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Detection of virulence factors of *Pseudomonas aeruginosa* from dog's wound infections by using biochemical and molecular methods

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Abstract
This investigation's goal was to find *Pseudomonas aeruginosa* virulence factors in canine wound infections. 100 samples were gathered and tested for eight virulence variables utilizing biochemical and molecular techniques for this purpose. According to the findings, women (72%) had the highest isolation ratio. The virulence factors were found in various ratios, with capsule being found in (16.6%) of them, amylase enzyme detected in (3.3%), hemolysin enzyme detected in (70%), protease detected in (10%), phospholipase enzyme detected in (83.3%), urease enzyme detected in (100%), DNAase enzyme detected in (80%), pigments production detected in (93.3%) from wound infections isolates, gal and lasA (86.6%), toxA (16.6%), pslA (96.6%),16SrRNA (100%). From this research, we conclude that dogs are more prone to bacterial infections.

Keywords: *Pseudomonas aeruginosa*, virulence factors, molecular methods

1. Introduction
A saprophytic and opportunistic pathogen, *Pseudomonas aeruginosa* is a G-bacterium that may infect both people and animals [2]. It causes pneumonia, dermatitis, mastitis, metritis, enteritis, and dermatitis in animals [16]. Despite the fact that *Pseudomonas aeruginosa* organisms have been found to be highly resistant to typical antibacterial substances like penicillin's and tetracyclines [19].
Pseudomonas aeruginosa has a variety of virulence factors, including hemolysin, proteases, ureases, and phospholipases, which break down immune system effector molecules. They also have components necessary for the cellular and tissue structure of the host [12, 21]. *Pseudomonas aeruginosa* has a solo polar flagellum that it uses to move, and by adhering to the asialyated glycolipid, it can attach to host epithelial cells [5].
Exotoxin A, which is present in both pathogenic and nonpathogenic strains of *Pseudomonas aeruginosa* and is located in its outer membrane proteins (toxA), prevents protein synthesis by ADP ribosylation of elongation factor 2 and consequently causes cell death [10].
(pslA): This gene was discovered in 517 genera and is a cluster that participates in cell-cell and/or cell-surface interaction in the formation of biofilms [11, 22].
The *Pseudomonas aeruginosa* gene (lasA), which is implicated in both proteolysis and elastolysis, was the first to be cloned and sequenced. Using genetic methods, its importance and function have been investigated [20].
(gal): cytoplasmic localization, present in both pathogenic and nonpathogenic strains 642 genera possessed this gene [20].
(16SrRNA): The majority of housekeeping genetic markers have been utilized to research bacterial phylogeny and taxonomy using 16S rRNA gene sequences for a variety of reasons [24].
These proteins may be a reliable factor for *Pseudomonas aeruginosa* fast identification in clinical samples because they are only present in this bacterium [7].
Consequently, the objectives of this study were to find *Pseudomonas aeruginosa* virulence factors in canine wound infections.

2. Materials and methods

Baghdad province served as the design hub for this study. The samples were collected from February to December 2022 from (Al-Shifa veterinary clinic, Mila veterinary clinic, dr. Shefa badrana clinic, Adhamiya veterinary clinic, Shabaad veterinary clinic\in the Adhamiya area), (Al-Farah veterinary clinic, dr. Muhannad veterinary clinic\ Al- Bayaa 20 th Street). This study included a total of 100 (28 male and 72 female) of dog's wound infection. The samples were obtained and delivered right away to the labs of Baghdad University and Al-Nahrain University for bacterial culturing and molecular technique.

2.1 Isolation of *Pseudomonas aeruginosa* from dog's wound infection

After transporting to the laboratory, the swabs were cultured on MacConkey agar, blood agar, nutrient agar and incubated at 37°C for 24 hours. After incubation, were subcultured on *Pseudomonas* chromogenic agar.

2.2 Identification of *Pseudomonas aeruginosa*

A. Colonial morphology

The form, color, diameter, and odor of the colonies of the bacterial isolates that grow on blood agar, MacConkey agar, nutritional agar, and Pseudomonas chromogenic agar were characterized [6].

B. Microscopical examination

One isolated colony was moved to a microscope slide, which was then properly treated and stained with Gram stain. Gram reaction, cell arrangement, and cell morphology were noted [9].

C. Biochemical tests

Oxidase test, catalase test, urease test, Lactose fermentation on MacConkey, Growth on Nutrient agar at 42 ˚C [8].

3. Results

The ratio of virulence factors were capsule: 16.6%, amylase enzyme: 3.3% (figure 6) , hemolysin enzyme: 70% (figure 2), protease: 10% (figure 3), phospholipase enzyme: 83.3% (figure 7), urease enzyme: 100% (figure 5), DNAase enzyme: 80% (figure 4), pigments: 93.3%. Table 1 & figure 13 demonstrated *Pseudomonas aeruginosa* virulence components that were identified from canine wound infections.

Table 1: *Pseudomonas aeruginosa* virulence components isolated from infections infected dog wounds

Samples of dog's wound infections (100)			
***Pseudomonas isolates* (30)**			
Virulence factors		**No**	**%**
1	Capsule	5	16.6%
2	Amylase enzyme	1	3.3%
3	Hemolysin enzyme	21	70%
4	Protease	3	10%
5	Phospholipase enzyme	25	83.3%
6	Urease enzyme	30	100%
7	DNAase enzyme	24	80%
8	Pigments	28	93.3%

3.1 Genetic methods used in detection of virulence factors

The gal and lasA genes were found in approximately 86.6% of Pseudomonas isolates, the toxA gene in 16.6%, the pslA gene in 96.6% of all Pseudomonas isolates, and the 16SrRNA gene in 100% of Pseudomonas isolates as shown in table 2. Figures 8 to 12 show the results of PCR products, and figure 14 displays the virulence factors of Pseudomonas.

Table 2: *Pseudomonas aeruginosa* virulence factors identified from canine wound infections

Samples of dog's wound infections (100)			
***Pseudomonas* isolates (30)**			
Virulence factors		**N0**	**%**
1	gal	26	86.6%
2	lasA	26	86.6%
3	toxA	5	16.6%
4	pslA	29	96.6%
5	16SrRNA	30	100%

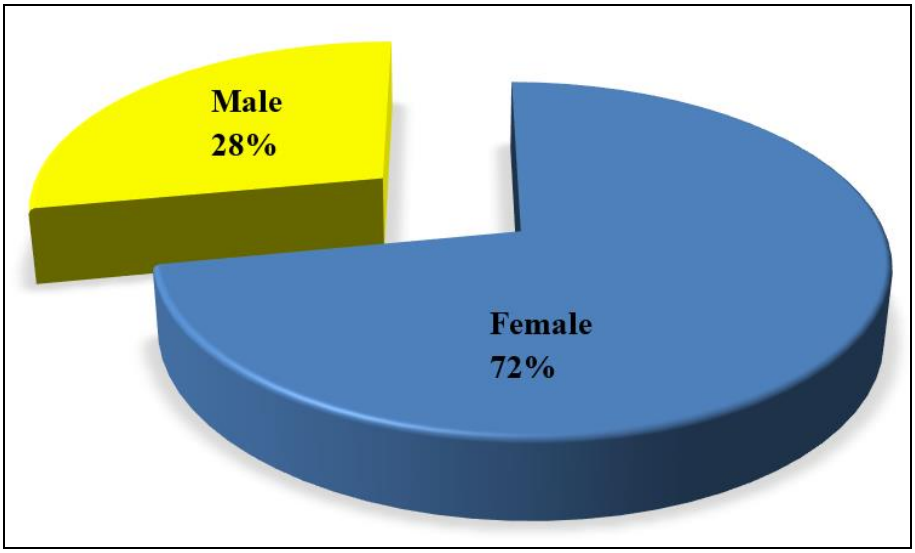


Fig 1: Percentage of *Pseudomonas aeruginosa* isolated from male and female dog's wound infections

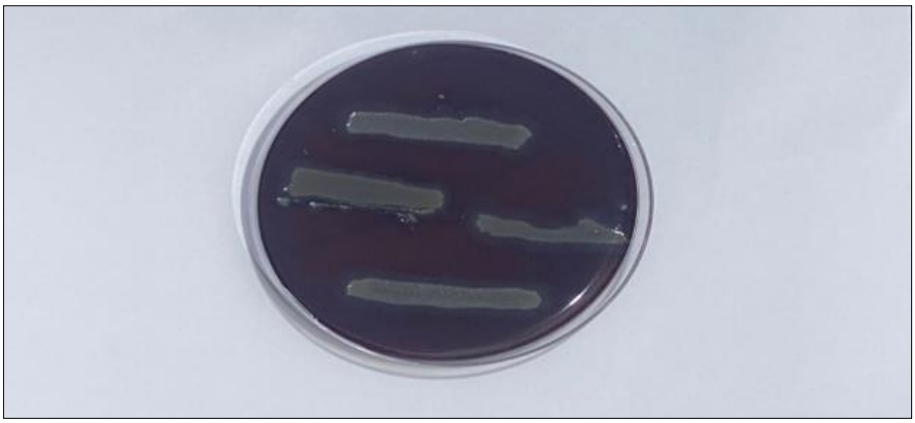


Fig 2: Hemolysin enzyme production

Fig 3: Protease enzyme production on skim milk agar

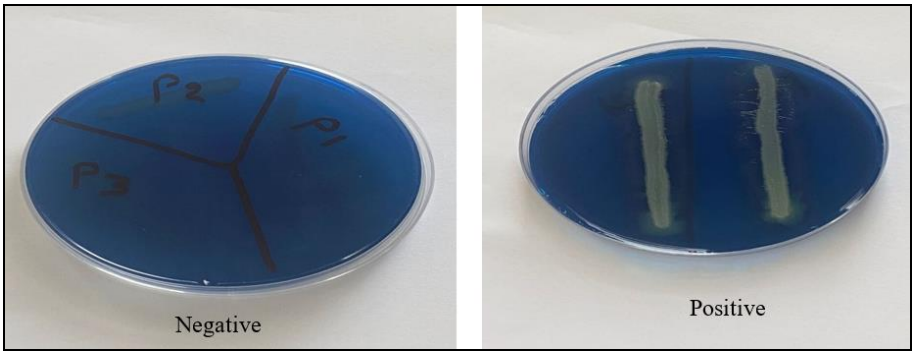


Fig 4: DNAase enzyme production

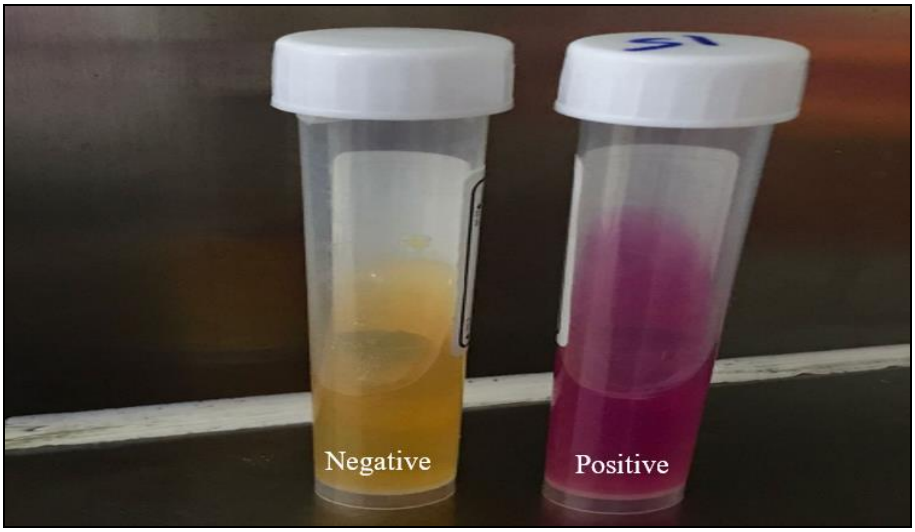


Fig 5: Urease enzyme production

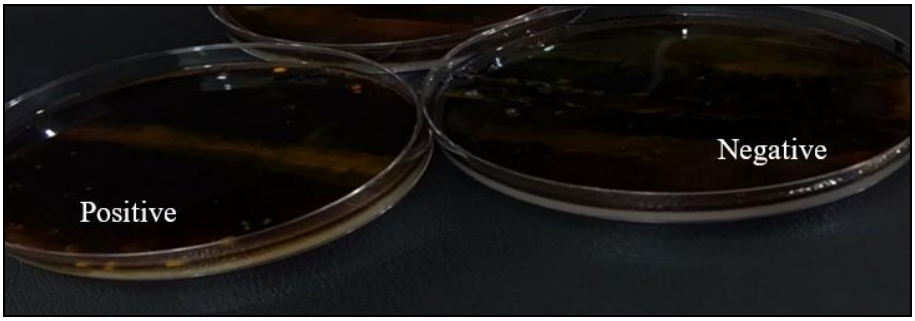


Fig 6: Amylase enzyme production

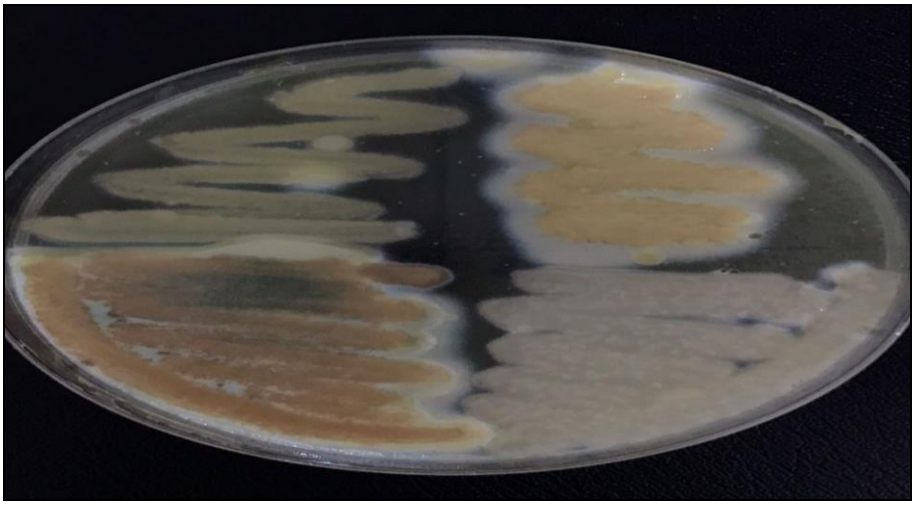
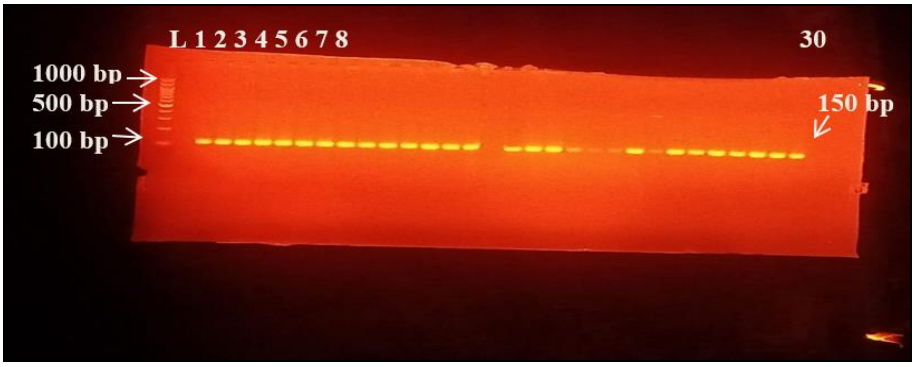
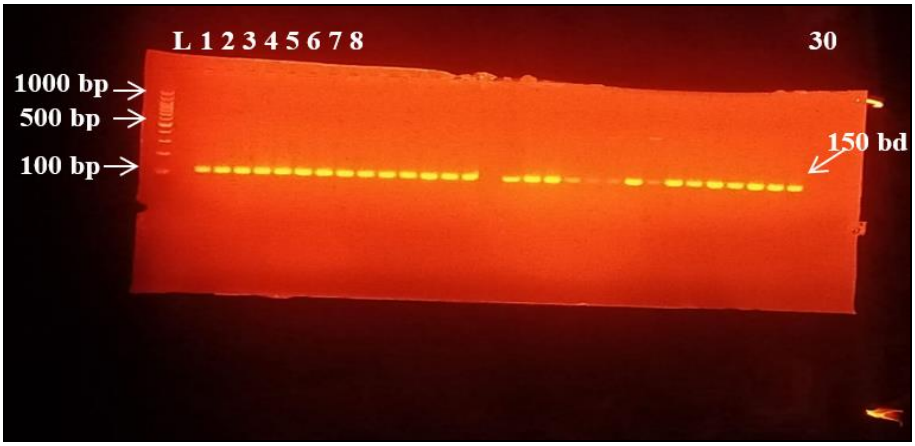


Fig 7: Phospholipase enzyme production



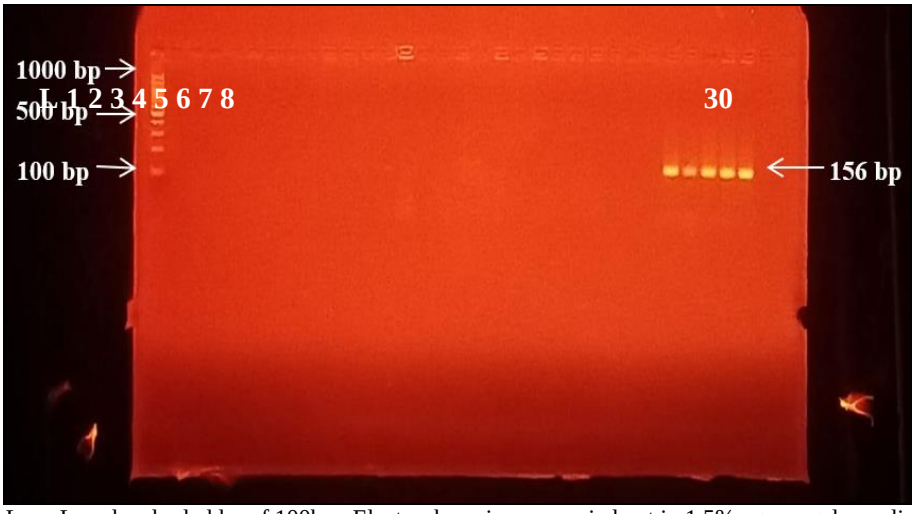
Lane L:molecular ladder of 100bp . Electrophorasis was carried out in 1.5% agaros gel supplied with ethidium bromide at 7V/cm for 90 minutes.

Fig 8: Agarose gel electrophoresis of amplified products of gal gene of *P. aeruginosa.* lanes 1-30 amplified product of gal gene (150 bp).



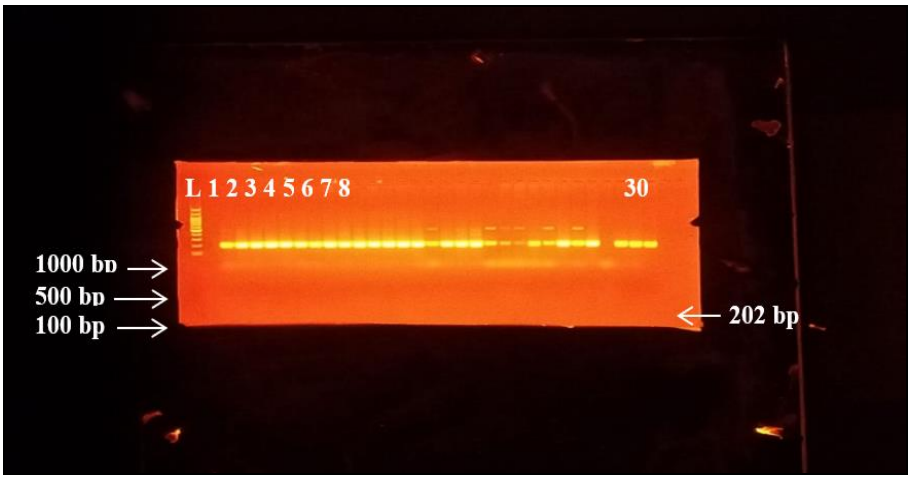
Lane L:molecular ladder of 100bp. Electrophorasis was carried out in 1.5% agaros gel supplied with Ethidium Bromide at 7V/cm for 90 minutes.

Fig 9: Agarose gel electrophoresis of amplified materials of lasA gene of *P. aeruginosa*. lanes 1-30 amplified product of lasA gene (150 bp).



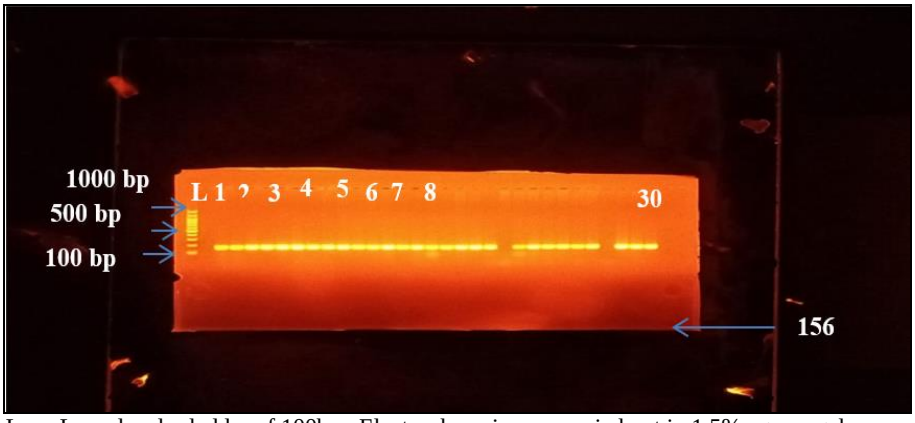
Lane L:molecular ladder of 100bp . Electrophorasis was carried out in 1.5% agaros gel supplied with Ethidium Bromide at 7V/cm for 90 minutes.

Fig 10: Agarose gel electrophoresis of amplified products of toxA gene of *P. aeruginosa*. Lanes 1-30 amplified product of toxA gene (156 bp).



Lane L: molecular ladder of 100bp. Electrophorasis was carried out in 1.5% agaros gel supplied with ethidium bromide at 7V/cm for 90 minutes.

Fig 11: Agarose gel electrophoresis amplified materials of pslA gene of P. *aeruginosa.* lanes 1-30 amplified product of pslA gene (202 bp) .



Lane L: molecular ladder of 100bp . Electrophorasis was carried out in 1.5% agaros gel supplied with Ethidium Bromide at 7V/cm for 90 minutes.

Fig 12: Agarose gel electrophoresis of amplified products of 16SrRNA gene of *P. aeruginosa.* lanes 1-30 amplified product of 16SrRNA gene (156 bp).

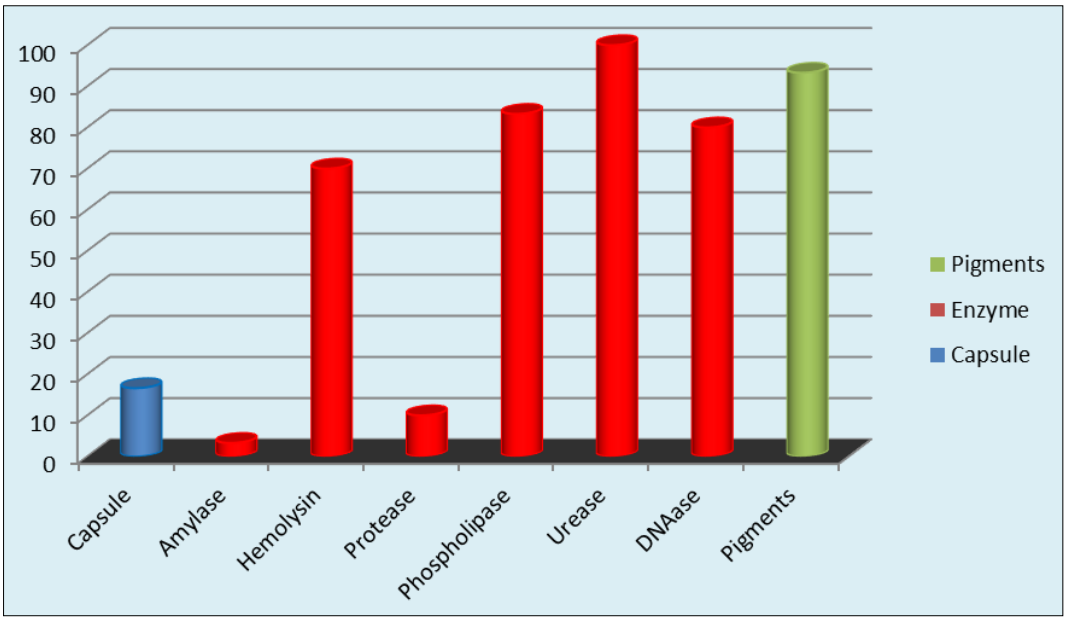


Fig 13: *Pseudomonas aeruginosa* virulence components isolated from infections infected dog wounds

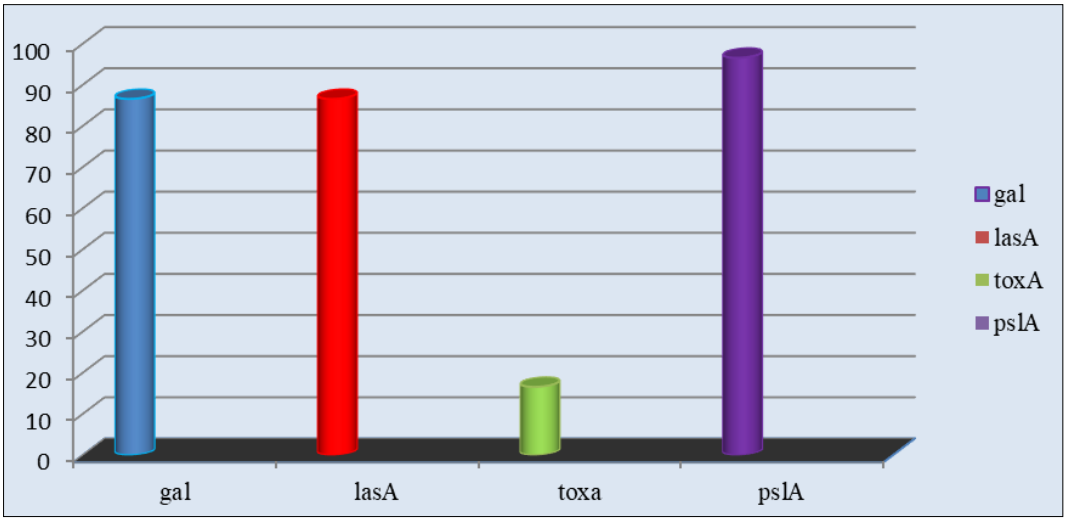


Fig 14: Virulence factors (genes) of *Pseudomonas aeruginosa* isolated from dog's wound infections

4. Discussion

In this investigation, P. aeruginosa was isolated from dog wound infections. Dog samples had the highest isolation rate, which was consistent with [15].

The isolation of *P. aeruginosa* from dog's wound infections, all the isolates have the ability to grow at 42C◦ but not at 4C◦ this result agreement [4]. Some isolates appeared to have capsule, this result differ from result showed by [1] were 0%, while [3] reported a 42.8% success rate. To protect bacteria from other types of immunological invasion, capsules play a significant role in preventing phagocytosis [17].

The detection of the hemolysin enzyme was 70%, in agreement with [15, 1] and differing from (5were 100%). 80% of samples tested positive for DNAase, which is a different outcome from the 15% who tested negative. The majority of isolates produced pigments when tested, which was in consistence with [13]. The virulence of P. aeruginosa is significantly influenced by pigments, which also lower host immunity [18]. As urease is an enzyme that is present in all isolates, it is able to divert urea into CO2 and NH3 and raise PH, all of which promote bacterial growth. This result varies from that reported by [15, 5] were negative. Also, the study's results for the protease detection test were favorable. By destroying host cells and impairing immune defenses like skin and mucous membranes, protease enzymes promote infection [14, 15]. In current study (gal, toxA, lasA, PslA, 16SrRNA) genes were detected in most isolates. The toxA gene was found in 16.6% of P. aeruginosa isolated from wound infections in dogs. ToxA is a naturally occurring genetic segment on the P. aeruginosa chromosome that controls the generation of exotoxin A, this study differs from what was reported [4, 23, 25, 26].

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