains different types of beneficial bacteria, such as Lactobacillus, but the other part is pathogenic and toxic, and its main source may be infected udder milk that has not been treated with heat (Bastam et al., 2021). While the bacteria were present in small numbers in fresh cow's milk, the reason is that the milk was not stored as it was milked and transferred directly to the laboratory (Mbye et al., 2020). The total count of coliform bacteria is important for detecting the presence of polluting intestinal bacterial species in the surrounding environment, the most important of which are *E.coli.*, *Enterococcus spp.*, *Klebsiella spp.*, and *Streptococcus spp.* The presence of these pathogenic bacteria in the human or animal body is evidence of fecal pollution of the surrounding environment, whether the stool is of human or animal origin (Soare et al., 2022).

Table 1. The total number of bacteria from food and environmental samples in the city of Mosul X 10^5 CFU/ml

Samples	1	2	3	4	5	6	7	8	9	10	11	12	%
Celery	95	81	109	53	13	133	138	125	34	130	138	39	90.67
Cabbage	8	11	10	6	2	12	82	43	10	87	133	92	41.3
Lettuce	98	4	3	88	172	83	94	39	19	44	19	182	70.42
Frozen minced meat	53	28	15	112	0	8	25	97	70	59	41	29	44.75
Non frozen minced meat	20	109	182	88	100	74	95	187	83	56	200	189	115.2
Chicken	52	192	17	36	16	40	62	6	21	3	136	190	64.25
Sausage	0	200	128	63	146	200	203	90	93	20	39	88	65
Fresh cow's milk	2	24	7	85	35	64	82	10	0	41	28	74	37.67
Fresh buffalo milk	65	118	93	112	147	45	193	12	98	16	118	155	97.7
Local cheese	30	140	150	44	112	196	183	209	37	80	143	96	118.3
Farm soil	32	196	38	45	164	80	46	17	97	36	178	39	80.67
Irrigation water	109	99	32	63	118	6	78	51	93	2	119	73	70.25

The results indicated that the highest percentage of bacteria was in celery, reaching 86.3% the highest value was in celery 250×10^5 CFU/ml, and the lowest percentage was in celery, which amounted to 28.1%, as shown in Table 2. Although both of them are leafy vegetables, celery is highly susceptible to pollution from the surrounding environment, since its leaves are small and exposed. As for the celery, samples were taken from the inner leaves of the plant, as it is less susceptible to contamination and does not allow most types of bacteria and molds to penetrate it (Swagato et al., 2015). From Table 1, we notice in the case of the total count of aerobic bacteria for environmental samples that soil samples contain a large number of bacteria, the highest value was 196×10^5 CFU/ml, these bacteria may be endemic (live and multiply in the soil) or intrusive (harmful bacteria caused by pollution of all kinds) (Lee

et al., 2021).

For the irrigation water samples, it was less polluted despite the fact that the water contained different types of bacteria that originally lived in the water and other types of harmful bacteria coming from factory waste and sewage (Zhang et al., 2021).

From Table 2, we note that the total number of coliform bacteria in the soil is also high, and the reason may be the use of organic fertilizers, poor reclamation of agricultural lands, or overgrazing and animal waste (Hilaire et al., 2022). While the irrigation water samples were less contaminated with coliform bacteria, because the water was taken from pumps that draw water from the river, meaning that the water is running and not static, so the percentage of contamination was less (Eissa et al., 2022).

Table 2. The total number of coliform bacteria from food and environmental samples in Mosul city * 10⁵ CFU/ml

Samples	1	2	3	4	5	6	7	8	9	10	11	12	%
Celery	58	20	13	19	88	128	29	38	82	261	250	50	86.3
Cabbage	0	0	2	18	0	6	95	33	82	7	12	83	28.17
Lettuce	60	10	1	32	18	37	35	77	28	56	67	20	36.75
Frozen minced meat	107	102	152	176	2	53	69	45	71	23	67	92	79.92
Non frozen minced meat	15	54	100	77	53	30	94	25	63	65	21	0	49.75
Chicken	81	5	7	18	24	19	0	0	12	189	25	18	33.17
Sausage	38	18	0	81	39	48	125	184	33	128	89	181	80.33
Fresh cow's milk	70	25	37	7	53	9	18	32	14	45	88	64	66.42
Fresh buffalo milk	65	49	112	84	124	98	35	66	36	63	9	56	38.5
Local cheese	43	2	100	15	65	72	5	89	71	45	28	70	50.42
Farm soil	87	154	21	83	29	0	33	25	65	47	11	178	61.08
Irrigation water	84	0	149	28	85	55	64	49	38	17	46	88	58.6

Table 3. Bacterial genera and species isolated from food and environmental samples

Samples & percentage	Percentage %	Isolates No.	Cabbage	Celery	Lettuce	Local cheese	Fresh cow's milk	Fresh buffalo milk	Sausage	Chicken	Frozen minced meat	Non-frozen minced meat	Farm soil	Irrigation water
<i>Staphylococcus lentus</i> (VITEK2)	4.86	7	0	2	1	1	0	0	1	0	0	2	0	0
<i>Staphylococcus saprophyticus</i> (VITEK2)	6.25	9	1	3	0	0	0	2	1	1	0	1	1	0
<i>Kocuria kristinae</i> (VITEK2)	9.02	13	0	2	3	0	0	1	1	1	1	3	2	0
<i>Enterococcus faecalis</i> (VITEK2)	28.4	41	6	3	2	0	3	4	4	2	3	3	7	4
<i>Enterococcus gallinarum</i> (VITEK2)	11.8	17	2	3	1	0	0	0	2	0	1	3	3	2
<i>Enterococcus casseliflavus</i> (VITEK2)	16.6	24	3	2	0	4	2	3	0	1	0	2	1	4
<i>Salmonella spp.</i>	9.02	13	0	0	0	0	0	1	5	4	0	1	2	0
<i>Streptococcus spp.</i>	9.7	14	1	0	0	1	1	4	2	3	0	1	1	0
<i>Escherichia coli.</i>	68.05	98	9	11	10	10	2	5	10	8	5	8	9	11
<i>Bacillus spp.</i>	15.2	22	3	5	2	1	0	3	4	0	0	1	2	1
<i>Staphylococcus aureus</i>	29.86	43	3	9	4	3	2	4	6	3	1	1	5	3
<i>Klebsiella spp.</i>	13.1	19	2	3	0	3	1	3	2	3	0	1	0	1
<i>Shigella spp.</i>	13.8	20	2	6	2	3	0	1	3	2	0	0	1	0
<i>Proteus spp.</i>	34.02	49	5	8	6	8	3	6	2	2	0	3	2	2

14 different genera and species of bacteria were identified according to the diagnostic keys adopted in the Bergey’s Manual of Systematic Bacteriology (Sneath et al., 1986). Fig. 1 and Table 3. show the diagnosed genera and species, their numbers and percentages, as the genus *Escherichia coli* predominated at a rate of 68.05%, while the lowest percentage was for the genus *Staphylococcus lentus* 4.86%, and the rest of the genera varied. Six species were diagnosed using the VITEK2 technique: *Staphylococcus (lentus and saprophyticus)*, *Kocuria kristinae*, *Enterococcus (gallinarum, faecalis and casseliflavus)*.

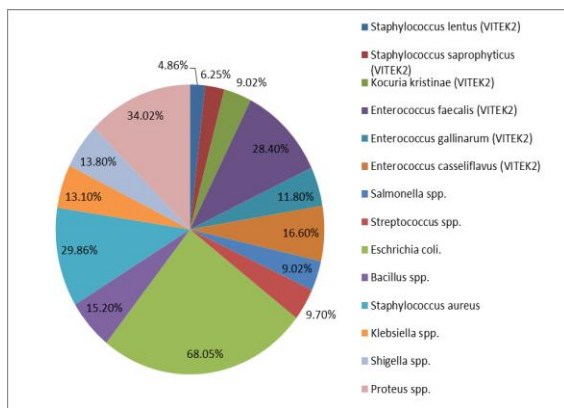


Fig. 1 Percentages of isolated bacterial genera and species

After the growth of bacterial colonies on the culture media, their phenotypic characteristics were recorded, such as shape (some

of bacteria were circular, regular, or irregular), color, where the colors varied between white, pink, yellow, blue-green, and black, and the texture was between sticky mucus to leathery hard (Woźniak et al., 2019). Shapes of bacteria were observed under the microscope. Gram-positive bacteria were observed to be spherical or bacilli. Whereas, Gram-negative bacteria were all rod-shaped and organized in chains or single (Hossain et al., 2019) as shown in Table 4.

Table 4. shows that the number of Gram-positive bacterial species and species isolated was 9 out of 14. The percentage of Gram-positive bacterial isolates was 64.28% and Gram-negative isolates were 35.71%, as shown in Table 5. The positive isolated bacterial genera are spore-forming, thermophilic, and antibiotic-resistant, and their susceptibility to resistance has recently increased due to the evolution of certain genes (Dimri et al., 2020).

One of the most important results obtained is the appearance of the bacteria *Staphylococcus aureus* which causes infection in dairy cows because it leads to a severe loss of milk, lower milk quality, and increased possibility of cow death (Okwu et al., 2019). This bacteria, when present in some foods, secrete toxins that are highly resistant to heat and are transmitted. These bacteria are introduced to food through methods and means of food preparation that do not observe safety and hygiene conditions. The toxin secreted by the bacteria in food causes severe vomiting and diarrhea (Tylor & Unakal., 2021). *Salmonella spp.* is the main cause of typhoid infection, and according to the World Health Organization (WHO) report in 2019, 9 million people are infected and more than 110 people die of typhoid every year (Nampota-Nkomba et al., 2023).

Table 4. Phenotypic and microscopic properties of bacterial species isolated from food and environment samples

Bacterial genera & species	phenotypic Properties			Microscopic Properties	
	Shape	Color	Media	Gram stain	Shape
<i>Staphylococcus lentus</i>	Regular circular	Yellow	M.S.A	Positive	spherical/cluster
<i>Staphylococcus saprophyticus</i>	Regular circular	Yellow	M.S.A	Positive	spherical/cluster
<i>Staphylococcus aureus</i>	Regular circular	Yellow	M.S.A	Positive	spherical/cluster
<i>Klebsiella spp.</i>	Regular circular	White	N.A	Negative	Bacilli
<i>Kocuria kristinae</i>	Irregular circular	White	B.A	Positive	spherical
<i>Enterococcus faecalis</i>	Regular circular	White	N.A	Positive	spherical
<i>Enterococcus gallinarum</i>	Regular circular	White	N.A	Positive	spherical
<i>Enterococcus casseliflavus</i>	Regular circular	White	N.A	Positive	spherical
<i>Salmonella spp.</i>	Regular circular	Black	S.S	Negative	Bacilli
<i>Streptococcus spp.</i>	Regular circular	Pink	M.S.A	Positive	spherical / rosary
<i>Escherichia coli.</i>	Regular circular	Pink	S.S	Negative	Bacilli
<i>Bacillus spp.</i>	Regular circular	Pink	M.A	Positive	Bacilli
<i>Shigella spp.</i>	Regular circular	Transparent with a pink focus	S.S	Negative	Bacilli
<i>Proteus spp.</i>	Regular circular	Transparent	M.A	Negative	Bacilli

E. coli bacteria are widely which found in various types of soil and water, and they coexist in the large intestine of humans and animals, most of its strains are harmless; however some of them can cause severe food poisoning resulting from the consumption of raw or undercooked minced meat products, unpasteurized milk and vegetables contaminated with feces (Denamur et al., 2021). *Bacillus spp.* bacteria cause food poisoning. The bacteria secrete two types of toxins, the first of which is characterized by diarrhea and the other by vomiting (Jawetz et al., 2019).

Shigella spp. leads to colitis of the rectal mucosa with concomitant malabsorption and the hallmark of bloodstained stools (Thomas et al., 2020). *Klebsiella spp.* causes pneumonia, urinary tract infections and wounds. It is caused by prolonged hospital stay, prolonged use of antibiotics, ventilators, intravenous catheters, and diabetes (Gorrie et al., 2022). *Enterococcus faecalis* is one of the most common species in humans. Enterococci colonize the gastrointestinal tract and harm the immune system, and are frequently present in the vagina or mouth (Krawczyk et al., 2021). *Proteus spp.* found in decomposing organic matter, wastewater, soil, mammalian intestines, human and animal feces, and are pathogens of urinary tract infections and infections, often in hospitals (Motahhir et al., 2017). The Figures below show the bacterial genera diagnosed in the study.

Table 5. Numbers and percentages of isolates relative to Gram stain

Bacterial genera	Numbers	Percentages %
Gram positive G+	9	64.28%
Gram negative G-	5	35.71%

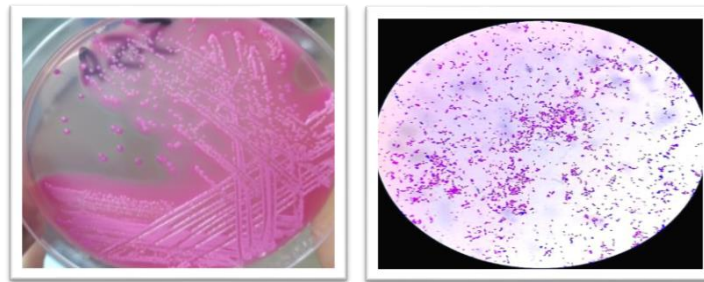


Fig. 2 *E. coli* bacteria on S.S medium and its colonies under the microscope

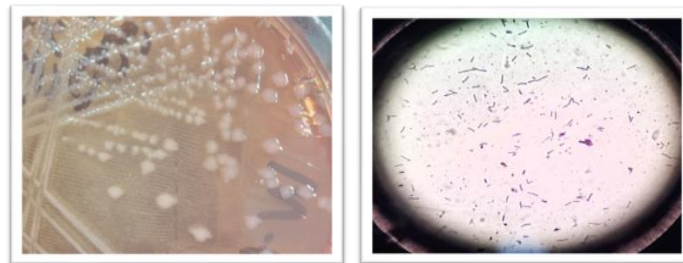


Fig. 3 *Shigella spp.* on S.S medium and its colonies under the microscope

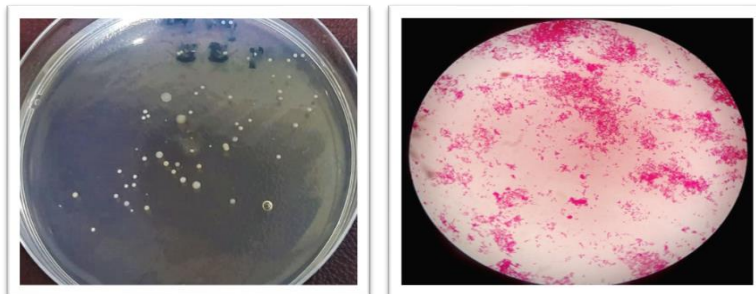


Fig. 4 *Klebsiella spp.* on N.A medium and its colonies under the microscope

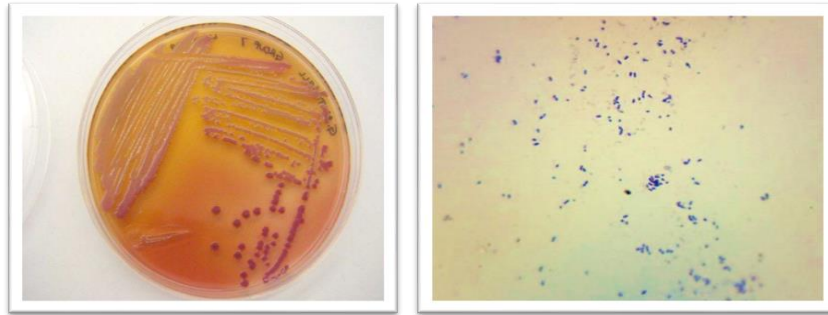


Fig. 5 *Streptococcus spp.* on M.S.A medium and its colonies under the microscope

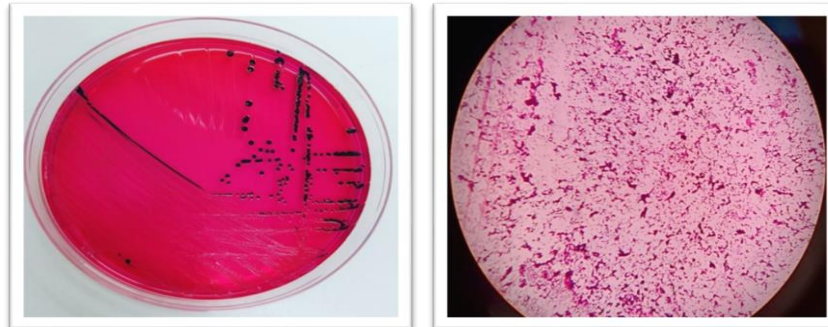


Fig. 6 *Salmonella spp.* on S.S medium and its colonies under the microscope

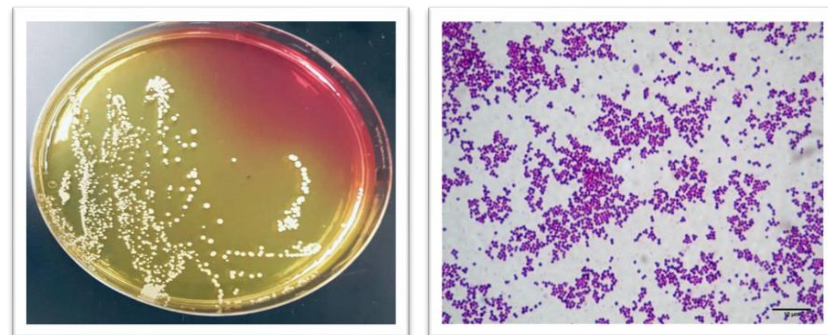


Fig. 7 *Staphylococcus aureus* on M.S.A medium and its colonies under the microscope

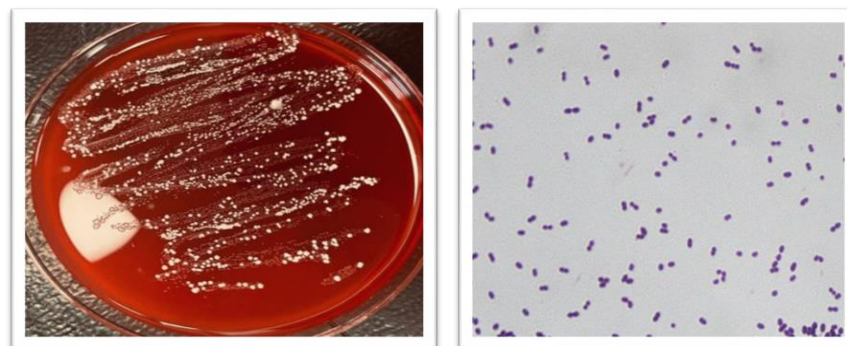


Fig. 8 *Kocuria kristinae* on B.A medium and its colonies under the microscope

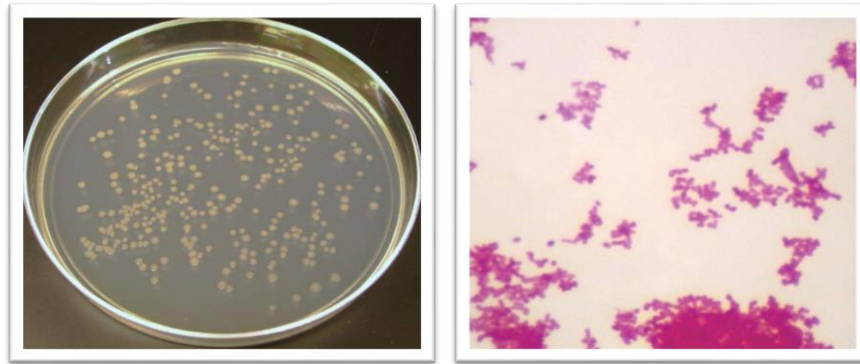


Fig. 9 *Enterococcus* spp. on N.A medium and its colonies under the microscope

Conclusion

The appearance of these multiple bacterial species in food and environmental samples is evidence that the wastewater discharged into the river has not been treated and transferred to agricultural lands to irrigate crops and thus pollute leafy vegetables. Contamination of food samples such as meat, poultry and processed meat with different types of bacteria results from human activity, lack of health awareness, and failure to clean cutting surfaces. Health conditions must be followed and the level of awareness of workers in the field of food manufacturing and marketing must be increased, as well as farmers should be trained to use modern methods to reduce pollution of agricultural lands, and finally the need to clean rivers and not to discharge sewage into the river.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

Funding support

The author declares that they have no funding support for this study.

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How to cite this article

Doori, H. L. and Hmoshi, R. M. (2023). Diagnostic study of some bacterial pollutants isolated from different food and environmental samples in the city of Mosul, Iraq. *Microbial Science Archives*, Vol. 3(3), 125-133. <https://doi.org/10.47587/MSA.2023.3308>

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