Genetic Characterization of *Pseudomonas Aeruginosa* Isolated from Fish, Environmental, and Human Sources

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Abstract

Pseudomonas aeruginosa has the ability to adapt to many situations. So, from El-Temsah Lake and fish farms in Ismailia governorate, samples were taken from fish that exhibited signs of septicemia and others that appeared to be healthy without any symptoms. Further, about 60 human samples were taken from patients at the Suez Canal University Hospital in Ismailia governorate, as well as 23 water samples from El-Temsah Lake for *P. aeruginosa* isolation and PCR confirmation. The bacteriological analysis revealed that *P. aeruginosa* was found in 33.4 % of all analyzed samples from various sources (Fish, human and water). By traditional cultivation, the prevalence of *P. aeruginosa* was 34.33 % in fish samples, 28.3 % in human samples, and 21 % in water samples. The PCR assay was used to validate *P. aeruginosa* isolates from various sources in a quick and precise manner. Genetic confirmation of 15 biochemically determined *P. aeruginosa* isolates was done using the *16S rDNA* sequence with a particular band at 956 base pair. This study proved that *P. aeruginosa* is widely distributed in all sources with different rates and it has a public health significance.

Key words: *P. aeruginosa,* fish, Water, Human.

Introduction

Lake Temsah, the study's principal location, is one of the main sites in Egypt where enormous numbers of migrating birds pass through, especially during the winter on their passage from Europe to Africa. It is also one of the most well-known wetlands in the Suez Canal region and a major tourist

attraction. ***(Varo, Serrano et al. 2002)***

Pseudomonas species are often separated from soil and water, or inhabit a variety of anatomical places, including plants, invertebrates, vertebrates, and humans. ***(Fadhil, Al-Marzoqi et al. 2016, Kaszab, Szoboszlay et al. 2016, Streeter and Katouli 2016).*** Environmental bacteria *P. aeruginosa* can be found in a variety of terrestrial and aquatic ecosystems. is owing to its wide metabolic adaptability, which promotes its spread, multiplication, and survival despite adverse conditions of temperature, hence improving its biological success and potential public health hazard***. (Deredjian, Colinon et al. 2014, Elshafiee, Nader et al. 2019).***

More than 120 species of Pseudomonas can be found in wet habitats such as water and soil populations, and they are dangerous to both people and animals. *P. aeruginosa* is the most common *Pseudomonas* strain linked to human illnesses. The bacteria is an opportunistic pathogen that primarily causes nosocomial infections in immunocompromised patients. Breathing machine pneumonia, catheter-related urinary tract illnesses, skin infections in severe burn cases, and septicemia are all prevalent nosocomial infections associated with *P. aeruginosa*, and their etiology has been demonstrated to be complex. ***(Streeter and Katouli 2016).***

By amplification of sequences specific to a particular organism, PCR provides the potential to quickly identify microbial species. ***(Khan and Cerniglia 1994) (Wahdan, Fadel et al. 1930).*** The use of a species-specific primer and a PCR assay to validate the isolates was more accurate and time-consuming. Genomic research is needed to confirm *Pseudomonas* spp, phylogenetic status***. (Franzetti and Scarpellini 2007).*** The *16S rDNA* gene can be sequenced to confirm the presence of *P. aeruginosa*. The *16S rDNA* methodology is a valuable tool for detecting bacteria quickly and precisely, and it can be used to substitute the time-consuming biochemical detection approach ***(Uma, Reddy et al. 2007).*** So, this study aimed to isolate *P. aeruginosa* in different sources and confirm the isolates genetically.

Materials and methods

Samples:

Samples from (liver, kidneys, spleen and gills) showed signs of septicemia were randomly collected from *Oreochromis niloticus*, *tilapia zilli and clarias gariepinus* from El-Temsah Lake and from fish farms in Ismailia

governorate. Thirty-two water samples were collected in bottles from El-Temsah Lake. The samples were transported alive in aerated plastic bags to the bacteriological lab at Suez Canal University's Faculty of Veterinary Medicine and submitted for clinical and bacteriological testing. Diverse clinical specimens from patients with a history of health-care-associated infection were gathered under aseptic circumstances and identified of *P. aeruginosa* at the microbiology lab of the faculty of medicine's laboratory.

Preparation and enrichment of the samples:

All of the specimens were sent to the lab in an icebox. Within two hours of delivery, all specimens were processed. In the specific instance of fish, the exterior of the investigated tissue was sterilized with a warm spatula, and one g of each liver, kidney, spleen, and gills was aseptically taken thick from these tissues and quickly inoculated in aseptic tubes containing 9 ml trypticase soya broth or peptone liquid, then incubated at 37°C for 24 h for refinement of samples.

Isolation, purification and identification of *pseudomonas* spp.

A loopful of incubated broth streaked on pseudomonas agar base media (Oxoid), on MacConkey's agar media (Oxoid). The plates were incubated at 37°C for 24hr ***(APHA, 1992).*** Suspected oxidase positive, non-lactose fermented colonies (pale colonies) on MacConkey's agar and blue – green or brown colonies on pseudomonas agar base media were sub cultured on Nutrient agar slant (semi-solid) tube and incubated at 37°C for 24hr. These pure colonies were used for identification of isolates according to **(MacFaddin 2000).** Oxidase, catalase, Indole, V.P, M.R and H2s production tests were used for identification.

PCR-based detection of *P. aeruginosa*

Fifteen samples from different sources were extracted by QIAamp DNA Mini Kit, Catalogue no.51304 as ***(Touihri, Bouzid et al. 2009).*** *P. aeruginosa 16S rDNA* (species specific sequence) was used for confirmation of isolates strains ***(Spilker, Coenye et al. 2004).*** About 12.5 µl Emerald Amp GT PCR master mix (2x premix) and 1µl from forward and reverse primers were adjusted in the reaction volume 251µl. Electrophoresis was done by using (1.5 g grade agarose in 100 ml TBE buffer) at 85 volts for 45 minutes.

Results

According to table (1), the bacteriological examination

revealed that 228 (33.4%) isolates out of 683 different samples were found positive for *P. aeruginosa*. Among different sources, the prevelance of *P. aeruginosa* in fish were 34.3 %, 28.3% and 21.7% in all examined fish, human and water samples respectively.

Appearance of light blue-green colonies on MacConkey's agar and dark brown colonies on pseudomonas agar were characteristic feature of *P. aeruginosa* isolates

Fifteen representatives, biochemically confirmed *P. aeruginosa* isolates were identified genetically by *16S rDNA* gene. Figure 1, Illustrated the positive results for amplification of 956bp fragment of *pseudomonas 16S rDNA* gene for 15 *P. aeruginosa* isolated from human, fish and water samples.

Table (1): *Prevalance of P. aeruginosa isolated from different sources:*

source of sample	No. of samples	Positive culture for *P. aeruginosa*	
		No	%
Fish	600	206	34.3
Human	60	17	28.3
Water	23	5	21.7
Total	683	228	33.4

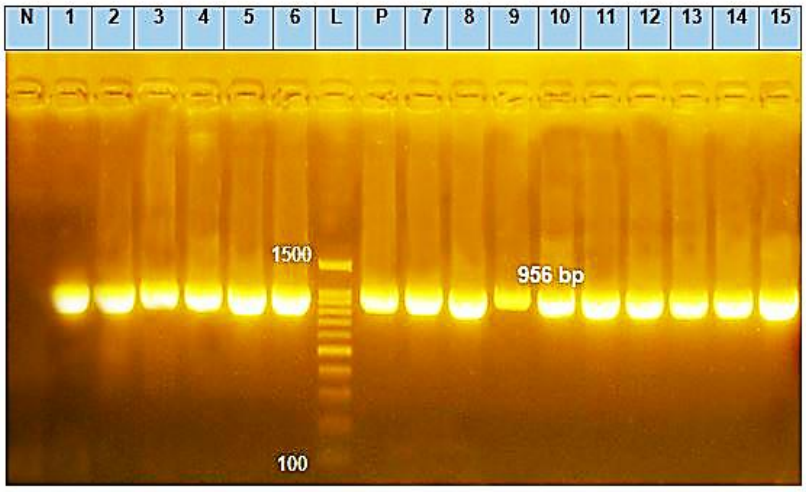

Figure 1: *Electrophoretic pattern of primers targeting P. aeruginosa 16S rDNA. Lane L: 100 bp molecular weight ladder. Lane P: positive control at 953bp (Reference strain from A. H. R. I, Dokki, Cairo, Egypt), Lane N:*

negative control (template without DNA). Lanes 1 to 5 were positive 956 bp bands of 16S rDNA gene from Human isolates of p. aeurginosa. Lanes 6 to 10 were positive from fish isolates of p. aeurginosa. Lanes 11 to 15 were positive from water isolates of p. aeurginosa.

Discussion

Pseudomonas species are by far the most common bacterial species found in soil, river water, and living creature skin all around the world. In hospitalized humans, animals, and plants, some species are pathogens or opportunistic pathogens. ***(Oh, Kim et al. 2019)***

The present results revealed that a total of 228 (33.4%) isolates out of 683 investigated samples were found positive for *P. aeruginosa*. Among different sources, the prevalence of *P. aeruginosa* was 34.33%, 28.3% and 21.7% in all examined fish, human and water samples respectively. And that result was less than that obtained by ***(Elshafiee, Nader et al. 2019)*** who detected that *P. aeruginosa* was isolated from humans with occurrence of 20% these samples were collected from people working on farms in the Giza Governorate. And also, was less than that obtained by ***(Mahmoud, Zahran et al. 2013)*** who detected that *P. aeruginosa* accounted for 19 % (54 of 283) of nosocomial infections in cases admitted to Menoufia University Hospital, Egypt. As regard to water isolates, that result was different to ***(Nasreen, Sarker et al. 2015)*** increasing predominance (32/52) who collected P. aeruginosa from various water sources such as pounds, lakes, and rivers (61.5 percent). Fish can acquire harmful germs from the natural aquatic environment because their bacterial load reflects the water quality in which they were captured. ***(Alawy, El-Tras et al. 2015) (Ismail, Wahdan et al. 2019).***

PCR-based techniques are simple, quick, and widely regarded as one of the most powerful technologies for bacterial identification, with specialized procedures created for a variety of significant bacterial diseases in aquaculture. ***(López, Navas et al. 2012).*** The present results in **Fig 1** revealed that the genotyping of *P. aeruginosa* using PCR targeting 16S rDNA gene sequencing were carried out for confirmation of 15 tested *P. aeruginosa* isolated from human, fish and water samples, which were identified biochemically. The electrophoresis of *P. aeruginosa* PCR product was shown with specific band at 956 base pair by using species-specific *P. aeruginosa* primer designed by ***(Spilker, Coenye et al. 2004)*** who designed *16S rDNA-based* PCR

assays that provide rapid of *Pseudomonas spp*. and help in its differentiation from other phylogenetically closely related *Pseudomonas spp*. And that results agree with ***(Magdy, El-Hady et al. 2014).*** Standard bacteriological methods were used to identify all *P. aeruginosa* isolates from clinical and environmental material, which were then verified using a *16S rDNA*-based PCR experiment. All isolates had a band that matched the P. aeruginosa unique band (956 bp***) (Fazeli, Akbari et al. 2012).*** The *16S rDNA* gene can be sequenced to confirm the presence of *P. aeruginosa.* The *16S rDNA* methodology is a valuable tool for detecting bacteria quickly and accurately, and it can be used to replace the time-consuming biochemical identification method. ***(Uma, Reddy et al. 2007).***

Conclusion:

Our results detected the high prevalence of *P. aeruginosa* among different sources, the prevalence of *P. aeruginosa* was 34.33%, 28.3% and 21.7% in all examined fish, human and water samples respectively.

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الكشف الجزيئي عن السيدوموناس ارجينوزا المعزولة من مصادر مختلفة

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السيدوموناس ارجينوزا لديها القدرة على التكيف مع العديد من المواقف. لذلك تم أخذ عينات منفصلة من بحيرة التمساح والمزارع السمكية بمحافظة الإسماعيلية من الأسماك التي ظهرت عليها علامات تسمم الدم وأخرى بدت صحية بدون أي أعراض. علاوة على ذلك ، تم أخذ حوالي 60 عينة بشرية من المرضى في مستشفى جامعة قناة السويس بمحافظة الإسماعيلية ، بالإضافة إلى 23 عينة مياه من بحيرة التمساح لعزل السيدوموناس ارجينوزا وتأكيد تفاعل البلمرة المتسلسل.أظهر التحليل البكتيري أن السيدوموناس ارجينوزا وجدت في 33.4٪ من جميع العينات التي تم تحليلها من مصادر مختلفة (الأسماك والبشر والماء). عن طريق الاستزراع التقليدي ، كان انتشار السيدوموناس ارجينوزا 34.33٪ في عينات الأسماك ، و 28.3٪ في عينات الإنسان ، و 21٪ في عينات المياه. تم استخدام اختبار PCR للتحقق من عزلات السيدوموناس ارجينوزا من مصادر مختلفة بطريقة سريعة ودقيقة. تم إجراء تأكيد وراثي لـ 15 عزلة من السيدوموناس ارجينوزا تم تحديدها كيميائيًا باستخدام تسلسل 16S rDNA مع نطاق معين عند القاعدة المزدوجة956.