University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Molecular characteristics of *P.aeruginosa* isolated from human sources and chickens.

Waleed Khalid Mathree Zainab Razzaq Zighir

College of Veterinary Medicine, Zoonotic Unit, University of Baghdad, Iraq.

Abstract

Samples were collected from different locations in Wasit Governorate, Iraq, 120human samplesTested from ear swabs, wounds, burns, feces and 153 chicken samples*P. aeruginosa* isolates. Samples were striped on blood andMcConkey agarSuspect colonies are grown on chromogenic media and surrounded by citram. She was isolated The determination is made according to colony morphology, Gram staining, and conventional biochemistry Interactions and analysis profile of Index 20E (API 20E). A PCR examination confirmed the presence of *P. aeruginosa* in 11.35%. (273/31), 17.5% (120/21) from human samples, and 6.53% (153/10) from chickens.The polymerase chain reaction demonstrated that 100% of the isolates were *P.Aeruginosa* and all human and chicken isolates carry the exoA and exoT gene and the mexR gene, at a percentage (100%). And the study of histopathology on the internal organs (heart, lung, liver and intestine) of infected chickens.

Keywords: Pseudomonas aeruginosa, molecular study, Pseudomonas aeruginosa inchickens

I. INTRODUCTION

Pseudomonas aeruginosa is the second most common pathogen isolated from patients among the Gram-negative pathogens reported for the Nosocomial Infectious Surveillance System (NNIS)(Shenoy *et al*., 2014).These bacteria have the ability to live in many environments such as soil, plants, animals, etc. Bacteria can cause a wide range of acute and chronic infectious diseases. The diseases caused by these bacteria affect both humans and animals and lead to many pathological effects (Mikkelsen *et al.*, 2011), humandiseases: pneumonia, cystic fibrosis, andbacteremia, And burn infections, wound infections, sepsis, urinary tract infections, ear infections, skin and soft tissues and other blood infections (Cholley *et al*., 2014), Pseudomonas species play an effective role in the poultry industry of all ages, especially in chicks resulting in respiratory infections, septicemia and deaths in both chickens and fetuses. And the peritoneal cavity, in addition to congestion of the internal organs and peripheral inflammation (Kheir and Awaad,1985). Infections range from 2 to 100% (Shahat *et al*., 2019). The most important cellular factors are; Flagellum pilus. non pilus adhesion. alginate. lipopoly - saccride, extracellular: protease,hemolysis, toxin Exo A, Exo enzyme S (EXOS), ExoenzymeT (ExoT), biocyanin produces more than 90% of *Pseudomonas aeruginosa* produces Exotoxin A which may be very aggressive protein , PCR is used to confirm isolates and detect three of the phenotypic factors in both human and chicken isolates (toxA, exoT, mexR) (Bjorn *et al*., 1977)

MATERIALS AND METHODS

This study was approved by the College's Ethical Research Committee, Veterinary Medicine, University of Baghdad, Ministry of Higher Education and Scientific Research. P. aeruginosa was isolated from human and poultry sources in Wasit governorate. Iraq from the beginning of October 2020 until the end of January 2021. Human samples FromSterilized ear swabs, burns and wounds. Burns were secured from hospitals, and chicken samples were obtained from chicken breeding fields in the governorate and chickens in rural villages. Samples were plotted on MacConkey agar and blood agar and incubated at 37 ° C for 24 h. Cultures were confirmed in selective chromogenic and cetrimide media. They were identified by colony, morphology, Gram stain, and biochemical reaction. Biochemical tests using the API 20E system (Forbes *et al.*, 1988). byCommercial Purification System (Genomic DNA Minni Kit Geneaid, Thailand) withSome minor adjustments. The concentrations and purity of the extracted DNA wereQuantify using a Nanodrop Spectrophotometer. Gel electrophoresis was used for Detection of DNA fragments stained with safe red dye visualized under UV light (Sambrook & Russell,2001). Agarose (2% g was prepared for DNA extraction and 2% g for PCR was added to 100 ml 1XTBE buffer, boiled and cooled at 50). ° C after pouring 2 µl of Red Safe dyeTrays. After polymerization for 30 minutes at room temperature, the comb was removed. After the agarose is hardened in the wells.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

The gels were placed in achamber filled with 1XTBE buffer. The gel pocket was completely covered by buffer. Then 10 µl of each The sample was mixed with 5 µl of loading dye solution loaded into wells in the gel. Polymerase chain reaction (PCR) was performed to identify *P. aeruginosa* in human and chicken specimens and to detect some virion factors.

Table(1) Primers used in the PCR assay.

Gens	Primer	Sequence	Ex pected Size (bp)	Reference
Exo A	EXo A-	TGCTGCACTACTCCATGGTC	190	Bahador *et al.*, (20
	EXo A-	ATCGGTACCAGCCAGTTCAG		
Exo T	EXo T-	5-AAT CGC CGT CCA ACT GCA TG G-3′)	153	Ajayi *et al.*, (20
	EXo T-	5 ′-TGT TCG CCG AGG TAC TGC TC 3′		
Mex R	Mexr -F	GCGCCATGGCCCATAT TCAG	637	Dapgh *et al.*, (20
	Mexr -R	GGCATTC GCCAGTAAGCGG		

Primers supplied by Alpha DNA company, Thailand were lyophilized and dissolved in sterile deionized D.W to give a final concentration 100 picomole/µl stored at -20C. PCR reaction mixture was set up in 20 µl consisting of 2 µl of PCR premix, 1 µl of each primer (final concentration was 10 picomole/µl) and 2 µl of DNA template completed to volume by sterile distilled water. PCR reaction tubes were mixed, vortexed and placed into PCR chain reaction thermal cycler. Conditions of polymerase chain reactions are shown in Table 2 below.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Table 2:Cycling conditions of PCR.

Gane	Initial denaturation	No. of cyc	Denaturatio	Annealin	Extensic	final Extensio
Exo A	94 °C for 5 min	35	94 °C for 30 sec	58 °C fo 50 sec	72 °C f 1 min	72 °C fc 10 min
Exo T	94 °C for 5 min	35	94 °C for 30 sec	58 °C fo 50 sec	72 °C f 1 min	72 °C fc 10 min
Mex	94 °C for 5 min	35	94 °C for 30 sec	55 °C fo 50 sec	72 °C f 7 min	72 °C fc 10 min

Before electrophoresis, PCR products for each well were loaded with 5µl sample.DNA ladders of 100bp and 1kb were rconcurrently with each electrophoresis runto determine size of PCR product. Electrophoresis was done at 5V for 1.5 hour for extracted genomic DNA and PCR products. After, agarose gel was removed from thetank and DNA bands were visualized by UV transilluminator and photographed on adigital camera.

II. RESULTS

We found that P. aeruginosa was present in 11.35%. (273/31), samples, distributed as120 human and 153 chickens. Isolates from humans comprised 17.5% (120/21) and the percentage of isolates from chickens 6.53% (153/10) as shown in Table 3, below.

Table 3: Distribution of *P. aeruginosa* isolates in human and chicken samples

Source	No. of total samples	No. of total isolates	Percentage (%)
Human sampl	120	21	17.5
chicken sampl	153	10	6.53
Total	273	31	11.35

The bacterial isolates were seeded on blood agar plates and MacConkey in aerobic conditions. On blood agar, *P. aeruginosa* colonies showed white to gray color and the bacteria were able to induce complete beta degradation in red blood cells, and this degradation appeared as a clear region surrounding the colonies (Hogardt and Heesemann, 2010). While in MacConkey agar, the colonies appeared smooth, round with a pale yellow (pale) coloration or diffuse pigments sometimes spread over the agar. Some colonies appeared in mucous or coarse form. The coarse shapes were of high proportions (Figures 1 and 2). On a selective medium such as *Pseudomonas medium*, *P. aeruginosa* isolates produced

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

pigments and showed distinct growth. The isolates had a blue-green color with rapid bacterial growth (Fig3), while in the middle of the stremid, the isolates showed a characteristic blue-green-yellow-green color (Fig.4)

Figure (1): *Pseudomonas aeruginosa* on MacConkey agar

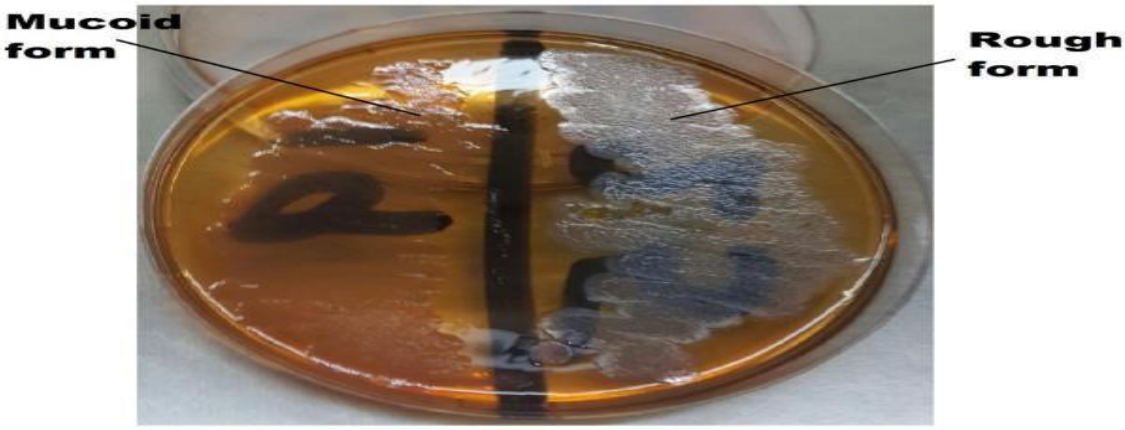


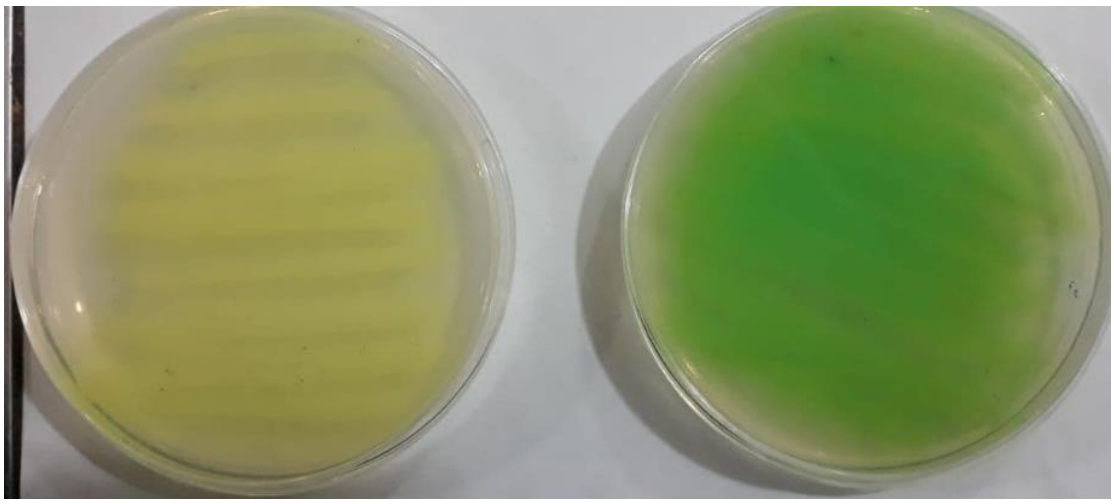
Figure (2): *Pseudomonas aeruginosa* on MacConkey agar shows mucoid and rough forms.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*



(1) (2)

Figure (3): *Pseudomonas aeruginosa* on Pseudomonas medium, (1): negative for pigment, and (2)positive for pigment

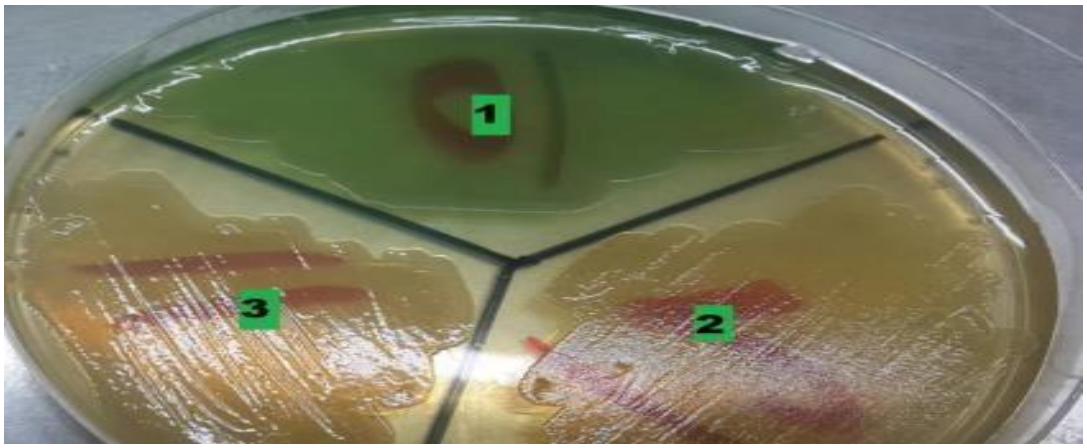


Figure (4): *Pseudomonas aeruginosa* on citrimide medium, (1)positive for pigment, and (2, 3): negative for pigment.

Microscopically, *P. aeruginosa* isolates appeared as Gram negative, small rods, and single, pairs or short chains (Lepanto *et al*., 2014). *Pseudomonas aeruginosa* positive results for oxidase , catalase tests and negative leads to urease and indol tests. it's ableto utilize citrate as a sole source for carbon and this bacterium is motile and show alkaline slant without change the TSI bottom and negative for H2S ,gas and negative to Gram's stain (Forbes *et al*., 1998). Identification of isolates was dependent on API 20E test as shown in Figure 5 below.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

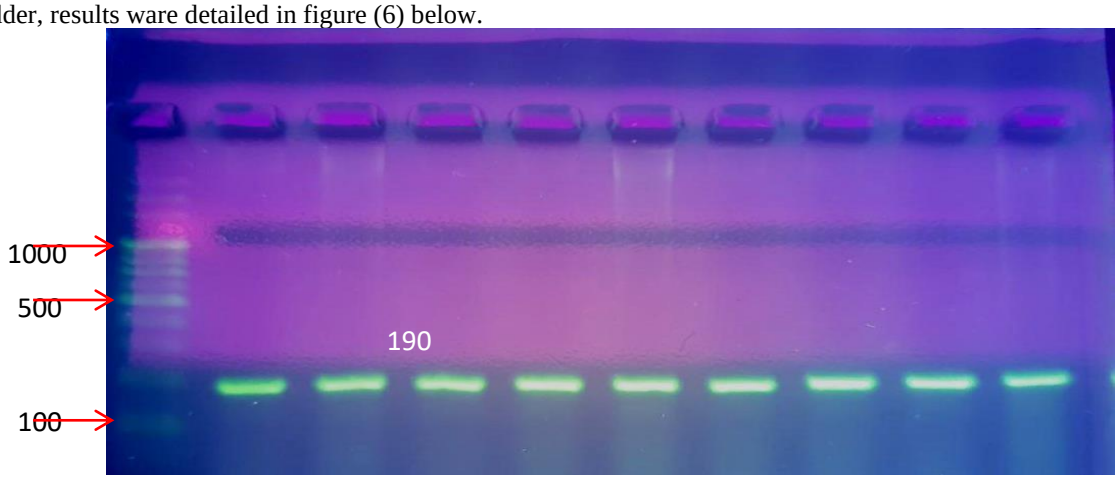
https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Figure (5): Results of *P. aeruginosa* on API 20E, (1): before inoculation and (2): after inoculation and incubation 24 h at 37 OC.

DNA was extracted from 31 isolates; Purity and concentration were confirmed with

Nanodrop. The purity of the isolated Pseudomonas aeruginosa was (1.7-2), and the concentration was between 50-360 ng / µl. DNA samples were taken from different sources and using an electrophoresis device, and the results were seen using ultraviolet light. Gel electrophoresis is used to identify the extracted DNA fragments (Brennan *et al.*, 2009)

Detection of Exo A gene: Amplification of this gene results in a product of size 190 bp as compared to the marker ladder, results ware detailed in figure (6) below.



University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

Figure (6) PCR product of Exo A gene, the band size 190 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm2. lx TBE buffer for1:30 hours. (A)represents human isolates,while (B) represents Chickens isolates

Detection of Exo T gene: Amplification of this gene results in a product of size 153 bp as compared to the marker ladder, results ware detailed in figure (7) below

Figure (7) PCR product of ExoT gene, the band size 153 bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. lx TBE buffer for1:30 hours. hours (A)represents human isolates,while (B) represents Chickens isolates.

Detection mexR gene: Amplification of this gene results in a product of size 637 bp as compared to the marker ladder, results ware detailed in figure (8) below

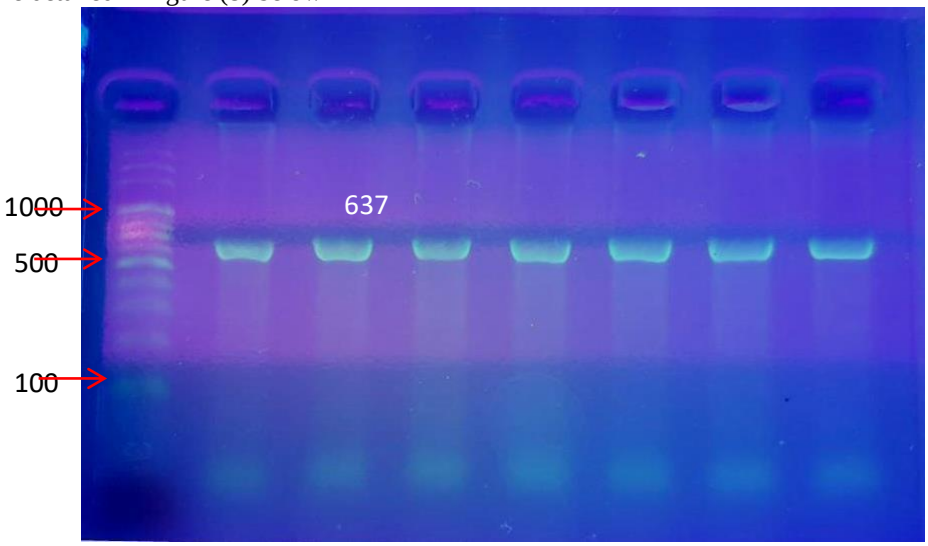
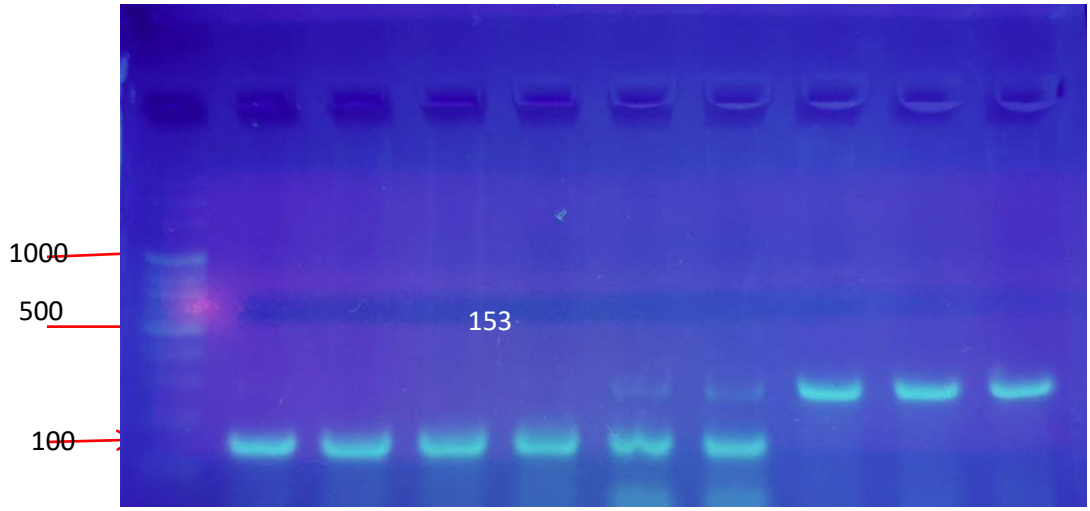


Figure (8) PCR product of mexR gene, the band size 637 bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. lx TBE buffer for1:30 hours. hours (A)represents human isolates,while (B) represents Chickens isolates.



University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

III. DISCUSSION

The percentage of ear isolates in our study, which is (12.19 %) It is identical if compared with (Al-Hadithi, 2007). Isolate from ear patients in Wasit hospitals in Iraq (12%), and be close if it is compared with what was isolated by (AL- Saeedi , 2018), from the ear isolates in the Medical City of Baghdad and Children's Hospital (8.33%). This percentage of ear isolates is less If it is compared with what was isolated by (AL - *Shafee* , 2018), who isolated (25.5%) of ear patients in Wasit hospitals,and by (Qader and Yaseen, 2012), who recorded 37.5% of the isolates of *pseudomonas aeruginosa* in the ear from patients in Hawler Hospitals in Iraq, the percentage of isolates from the ear in our study is also less than it was isolated by (Al-Charrakh *et al*., 2016). who Isolation of what (21.3%) from public and private hospitals in Baghdad, Iraq, and less than (Gad *et al*., 2007) who isolated (20%) of *Pseudomonas aeruginosa* isolates from ear patients in Egypt. *Pseudomonas aeruginosa* isolates from ear patients in our study (12.19%) would be higher if compared with what was isolated by (Hassan *et al.*, 2012), who recorded what was isolated (1.81%) from three hospitals in Iraqi Kurdistan in the governorates of Sulaymaniyah and Erbil. Also, the isolation rate was recorded. In our study, a higher rate than that recorded by Golshani and Sharifzadeh, who recorded a percentage (3%) of ear patients in Isfahan, Iran, 2013.The percentage of *Pseudomonas aeruginosa* isolates in the burn samples in our study (37.03%) is comparable if or comparing it with the results of the (Gad *et al.*, 2007) study, which recorded the percentage (33%) of burn patients in Wasit hospitals. As for(37%) in our study it is higher if It was compared with the results of the (AL - Shafee ,2018), study, which scored 25% of burn patients in Wasit hospitals, and higher than the isolation of (Hassan *et al.*, 2012),who recorded (10.9%) of the isolates *of pseudomonas aeruginosa* for patients suffering from burns in Iraqi Kurdistan. And it is higher than the results of a study (Al-Graibawi *et al.*, 2016), who recorded (13.75%) of the false isolates p. Aeruginosa, which was isolated from burns and wounds in Iraq. However, the results of our study are less than the results of a study to (AL-Kaisse, 2013).which recorded (41%) of burn patients in Baghdad hospitals.In our study, the percentage of isolates of *Pseudomonas aeruginosa* in wound samples is (15.78%), which is similar if compared with the results of the study (AL-Kadhmi *et al*., 2016) ,which isolated (14.61%) from wound infections in Baghdad hospitals. The percentage of what was isolated from patients suffering from wounds in Wasit hospitals is (13.3%). As for the percentage of our study of isolates of *Pseudomonas aeruginosa* in wound samples (15 78%), it is less than what was shown in the results of the study (AL- Saeedi , 2018)where the percentage of what was isolated was (28.8) %) Of patients in Wasit hospitals. And less than the results of (AL-Mayyahi, 2018). where (22%) were patients suffering wounds in Wasit hospitals. But the isolation rate in our study is lower if it is compared with the results of the study (Al-Rubaiee, 2009).the percentage was (45%) in Baghdad hospitals, and less than the percentage of (AL-Kaisse, 2013) (41%), which were isolated from wounds and burns. In Baghdad hospitals, the percentage recorded in (Al-Azawi, 2013) is 31.8%, which was isolated from patients suffering from wounds in Diyala hospitals.In our study, stool samples from patients suffering from diarrhea in Wasit hospitals are (9.09%), which are identical if compared with the percentage of the (AL- Saeedi, 2018)study, which was (6.5%) from Wasit hospitals. This percentage is less in the two studies than in the (Hayford, 2017) study. Who isolated a percentage (39.7%) of patients in hospitals in Ghana. From this it is evident that the highest percentage of *pseudomonas aeruginosa* isolates was in burn samples (37.03%), followed by the rates of wound isolates (15.78%), then ear isolates (12.19%), and most recently stool isolates were in patients with diarrhea (9.09%).In this study *Pseudomonas aeruginosa* isolates in 100 samples of broiler chicken that were collected from swabs from the oral cavity, feces, and internal organs of sick chickens after anatomy(heart, liver and intestinal) dissections were zero, and thus they are similar if compared with the results of the (Shahat *et al.*, 2019) study in Egypt. *Pseudomonas aeruginosa* isolates in 53 of the samples of chickens that live in rural areas and of different ages, the percentage of isolation was 18.18, which is a high percentage compared with meat chickens, in addition to not being mentioned in previous research, and the reason for this high percentage may be due to the lack of regular administration of medicines and vaccines. In rural chickens as in meat chickens, it is added to the difference in nutrition in rural chickens, where mothers have a role in raising the young and the mother's participation in cutting large pieces of food for the young by the mouth to make it easier for the young to swallow and since *Pseudomonas aeruginosa* lives in the intestines naturally and is transmitted to the young and is opportunistic when creating conditions Thus, rural chickens differ from broiler chicken that hatch with incubators, so they do not receive nourishment from mothers. The bacterial isolates were seeded on blood agar plates and MacConkey in anaerobic conditions. And on a selective medium such as Pseudomonas medium , the results were similar to previous studies conducted by(Ilham and AL-Rubaye,2015), as well as the results of studies that he conducted (Saadoon , 2019)in Iraq, which used sterimide agar, as a pseudo-medium. And other modalities for the initial diagnosis of Pseudomonas disease in their study.

University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

IV. REFERENCES

.Al-Azawi, Z. H. (2013).Antimicrobial Susceptibility Patterns of
Aerobic Bacterial Species of Wound Infections in Baquba General Teaching Hospital-Diyala . Diyala Journal of Medicine.4: 94 -100.

.Al-Charrakh, A.H.; Al-Awadi, S.J. and Mohammed, A.S. (2016).
Detection of Metallo-β-Lactamase Producing *Pseudomonas aeruginosa* Isolated from Public and Private Hospitals in Baghdad, Iraq. Acta Medica Iranica, 2 (54): 107-113.

.Al-Graibawi, M.A.A. and Ati, A.T. (2016). The efficacy of prepared specific *Pseudomonas aeruginosa* transfer factor to protect mice against experimental challenge. Adv Anim Vet Sci, 4: 33-128.

.AL-Kadhmi,N.A., AL-Thwaini,A.N., AL-Turk ,W.A.
and ALtaif, K.I.(2016). Studies on the Multidrug
Resistance to Pseudomonas aeruginosa Isolated from Infected Wounds. Int.J.Curr.Microbiol. 5(5): 963-970.

.AL-Kaisse,A.A.S.(2013).Molecular detection of
OXA-4, OXA-10 and VEB-1 genes in *Pseudomonas aeruginosa* isolated from burn's wound patients. International Journal of Pharmacy and Biological Sciences ,3:299-307.

.Al-Hadithi, A.B. (2007). Isolation and identification of
Pseudomonas aeruginosa from humans and animals, (MSc. Thesis, University of Baghdad).

.Ajayi ,T.; Allmond,L.; R.;Sawa, T. and Wiener-Kronish, J.P. (2003). Single-Nucleotide-Polymorphism Mapping of the *Pseudomonas aeruginosa* Type III Secretion Toxins for Development of a Diagnostic Multiplex PCR System. J.Clin.Microbiol., 41(8): 3526-3531

.AL-Mayyahi ,A.W.J.(2018). Detection of (exoT,exoY,exo SandexoU)Genes inPseudomonasaeruginosaIsolatefrom Different Clinical Sources. M.SC. Thesis, College of Science, University of Baghdad .

.Al-Rubaiee, L. (2009). The Role of *Pseudomonas aeruginosa* in chronic suppurative otitis media infection (Thesis M. Sc.; Medicine collage, University
of Baghdad).

.AL- Saeedi , R.H.A (2018) molecular detection of som virulence genes in P. aeruginosa clinical isolates , University of Wasit

.AL - *Shafee , A.A.J (2018) molecular and Bacteriological study of Pseudomonas aeruginosa Isolated from Human and milk of cows , University of Baghdad .*

.Bahador, N., Shoja, S., Faridi, F., Dozandeh-Mobarrez, B.,
Qeshmi, F. I., Javadpour, S., & Mokhtary, S. (2019). Molecular detection of virulence factors and biofilm formation in Pseudomonas aeruginosa obtained from different clinical specimens in Bandar Abbas. Iranian journal of microbiology, 11(1),
25.

.Bjorn, M.J.; Vasil, M.L.; Sadoff, J.C. and Iglewski,
B.H. (1977). Incidence of exotoxin production by *Pseudomonas* species. Infection and immunity, 1(16): 362- 366.

.Cholley, P.; Ka, R.; Guyeux, C.; Thouverez, M.; Guessennd, N.; Ghebremedhin, and Hocquet, D. (2014). Population structure of clinical *Pseudomonas aeruginosa* from west and central African countries. PloS one, 9(9).

.Dapgh, A. N., Hakim, A. S., Abouelhag, H. A., Abdou, A.
M. , & Elgabry, E. A. (2019). Detection of virulence and multidrug resistance operons in Pseudomonas aeruginosa isolated from Egyptian Baladi sheep and goat. *Veterinary world*, *12*(10), 1524.

.Gad, G.F.; El-Domany, R.A.; Zaki, S. and Ashour, H.M. (2007).
Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. Journal of antimicrobial chemotherapy, 5(60): 1010-1017.

.Forbes, B. A.; Sahm, D. F. and Weissfeld, A.S.
(1998). Pseudomonas, Burkholderia, and similar organisms. Baily and Scott's Diagnostic Microbiology,3: 448-461.

.Hassan, K.I.; Rafik, S.A. and Mussum, K. (2012). Molecular identification of *Pseudomonas aeruginosa* isolated from Hospitals in Kurdistan region. Journal of Advanced Medical Research, 2(3): 90-98.

.Hayford, O. (2017). *Isolation and characterisation of multidrug resistant Pseudomonas aeruginosa from clinical, environmental and poultry litter sources in Ashanti region of Ghana. Mv.Sc* (Doctoral dissertation,Thesis, Kwame Nkrumah Unof Science and Technology, Kumasi-Ghiversity ana).

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 14, Issue 1 (2025) PP 62-72

https://jam.utq.edu.iq/index.php/main *https://doi.org/10.54174/utjagr.v13i1.323*

.Hogardt, M. and Heesemann, J. (2010). Adaptation of *Pseudomonas aeruginosa* during persistence in the cystic fibrosis lung.International Journal of Medical Microbiology,300(8):557562.

.Ilham Ab.H.K.AL-Rubaye,I.A.H.K .(2015).
Frequency of *blaOxa10* Beta-lactamase gene in *Pseudomonas aeruginosa* isolated from different clinical swabs. Iraqi Journal of Science, 56 (4): 34053412

.Kheir El-Din, A.W., Awaad, M.H.H. (1985): Pseudomonas infection in turkeys. Vet. Med. J., 33 (2), 211-218.

.Lepanto, P.; Lecumberry, F.; Rossello, J., and Kierbel, A. (2014).
A confocal microscopy image analysis method to measure adhesion and internalization of *Pseudomonas aeruginosa* multicellular structures into epithelial cells. Molecular and Cellular Probes, 28(1): 1-5.

.Mikkelsen, H., McMullan, R., & Filloux, A. (2011). The Pseudomonas aeruginosa reference strain PA14 displays increased virulence due to a mutation in ladS. *PloS one*, *6*(12), e29113.

.Qader, S.N., and Yaseen, M.A. (2012). Management of acute otitis externa using aural wick versus local drops. Zanco. J. Med. Sci, 16(3):187-193.

.Qader, S.N., and Yaseen, M.A. (2012). Management of acute otitis externa using aural wick versus local drops. Zanco. J. Med. Sci, 16(3):187-193.

.Rabb, S., Holden, M. J., Winchester, M. R. and Turk,G.C. (2009). Potential primary measurement tool for the quantification of DNA. Anal Chem. (81):3414–3420.

.Ruwaida Kh. Frayyeh and Zainab R. Zghair (2023). Extraction of pyocyanin from Pseudomonas aeruginosa that isolated from dog with otitis. Acta BiomedicaVol. 94,2, pp.1-5.

.Sambrook, J. R., & Russell, D. (2001). DW. 2001 Molecular cloning: a laboratory manual. Quarterly Review of Biology, 76(3), 348-349

.Shahat, H. S., Mohamed, H., Al-Azeem, A., Mohammed, W., & Nasef, S. A. (2019). Molecular detection of some virulence genes in Pseudomonas aeruginosa isolated from chicken embryos and broilers with regard to disinfectant resistance. *SVU-International Journal of Veterinary Sciences*, *2*(2), 52-70

.Shenoy,R.; V Shetty,V.; A Lamsal,A.; Lamichhane,P. and Pokhrel,S. (2014). Multi-drug Resistant *Pseudomonas aeruginosa* Isolated from Intensive Care Burn. International Journal of Biomedical Research.5(4):1-6.

.Zamn S. Saadoon and Zainab R. Zghair (2019).MOLECULAR DETECTION OF PSEUDOMONAS *AERUGINOSA* BY USING *ALGD, PLCH* AND *LASB* GENES AND PATHOLOGICAL STUDY OF THE VIRULENT ISOLATE FROM HUMAN BLOOD.*Plant Archives* Vol. 19, Supplement 2, pp. 1633-1639.