Official Journal Issued by Faculty of Veterinary Medicine	**Benha Veterinary Medical Journal** Journal homepage: https://bvmj.journals.ekb.eg/	Since 1990

Original Paper

Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) technique for identification of *Pseudomonas aeruginosa* isolated from broiler and human.

Marwa M. Elsayed, Ashraf A. Abd El Tawab, Fatma I. Elhofy, Amira M. Rizk
Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University

ARTICLE INFO	ABSTRACT
	Pseudomonas aeruginosa is an opportunistic pathogen that is ubiquitously distributed throughout the environment, particularly in moist habitats. It causes severe economic losses in poultry industry. It also causes serious diseases in human especially in nosocomial patients lead to high death rates. The main objective in this study is to explain the prevalence rates of *P. aeruginosa* in broilers and humans. So**,** the samples were collected from 100 broiler chicken at different age groups: one day old chicks n=50, 14 days old chickens n=25 and 28 days old chickens n=25. From each chick, different organs were collected (liver, gall bladder, intestine, lungs, cloacal swabs, n=100 each). Yolk sac samples were collected only from one day old chicks (n=50). Thirty samples were collected from human: urine samples from patients complain from urinary tract infections (UTIs) (n=17) and pus swabs from abscesses (n=13) at Qalyubia Governorate, Egypt. The results of bacteriological examinations revealed that *P. aeruginosa* was recovered from; 47/100 chickens (47%), 5/17 (29.41%) urine samples, and 3/13 (23.07%) pus swabs. A Total of 55 isolates (47 isolates from broilers and 8 isolates from human) were identified by MALDI-TOF MS. It concluded that MALDI-TOF MS identification were 24/55 (43.63%), while by conventional method were 55/100 (55%); (from broilers and human), so MALDI-TOF MS is more accurate than conventional method.

1. INTRODUCTION

Pseudomonas aeruginosa is a bacteria that causes high rates of illness and mortality in chickens and other animals, especially those that are young, leading to significant financial losses (Labib and Roshdy., 2021). It was identified as Gram-negative, motile, non-spore-forming, and non-capsulated aerobic bacillus. Their isolates have the ability to grow in aerobic environments, forming non-lactose fermenter colonies on MacConkey's agar and β-zone of hemolysis on blood agar. The organism produces a greenish pigment with a fruity odor on the selective Pseudomonas agar base. The most common serotypes of *P. aeruginosa*, according to a serological analysis, were A, B, D, F, H, K, L, and M (Abd El-Ghany, 2021). Pseudomonas aeruginosa's pathogenicity in birds is primarily associated with respiratory and septicemic infections, sinusitis, keratitis, keratoconjunctivitis, and hatchery embryo fatality rates. *Pseudomonas aeruginosa* was the most common cause of avian mortality, particularly in chickens. The sickly chicks had a significant mortality rate and were at risk for respiratory conditions, swelling of the internal organs, pericarditis, and perihepatitis. *P. aeruginosa* infection in chickens is linked to respiratory symptoms, diarrhea, and septicemia (Dinev et al., 2013; Shukla and Mishra., 2015). *P. aeruginosa* is a common environmental bacterium that can infect people when they are not at risk. This bacterium is very adaptable to varied growing environments because of the abundance of metabolic pathways and regulatory genes. It is particularly difficult to eliminate from infected people,

especially lung infections in cystic fibrosis patients, due to its dietary diversity, a wide variety of virulence factors, and strong antibiotic resistance (Wu et al., 2015). It represents one of the most frequent causes of healthcare-associated infections, and are linked to longer hospital stays, higher expenses, and higher rates of morbidity and mortality (Zafer et al., 2014). MALDI-TOF MS has recently been used in normal microbiological laboratories since it produces results in just a few hours. Although the devices are still pricey, reagent costs are inexpensive, and bacterial identification can be done in large part automatically. By contrasting the resulting mass spectra with those from a reference library, the bacteria are identified (Gwida et al., 2014). Protein profiles provide the basis for MALDI-TOF MS. A mass spectrum of a tested organism is compared to reference spectra in the databases to find the closest match (Chean et al., 2014). So, the current research is focused on study the prevalence of *Pseudomonas aeruginosa* isolated from broilers and human based on comparison between conventional method that depend on cultivation and biochemical tests and MALDI-TOF MS in the bacterial identification.

2. MATERIAL AND METHODS

2.1. Sampling:
The samples were collected from 100 broiler chicken at different age groups: one day old chicks n=50, 14 days old

---

* Corresponding author: marwa.mohamed20@fvtm.bu.edu.eg

chickens n=25 and 28 days old chickens n=25. From each chick, different organs were collected (liver, gall bladder, intestine, lungs, cloacal swabs, n=100 each). Yolk sac samples were collected only from one day old chicks (n=50). The samples from humans included urine samples (n=17) and pus swabs (n=13), they were collected from human (Ethical number: BUFVTM35-10-22) at Qalyubia Governorate. All samples were collected under complete aseptic conditions from Qalyubia Governorate, Egypt from July to October 2021. They were sent to the Microbiology laboratory at Faculty of Veterinary Medicine, Benha University, in a sterile ice box as soon as possible to avoid the bacterial contamination.

2.2. Culture media and conventional diagnostic tests for Pseudomonas aeruginosa: (Shukla and Mishra, 2015):

All samples from broilers (liver, gall bladder, intestine, lungs, cloacal swabs, and yolk sac) and human (urine and pus swabs samples) were inoculated into nutrient broth (Himedia, india) in a separate tube and incubated at 37 °C for 24 hours. A loopful from each tube was streaked onto selective media for *Pseudomonas aeruginosa*, Cetrimide agar (Himedia, india). The grown colonies were subculture on MacConkey's agar (Himedia, india) and incubated at 37 °C for 24 hours, to observe the non-lactose fermenting colonies. The suspected colonies were streaked into nutrient agar, (Himedia, india) and incubated at 37 °C for 24 hours, to detect the pigment production. Then the suspected colonies were taken for further identification by microscopic examination and biochemical tests

2.2.1. Morphological identification: (Cruickshank et al., 1975):

Bacterial films were made from the purified colonies, stained with Gram's stain then examined under the light microscope to study the bacterial morphology.

2.2.2 Biochemical identification: (Cruickshank et al., 1975):

The biochemical tests that performed on the grown colonies were Oxidase, Catalase, Urea hydrolysis, Citrate utilization, Triple sugar iron agar (TSI), Indole production, Methyl red (MR) and Voges proskauer test (VP), (Himedia, india).

2.3. Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS): (Khater et al., 2021)

This technique was applied in Friedrich – Loeffler - Institute, Jena, Germany. Amies agar gel with charcoal transfer swabs (Thermo-Fisher Scientific, Germany) were used to capture only pure bacterial colonies, which were then transmitted straight for species identification using the MALDI-TOF MS assay. Bacterial swabs were cultured on blood agar plates with 7.5% blood and incubated for 24-48 hours at 37° with (5% CO_2). A single fresh colony from each plate was suspended in 300 μl of HPLC-grade water in a 1.5 ml Eppendorf tube and thoroughly homogenized using a vortex. Then, 900 μL of 100% ethanol was added to each tube to inactivate the microorganisms, and the tubes were vortexed once more. Each sample's protein was extracted using the methods previously described. Bacterial pellets that had been inactivated were recovered by centrifuging for two minutes at 11,000 g. After being air-dried to remove any remaining ethanol, the pellets were reconstituted in 50 μL of 70% formic acid and 50 μL of acetonitrile. The samples were centrifuged at 11,290g for 5 minutes at room temperature after being sonicated (100% amplitude, 1.0 duty cycle) for 1 minute on ice with the samples. The clear supernatant was then collected. One μL of each supernatant was spotted onto the MALDI target (Bruker Daltonik, Bremen, Germany's MSP 96 target polished steel MicroScout Target plate), dried by air, and then covered with 1.0 μL of a saturated-cyano-4-hydroxycinnamic acid matrix solution (in 50% acetonitrile and 25% trifluoroacetic acid). A MicroflexLT (Bruker Daltonics, Bremen, Germany) apparatus and MBT Compass Explorer 4.1 software were used to perform the MALDI measurements. The log score value of 0–3 recommended by the MALDI Biotyper manufacturer for species identification was followed by MALDI-TOF MS, only bacterial species with score values of 2.300 or higher were regarded as accurately identifying bacteria. The analysis excluded isolates identified with score values less than 2.300.

3. RESULTS

### 3.1. Prevalence of Pseudomonas aeruginosa in broilers and human:

The results of bacteriological examinations revealed that *P. aeruginosa* was recovered from 47/100 chickens (47%), While its isolation rate of from 550 chicken samples (liver, gall bladder, intestine, lungs, cloacal swabs, and yolk sac) were 105/550 (19.09%). The prevalence rate from liver, gall bladder, intestine, lungs, cloacal swabs and yolk sac were 23/100 (23%), 18/100 (18%), 23/100 (23%), 21/100 (21) %, 14/100 (14%) and 6/50 (12%) respectively. While the prevalence rate of P. *aeruginosa* from human samples were 8/30 (26.66%) including 5/17 (29.41%) urine samples, and 3/13 (23.07%) pus swabs. (Table 1 and 2).

Table 1 The prevalence of *P. aeruginosa* from broiler chicken and organs.

Type of samples	Recently dead chickens						Diseased chickens						Apparent healthy chickens					
	1 day old n=20	%	14 days old n=10	%	28 days old n=10	%	1day old n=20	%	14 days old n=10	%	28 days old n=10	%	1 day old n=10	%	14 days old n=5	%	28 days old n=5	%
Liver (n=100)	6	30	5	50	1	10	3	15	2	20	2	20	-	-	-	-	4	80
Gallbladder (n=100)	7	35	2	20	2	20	2	10	3	30	1	10	-	-	1	20	-	-
Intestine (n=100)	5	25	4	40	3	30	5	25	2	20	1	10	-	-	-	-	3	60
Lungs (n=100)	7	35	4	40	2	20	1	5	1	10	4	40	2	20	-	-	-	-
Cloacal swabs (n=100)	5	25	3	30	1	10	3	15	1	10	1	10	-	-	-	-	-	-
Yolk sac (n=50)	5	25	-	-	-	-	1	5	-	-	-	-	-	-	-	-	-	-

Table 2 The Percentage of *P. aeruginosa* from human samples.

Type of sample	Number of isolates	Percentage
Urine(n=17)	5	29.4%
Pus swabs (n=13)	3	23.07%
Total (n=30)	8	26.6%
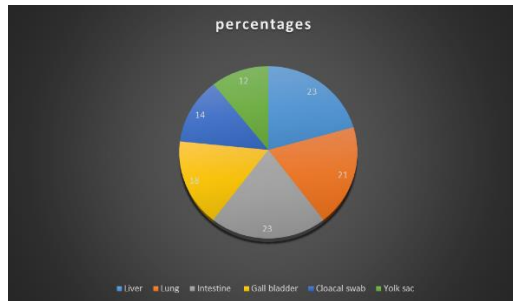
Figure 1 Percentages of *P. aeruginosa* in different organs in broilers.
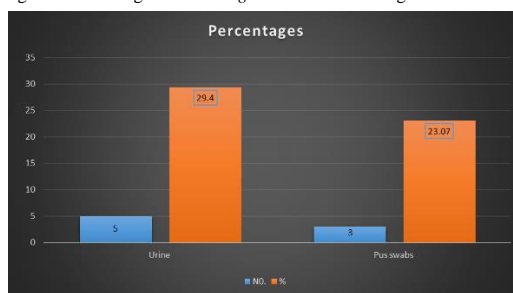
Figure 2 Percentages of *P. aeruginosa* in urine and pus swabs of human samples.

3.2. The results of conventional method of Identification of *P. aeruginosa* isolates:

On Cetrimide agar, the colonies of the isolates were smooth (when fresh) but become mucoid when the slime layer formed and produce yellow green to blue pigment in the media. On MacConkey's agar, the colonies were large and flat, and the media take yellow coloration due to non-lactose fermenting activity. While on nutrient broth there was greenish coloration. On nutrient agar, there were convex, large irregular colonies with green pigment production in the media and fruity smell like grapes. The microscopic examination showed Gram negative bacilli arranged singly or in pairs, rod shape with bulged sides and rounded ends, non-spore forming and non-capsulated, but some strains possess a slime layer. By biochemical identification, the suspected colonies gave positive results with Oxidase, Catalase, Urea hydrolysis and Citrate utilization test. While gave negative results with Triple sugar iron agar (TSI), Indole production, Methyl red (MR) and Voges proskauer test (VP).

3.3. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique:

A total of 55 isolates (47 isolates from broilers and 8 isolates from human) were identified by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). *P. aeruginosa* were identified in 24/55 strains (from broilers and human) (43.63%), The prevalence rates of *P. aeruginosa* from broilers were 21/47 (44.68%); liver 4/21 (19.04%), gall bladder 4/21 (19.04%), intestine 3/21 (14.28%), lungs 3/21 (14.28%), cloacal swabs 5/21 (23.80%) and yolk sac 2/21 (9.52%), with score range (2.300

to 2.450). While, from human samples were 3/8 (37.5%); 1/3 urine samples (33.33%), and 2/3 pus swabs (66.66%) with score range (1.840 to 2.420) (Table 3 and 4). The other *Pseudomonas* species strains were 17/55 (30.90%), while the other non-*Pseudomonas* species strains were 14/55 (25.45%).

Table 3 The number and percentage of *Pseudomonas aeruginosa* strains in broilers by (MALDI-TOF MS) technique.

Broilers		
Type of sample	No.	%
Liver	4	19.04
Gall bladder	4	19.04
Intestine	3	14.28
Lungs	3	14.28
Cloacal swabs	5	23.80
Yolk sac	2	9.52

% to total number of *P. aeruginosa* strains in broilers n=21.

Table 4 The number and percentage of *Pseudomonas aeruginosa* strains in human by (MALDI-TOF MS) technique.

Human		
Type of sample	No.	%
Urine	1	33.33
Pus swabs	2	66.66

% to total number of *P. aeruginosa* strains in human n= 3

4. DISCUSSION

The pathogenicity of *P. aeruginosa* in birds is mostly linked to respiratory and septicemic infections, sinusitis, keratitis, keratoconjunctivitis, and hatchery embryo mortality rates (Dinev et al., 2013).
The diseased chicks had a high mortality rate and were susceptible to pericarditis, perihepatitis, pulmonary diseases, and internal organ emphysema, Septicemia, diarrhoea, and respiratory symptoms are all brought on by the *P. aeruginosa* infection in chickens (Shukla and Mishra., 2015).
Pseudomonas aeruginosa could infect people even when they are not at risk. Because there are so many metabolic pathways and regulatory genes in this bacterium, it could grow in a wide range of settings. Due to its diverse diet, numerous virulence factors, and significant antibiotic resistance, this bacterium is very challenging to get rid of from infected persons, especially lung infections in cystic fibrosis patients (Wu et al., 2015).
It represents one of the most prevalent causes of healthcare-associated infections, is in charge of urinary tract, respiratory, and surgical site infections. This opportunistic infection is thought to pose a major health danger, especially to those who are immunocompromised. Infections caused by isolates of MDR *P. aeruginosa* are also associated with increased rates of morbidity and mortality, longer hospital stays, and greater costs (Zafer et al., 2014).
In the current study, the bacteriological examination of total 100 examined chickens showed presence of *Pseudomonas aeruginosa* in 47 chicken (47%) collected from different farms. This result agree with Mo'men et al., (2018), but higher than those of Shukla and Mishra.,(2015), Shahat et al., (2019), Hassan et al.,(2020), and Abd El-Ghany,(2021). Between the age groups, those *Pseudomonas aeruginosa* isolates (47) were from one day old chicks 25/47 (53.2%), fourteen days old chicken 13/47(27.7%) and 28 days old chicken 9/47 (19.1%). Those results nearly similar to (Kebede., 2010) but higher than (Mohamed., 2004). The isolation rates from liver, gall bladder, intestine, lungs, cloacal swabs, and yolk sac were 23%, 18%, 23%, 21%, 14% and 12% respectively. The isolation rates were highest

from liver and intestine, This finding similar to Shukla and Mishra., (2015) and Algammal et al., (2023) followed by lungs, gall bladder, cloacal swabs and yolk sac, These were agreed with (El-sadda et al., 2021). Total of 30 samples were collected from human patients complain from UTIs and abscesses from hospitals at Qalyubia Governorate, Egypt. The prevalence rates of *Pseudomonas aeruginosa* that collected from urine and pus swabs samples were 5/17 (29.4%) and 3/13 (23.07%). This came in harmony with (Tanwar et al., 2014).

The isolates were identified morphologically as Gram negative bacilli, rod-shaped with rounded ends and bulged sides, flagellated with polar flagella, non-spore-forming, and non-capsulated. When grown on culture medium, the colonies were smooth (when new) then later turned into mucoid colonies and produced yellow green to blue pigment on Cetrimide agar. However, on MacConkey's agar, there were large, flat, and non-lactose fermenter colonies and yellow color media. On nutrient broth gave green coloration. On nutrient agar grew as Convex, large, and irregular colonies with green pigment synthesis in the media and a fruity aroma resembling grapes. It was successfully identified biochemically using Oxidase, Catalase, Urea hydrolysis and Citrate utilization test. While gave negative results with Triple sugar iron agar (TSI), Indole production, Methyl red (MR) and Voges proskauer test (VP). These results were agreed with Mohamed., (2004)and Shukla and Mishra., (2015).

In clinical diagnostics, (MALDI-TOF MS) had become a widely used and essential component of the workflow for microbial identification (Welker et al., 2019).

The results of identification of Pseudomonas *aeruginosa* by MALDI-TOF MS were more accurate, as the number of strains from broilers that confirmed to be *Pseudomonas aeruginosa* became 21/47 (44.68%), While human samples were 3/8 (37.5%): from urine samples 1/3 (33.33%), and from pus swabs 2/3 (66.66%). For the best of our knowledge, there were no previous studies in using MALDI-TOF MS in identification of *Pseudomonas aeruginosa.* This technique is not common in Egypt because of its high cost and in-availability of MALDI-TOF MS apparatus in most laboratories. However, MALDI-TOF MS is more reliable in microbiology laboratories as it more accurate than conventional method of identification that based on culture characters and biochemical tests, save the time and efforts as the result produced within minutes instead of many days by conventional method and performs many samples in short time.

The variations between the results of other researchers and this work due to many reasons and different factors such as sources, season of sample collection, sanitary and hygienic measures, and geographical variation. These previous reasons cause fluctuations in the results.

5. CONCLUSION

It was concluded that MALDI-TOF MS is a helpful and more specific technique for bacterial identification, so it launches the green light for its application in Microbiology Laboratories on large scale.

6. REFERENCES

1. Al-Adl, M. M., Abd El- Tawab, A. A., El-Hofy, Fatma I., Khater, Dalia F. 2018. Virulence, Resistance Genes Detection and Sequencing of gyrA Gene of Pseudomonas aeruginosa Isolated from Chickens and Human in Egypt. Nature and Science, 16(2) :32-39**.**

2. Abd el-ghany, W. A. 2021. Pseudomonas aeruginosa infection of avian origin: Zoonosis and one health implications. Vet World, 14(8):2155-2159**.**

3. Algammal, A. M., Eidaroos, N. H., Alfifi, K. J., Alatawy, M., Al-Harbi, A. I., Alanazi, Y. F., Ghobashy, M. O., Khafagy, A. R., Esawy, A. M., El-Sadda, S. S. 2023. opr L Gene Sequencing, Resistance Patterns, Virulence Genes, Quorum Sensing and Antibiotic Resistance Genes of XDR Pseudomonas aeruginosa Isolated from Broiler Chickens. Infection and Drug Resistance, 16: 853- 867**.**

4. Chean, R., Kotsanas, D., Francis, M.J., Palombo, E.A., Jadhav, S.R., Awad, M.M., Lyras, D., Korman, T.M. et al. (2014). Comparing the identification of Clostridium spp. by two Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry platforms to 16S rRNA PCR sequencing as a reference standard: a detailed analysis of age of culture and sample. Anaerobe, 30: 85–89**.**

5. Cruickshank, R., Duduid, J.P. Maimion B.P. and Swain, R.H. 1975. Medical Microbiology, The Practice of Medical Microbiology. 12th Edition, vol. 11 Churchill Livingstone limited, Edinburgh, London, and New York**.**

6. Dinev, I., Denev, S. and Beev, G. 2013. Clinical and morphological studies on spontaneous cases of Pseudomonas aeruginosa infections in birds. Pakistan Veterinary Journal, 33(3): 398-400**.**

7. El-demerdash, Ghada, O., EL-shafei, A.A., Mahmoud, Amr, E. M., Roshdy, Heba. 2021. Potential risk of pseudomonas infection in broiler chickens with detection of the antibiotic resistant genes. Egyptian Poultry Science Journal, 40(4): 783-792**.**

8. El-Oksh, A. S., Elmasry, D. M. and Ibrahim, G. A. 2022. Effect of garlic oil nanoemulsion against multidrug resistant Pseudomonas aeruginosa isolated from broiler. Iraqi Journal of Veterinary Sciences, 36(4): 877-888**.**

9. El-Sadda, S. S., Esawy, A., Eltarabili, R. M. and Khafagy, A. 2021. Molecular Characterization and Genetic Analysis of Pseudomonas aeruginosa Recovered from Broiler Chickens.Suez Canal Veterinary Medical Journal 26(1): 61-77**.**

10. Gwida, M., Hotzel, H.E., Geue, L., and Tomaso, H. 2014. Occurrence of Enterobacteriaceae in Raw Meat and in Human Samples from Egyptian Retail Sellers. International Scholarly Research Notices, 2014: 6 ID 565671

11. Hassan, W. H., Ibrahim, A. M. K., Shany, S. A. S., and Salam, H. S. H. 2020. Virulence and resistance determinants in Pseudomonas aeruginosa isolated from pericarditis in diseased broiler chickens in Egypt. Journal of advanced veterinary and animal research, 7(3): 452–463**.**

12. Khater, Dalia F., Lela, Radwa A., El-Diasty, M., Moustafa S. A., Wareth, G. 2021. Detection of harmful foodborne pathogens in food samples at the points of sale by MALDT-TOF MS in Egypt. BMC Research Notes, 14:112

13. Kebede, F. 2010. Pseudomonas infection in chickens. Journal Veterinary Medicine and Animal Health, 2 (4): 55-58**.**

14. Mohamed, Heba A. E. 2004. Some studies on Pseudomonas species in chicken embryos and broilers in Assiut governorate. Assiut University Bulletin for Environmental Research, 7(1): 23-30**.**

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

16. Shukla, S. and Mishra, P. 2015. Pseudomonas aeruginosa infection in broiler chicks in Jabalpur. International journal of extensive research, 6: 37-39**.**

17. Tanwar, J., Das, S., Fatima, Z. and Hameed, S. 2014. Multidrug resistance: an emerging crisis. Interdisciplinary Perspectives on Infectious Diseases.2014: 541340

18. Welker, M., Belkum, A.V., Girard, Victoria, Charrier, J.P., Pincus, D. 2019. An update on the routine application of MALDI-TOF MS in clinical microbiology, Expert Review of Proteomics, 16(8) 695-710.

19. Wu, W., Jin, Y., Bai, F. and Jin, S. 2015. Pseudomonas aeruginosa. Molecular Medical Microbiology. (Second Edition). Chapter 41, Pages :753-767.

20. Zafer, M. M., Amin, M., El Mahallawy, H., Ashour, M. S. E.-D. and Al Agamy, M. 2014. First report of NDM-1-producing Pseudomonas aeruginosa in Egypt. International Journal of Infectious Diseases, 29: 80-81.