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Detection of virulence factors of Pseudomonas aeruginosa Isolated from Hospital Environment Samples

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Abstract

The aim of this research is to study the effectiveness of Pseudomonas aeruginosa bacteria isolated from hospital environment samples to produce Protease, Lipase, Amylase, Urease, DNase and Hemolysine. One hundred samples were were collected from Al-Alam Hospital during July 2020 till the end of January 2021 to isolate and identify P.aeruginosa from different environmental sources in the hospital including the operating theaters, patients' beds and surgical kits. Twenty eight isolates were found to be belonging to P. aeruginosa, which mean an isolate rate of (28%) of the total samples. The diagnosis was carried out based on phenotypic characteristics and biochemical tests. P. aeruginosa was found in (11) out of (41) operating theaters samples, (9) out of (32) hospitals' beds samples and (8) out of (27) surgical kits samples. This means that isolates from the surgical kits constituted the highest percentage of (29.6%) of the total samples, while the lowest percentage of (26.8%) was obtained from the operating theaters samples. The results showed that (35.7%) of total isolates were Hemolysin producer on blood agar, It was also found that (57.1%) of total isolates were Amylase producer on Starch agar, (67.8%) of total isolates were Protease and Lipase producer on Skim milk agar and on Rhan agar, (21.4%) of total isolates were Urease producer on Urea agar and (53.5%) were DNase producer on a DNase agar medium.

Keywords: Pseudomonas aeruginosa, Hospital Environment Samples, virulence factors.

Introduction

P. aeruginosa is an important opportunistic pathogen causing life-threatening acute infections in individuals with compromised immune systems. It is also the most common cause of chronic mental infections and skin infections, and the main cause of morbidity and death in patients with hereditary cirrhosis (Kostylev *et al*., 2019).

Pseudomonas Gram-negative bacteria appear as short chains or single cells, or may be straight or curved bacilli, moving by a single polar flagellum. P. aeruginosa are endemic in different environments such as soil, water, on surfaces, etc. (Chevallereau, 2017).

Pseudomonas transmitted mainly by dust-borne, tap water and bed sheets. Moreover, patients with chronic skin lesion are considered as a source for others lying in the same hall, as well as the operating theater bed, surgical instruments, floors, walls, cupboards, lighting, anesthesia device, ventilation and sterilizers. Doctors, nurses, other staff and visitors also take part in the process of transmitting these bacteria by direct contact or by using some multi-use uniforms (Nikbin *et al*., 2012; Inglisa *et*. *al*., 2009).

Diseases caused by P. aeruginosa bacteria depend on their possession of multiple virulence factors that enable them to break down tissues and invade the bloodstream. Among the most important of these factors are Protease, DNase and Hemolysine (Wilhel *et*. *al*., 1999; Ernst, 1999).

Hospital infection is one of the main problems facing health care staff, especially those who deal with serious surgical cases as a result of bacterial contamination in the surgical operating theaters. This in turn leads to the spread of hospital infections among patients too (Iiyama *et*. *al*., 2017). The spread of these infections has many dangerous effects, including an increase in the morbidity and mortality rate, the prolonged stay of the patient in the hospital and the increased need to use antibiotics that have an effective effect on the bacteria (Finnan *et al*., 2004).

Materials and Methods

1. Samples Collection

The samples of this study were collected from many environmental sources using cotton swab under supervision of a specialist physician. Then each sample was activated on a nutrient agar, incubated, taken with a tube and be carefully taken out of the tube so as not to touch the tube wall, and finally transplanted on a special plate for the growth of P. aeruginosa for the purpose of purification.

It is isolated from environmental cases, (100) environmental samples from different places of the hospital environment. The sample collection period lasted from the end of July (2020) to the end of January (2021).

2. Preparation of Culture Media

Readymade culture media were prepared according to the manufacturer's instructions.. Three types of media were prepared as follows:

2.1 Blood Agar

This medium was prepared according to (Rutala W.A., 1996).

2.2 Cetrimide Agar

This medium was prepared according to (Holt *et al.*, 1984).

2.3 Skim Milk Agar

This medium was prepared according to (Kalai, 2009).

3. Isolation of P. aeruginosa

After the samples were collected by swabs, they were cultured by loops using the spreader method on blood agar and MacConkey agar. The plates were incubated at 37°C for 24 hours. After the incubation period, the results are read in which P. aeruginosa was found not to ferment lactose sugar. Then, the developing bacterial colonies were transferred to Pseudomonas agar medium and the dishes were incubated at 37°C for 24 hours. The isolates carrying the characteristics of P. aeruginosa were selected and re-cultivated on a Cetrimide agar, which is regarded as a special medium for P. aeruginosa, and then incubated at 37°C for 24 hours. These colonies were also purified more than once to obtain good, pure isolates for the purpose of diagnosis confirmatory of P. aeruginosa.

4. Diagnosis of Bacterial Isolates

Bacterial isolates were diagnosed based on cultural and microscopic characteristics as well as biochemical tests as follows:

4.1 Cultural Characteristics

In order to study the agronomic characteristics of the bacterial isolates, the bacterial isolates ability to grow was tested at first on a group of culture media, including Pseudomonas agar, special Pseudomonas agar, Cetrimide agar, and selective P. aeruginosa according to (Brooks *et. al.*, 2007). They were also cultured on MacConkey agar and Blood base agar in order to diagnose cultural characteristics in terms of colony color and shape.

4.2 Microscopic Examination

A microscopic examination of the developing bacterial cells was carried out and stained with a gram stain solutions according to (Brooks *et. al.*, 2007).

4.3 Biochemical Tests

In order to diagnose the isolates at the species level, the purified colonies were subjected to two types of biochemical tests, Oxidase test and Catalase test According to (Rahman, 2006).

5. Determination of Virulence Factors

5.1 Hemolysin Production

According to (Wisplinghoff, 2017) , the ability of the isolates to produce bacterial hemolysin and ability of P. aeruginosa bacteria to analyze blood.

5.2 Protease Production

According to (Cruickshank *et al.*, 1975), the ability of the isolates to produce bacterial Protease and ability of P. aeruginosa bacteria to analyze protein .

5.3 DNase Production

According to (Heidi *et. al.*, 2010), the ability of the isolates to produce bacterial DNase and ability of P. aeruginosa bacteria to hydrolyze DNA .

5.4 Lipase Production

According to (Ghafil & Hassan, 2014), the ability of the isolates to produce bacterial Lipase and ability of P. aeruginosa bacteria degradation of lipids .

5.5 Amylase Production

According to (Sivaramakrishnan, 2007), the ability of the isolates to produce bacterial Amylase and ability of P. aeruginosa bacteria to hydrolysis starch .

5.6 Urease Production

According to (Qassem, 2006), the ability of the isolates to produce bacterial Urease and ability of P. aeruginosa bacteria to hydrolysis urea (ammonia).

6. IMViC Tests

6.1 Indole test

The indole test is used to investigate the ability of bacteria to break down the amino acid tryptophan and convert it to indole, which is considered as complex with Kovac's reagent (Sundström *et .al*., 1996).

6.2 Red methylation test

The red methylation test is used to investigate the ability of bacteria to ferment glucose sugar into a number of acids, leading to a decrease in the acidity function. The color of the medium does not change to red when adding the reagent, which is an indication of the non-fermentation of sugar (Cottell *et. al.*, 2009).

6.3 Voges Proskauer Test

The Voges Proskauer test is used to investigate the ability of bacteria to partially ferment glucose sugar, producing acetyl methyl carbinol and 2,3-butylene glycol, which give a red color when the reagent is added (Cottell *et. al.*, 2009).

6.4 Citrate Utilization test

This test is used to investigate the ability of bacteria to consume citrate as the only carbon source in Simmons citrate agar so the medium turned blue.

Results and Discussion

1. Isolation and Identification

The results of isolation and diagnosis of environmental samples showed the presence of bacterial growth belonging to the species P. aeruginosa in (28) out of (100) samples of environmental sources, including (11) out of (41) isolates from the operating theatre, and(9) out of (32) isolates from the hospitalized beds, and (8) out of (27) isolates from the surgical kit, as listed in Table (1).

Table 1: Environmental P. areuginosa isolates according to sources

Type of samples	Total Environmental samples	P. aeruginosa isolates	Percentages
Hospitalized beds	32	9	28%
Operating theatre	41	11	26.8%
Surgical kit	27	8	29.6
Total	100	28	28%

2. Agricultural Characteristics

2.1 Growth on Cetrimide Agar

It appears in the form of green colonies as shown in Fig. (1).

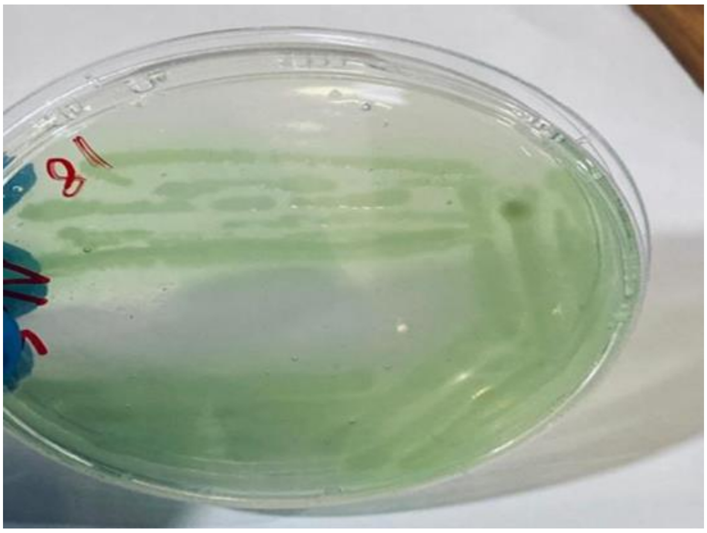

Figure 1: P. aeruginosa green colonies grown on Cetrimide medium

2.2 Growth on Nutrient Agar

It produce (80%) pyocyanin when grown on Nutrient agar medium. Large colonies with little convexity and flat edges were observed as shown in Fig. (2).

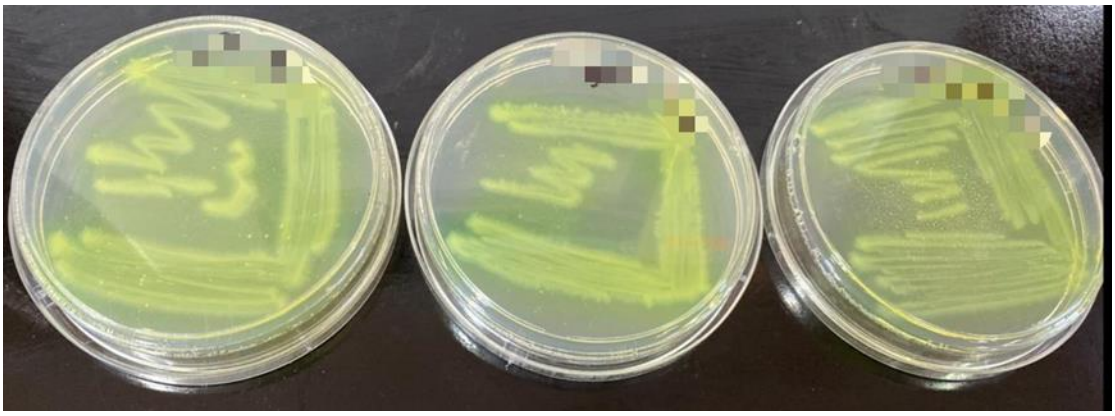

Figure 2: P. aeruginosa colonies grown on Nutrient medium

2.3 Growth on Blood Agar

Transparent halo appeared around the colonies that were grown on a stimulating enrichment medium as shown in Fig. (3).

Figure 3: P. aeruginosa colonies grown on Blood medium

2.4 Growth on Skim Milk agar

The ability of the isolates to produce protease enzyme was investigated using Skim Milk agar medium as shown in Fig. (4).

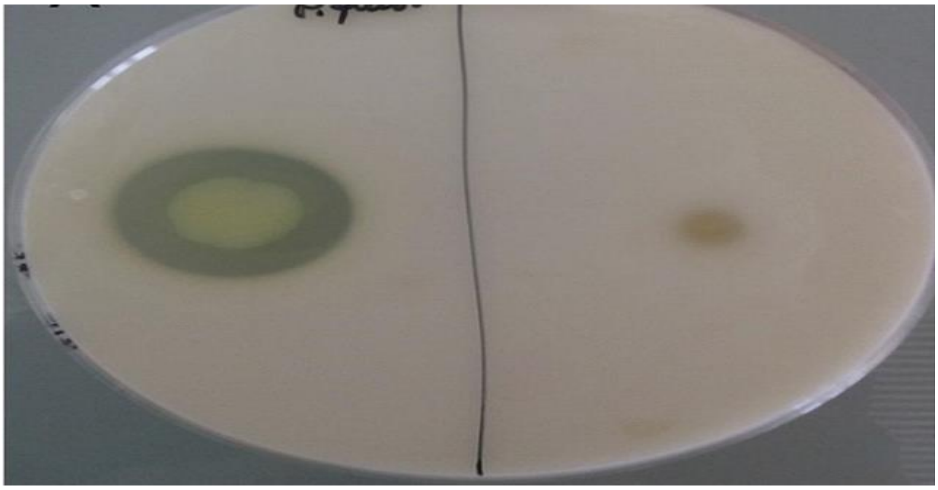

Figure 4: P. aeruginosa colonies grown on Skim Milk agar

2.5 Production of the deoxyribonucleic acid (DNase) enzyme

The results of this research showed the ability of the isolates to produce this enzyme as shown in Fig. (5). Transparent halo appeared around the bacterial colonies cultivated on a DNase agar medium after adding HCL.

Figure 5: Production of the deoxyribonucleic acid (DNase) enzyme

2.6 Production of the Lipase enzyme

The ability of the isolates to produce Lipase enzyme was investigated using Rhan agar medium as shown in Fig. (6).

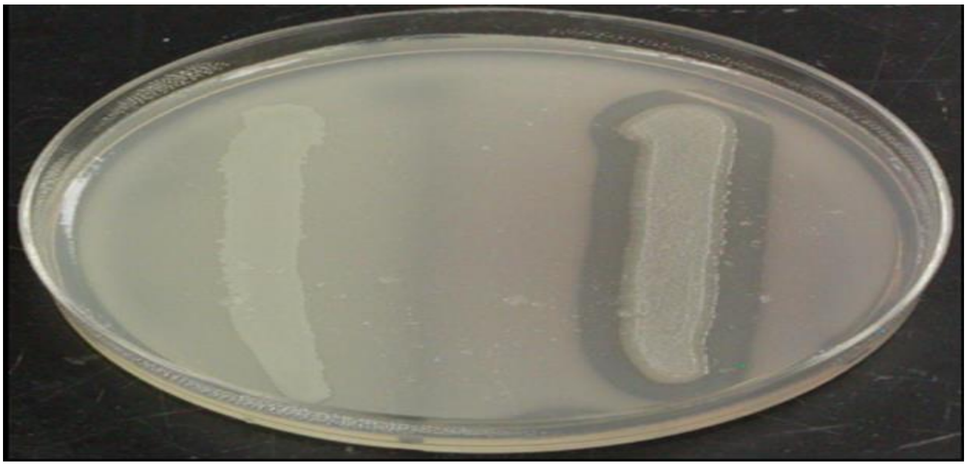

Figure 6: Production of the Lipase enzyme on Rhan agar medium

2.7 Production of the Amylase enzyme

The ability of the isolates to produce Amylase enzyme was investigated using Starch agar medium as shown in Fig. (7).

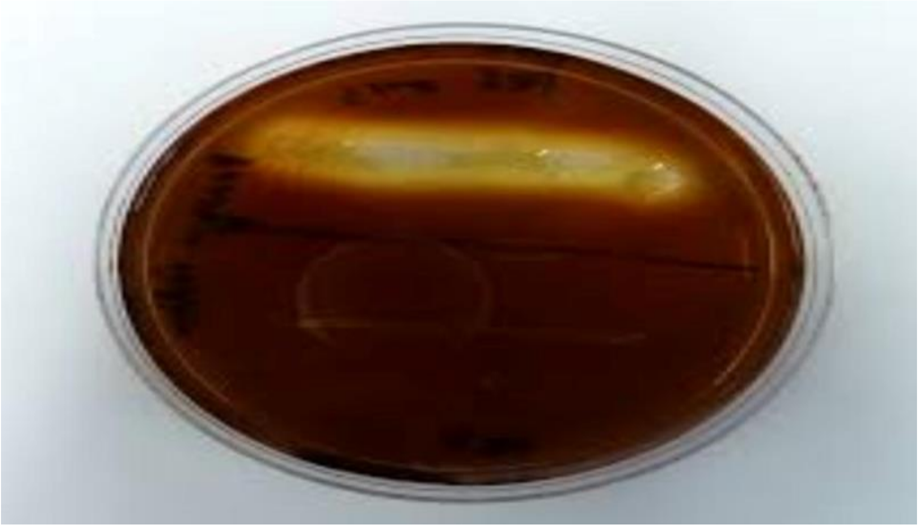

Figure 7: Production of the Amylase enzyme on Starch agar

2.8 Production of the Urease enzyme

The ability of the isolates to produce Urease enzyme was investigated using Urea agar base as shown in Fig. (8).

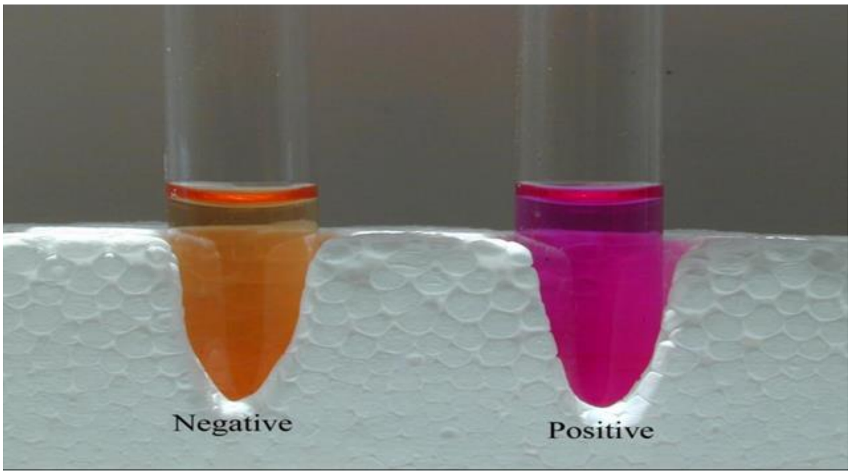

Figure 8: Production of the Urease enzyme on Urea agar

Virulence factors

The results showed that (35.7%) of total isolates were Hemolysin producer on blood agar,It was also found that (67.8%) of total isolates were Protease producer on Skim milk agar, (53.5%) were DNase producer, (67.8%) were Lipase producer, (57.1%) were Amylase producer, (21.4%) were Urease producer, Table (2)

Table (2) shows a summary of the Virulence factors found in this research in the P. aeruginosa isolates out of 23 Clinical samples.

Table 2: P. aeruginosa isolates out of 28 environmental samples

Virulence factors	No.	percentage
Hemolycin	10	35.7%
Protease	19	67.8%
DNase	15	53.5%
Lipase	19	67.8%
Amylase	16	57.1%
Urease	6	21.4%

3. Results of the IMViC Tests

All isolates were found to be negative for indole assay, red methylation test and Voges Proskauer test. And All isolates were found to be positive in the citrate reduction test. Table (3) shows the results of the biochemical tests that were conducted for the isolates under study.

Table 3: Phenotypic and biochemical examinations of the isolates

No.	Type of Test	Results
1	Oxidase	+
2	Gram-Stain	-
3	Catalase	+
4	Indole Production	-
5	Red Methyl	-
6	Voges Proskauer	-
7	Citrate	+
8	Hemolycin	B
9	Cetrimide Agar	+

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