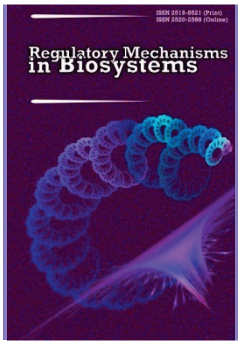

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Antibiotic resistance of bacterial cultures isolated from the feral pigeon (*Columba livia*) and starling (*Sturnus vulgaris*) at a solid waste landfill

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Resistance to antibiotics is well-known global phenomenon. There are places contributing to the development of antibiotic resistance such as waste landfills, especially ones that accept medical waste which did not undergo disinfection and livestock waste with bacteria not sensitive to antibiotics. An extensive system of transfer of antibiotic resistant microorganisms is formed on these territories (zoochory, groundwater, transport etc.). The aim of the research was to determine the species composition of bacteria isolated from birds of Derhachi municipal solid waste landfills in Kharkiv city, Ukraine. Also, we determine the sensitivity of bacterial isolates to a number of standard antibiotic drugs. We collected droppings of feral pigeons (*Columba livia* Gmelin, 1789; Columbidae) and starlings (*Sturnus vulgaris* Linnaeus, 1758; Sturnidae) during the winter period in 2020/2021; both species are dominants of waste landfills. We isolated 15 bacteria species of 4 families by bacteriological methods (growing on simple and selective media and identification by biochemical properties): Enterobacteriaceae (*Enterobacter asburiae*, *E. dissolvens*, *E. cancerogenus*, *E. cloacae*, *E. sakazakii*, *Escherichia coli*, *Klebsiella terrigena*, *K. ornithinolytica*, *Citrobacter freundii*, *Proteus mirabilis*), Yersiniaceae (*Serratia ficaria*, *S. rubidaea*, *S. entomophila*), Morganellaceae (*Providencia stuartii*) and Pseudomonadaceae (*Pseudomonas aeruginosa*). Sensitivity was determined by the disk-diffusion method to 18 antibiotics. Ten isolates turned out to be multiresistant-resistant to three or more classes of antimicrobial drugs. A promising direction for future research is the determination of the pathogenicity of the isolates and checking the roles of birds of Derhachi solid waste landfills as reservoirs of pathogens. Currently, it can be assumed that large concentrations of synanthropic birds (especially those that forage on solid waste landfills) with a high probability are reservoirs of many bacteria, in particular those that have developed resistance to drugs.

Keywords: synanthropic birds; birds as potential reservoirs of pathogens; zoochory; resistance to antibiotics; trophic connections.

Introduction

Rapid global population growth and urbanization have led to a significant increase in the amount of municipal solid waste, which is not segregated and is mainly sent to landfills for storage (Hussein et al., 2018). According to UN reports, waste production is expected to continue to increase to 6.1 million metric tons per day of solid household waste production by 2025 (UN Environment Programme, 2017). Landfilling without prior separation and depollution leads to soil and groundwater contamination due to seepage of toxic leachate such as toxic organic pollutants, antibiotics, pharmaceuticals and personal care products and heavy metals too. The latter can penetrate during rain through disposable waste and contaminate soil and groundwater (Kwon et al., 2017; Yu et al., 2020).

Solid waste landfills are characterized by higher temperature and accessible food waste, which is so attractive for different animal species. The aggregation of animals that often feed on waste definitely affects their biology, morbidity and transmission of infections to different settlements (Muzyka & Stegniy, 2012). Birds are dominants of solid waste landfills (Dementieieva, 2021). They can be potentially very dangerous to humans and animals by transmitting multi-resistant strains of microorganisms through faecal deposits which enter through the water etc. (Cunha et al., 2019). Thus, investigation in Nebraska (USA) on the territories of solid and liquid municipal waste showed that significant contamination with antimicrobial-resistant bacteria and genes is common and they are from areas affected by animal husbandry and household waste (Agga et al., 2015). Due to the interaction of synanthropic birds with wild animals, natural foci of dangerous bacterial infections are formed (Muzyka, 2013; Mikheev, 2018), the causative agents of which have resistance to many antibiotics and have significant genetic plasticity (Gljebova, 2014). At least 80 species of wild animals have been identified, most of which are birds that carry the bacteria from the Enterobacteriaceae family. These bacteria produce mediated resistance that can be transmitted to farm animals and humans (Wang et al., 2017). The role of birds in the transmission of highly pathogenic avian influenza (flu) viruses (Shriner & Root, 2020) and salmonellosis (*Salmonella* spp.) (Martín-Maldonado et al., 2020) during migration has been proven. Wild birds and mammals can be key and important reservoir hosts and potential vectors for the spread of antibiotic-resistant bacteria and genetic determinants (Carroll et al., 2015). The searching for food by birds among the waste leads to the transfer of antibiotic-resistant *Escherichia coli* (Guenther et al., 2010; Höfle et al., 2020).

Synanthropic birds also participate in the spreading of ornithosis (birds' psittacosis) which is associated with an intracellular parasite of the family Chlamydiaceae *Chlamydia psittaci* (Stephens et al., 2009; Romanyshyna & Skrypnyk, 2014). Meyer was the first who described two cases of human infection from synanthropic pigeons in 1941. Human infection usually occurs through direct contact with infected birds or aerogenously, including inhalation of infected dust (Clayton & Price, 1999; Soroka & Sidorenko, 2013). The feral pigeon is often mentioned as a potential vector in disease transmission. Synanthropic pigeons gather in larger flocks, especially in garbage dumps (Muzyka & Stegniy, 2012;

Lyulin & Fedorova, 2016). Investigations of pigeons as vectors of pathogens, often enteropathogenic *E. coli*, confirm the importance of the issue of the danger of anthropogenic activity for birds and, as a result, for humans through avian transmitton of pathogens (de Oliveira et al., 2018; Panikar et al., 2018; Cunha et al., 2019). Starlings also often become objects of microbiological research as reservoirs of various kinds of antibiotic-resistant bacteria, especially *E. coli* (Colles et al., 2009; Kauffman & LeJeune, 2011). It is known that large flocks of these birds increase the risk of transmission of enteric diseases to farm animals, including widespread *E. coli* O157:H7 on dairy farms (Colles et al., 2009; Kauffman & LeJeune, 2011; Swirski et al., 2013; Carlson et al., 2015; Cunha et al., 2019; Carlson et al., 2020).

It is well known that antibiotics are commonly used around the world in livestock farming or raising animals for the food industry. Manure from farm animals is used to produce humus, which can play a key role in the transfer of antibiotic-resistant bacteria to the natural environment. Moreover, birds that feed on animal corpses and predators can be directly exposed to the action of active antibiotics when feeding on the remains of farm animals that end up in landfills (Blanco & Bautista, 2020). Therefore, the use of antimicrobials in animal husbandry is one of the reasons for the spread of antimicrobial resistance (AMR). Interactions between wildlife and livestock, acquisition of associated bacteria and AMR genes, and subsequent dispersal of wildlife across the landscape play important roles in AMR ecology. The investigation in the USA showed the priority of AMR phenotypes and genotypes *E. coli* isolated from the gastrointestinal tract of starlings and underlined multidrug resistance in the majority of *E. coli* isolates with most isolates showing resistance to six or more antibiotic classes (Chandler et al., 2020).

The spreading of clinical antimicrobial resistance is a global problem. Antimicrobial-resistant infections affect humans especially because they challenge the treatment of infectious diseases, in particular through the emergence of resistant forms of *E. coli* worldwide (Wang et al., 2017). The role of opportunistic pathogens among the causative agents of infectious diseases of birds is rapidly increasing. Such microorganisms, which most often circulate in various associations, lead to decrease in the body's resistance. The human body's resistance is sharply reduced compared to monoinfections, which negatively affects immunological reactivity. In such cases it becomes difficult to establish a diagnosis and timely implementation of antiepizootic measures in poultry farms.There is an opinion that the key focus of scientists on prevalence of pathogens likely overestimates the probability of transmission of enteric pathogen from wild birds to humans because in order to cause infection the pathogen should survive long enough at an infectious dose and simultaneously be a strain capable of colonizing humans. Moreover, most data have been obtained from common synanthropic bird species, for example pigeons and starlings. It is very important to take into account a complex of phenomena such as duration and intensity of bacterial release and survival of bacteria in the external environment in bird feces (Wang et al., 2017). However, landfills are characterized by condition of unimpeded circulation of bacterial contamination (Smith et al., 2020). The danger of solid waste landfills as a reservoir of pathogens has been of interest to researchers since the end of the last century (Clayton & Price, 1999). The bacteria-dispersing fauna include various insects and hundreds of bird species of terrestrial and wetland complexes, which during many years create a powerful base for the accumulation and transfer of bacteria (Rusev et al., 2011). Derhachivskyi is one of the largest solid waste landfills in Kharkiv region, which receives waste from the administrative center – Kharkiv city (municipality). Avifauna of landfills is characterized of high diversity and consists of 72 species of birds from 25 families (Dementieieva, 2021). Our investigation of avifauna at the Derhachi landfill site showed that the feral pigeon (*Columba livia*) and starling (*Sturnus vulgaris*) are dominant species. These synanthropic birds gather in the territories of urban landscapes, settlements and solid waste landfills and can represent a potential danger as sources of pathogens of infectious diseases (Muzyka & Stegniy, 2012). During daytime movements within a short distance these birds are able to visit poultry farms and settlements. During such regular movements from one object to another the transmission of infectious agents becomes possible (Muzyka, 2013). Feral pigeons of Derhachi landfill form a stable group of approximately 300 individuals. It makes this species highly interesting for microbiological investigation into the presence of pathogens as potentially infection is associated with their being at the landfill. By contrast, starlings visit the territory of the landfill only in the winter period (from the last decade of November to the first decade of March) in groups of 35 to 1,000 individuals. The ecological features of this species are dynamically transformed. The data of past centuries showed that previously starlings did not spend the winter in Kharkiv region (Somov, 1897; Gladkov & Rustamov, 1965). Birds started to overwinter on landfills because these places are characterized with a more comfortable microclimate during winter (higher temperature due to the decay of waste), which is confirmed by observations of communal roosting of starlings during winters in Kharkiv city (Brezgunova, 2013). Thus, this species is of great interest for investigation as a possible reservoir of pathogenic bacteria. The aim of the study was to carry out bacteriological research of droppings of starlings and pigeons on the territory of Derhachi landfill with isolation and identification of bacterial cultures, determination of their antibiotic resistance in order to prevent outbreaks of infectious diseases that can be spread to settlements and distributed among animals and people.

Materials and methods

During the winter period of 2019–2020 and 2020–2021 the investigation of the circulation of pathogens of bacterial diseases of feral pigeon and starling on the territory of the active solid waste landfill of Derhachi district and its surroundings was conducted. Bacteriological studies were carried out in the Department for the Study of Poultry Diseases of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" in 2020–2021.

For bacteriological studies, 10 samples of pigeon feces were taken in 2020 and 20 samples each from pigeons and starlings in 2021. Biological material (feces) was collected in cryotubes with medium (Brain heart infusion broth, Sigma-Aldrich) with the addition of 15% glycerol. The samples were transported and stored in liquid nitrogen. In the laboratory the samples were stored at a temperature of minus 80 °C for future research. After thawing, the samples were cultured in liquid enrichment and selective nutrient media (buffered peptone water, lactose-peptone medium) incubated in aerobic conditions at 37 ± 1 °C for 16–18 hours with subsequent transfer to dense differential-diagnostic media (Endo and Ploskirev's media, nutrient agar, ISO 4730:2004, 2012).

Biochemical properties of the isolated cultures were studied using a small variegated series; species identification was carried out by tests which were recommended in "Determinant of Berghi bacteria" (Hoult et al., 1997). Determination of the sensitivity of bacterial cultures to antibiotics was carried out by the disk diffusion method according to the generally accepted method using a set of disks by "Himedia": ampicillin 10 mg, amoxiclav 10 mg, amoxicillin 10 mg, cefazolin 30 mg, cefepime 30 mg, imipenem 10 mg, meropenem 10 mg, cefoperazone/sulbactam 75/30 mg, aztreonam 30 mg, gentamicin 10 mg, amikacin 30 mg, ciprofloxacin 5 mg, ofloxacin 5 mg, enrofloxacin 10 mg, doxycycline 30 mg, colistin 10 mg, chloramphenicol 30 mg.

Results

Based on the results of research on pigeon faecal samples collected in 2020 it was established that the majority of isolated bacterial cultures (84.6%) belonged to the Enterobacteriaceae: *E. coli* (n = 6, 46.1% from all isolates), *Enterobacter dissolvens* (n = 4, 30.7%), *Citrobacter freundii* (n = 1, 7.6%). The culture of *Pseudomonas aeruginosa* from family Pseudomonadaceae (n = 2, 15.4%). Our investigation of antibiotic resistance showed that 90.9% of the isolated cultures of bacteria from the Enterobacteriaceae were resistant to ampicillin, doxycycline, 81.8% – to cefazolin and 36.4% to colistin (Table 1). All isolates showed sensitivity to gentamicin and chloramphenicol. The lowest sensitivity to drugs (up to 3–4 out of 6 studied) among cultures was determined in isolates of *E. dissolvens* and *E. coli*. Two isolates of *P. aeruginosa* from the Pseudomonadaceae were insensitive to carbapenems (Table 2).

Our results which were based on material from pigeons in 2021 confirmed that most of the isolated cultures (80%) were bacteria from the

Enterobacteriaceae: *Enterobacter* spp. (n = 8, 40% of all isolates from pigeons), *Klebsiella* spp. (n = 4, 20%), *E. coli* (n = 4, 20%). But we have found some differences among cultures which have changed. Instead of *P. aeruginosa* from the family Pseudomonadaceae, pathogens from Yersiniaceae (*Serratia ficaria* (n = 1, 5%), *S. rubidaea* (n = 2, 10%)) and Morganellaceae (*Providencia stuartii* (n = 1, 5%)) were found (Table 3).

Table 1
Sensitivity of cultures Enterobacteriaceae
isolated from pigeon to antibacterial drugs in 2020

Antibiotic	*E. coli,* n=6			*E. dissolvens* n=4			*C. freundii* n=1		
	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive
Ampicillin	6	0	0	4	0	0	0	1	0
Gentamicin	0	0	6	0	0	4	0	0	1
Chloramphenicol	0	0	6	0	0	4	0	0	1
Doxycycline	6	0	0	4	0	0	0	1	0
Cefazolin	5	0	0	4	0	0	0	0	1
Kolistyn	3	0	3	2	0	2	0	0	1

Table 2
Sensitivity of cultures *P. aeruginosa* (n = 2)
isolated from pigeons to antibactibiotics in 2020

Antibiotic	Sensitive	Insensitive
Cefepime	2	0
Imipenem	0	2
Meropenem	0	2
Gentamicin	2	0
Amikacin	2	0
Ciprofloxacin	2	0
Cefoperazone/sulbactam	2	0

Comparing the results of two stages of bacteriological researches of pigeon droppings, the following differences are visible. In 2020, we discovered 4 species of bacteria from the Enterobacteriaceae and Pseudomonadaceae families; in 2021 – 10 types of microorganisms from the families Enterobacteriaceae, Yersiniaceae and Morganellaceae. Isolates of *Enterobacter* bacteria were detected both in 2020 (4 isolates, 30.7%) and in 2021 (8 isolates, 40.0%); *E. coli* was present in 6 samples (46.1%) in 2020 and in 4 samples (20.0%) in 2021. Bacteria from family Enterobacteriaceae: *Enterobacter* spp. (n = 11, 33.3%), *C. freundii* (n = 2.6%), *K. ornithinolytica* (n = 5, 15.1%), *E. coli* (n = 1, 3.0%), *P. mirabilis* (n = 2, 6.0%); Yersiniaceae: *Serratia entomophila* (n = 2, 6.0%) were isolated from starlings in 2021. In addition to these findings *P. aeruginosa* cultures from the Pseudomonadaceae were isolated in 50% of the samples (n = 10, 30.3% of all isolates). In general, the species composition of bacterial isolates from the droppings of pigeons and starlings consisted of 15 species from four families.

Table 3
Prevalence profile of isolated cultures from bird droppings in 2021

Isolated cultures	Isolated cultures from feral pigeons		Isolated cultures from starlings	
	number of isolates	%	number of isolates	%
P. aeruginosa	0	0	10	30.3
Enterobacter cancerogenus	1	5	6	18.2
Klebsiella ornithinolytica	2	10	5	15.2
E. coli	4	20	1	3.0
Enterobacter asburiae	4	20	0	0.0
E. sakazakii	0	0	4	12.1
Enterobacter dissolvens	2	10	1	3.0
Proteus mirabilis	0	0	2	6.1
C. freundii	0	0	2	6.1
Klebsiella terrigena	2	10	0	0.0
S. rubidaea	2	10	0	0.0
P. stuartii	1	5	0	0.0
S. ficaria	1	5	1	3.0
S. entomophila	0	0	1	3.0
Enterobacter cloacae	1	5	0	0.0

We found out that 100% of isolates from pigeons from the Enterobacteriaceae family were resistant to ampicillin and amoxiclav, 87.5% to doxycycline, 50% to amoxicillin. An isolate of the Yersiniaceae family *Serratia rubidaea* had resistance to amoxiclav, amoxicillin, imipenem and doxycycline and up to 25% broad-spectrum antibiotic drugs in general. An isolate of the Morganellaceae family *Providencia stuartii* showed resistance to ampicillin, amoxiclav, cefepime and aztreonam (Table 4).

Ten species of microorganisms (all from the Enterobacteriacea family) from 33 isolates from starlings were tested for antimicrobial susceptibility. It was established that 100% of isolates were insensitive to ampicillin, 90% to amoxiclav, 80% to amoxicillin and doxycycline, 60% to chloramphenicol and colistin. Such indicators of resistance to a number of antibiotics cause considerable concern (Table 5).

Table 4
Sensitivity of cultures isolated from feral pigeons to antibactics in 2021

Antibiotics	*E. spp.,* n = 6			*K. ornithinolytica.,* n = 1			*E. coli,* n = 1			*C. freundii,* n = 1			*P. mirabilis,* n = 1		
	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive
Ampicillin	6	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Amoxiclav	5	0	1	1	0	0	1	0	0	1	0	0	1	0	0
Amoxicillin	4	1	1	1	0	0	1	0	1	1	0	0	1	0	0
Cefepime	0	1	5	0	1	0	0	0	1	0	0	1	0	0	1
Imipenem	3	0	3	0	0	1	0	0	1	0	1	0	0	0	1
Meropenem	0	0	6	0	0	1	0	0	1	0	0	1	0	0	1
Cefoperazone / Sulbactam	0	0	6	0	0	1	0	0	1	0	0	1	0	0	1
Aztreonam	0	1	5	0	0	1	0	0	1	0	0	1	0	0	1
Gentamicin	1	0	5	0	0	1	0	0	1	0	0	1	0	0	1
Amikacin	1	0	5	0	0	1	0	0	1	0	0	1	0	0	1
Ciprofloxacin	2	1	3	0	0	1	1	0	0	0	0	1	0	0	1
Ofloxacin	3	0	3	0	0	1	1	0	0	0	0	1	0	1	0
Enrofloxacin	2	0	4	0	0	1	1	0	0	0	0	1	0	0	1
Doxycycline	5	0	1	1	0		1	0	0	0	1		1	0	0
Colistin	4	0	2	0	0	1	1	0	0	0	0	1	1	0	0
Chloramphenicol	4	0	2	0	0	1	1	0	0	0	0	1	1	0	0

Discussion

Pigeons are permanent residents of landfills and part of their avifauna, that is why they are constantly exposed to the direct and indirect influence of a specific ecological situation. Starlings visit the landfill periodically and in large flocks in accordance with the onset of cold weather. Our bacteriological research of droppings of pigeons and starlings and subsequent determination of the sensitivity of isolated cultures to antimicrobial drugs showed that birds are carriers of microorganisms of the families Enterobacteriaceae, Yersiniaceae, Morganellaceae and Pseudomonadaceae. We found *S. ficaria* and *S. rubidaea* (Yersiniaceae). These two species were considered harmless environmental bacteria for a long time, until it was discovered that the most common species of this genus, *Serratia marcescens*, is one of the conditionally pathogenic microorganisms of many animals, including humans (Zon & Ivanovskaya, 2018). In humans these microorganisms are mainly associated with nosocomial infections, and can also cause urinary tract infections, pneumonia, and endocarditis. *P. aeruginosa* is a type of gram-negative motile rod-shaped bacteria. It can be present in water, soil and is conditionally pathogenic for humans. Treatment is complicated by its high natural resistance to antibiotics (Vorobyov & Bykov, 2003). This makes it particularly resistant even to large doses of antibiotics. A biofilm formed in this way, for example, protects the entire colony from the ingress of harmful substances, including antibiotics, which greatly complicates treatment.

Enterobacteriaceae is a large family of bacteria that includes well-known pathogens such as *Salmonella*, *E. coli* etc. Some members of the family are part of the normal intestinal microflora and can be found in the intestines of humans and many other animals, while others live in soil, water or can be found on various plants and animals. The best-studied bacterium *E. coli* was recorded during the study. It resides in the lower intestines of mammals, commonly known as intestinal flora (Gostev, 2010). The presence of these species on the surface of water is a general indicator that water contaminated with feces.

E. coli deserves special attention as the object of numerous studies in the world. Thus in Poland, separate smears of cloaca of mallard (*Anas platyrhynchos*) and European herring gull (*Larus argentatus*), as well as samples of other waterbird feces, were examined for *E. coli* content (2008–2009). *E. coli* isolates were tested for sensitivity to 12 antimicrobial agents by the disk diffusion method, as in our study. Nine quinolone-resistant *E. coli* isolates with qnrS genes were found and characterized. Even if wild bird populations are not directly affected by antibiotic therapy, it was reported that antibiotic-resistant strains of *E. coli*, including strains with different resistance genes, are found in the feces of wild birds on the Baltic Sea coast. The isolates were resistant to ampicillin, amoxicillin and ciprofloxacin, as in our studies. Moreover, in some cases, the isolates carried resistance genes. This indicates the deep penetration into ecosystems in particular through translocation by wild birds that feed on landfills (Literak et al., 2010; Handrova & Kmet, 2019). The numerous researches underline the important role of waste separation and mandatory composting of food waste, decontamination of medical and pharmaceutical, as well as livestock waste, chemical compounds of pesticides, hormones, flame retardant and plasticizers, with the purpose of reducing the supply of microbiological contamination of the environment and reducing of antibiotic resistance as well (Marcelino et al., 2019; García et al., 2020).

Research evidence suggests that the biology and ecology of the host) including the level of anthropogenic influence on the territory where wild animals feed and rest, are among the key factors (Gljebova, 2014). Some of the main anthropogenic sources of AMR bacteria in the wild are landfills, insufficiently treated sewage flowing into rivers and lakes, and waste from livestock farms.

The fact that municipal solid waste landfills are absolutely reservoirs of the uncontrolled development process of antibiotic resistance genes (ARG) is emphasized by specific studies at the Narman landfill (Turkey), where samples were taken not from birds but directly from the landfill's waste. The results showed that the tested genes were detected in all landfill samples. Antibiotic-resistant bacteria were found in all areas of the landfill (mainly in waste of animal origin and clinical waste) as well as in the surface of water of the streams near the landfill. As a result, the authors underline that birds, insects and other animals are responsible for transmission (Hiltunen et al., 2017). Even if people control the medical use of antibiotics, we receive them including ARG from food, water and air. Currently, this system consists of the uncontrolled use of antibiotics in various economic sectors and medicine (microbiological contomination of the environment – the impact of the environment on a person). This problem should be solved because it threatens the medical achievements of the last century (Sivri et al., 2019).

Table 5
Sensitivity of cultures isolated from starlings to antibiotics in 2021

Antibiotics	*E. coli,* n = 3			*K. spp.,* n = 3			*E. spp.,* n = 2			*P. stuartii,* n = 1			*S. rubidaea,* n = 1		
	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive	insensitive	moderately sensitive	sensitive
Ampicillin	3	0	0	3	0	0	2	0	0	1	0	0	0	0	1
Amoxiclav	3	0	0	3	0	0	2	0	0	1	0	0	1	0	0
Amoxicillin	3	0	0	0	3	0	1	0	1	0	0	1	1	0	0
Cefepime	0	0	3	1	0	2	0	0	2	1	0	0	0	0	1
Imipenem	1	0	2	3	0	0	1	1	0	1	0	0	1	0	0
Meropenem	0	0	3	0	1	2	0	0	2	0	0	1	0	0	1
Cefoperazone / Sulbactam	0	0	3	0	0	3	0	0	2	0	0	1	0	0	1
Aztreonam	0	0	3	1	0	2	0	0	2	1	0	0	0	0	1
Gentamicin	0	0	3	0	0	3	0	0	2	0	0	1	0	0	1
Amikacin	0	0	3	1	0	2	0	0	2	0	0	1	0	0	1
Ciprofloxacin	0	0	3	1	0	2	1	0	1	0	0	1	0	0	1
Ofloxacin	0	0	3	0	1	2	1	0	1	0	0	1	0	0	1
Enrofloxacin	0	0	3	0	0	3	1	0	1	0	0	1	0	0	1
Doxycycline	3	0	0	2	0	1	2	0	0	0	0	1	1	0	0
Colistin	0	0	3	0	0	3	0	0	2	0	0	1	0	0	1
Chloramphenicol	1	0	2	0	0	3	0	0	2	0	0	1	1	0	0

Another potentially dangerous fact was identified in China. The aim of investigation was detecting baseline natural variations in antibiotic resistance genes (ARGs) in soil without anthropogenic activity over decades. A total of 196 ARG subtypes were detected in soil and rhizosphere. ARG diversity remained stable over 69 years, likely due to the alkaline pH of the soil rather than antibiotics. It is important that birds are given the key role in the increase in ARG after 86 years. It deserves special attention that ARG-containing pathogenic bacteria are transmitted by migratory birds to the territory with an 86-year history of soil formation near settlements. This study revealed slow changes in ARG as soil matured without anthropogenic activity over decades (Torres et al., 2020).

Another problem that has not received much attention by scientists is the combined effect of antibiotics and other compounds, such as heavy metals and biocides (present in the environment) on nature.

Further research is needed to understand the role of wild birds and other animals in the transmission of clinically relevant resistant bacteria, bacterial genes and to assess the level of potential opportunities. Future research should focus on human-animal-environment interaction. It is clearly crucial to minimize the transmission of bacteria from livestock and humans to the environment. Proper waste management and decontamination of medical, pharmaceutical, and other bacterially or chemically contaminated waste will help (Tang et al., 2021).

Conclusion

Our results showed that the birds of solid waste landfills especially their large flocks play an important ecological role in the epidemiological situation and can carry and transmit a wide range of antibiotic-resistant bacteria. Contamination of food or water by bird feces can serve as one of the routes of transmission of antibiotic-resistant pathogens to farm animals and humans. During our laboratory research in 2020 and 2021 (winter time) a total of 15 types of bacteria from 4 families were isolated from pigeons and starlings that feed at the solid waste landfill in Kharkiv: Enterobacteriaceae (*E. asburiae*, *E. coli*, *E. dissolvens*, *K. terrigena*, *K. ornithinolytica*, *E. cancerogenus*, *E. cloacae*, *E. sakazakii*, *C. freundii*, *P. mirabilis*), Yersiniaceae (*Serratia ficaria*, *S. rubidaea*, *S. entomophila*), Morganellaceae (*P. stuartii*) and Pseudomonadaceae (*P. aeruginosa*). Moreover, we found out that some of the cultures were multiresistant, although the birds did not receive antibiotics in nature.

Of all 66 isolates from starlings and feral pigeons during the two parts of the research, 33 isolates were tested for sensitivity to antimicrobial drugs. The highest resistance was determined to ampicillin (penicillins) – 87.9% (n = 29) and doxycycline (tetracyclines) – 78.8% (n = 26); 57.6% (n = 19) isolates were insensitive to amoxiclav (penicillins), 39.4% (n = 13) and 36.4% (n = 12) to amoxicillin (penicillins) and imipenem (carbapenems), respectively. We found that 10 isolates (all from Enterobacteriaceae) were multi-resistant/resistant to three or more classes of antibiotics. Such indicators of antimicrobial resistance to a number of antibacterial drugs cause great concern. Especially alarming is the fact that cultures from the Enterobacteriaceae, isolated from both species of birds, had absolute insensitivity to a number of drugs and a low threshold of reaction to others.

Its important that the sampling of 2021 took place during severe frosts. However, this did not affect the results and did not destroy the pathogenic bacteria. A greater species diversity of microorganisms and their greater resistance to drugs were determined in bird droppings in 2021. So large concentrations of synanthropic birds, and especially those that feed on solid waste landfills, are likely to be reservoirs and can transmit many bacteria species including those that have developed resistance to antibiotics. This problem is about ecological awareness and conscientious waste management. It is worth underling to the public the importance of taking antibiotics only according to the doctors' recommendations and necessarily in compliance with the dose and course of treatment. The problems of waste management should be solved at the national level: landfilling and storage of waste in landfills and access to them by wild birds is one of the key factors in the evolution of antibiotic resistance in the world.

The authors consider that there is no conflict of interest.

References

Agga, G. E., Arthur, T. M., Durso, L. M., Harhay, D. M., & Schmidt, J. W. (2015). Antimicrobial-resistant bacterial populations and antimicrobial resistance genes obtained from environments impacted by livestock and municipal waste. PLoS One, 10(7), e0132586.

Ahlstrom, C. A., Bonnedahl, J., Woksepp, H., Hernandez, J., Olsen, B., & Ramey, A. M. (2018). Acquisition and dissemination of cephalosporin-resistant *E. coli* in migratory birds sampled at an Alaska landfill as inferred through genomic analysis. Scientific Reports, 8, 7361.

Blanco, G., & Bautista, L. M. (2020). Avian scavengers as bioindicators of antibiotic resistance due to livestock farming intensification. International Journal of Environmental Research and Public Health, 17(10), 3620.

Brezgunova, O. A. (2013). Night roosts of wintering starlings (*Sturnus vulgaris*) in Kharkiv city. Branta, 16, 120–126.

Carlson, J. C., Chandler, J. C., Bisha, B., LeJeune, J. T., & Wittum, T. E. (2020). Bird-livestock interactions associated with increased cattle fecal shedding of ciprofloxacin-resistant *Escherichia coli* within feedlots in the United States. Scientific Reports, 10, 10174.

Carlson, J. C., Hyatt, D. R., Bentler, K., Mangan, A. M., Russell, M., Piaggio, A. J., & Linz, G. M. (2015). Molecular characterization of *Salmonella enterica* isolates associated with starling–livestock interactions. Veterinary Microbiology, 179, 109–118.

Carroll, D., Wang, J., Fanning, S., & McMahon, B. J. (2015). Antimicrobial resistance in wildlife: Implications for public health. Zoonoses and Public Health, 62(7), 534–542.

Chandler, J. C., Anders, J. E., Blouin, N. A., Carlson, J. C., LeJeune, J. T., Goodridge, L. D., Wang, B., Day, L. A., Mangan, A. M., Reid, D. A., Coleman, S. M., Hopken, M. W., & Bisha, B. (2020). The role of European starlings (*Sturnus vulgaris*) in the dissemination of multidrug-resistant *Escherichia coli* among concentrated animal feeding operations. Scientific Reports, 10, 8093.

Clayton, D. H., & Price, R. D. (1999). Taxonomy of New World *Columbicola* (Phthiraptera: Philopteridae) from the Columbiformes (Aves), with descriptions of five new species. Annals of the Entomological Society of America, 92(5), 675–685.

Colles, F. M., McCarthy, N. D., Howe, J. C., Devereux, C. L., Gosler, A. G., & Maiden, M. C. J. (2009). Dynamics of *Campylobacter* colonization of a natural host, *Sturnus vulgaris* (European Starling). Environmental Microbiology, 11(1), 258–267.

Cunha, M. P. V., Oliveira, M. C. V., Oliveira, M. G. X., Menão, M. C., & Knöbl, T. (2019). CTX-M-producing *Escherichia coli* isolated from urban pigeons (*Columba livia domestica*) in Brazil. The Journal of Infection in Developing Countries, 13(11), 1052–1056.

de Oliveira, M. C. V., Camargo, B. Q., Cunha, M. P. V., Saidenberg, A. B., Teixeira, R. H. F., Matajira, C. E. C., Moreno, L. Z., Gomes, V. T. M., Christ, A. P. G., Barbosa, M. R. F., Sato, M. I. Z., Moreno, A. M., & Knöbl, T. (2018). Free-ranging synanthropic birds (*Ardea alba* and *Columba livia domestica*) as carriers of *Salmonella* spp. and diarrheagenic *Escherichia coli* in the vicinity of an urban zoo. Vector-Borne and Zoonotic Diseases, 18(1), 65–69.

Dementieieva, Y. Y. (2021). Ornithofauna of solid waste landfills of the Kharkov city. Visnyk Cherkaskogo Universytetu, 1, 26–36.

García, J., García-Galán, M. J., Day, J. W., Boopathy, R., White, J. R., Wallace, S., & Hunter, R. G. (2020). A review of emerging organic contaminants (EOCs), antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in the environment: Increasing removal with wetlands and reducing environmental impacts. Bioresource Technology, 307, 123228.

Gladkov, N. A., & Rustamov, A. K. (1965). The main problems of studying birds of the cultural landscape. In: Modern problems of ornithology. Frunze. P. 111–156 (in Russian).

Gliebova, K. V. (2014). The role of the wild birds as natural sources of bacterial diseases. Scientific and Technical Bulletin of State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology, 15(2–3), 119–122.

Gostev, V. V., & Sidorenko, S. V. (2010). Bacterial biofilms and infections. Journal Infectology, 2(3), 4–15.

Guenther, S., Grobbel, M., Lübke-Becker, A., Goedecke, A., Friedrich, N. D., Wieler, L. H., & Ewers, C. (2010). Antimicrobial resistance profiles of *Escherichia coli* from common European wild bird species. Veterinary Microbiology, 144, 219–225.

Handrova, L., & Kmet, V. (2019). Antibiotic resistance and virulence factors of *Escherichia coli* from eagles and goshawks. Journal of Environmental Science and Health, Part B, 54(7), 605–614.

Hiltunen, T., Virta, M., & Laine, A.-L. (2017). Antibiotic resistance in the wild: An eco-evolutionary perspective. Philosophical Transactions of the Royal Society B: Biological Sciences, 372, 1712.

Höfle, U., Jose Gonzalez-Lopez, J. Camacho, M. C., Solà-Ginés, M., Moreno-Mingorance, A., Manuel Hernández, J., De La Puente, J., Pineda-Pampliega, J., Aguirre, J. I., Torres-Medina, F., Ramis, A., Majó, N., Blas, J., & Migura-Garcia, L. (2020). Foraging at solid urban waste disposal sites as risk factor for cephalosporin and colistin resistant *Escherichia coli* carriage in white storks (*Ciconia ciconia*). Frontiers in Microbiology, 11, 01397.

Hoult, J., Kryga, N., & Snyt, P. (1997). Determinant of Berghi bacteria. Mir, Moscow. Vol. 1.

Hussein, I., Abdel-Shafy, M., & Mansour, S. M. (2018). Solid waste issue: Sources, composition, disposal, recycling, and valorization. Egyptian Journal of Petroleum, 27(4), 1275–1290.

Jing, W., Ma, Z.-B., Zeng, Z.-L., Yang, X.-W., Huang, Y., & Liu, J.-H. (2017). The role of wildlife (wild birds) in the global transmission of antimicrobial resistance genes. Zoological Research, 38(2), 55–80.

Kauffman, M. D., & LeJeune, J. (2011). European starlings (*Sturnus vulgaris*) challenged with *Escherichia coli* O157 can carry and transmit the human pathogen to cattle. Letters in Applied Microbiology, 53(6), 596–601.

Kwon, M. J., Yun, S.-T., Ham, B., Lee, J.-H., Oh, J.-S., & Jheong, W.-W. (2017). Impacts of leachates from livestock carcass burial and manure heap sites on groundwater geochemistry and microbial community structure. PLoS One, 12(8), 0182579.

Literak, I., Dolejska, M., Janoszowska, D., Hrusakova, J., Meissner, W., Rzyska, H., Bzoma, S., & Cizek, A. (2010). Antibiotic-resistant *Escherichia coli* bacteria, including strains with genes encoding the extended-spectrum beta-lactamase and QnrS, in waterbirds on the Baltic Sea coast of Poland. Applied and Environmental Microbiology, 76(24), 8126–8134.

Lyulin, P., & Fedorova, O. (2016). Prevalence, seasonal and age-related dynamics of Pigeons' ascaridosis in Kharkiv and its suburbs. Theoretical and Applied Veterinary Medicine, 4(2), 68–73.

Marcelino, V. R., Wille, M., Hurt, A. C., González-Acuña, D., Klaassen, M., Schlub, T. E., Eden, J.-S., Shi, M., Iredell, J. R., Sorrell, T. C., & Holmes, E. C. (2019). Meta-transcriptomics reveals a diverse antibiotic resistance gene pool in avian microbiomes. BMC Biology, 17, 31.

Martín-Maldonado, B., Vega, S., Mencía-Gutiérrez, A., Lorenzo-Rebenaque, L., de Frutos, C., González, F., Revuelta, L., & Marin, C. (2020). Urban birds: An important source of antimicrobial resistant *Salmonella* strains in Central Spain. Comparative Immunology, Microbiology and Infectious Diseases, 72, 101519.

Mikheev, A. O. (2018). Bird migrations and the spread of infectious diseases (literature review). World Science, 6(34), 6–13.

Muzyka, D. V. (2013). Wild birds as one of the main distribution factors of birds, animals and people pathogens. Veterinary Medicine, 97, 34–36.

Muzyka, D. V., & Stegniy, B. T. (2012). Wild birds as one of the main factors of distribution of agents of avian, animal and human infections. Veterinary Medicine, 96, 222–224.

Nuket, S., Bagcigil, A. F., Celik, B., Azaz, D., & Gungor, Y. (2019). The antibiotic resistance genes in *Escherichia coli* isolates from narman landfill (nl) area in Erzurum, Turkey. Fresenius Environmental Bulletin, 28(2), 803–808.

Panikar, I., Susol, R., & Kolomak, I. (2018). Morphological changes in colibacillosis of pigeons. Bulletin of SNAU, 42, 64–67.

Romanyshyna, J. R., & Skrypnyk, V. G. (2014). The discovery of *Chlamydia avium* in synanthropic pigeons in Ukraine. Veterynarna Virusologija ta Mikrobiologija, Problemy Biobezpeky ta Biozahystu, 99, 65–68.

Rusev, I. T., Zakusylo, V. N., & Vynnyk, V. D. (2011). Ecological and faunistic background of arboviruses circulation in north-west coast of Black Sea. Visnyk of Dnipropetrovsk University, Biology, Medicine, 2(2), 95–109.

Shriner, S. A., & Root, J. J. (2020). A review of avian influenza A virus associations in synanthropic birds. Viruses, 12(11), 1209.

Smith, O. M., Snyder, W. E., & Owen, J. P. (2020). Are we overestimating risk of enteric pathogen spillover from wild birds to humans? Biological Reviews, 95(3), 652–679.

Somov, N. N. (1897). Ornithological fauna of the Kharkov Province. Kharkov, A. Darre.

Soroka, N. I., & Sidorenko, I. V. (2013). Fauna of chewing lice of the order Phthiraptera, suborders Ischnocera and Amblycera, of the rock pigeon (*Columba livia*) in Ukraine. Vestnik Zoologii, 47(3), 211–217.

Stephens, R., Myers, G., Eppinger, M., & Bavoil, P. (2009). Divergence without difference: Phylogenetics and taxonomy of *Chlamydia* resolved. FEMS Immunology and Medical Microbiology, 55, 115–119.

Swirski, A. L., Pearl, D. L., Williams, M. L., Homan, H. J., Linz, G. M., Cernicchiaro, N., & LeJeune, J. T. (2013). Spatial epidemiology of *Escherichia coli* O157:H7 in dairy cattle in relation to night roosts of *Sturnus vulgaris* (European starling) in Ohio, USA (2007–2009). Zoonoses and Public Health, 61(6), 427–435.

Tang, X., Shen, M., Zhang, Y., Zhu, D., Wang, H., Zhao, Y., & Kang, Y. (2021). The changes in antibiotic resistance genes during 86 years of the soil ripening process without anthropogenic activities. Chemosphere, 266, 128985.

Torres, R. T., Carvalho, J., Cunha, M. V., Serrano, E., Palmeira, J. D., & Fonseca, C. (2020). Temporal and geographical research trends of antimicrobial resistance in wildlife – A bibliometric analysis. One Health, 11, 100198.

Vorobyov, A. A., & Bykov, A. S. (2003). Atlas of medical microbiology, virology and immunology. Medical Information Agency, 60, 236.

Yu, X., Sui, Q., Lyu, S., Zhao, W., Liu, J., Cai, Z., Yu, G., & Barcelo, D. (2020). Municipal solid waste landfills: An underestimated source of pharmaceutical and personal care products in the water environment. Environmental Science and Technology, 54(16), 9757–9768.

Zon, G. A., & Ivanovskaya, L. B. (2018). Current epizootic state on leptospirosis of cattle in Sumy Oblast. Veterinary Biotechnology, 32(2), 193–201.