Diversity and Characterization of the Bacterial Communities of Different Water Resources in Egypt Concerning Contamination Type

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Abstract

The habitat of microorganisms is different from one to another, some are naturally found in water environment, others are introduced due to different sources of contamination. The present study aimed to characterize the bacterial community of diverse water resources in Egypt in relation to the contamination source pursued them. So, water samples were collected from seven different water resources; groundwater, Nile River water, agricultural drainage water (El-Hussieniya drain), sewage contaminated drain (Bahr El-Baqr drain), industrial wastes contaminated drain (Al-Khadrawiya drain), fresh water Lake (Nasser Lake) and brackish water Lake (Qaroun Lake).

A total of 2218 bacterial isolates were collected, purified and identified by means of biochemical reactions and 16S rDNA sequence analysis to 21 bacterial genera. The sewage contaminated drain water (Bahr El-Baqr drain) has the highest diverse bacterial community, while the fresh water (Nasser) Lake has the lowest diverse community due to lake of contamination. In addition, *Bacillus, Pseudomonas and Staphyllococcus* were the most popular genera, where they have been detected in all types of water resources. The less popular genera were *Yersinia, Klebsiella, Aerumonas, Shigella, Salmonella, Cardiobacterium, Arthrobacter and Streptobacillus*, where they all have been detected once.

Key words: Diversity, Bacterial Communities, bacterial genera

Introduction

The term diversity describes heterogeneity within a system. As applied to ecology it refers to (i) the total number of species present in an ecosystem and (ii) the number of individuals of each species present in that ecosystem. When dealing with microbes individuals can only be identified using culture techniques. The selectivity of culturing microorganisms in the laboratory automatically introduces bias into measuring diversity. Even with the limitations imposed by the selectivity bias, it is evident that determining the number of individuals of each species present is not a practical undertaking (Barton and Northup, 2011) Another widely used technique for estimating microbial diversity assumed by (Hirsch and Rades–Rohkohl, 1983; Stetzenbach *et al.,* 1986 and Pedersen and Ekendahl, 1990) to recover microorganisms present in a given water resource, counting them and identifying them, using physiologic criteria. For example, Pedersen and Ekendahl (1990) recovered four species of bacteria from a depth of 463 meters in a granitic aquifer in Sweden. The total plate count of this sample was 3.9×10^4 cells/ml. Of this total, 3.2×10^4 cells were identified as *Pseudomonas fluorescens.* These data showed that most of the viable cells present at that sampling depth were from a single species. That is to say, the observed diversity was fairly low. In a similar study conducted by Stetzenbach *et al.* (1986) who identified bacteria isolated from groundwater produced from deep wells in Tucson, Arizona. They found that 70% of the total viable microorganisms present were members of the genus *Acinetobacter.* Almost 10% of the microorganisms present were an un-identified pigmented organism. It is evident that as in the example given by Pedersen and Ekendahl (1990) the diversity of the groundwater flora is fairly low.

The objective of the present study is to characterize the bacterial community of seven selected water resources and interpret the results with types and sources of contamination.

Materials and Methods

2.1 Location of samples and description of sites
2.1.1 Groundwater samples

Five shallow groundwater samples have been collected from hand pumps in some rural areas, locations and sites description are given in (Table 1).

Table 1. Locations and sites descriptions of groundwater samples

Sample No	Code	Location	Description
1	G1	Aghour Assughra village – Qaluybia Governorate	Hand pump of depth 30 m, at a rural house
2	G2	Aghour Assughra village – Qaluybia Governorate	Old hand pump of depth 35 m, not in use since a long time
3	D	Darawa village – Munofia Governorate	Hand pump of depth 35 m, at a rural house
4	MG	Al Kharqaniyyah village – Qaluybia Governorate	Hand pump of depth 60 m, at a rural house
5	Br	Al Baradah village - Qaluybia Governorate	Hand pump of depth 20 m, at a rural house

2.1.2 River Nile water samples
Five water samples from River Nile have been collected from different sites, full description of sites and locations were given in (Table 2).

2.1.3 Drains water samples
Three drains were selected, one of them is receiving agricultural wastewater, the second is receiving domestic wastes, and the third is receiving industrial wastes. The full description of all the selected drains was illustrated in (Table 3).

Table 2. Locations and sites descriptions of Nile River water samples

Sample No	Code	Location	Description
1	AS	River Nile at Aswan, after the high dam – Aswan Governorate	Beginning of River Nile upstream in Egypt
2	IM	River Nile at Al-Wasta city – Beni Suef Governorate	End of upper Egypt region
3	BH	River Nile at Bahadh village - Qaluybia Governorate	Damietta branch
4	NI	River Nile at Al Qanatir Al Khayriyyah - Qaluybia Governorate	Damietta branch
5	Ds	River Nile at Desouk – Kafr El Sheikh Governorate	Rosetta branch after the discharge of El-Rahawy drain

Table 3. Locations and sites descriptions of drains water samples

Sample No	Code	Location	Description
1	Hu	**Sahl El-Husseiniya drain.** Samples collected from the drain before connecting with Bahr El-baqar drain	Receives agricultural wastewater from Sahl El-Husseiniya city, Alsharqia Governorate
2	BR	**Bahr El-Baqar drain.** Samples were collected from the drain at Port Saied Governorate, before connecting with Manzala lake	It is considered as one of the most polluted drains in Egypt (Abdel-Shafy and Aly, 2002). Discharge about 2.3 BCM/year to Manzala lake. It is passing through four highly populated Governorate; Qalubeya, Sharkia, Ismailia and Port Said. Receives agricultural, domestic and industrial wastewaters.
3	Kh	**Al-Khadrawiya drain.** Samples collected from Quesna industrial region, Munofia Governorate	Receives mainly industrial wastewater from factories pipes extended from Quesna industrial zone, Munofia Governorate

2.1.4 Lakes water samples
Two Lakes were selected; Nasser Lake (as an example of fresh water Lake), and Qaroun Lake (as an example of brackish water Lake), as shown in (Table 4).

Table 4. Locations and sites descriptions of Lakes water samples

Sample No	Code	Location	Description
1	NL	Nasser Lake, Aswan Governorate	It was generated by the construction of the Aswan High Dam between January 1964 and June 1968 (Salih et al. 2019). The area of the Lake is about 5000 km². The mean depth is 90 m and the maximum width is about 60 km (Farhat and Aly 2018).
2	QL	Qaroun lake, Al-Fayoum Governorate	Brackish water lake, receives discharges contaminated with agricultural, industrial and sewage wastes from El-Bats and El-Wadi drains without prior treatments (Derbala, 2018).

2.2 Diversity and characterization of the bacterial community of each water resource

2.2.1 Isolation, purification and pre-identification of bacterial isolates

Total viable bacterial count has been performed for each water resource. The growing colonies of each plate were differentiated and categorized according to the cultural characteristics, and then they were purified and maintained for further identification. The purified isolates were subjected to pre-identification, which included culture characteristics, Gram stain, biochemical characterization according to Bergey's manual of systematic bacteriology (Krieg and Holt, 1986; Sneath et al. 1986) They were classified to groups according to their Gram reactions; Gram positive cocci, Gram negative rods and Gram positive rods.

2.2.2 Full identification of the bacterial community of each water resource

The pre-identified isolates have been subjected to 16S rDNA sequencing for confirming their identification. DNA was extracted using the GeneJet genomic DNA extraction kit (Thermo K0721). PCR amplification of the 16S rDNA gene was performed using 10 ng of genomic DNA in 20 μl of 1×Amplitaq (Perkin-Elmer, 940 Winter Street, Waltham, Massachusetts 02451, USA) buffer (10 mmol/l Tris - HCl, 50 mmol/l KCl, 1.5 mmol/l MgCl2 , 0.001% gelatin) with 150 ng each of primers, F: 5 AGA GT T TG A TC C TGG CTC AG-3'and R: 5 GGT T AC C TT GTT ACG ACT T-3', 250 μmol/l of dNTPs, and IU of Amplitaq (Perkin-Elmer). Reaction mixtures of PCR were incubated at 95°C for 3 min, then cycled 35 times as follows: annealing for 30 sec at 95°C, and at 72°C for 4 min. Annealing temperature was 60°C for the first five cycles, 55°C for the next five cycles, and 50°C for the last 25 cycles. Finally, the mixtures were incubated at 72°C for 10 min and at 60°C for 10 min; 2.0 μl of each amplification mixture was analyzed by agarose gel (1.0% w/v) electrophoresis in a TAE buffer (0.04 mol/l Tris-acetate, 0.001 MEDTA) containing 0.5 mg/ml (w/v) ethidium bromide. Sequencing was carried out at Biomolecular Research Services (GATC Biotech, Konstanz, Germany) using ABI 3730 l× DNA sequencer with the same forward and reverse primers. The sequences obtained in this study were aligned to the GenBank database with the maximum similarity and identity using MEGA 6 database.

2.3 Statistical analysis of data

The predominated genera in each community, the most and the seldom present genera, the percentage of each group of bacteria and the percentage of each species were determined using STATISTICA software, ver 4.
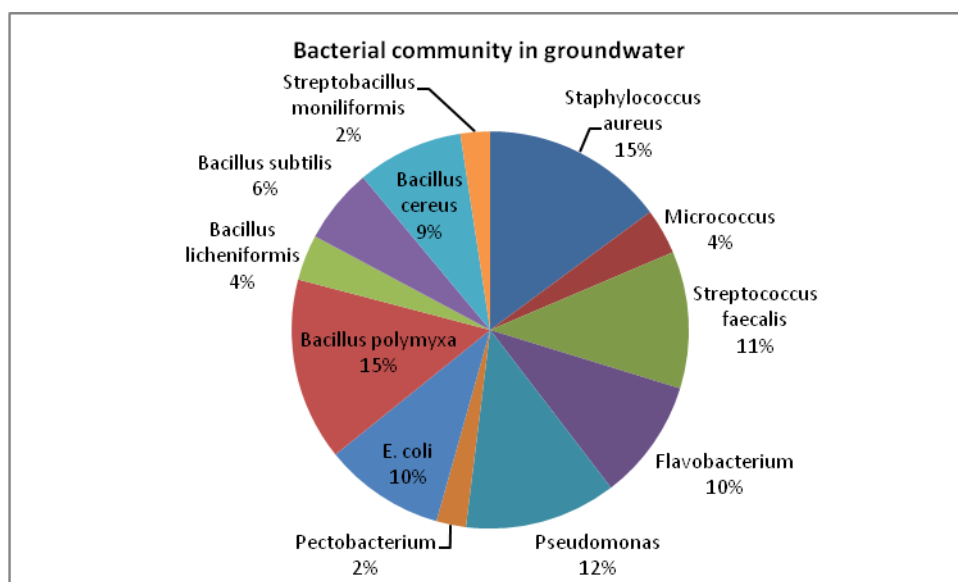
Results and Discussion

3.1 Diversity and characterization of the bacterial community in Groundwater

The identified isolates in groundwater are characterized as Gram-positive rods (35.8%), Gram-positive cocci (29.6%) and Gram-negative rods (34.6%) as illustrated in table (5) and fig (1). In addition, *Staphylococcus aureus* and *Bacillus polymyxa* recorded the highest number of isolates in the bacterial community, while *Pectobacterium* and *Streptobacillus moniliformis* recorded the lowest number. As the groundwater tested in the present work was free from sewage or industrial contamination (Chapelle, 2001), the bacterial community is considered of moderate diversity.

Table 5. Characterization of the bacterial community in groundwater

Bacterial group	Identified bacterial community in groundwater	No. of isolates	Isolate (%) in the community	Group (%) in the community
Gram positive cocci	*Staphylococcus aureus*	12	14.8	
	Micrococcus	3	3.7	29.6
	Streptococcus faecalis	9	11.1	
Gram negative rods	*Flavobacterium*	8	9.8	
	Pseudomonas	10	12.3	34.6
	Pectobacterium	2	2.4	
	E. coli	8	9.8	
Gram positive rods	*Bacillus polymyxa*	12	14.8	
	Bacillus licheniformis	3	3.7	
	Bacillus subtilis	5	6.1	35.8
	Bacillus cereus	7	8.6	
	Streptobacillus moniliformis	2	2.4	
Total		**81**		

Fig (1): Characterization of the bacterial community in groundwater

3.2 Diversity and characterization of bacterial community in Nile River water
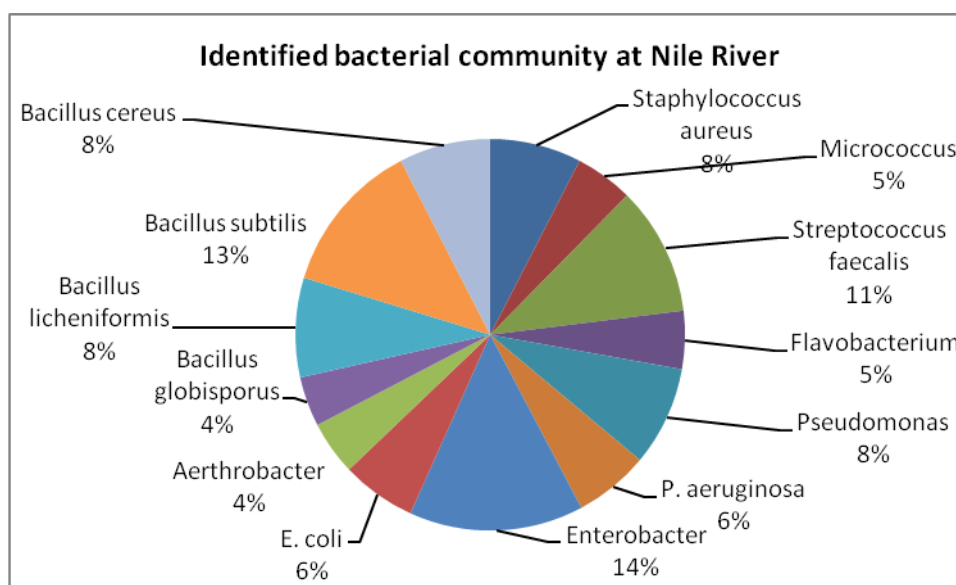
The identified isolates in Nile River water are characterized as Gram-negative rods (39.6%) Gram-positive rods (36.9%) and Gram positive cocci (22.9%) as illustrated in table (6) and fig (2). It was noticed that genus *Bacillus* (constitutes 32.5% of the community) and *Pseudomonads* (constitute 14.3% of the community) approximately comprise the half of the bacterial community of Nile River water, as they are normal inhabitants of soil and water, so, the contamination of such water is of acceptable ranges, and the diversity of bacterial community in such water resource is considered moderate.

3.3 Diversity and characterization of bacterial community in agricultural drainage water

The Agricultural drain water was characterized by a high number of Gram negative rods (67.5%) of the community, while the Gram positive cocci (18%) and the Gram positive rods (14.3%), as indicated in table (7) and fig (3). The majority of the community is included in Gram negative bacteria, nevertheless, there were only 6 genera have been identified. In addition, there are 2 genera of Gram positive cocci and 1 genus in Gram positive rods have been identified. So, the bacterial community in the tested agriculture drain water is considered of moderate diversity.

Table 6. Characterization of the bacterial community in Nile River water

Bacterial group	Identified bacterial community in Nile River water	No. of isolates	Isolate (%) in the community	Group (%) in the community
Gram positive cocci	*Staphylococcus aureus*	22	7.5	
	Micrococcus	14	4.8	22.9
	Streptococcus faecalis	31	10.6	
Gram negative rods	*Flavobacterium*	14	4.8	
	Pseudomonas	24	8.2	
	P. aeruginosa	18	6.1	39.6
	Enterobacter	42	14.4	
	E. coli	18	6.1	
	Arthrobacter	13	4.4	
Gram positive rods	*Bacillus globisporus*	12	4.1	
	Bacillus licheniformis	24	8.2	36.9
	Bacillus subtilis	37	12.7	
	Bacillus cereus	22	7.5	
	Total	**291**		

Fig (2): Characterization of the bacterial community in Nile River water

Table 7. Characterization of the bacterial community in agricultural drain water

| Bacterial Groups	Identified bacterial community at Al-Husseiniya drain	No. of isolates	Isolate (%) in the community	Group (%) in the community
Gram positive cocci	*Staphylococcus aureus*	34	8.2	18.2
	Streptococcus bovis	41	9.8	
Gram negative rods	*Flavobacterium*	28	6.7	
	Pseudomonas	55	13.2	
	P. aeruginosa	34	8.1	
	Citrobacter	33	7.9	67.5
	Enterobacter	44	10.5	
	E. coli	65	15.6	
	Pectobacterium	22	5.2	
Gram positive rods	*Bacillus cereus*	28	6.7	14.3
	Bacillus licheniformis	32	7.6	
	Total	**416**		
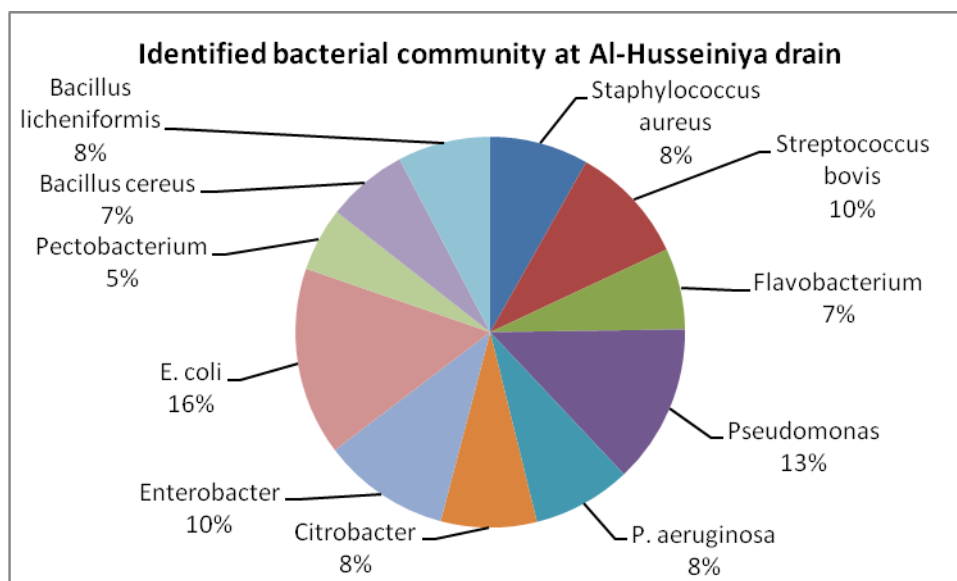
Fig (3): Characterization of the bacterial community in agricultural drainage water

3.4 Diversity of bacterial community in sewage contaminated drains
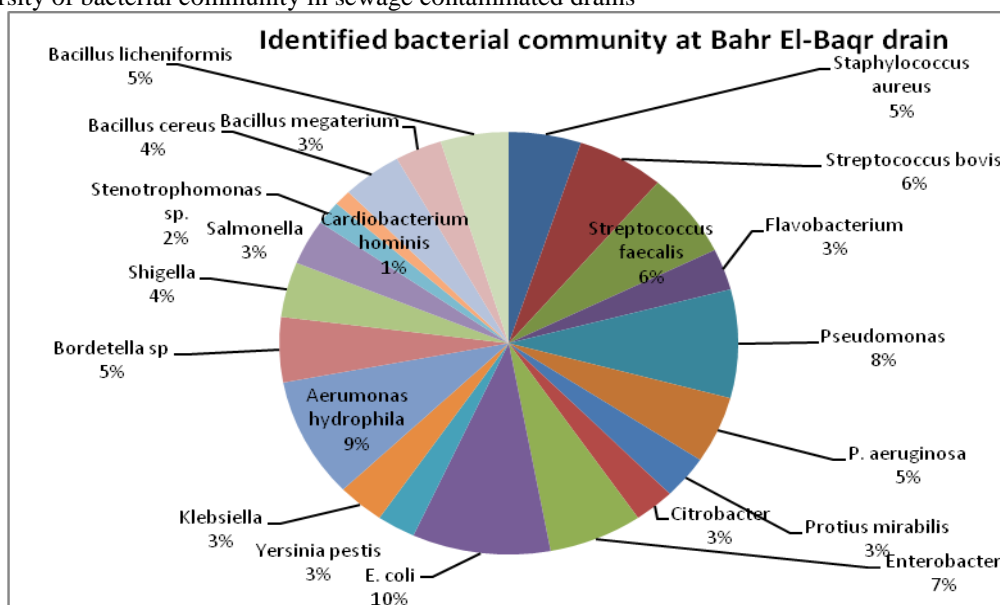

Fig (3): Characterization of the bacterial community in sewage drainage water

The majority of the bacterial isolates in sewage contaminated drainage water (Bahr El-baqr drain) were belonged to Gram negative rods, where they constitute 67.9% of the bacterial community. The identified bacterial community is given in table (8) and figure (5) where it contains 14 genera of Gram negative bacteria most of them belong to enterobacteriacea family due to sewage contamination. In addition, there are some genera have been rarely isolated like *Stenotrophomonas* and *Cardiobacterium*. The presence of *Shigella* is indicative for the renewable source of contamination of sewage. In conclusion, the bacterial community of Bahr El-Baqr drainage water as an example of sewage contaminated water is considered of high diversity.

Table 8. Characterization of the bacterial community in sewage contaminated drain

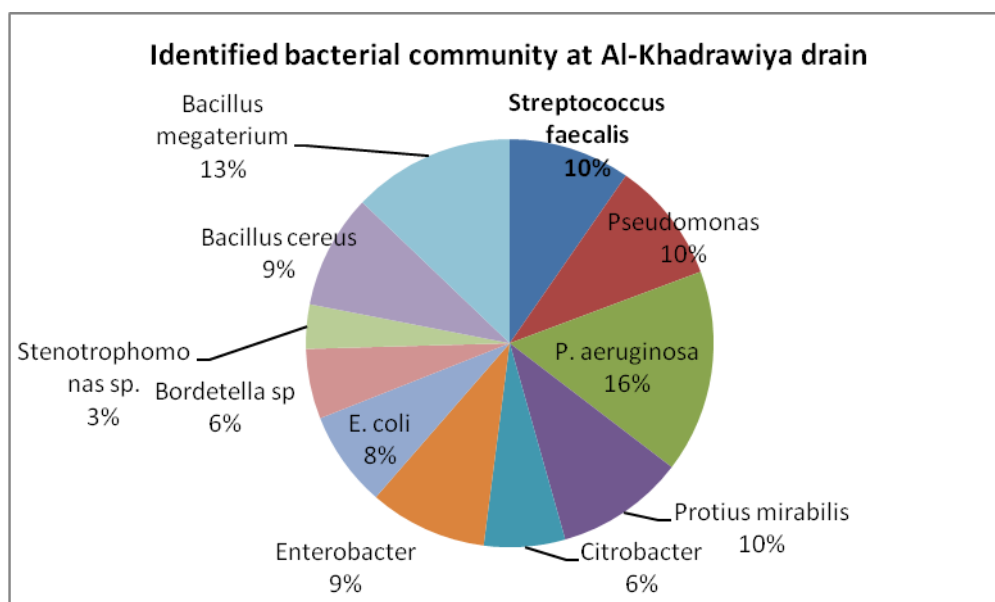| Bacterial Groups	Identified bacterial community at Bahr El-Baqr drains	No. of isolates	Isolate (%) in the community	Group (%) in the community
Gram positive cocci	*Staphylococcus aureus*	34	5.0	
	Streptococcus bovis	41	6.0	17.2
	Streptococcus faecalis	43	6.3	
	Flavobacterium	21	3.1	
	Pseudomonas	55	8.0	
	P. aeruginosa	34	5.0	
	Protius mirabilis	22	3.2	
	Citrobacter	19	2.8	
	Enterobacter	44	6.4	
	E. coli	65	9.5	
Gram negative rods	Yersinia pestis	18	2.6	67.9
	Klebsiella	22	3.2	
	Aerumonas hydrophila	61	8.9	
	Bordetella sp	33	4.8	
	Shigella	28	4.1	
	Salmonella	23	3.4	
	Stenotrophomonas sp.	12	1.8	
	Cardiobacterium hominis	8	1.2	
	Bacillus cereus	28	4.1	
Gram positive rods	*Bacillus megaterium*	22	3.2	14.9
	Bacillus licheniformis	32	4.7	
	Bacillus subtilis	20	2.9	
	Total	**685**		

3.5 Diversity and characterization of bacterial community in industrial wastes contaminated drainage water

The bacterial community of the industrially contaminated drain water (Al-Khadrawiya drain) was characterized by a high number of Gram negative rods (68.4%) as indicated in table (9) and fig (5). The bulk of the community is included in two genera; *Pseudomonas* (25.7%) and *Bacillus* (21.8%), these two prompt genera have been reported in the literature as strong bio-removers for chemical contamination of water so, they could predominate the community (Ewida, 2014; Jahin et al, 2014; Chuahan et al, 2016; Pathak et al, 2016; Ewida, 2020; Ewida et al, 2021). The total number of identified genera in the community was 9, so, it is considered of moderate diversity.

Table 9. Characterization of the bacterial community in industrial wastes contaminated drain

Bacterial Groups	Identified bacterial community at Al-Khadrawiya drain	No. of isolates	Isolate (%) in the community	Group (%) in the community
Gram positive cocci	*Streptococcus faecalis*	33	9.6	9.6
	Pseudomonas	33	9.6	
	P. aeruginosa	55	16.1	
	Protius mirabilis	35	10.2	
Gram negative rods	*Citrobacter*	22	6.4	68.2
	Enterobacter	32	9.3	
	E. coli	26	7.6	
	Bordetella sp	19	5.5	
	Stenotrophomonas sp.	12	3.5	
Gram positive rods	*Bacillus cereus*	31	9	21.8
	Bacillus megaterium	44	12.8	
	Total	**342**		

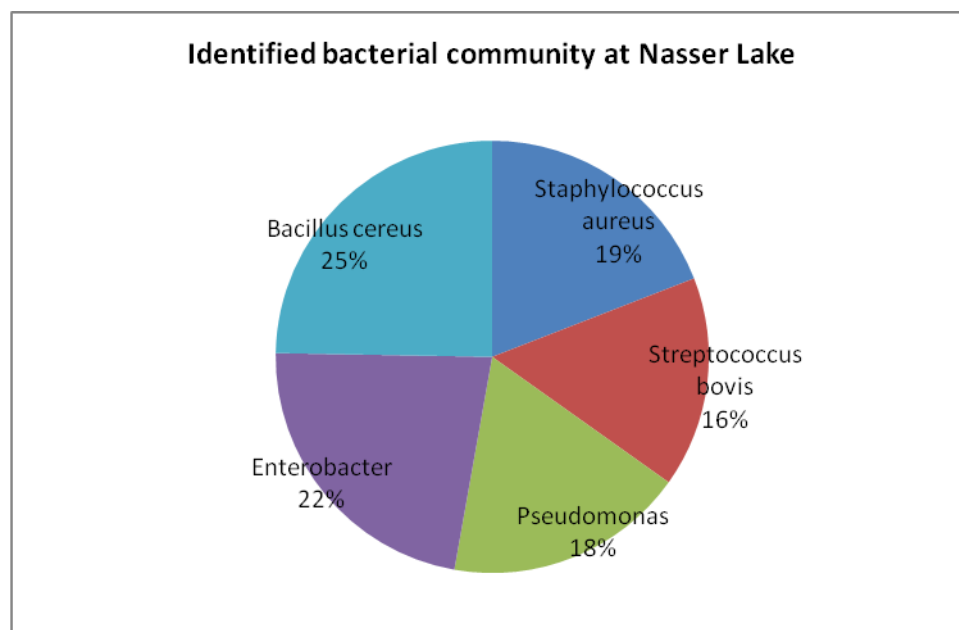**Fig (5): Characterization of the bacterial community in industrial wastes contaminated drain water**

3.6 Diversity and characterization of bacterial community in fresh water Lake

Small number of isolates has been collected from Nasser Lake water, which confirms that the bacterial community at this Lake is considered of low diversity. Nevertheless, the three bacterial groups were detected; Gram positive cocci represented genus *Staphylococcus aureus and Streptococcus bovis* which constituted 34.8% of the total number of isolates, the Gram-negative rods constituted 40.4% of total number of isolates. The percentage of Gram-positive rods was 24.7% including only one species (*Bacillus cereus*).

Table 10. Characterization of the bacterial community in fresh water Lake (Nasser Lake)

Bacterial Groups	Identified bacterial community at Nasser Lake	No. of isolates	Isolate (%) in the community	Group (%) in the community
Gram positive cocci	*Staphylococcus aureus*	17	19.1	34.8
	Streptococcus bovis	14	15.7	
Gram negative rods	*Pseudomonas*	16	17.98	40.4
	Enterobacter	20	22.47	
Gram positive rods	*Bacillus cereus*	22	24.7	24.7
	Total	**89**		

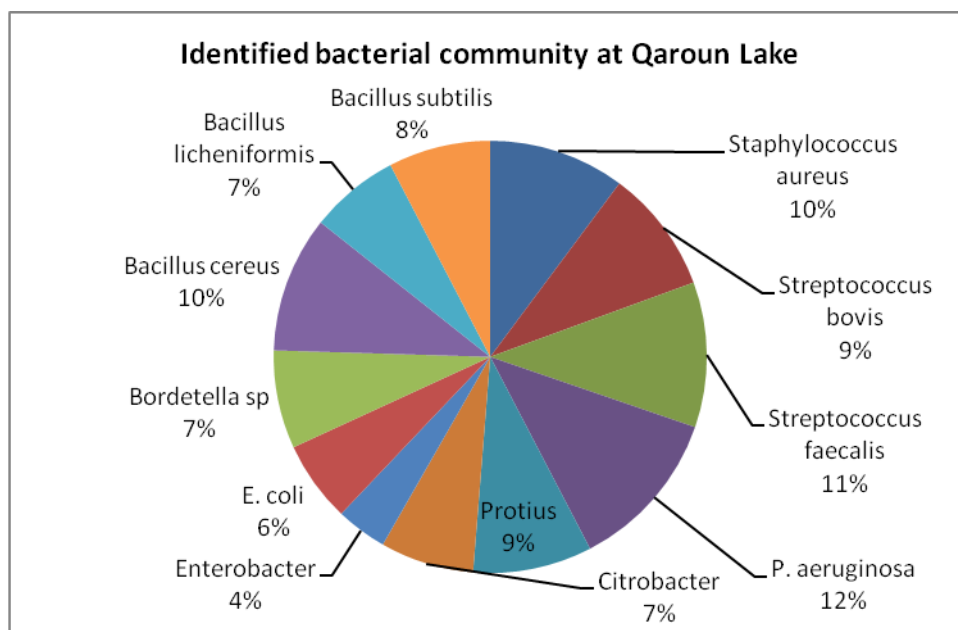**Fig (6): Characterization of the bacterial community in Nasser Lake water**

3.7 Diversity and characterization of bacterial community in brackish water Lake

The identified isolates in brackish water Lake are characterized as Gram-negative rods (45.2%), Gram-positive cocci (30.2%) and Gram-positive rods (24.5%) as illustrated in table (11) and fig (7). The presence of streptococci in high content of the identified isolates (about one-fifth of the community) might be attributed to the presence of thousands of migratory birds which always rest at Qaroun Lake during their winter migration (Derbala, 2018). In general, the bacterial community at Qaroun Lake is considered of moderate diversity. It is clear that the Gram negative bacteria were found to constitute the highest percentages of the bacterial communities in each studied water resource, these results are in agreement with those obtained by Balkwill and Ghiorse (1985) who studied the bacterial community at a shallow water table aquifer in Oklahoma; they reported that about two-thirds of the isolated bacteria were Gram negative. In a carbonate aquifer in the Atlantic coastal plain (Chapelle *et al.*, 1988), about 70% of the isolates recovered were Gram negative. Heikal (2000) studied the bacteriological quality of the groundwater at El-Qanater village, Kalubiya governorate, a total of 500 isolates were collected from 26 groundwater wells. He reported that aerobic Gram negative rods were constitute 46%, while the facultative anaerobic Gram negative rods constituting 16%, and Gram positive cocci, as well as, Gram positive rods constituting 26% and 8%, respectively.

Table 11. Characterization of the bacterial community in brackish water Lake (Qaroun Lake)

Bacterial Groups	Identified bacterial community at Qaroun Lake	No. of isolates	Isolate (%) in the community	Group (%) in the community
Gram positive cocci	*Staphylococcus aureus*	32	10.2	
	Streptococcus bovis	29	9.2	30.2
	Streptococcus faecalis	34	10.8	
Gram negative rods	*P. aeruginosa*	38	12.1	
	Protius	28	9	
	Citrobacter	22	7	45.2
	Enterobacter	12	3.8	
	E. coli	19	6	
	Bordetella sp	23	7.3	
Gram positive rods	*Bacillus cereus*	32	10.2	
	Bacillus licheniformis	21	6.6	24.5
	Bacillus subtilis	24	7.6	
	Total	**314**		

Fig (7): Characterization of the bacterial community in Qaroun Lake water

Conclusion

Seven different water resources were selected to carry out the study; groundwater, Nile River water, agricultural drain water (El-Hussieniya drain), sewage contaminated drain (Bahr El-Baqr drain), industrial wastes contaminated drain (Al-Khadrawiya drain), fresh water Lake (Nasser Lake) and brackish water Lake (Qaroun Lake). A total of 2218 bacterial isolates were collected, purified and identified to 21 bacterial genera. The most popular genera were *Bacillus, Pseudomonas and Staphyllococcus*, where they present in all types of water resources. The less popular genera were *Yersinia, Klebsiella, Aerumonas, Shigella, Salmonella, Cardiobacterium, Arthrobacter and Streptobacillus*, where they all are present once. Furthermore, the sewage contaminated

drain water has the highest diverse community, while the fresh water (Nasser) Lake has the lowest diverse community.

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الملخص العربى

يختلف مواطن الكائنات الحيه الدقيقه من مكان لاخر ، وبعضها موجود بشكل طبيعى فى البيئه المائيه، ويتم ادخال البعض الاخر بسبب مصادر التلوث المختلفه . تهدف هذه الدراسه الحالية إلى توصيف المجتمع البكتيري لموارد المياه المتنوعة في مصر فيما يتعلق بمصدر التلوث الذي المقصود . لذلك ، تم جمع عينات المياه من سبعة مصادر مائية مختلفة ؛ المياه الجوفية ، مياه نهر النيل ، مياه الصرف الزراعي (مصرف الحسينية) ، الصرف الصحي الملوث (مصرف بحر البقر) ، الصرف الملوث بالنفايات الصناعية (مصرف الخضراوية) ، بحيرة المياه العذبة (بحيرة ناصر) وبحيرة المياه معتدلة الملوحة (بحيرة قارون).تم جمع عدد 2218 عزله بكتيريه بكتيرية وتنقيتها وتحديدها عن طريق التفاعلات الكيميائية الحيوية وتحليل تسلسلى

وتمت التصفيه الى 21عزله (جنس بكتيرى) تحتوي مياه الصرف الملوثة بمياه الصرف الصحي (مصرف بحر البقر) على اعلى مجتمع بكتيرى متنوع، بينما تحتوى بحيره ناصر (مياه عذبه) على اقل مجتمع متنوع بسبب تلوث البحيره.

. بالإضافة إلى ذلك ، كانت الاجناس الاكثر شيوعا ، حيث تم اكتشافها فى جميع الانواع هى:

Bacillus, Pseudomonas and Staphyllococcus

وكانت الاجناس الاقل شيوعا هى :

*Yersinia*و *Klebsiella* و *Aerumonas* و *Shigella* و *Salmonella* و *Cardiobacterium* و *Arthrobacter* و*Streptobacillus* حيث تم اكتشافهم جميعا.