Bacteriological Diagnosis in Wild Bird Pathology: A Practical Guide from Sample Collection to Antibiogram Interpretation

Diagnosticul bacteriologic în patologia păsărilor sălbatice: ghid practic de la recoltarea probelor la interpretarea antibiogramei

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Abstract

Wild birds are increasingly relevant in veterinary practice, wildlife rehabilitation and infectious disease surveillance, particularly in relation to bacterial pathogens and antimicrobial resistance (AMR). However, bacteriological diagnosis in these species is often difficult to interpret, because bacterial isolation may reflect true infection, opportunistic colonisation, normal microbiota, environmental contamination or asymptomatic carriage. This article was designed as a narrative review and practical guide for veterinarians, pathologists, microbiologists and personnel involved in the clinical care, rehabilitation or post-mortem examination of wild birds. The review summarises the main contexts in which bacteriological investigation is indicated, the principles of sample collection from live and dead birds, the role of bacterial culture and identification, and the interpretation of antimicrobial susceptibility testing (AST). Particular attention is given to the diagnostic relevance of sample origin, lesion compatibility, purity of bacterial growth and the distinction between clinical infection and epidemiological carriage. The article also discusses AMR in wild birds and its implications for treatment decisions, biosecurity and environmental surveillance. Overall, reliable bacteriological diagnosis in wild bird pathology requires appropriate sampling, correct handling and transport, complete clinical information and cautious interpretation of culture and antibiogram results. AST should guide therapy only when the isolate is clinically relevant and should be integrated with responsible antimicrobial use.

Rezumat

Păsările sălbatice prezintă o importanță tot mai mare în practica veterinară, reabilitarea faunei sălbatice și supravegherea bolilor infecțioase, în special în contextul agenților bacterieni și al rezistenței antimicrobiene. Totuși, diagnosticul bacteriologic la aceste specii este adesea dificil de interpretat, deoarece izolarea unei bacterii poate reflecta infecție reală, colonizare oportunistă, microbiotă normală, contaminare de mediu sau portaj asimptomatic. Acest articol este conceput ca un review narativ și ghid practic pentru medicii veterinari, patologii, microbiologii și personalul implicat în îngrijirea clinică, reabilitarea sau examinarea post-mortem a păsărilor sălbatice. Lucrarea sintetizează principalele situații în care este indicată investigația bacteriologică, principiile recoltării probelor de la păsări vii și moarte, rolul culturii și identificării bacteriene, precum și interpretarea testării sensibilității antimicrobiene. Se acordă atenție relevanței originii probei, compatibilității cu leziunile, purității culturii bacteriene și diferențierii dintre infecția clinică și portajul epidemiologic. De asemenea, sunt discutate rezistența antimicrobiană la păsările sălbatice și implicațiile acesteia pentru tratament, biosecuritate și supravegherea mediului. În concluzie, diagnosticul bacteriologic fiabil necesită recoltare adecvată, manipulare și transport corecte, informații clinice complete și interpretarea prudentă a culturii și antibiogramei.

1. Introduction

Wild birds are increasingly recognised as relevant hosts in veterinary pathology, wildlife rehabilitation and infectious disease surveillance.

Because they occupy diverse habitats and often interact with urban, agricultural, aquatic and rehabilitation environments, they may carry bacterial agents of clinical, zoonotic and ecological importance.

In this context, bacteriological diagnosis is useful not only for individual clinical cases, but also for mortality investigations and monitoring of antimicrobial resistance (AMR) in wildlife populations (1-4).

The interpretation of bacteriological findings in wild birds is often challenging.

A positive culture may reflect true infection, opportunistic overgrowth, asymptomatic carriage, normal microbiota or environmental contamination, depending on the sample type, clinical context and laboratory method used.

This is particularly important for samples such as cloacal swabs and faeces, which are useful for surveillance but may not have the same diagnostic value as deep lesion samples, organ samples or intestinal mucosa collected during necropsy (5–7).

Enterobacteriaceae, especially *Escherichia coli* and *Salmonella enterica*, are among the most frequently investigated bacteria in wild birds, together with other opportunistic genera such as *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp. and *Hafnia* spp.

Several studies have also reported *Campylobacter* spp., particularly in birds of prey and water-associated species.

These bacteria may act as pathogens, commensals or indicators of environmental exposure, which makes interpretation dependent on both microbiological and clinical evidence (3–5).

AMR adds another layer of complexity. Wild birds may acquire resistant bacteria from wastewater, agricultural areas, landfills, livestock-associated environments or other anthropogenic sources, and may contribute to the environmental circulation of resistance determinants.

However, antimicrobial susceptibility testing (AST) should be applied and interpreted carefully, because only clinically relevant isolates, not contaminants or normal flora, should guide therapeutic decisions (2,4,8,9).

Accurate bacteriological diagnosis therefore depends on a coherent workflow, beginning with appropriate case selection and sample collection and ending with clinically responsible interpretation of culture and antibiogram results.

The aim of this narrative review and practical guide is to provide a concise framework for veterinarians, pathologists, microbiologists and wildlife rehabilitation personnel involved in bacteriological diagnosis in wild bird pathology.

1.1. Literature Search Strategy

This article was designed as a narrative review and practical guide. The literature was selected based on its relevance to bacteriological diagnosis, sample collection, bacterial pathogens, AST and AMR in wild birds.

Scientific articles, practical guidelines and veterinary manuals were considered, with priority given to studies involving wild birds, wildlife rehabilitation centres, bacteriological culture, post-mortem sampling and AMR surveillance.

The review does not aim to provide a systematic or meta-analytical assessment, but rather to integrate available evidence into a practical framework for clinicians, pathologists, microbiologists and wildlife rehabilitation personnel.

Because the objective was educational and practice-oriented, no formal PRISMA screening, risk-of-bias assessment or meta-analysis was performed.

Relevant publications were identified through searches in PubMed, Web of Science, Google Scholar and reference lists of selected papers, using combinations of terms such as "*wild birds*", "*bacteriological diagnosis*", "*sample collection*", "*Enterobacteriaceae*", "*antimicrobial susceptibility testing*", "*antibiogram*" and "*AMR*".

2. Bacterial Agents in Wild Birds: Clinical and Epidemiological Context

Bacterial infections in wild birds may be encountered in several clinical contexts, including traumatic wounds, pododermatitis, respiratory disease, enteritis, septicaemia, post-surgical complications, rehabilitation-associated infections and mortality investigations.

In many cases, however, bacterial isolation does not necessarily indicate primary disease, as wild birds may also carry commensal, opportunistic or environmentally acquired bacteria without overt clinical signs.

Therefore, the diagnostic value of a bacterial isolate depends strongly on the clinical presentation, lesion type, sample origin and quality of sampling.

Wild birds are also relevant from an epidemiological perspective because they occupy diverse ecological niches and may interact with environments influenced by human activity, such as agricultural areas, wastewater systems, landfills, urban habitats and wildlife rehabilitation centres.

These interfaces may facilitate exposure to enteric bacteria and antimicrobial-resistant strains of human, livestock or environmental origin.

Migratory and synanthrophic species, such as gulls, pigeons, corvids and starlings, are particularly important in this context because they may contribute to the environmental circulation of bacteria and resistance determinants (2-4).

Among the bacterial groups most frequently reported in wild birds, *Enterobacteriaceae* are of particular importance. *Escherichia coli* and *Salmonella enterica* are the most commonly investigated species, while other genera such as *Klebsiella*, *Enterobacter*, *Proteus*, *Citrobacter* and *Hafnia* are also reported in cloacal, faecal and post-mortem samples.

These bacteria may act as commensals, opportunistic pathogens, zoonotic agents or indicators of environmental contamination and AMR, depending on the host species and sampling context (3-5).

Other enteropathogenic bacteria, particularly *Campylobacter spp.*, have also been detected in wild birds, including birds of prey.

In a study on raptor carcasses from southern Italy, *Campylobacter spp.* were more frequently detected than *E. coli* or *Salmonella spp.*, suggesting that the bacterial spectrum identified in wild birds may vary according to host group, diagnostic target and sampling method (5).

This reinforces the need to select bacteriological investigations according to the suspected disease process rather than applying a uniform approach to all cases.

Bacterial findings in wild birds should be interpreted as part of a broader diagnostic framework.

The same bacterial species may have different significance when isolated from faeces, a cloacal swab, a superficial wound, a deep lesion, blood, intestinal mucosa or an internal organ collected during necropsy.

For this reason, bacteriological diagnosis in wild bird pathology requires integration of clinical signs, gross lesions, sample type, culture result and antimicrobial susceptibility data, rather than relying on bacterial isolation alone.

3. When bacteriological diagnosis is indicated ?

Bacteriological diagnosis is indicated when bacterial infection is clinically suspected, when antimicrobial treatment is being considered, or when the result may influence case management, biosecurity or rehabilitation decisions.

In wild birds, this is particularly relevant in traumatic wounds, deep soft-tissue infections, pododermatitis, abscesses, osteomyelitis, respiratory disease, enteritis, septicaemia and unexplained mortality.

In rescued birds of prey, trauma and wound contamination are frequent clinical contexts, and early assessment is important because successful rehabilitation depends on complete recovery and return to normal function (10,11).

Sampling should ideally be performed before antimicrobial therapy is started, especially in severe, recurrent, systemic or poorly responsive infections.

In these cases, bacterial culture and AST may help distinguish empirical treatment failure from inappropriate antimicrobial choice, resistant bacteria, poor tissue penetration or non-bacterial disease.

However, bacteriology should not be requested indiscriminately, because samples collected from inappropriate sites, poorly handled specimens, or cultures performed on contaminants or normal flora may produce misleading results (8,12,13).

Bacteriological investigation is also useful in post-mortem examinations, especially when gross lesions suggest septicaemia, enteritis, pneumonia, air sacculitis, hepatitis, peritonitis, osteomyelitis or focal suppurative lesions. In mortality investigations, post-mortem sampling should be integrated into a complete necropsy protocol, because infectious bacterial disease must often be differentiated from trauma, intoxication, viral infection, parasitic disease or other causes of death (5,12).

Internal organs, intestinal mucosa and deep lesions generally provide more meaningful diagnostic information than superficial swabs, provided that samples are collected aseptically, promptly and in relation to the observed lesions (5,12).

In wildlife rehabilitation centres, bacteriological testing may also be indicated when several birds develop similar clinical signs, when hospital-associated infections are suspected, or when zoonotic bacteria such as *Salmonella enterica* are detected. Birds admitted to rehabilitation centres may carry enteric bacteria and antimicrobial-resistant strains, creating potential risks for other birds, personnel and the rehabilitation environment (3, 5). In such situations, bacteriology supports not only individual treatment but also hygiene, quarantine and biosecurity decisions.

Bacteriological diagnosis may also be requested for epidemiological surveillance, particularly in studies focused on AMR or zoonotic bacteria in wild bird populations.

Cloacal swabs and faecal samples are commonly used for this purpose because they are practical and minimally invasive, but their results should be interpreted mainly as evidence of bacterial carriage or environmental exposure rather than as proof of clinical infection.

This distinction is important because non-invasive samples may differ from intestinal or tissue samples, and prevalence estimates are strongly influenced by sampling strategy, host species, laboratory methods and study design.

Therefore, the indication for bacteriological testing should always be clearly defined before sampling, whether the objective is diagnosis of disease, treatment guidance, mortality investigation, outbreak management or surveillance (2-4, 6, 7).

4. Sample Collection in Wild Birds: Live-Bird and Post-Mortem Approaches

4.1. General principles

Sample collection is a critical step in bacteriological diagnosis, because the reliability of culture and antimicrobial susceptibility results depends strongly on the quality, origin and handling of the specimen.

Sampling should be guided by a clear diagnostic question: confirmation of bacterial infection, treatment guidance, mortality investigation, outbreak management or surveillance.

Whenever possible, samples should be collected before antimicrobial therapy is started and from sites that are most likely to reflect the disease process, rather than from easily accessible but poorly informative surfaces (8,12,13).

In live wild birds, sampling must also consider animal welfare, handling stress and personnel safety.

Birds should be restrained for the shortest time possible, using appropriate personal protective equipment and trained personnel, especially when birds of prey, sick birds or potentially zoonotic infections are involved.

The choice of sample must balance diagnostic value with invasiveness, particularly in debilitated, endangered or rehabilitation cases (11,12,14). The choice of sample should be guided by the clinical context and by the diagnostic question.

Table 1 summarises the main types of samples that may be considered in common clinical, post-mortem and surveillance situations in wild bird bacteriology. These recommendations should be adapted to the bird's condition, the suspected disease process and the facilities available for safe handling and transport.

Table 1.
Recommended bacteriological samples according to clinical context in wild birds.

Clinical context	Preferred sample	Alternative sample	Observations	References
Traumatic wounds and deep soft-tissue lesions	Aspirate, deep tissue fragment, purulent material, lesion-associated sample	Swab collected after gentle surface cleaning	Deep samples are preferable; superficial swabs may reflect contamination or colonising flora.	(8, 13)
Pododermatitis	Deep lesion sample, purulent material, tissue from the active margin of the lesion	Swab after surface cleaning, if tissue sampling is not possible	Interpret together with lesion severity and chronicity; mixed flora is common.	(8, 13)
Abscesses	Aspirated pus, abscess wall tissue, deep lesion sample	Swab from freshly opened abscess cavity	More useful for AST when a predominant bacterial isolate is recovered.	(8, 13)
Osteomyelitis or septic arthritis	Bone sample, joint aspirate, synovial fluid, lesion-associated tissue	Deep swab from affected area, if tissue or fluid cannot be obtained	Usually clinically relevant when compatible lesions are present; good candidate for AST.	(8, 13)
Respiratory disease	Choanal, oropharyngeal or tracheal swab, depending on lesion location	Lung or air sac sample at necropsy	Routine aerobic culture may be insufficient; the laboratory should be informed if specific pathogens are suspected.	(5, 12)
Enteritis or diarrhoea	Fresh faeces, cloacal swab, intestinal content	Intestinal mucosa at necropsy	Enteric samples may indicate carriage, shedding or dysbiosys; correlate with clinical signs and lesions.	(3, 5, 7)
Septicaemia or systemic infection	Internal organs at necropsy, especially liver, spleen, heart blood and lung	Blood culture in live birds, if clinically feasible	Pure or predominant growth from internal organs is more meaningful than superficial isolation.	(8, 12)
Unexplained mortality	Internal organs, intestinal mucosa and visible lesions collected during necropsy	Cloacal swab if necropsy samples are unavailable	Bacteriology should be integrated with necropsy findings, not interpreted alone.	(5, 12)
Zoonotic suspicion, especially *Salmonella* spp. or *Campylobacter* spp.	Cloacal swab, fresh faeces, intestinal content or intestinal mucosa	Environmental swabs from housing or rehabilitation areas, if outbreak investigation is needed	Positive results may have biosecurity relevance even without clear clinical disease.	(3–5)
AMR surveillance	Cloacal swab or fresh faecal sample	Environmental samples or post-mortem intestinal samples	Results usually reflect carriage or environmental exposure, not necessarily active infection.	(2, 4, 6)
Rehabilitation-centre outbreak or repeated similar cases	Samples from clinically affected birds, lesion-associated samples, faeces/cloacal swabs, and environmental samples when appropriate	Post-mortem samples from dead birds	Interpretation should support both individual treatment and hygiene, quarantine and biosecurity decisions.	(3–5)
Culture after treatment failure	Sample from the active lesion or suspected infection site before changing therapy	Repeat sample from a better anatomical site, if the first sample was superficial or mixed	Helps distinguish AMR from poor sample choice, poor drug penetration or non-bacterial disease.	(8, 13)

4.2. Live-bird sampling

In live wild birds, cloacal swabs and fresh faecal samples are commonly used for bacteriological screening, especially in studies focused on enteric bacteria and AMR.

These samples are practical, minimally invasive and suitable for surveillance, but they mainly reflect carriage, shedding or environmental exposure rather than confirmed clinical disease.

Their diagnostic value increases when they are interpreted together with clinical signs, history, species, sampling context and laboratory method (2–4, 7).

Fresh faecal sampling is particularly useful when direct handling should be reduced or repeated sampling is needed.

Non-invasive collection methods can minimise stress and reduce contamination if the sample is collected rapidly and without prolonged contact with soil, bedding or cage surfaces.

Cloacal swabs, by contrast, require handling but may provide a more standardised sample from the distal intestinal tract.

However, neither faecal samples nor cloacal swabs should be overinterpreted as evidence of systemic infection in the absence of compatible clinical or pathological findings (7,15,16).

Oropharyngeal, choanal or tracheal swabs may be indicated when respiratory disease, oral lesions, sinusitis or upper airway infection is suspected. The size of the swab must be adapted to the bird, and sampling should avoid unnecessary trauma.

Although many field manuals describe these techniques in the context of avian influenza surveillance, the same principles of atraumatic sampling, correct swab selection, labelling and rapid transport are relevant to bacteriological investigations (12).

For wounds, abscesses, pododermatitis, osteomyelitis or deep soft-tissue infections, superficial swabs are often less informative because they may recover contaminants or colonising flora.

When possible, samples should be obtained from deep tissue, aspirated material, purulent exudate or the active margin of the lesion after gentle surface cleaning.

These samples are more likely to identify clinically relevant bacteria and are more appropriate for AST than mixed superficial flora (8,13).

Blood sampling for bacteriology is not routine in wild birds, but it may be considered when septicaemia is suspected and when the bird's condition allows safe collection.

Blood collection must respect species size, clinical status and maximum safe volume.

Field sampling guidance generally recommends using the smallest volume necessary for the intended test and avoiding maximal blood collection, especially in small or debilitated birds (12,17).

4.3. Necropsy sampling

Post-mortem bacteriological sampling is indicated when bacterial disease is suspected at necropsy, particularly in cases of septicaemia, enteritis, pneumonia, airsacculitis, hepatitis, peritonitis, osteomyelitis, infected traumatic lesions or unexplained mortality.

Necropsy sampling should be performed as soon as possible after death, before extensive autolysis or environmental contamination occurs, and should be integrated into a complete pathological examination rather than performed as an isolated laboratory request (5, 12).

Internal organs and deep lesions generally have greater diagnostic value than superficial post-mortem swabs, especially when collected aseptically and in direct relation to gross lesions.

Depending on the case, useful samples may include liver, spleen, heart blood, lung, air sacs, intestine or intestinal mucosa, yolk sac in nestlings, joints, bone, brain or focal suppurative lesions.

When enteric pathogens are suspected, intestinal content or mucosal swabs may be appropriate; when systemic infection is suspected, multiple internal organs should be sampled separately (5,12).

The interpretation of post-mortem culture must consider the time since death, storage conditions, autolysis, carcass integrity, and necropsy findings.

Bacteria recovered from decomposed carcasses or exposed surfaces may reflect post-mortem invasion or contamination rather than ante-mortem disease. Conversely, the isolation of a plausible pathogen in pure or predominant growth from internal organs or lesions, especially when supported by compatible gross or histological lesions, is more suggestive of clinical significance (5,8,12).

The main sampling options in live and necropsied wild birds are presented in Figure 1.

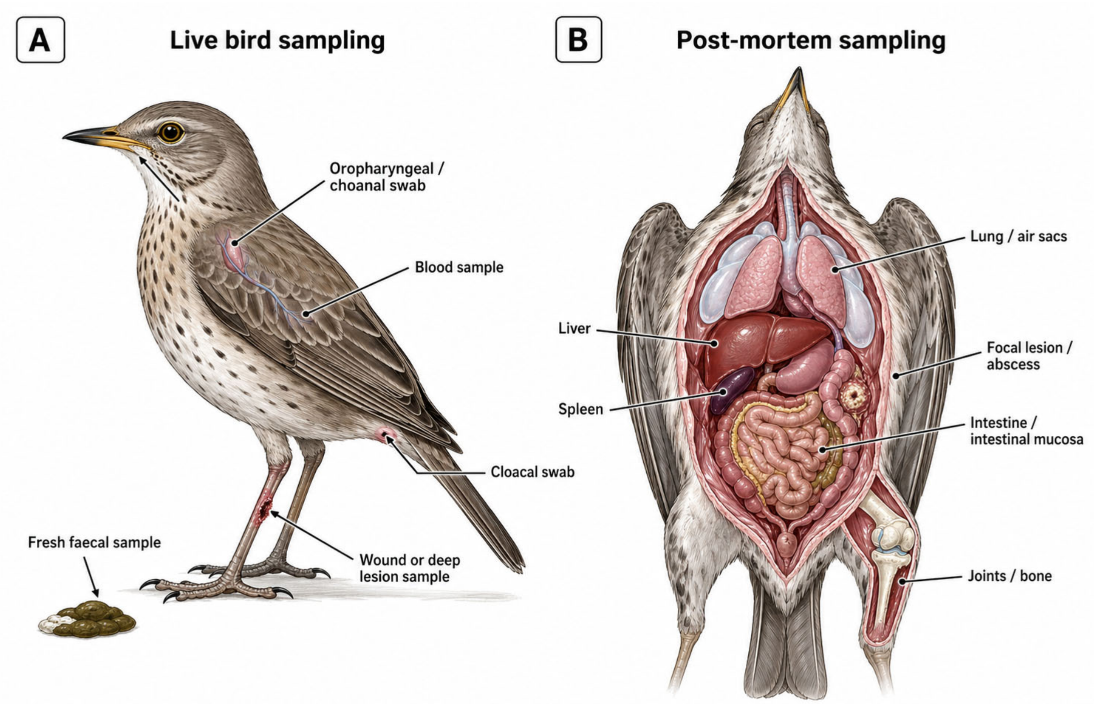

Figure 1. Main sample types used for bacteriological diagnosis in live and necropsied wild birds (Processed with ChatGPT 5.5.)

4.4. Sample handling and transport

Correct handling, labelling and transport are essential for maintaining sample integrity.

Each specimen should be placed in an appropriate sterile container or transport medium, labelled with a unique identification code and accompanied by relevant metadata, including species, age or life stage if known, sampling site, date, clinical signs, gross lesions, previous antimicrobial exposure and diagnostic question.

Poor labelling or incomplete clinical information can seriously limit interpretation, even when laboratory procedures are technically correct (12,13,17).

Most bacteriological samples should be transported to the laboratory as rapidly as possible, generally under refrigerated conditions unless specific laboratory instructions indicate otherwise.

Swabs should not be allowed to dry, and tissues should be submitted in sterile containers without formalin when culture is required. If histopathology is also needed, separate tissue samples should be fixed in formalin.

Clear separation of samples for microbiology and pathology avoids loss of diagnostic material and improves the value of combined interpretation (12,13).

Overall, sample collection in wild birds should be selective, purposeful and adapted to the clinical or epidemiological objective.

The most useful bacteriological result is obtained when an appropriate sample is collected aseptically, transported correctly and interpreted in relation to the bird, the lesion and the diagnostic question. A simplified decision tree for sample selection and handling is shown in Figure 2.

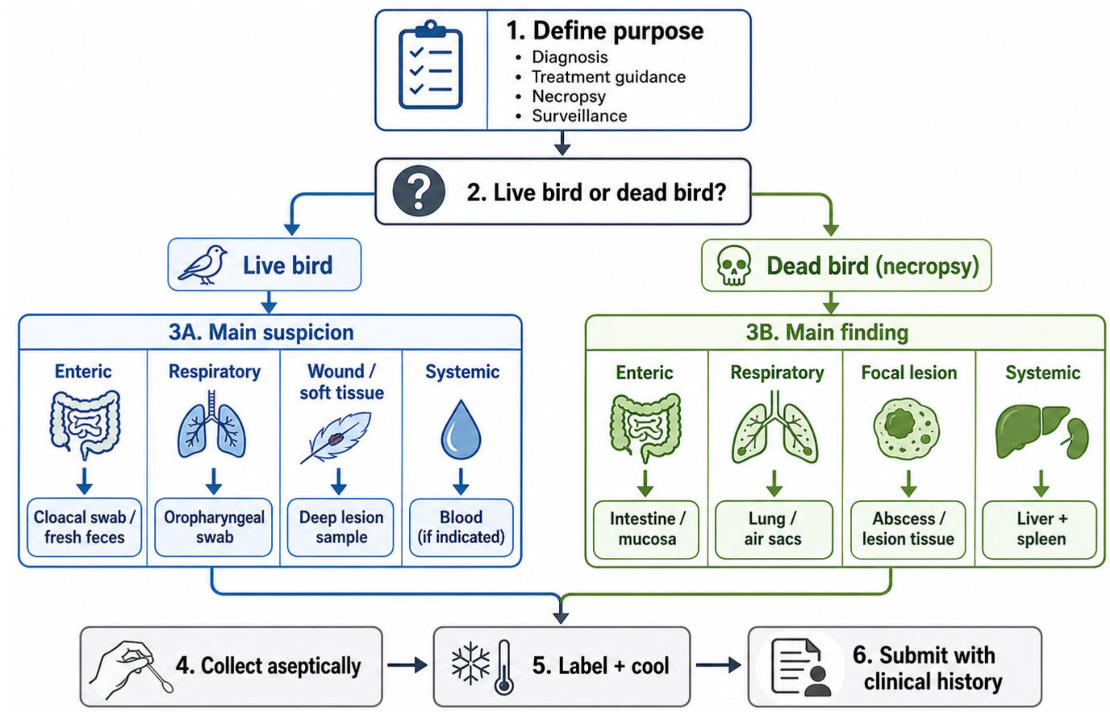

Figura 2. Decision tree for sample selection and handling (Processed with ChatGPT 5.5.)

5. Bacterial Culture and Identification

Bacterial culture remains a central diagnostic tool in wild bird pathology because it allows the isolation of viable bacteria and, when appropriate, subsequent AST.

However, culture results are meaningful only when interpreted in relation to the sample type, clinical history and lesions observed; a cloacal swab, faecal sample, superficial wound swab and internal organ sample do not have the same diagnostic value (3,5,8,12).

The laboratory approach should be adapted to the suspected condition and the submitted sample.

Enteric investigations may involve faeces, cloacal swabs, intestinal content or intestinal mucosa, while respiratory disease requires samples from the upper or lower respiratory tract, and deep infections are better investigated using aspirates, tissue fragments or lesion-associated samples rather than superficial swabs.

During necropsy, liver, spleen, lung, intestine, heart blood and focal lesions may be sampled separately when systemic or localised bacterial infection is suspected (3,5,12).

Routine bacteriology generally involves inoculation onto general and selective culture media, followed by incubation under conditions suitable for the suspected bacteria.

In wild bird studies, MacConkey agar has been used for *Enterobacteriaceae*, selective enrichment and plating for *Salmonella spp.*, and specific enrichment with microaerophilic incubation for *Campylobacter spp.*; therefore, the laboratory should be informed when particular pathogens are suspected, because routine aerobic culture alone may be insufficient (3,5).

Bacterial identification may rely on colony morphology, Gram staining, biochemical testing, commercial identification systems, serotyping or PCR, depending on the laboratory capacity and diagnostic aim.

In published studies on wild birds, *Enterobacteriaceae* were commonly identified by standard culture and biochemical methods, *Salmonella* isolates were further serotyped, and *Campylobacter* identification was supported by PCR-based methods.

More advanced approaches, such as MALDI-TOF MS or whole-genome sequencing, may improve epidemiological interpretation, especially in AMR surveillance, but are not always available in routine diagnosis (3–5).

Mixed cultures are frequent in cloacal, faecal and contaminated wound samples and may reflect normal flora, environmental contamination or polymicrobial infection.

For this reason, the laboratory report should be interpreted together with the sample origin, lesion type and diagnostic question, and AST should be reserved for isolates considered clinically relevant rather than contaminants or normal microbiota (6–8).

Culture-negative results should also be interpreted cautiously.

They do not always exclude bacterial infection, especially when sampling is delayed, the submitted specimen is inappropriate, transport conditions are suboptimal, fastidious organisms are involved or antimicrobial exposure has occurred.

Conversely, bacterial growth from a low-quality or contaminated specimen should not be overinterpreted as proof of disease (8,12,13).

Bacterial culture and identification are most useful when the diagnostic objective is clearly defined, the sample is appropriately selected and handled, and the result is interpreted in clinical or pathological context (Figure 3).

In wild bird pathology, the key question is not only which bacterium was isolated, but whether it is likely to be relevant for treatment, rehabilitation, biosecurity or surveillance (2–4, 8).

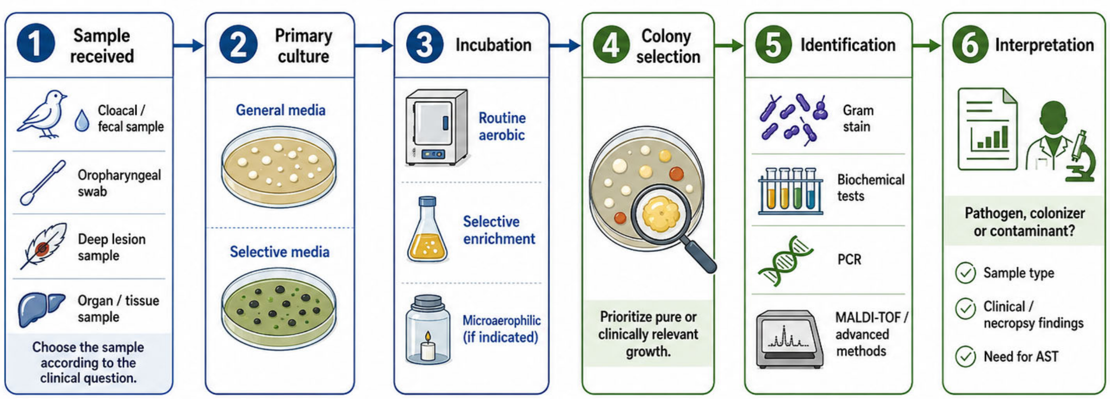

Figure 3. Simplified workflow for bacterial culture and identification in wild bird samples (Processed with ChatGPT 5.5.)

6. Interpretation of Bacteriological Results

The interpretation of bacteriological results in wild birds should begin with the diagnostic question for which the sample was collected.

A positive culture is not automatically equivalent to bacterial disease, particularly when the isolate comes from faeces, cloacal swabs, superficial wounds or samples exposed to environmental contamination. The same organism may represent normal carriage, opportunistic colonisation, contamination or true infection, depending on the sample origin, host condition, clinical signs and associated lesions (3,5,8)

Sample origin is one of the most important determinants of diagnostic value.

Enteric bacteria isolated from cloacal swabs or faecal samples are useful for surveillance and AMR monitoring, but they usually indicate carriage or shedding rather than systemic infection. In contrast, isolation of a plausible pathogen from a deep lesion, abscess, joint, bone or internal organ collected aseptically during necropsy is more likely to be clinically meaningful, especially when it corresponds with gross or histological lesions (5,7,12).

The type of bacterial growth should also be considered.

Pure or predominant growth from a normally sterile site, such as liver, spleen, heart blood, joint, bone or deep tissue, is more suggestive of clinical relevance than mixed growth from a superficial or contaminated sample.

Mixed cultures from cloacal, faecal or superficial wound samples are common and may reflect intestinal flora, environmental bacteria or sampling contamination rather than a primary infectious process.

Therefore, mixed growth should be interpreted cautiously and, when necessary, repeat sampling from a more appropriate site should be considered (6,8,13).

Some bacteria require particularly careful interpretation because they may act as either commensals or pathogens.

Escherichia coli, for example, is frequently isolated from wild birds and may represent normal intestinal carriage, opportunistic infection, septic disease or an indicator organism for AMR.

Similarly, *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Hafnia* may be clinically relevant in some contexts but may also reflect environmental or intestinal flora.

Their significance depends on the site of isolation, lesion compatibility, purity of growth and the clinical status of the bird (2–4).

By contrast, detection of recognised zoonotic or enteropathogenic bacteria, such as *Salmonella enterica* or *Campylobacter jejuni*, has broader clinical and epidemiological relevance.

These agents may be associated with disease, asymptomatic carriage or environmental dissemination, and their detection may have implications for biosecurity in rehabilitation centres, personnel safety and surveillance programs. In birds of prey, Gargiulo et al., 2018 reported *Campylobacter spp.*, *E. coli* and *Salmonella spp.* in carcasses examined in southern Italy, illustrating the need to interpret pathogen detection within both pathological and epidemiological contexts.

Antimicrobial susceptibility results should only be interpreted after the clinical relevance of the isolate has been assessed.

An antibiogram performed on a contaminant, coloniser or mixed normal flora may encourage inappropriate antimicrobial use.

Conversely, susceptibility testing is valuable when a clinically relevant organism is isolated from a compatible lesion or normally sterile site, especially in severe, recurrent or treatment-resistant infections.

This distinction is essential for antimicrobial stewardship and is consistent with the recommendation that AST should target potential pathogens rather than contaminants or normal flora (8).

Negative culture results also require cautious interpretation.

A negative result may reflect absence of viable bacteria, but it may also result from unsuitable sampling, poor transport, prior antimicrobial exposure, delayed necropsy, overgrowth by contaminants or failure to request appropriate culture conditions for fastidious organisms.

Therefore, a negative culture should be interpreted alongside clinical and pathological findings, rather than used alone to exclude bacterial disease (5,12,13).

The interpretation of bacteriological findings in wild birds should follow a contextual approach: first evaluate the sample quality and origin, then assess the type and purity of bacterial growth, then correlate the isolate with lesions and clinical signs, and only then decide whether AST and treatment decisions are justified (Figure 4).

This approach helps distinguish clinically relevant infection from colonisation or contamination and reduces the risk of unnecessary antimicrobial use (4,6,8).

7. Antibiogram and Antimicrobial Susceptibility Testing

AST is indicated when a clinically relevant bacterial isolate has been recovered and antimicrobial treatment is being considered. In wild birds, this step is particularly useful in severe infections, recurrent cases, deep wounds, osteomyelitis, septicaemia, respiratory infections, post-surgical complications and infections that do not respond to empirical therapy.

However, susceptibility testing should not be performed or interpreted automatically for every isolate, especially when bacteria are recovered from mixed faecal, cloacal or superficial samples without clear clinical relevance (3,5,8).

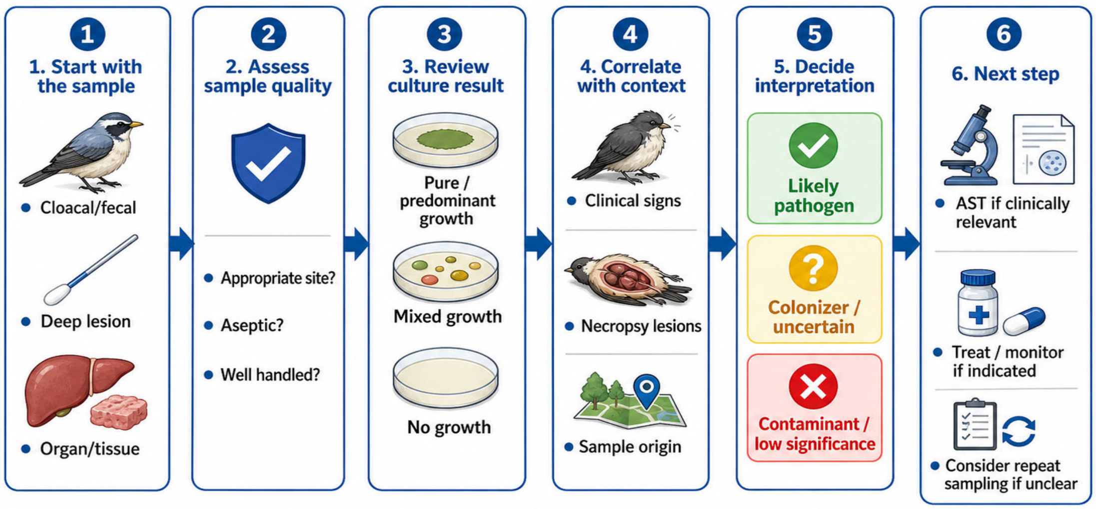

Figura 4. Simplified interpretation pathway for bacteriological results in wild birds (Processed with ChatGPT 5.5.)

The antibiogram must be interpreted only after the isolate itself has been evaluated.

If the bacterium is likely to be a contaminant, coloniser or part of the normal microbiota, its susceptibility profile should not guide treatment.

Conversely, if the organism is isolated in pure or predominant growth from a deep lesion, normally sterile site, internal organ or lesion-compatible sample, AST may provide useful therapeutic guidance.

This distinction is essential because inappropriate interpretation of antibiograms may promote unnecessary or poorly targeted antimicrobial use (4,6,8).

The most frequently used methods for AST include disk diffusion and dilution-based methods, such as broth microdilution or minimum inhibitory concentration determination.

In studies on wild birds, disk diffusion has commonly been used to test isolates of *Enterobacteriaceae* against antimicrobial classes such as β-lactams, tetracyclines, aminoglycosides, sulfonamides, quinolones and fluoroquinolones.

The use of recognised interpretive systems, such as CLSI or EUCAST criteria, improves standardisation, but species-specific breakpoints for wild birds and avian pathogens may be unavailable or limited (2-4,8).

A result reported as susceptible, intermediate or resistant reflects in vitro bacterial behaviour under standardised laboratory conditions.

It does not automatically predict clinical success or failure in a wild bird patient.

Treatment outcome also depends on the bird species, dose, and route of administration, drug safety, pharmacokinetics, tissue penetration, and infection site, severity of disease, immune status, dehydration, concurrent trauma and possibility of release after rehabilitation.

Therefore, the antibiogram should support, but not replace, clinical judgement (8,11,12).

The absence of validated breakpoints for many wild bird species is a major limitation. In practice, laboratories may apply interpretive criteria developed for other animal species, humans or bacterial groups, which can introduce uncertainty.

For this reason, antibiogram interpretation should be cautious, especially when dealing with unusual hosts, rare bacterial species, mixed cultures or isolates from surveillance samples.

When the clinical meaning of the isolate is unclear, repeating sampling or consulting a microbiologist may be more appropriate than initiating broad-spectrum antimicrobial therapy (4,6,8).

Antibiotic choice should also consider antimicrobial stewardship.

Broad-spectrum and critically important antimicrobials should not be selected solely because they appear active in vitro, particularly when narrower, safer or more appropriate alternatives exist.

In wildlife rehabilitation, treatment decisions should also consider individual welfare, prognosis, biosecurity, public health relevance and the risk of releasing birds carrying resistant bacteria.

The detection of resistant *Enterobacteriaceae* in wild birds, including *E. coli* and *Salmonella enterica*, reinforces the need for prudent antimicrobial use and careful interpretation of susceptibility profiles (2–4, 9).

Antibiogram results may also have epidemiological relevance when resistant bacteria are detected in surveillance studies or rehabilitation centres.

However, such findings should be distinguished from therapeutic indications.

A resistant *E. coli* isolate from a cloacal swab may be important for AMR monitoring, but it does not necessarily justify antimicrobial treatment of the bird.

Conversely, the same organism isolated from a septic lesion or internal organ may be clinically relevant and may require targeted therapy.

This distinction between surveillance value and clinical value is central to responsible antibiogram interpretation in wild bird bacteriology (2–4,8).

AST should answer a practical clinical question: whether a relevant bacterial isolate is likely to respond to a safe and appropriate antimicrobial option in the specific patient and lesion context.

The antibiogram should therefore be interpreted together with sample origin, culture purity, clinical signs, necropsy findings, drug safety and stewardship principles, rather than as a stand-alone prescription tool (4,6,8).

A simplified pathway for deciding when AST is appropriate and how antibiogram results should be interpreted in wild bird bacteriology is presented in Figure 5.

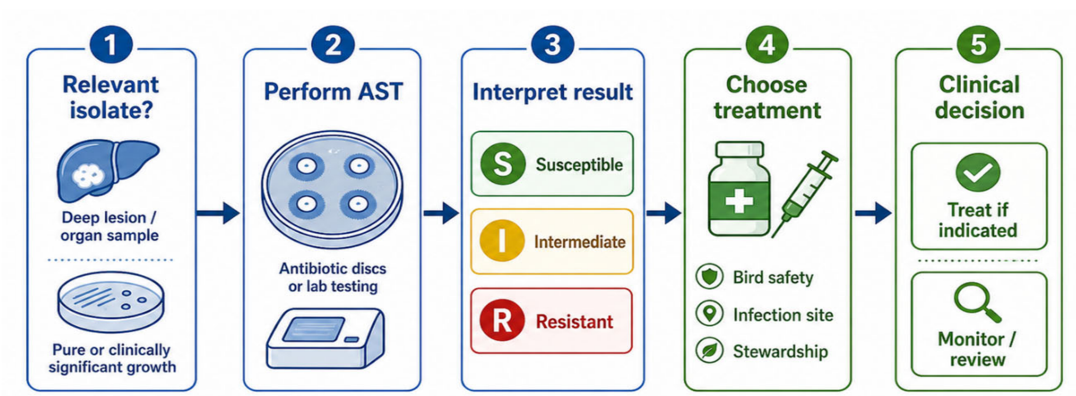

Figure 5. Antimicrobial susceptibility testing (AST) interpretation pathway in wild bird bacteriology (Processed with ChatGPT 5.5.)

8. Antimicrobial Resistance in Wild Birds

While the previous section focused on the clinical interpretation of AST, this section addresses AMR as an ecological and epidemiological issue in wild bird populations.

AMR in wild birds is increasingly recognised as an environmental and public health concern, rather than only a clinical issue.

Wild birds are not usually exposed to antimicrobials directly, but they may acquire resistant bacteria through contact with wastewater, agricultural land, livestock environments, landfills, urban areas or contaminated aquatic habitats.

For this reason, they are often regarded as reservoirs, vectors or sentinels of AMR circulating at the wildlife – livestock – human - environment interface (2–4,18).

The most frequently investigated resistant bacteria in wild birds belong to the family *Enterobacteriaceae*, especially *Escherichia coli* and *Salmonella enterica*.

Other genera, such as *Klebsiella*, *Enterobacter*, *Proteus*, *Citrobacter* and *Hafnia*, may also be reported, particularly in studies using cloacal swabs, faecal samples or post-mortem material.

A recent systematic review of European wild birds found that *E. coli* and *S. enterica* were the most frequently reported *Enterobacteriaceae*, with resistance commonly involving β-lactams, tetracyclines and fluoroquinolones (2–4,9).

The prevalence and profile of resistance vary according to bird species, ecological niche, feeding behaviour, geographical area and sampling method.

Gulls, pigeons, corvids, starlings, waterbirds and raptors are frequently discussed because many of these species interact with anthropogenic environments, including refuse sites, agricultural areas, sewage-affected waters and rehabilitation centres.

Migratory species may also contribute to the long-distance movement of resistant bacteria or resistance genes, although direct transmission pathways are often difficult to prove (3,4,6,14).

In clinical and rehabilitation contexts, AMR is relevant because resistant bacteria may complicate treatment, increase the need for targeted therapy and raise biosecurity concerns.

However, the detection of a resistant bacterium in a cloacal or faecal sample does not automatically mean that the bird requires treatment.

Such isolates may indicate carriage or environmental exposure rather than disease.

Therefore, the clinical importance of AMR findings depends on the same principles used for culture interpretation: sample origin, lesion compatibility, purity of growth and the condition of the bird (3–5, 8).

Several studies have reported multidrug-resistant isolates from wild birds.

Giacopello et al. (2016) found multidrug resistance among *Enterobacteriaceae* isolated from wild birds admitted to a rescue centre in Sicily, while Carroll et al. (2015) detected multidrug-resistant *E. coli* phenotypes in wildlife samples from Ireland and identified resistance-associated genes such as *blaTEM*, *strA*, *tet(A)* and *tet(B)*.

These findings support the idea that wild birds can carry not only resistant bacteria but also mobile genetic determinants of resistance (2, 3).

AMR findings in wild birds should also be interpreted cautiously because studies differ widely in sampling design, bacterial isolation methods, antimicrobial panels, interpretive criteria and molecular confirmation.

Some investigations report prevalence based on individual birds, whereas others report bacterial isolates or resistance genes, which makes direct comparison difficult.

This methodological heterogeneity is a major limitation in the interpretation of AMR data from wild bird populations and supports the need for harmonised sampling and laboratory protocols (4,6,8).

From a practical point of view, AMR surveillance in wild birds should not be separated from responsible antimicrobial use.

Culture and susceptibility testing are valuable when they support targeted treatment of clinically relevant infections, but they should not encourage unnecessary therapy for colonisation or carriage.

In rehabilitation centres, prudent antimicrobial use, hygiene, quarantine, environmental cleaning and careful monitoring are essential to reduce the risk of selecting or spreading resistant bacteria (2-4,8).

Wild birds should be considered both clinical patients and ecological sentinels in the context of AMR.

Their bacterial flora may reflect local environmental contamination, exposure to human or livestock-associated sources, migratory movements and rehabilitation-associated factors.

For this reason, AMR results in wild birds should be interpreted at two levels: their relevance for the individual bird and their broader significance for environmental and public health surveillance (2,4,9,18).

9. Practical Recommendations for Clinicians, Pathologists and Rehabilitation Centres

Bacteriological diagnosis in wild birds should be requested with a clear purpose.

Before sampling, the clinician or pathologist should define whether the objective is individual treatment, confirmation of suspected bacterial disease, post-mortem investigation, outbreak management, biosecurity assessment or AMR surveillance.

This distinction is important because the same sample type may have different value depending on the diagnostic question; for example, a cloacal swab may be useful for surveillance, whereas a deep lesion sample or internal organ is more informative for clinical diagnosis (3,5,8,12).

Whenever possible, samples should be collected before antimicrobial therapy and from the most relevant anatomical site.

Deep tissue, aspirates, purulent material, internal organs and lesion-associated samples are generally preferable to superficial swabs when bacterial disease is suspected.

In contrast, faecal samples and cloacal swabs are better suited for enteric screening, carrier detection or AMR monitoring, and should not be interpreted automatically as evidence of active infection (4,7,12,13).

Clinicians should provide the laboratory with complete contextual information, including bird species, sample type, anatomical site, clinical signs, gross lesions, treatment history, rehabilitation status and suspected diagnosis.

This information helps the laboratory prioritise relevant isolates and avoid unnecessary AST on contaminants or normal flora. Incomplete submission forms may reduce the practical value of even technically correct laboratory results (8,12,13).

For pathologists, bacteriology should be integrated into the necropsy plan rather than performed as an isolated add-on.

Samples should be taken aseptically and early during necropsy, especially from internal organs, intestinal mucosa, air sacs, joints, bone or focal lesions that correspond with gross findings.

When both bacteriology and histopathology are needed, separate samples should be submitted: fresh sterile tissue for culture and formalin-fixed tissue for microscopic examination (5,12).

In rehabilitation centres, bacteriological testing should be linked to hygiene and biosecurity decisions.

Birds admitted with wounds, diarrhoea, respiratory signs, severe debilitation or previous antimicrobial exposure should be managed with appropriate isolation and handling precautions when indicated. The detection of zoonotic or resistant bacteria should prompt review of cleaning protocols, cage hygiene, staff protection, quarantine procedures and antimicrobial use, rather than relying only on treatment of the individual bird (2–5).

AST should be reserved for isolates that are likely to be clinically relevant. An antibiogram from a deep lesion, internal organ or pure culture associated with compatible disease may guide therapy, whereas an antibiogram from mixed cloacal or superficial flora may lead to inappropriate antimicrobial selection.

Treatment decisions should also consider bird species, drug safety, lesion location, tissue penetration, prognosis, legal restrictions and the possibility of release after rehabilitation (4,8,11).

Negative or ambiguous culture results should not be interpreted in isolation. If bacterial disease remains likely, clinicians and pathologists should review the sample type, timing of collection, transport conditions, previous antimicrobial exposure and requested culture methods.

In selected cases, repeat sampling from a better site, additional histopathology or targeted molecular testing may be more useful than empirical broad-spectrum therapy (8,12,13).

Good practice in wild bird bacteriology requires close communication between clinicians, pathologists, microbiologists and rehabilitation personnel.

The most useful laboratory result is obtained when sampling is purposeful, specimens are well handled, the laboratory receives adequate clinical information and the final interpretation distinguishes infection from colonisation, contamination or epidemiological carriage (3,4,6,8).

The main practical recommendations for clinicians, pathologists and rehabilitation centres involved in wild bird bacteriology are summarised in Figure 6.

Because bacteriological investigation in wild birds may involve zoonotic agents and antimicrobial-resistant bacteria, practical recommendations should always be accompanied by appropriate biosafety measures.

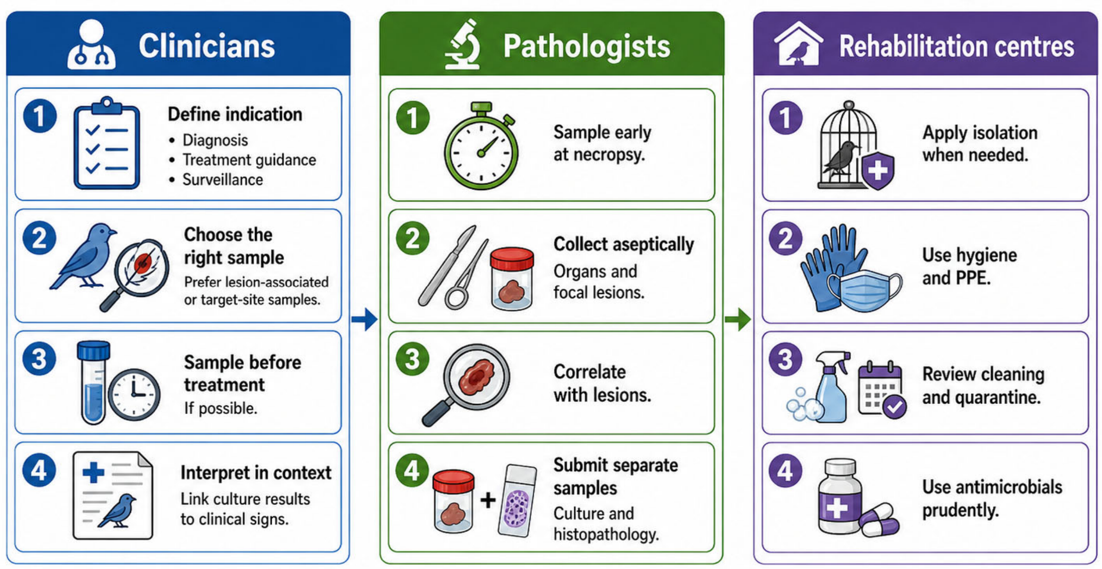

Figure 6. Key recommendations for clinicians, pathologists and rehabilitation centres involved in wild bird bacteriology (processed with ChatGPT 5.5.)

10. Biosafety Considerations in Wild Bird Bacteriology

Biosafety is an essential component of bacteriological investigation in wild birds, particularly when sick, injured or dead birds are handled.

Even apparently healthy wild birds may carry infectious agents without visible clinical signs, and field or rehabilitation-centre personnel may be exposed to bacteria through faeces, oral secretions, respiratory droplets, wound exudate, carcasses, contaminated bedding or sampling equipment.

Therefore, basic protective measures should be applied whenever wild birds are examined, sampled or necropsied (3,12).

Personal protective equipment should be adapted to the level of risk. For routine handling and sampling, gloves, hand hygiene and careful avoidance of direct contact with faeces or body fluids are minimum requirements.

When birds are sick, moribund, dead, involved in mortality events or suspected of carrying zoonotic agents, additional protection such as masks, eye protection, disposable gowns or coveralls should be used.

The same protective equipment should not be used between different sampling sites, enclosures or groups of birds, because inadequate PPE management may contribute to pathogen transfer between individuals, facilities or wild and domestic bird populations (12,19).

Special attention should be given to zoonotic and enteropathogenic bacteria, especially *Salmonella enterica*, *Campylobacter jejuni* and antimicrobial-resistant *Enterobacteriaceae*.

Studies in wild birds and birds admitted to rehabilitation centres have shown that these animals may carry enteric bacteria of clinical and public health importance, including multidrug-resistant isolates.

Such findings do not always indicate disease in the bird, but they are relevant for staff protection, environmental hygiene and prevention of bacterial dissemination within rehabilitation facilities (3-5).

In rehabilitation centres, biosecurity should include separation of newly admitted or clinically suspect birds, regular cleaning and disinfection of cages and work surfaces, safe disposal of faeces and contaminated bedding, and strict hand hygiene between cases.

Sampling instruments, transport containers and necropsy surfaces should be cleaned or disinfected appropriately after use.

Birds with diarrhoea, infected wounds, respiratory signs, severe debilitation or previous antimicrobial exposure should be managed with increased caution, especially when several birds show similar clinical signs or when resistant bacteria are detected (3,5).

Post-mortem examination requires additional precautions because carcasses may be contaminated externally and internally, and bacterial overgrowth may occur after death.

Necropsies should be performed in an appropriate area, using gloves, protective clothing and procedures that minimise aerosolization, splashing and environmental contamination.

Fresh tissues intended for bacteriology should be collected aseptically, while contaminated instruments or surfaces should not be allowed to compromise subsequent samples.

When zoonotic disease, unusual mortality or highly contagious infection is suspected, local veterinary or wildlife authorities should be contacted and sampling should follow applicable official guidance (12).

Overall, biosafety in wild bird bacteriology protects both people and animals. It reduces occupational exposure, prevents cross-contamination between samples, limits the spread of pathogens within rehabilitation centres and improves the reliability of bacteriological results.

For this reason, biosafety should not be considered separate from diagnosis, but rather as part of the same workflow that includes case selection, sampling, culture, interpretation and antimicrobial stewardship.

11. Limitations of this study

This review is narrative in nature and does not follow a systematic review or meta-analysis protocol.

The available literature is heterogeneous in terms of bird species, sample types, laboratory methods, antimicrobial panels and interpretation criteria.

Therefore, direct comparison of prevalence or resistance rates between studies should be made with caution.

In addition, many recommendations are extrapolated from general veterinary bacteriology, avian practice and wildlife disease investigation, because species-specific protocols and breakpoints for wild birds remain limited.

12. Conclusions

Bacteriological diagnosis in wild birds should be based on a clear diagnostic purpose, appropriate sampling and careful interpretation of results.

A positive culture does not always indicate disease, especially when obtained from faeces, cloacal swabs or superficial lesions.

Culture and antibiogram results should be correlated with clinical signs, necropsy findings, sample quality and lesion type.

Antimicrobial susceptibility testing (AST) should guide treatment only when the isolate is clinically relevant.

Reliable bacteriological diagnosis requires correct sampling, good laboratory communication and responsible antimicrobial use.

Note:

Figures 1-6 were edited and optimized using the IA ChatGPT v.5.5 program. The scientific content and accuracy of the data were fully verified by the authors.

References

1. Benskin, C. McW. H., Wilson, K., Jones, K. and Hartley, I. R. (2009) Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biological Reviews* 84, 349–373.
2. Carroll, D., Wang, J., Fanning, S. and McMahon, B. J. (2015) Antimicrobial Resistance in Wildlife: Implications for Public Health. *Zoonoses Public Health* 62, 534–542.
3. Giacopello, C., Foti, M. and Mascetti, A. (2016) Antimicrobial resistance patterns of Enterobacteriaceae in European wild bird species admitted in a wildlife rescue centre. *Veterinaria Italiana* 139–144.
4. Cocoș, D.-I., Dumitrescu, E., Muselin, F., Brezovan, D., Degi, J., Boldura, O.-M. and Cristina, R. T. (2025) Prevalence and Antimicrobial Resistance of Enterobacteriaceae in Wild Birds Across Europe: A Systematic Review. *Antibiotics* 14, 905.
5. Gargiulo, A., Fioretti, A., Russo, T. P., Varriale, L., Rampa, L., Paone, S., De Luca Bossa, L. M., Raia, P. and Dipineto, L. (2018) Occurrence of enteropathogenic bacteria in birds of prey in Italy. *Lett Appl Microbiol* 66, 202–206.
6. Vogt, N. A., Stevens, C. P. G., Pearl, D. L., Taboada, E. N. and Jardine, C. M. (2020) Generalizability and comparability of prevalence estimates in the wild bird literature: methodological and epidemiological considerations. *Animal Health Research Reviews* 21, 89–95.
7. Zhou, T., Bodawatta, K. H. and Jiang, A. (2023) A network meta-analysis on comparison of invasive and non-invasive sampling methods to characterize intestinal microbiota of birds. *Avian Research* 14, 100086.
8. Watts, J. L., Sweeney, M. T. and Lubbers, B. V. (2018) Antimicrobial Susceptibility Testing of Bacteria of Veterinary Origin. *Microbiol Spectr* 6,
9. Mbuthia, C. W. and Hoza, A. S. (2025) Wild birds as potential reservoirs of antimicrobial-resistant Escherichia coli: a systematic review. *Front Microbiol* 16, 1615826.
10. Heatley, J., Marks, S., Mitchell, M. and Tully, T. (2001) Raptor Emergency and Critical Care: Assessment and Examination. *Compendium on Continuing Education for the Practicing Veterinarian* 23, 442–448.
11. State of New South Wales and Department of Planning and Environment (2022) Initial treatment and care guidelines for rescued birds of prey. Parramatta.
12. 12. FAO (2007) Chapter 5. Disease sampling procedures. In *Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques.* FAO Animal Production and Health Manual, No. 5. Rome.
13. IDEXX REFERENCE LABORATORIES (2025) Specimen Collection Guidelines. Available at: https://www.idexx.com/files/06-0013212-06-irl-specimen-guide-en-us.pdf. Accessed on 27 April 2026.
14. Fair, J. M., Paul, E., Jones, J., Clark, A. B., Davie, C. and Kaiser, G. (2010) Guidelines to the Use of Wild Birds in Research. Third

Edition. The Ornithological Council, Washington, D.C.

15. Knutie, S. A. and Gotanda, K. M. (2018) A Non-invasive Method to Collect Fecal Samples from Wild Birds for Microbiome Studies. *Microb Ecol* 76, 851–855.

16. Borrelli, L., Minichino, A., Pace, A., Dipineto, L. and Fioretti, A. (2020) Fecal Sample Collection Method for Wild Birds-Associated Microbiome Research: Perspectives for Wildlife Studies. *Animals* 10, 1349.

17. Evers, D., Savoy, L., DeSorbo, C., Regan, K., Persico, C. and Sayers, C. I. (2021) Bird field sampling methods: collection of tissues for mercury analysis. Biodiversity Research Institute, Portland, Maine, USA.

18. Skarżyńska, M., Leekitcharoenphon, P., Hendriksen, R. S., Aarestrup, F. M. and Wasyl, D. (2020) A metagenomic glimpse into the gut of wild and domestic animals: Quantification of antimicrobial resistance and more. *PLoS One* 15, e0242987.

19. Friend, M., Franson, J. C. and Ciganovich, E. A. (1999) Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds. U.S. Department of the Interior, U.S. Geological Survey.