DOI: 10.34101/ACTAAGRAR/2/11523

Does the Hooded Crow (*Corvus cornix*) harbour vancomycin-resistant enterococci in Hungary?

Isma Benmazouz[1,2*] – László Kövér[2] – Gábor Kardos[3]

[1]Animal Husbandry Doctoral School. University of Debrecen, Hungary
[2]Department of Nature Conservation Zoology and Game Management, University of Debrecen, Hungary
[3]Institute of Metagenomics, University of Debrecen, Hungary
*****Correspondence: benmazouz.isma@agr.unideb.hu

SUMMARY

Vancomycin resistant enterococci (VRE) are high priority healthcare associated bacteria with a potential for zoonotic transmission. Thus, its emergence outside health establishments is a major concern. In order to study the prevalence of VRE in wildlife, we collected 221 faecal samples from free-ranging Hooded Crow (Corvus cornix) from urban and rural habitats in Hungary, from March to August 2020. The screening for resistant enterococci was done using bile esculin azide (BEA) agar supplemented with Vancomycin, specific to the screening of VRE. None of the samples from either habitat types yielded VRE. It seems that Hooded Crows from Hungary do not necessarily constitute a reservoir of VREs at present. Nonetheless, a continuous surveillance of VRE in wildlife would be judicious.

Keywords: *antimicrobial resistance, wild bird, antibiotics, One Health.*

INTRODUCTION

The appearance of antimicrobial resistant bacteria (AMRB) in the wild was documented as early as 1975, when Sato (1987) found multi-resistant *E. coli* strains in pigeons. Since then, continuous reports of such occurrence have been published, especially in wild birds (Wang et al., 2017) including resistant enterococci (Murray, 2000). The genus Enterococcus includes a number of species normally found in the intestinal tract of humans, mammals, birds, reptiles and insects (Abriouel et al., 2008; Wada et al., 2019). These Gram-positive bacteria (GPB) can be opportunistic and cause serious infections like cystitis, bacteraemia and sepsis (Simjee, 2006; Fisher and Phillips, 2009). Enterococci are naturally resistant to several antimicrobials (e.g., penicillin, cephalosporins, macrolides and clindamycin) and can rapidly acquire resistance following the clinical introduction of antimicrobial agents (reported by Ahmed and Baptiste, 2018) and thus cause nosocomial infections, usually occurring in hospital care. Antibiotic resistance observed in these microorganisms has emerged as a serious global health issue, among them the most important is resistance to vancomycin (Delpech et al., 2019), which is listed as essential to medicine by the World Health Organization (WHO) and classified as critical to human health (WHO, 2021). Vancomycin is aglycopeptide antibiotic mainly reserved for the treatment of healthcare associated infections caused by antimicrobial resistant GPB (Fiore et al., 2019). Considered as a drug of last resort, the appearance of resistant bacteria to this essential drug is an alarming phenomenon.

Described for the first time in hospital environments (Willems et al., 2005; Fisher and Phillips, 2009), VRE have since been found in domestic animals and livestock (Pomba et al., 2017; Ramos et al., 2012; Wada et al., 2021). Similarly to many emerging AMRB, the occurrence of VRE outside inpatient care facilities is believed to have been facilitated by extensive use of similar antimicrobials in human and veterinary medicine as well as antibiotic use for growth promotion in farm animals (Bager et al., 1997; Buetti, et al., 2019). For instance, in Europe, the use of avoparcin, a vancomycin like antibiotic as a feed supplement for livestock, was shown to be fundamental for VRE emergence in food producing animals (Kruse et al., 1999; Werner et al., 2020; Ünal et al., 2020). Consequently, in 1997, the European Union banned the use of avoparcin, leading to decreased occurrence of VRE in farm animals but not to its disappearance (Ahmed and Baptiste, 2018). Instead, VRE occurrence is still arising and reports of these bacteria in wildlife have been documented since. For instance, VRE strains were isolated in free ranging gulls (Sjölund et al., 2008; Drobni et al., 2009) as well as free ranging crows and ravens (Oravcova et al., 2013; Oravcova et al., 2014; Oravcova et al., 2016).

Vancomycin resistance in enterococci results from the modification of the peptidoglycan synthesis pathway encoded by eight different genes: vanA, vanB, vanC, vanD, vanE, vanG, vanL and vanM (Arias et al., 2012). Such resistance is acquired by obtaining genetic materials encoding vancomycin resistance in the form of plasmids or transposons. In addition, considering the latest reports on AMR in wildlife, it seems that the dissemination of AMR is often closely linked to human related activities (Wu et al., 2018), highlighting the important intricacies of AMR in wildlife as well as the undeniable interconnection between humans, environment, and wildlife in line with the One Health principle (reported by Wang et al., 2017). Therefore, wild animals may pick-up AMRB strains from different contaminated anthropogenic surfaces (Dolejska et al., 2007). Several authors have found that different wild bird populations from urban areas carry AMR strains (Dolejska et al., 2007; Bonnedahl et al., 2009; Silva et al., 2011) including VRE strains (Sjölund et al., 2008; Drobni et al., 2009; Oravcova et al., 2013, 2014, 2016).

DOI: 10.34101/ACTAAGRAR/2/11523

Previous studies have reported the presence of potential pathogens in the Hooded Crow (*Corvus cornix*) (Ferrazzi et al., 2007), a Eurasian bird species, widely spread across Northern, Eastern, and South-eastern European cities, and some parts of the Mid-East. Like other Corvidae, Hooded Crows may harbour important AMRB as previously reported (Sjölund et al., 2008; Oravcova et al., 2013, 2014; Oravcova et al., 2016), however, there is no information regarding the carriage of VRE amongst these highly urbanized creatures. The aim of this study was to explore the occurrence of VRE, in resident Hooded Crows from two different habitat types in Hungary.

MATERIALS AND METHODS

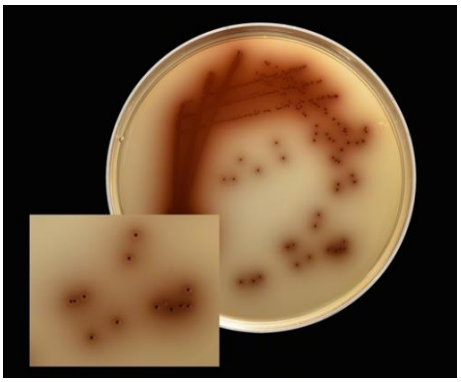
Study area

Hooded Crows were captured in several locations in the northern part of the city of Debrecen, the second largest city after Budapest, situated in the northern Great Plain region near the Hortobágy National Park. Rural individuals were trapped in Balmazújváros, a rural town 40 km away from Debercen and Szakoly, located 25 km away. Additional urban crows were also captured at the Budapest zoo.

Sample collection and analysis:

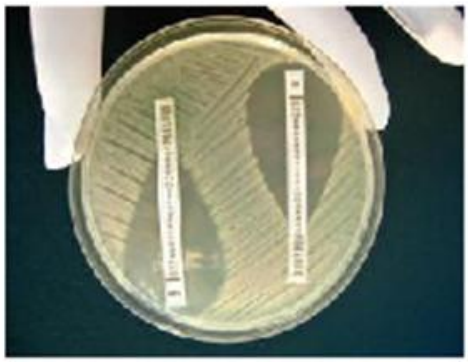
In total, 221 faecal samples were collected directly from the cloaca of free ranging Hooded Crows using sterile cotton swabs than cultured on a blood agar and a bile esculin azide (BEA) agar supplemented with vancomycin. After a 24 h incubation at 37 ºC, each colony showing brown to black colour in the surrounding medium was considered a possible Enterococcus and was transferred onto blood agar (*Figure 1*). After a 24 h incubation at 37 ºC, isolated colonies were identified using the MALDI-TOF (Matrix-assisted laser desorption/ionization – Time of Flight) Mass Spectrometry.

Figure 1: **VRE growth on a bile esculin azide (BEA) agar with vancomycin colonies showing brown to black colour in the surrounding medium (www.microbiologyinpictures.com)**



Vancomycin resistance was confirmed using vancomycin and teicoplanin MICs test strips (Liofilchem, Roseto degli Abruzzi, Italy) placed on Mueller-Hinton agar plates and incubated for 18 to 24h at 37 ºC (*Figure 2*).

Figure 2: **Vancomycin and Teicoplanin MICs test stripes on a Mueller Hinton agar**



RESULTS AND DISCUSSION

None of the Hooded Crow samples yielded VRE indicating that VRE prevalence is very low in the sampled populations in either habitats, although previous reports on VRE carriage in the Corvus species (Oravcoca et al., 2013; Oravcoca et al., 2014) demonstrate that these birds may play a role as reservoirs of VRE.

The emergence of vancomycin resistant bacteria outside hospital settings was first described in farm animals, in the mid-1990s when, VRE strains were isolated from farm animals in several locations in Europe (Ahmed and Baptiste, 2018). This occurrence was shown to be associated with the widespread use of avoparcin in farm animals (Bager et al., 1997). However, in spite of the EU's 1997 prrohibition of avoparcin use, VRE still occur (Borgen et al., 2000; Nilsson et al., 2009). On the other hand, North American countries never approved the use of avoparcin, which could explain their low VRE prevalence in livestock (Simjee et al., 2006; Lanthier et al., 2010) despite being frequent in hospital acquired infections in those countries, suggesting that spread of VRE in animals may be caused by the use of animal growth promoters and not of drug overuse in humans.

More recently, and with the increase nosocomial infections caused by VRE around the world, reports of these microorganisms in wildlife have surfaced. For example, in Canada, a single isolate, out of 100 enterococci sampled from American crows (*Corvus brachyrhynchos*), was found to be resistant to vancomycin in Canada (Oravcoca et al., 2014), while a few more VRE isolates were detected in the USA (Oravcoca et al., 2013). Thus, VREs occurring in wildlife might originate from different human related sources such as wastewater, or landfills, where wild birds are in close contact with potentially human-contaminated objects or foods (Cole et al., 2005; Dolejska et al., 2007; Allen et al., 2016). Subsequently, the prevalence of AMR bacteria in wildlife is generally

DOI: 10.34101/ACTAAGRAR/2/11523

associated with anthropogenic settings (Bonnedahl et al., 2009; Alroy and Ellis, 2011).

Nonetheless, VRE incidence in healthcare facilities is a growing worldwide health concern that requires continuous surveillance efforts. In 2012, a research conducted at the University of Debrecen revealed a low incidence of VRE in Debrecen's hospital settings (Dombrádi et al., 2012), while more recent reports showed an increase (Melegh et al., 2018; Ayobami et al., 2020; Werner et al., 2020). Considering its growing occurrence in the wild (Radimersky et al., 2010; Oravcova et al., 2016, 2017), it is advisable that VRE surveillance efforts should be integrated into the One Health Concept.

Additionally, the Hooded Crow shows a widespread distribution in Hungary, and is frequent within urban environments. It occupies different ecological niches in urban areas and shows a continuous population increase (Kövér et al., 2015).

Moreover, though this study revealed an absence of VRE among free ranging Hooded Crows from both rural and urban areas, Considering the multi-resistance reported in hospital-derived strains and the rise of VRE in wintering rooks in Europe (Oravcova et al., 2013, 2016), a potential environmental spread of VRE is foreseeable, warranting monitoring of urban bird populations.

STUDY LIMITATIONS

Since the present study focuses exclusively on the presence of VRE strains in Hooded Crows. It is highly unlikely to generalize such findings on a large scale when it comes to AMRB. Further investigations on the prevalence of AMRB in different wild species residing urban settlements like city pigeons (*Columba livia*) is urged.

ACKNOWLEDMENTS

The Stipendium Hungaricum Program of the Tempus Public Foundation provided funding for this study under grant number SH-00355-004/2019.

REFERENCES

Abriouel, H.–Omar, N.B.–Molinos, A.C.–Lopez, R.L.–Grande, M.J.–Martinez-Viedma, P.–Ortega, E.–Martínez-Cañamero, M.–Galvez, A. (2008): Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples. Int J Food Microbiol. 123:38e49.

Ahmed, M.O.–Baptiste, K.E. (2018): Vancomycin-resistant enterococci: a review of antimicrobial resistance mechanisms and perspectives of human and animal health. Microbial Drug Resistance, 24(5), 590–606.

Alroy, K.–Ellis, J.C. (2011): Pilot study of antimicrobial-resistant *Escherichia coli* in herring gulls (*Larus argentatus*) and wastewater in the Northeastern United States. J. Zoo. Wildl. Med. 42, 160–163.

Ayobami, O.–Willrich, N.–Reuss, A.–Eckmanns, T.–Markwart, R. (2020): The ongoing challenge of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* in Europe: an epidemiological analysis of bloodstream infections. Emerging Microbes and Infections, 9(1), 1180–1193.

Bonnedahl, J.–Drobni, M.–Gauthier-Clerc, M.–Hernandez, J.–Granholm, S.–Kayser, Y.–Melhus, A.–Kahlmeter, G.–Waldenström, J.–Johansson, A.–Olsen, B. (2009): Dissemination of Escherichia coli with CTX-M type ESBL between humans and yellow-legged gulls in the south of France. PLoS One, 4(6): e5958, doi: 10.1371/journal.pone.0005958.

Buetti, N.–Wassilew, N.–Rion, V.–Senn, L.–Gardiol, C.–Widmer, A.–Marschall, J. (2019): Emergence of vancomycin-resistant enterococci in Switzerland: a nation-wide survey. Antimicrobial Resistance and Infection Control, 8(1), 1–5.

Bager, F.–Madsen, M.–Christensen, J.–Aarestrup, F.M. (1997): Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. Preventive veterinary medicine, 31(1–2), 95–112.

Delpech, G.–Allende, L.G.–Sparo, M. (2019): Mobile genetic elements in vancomycin-resistant *Enterococcus faecium* population. In Pathogenic Bacteria. IntechOpen.

Dolejska, M.–Cizek, A.–Literak, I. (2007): High prevalence of antimicrobial-resistant genes and integrons in *Escherichia coli* isolates from black-headed gulls in the Czech Republic. Journal of Applied Microbiology, 103(1), 11–19.

Dombrádi, Z.–Dobay, O.–Nagy, K.–Kozák, A.–Dombrádi, V.–Szabó, J. (2012): Prevalence of vanC vancomycin-resistant enterococci in the teaching hospitals of the University of Debrecen, Hungary. Microbial Drug Resistance, 18(1), 47–51.

Drobni, M.–Bonnedahl, J.–Hernandez, J.–Haemig, P.–Olsen, B. (2009): Vancomycin-resistant enterococci, point barrow, Alaska, USA. Emerging infectious diseases, 15(5), 838.

Ferrazzi, V.–Moreno Martin, A.–Lelli, D.–Gallazzi, D.–Grilli, G. (2007): Microbiological and serological monitoring in hooded crow (*Corvus corone cornix*) in the region Lombardia, Italy. Italian Journal of Animal Science, 6(3), 309–312.

Fiore, E.–Van Tyne, D.–Gilmore, M.S. (2019): Pathogenicity of Enterococci. Microbiol. Spectr. 7.

Fisher, K.–Phillips, C. (2009): "The ecology, epidemiology and virulence of Enterococcus". Microbiology. 155 (Pt 6): 1749–57. doi:10.1099/mic.0.026385-0. PMID 19383684.

Kövér, L.–Gyüre, P.–Balogh, P.–Huettmann, F.–Lengyel, S., and Juhász, L. (2015): Recent colonization and nest site selection of the Hooded Crow (*Corvus corone cornix* L.) in an urban environment. Landscape and Urban Planning, 133, 78–86.

Kruse, H.–Johansen, B.K.–Rørvik, L.M.–Schaller, G. (1999): The use of avoparcin as a growth promoter and the occurrence of vancomycin-resistant Enterococcus species in Norwegian poultry and swine production. Microb Drug Resist. 5(2):135-9.

Lanthier, M.–Scott, A.–Lapen, D.R.–Zhang, Y.–Topp, E. (2010): Frequency of virulence genes and antibiotic resistance in Enterococcus spp. isolates from wastewater and feces of domestic mammals and birds, and wildlife. Can J Microbiol 56: 715–729.

DOI: 10.34101/ACTAAGRAR/2/11523

Melegh, S.–Nyul, A.–Kovács, K.–Kovács, T.–Ghidán, Á.–Dombrádi, Z.–Szabó, J.–Berta, B.–Lesinszki, V.–Pászti, J.–Tóth, Á.–Mestyán, G. (2018): Dissemination of VanA-Type *Enterococcus faecium* Isolates in Hungary. Microbial Drug Resistance. 24(9), 1376:1390. http://doi.org/10.1089/mdr.2017.0296

Nilsson, O.–Greko, C.H.–Bengtsson, B. (2009): Environmental contamination by vancomycin resistant enterococci (VRE) in Swedish broiler production. Acta Vet Scand 51: 49–55.

Oravcova, V.–Ghosh, A.–Zurek, L.–Bardon, J.–Guenther, S.–Cizek, A.–Literak, I. (2013): Vancomycin-resistant enterococci in rooks (*Corvus frugilegus*) wintering throughout Europe. Environmental microbiology, 15(2), 548–556.

Oravcova, V.–Zurek, L.–Townsend, A.–Clark, A.B.–Ellis, J.C.–Cizek, A.–Literak, I. (2014): A merican crows as carriers of vancomycin-resistant enterococci with vanA gene. Environmental microbiology, 16(4), 939–949.

Oravcova, V.–Hadelova, D.–Literak, I. (2016): Vancomycin-resistant Enterococcus faecium with vanA gene isolated for the first time from wildlife in Slovakia. Veterinary microbiology, 194, 43–47.

Oravcova, V.–Svec, P.–Literak, I. (2017): Vancomycin-resistant enterococci with vanA and vanB genes in Australian gulls. Environmental microbiology reports, 9(3), 316–318.

Pelletier Jr, L.L. (1996): Microbiology of the circulatory system. Medical Microbiology. 4th edition.

Pomba, C.–Rantala, M.–Greko, C.–Baptiste, K.E.–Catry, B.–Van Duijkeren, E.–Mateus, A.–Moreno, M.A.–Pyörälä, S.–Ružauskas, M.–Sanders, P.–Teale, C.–Threlfall, E.J.–Kunsagi Z.–Torren-Edo, J.–Jukes, H.–Törneke, K. (2017): Public health risk of antimicrobial resistance transfer from companion animals, Journal of Antimicrobial Chemotherapy. 72(4), 957–968. https://doi.org/10.1093/jac/dkw481

Radhouani, H.–Silva, N.–Poeta, P.–Torres, C.–Correia, S.–Igrejas, G. (2014): Potential impact of antimicrobial resistance in wildlife, environment and human health. Frontiers in microbiology, 5, 23.

Radimersky, T.–Frolkova, P.–Janoszowska, D.–Dolejska, M.–Svec, P.–Roubalova, E.–Cikova, P.–Cizek, A.–Literak, I. (2010): Antibiotic resistance in faecal bacteria (*Escherichia coli*, *Enterococcus* spp.) in feral pigeons. Journal of Applied Microbiology, 109: 1687–1695. https://doi.org/10.1111/j.1365-2672.2010.04797.x

Ramos, S.–Igrejas, G.–Rodrigues, J.–Capelo-Martinez, J.L.–Poeta, P. (2012): Genetic characterisation of antibiotic resistance and virulence factors in vanA-containing enterococci from cattle, sheep and pigs subsequent to the discontinuation of the use of avoparcin. The Veterinary Journal, 193(1), 301–303.

Sato, G.–Oka, C.–Asagi, M.–Ishiguro, N. (1978): Detection of conjugative R plasmids conferring chloramphenicol resistance in Escherichia coli isolated from domestic and feral pigeons and crows. Zentralbl Bakteriol Orig A. 1978;241:407–17.

Silva, N.–Igrejas, G.–Rodrigues, P.–Rodrigues, T.–Gonçalves, A.–Felgar, A.C.–Pacheco, R.–Gonçalves, D.–Cunha, R.–Patrícia Poeta (2011): Molecular characterization of vancomycin-resistant enterococci and extended-spectrum β-lactamase-containing *Escherichia coli* isolates in wild birds from the Azores Archipelago. Avian Pathology, 40(5), 473–479. DOI: 10.1080/03079457.2011.599061

Simjee, S.–Jensen, L.B.–Donabedian, S.M.–Zervos, M.J. (2006): Enterococcus. In Antimicrobial Resistance in Bacteria of Animal Origin. F.M. Aarestrup (ed.). Washington, DC, USA: ASM Press, pp. 315–323.

Sjölund, M.–Bonnedahl, J.–Hernandez, J.–Bengtsson, S.–Cederbrant, G.–Pinhassi, J.–Kahlmeter, G.–Olsen, B. (2008): Dissemination of multidrug-resistant bacteria into the Arctic. Emerg Infect Dis. 14(1):70-2. doi: 10.3201/eid1401.070704. PMID: 18258081; PMCID: PMC2600168.

Ünal, N.–Bal, E.–Karagöz, A.–Altun, B.–Koçak, N. (2020): Detection of vancomycin-resistant enterococci in samples from broiler flocks and houses in Turkey. Acta Veterinaria Hungarica, 68(2), 117–122.

Wada, Y.–Harun, A.B.–Yean, C.Y.–Zaidah, A.R.–Wada, Y. (2019): Vancomycin-resistant Enterococcus: Issues in human health, animal health, resistant mechanisms and the Malaysian paradox. Adv. Anim. Vet. Sci, 7(11), 1021–1034.

Wada, Y.–Irekeola, A.A.–EAR., E.N.S.–Yusof, W.–Lih Huey, L.–Ladan Muhammad, S.–Harun, A.–Yean, C.Y.–Zaidah, A.R. (2021): Prevalence of Vancomycin-Resistant Enterococcus (VRE) in Companion Animals: The First Meta-Analysis and Systematic Review. Antibiotics, 10 (2), 138. https://doi.org/10.3390/antibiotics10020138

Wang, J.–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.

Werner, G.–Neumann, B.–Weber, R.–Kresken, M.–Wendt, C.–Bender, J.K.–Becker, K.–Borgmann, S.–Diefenbach, A.–Hamprecht, A.–Hogardt, M.–Wichelhaus, T.–Kemp, V.–Huebner, N.–Kaasch, A.–Geginat, G.–Kohnen, W.–Menzer, A.–Krause, T.–Miethke, T.–Pranada, F.–Radojn, F.–Tobisch, S.–Jansen, V.–Regnath, T.–Bührlen, U.–Schneider-Brachert, W.–Schwarz, R.–Luemen, M.–Skov, R.–Thuermer, A.–von Baum, H.–Weig, M.–Uwe, G.–Zabel, L.–von Wulffen, H.–Döring, S. (2020): Thirty years of VRE in Germany–"expect the unexpected": The view from the National Reference Centre for Staphylococci and Enterococci. Drug Resistance Updates, 53, 2020, 100732, ISSN 1368–7646. https://doi.org/10.1016/j.drup.2020.100732.

Willems, R.J.–Top, J.–Van Santen, M.–Robinson, D.A.–Coque, T.M.–Baquero, F.–Grundmann, H.–Bonten, M.J. (2005): Emerg Infect Dis. 11(6):821-8.

Wu, J.–Huang, Y.–Rao, D.–Zhang, Y.–Yang, K. (2018): Evidence for environmental dissemination of antibiotic resistance mediated by wild birds. Front. Microbiol. 9.