ULST Timisoara
Multidisciplinary Conference on Sustainable Development
15-16 May 2025

Isolation and identification of bacterial species in white stork (*Ciconia ciconia*) in Romania

Andrei Alexandru IVAN[1], Iulia BUCUR[1,*], Alex Cristian MOZA[1,*], Miruna PRIPOREANU-GRITU[2], Ileana NICHITA[1], Dorin Alexandru VIZITU[1], Radu-Valentin GROS[1] and Emil TÎRZIU[1]

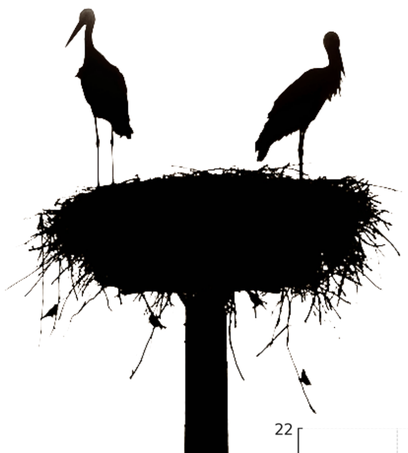
1 University of Life Sciences "King Mihai I" from Timișoara, Faculty of Veterinary Medicine, 300645, Calea Aradului, no. 119, Timișoara, Romania
2 "Lucian Blaga" University of Sibiu, Faculty of Sciences, 550012, Str. Dr. I. Ratiu, No. 5-7, Sibiu, Romania
* Correspondence: I.B: iulia.bucur@usvt.ro; A.C.M.: alex.moza@usvt.ro

Abstract: *The white stork (Ciconia ciconia) is a protected species within the European community. In Romania, associations and rehabilitation centers play a vital role in conserving the species and aiding the recovery of injured or orphaned individuals. This study aims to contribute to a better understanding of the bacterial microbiota associated with this species, particularly in the context of captivity and physiological stress. To achieve this, 44 biological samples were collected from 22 white storks under the care of a wildlife protection association in Sibiu County, and sub-sequently analyzed. The birds originated from various regions of the country and were rescued following incidents such as nest falls, electrocution, abandonment, or poaching. The primary objective of this study was the isolation and identification of pure bacterial strains from the collected samples. Identification was performed using VITEK®2 Compact, resulting in a number of 52 isolated bacterial strains, including seven different species. The findings offer relevant data for understanding the commensal and potentially pathogenic bacterial microbiota of these birds, contributing to the improvement of hygiene, prophylaxis, and treatment measures in wildlife rehabilitation centers. Additionally, due to the mobility and feeding behavior of the white stork, these birds may play a key role in spreading bacterial species with zoonotic potential into the environment, acting as a vector and source of infection for humans. Consequently, this study opens new research opportunities regarding the risks associated with pathogen transmission and the phenomenon of antibiotic resistance observed in this bird species.*

Introduction

In Romania, injured or orphaned juvenile white storks are frequently rescued and admitted to wildlife rehabilitation centers (WRCs), particularly during the vulnerable post-fledging period. These circumstances make WRCs an ideal setting for examining host-microbe interactions in captive white storks undergoing physiological stress. As a result, this species serves as a valuable model for studying both commensal and potentially pathogenic bacteria.

Our study aimed to assess the diversity of culturable bacterial strains in white storks admitted to a Romanian wildlife protection center. More specifically, we sought to explore how captivity may influence the presence of bacteria with zoonotic potential, and to evaluate the broader role of WRCs as sentinel sites for wildlife pathogen surveillance.



Materials and methods

The samples analyzed in this study were collected from a single wildlife rehabilitation center located in Sibiu County, Romania. The white storks (*Ciconia ciconia*) included in the study were randomly selected from among individuals admitted to the center, which receives birds originating from multiple counties across the country. These individuals were rescued under various circumstances, including nest falls, electrocution, abandonment, and poaching.

A total of 44 samples were collected from 22 white storks. From each bird, two samples were obtained: one oral swab and one cloacal swab. Upon arrival at the laboratory, the samples were introduced into nutrient meat broth and incubated at 37 °C for 24 hours to support bacterial growth. Following incubation, each sample was inoculated onto selective and differential media appropriate to the swab collection site (Figure 1-5). Distinct colonies were selected based on the color observed on the specific media and their morphological characteristics, and were repeatedly subcultured to obtain pure bacterial isolates. The resulting bacterial colonies were examined both macroscopically, by assessing colony morphology directly on the agar surface, and microscopically, through the preparation of Gram-stained smears.

Figure 1. Presumptive *E. coli* colonies grown on Levine Agar

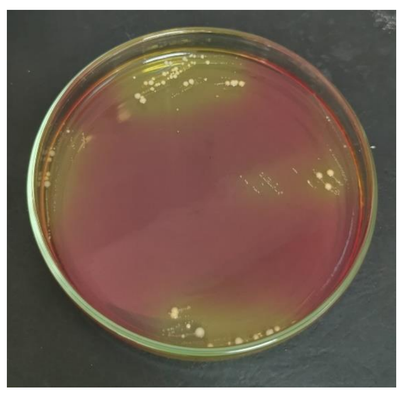


Figure 2. Mannitol-positive *Staphylococcus* spp. growing on Mannitol Salt Agar

Figure 3. Presumptive *Salmonella* spp. colonies grown on Salmonella Brilliance Agar

Figure 4. Presumptive *E. coli* colonies grown on TBX Agar

Figure 5. Colonies of *Pseudomonas* spp. on Nutrient Agar

Following the isolation of pure bacterial colonies, species-level identification of the isolates was conducted using the VITEK automated system. A total of 52 bacterial strains were successfully isolated and identified through this method, representing seven distinct species. The data obtained through VITEK analysis served as the foundation for interpreting the composition and diversity of the culturable microbiota associated with the sampled white storks.

Results and discussions

In total, 52 bacterial strains were isolated and identified using 44 samples collected during the examination of 22 white storks (*Ciconia ciconia*), including 22 oral and 22 cloacal swabs. The isolates comprised seven distinct bacterial species, including both commensal and potentially pathogenic bacteria.

From the oral swabs (Figure 6), the predominant species identified was *Staphylococcus sciuri*, with 18 isolates (81.8% of the oral samples), followed by *Staphylococcus simulans* with 4 isolates (18.2%), and *Pseudomonas aeruginosa*, also with 4 isolates (18.2%). These bacterial species are commonly associated with skin, mucosal, or environmental colonization but can act as opportunistic pathogens, particularly in immunocompromised individuals.

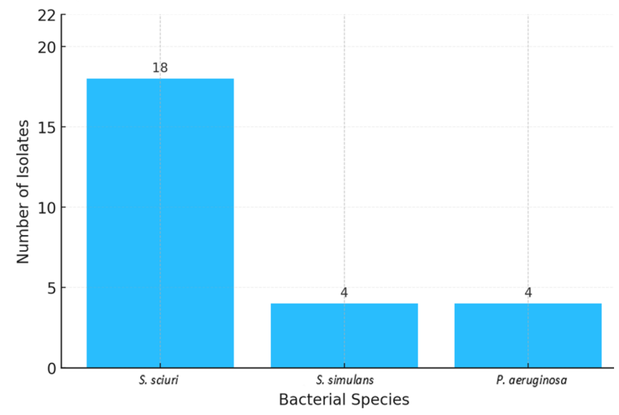


Figure 6. Bacterial isolates from oral swabs

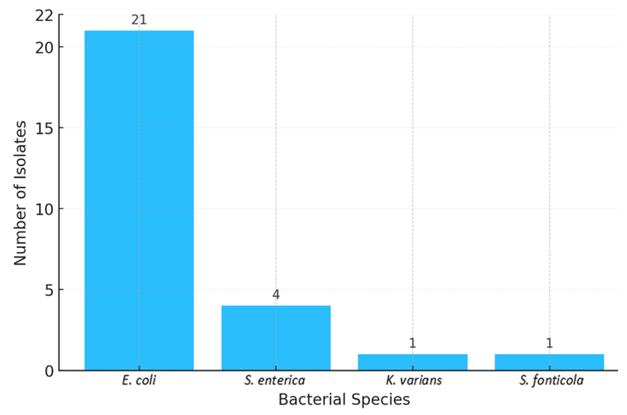


Figure 7. Bacterial isolates from cloacal swabs

Analysis of the cloacal swabs (Figure 7) revealed *Escherichia coli* in 21 out of 22 samples (95.5%), indicating its consistent presence within the intestinal microbiota. *Salmonella enterica* was identified in 4 cloacal samples (18.2%), while *Kocuria varians* and *Serratia fonticola* were each isolated from one sample (4.5%). Notably, *S. enterica* and *E. coli* include strains with zoonotic potential, capable of causing gastrointestinal infections.

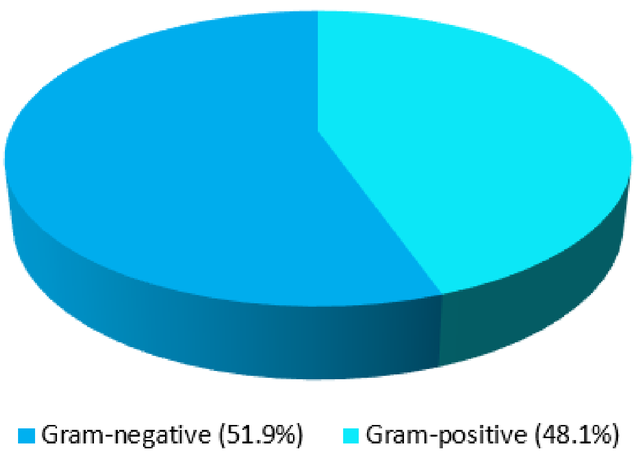


Figure 8. Distribution of bacterial isolates based on Gram staining characteristics

Among the 52 bacterial isolates, classification based on Gram staining revealed a predominance of Gram-negative bacteria (Figure 8). Specifically, 27 isolates (51.9%) were identified as Gram-negative species, including *E. coli*, *S. enterica*, *S. fonticola*, and *P. aeruginosa*. In contrast, 25 isolates (48.1%) were Gram-positive bacteria, represented by *S. sciuri*, *S. simulans*, and *K. varians*.

The high prevalence of *E. coli* and the detection of *Salmonella* and *P. aeruginosa* highlight the potential public health risks posed by these birds, especially considering their mobility, scavenging behavior, and frequent contact with human-modified environments, such as landfills or urban nesting areas. White storks may act as biological vectors, contributing to the environmental dissemination of pathogenic or antimicrobial-resistant bacteria across.

Conclusions

Overall, our findings confirm the important ecological role of the white stork as a vector and reservoir of potentially pathogenic and antimicrobial-resistant bacteria.

Given their ecological habits and migratory patterns, these birds represent a significant public health concern, acting as biological carriers capable of transferring antimicrobial resistance and pathogenic bacteria across continents. Surveillance of bacterial pathogens in this species is, therefore, essential not only for avian conservation efforts but also for the development of One Health strategies aimed at monitoring and mitigating the spread of infectious diseases at the wildlife-human-environment interface.