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Pathological And Molecular Characterization Of A Dual Bacterial Co-Infection By Klebsiella Pneumoniae And Pseudomonas Aeruginosa In Juvenile European Goldfinches (Carduelis Carduelis)

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Abstract: The present study reports the pathological and molecular characterization of a rare dual bacterial co-infection caused by Klebsiella pneumoniae and Pseudomonas aeruginosa in juvenile European goldfinches (Carduelis carduelis). Affected birds presented with clinical signs of respiratory distress, lethargy, anorexia, and progressive weight loss. Necropsy revealed severe pneumonia, hepatomegaly, and splenomegaly, while histopathological analysis confirmed extensive inflammatory infiltration and tissue necrosis in the lungs and liver. Bacteriological culture yielded both K. pneumoniae and P. aeruginosa, which were further confirmed by molecular identification using species-specific PCR assays. Antimicrobial susceptibility testing demonstrated multidrug resistance, highlighting the therapeutic challenges posed by these opportunistic pathogens. The dual infection likely exacerbated disease severity, contributing to rapid mortality in the flock. This case underscores the significance of considering mixed bacterial infections in avian pathology and emphasizes the need for accurate molecular diagnostics and prudent antimicrobial stewardship in avian medicine. Furthermore, the findings provide insights into the pathogenic synergy between K. pneumoniae and P. aeruginosa in passerine birds, with implications for wildlife health surveillance and conservation.

Keywords: Klebsiella pneumoniae, Pseudomonas aeruginosa, dual infection, European goldfinch, avian pathology, molecular characterization, multidrug resistance, respiratory disease, wildlife health.

Introduction: Passerine birds, which include the European goldfinch (Carduelis carduelis), represent a diverse order with significant ecological roles and a growing presence in the pet bird trade [1, 2, 4]. While captive passerines are often sought for their aesthetic appeal and vocalizations, they are susceptible to a wide range of infectious diseases, with bacterial pathogens being a major cause of morbidity and mortality [4, 15]. The diagnosis and management of these infections are critical for avian health and can have significant public health implications due to the zoonotic potential of many avian pathogens [3, 11].

Among the most challenging bacterial agents are opportunistic pathogens like Klebsiella pneumoniae and Pseudomonas aeruginosa. Both are Gram-negative bacteria with a global distribution that are commonly associated with environmental contamination and can colonize various animal species, including poultry and pet birds [5, 24]. Klebsiella pneumoniae is a known cause of respiratory disease, yolk sac infections, and septicemia in several avian species [25, 26, 27], while Pseudomonas aeruginosa is notorious for causing necrotizing lesions, septicemia, and respiratory granulomas [28, 29]. The increasing prevalence of antimicrobial resistance in these pathogens is a serious concern, as it limits treatment options and facilitates the transfer of resistance genes between different bacterial populations, creating a significant "One Health" challenge [5, 6, 7].

The complexity of bacterial infections is further compounded by the phenomenon of co-infection, where two or more pathogenic agents simultaneously infect a host [33, 40]. The synergistic interactions between these pathogens can lead to more severe disease outcomes, enhanced virulence, and increased resistance to host defenses and antibiotic treatments [41, 42]. In co-infections, the presence of one pathogen can disrupt the host's normal gut microbiota, facilitating colonization by a second pathogen [16, 17, 18, 19, 20]. Additionally, interspecies communication mechanisms, such as quorum sensing, can promote biofilm formation and enhance antibiotic tolerance, complicating therapeutic interventions [43, 44, 45]. To date, reports of dual infections by K. pneumoniae and P. aeruginosa in passerine birds are rare.

This study aims to describe the pathological findings and molecular characteristics of a fatal dual co-infection with K. pneumoniae and P. aeruginosa in a cohort of nestling European goldfinches. By providing a detailed account of this case, we hope to improve diagnostic awareness and contribute to a better understanding of the pathology and clinical significance of such co-infections in avian medicine.

Methods

Case Description and Sample Collection

Three deceased nestling European goldfinches (approximately 10 days old) were submitted to a veterinary pathology laboratory with a history of progressive lethargy, respiratory distress, and emaciation. The nestlings were part of a clutch found in a domestic setting. A full necropsy was performed immediately upon arrival to assess gross pathological changes [8, 21].

Necropsy and Histopathology

During post-mortem examination, a detailed record of all gross pathological findings was made. Tissue samples from major organs, including the liver, spleen, lungs, and kidneys, were collected, immediately fixed in 10% neutral-buffered formalin, and embedded in paraffin [8]. Sections were cut at 4 µm and stained with hematoxylin and eosin (H&E) for histopathological examination under a light microscope. Special stains were not used.

Bacteriological Isolation and Identification

Swabs were collected aseptically from the liver, lungs, and yolk sac of each nestling. The swabs were inoculated onto various bacteriological media, including blood agar, MacConkey agar, and specific selective media for Pseudomonas species. The plates were incubated aerobically at 37 ◦C for 24–48 hours. Morphologically distinct colonies were subcultured for pure isolation and subjected to standard biochemical tests for preliminary identification [15, 21].

Molecular Characterization

To confirm the bacterial species and rule out co-infection with common avian viruses, molecular analysis was performed. Bacterial DNA was extracted from the isolated pure cultures using a commercial DNA purification kit. Polymerase Chain Reaction (PCR) assays were conducted to amplify species-specific gene targets for both K. pneumoniae and P. aeruginosa [30, 31, 32]. For K. pneumoniae, the rpoB gene was targeted, and for P. aeruginosa, the ecfX gene was utilized [31, 32]. In addition, reverse transcription PCR (RT-PCR) and real-time PCR assays were performed on tissue homogenates to test for common avian viral pathogens, including Avian avulavirus 1, Avian Bornavirus, Avian Polyomavirus, and Circovirus, to exclude a viral etiology [34, 35, 36, 37].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on the isolated bacterial strains using the standard Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of antibiotics commonly used in veterinary medicine was tested to determine the resistance profile of each isolate [5, 22].

Results

Gross Pathological Findings

Necropsy revealed severe, multifocal to coalescing fibrinous inflammation of the air sacs, consistent with airsacculitis. The lungs were congested and edematous, with areas of consolidation. The liver appeared swollen, with multiple discrete white-to-yellow granulomatous foci visible on the surface and within the parenchyma. The spleen was moderately enlarged (splenomegaly), and the kidneys were pale with petechial hemorrhages. One of the nestlings also presented with an infected, unabsorbed yolk sac containing a caseous exudate.

Histopathological Findings

Microscopic examination of the lungs showed severe heterophilic pneumonia and marked airsacculitis, with infiltration of heterophils and macrophages. The alveolar septa were thickened, and numerous bacterial colonies were observed within the air spaces. The most striking finding in the liver was the presence of multiple, well-demarcated granulomas, characterized by a central core of necrotic debris and bacterial colonies, surrounded by a cuff of epithelioid macrophages and multinucleated giant cells. In some areas, these granulomas coalesced to form larger lesions. The spleen showed marked lymphoid depletion and diffuse histiocytic proliferation.

Bacteriological and Molecular Findings

Bacteriological cultures from the lungs, liver, and yolk sac consistently yielded both Klebsiella pneumoniae and Pseudomonas aeruginosa. The two species were identified based on colony morphology, biochemical tests, and confirmed by molecular methods. PCR assays targeting species-specific genes (rpoB and ecfX) confirmed the presence of both organisms in all tested samples [31, 32]. All tissue samples tested negative for Avian avulavirus, Avian Bornavirus, Polyomavirus, and Circovirus [34, 35, 36, 37].

Antimicrobial Susceptibility Profile

The isolated strains of K. pneumoniae and P. aeruginosa demonstrated a multidrug-resistant profile. Both strains were resistant to several broad-spectrum antibiotics, including ampicillin, tetracycline, and chloramphenicol, which are commonly used in avian medicine. The isolates showed some susceptibility to aminoglycosides (e.g., amikacin) and fluoroquinolones (e.g., enrofloxacin), although the resistance patterns varied slightly between the two bacterial species.

Discussion

This report documents a fatal case of co-infection by K. pneumoniae and P. aeruginosa in nestling European goldfinches, providing the first detailed pathological findings of this dual infection in this species. The severity of the lesions, including granulomatous hepatitis and fibrinous airsacculitis, highlights the significant synergistic pathogenicity of these two organisms [33, 40]. The co-infection likely exacerbated the pathological outcome, as has been observed in other animal models and in-vitro studies [41, 42]. The presence of both organisms in multiple organs, including the yolk sac, suggests a systemic, septicemic process [27, 28].

The probable route of transmission in this case was environmental or parental [46, 47]. Nestlings are particularly vulnerable to infections from their immediate environment, which can be a reservoir for opportunistic bacteria [48]. Contamination of eggs or nests with fecal material from parents or other birds could have led to the initial infection [12, 13, 14, 47]. The avian gut microbiota plays a crucial role in preventing colonization by pathogens, but stress, poor sanitation, and the underdeveloped immune system of nestlings can disrupt this balance, allowing opportunistic pathogens to thrive [17, 18, 19]. The presence of the unabsorbed yolk sac provided an ideal growth medium for the bacteria, likely serving as a source of systemic infection.

The histopathological findings were consistent with the known pathology of these agents. The granulomas in the liver are a hallmark of chronic inflammatory reactions to pathogens like Klebsiella and are a common finding in avian bacterial diseases [15, 27]. Conversely, the extensive necrotizing and purulent inflammation in the lungs and air sacs is characteristic of Pseudomonas infection [38, 39, 53]. This dual presentation suggests a complex pathological process where both bacteria contributed to the overall disease picture. The molecular identification of both species confirms their roles as co-infecting agents, and the negative results for viral pathogens emphasize the primary bacterial nature of this outbreak.

The finding of multidrug resistance in both bacterial isolates is particularly concerning, underscoring the broader issue of antimicrobial resistance in wildlife and captive birds [5, 6, 7, 24]. Pet and wild birds can act as sentinels and reservoirs for resistant bacteria, which can be transmitted to humans [3, 11]. The use of molecular

techniques for rapid and accurate diagnosis is essential for guiding effective treatment and implementing biosecurity measures to prevent the spread of such resistant strains [49, 50, 51, 52]. This case highlights the need for continued surveillance of bacterial pathogens in passerine birds and a proactive "One Health" approach to managing antimicrobial resistance [11].

Conclusion

In conclusion, this study documents the pathological and molecular findings of a fatal dual infection by Klebsiella pneumoniae and Pseudomonas aeruginosa in European goldfinch nestlings. The severity of the pathological lesions and the multidrug-resistant nature of the isolates underscore the significant threat posed by these opportunistic pathogens, especially in co-infection scenarios. This report serves as a reminder of the importance of detailed diagnostic investigations in avian pathology and the need for a comprehensive understanding of co-infection dynamics to improve clinical outcomes in avian patients and protect public health.

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