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In vitro efficacy of antibiotics and bacteriophages against *Pseudomonas aeruginosa* isolates from clinically affected captive falcons in Dubai, United Arab Emirates

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ABSTRACT

Antimicrobial resistance (AMR) poses a significant global threat to human and animal health. This requires extensive research in order to understand the implications on health, pathogenicity and diseases in various species.

Falcons play a crucial role in the United Arab Emirates (UAE) as part of the Arab cultural heritage. In falcons, AMR research is essential for the benefit of veterinary medicine, public health and the environment. The primary objective of this study was to assess the *in vitro* efficacy of antibiotics against *Pseudomonas aeruginosa* isolates from clinically affected captive falcons in Dubai, UAE and investigate the possibility of using bacteriophages as an alternative treatment option. To achieve this, *P. aeruginosa* isolates were tested by antibiogram and phagogram. The results provide valuable information on effectiveness and possible treatment options. Furthermore, demonstrating a high resistance of *P. aeruginosa* in falcons in veterinary-only drugs including enrofloxacin and marbofloxacin, while antibiotics are listed on the WHO AWaRe (access, watch, reserve) monitoring list such as ceftazidime, ciprofloxacin and piperacillin/tazobactam show good sensitivity.

Bacteriophages, as natural viruses that lyse bacteria, have gained attention as an alternative therapeutic tool to combat bacterial infections, particularly those caused by antibiotic resistant strains. The *in vitro* efficacy shows that commercially available bacteriophage preparations for therapeutic use might provide an alternative to antibiotics in falcons. Nevertheless, the *in vivo* efficacy might differ from the *in vitro* results, and regulatory difficulties currently restrict therapeutic use.

From a One Health perspective, this study explores AMR in falcons asf potential sentinel for AMR due to their close contact with humans, frequent antimicrobial exposure, and shared environment. It also shows possibilities to approach AMR by innovative strategies such as bacteriophage therapy. It also shows the need for effective surveillance, responsible antimicrobial use *via* antibiotic stewardship and control not only in human, but also in veterinary medicine. Emphasising the connectivity between human, animal and environment health is of importance under the One Health approach and is essential to combat AMR.

1. Introduction

1.1. Antimicrobial resistance

The ability of bacteria to survive the effects of medications that were previously effective in treating infections is called antimicrobial resistance (AMR). AMR is increasing worldwide and has been addressed by the World Health Organization (WHO) as a major global health concern not only affecting human, but also animal health and the environment [1]. AMR must be tackled with a One Health approach and under a combined umbrella of WHO, World Organisation for Animal Health (WOAH), Food and Agriculture Organization (FAO) and United Nations Environment Programme (UNEP). A quadripartite joint secretariat of the four institutions has been created, which coordinates the

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surveillance of AMR, promoting research, education, antimicrobial stewardship and encouraging development of new antibiotics, as well as promoting alternative treatments [2].

Multiple factors have contributed to the rise of AMR, including overuse and misuse of antibiotics, inadequate hygiene measures and a lack of control. In the Middle East, especially in the United Arab Emirates (UAE) control of antibiotics is poorly regulated. Most antibiotics are available without prescription [3,4]. In veterinary medicine, especially in falcon medicine, this has encouraged owners to initiate treatments on their animals without clinical examinations and further diagnostics from veterinarians. The use of second-line or antibiotics reserved for human medicine is common because these drugs are easily accessed. Additionally, poor awareness of AMR and poor antimicrobial stewardship results in an increasing risk of multidrug resistant (MDR) isolates within *Pseudomonas aeruginosa* infections [5].

Therefore, as falconry plays a significant role in the Middle East, it is important to evaluate the role of falcons as being carriers of MDR bacteria such as *P. aeruginosa.*

1.2. Falconry in the UAE

Falconry represents an important cultural, social and economic tradition in the UAE, where falcons are commonly kept in captivity for hunting, breeding and recently racing [6]. This practice is officially recognized by the UNESCO as an Intangible Cultural Heritage of Humanity [7]. Falcons receive intensive veterinary attention and specialized falcon hospitals and clinics are widely available. Close and frequent contact between falcons, their handlers, veterinarians and the surrounding environment may facilitate the exchange of pathogenic and antimicrobial-resistant bacteria. Therefore, falcons may act as potential reservoirs and sentinels for multidrug-resistant organism, highlighting their relevance within a One Health framework in the region.

1.3. Pseudomonas aeruginosa

P. aeruginosa is a rod-shaped, ubiquitous, Gram-stain-negative opportunistic bacterium, that has been listed since 2017 on WHO catalogue as one of the “priority pathogens” with high risk to human health [8]. It is one of the main pathogens responsible for nosocomial, acute and chronic infections in humans and animals. It is difficult to treat due to several virulence factors: its' capability to adhere surfaces, to form biofilms and its' tendency to develop resistance to multiple antibiotics. There are three different resistance factors in *P. aeruginosa* described [9,10]. The intrinsic resistance due to low permeability of the outer membrane, efflux pumps and production of β-lactamase, the acquired resistance due to horizontal genetic transfer mechanisms and mutation, as well as the adaptive resistance by change of gene expression caused by exposures to different antibiotics and environmental stressors [10,11].

P. aeruginosa infections in falcons are commonly reported possibly due to combined factors of poor husbandry and management practices especially regarding hygiene and nutrition. This is due to a marked increase in the captive falcon population under poor management and no antibiotic stewardship. Falcons are often presented with both acute and chronic upper and lower respiratory tract infections clinically manifested as glossitis, sinusitis, otitis, ingluvitis, tracheitis, airsacculitis and pneumonia [12,13]. Close contact of falconry birds with humans may facilitate bidirectional exchange of strains between birds, humans and the environment [14]. Addressing AMR requires a multi-layered One Health approach. Besides surveillance and monitoring of AMR patterns, it is important to investigate alternative treatment strategies. One of the most promising alternative treatments attracting interest is bacteriophage therapy [15].

1.4. Bacteriophages

Bacteriophages are viral pathogens of bacteria that are recognized as the most abundant biological agent in nature, infecting and lysing specific species of bacteria [16]. There are two types of bacteriophages: lytic (virulent) bacteriophages, that kill by lysing the bacterial cell wall, and lysogenic (temperate) bacteriophages, which integrate their genetic material into the bacterial genome [17,18]. Initially discovered in 1915 by Frederick W. Twort, who found “transparent material” that stopped or hindered bacterial growth, they were named bacteriophages by Felix d'Herelle in 1917 [19,20] Since then, bacteriophages have been used for treatment in human medicine mainly in Eastern Europe [21,22]. Since the 1940's, the Western world focused on using and developing antibiotics [17].

In the Western world, the adoption of bacteriophage therapy has been hampered by a lack of robust evidence from clinical trials not only in humans, but also in animals [23,24]. Additionally, the absence of treatment protocols and a lack of a regulatory framework is limiting their use [23,25].

Bacteriophage therapy has been suggested as a leading alternative in the fight against AMR reducing antibiotic usage because of its efficiency in lysing bacterial cells *via* utilizing bacterial cell machinery for replication, releasing new bacteriophages [26].

This mechanism is used also against *P. aeruginosa,* where specific bacteriophages can be selected, isolated and multiplied, to then be applied in therapeutic concentrations. Bacteriophages are host-specific, infecting and lysing the pathogen causing the infection. Previous studies illustrate the safety and specificity of bacteriophages as therapeutic agents as they don't interfere with commensal bacteria of the host microbiome [27]. Different preparations are available such as mono-suspensions (a single bacteriophage) and multi-suspensions, made into mixtures, so called phage cocktails or bacteriophage preparations (BP).

The promising characteristics of bacteriophages, and the lack of scientific studies regarding their use in treatment of different species gain the attention as a novel therapeutic strategy. Therefore, we investigated the *in vitro* efficacy of antibiotics and commercially available bacteriophages against *P. aeruginosa* isolates from clinically affected falcons in Dubai, UAE. This can further be used to investigate bacteriophages efficacy *in vivo*, promoting it as a potential novel therapy for MDR *P. aeruginosa* in falcons, providing a suitable therapeutical approach under the One Health concept.

2. Material and methods

2.1. Ethical approval

Ethical approval (Nr. OS09-22) for this study was granted by the Animal Welfare and Ethical Review Board (Animals (Scientific Procedures) Act, 1986) (AWERB) at the University of Edinburgh, Scotland.

2.2. Study population and isolate collection

In order to investigate the *in vitro* efficacy of antibiotics and bacteriophage preparations (BP) in *P. aeruginosa* isolates from clinically affected falcons, a total of 46 samples were collected from October 2022 to February 2023 including 29 gyrfalcons (*Falco rusticolus),* 14 gyr peregrine hybrid falcons *(F. rusticolus x F. peregrinus)* and 3 peregrine falcons *(F. peregrinus*). The samples were collected from falcons presented with different clinical conditions at different private falcon clinics in Dubai, UAE. Samples were obtained during routine examinations by crop swabs and were cultured on sheep blood agar (BA) (Medysinal FZCO, Dubai, UAE) and MacConkey agar (Medysinal FZCO, Dubai, UAE) for 24 h at 37 °C before evaluation with Vitek 2 Compact (Biomerieux S.A., Marcy l'Etoile, France). *P. aeruginosa* isolates were collected and stored at −20 °C *via* cryopreservation on beads (Microbank, ProlabDiagnostics, Richmond Hill, Canada) for subsequent

antibiotic sensitivity testing (AST) (antibiogram) and for testing efficacy of BP by phagogram.

2.3. Antibiotic sensitivity testing (Antibiogram)

AST was performed according to the standard disc diffusion methodology described in Clinical Veterinary Microbiology [28] using Mueller Hinton (MH) agar (Oxoid Ltd., Basingstoke, United Kingdom). Bacterial material was resuspended in 0.9 % sodium chloride to achieve 0.5 McFarland turbidity and completely streaked evenly on MH agar. Antibiotic sensitivity discs (Oxoid Ltd., Basingstoke, United Kingdom, and Mast Group Ltd., Bootle, United Kingdom) used for analysis were Amikacin 30 µg (AK), Azithromycin 15 µg (AZM), Cephalexin 30 µg (LEX), Ceftazidime 30 µg (CAZ), Ciprofloxacin 10 µg (CIP), Enrofloxacin 5 µg (ENR), Gentamycin 10 µg (CN), Marbofloxacin 5 µg (MAR), Piperacillin-Tazobactam 100 µg (PR). The choice of antibiotics used for AST was made according to antibiotics routinely used in falcon clinics in UAE.

After 18–24 h incubation at 37 °C, the standard disc diffusion method was done according to Clinical Veterinary Microbiology [28] by measuring the zone diameter and sensitivity was scored as sensitive (S), intermediate (I) or resistant (R), respectively.

2.4. Bacteriophage sensitivity testing (Phagogram)

Bacteriophage sensitivity testing was done by the top agar method according to the Eliava Institute, Georgia using 100 µl of the overnight culture of *P. aeruginosa* isolates and mixed with 4 ml of 65 °C degrees preheated TSB agar and poured onto TSB agar plates as top layer. After the top agar layer dried, 10 µl of different commercially available bacteriophage preparations (Table 1) were pipetted onto the top agar. Once the surface dried, the plates were incubated at 37 °C overnight. The plates were examined for the presences of lysis zones. The absence of lysis zones was scored with resistant (R) and the presence of lysis zones as individual plaques (IP), semi confluent lysis (SCL) or clear lysis (CL), (Fig. 1) [29].

The used BP, their details and origins in this study are shown in Table 1.

Table 1
Details on bacteriophage preparations (BP) and origins.

Phage preparation	Details	Origin
Pyo bacteriophage (Pyo)	Bacteriophage preparation: *Staphylococcus* (10^5), *Streptococcus* (10^5), *Proteus* (10^5), *Escherichia coli* (10^5), *Pseudomonas* (10^5)	Eliava Institute, 3 Levan Gotua Street, Tbilisi, Georgia
Intesti bacteriophage (Intesti)	Bacteriophage preparation: *Staphylococcus* (10^5), *Enterococcus* (10^5), *Proteus* (10^5), *Shigella* (10^5), *Salmonella* (10^5), *Escherichia coli* (10^5), *Pseudomonas aeruginosa* (10^5)	Eliava Institute, Levan, 3 Gotua Street, Tbilisi, Georgia
188 *Pseudomonas aeruginosa*[a] (188)	No information	Eliava Institute, Levan, 3 Gotua Street, Tbilisi, Georgia
Floraphage (Flora)	Prebiotic bacteriophage 1,000,000 PFUs	Arthur Andrew Medical, 8350 E. Raintree Dr., Scottsdale, AZ, United States
Florassist GI with phage technology (GI)	Bacteriophage preparation: Tetra Phage Blend 15 mg LH01 - *Myoviridae* LL5 - *Siphorviridae* T4D - *Myoviridae* LL12 - *Myoviridae*	Lifeextension, 900 N. Federal Hwy, Fort Lauderdale, FL, United States

[a] This bacteriophage preparation was specifically prepared based on previous *P. aeruginosa* isolates from affected falcons.

3. Results

3.1. Antibiotic sensitivity testing (Antibiogram)

To investigate *the* susceptibility *of P. aeruginosa* to antibiotics used clinically, 46 swabs from *P. aeruginosa* infected falcons were cultured and isolated to perform an AST. Detailed results are shown in Table 2 (Supplementary Information).

Of the nine antibiotics tested in this study, the most common susceptibility was demonstrated for ciprofloxacin (CIP) at 96 %, followed by ceftazidime (CAZ) at 91 % and piperacillin/tazobactam (PR) at 80 % (Table 3). Marbofloxacin (MAR), one of the most commonly used antibiotic for falcons in the UAE, showed low sensitivity (37 %). Within the group of aminoglycosides, amikacin (AK) showed significant higher sensitivity than gentamycin (CN). There was 100 % resistance to cephalexin (LEX).

3.2. Bacteriophage sensitivity testing (Phagogram)

To investigate the *in vitro* efficacy of different BP against *P. aeruginosa*, a bacteriophage sensitivity test was performed by a phagogram, analysing the rate and pattern of lysis zones (Fig. 1). Detailed results are shown in Table 2 (Supplementary information).

The highest *in vitro* efficacy was observed by the BP 188 from the Eliava Institute Georgia with 80 % efficacy resulting in a morphology of CL followed by BP Intesti with 46 % *in vitro* efficacy with CL morphology and BP Pyo with 20 % *in vitro* efficacy with CL morphology. The two American produced BP Flora and GI showed much lower efficacy: Flora proved to have mainly IP morphology (48 %), but only 2 % CL morphology, while GI was mainly resistant (83 %) to most of the *P. aeruginosa isolates*. The phagogram results are summarized in Fig. 2.

4. Discussion

This is the first study reporting the *in vitro* susceptibility of *P. aeruginosa* isolates against antibiotics and BP in clinically affected falcons in Dubai, UAE.

P. aeruginosa plays a major role in human and veterinary medicine in nosocomial infections and immunocompromised individuals. Therefore, it is an important pathogen to monitor under the One Health concept not only for current but also for alternative treatment possibilities. *P. aeruginosa* is commonly associated with respiratory infections in avian species in the UAE [30–32], and has been reported in falcons [12,13] Currently, there is no published data on the incidence of *P. aeruginosa* or the AMR situation of the pathogen in falcons in the UAE. Therefore this study aimed to investigate AMR in *P. aeruginosa* and to look at bacteriophage therapy as an alternative treatment under the umbrella of a One Health approach.

To identify effective antibiotics for treating *P. aeruginosa* infections this study analysed 46 samples from clinically affected falcons. From the nine antibiotics commonly used in falcons in the UAE, the results show (Table 2) that only two veterinary antibiotics ENR and MAR show an effect of 11 % and 37 % of the tested isolates. The low efficacy of MAR might be to an overuse as first choice antibiotic for falcons with respiratory infections. The resistance results of ENR with 52 % of the falcon isolates tested is similar to the results found in wild raptors in Spain with 57 % resistance against ENR [33]. Even though older data on falcons is not available, a study done in Dubai in 1998 on a prey species of falcons, the houbara bustard (*Chlamydotis macqueenii*), showed 19 % of isolates were ENR resistant [31]. The low sensitivity to drugs such as MAR and ENR could be an indication for higher use and abuse.

However, the results of this study (Table 2) show that PR (80 %), CAZ (91 %), and CIP (96 %) are the most effective agents for treating *P. aeruginosa*. This correlates with results from the houbara bustard (*Chlamydotis macqueenii)*, that found 100 % of the isolates sensitive to PR [31]. The study of wild raptors found no resistance to CIP and CAZ in

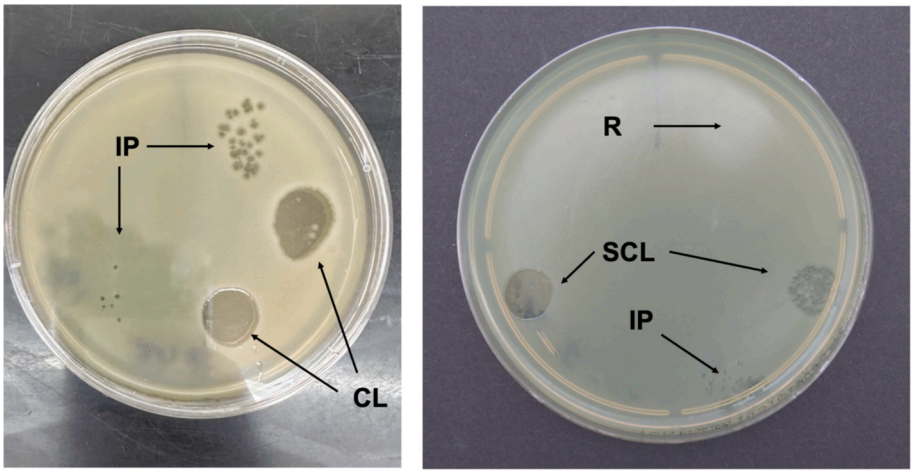

Fig. 1. Different morphologies of lysis zones by BP on *P. aeruginosa* growth (Phagogram) are shown: resistant (R), individual plaques (IP), semi confluent lysis (SCL) and clear lysis (CL).

Table 3
Results of antibiotic sensitivity testing (AST) (Antibiogram). Percentage of *P. aeruginosa* isolates from infected falcons ($n = 46$) being sensitive, intermediate or resistant to the antibiotics are indicated.

Antibiotic class	Antibiotic	Sensitive (S)	Intermediate (I)	Resistant (R)
Aminoglycosides	AMIKACIN (AK)	46 %	48 %	7 %
	GENTAMYCIN (CN)	4 %	50 %	46 %
β-lactam antibiotics	PIPERACILLIN/TAZOBACTAM (PR)	80 %	11 %	9 %
Cephalosporins	CEFTAZIDIME (CAZ)	91 %	4 %	4 %
	CEPHALEXIN (LEX)	0 %	0 %	100 %
Fluroquinolones	CIPROFLOXACIN (CIP)	96 %	0 %	4 %
	ENROFLOXACIN (ENR)	11 %	37 %	52 %
	MARBOFLOXACIN (MAR)	37 %	43 %	20 %
Macrolide antibiotics	AZITHROMYCIN (AZM)	7 %	13 %	80 %

the *P. aeruginosa* isolates [33]. This is a similar pattern to our study which showed 4 % resistance in CIP and CAZ and 9 % in PR.

Therefore, it will come as no surprise that veterinarians use antibiotics such as CIP, CAZ and PR, which are the main human drugs used to treat *P. aeruginosa* but are on WHO AWaRe (access, watch, reserve) monitoring list categorized as "watch" due to higher resistance potential [34]. These antibiotics are recommended to be especially highlighted in stewardship programs, which are currently lacking, especially in veterinary medicine in the UAE and the Middle East in general [34].

From a One Health perspective, AST results of this study show that drug-resistance, especially to veterinary only drugs, should be seen as a concern in falconry medicine in the UAE, and that an increasing incidence of *P. aeruginosa* in falcons should be evaluated under a One Health approach. Not only because *P. aeruginosa* is listed as one of the "priority pathogens" for human health but also because it is an important pathogen to monitor under the One Health concept [8]. Falcons may therefore be considered potential sentinel species for the presence and emergence of antimicrobial-resistant *P. aeruginosa* at the animal–human-interface. Given the close and frequent contact between falcons, their handlers, veterinarians and the shared environment, the findings in this study have potential implications beyond animal health. Although *P. aeruginosa* is an opportunistic pathogen commonly found in environmental reservoirs, close animal–human interactions may facilitate bidirectional transmission of strains, including multidrug-resistant isolates. This relevance is underscored by hospital based studies from the UAE reporting *P. aeruginosa* as a frequent cause of human infections, including multidrug-resistant strains associated with increased morbidity and mortality in clinical settings [5]. At present, there are no data on the carriage of MDR strains in falcon handlers or veterinarians. Therefore, it remains unknown whether falconry-associated exposure contributes to increased human carriage or infection risk. Addressing this knowledge gap would require targeted epidemiological studies investigating *P. aeruginosa* prevalence and resistance profiles in humans in close contact with falcons and comparing them with avian isolates. Such integrated investigations would considerably strengthen the understanding of falcons as potential sentinels or reservoirs within a One Health framework.

With the issues of rising AMR worldwide, it is not only important to highlight the danger of AMR but also to evaluate alternative treatments such as antimicrobial proteins, bacteriophages, probiotics and plant-based treatments, to avoid further drug resistance [35]. Among these, bacteriophage therapy is discussed as a foremost alternative [26]. Therefore, this study evaluated the *in vitro* bacteriophage susceptibility of the *P. aeruginosa* falcon isolates.

The phagogram results proved that BP show *in vitro* efficacy against the *P. aeruginosa* isolates. It is evident that BP purchased from the Eliava Institute in Georgia have a high efficacy *in vitro*: BP 188 shows the lysis zone CL in 80 % of the samples, BP Intesti in 46 % and BP Pyo in 20 %, compared to BP Flora and BP GI, which were ordered from the United States, with neither of these two producing CL zones. Nevertheless, BP Flora showed 50 % IP, proving that there is still some *in vitro* effect against half of the *P. aeruginosa* isolates, while the other half showed resistance. With CL results of 80 % in the BP 188, this BP shows similar *in vitro* effect as the antibiotic sensitivity of PR.

The higher efficacy of the BP from Georgia compared to the US BP may be due to the concentration/titre of bacteriophages in the Georgian BP and the fact that they have specifically been developed as therapeutic agent against *P. aeruginosa* isolates. Even though the American BP are sold as supplements and do not claim to contain bacteriophages against *P. aeruginosa*, they still show efficacy *in vitro*.

The data collected in this study indicate that bacteriophages could be considered as a treatment alternative, as most of the *P. aeruginosa*

Table 2
Summary of the tested isolates from affected falcons. Results of the antibiogram and phagogram are shown.

		Antibiogram results									Phagogram results				
Nr	Species	ENR (5 µg)	CIP (10 µg)	CAZ (30 µg)	LEX (30 µg)	CN (10 µg)	AK (30 µg)	MAR (5 µg)	AZM (15 µg)	PR (100 µg)	188	INTESTI	PYO	FLORA	GI
1	Gyr	R	R	R	R	R	I	I	R	I	CL	R	R	R	R
2	Gyr	I	S	S	R	R	I	I	R	S	CL	IP	R	R	R
3	Gyr	R	S	S	R	R	I	I	R	S	CL	R	R	IP	IP
4	Gyr	I	S	S	R	R	I	I	I	I	SCL	CL	IP	IP	R
5	Gyr	R	S	S	R	R	R	I	S	S	CL	CL	IP	IP	R
6	GxP	R	S	S	R	R	I	I	R	S	CL	SCL	IP	R	R
7	Gyr	R	S	S	R	I	S	S	R	R	CL	CL	CL	IP	R
8	Gyr	I	S	S	R	R	S	I	I	S	CL	R	R	IP	R
9	Gyr	I	S	S	R	R	S	R	I	S	CL	R	R	IP	IP
10	GxP	S	S	S	R	I	S	S	R	S	CL	CL	CL	IP	IP
11	GxP	R	S	S	R	R	I	S	R	S	CL	IP	R	R	R
12	Gyr	R	S	S	R	I	S	R	R	S	CL	CL	IP	R	R
13	GxP	R	S	S	R	R	R	I	R	S	CL	CL	CL	R	R
14	Peregrine	R	S	S	R	R	I	I	R	S	SCL	CL	CL	R	R
15	Gyr	I	S	S	R	R	S	I	S	S	CL	CL	IP	R	R
16	Peregrine	R	S	S	R	R	I	S	R	S	SCL	R	CL	SCL	R
17	GxP	R	S	S	R	I	S	R	R	R	CL	R	R	IP	R
18	Gyr	I	S	S	R	I	S	I	R	S	R	R	R	R	R
19	Gyr	R	S	S	R	R	I	I	R	S	SCL	R	R	R	R
20	Gyr	R	S	S	R	I	R	R	R	S	CL	SCL	R	R	R
22	Gyr	I	S	S	R	S	S	S	R	S	CL	CL	IP	IP	R
23	Gyr	R	S	S	R	S	S	R	R	R	SCL	R	R	R	R
24	Gyr	s	S	S	R	I	S	I	R	S	CL	SCL	R	IP	IP
26	GxP	R	S	S	R	I	I	I	R	S	SCL	R	R	IP	R
27	GxP	I	S	S	R	R	I	I	I	S	R	R	R	R	R
28	GxP	R	S	S	R	R	I	I	R	S	CL	R	R	IP	IP
30	Gyr	R	S	S	R	I	I	S	R	S	CL	CL	CL	R	IP
31	GxP	I	S	S	R	I	S	S	R	S	CL	SCL	R	IP	R
32	GxP	S	S	S	R	I	S	S	R	S	CL	CL	CL	CL	R
33	Peregrine	I	S	S	R	I	S	S	R	S	CL	CL	CL	IP	IP
34	Gyr	R	S	S	R	I	S	S	R	S	CL	SCL	SCL	SCL	SCL
35	Gyr	I	S	I	R	I	I	S	R	S	CL	R	R	R	R
36	Gyr	I	S	S	R	I	S	S	I	S	CL	R	R	IP	R
37	Gyr	I	S	S	R	I	I	S	R	S	CL	CL	IP	R	R
38	Gyr	R	S	R	R	R	I	R	R	I	CL	CL	IP	R	R
39	GxP	I	S	S	R	R	I	I	R	S	CL	CL	R	R	R
40	Gyr	R	S	S	R	I	S	R	R	I	R	R	R	R	R
42	GxP	R	S	S	R	I	S	R	R	R	CL	R	R	IP	R
45	Gyr	R	R	S	R	R	S	R	I	I	CL	CL	IP	IP	R
46	Gyr	S	S	S	R	I	S	S	R	S	CL	CL	CL	R	R
47	GxP	R	S	S	R	R	I	I	R	S	CL	CL	R	IP	R
48	Gyr	I	S	S	R	I	I	I	R	S	CL	CL	IP	IP	R
49	Gyr	I	S	S	R	I	I	S	R	S	CL	CL	IP	IP	R
50	GxP	R	S	I	R	R	I	I	S	S	CL	CL	IP	R	R
51	Gyr	s	S	S	R	I	I	S	R	S	CL	R	R	IP	R
52	Gyr	I	S	S	R	I	S	S	R	S	CL	R	R	IP	R

isolates collected showed susceptibility to one of BP.

In recent years bacteriophages are being increasingly considered as potential treatments for bacteria with MDR strains such as *P. aeruginosa* MDR strains [36]. Nevertheless, bacteriophages might not be considered the ideal solution. They are more specific than antibiotics, which means the spectrum they can be used for is smaller [27].As with antibiotics, they must first be tested *in vitro* for efficacy against the specific isolates of interest. In addition, it must be considered that *in vitro* results of BP may not necessarily have *in vivo* the same effect in falcons. Besides this, bacteriophages can also develop resistance similar to antibiotics during therapy [37]. To overcome the development of resistance, it is suggested to not use single bacteriophages but a mixture of bacteriophages (the BP/ cocktails) and to adapt the BP periodically.

In contrast to Eastern European countries bacteriophages are only discussed as potential therapy in the Western world, as a lack of clinical trials and regulatory challenges hinders the implementation [25]. While adverse reactions to antibiotics are well studied, the safety of the use of bacteriophages might be different [23]. One of the major arguments against bacteriophage therapy is that these are live organisms that can reproduce [38]. But till now bacteriophage therapy is describes as having very low side effects, possibly mainly because bacteriophages interact with bacterial cells and not with mammalian cells [39].

This study has several limitations. AMR was assessed solely by phenotypic susceptibility testing, and molecular analyses such as whole-genome sequencing were not performed to confirm the resistance mechanisms or correlate phenotypic findings with genotypic data. The sample size was limited to clinically affected falcons from a single clinic and geographic region, which may not fully represent the broader falcon population in the UAE. Only commercially available bacteriophage preparations were tested, and no phage isolation was conducted. In addition, no samples from humans in contact with falcons were included; therefore, potential zoonotic transmission or increase human carriage of *P. aeruginosa* could not be assessed. Furthermore, bacteriophage efficacy was elevated only *in vitro*, and results may not directly translate to *in vivo* effectiveness or clinical outcomes. Despite these limitations, this study provides valuable baseline data and supports considering bacteriophages as an alternative treatment for managing AMR in falcons with *P. aeruginosa* infections. This study should invite to further investigations and clinical trials to investigate bacteriophage therapy as an option not only for falcons, but also for other animals and humans under a One Health framework.

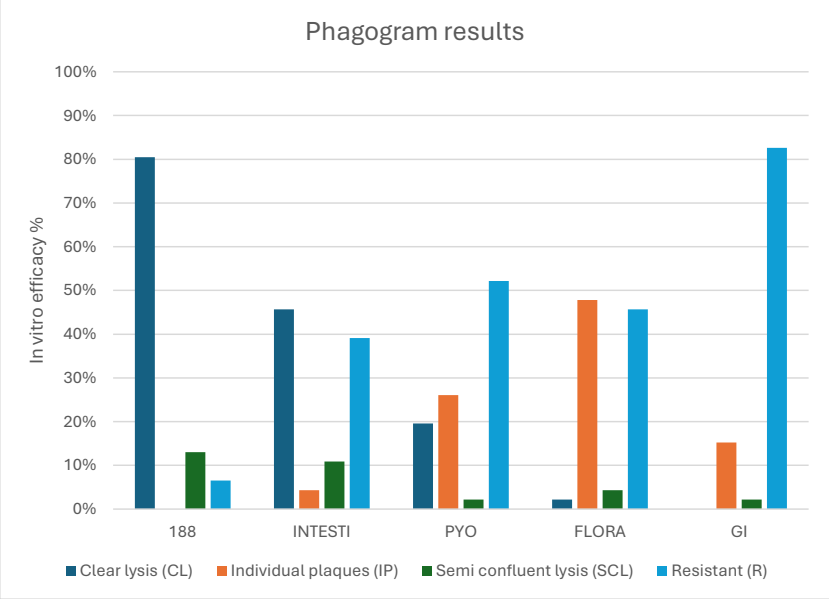

Fig. 2. Results of *in vitro* efficacy of BP (Phagogram). Proportion of *P. aeruginosa* isolates from falcons ($n = 46$) indicating clear lysis (CL), semi confluent lysis (SCL), individual plaques (IP) or being resistant (R).

5. Conclusion

From a One Health perspective, with increasing incidences of AMR worldwide, it is important to monitor antibiotic sensitivity not only in humans and food production animals or pets, but also in other species. Falcons, which play an important role in the UAE and have close contact with humans and the environment, may serve as valuable sentinel species for local surveillance of MDR in *P. aeruginosa.* In addition, evaluating alternative treatments such as bacteriophage therapy against *P. aeruginosa* in falcons might be in future be transferable to other species or humans, not only in Eastern European countries, but also in the Western world. In this interlinked world, addressing health concerns such as AMR through an integrated One Health approach is of utmost importance.

Author Contributions Statement

Christiana Hebel conceived and designed the study, coordinated sample collection, performed the microbiological analyses, interpreted the data, and drafted the manuscript.

Tom Bailey contributed to study design and critically reviewed the manuscript.

Eva M. Kalbhenn supervised the bacteriophage sensitivity testing and contributed to interpretation of the phagogram data.

G.K. Paterson contributed to data interpretation, provided methodological and scientific input, and critically revised the manuscript for intellectual content.

Ulrich Wernery contributed to study conception, provided institutional support and resources, and critically reviewed the manuscript.

CRediT authorship contribution statement

C. Hebel: Writing – original draft. **T. Bailey:** Writing – review & editing, Supervision. **E.M. Kalbhenn:** Writing – review & editing, Methodology. **G.K. Paterson:** Writing – review & editing, Supervision. **U. Wernery:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2025.101316.

Data availability

Data will be made available on request.

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