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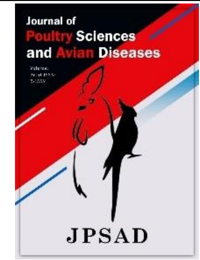

Molecular and Biochemical Characterization of Pseudomonas aeruginosa Isolated from a Turkey Flock

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A B S T R A C T

Pseudomonas aeruginosa is a Gram-negative, aerobic, predominantly motile, non-spore-forming, catalase-positive bacterium responsible for nosocomial infections, food and waterborne diseases in the human population, and infection of different organs in avian species. Because of the emergence of multidrug-resistant isolates in recent years, the occurrence of infection in pet avian species or poultry can lead to severe infection in the human population. This study reports an infection in turkey poults related to *Pseudomonas aeruginosa*. Overall, eight 4-day-old dead turkey chicks were presented to SANA Avian Hospital to diagnose the cause of death. For this purpose, liver and bone marrow were sampled and cultured on blood agar after necropsy. Then, other complementary biochemical tests were performed to achieve a primary diagnosis. After the primary diagnosis of *Pseudomonas aeruginosa* infection, at the next step, PCR was used for definitive diagnosis and evaluation of virulence factors. Finally, the agar disk diffusion method determined antimicrobial resistance to some common medications, and Clinical and Laboratory Standards Institute guidelines interpreted the results.

Keywords: *Pseudomonas aeruginosa; Iran; Turkey Poult; PCR; Microbial Culture; Antimicrobial resistance*

1 Introduction

Genus Pseudomonas consists of rod-shaped, Gram-negative, aerobic, predominantly motile, non-spore-forming, catalase-positive bacteria. The most significant human and animal pathogen in this genus is *Pseudomonas aeruginosa* (1). This bacterium is important in humans because of nosocomial infections, food and waterborne diseases, and spoilage (1, 2). In livestock and companion animals, there are also reports of different diseases (3, 4). In various avian species, there are reports of systemic diseases, airsacculitis, sinusitis, keratitis, keratoconjunctivitis, death of embryos and hatchlings, yolk sac infection, and septicemia in young birds (5, 6).

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In pet birds and poultry, *Pseudomonas aeruginosa*, usually an opportunistic pathogen, causes primarily upper and lower respiratory tract infections such as rhinolith, sinusitis, tracheitis, and airsacculitis (7). The infections can cause mortality and impose economic losses and can be a potential source of human infections. Because the bacterium has different virulence factors, the most important is antimicrobial resistance; possible human infections can lead to severe outcomes (2, 7). Other known mechanisms for antimicrobial resistance are producing extra-cellular enzymes, decreased cell permeability, and efflux pumps (2, 7).

The base of diagnosis for infection is mostly isolation and molecular techniques (7, 8). Overall, the prevalence of *P. aeruginosa* infection in poultry and pet birds in Iran and the evaluation of virulence factors are poorly investigated. In this study, we report a case of infection in a turkey poult flock and evaluate the virulence profile of the isolate. This is the first report of *P. aeruginosa* infection in a turkey poult flock in Iran.

2 Case presentation

Following the report of sudden mortality in a Turkey poults farm, eight dead cases of 4-day-old turkey poults were referred to SANA Avian Hospital for diagnosis of the underlying cause. First, Necropsy was done, and then microbial cultures from bone marrow and liver were performed on the blood agar (HIMEDIA, India) and MacConkey agar (Merk, Germany) growth media. After 24 hours of incubation at 37 ^{oC} (7), colonies had specific odor and beta hemolysis on blood agar. Then, more biochemical tests were done for the primary diagnosis of the isolate. The results were positive for oxidase and motility, nitrate reduction, pigment, hemolysis, and growth at 42 ^{oC}; MacConkey agar was seen. Glucose fermentation, urease, and maltose test were negative. The result for mannitol was also variable. It should be added that biochemical tests for the Entrobactiacae group were negative.

PCR was performed for a definitive diagnosis after primary verification with culture and biochemical tests. For this purpose, genomic extraction was done using a DNA extraction kit (MBST, Iran) from two or three fresh colonies according to the manufacturing procedure. Then, a simplex PCR was carried out to identify the *Pseudomonas aeruginosa* by the PCR master kit (Biotech Rabbit, Germany), using two primers targeting 16S rDNA variable regions 2 and 8 (V2 and V8), respectively by MiniAmp Plus thermocycler (Thermo Fisher Scientific, USA) (Table 1)(9). PCR thermal conditions consisted of 5 minutes of denaturation (95 ^{oC}; 1 cycle), followed by 35 cycles of denaturation (95 ^{oC}; 30 seconds), annealing (58 ^{oC}; 45 seconds) and extension (72 ^{oC}; 1 minute) and one cycle of final extension (75 ^{oC}; 5 minutes) (Figure 1).

Table 1. Primers used for PCR (9)

Primer	Sequence	Target Gene	Annealing temp (°C)	Product size (bp)	Ref.
PA-SS-F	GGGGGATCTTCGGACCTCA	*16S rDNA*	58 ^{oC}	956	
PA-SS-R	TCCTTAGAGTGCCCACCCG				
lasB F	GGAATGAACGAAGCGTTCTCCGAC	*las*B	60 ^{oC}	284	
lasB R	TGGCGTCGACGAACACCTCG				
toxA F	CTGCGCGGGTCTATGTGCC	*tox*A	60 ^{oC}	270	
toxA R	GATGCTGGACGGGTCGAG				
exoS F	CGTCGTGTTCAAGCAGATGGTGCTG	*exo*S	60 ^{oC}	444	
exoS R	CCGAACCGCTTCACCAGGC				
plcH F	GCACGTGGTCATCCTGATGC	*plc*H	60 ^{oC}	608	
plcH R	TCCGTAGGCGTCGACGTAC				
plcN F	TCCGTTATCGCAACCAGCCCTACG	*plc*N	60 ^{oC}	481	
plcN R	TCGCTGTCGAGCAGGTCGAAC				

Then, the PCR products were run at 2% agarose gel at 100 V for 90 min in 1x TBE buffer and pictures were taken (Figure 1 and Figure 2).

Then, the simplex PCR test was done using the already published primers for the most important virulent targeted genes (9). In this step, the PCR thermal condition was the same as the previous step except for the annealing temperature (60 ^{oC} instead of 58 ^{oC}) (Figure 2).

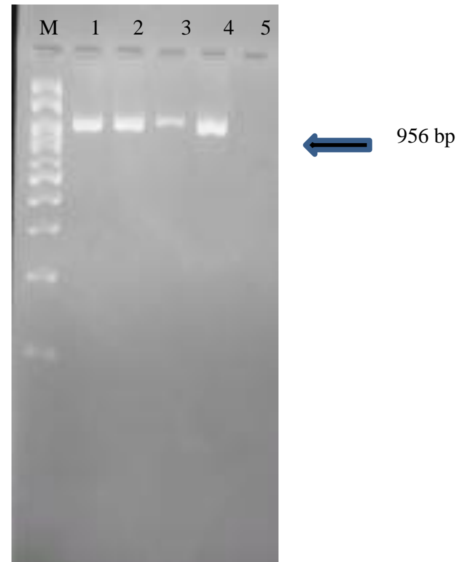

Figure 1. Amplification products of species-specific 16S rDNA in Pseudomonas aeruginosa.

Lane M Marker100,1-2 culture sample,3-4 positive control, five negative control

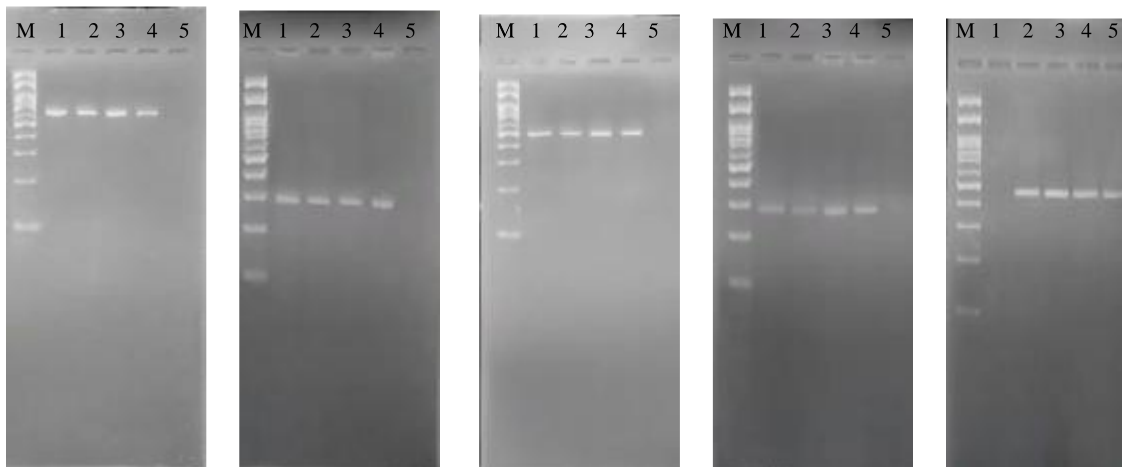

Figure 2. Amplicons for different virulent genes of the isolate.

A: Lane M Marker100,1-2 culture sample,3-4 positive control, five negative control (plcH,608bp); B: Lane M Marker100, 1-2 culture sample,3-4 positive control, five negative control (lasB,283bp); C: Lane M Marker100, 1-2 culture sample,3-4 positive control, five negative control (plcN,481bp); D: Lane M Marker100,1-2 culture sample,3-4 positive control, five negative control (toxA,270bp); E: Lane M Marker100,1 negative control,2-3 culture sample,4-5 positive control (exoS,444bp)

After confirmation of *Pseudomonas aeruginosa* by PCR, the agar disk diffusion method checked antimicrobial resistance to some common medications. Then, Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret the results (7).

3 Discussion

Pseudomonas aeruginosa is a cause of nosocomial infections, food and waterborne diseases in the human population, and upper and lower respiratory tract infections in avian species (1, 2, 5, 6). It also affects human and animal food spoilage, especially meat stored at cold temperatures (10). The distinct blue to-green colonies are the reason for naming this bacterium (pseudo is 'false,' monas is 'single unit,' “Aeruginosa” is from the Latin word “aerūgō,” means 'greenish-blue' or 'rusty copper') (2, 10). It has different virulence factors that can help the bacteria to cause severe infections in human and avian species. Some virulence

factors are lipopolysaccharides, flagella, pili type IV, exotoxin A, exo-proteases, phospholipase C, chromophores, siderophores, and antibiotic resistance (2, 7).

The infection in avian species can be a source of disease for the human population. This problem is more important for multidrug-resistant (MDR) species (2, 7). There is some research on MDR species in Iran, and one of them is a systematic review and meta-analysis by Vaez et al. In this study, *Pseudomonas aeruginosa* from different sources in Iran was evaluated. Based on the results, 58 percent of the isolates were MDR. In this study, the highest and lowest prevalence of MDR *isolates* were observed in Tehran (100%) and Zahedan (16%), respectively, and the highest rate of resistance was against ceftazidime (50%) and amikacin (50%) (11). These results are in contrast with the present study in which the isolate was susceptible to amikacin.

Unfortunately, there are limited studies on *P. aeruginosa* in avian species in Iran. Still, one of the reliable studies was done by Meamar et al. on 126 companion birds from different orders (7). In this study, swabs took samples from the eye or choanal slit of birds with upper or lower respiratory tract signs. A total of seven cases were positive for *P. aeruginosa*. The highest resistance to antibiotics was to neomycin, kanamycin, rifampicin, and vancomycin (100% of isolates) and colistin (57% of isolates), respectively (7). These results agree with our results from the neomycin resistance point of view but contrast with the colistin resistance point of view.

There are some reports of P. aeruginosa infection in turkeys worldwide, one of them is a study by Stafseth et al. at Michigan State (12). In this outbreak in 1938, in approximately 19,000 turkeys, the morbidity rate was nearly 50 percent, and the mortality was very low, but there was no antimicrobial resistance evaluation data for this study (12). Another report by Hafez et al. in 1987, in which 5-10 percent of eight turkey flocks with a total population of 50,000 birds were affected with signs of lameness at 10-14 days (13). In the first three weeks of this disease outbreak, mortality was 2.3-4 percent in males and 1.8-2.8 percent in female turkey poults. The isolates were resistant to furazolidone, sulfonamide, chlortetracycline, erythromycin, and chloramphenicol (13). Resistance to erythromycin is in agreement with our results. Another study on different avian species, including turkeys in Egypt showed MDR isolates resistant to kanamycin, amoxicillin, amoxicillin-clavulanic acid, neomycin, chloramphenicol, vancomycin, cefotaxime clavulanic acid, lincomycin-spectinomycin, co-trimoxazole, cefoxitin, gentamycin, and doxycycline, but susceptible to silver nanoparticles (14). Resistance to neomycin, amoxicillin, and doxycycline is like our study, but resistance to lincospectin and gentamycin are in contrast.

At last, *P. aeruginosa* in avian species can potentially source human infections. There is little published data on avian species in Iran, and there is a need for further evaluation. This study is the first report of *P. aeruginosa* infection in turkey poults in Iran. Aside from isolation and identification, there is an evaluation of the drug resistance profile of this isolate. The results showed that the isolate is resistant to amoxicillin, ampicillin, ceftiofur, doxycycline, erythromycin, florfenicol, neomycin, sulfonamide + trimethoprim but susceptible to amikacin, colistin, Difloxacin, enrofloxacin, gentamicin, Lincospectin. The resistance to fosbac and tetracycline was also intermediate.

Conflict of Interest

The authors declared no conflicts of interest.

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Authors' Contributions

All authors contributed equally to this study.

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Data Availability Statement

Data are available from the first author upon reasonable request.

Ethical Considerations

This study was reviewed and approved by the Institutional Animal Ethics Committee of the SANA Research Institute for Avian Health and Diseases under the approval code IAEC–SANA–003/2023. All procedures involving animals followed national and institutional guidelines for the care and use of laboratory and avian species.

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