Prevalence of *Pseudomonas* and *Vibrio* Species in Shellfish with Special Reference to Their Susceptibility to Antibacterial Agents, Toxigenic and Virulent Genes

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Abstract

A total of 250 samples were randomly taken from the Port-Said markets, 125 from each of the seemingly healthy Om EL-Khloul "*Donax trunculus anatinus*" and Baclawese "*Tartufo di mare*" samples. and subjected to bacteriologically, sensitivity to some antibacterial agent and molecular examination. The prevalence of *Pseudomonas* and *Vibrio* species from the examined Om EL-Khloul samples were 35/125 (28.00%) and 30/125 (24.00%) respectively, while that of Baclawese were 52/125 (41.60%) and 40/125 (32.00%) respectively. Biochemical and API identification of the isolates revealed that *Pseudomonas* species were identifies as *P. aeruginosa, P. putida and P. florescent* while that of *Vibrio* species were *V. parahaemolyticus, V. vulanificus, V. anguillarum, V. alginolyticus* and *V. metschnikovil.* Serological typing of *P. aeruginosa* were *P. aeruginosa* polyvalent III Group E and *P. aeruginosa* polyvalent III Group F. while *V. parahaemolyticus* were identified as O3:K6, O4:K68, O4:K6, O1:K25 and O11:K36. *Pseudomonas* serovar resist to most studied antibacterial except gantamin , doxycycline and tobramycin . Most of *Vibrio* serovar resist to studied antibacterial but sensitive to a lesser extent for Amoxicillin, Chloramphinicol and Norfloxacin. Molecular study showed that 16S rRNA was detected in

Pseudomonas and *Vibrio* species. Study of the virulence and toxigenic genes showed that *toxA* and *oprL* genes were detected in *Pseudomonas aeruginosa* and *tdh* and *recA* genes in *Vibrio parahaemolyticus.*

Keywords: Shellfish-*Pseudomonas* -*Vibrio* species

Introduction

The great majority of bacterial species linked to aquatic creatures can be attributed to the aquatic environment. While gram-negative bacteria account for the majority of fish pathogens, both gram-positive and gram-negative bacteria are possible zoonotic pathogens that are associated with aquatic species. **(Lowry and Smith, 2007)**.

The presence and concentration of microorganisms in bivalve vary temporarily within the same habitat ***(Lindstrom, 2001)*** as well as among different habitats ***(Yannarell and Triplett, 2004)*** due to environmental factors that come to play individually or synergistically role ***(Hahn, 2006*** **and Anacleto et al.; 2013).** The aquatic environment is responsible for the vast majority of bacterial species that have been connected to aquatic animals. Both gram-positive and gram-negative bacteria have the potential to be zoonotic pathogens linked to aquatic animals, while gram-negative bacteria are the predominant cause of disease in fish**. (International Commission on Microbiological Specifications for Foods, 2005).** For instance, freshwater species are more frequently linked to pseudomonas and Vibrio species, while marine species of aquatic organisms are typically related with Vibrio spp.. **(Lowry and Smith, 2007)**.

16S ribosomal DNA (rDNA) sequencing information was used to identify the pseudomonads genotypically and determine their evolutionary relationships**. (Anzai, et. al., 2000).** One of the most critical virulence genes is *tox*A **(Auda, et al., 2015),** while *OprL* is an outer membrane lipoprotein which has been implicated in efflux transport systems, as well as affecting cell permeability **(*De Vos, et. al., 1997*)** Moreover, 16S rRNA gene sequencing provides precise family and genus-level identification of Vibrio spices**.** Moreover, 16S rRNA gene sequencing provides precise family and genus-level identification of Vibrio spices., (***Thompson, et. al., 2004).***

The *tdh* is considered one of the major pathogenic virulence factors of *Vibrio parahaemolyticus* (***Hsern, et, al., 2015*****).** It has been proposed that the recA gene analysis is a valuable marker for determining the genetic relatedness of Vibrios. (***Thompson et al. 2004***) .Thus, the aim of this work was investigating the bacteriological studies of Baclawese and Om EL-Khloul

through the isolation, identification of *Pseudomonas* and *Vibrio* species biochemically, API typing and serologically by slide agglutination test. Susceptibility of the isolates for antibacterial agents and molecular studied were discussed.

Materials and methods

1. Samples: collection:

Two hundred and fifty Om EL-Khloul "*Donax trunculus anatinus*" and baclawese "*Tartufo di mare*" samples, each with 125 appearances of health, were randomly selected from Port-Said markets. Every sample was carefully identified, sealed in a sterile plastic bag, and sent straight to the lab in a chilled container.

2. Bacteriological examination:

2.1. Isolation and Identification of *Pseudomonas* species:

Aseptically, a representative 25-gram sample from each of the samples under examination was collected, and it was then homogenized in 225 ml of 0.1% peptone water with salt (Na Cl, 0.85% "wt/vol") in a stomacher bag (Seward Medical, London, UK). On the surface of a different, labeled petri plate containing CFC (cetramid, fucidin, cephaloridine) agar medium, pipette 1 ml of the homogenate was applied. Subsequently, all petri plates were turned upside down, incubated for 24 to 48 hours at 25°C, and checked for possible colonies of Pseudomonas species using both white and ultraviolet light. **(*APHA, 2004*).** Following the inversion of each petri plate, it was incubated at 25°C for 24 to 48 hours, and any potential Pseudomonas species colonies were inspected using both white and ultraviolet light.**.** ***Finegold and Martin (1982) and Bergey and John (1984).***

2.2. Isolation and Identification of *Vibrio* species:

Aseptically remove a representative 25 g sample from each of the bivalve samples that were analyzed. Then, transfer the sample to a stomacher bag (Seward Medical, London, UK) with 225 ml of alkaline saline peptone water and homogenize it for 60 seconds to create a 10-1 dilution. After that, divide the homogenate into two parts. One portion was incubated for 6 hours at 41.5°C ±1 °C, while the other part was incubated for 6 hours at 37°C ± 1 °C.. After transferring 1 ml of the two culture portions (removed from the surface) into each of the three tubes holding 10 ml of ASPW, the first three tubes were incubated at 41.5 °C ± 1 h °C for 18 h ± 1 h, while the second three tubes were incubated at 37 °C ± 1 h °C for 18 h ± 1 h. Introduce the microorganisms onto the surface of TCBS and Vibrio agar plates from each cultivated tube used for the primary and secondary selective enrichment. Then, let the TCBS agar plate sit at 37°C for 24 hours ± 3 hours and the Vibrio agar plate at 35–37°C for 18–24 hours. Following incubation, check if typical colonies are present on the

plates. (***ISO:21872-1:2017/Amd1:2023*).**

2.3. Identification of the Isolated bacteria:

2.3.1. Biochemical identification

Further biochemical identification of a suspected Pseudomonas species colony that was gram negative, oxidase positive, and catalase positive was carried out in accordance to ***Finegold and Martin (1982), Bergey and John (1984) and Holtz et al., (2000*)**. Although the likely Vibrio species colony was recognized using visual and biochemical methods in accordance with ***Elliot et al., (2001), Yukiko, et al., (2001), Bergey's Manual of Systematic Bacteriology (2005) and (ISO:21872-1:2017/Amd1:2023).***

2.3.2. API (Analytical Profile Index) 20 NE:

The isolated *Pseudomonas* and *Vibrio* species were identified by API 20NE and the reactions were read according to the Reading table and Analytical Profile Index of the manufactured. (***BioMérieux SA F-69280 Marcy l'Etoile France)***

2.3.3. Serological Identification of the Isolated bacteria by Slide Agglutination test:

using the slide agglutination test, which is dependent on the presence of Pseudomonas aeruginosa O-antigens. and done by using antisera according to the instructions of the manufacturer DENKA SEIKEN Co. Ltd and presence of O-antigens and K-antigens of *V. parahaemolyticus* and done by using antisera according to the instructions of the manufacturer MAST®ASSURE, United Kingdom, Mast Group Ltd.

3. Sensitivity test for the isolated bacteria:

Four to five colonies of each phenotypic and genotypic identified strain were picked from overnight growth colony on nutrient agar slant and inoculated into Muller-Hinton broth then incubated at 35 ˚C for 16-18 hr. Oxoid Co. discs were gradually added to the swabbed culture and incubated for 16 to 18 hours at 35°C. We assessed the generated zone of inhibition and compared it to the manufacturer's instructions. The interpretation of results according to ***CLSI (2015).***

4. Molecular diagnosis (PCR for the virulent and toxigenic genes):

4.1. Extraction of DNA According to QIAamp DNA mini kit instructions

4.2. Preparation of PCR Master Mix for cPCR according to **Emerald Amp GT PCR mastermix (Takara)** Code No. **RR310A**kit as

4.3. Cycling conditions of the primers during cPCR

Temperature and time conditions of the two primers during PCR as shownin Table1

4.4. DNA Molecular weight marker: By pipetting up and down, the ladder was gently mixed. Immediately loaded were 6 µl of the necessary ladder (100bp).

4.5. Agarose gel electrophoreses according to ***Sambrook et al., (1989)*** with modification.

Table (1): *Conditions for primer cycling during cPCR.*

Target bacteria	Gene	Length of amplified product (bp)	Denaturation in primary phase	Denaturation in secondary phase	Annealing	Extension	No. of cycles	Final extension
Pseudomonas	*16S rRNA*	618	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
	toxA	396	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
	oprL	504	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
Vibrio	*16S rRNA*	663	94˚C 5 min.	94˚C 30 sec.	56˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
	tdh	373	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
	recA	793	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Results

The results presented in table (2) revealed that the **prevalence of** *Pseudomonas* species was 35/125 (28.00%) and 52/125 (41.60%) in the examined Om EL-Khloul *"Donax trunculus anatinus* and Baclawese *"Tartufo di mare"* respectively and statistically non-significant p vale> 0. 05. While that of *Vibrio* species was 30/125 (24.00%) and 40/125 (32.00%) in the examined Om EL-Khloul *Donax trunculus anatinus* and Baclawese *"Tartufo di mare"* sample respectively but statistically non-significant p vale> 0.05.

The biochemical identification and API typing of the isolated bacteria in table (3) revealed that *Pseudomonas* species were identified as *pseudomonas aeruginosa, pseudomonas putida and pseudomonas florescent* in Om EL-Khloul *Donax trunculus anatinus"* with a prevalence of 21 (24.14%), 9 (10.34%) and 5 (5.75%), respectively while that of Baclawese *"Tartufo di mare"* were 31 (35.63%), 9 (10.34%) and 12 (13.79%) respectively. Statistically non-significant differences between Om EL-Khloul and Baclawese among *Pseudomonas* spp pvalue > 0.05.

The identified *Vibrio* species by biochemical identification and API typing showed in in table (4) were *Vibrio parahaemolyticus, Vibrio vulanificus, Vibrio anguillarum, Vibrio alginolyticus* and *Vibrio metschnikovil* in a prevalence of 10 (14.29%), 6 (8.57%), 5 (7.14%), 4 (5.71%) and 5 (7.14%) for Om EL-Khloul "*Donax trunculus anatinus"* respectively while that in case of Baclawese "*Tartufo di mare"* were 11(15.71%), 7 (10.00%), 6 (8.57%), 6 (8.57%) and 10 (14.29%) respectively. Statistically non-significant differences between Om

EL-Khloul and Baclawese among *Vibrio* spp**.**

Concerning the serological typing of the isolated *Pseudomonas aeruginosa* presented in table (5) illustrated that *Pseudomonas aeruginosa* were typed as *Pseudomonas aeruginosa* polyvalent III Group E (corresponding to O11) with a prevalence of 17 (32.69%) and 23 (44.23%) and *Pseudomonas aeruginosa* polyvalent III Group F (corresponding to O4) with a prevalence 4 (7.69%) and 8 (15.38%) in Om EL-Khloul "*Donax trunculus anatinus"* and Baclawese "*Tartufo di mare"* respectively.

Table (6) showed that isolated *V. parahaemolyticus were serotyped as* O3:K6, O4:K68, O4:K6, O1:K25 and O11:K36 with a prevalence of 5 (23.81%), 2 (9.52%), 1 (4.76%), 1 (4.76) and 1 (4.76) respectively for Om EL-Khloul *"Donax trunculus anatinus"* while that of Baclawese "*Tartufo di mare"* were 4 (19.05%), 4 (19.05%), 2 (9.52%), 1 (4.76%), 0 (0.00%) respectively.

In Table (7) isolates showed that 100% sensitivity against Doxycycline while intermediate sensitivity toward Norfloxacin 51.4% (Om EL-Khloul) and 51.9% (Baclawese) while 100% toward cefoperazone on the other hand isolates showed resistance 85.7%(om elkhloul) ,76.9% (Baclawese) toward Vancomycin and 94.3%(om elkhloul) ,92.3% (Baclawese) toward Ampicilin

In table (8) showed that most of *Vibrio parahaemolyticus* were resist to the studied antibacterial but sensitive to a lesser extent for Amoxicillin, Chloramphenicol and Norfloxacin with a prevalence of 33.3% (10/30), 50% (15/30) and 20% (6/30) respectively in Om EL-Khloul *"Donax trunculus anatinus"* and with a prevalence of 37.5% (15/40), 50% (20/40) and 47.5% (19/40) respectively in Baclawese "*Tartufo di mare"*

The virulence and toxigenic gene of the strains confirmed by PCR showed in table (9) revealed that the *Pseudomonas aeruginosa* were harbored *toxA* and *oprL* genes in 100% (5/5) of examined *Pseudomonas aeruginosa* while tdh and *recA* genes of the *Vibrio parahaemolyticus* were detected in 100% (5/5) of the examined strain.

Table (2): *Prevalence of Pseudomonas species and Vibrio species, recovered from the examined samples*

Items	Type of samples									P value
	Om EL-Khloul "Donax trunculus anatinus"			Baclawese *"Tartufo di mare"*			Total samples			
	+ve	-ve	Total	+ve	-ve	Total	+ve	-ve	Total	
***Pseudomonas* species**	**35**	**90**	**125**	**52**	**73**	**125**	**87**	**163**	**250**	**0.06837 NS**
***Vibrio* species**	**30**	**95**	**125**	**40**	**85**	**125**	**70**	**180**	**250**	**0.232 NS**

Table (3): *Prevalence of pseudomonas species identified biochemically and by API 20 NE typing isolated from the examined samples (n=87)*

Isolated bacteria	Total strains (N=87)	Strain from examined samples				P value
		Om EL-Khloul *"Donax trunculus anatinus*		Baclawese *"Tartufo di mare"*		
	No. (%)	No.	%	No.	%	
Pseudomonas aeruginosa	52 (59.77)	21	24.14	31	35.63	0.1655 ^{NS}
Pseudomonas putida	18 (20.69)	9	10.34	9	10.34	-
Pseudomonas florescent	17 (19.54)	5	5.75	12	13.79	0.08956 ^{NS}
Total	87 (100)	35	40.23	52	59.77	

Table (4): *Prevalence of Vibrio species identified biochemically and by API 20 NE typing isolated from the examined samples (n=70)*

Isolated bacteria	Total strains (N=70)	Strain from examined samples				P value
		Om EL-Khloul *"Donax trunculus anatinus*		Baclawese *"Tartufo di mare"*		
	No. (%)	No.	%	No.	%	
Vibrio parahaemolyticus	21 (30%)	10	14.29	11	15.71	0.8273 ^{NS}
Vibrio vulanificus	13 (18.6%)	6	8.57	7	10.00	0.7815 ^{NS}
Vibrio anguillarum	11 (15.7%)	5	7.14	6	8.57	0.763 ^{NS}
Vibrioalginolyticus	10 (14.3%)	4	5.71	6	8.57	0.5271 ^{NS}
Vibrio metschnikovil	15 (21.4%)	5	7.14	10	14.29	0.1967 ^{NS}
Total	70 (100%)	30	42.86	40	57.14	

Table (5): *Sero-prevalence* ***of pseudomonas aeruginosa serotyping isolated from the examined samples (n=52).***

Isolated bacteria	Serotypes		Total strain (N=52)	Sero-prevalence of examined samples			
	Group	Corresponding O antigen (Habs 1957)		Om EL-Khloul *"Donax trunculus anatinus*		Baclawese *"Tartufo di mare"*	
			No. (%)	No.	%	No.	%
Pseudomonas aeruginosa polyvalent III	E	O11	40 (76.92)	17	32.69	23	44.23
	F	O4	12 (23.08)	4	7.69	8	15.38
Total			52 (100)	21	40.38	31	59.62

Table (6): *Sero-prevalence of* V. parahaemolyticus *isolates from* ***the examined samples (n=21).***

Isolated bacteria	Serotypes	Total strain (21 in No.)	Sero-prevalence of examined samples			
			Om EL-Khloul *"Donax trunculus anatinus*		Baclawese *"Tartufo di mare"*	
		No. (%)	No.	%	No.	%
Vibrio parahaemolyticus	O3:K6	9 (42.86)	5	23.81	4	19.05
	O4:K68	6 (28.57)	2	9.52	4	19.05
	O4:K6	3 (14.29)	1	4.76	2	9.52
	O1:K25	2 (9.52)	1	4.76	1	4.76
	O11:K36	1 (4.76)	1	4.76	0	0.00
Total		21 (100)	10	47.62	11	52.38

Table (7): *Antibacterial susceptibility of the isolated Pseudomonas aeruginosa recovered from the examined Om EL-Khloul "Donax trunculus anatinus" (n=35) and Baclawese "Tartufo di mare" samples (n= 52)*

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Antimicrobial agent	Abbreviation Symbol	Disc potency	Zone of inhibition (mm)											
			Om EL-Khloul "Donax trunculus anatinus"						Baclawese *"Tartufo di mare"*					
			S		I		R		S		I		R	
			No	%	No	%	No	%	No	%	No	%	No	%
1 -**Vancomycin**	VA	**30 µg**	**0**	**0**	**5**	**14.3**	**30**	**85.7**	**0**	**0**	**12**	**23.1**	**40**	**76.9**
2- **Gentamicin**	CN	**10 µg**	**29**	**82.8**	**6**	**17.1**	**0**	**0**	**10**	**19.2**	**0**	**0**	**42**	**80.8**
3- **Chloramphinicol**	C	**30 µg**	**15**	**42.9**	**20**	**57.1**	**0**	**0**	**12**	**23.1**	**0**	**0**	**40**	**76.9**
4- **Ampicillin**	AM	**10 µg**	**2**	**5.70**	**0**	**0**	**33**	**94.3**	**4**	**7.7**	**0**	**0**	**48**	**92.3**
5- **Streptomycin**	S	**10 µg**	**5**	**14.3**	**0**	**0**	**30**	**85.7**	**2**	**3.8**	**0**	**0**	**50**	**96.2**
6- **Doxycycline**	Do	**30 µg**	**35**	**100**	**0**	**0**	**0**	**0**	**52**	**100**	**0**	**0**	**0**	**0**
7- **Cefoperazone**	CFP	**75 µg**	**0**	**0**	**35**	**100**	**0**	**0**	**0**	**0**	**52**	**100**	**0**	**0**
8- **Ceftriaxone**	CRO	**30 µg**	**0**	**0**	**0**	**0**	**35**	**100**	**0**	**0**	**0**	**0**	**52**	**100**
9- **Tobramycin**	Tob	**10 µg**	**18**	**51.4**	**0**	**0**	**17**	**48.6**	**0**	**0**	**0**	**0**	**52**	**100**
10- **Amoxicillin**	A x	**25 µg**	**20**	**57.1**	**0**	**0**	**15**	**42.9**	**15**	**28.8**	**0**	**0**	**37**	**71.2**
11- **Norfloxacin**	NOR	**10 µg**	**17**	**48.6**	**18**	**51.4**	**0**	**0**	**25**	**48.1**	**27**	**51.9**	**0**	**0**

Table (8): *Antibacterial susceptibility of the isolated Vibrio parahaemolyticus recovered from the examined Om EL-Khloul"Donaxtrunculus anatinus" (n=30) and Baclawese "Tartufo di mare" samples (n= 40)* **.**

Antimicrobial agent	Abbreviation Symbol	Disc potency	Zone of inhibition (mm)											
			Om EL-Khloul "Donax trunculus anatinus"						Baclawese *"Tartufo di mare"*					
			S		I		R		S		I		R	
			No	%	No	%	No	%	No	%	No	%	No	%
1-Amoxicillin	A x	25 µg	10	33.3	0	0	20	66.7	15	37.5	0	0	25	62.5
2-Trimethoprim / sulphamethoxazole	SXT	1.25+23.75 µg	0	0	2	6.7	28	93.3	0	0	4	10	36	90
3-Cefatoxim	CTX	30 µg	0	0	0	0	30	100	0	0	0	0	40	100
4-Gentamicin	CN	10 µg	0	0	0	0	30	100	0	0	0	0	40	100
5-Chloramphinicol	C	30 µg	15	50	0	0	15	50	20	50	0	0	20	50
6-Ampicillin	AM	10 µg	0	0	0	0	30	100	0	0	0	0	40	100
7-Erythromycin	E	15 µg	0	0	15	50	15	50	0	0	20	50	20	50
8-Streptomycin	S	10 µg	0	0	4	13.3	26	86.7	0	0	6	15	34	85
9-Tetracyclin	TE	30 µg	0	0	6	20	24	80	0	0	5	12.5	35	87.5
10-Norfloxacin	NOR	10 µg	6	20	24	80	0	0	19	47.5	21	52.5	0	0

Table (9): *Prevalence of some genes detected in the isolated bacteria serovar*

Isolated bacteria	Gene	1	2	3	4	5	Total	%
Pseudomonas aeruginosa	***16S rRNA***	+ve	+ve	+ve	+ve	+ve	5/5	100
	toxA	+ve	+ve	+ve	+ve	+ve	5/5	100
	oprL	+ve	+ve	+ve	+ve	+ve	5/5	100
Vibrio parahaemolyticus	***16S rRNA***	+ve	+ve	+ve	+ve	+ve	5/5	100
	tdh	+ve	+ve	+ve	+ve	+ve	5/5	100
	recA	+ve	+ve	+ve	+ve	+ve	5/5	100

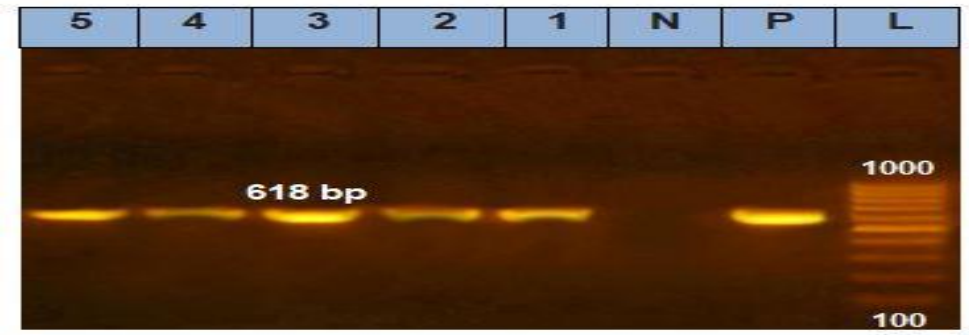

Figure (1): Agarose gel electrophoresis of PCR products after amplification of *16S rDNA* gene for *Pseudomonas aeruginosa*.
N=negative control (Salmonella typhimurium)
P=positive control Psedomonas aeruginosa (ATCC® 27853™)
L=ladder (100 bp DNA ladder)
all lanes showed positive results confirmed that all Psedomonas aeruginosa isolates were positive for *16S rDNA* gene at 618 bp .

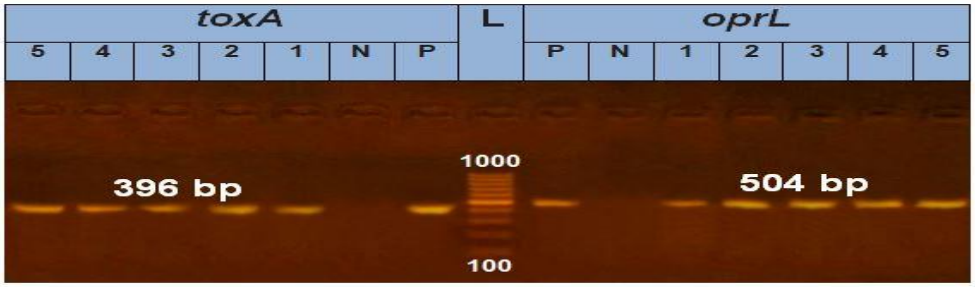

Figure (2): Agarose gel electrophoresis of PCR products after amplification of *toxA* and *oprL* genes for *Pseudomonas aeruginosa*.
N=negative control (Salmonella typhimurium) P=positive control (Psedomonas aeruginosa (ATCC® 27853™)
L=ladder (100 bp DNA ladder).
lanes (1-5) on right side showed positive results confirmed that all Pseudomonas aeruginosa were positive for *oprL* gene at 504 bp). & lanes (1-5) on left side showed positive results confirmed that all pseudomonas aeruginosa isolates were positive for *toxA* gene at 396 bp.

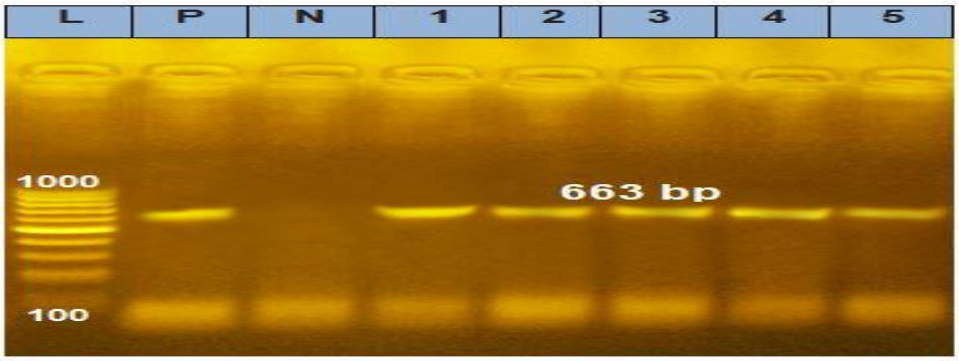

Figure (3): Agarose gel electrophoresis of PCR products after amplification of *16S rRNA* gene for *Vibrio parahaemolyticus*,
N=negative control (Salmonella typhimurium) P=positive control *Vibrio parahaemolyticus* (ATCC® 17802 ™) L=ladder(100bp DNA ladder)
lanes (1-5) showed positive results confirmed that all Vibrio parahaemolyticus were positive for *16S rRNA* gene at 663 bp.

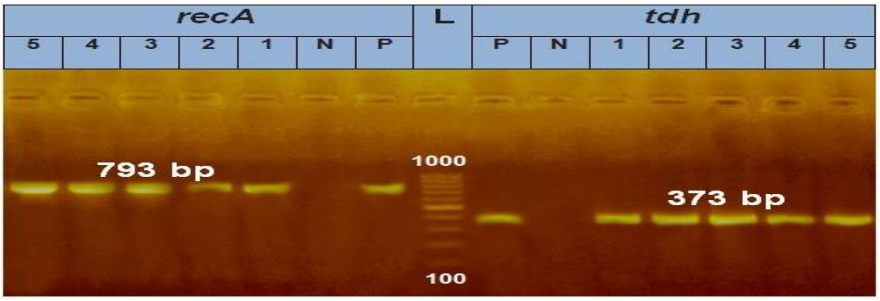

Figure (4): Agarose gel electrophoresis of PCR products after amplification of *recA and tdh* genes for *Vibrio parahaemolyticus* N=negative control (Salmonella typhimurium) P=positive control *Vibrio parahaemolyticus* (ATCC® 17802 ™) L=ladder(100bp DNA ladder) lanes (1-5) on right side showed positive results confirmed that all Vibrio parahaemolyticus were positive for tdh gene at 373 bp & lanes(1-5) on left side showed positive results confirmed that all Vibrio parahaemolyticus were positive for recA gene at 793 bp .

Discussion

Marine bivalves collect a lot of microorganisms, both gram-positive and gram-negative, and there aren't many differences in this flora across bivalves from different places. ***(McHenery and Birkbeck 1985).***

The results presented in table (2) revealed that the prevalence of *Pseudomonas* species was 35/125 (28.00%) and 52/125 (41.60%) in the examined Om EL-Khloul *"Donax trunculus anatinus* and Baclawese *"Tartufo di mare"* respectively and displayed a statistically significant result. These outcomes were marginally comparable to the outcomes recorded by ***Kueh and Chan (1985) and Udoekonget. al., (2021)*** who found that the *Pseudomonas* species prevalence was 30.8%and 32.6% respectively but higher than the results recorded by ***Salgueiro et. al., 2021*** who found that the prevalence of *Pseudomonas* species in 2 aquacultures of mussel's farms were 5.6% and 11.8% in summer but in autumn was 5.6% in one aquaculture farm. While that of *Vibrio* species was 30/125 (24.00%) and 40/125 (32.00%) in the examined Om EL-Khloul *Donax trunculus anatinus* and Baclawese *"Tartufo di mare"* sample respectively but statistically non-significant. The outcomes agreed with these results. recorded by ***Di Pinto et al., (2008)*** *and* ***Rosec et al., (2012)*** who found that the prevalence of *V. parahaemolyticus* were 32.6% in Mussel and 31.6% in Oyster but lower than the results recorded by ***Tang et. al., (2014)*** who found that prevalence of green mussels, carpet clam and cockles were 55%, 80% and 40% respectively. However, these outcomes were more than that

recorded by ***Di Pinto et. al., (2012)*** and ***Khouadja et al., (2013)*** who recorded that *V. parahaemolyticus*, was detected with a prevalence of 10.8% (21/195) and % 10.0 (2/20) in shellfish respectively.

The biochemical identification and API typing of the isolated bacteria in table (3) demonstrated that Pseudomonas species were recognized. as *pseudomonas aeruginosa, pseudomonas putida and pseudomonas florescent* in Om EL-Khloul *Donax trunculus anatinus"* with a prevalence of 21 (24.14%), 9 (10.34%) and 5 (5.75%), respectively while that of Baclawese *"Tartufo di mare"* were 31 (35.63%), 9 (10.34%) and 12 (13.79%) respectively. Statistically non-significant differences between Om EL-Khloul and Baclawese among *Pseudomonas* spp. except *P. florescent.* statistically significant. The recorded results concurred with findings by ***Noor El-Deen, et. al., 2023*** who found that *Pseudomonas* species were identified as *pseudomonas aeruginosa, pseudomonas putida and pseudomonas florescent* and *anguilliseptica.* But these results varied with the results recorded by ***Abd El-Tawab, et. al., 2019*** who identified *Pseudomonas* species as *Pseudomonas aeruginosa* strains and *Pseudomonas fluorescens* strains with an incidence 67.4% and 32.6% respectively.

The identified *Vibrio* species by biochemical identification and API typing showed in in table (4) were *Vibrio parahaemolyticus, Vibrio vulanificus, Vibrio anguillarum, Vibrio alginolyticus* and *Vibrio metschnikovil* in a prevalence of 10 (14.29%), 6 (8.57%), 5 (7.14%), 4 (5.71%) and 5 (7.14%) for Om EL-Khloul "*Donax trunculus anatinus"* respectively while that in case of Baclawese "*Tartufo di mare"* were 11(15.71%), 7 (10.00%), 6 (8.57%), 6 (8.57%) and 10 (14.29%) respectively. Statistically non-significant differences between Om EL-Khloul and Baclawese among *Vibrio* spp. The outcomes differed from the ones that were documented. by ***Ibrahim et. al., (2018)*** who identified *Vibrio* spp in shellfish . were identified as *V. parahaemolyticus, mimicus, V. alginolyticus, V. vulnificus, V. fluvialis* and *V. cholera* with a prevalence of 16%, 12%, 8%, 4%, 8% and 4% respectively.

Concerning the serological typing of the isolated *Pseudomonas aeruginosa* presented in table (5) illustrated that *Pseudomonas aeruginosa* were typed as *Pseudomonas aeruginosa* polyvalent III Group E (corresponding to O11) with a prevalence of 17 (32.69%) and 23 (44.23%) and *Pseudomonas aeruginosa* polyvalent III Group F (corresponding to O4) with a prevalence 4 (7.69%) and 8 (15.38%) in Om EL-Khloul "*Donax trunculus anatinus"* and Baclawese "*Tartufo di mare"* respectively. These results disagreed with the results recorded by ***Shahat, et. al.,***

2019 who found that the serological identification of *Pseudomonas* spp. was subtended to *Pseudomonas. aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas* fluorescens, *Pseudomonas putida* and Pseudomonas *fragi* with an incidence of 20%, 22%, 31%, 20% and 507% respectively. Furthermore, *Pseudomonas. aeruginosa* were divided into 4 serotypes *Pseudomonas aeruginosa* O2, O6, O10 and O11.

Table (6) showed that isolated *V. parahaemolyticus were serotyped as* O3:K6, O4:K68, O4:K6, O1:K25 and O11:K36 with a prevalence of 5 (23.81%), 2 (9.52%), 1 (4.76%), 1 (4.76) and 1 (4.76) respectively for Om EL-Khloul *"Donax trunculus anatinus"* while that of Baclawese "*Tartufo di mare"* were 4 (19.05%), 4 (19.05%), 2 (9.52%), 1 (4.76%), 0 (0.00%) respectively. The outcomes matched the ones that were noted by ***Vongxay et al., 2008*** who serotyped the isolated *V. parahaemolyticus* as O3:K6 in seafood and clinical specimen and. However, these outcomes did not agree with the results that were noted by ***Chang et al., 2011*** who serotyped the *V. parahaemolyticus* into O1, O2, O3, O4, O5, O6, O7, O8, O10, and O11 with a prevalence 29.8%, 13.8%, 17.0%, 12.8%, 16.0%, 1.06%, 1.06%,1.06%, 2.1% and 5.3% in oyster-growing environment respectively.

In table (7) showed the sensitivity of *Pseudomonas aeruginosa* isolated from Om EL-Khloul *"Donax trunculus anatinus"* samples to Gentamicin, Doxycycline, Tobramycin and Amoxicillin with a percentage of 82.8%, 100%, 51.4%, and 57.1% respectively but intermediate sensitive to Chloramphenicol, Cefoperazone and Norfloxacin with percentage of 57.1%, 100% and 51.4% respectively, meanwhile resistance to Vancomycin, Ampicillin, Streptomycin and Ceftriaxone with a percentage of 85.7%,94.3%, 85.7% and 100% respectively. While that of Baclawese *"Tartufo di mare"* were sensitive to Doxycycline with a percentage of 100. but intermediate sensitive to Cefoperazone and Norfloxacin with percentage of 100% and 51.9% respectivelymeanwhile resistance to Vancomycin, Gentamicin, Chloramphinicol, Ampicillin, Streptomycin, Ceftriaxone, Tobramycin and Amoxicillin with a percentage of 76.9%, 80.8 %, 76.9%, 92.3%, 96.2%, 100%, 100% and 71.2% respectively. These results were agreed with the results recorded by ***Udoekong et. al., (2021)*** who found that *Pseudomonas aeruginosa* were resistant to ceftriaxone and chloramphenicol with a percentage 100% but disagreed with our results of Om EL-Khloul *"Donax trunculus anatinus"*.

The results in table (8) showed that most of *Vibrio parahaemolyticus* were resist to the studied antibacterial but sensitive to a lesser extent for Amoxicillin, Chloramphenicol and Norfloxacin

with a prevalence of 33.3% (10/30), 50% (15/30) and 20% (6/30) respectively in Om EL-Khloul *"Donax trunculus anatinus"* and with a prevalence of 37.5% (15/40), 50% (20/40) and 47.5% (19/40) respectively in Baclawese "*Tartufo di mare"*. The outcomes match the ones that were noted by ***Lopatek et. al., (2022)*** who found *V. parahaemolyticus* isolates were resistant to ampicillin, streptomycin, gentamicin ciprofloxacin and tetracycline with a prevalence of 77.3%, 64.0%, 12.8%, 1.7%and 0.8% respectively. And all isolated analyzed *V. parahaemolyticus* isolates were sensitive to chloramphenicol. but disapproved of the recorded results by ***Tang et, al., (2014)*** who found that all *V. parahaemolyticus* isolates were susceptible to Tetracycline and Gentamycin with a prevalence 100% for both antibacterial agents and resistance to Norfloxacin with a prevalence 1.82%.

The difference between these results and those reported by other authors could be explained by human activities changing water ecosystems, which can lead to the transfer of antibiotic-resistant genes between microbial species and the persistence of antibiotics in bivalves and shellfish **(*Gufe et al., 2019*).**

The results of molecular studied in table (9) and Fig. (1and 3) demonstrated the presence of 16S rRNA in 100% (5/5) of Vibrio parahaemolyticus and Pseudomonas aeruginosa, as advised by ***Tarr et. al., (2007) and Mason et. al., (2001)*** respectively.

The virulence and toxigenic gene of the strains confirmed by PCR showed in table (9) and Fig. (2) revealed that the *Pseudomonas aeruginosa* were harbored *toxA* and *oprL* genes in 100% (5/5) of examined *Pseudomonas aeruginosa*. These outcomes agreed with the documented outcomes by ***Algammal, et al., 2020 and Yaseen et. al., (2020)*** who disagreed with the published results despite discovering that the toxA and oprL genes were present in every isolated Pseudomonas species. by ***Salgueiro et. al., (2021*)** who discovered that *P. aeruginosa* harbored *toxA* genes with a prevalence 95.4%. The results of the virulence and toxigenic gene of *Vibrio parahaemolyticus* in table (9) and Fig. (4) revealed that *tdh* and *recA* genes of the *Vibrio parahaemolyticus* were detected in 100% (5/5) of the examined strain. These results matched with results documentd by ***Banerjee, and FarBer (2017*)** who found that *Vibrio parahaemolyticus* harbored *tdh* with a prevalence of 100%. But disagreed with results recorded by ***Kang et al., (2017) and Mokaet al., (2019)*** who *discovered that every Vibrio species isolate tested negative for the tdh gene, and the findings documented by* ***El-Tawab et. al., (2021)*** who detected the *recA* genes from *Vibrio parahaemolyticus* with an incidence of 60% of the isolates. *The differences between this conclusion and the findings*

documented by other authors could be related to the pathogenic character of the bacteria, tracking their abundance and species dispersion. ***Joseph, et. al. (2013)***.

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مدي انتشار انواع من بكتريا السودوموناس و الفيبريو في المحار ذات الصدفتين مع الاشارة الي الحساسية لمضات البكتريا والجينات السامة والضارة لتلك البكتريا

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الملخص العربي

تم جمع 125 عينة عشوائية صالحة ظاهريا من كل من أم الخلول والبكلاويزمن أسواق مدينة بورسعيد وتم دراستها بكتريولوجيا للكشف عن وجود انواع من *السودوموناس والفيبريو* ودراسة حساسية هذه الميكروبات تجاه بعض المضادات البكتيرية و التعرف الجزيئي لهذه الميكروبات و بعض الجينات المسببة للأمراض والجينات ذات السمية في تلك الميكروبات. واظهرت النتائج أن انواع من ميكروبات *السودوموناس والفيبريو* في عينات أم الخلول كانت بنسبة 125/35(28.00%) &125/30 (24.00%) على التوالي، بينما كانت في عينات البكلويز125/52 (41.60%) &125/40 (32.00%) على التوالي. و اظهر التصنيف البيوكيميائي و كذلك التصنيف باستخدام API ان تصنيف ميكروبات السودوموناس المعزولة كانت *سودوموناس ايروجينوزا & سودوموناس بوتيدا & سودوموناس فلورسنت* في عينات أم الخلول و البكلويز وتم تصنيف ميكروب الفيبريو الي فيبريو باراهيموليتكس & فيبريو فيلنيفكس & فيبريو انجيوليرم & فيبريو الجينوليتكس & فيبريو ميتشنيكوفيل في العينات موضع الدراسة.

تم تصنيف عترات السودوموناس ايروجينوزا سيرولوجيا الي سودوموناس ايروجينوزا polyvalent III Group E (O11)& سودوموناس ايروجينوزا polyvalent III Group F (O4) في عينات أم الخلول و البكلويز. وتم تصنيف ميكروب فيبريو باراهيوليتكس الي O3: O11: K36 &O1: K25 &O4: K6&O4: K68&K6 في عينات أم الخلول و البكلويز.
وبدراسة حساسية الميكروبات المعزولة تجاه بعض المضادات البكتيرية ووجدت أن ميكروبات *السودومونس ايروجينوزا* حساسة لمعظم مضادات البكتيرية موضع الدراسة بينما ميكروبات الفيبريو باراهيموليتكس فكانت مقاومة لمعظم المضادات البكتيرية.
تم تحديد 16S rRNA في ميكروبات *السودومونس ايروجينوزا* و*الفيبريوباراهيموليتكس* بنسبة 100 ٪ (5/5) وكذلك جينات الضراوة والجينات ذات السمية موضع الدراسة في تلك الميكروبات بواسطة تفاعل البوليميريز المتسلسل (PCR).