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# Absence of vancomycin-resistant enterococci among highly ESBL-positive crows (*Corvus splendens*) foraging on hospital waste in Bangladesh

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***Background***: Vancomycin-resistant enterococci (VRE) have emerged as a growing problem in hospitals; however, domesticated animals, poultry, and wild birds are acting as potential reservoirs. There is a knowledge gap in the Epidemiology of VRE from Bangladesh.
***Methods***: To study the prevalence of VRE and the mechanisms of resistance implicated among wild birds, 238 fecal samples were collected in 2010 from house crows (*Corvus splendens*) foraging on hospital waste in Bangladesh. Fecal samples were screened by analyzing color change in broth and screening for *vanA* and *vanB* resistant genes by PCR.
***Results***: Neither *vanA* nor *vanB* genes were detected from the fecal samples. The house crow does not seem to constitute a reservoir for VRE.
***Conclusion***: The zero prevalence is an indication that foraging on hospital waste does not constitute a major risk of VRE carriage in house crows and this is the first study to focus on the prevalence of VRE from wild birds in Bangladesh.

Keywords: *VRE*; *ESBL*; *house crow*; *hospital waste*; *Bangladesh*

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Different enterococci species are normally found as normal flora in human, mammals, birds, reptiles, and insects (1). The emergence and rapid spread of antibiotic resistance in enterococcus has become a serious public health concern. Vancomycin is a glycopeptide antibiotic, which is considered as the last lines of defense against many multiresistant Gram-positive cocci. Vancomycin-resistant enterococci (VRE) are one of the organisms responsible for hospital-acquired infections with a high morbidity and mortality in humans (2, 3). Resistance to vancomycin is caused by a series of genes (classified by the prefix *van*) that encode an enzyme important for the cell wall. There have been nine genes described in different species of enterococci: *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN* (4–7). However, *E. faecium* and *E. faecalis* carrying *vanA* or *vanB* gene have received the most attention in relation to human health care because of their clinical importance, transferability potential, and abundance in clinical isolates (8–10).

VRE have been reported in the hospitals of many countries throughout the world and spread is associated with poor hospital hygiene practice (8, 11). Different livestock species in different countries have been reported as potential reservoirs for vancomycin-resistant determinants (12, 13). VRE carrying the *vanA* gene were found in wild birds, including glaucous gulls (*Larus hyperboreus*) and wintering rooks (*Corvus frugilegus*) (14, 15). Different livestock species and/or humans were thought to be sources of VRE in free-living wild bird populations (15–17). Previously, we reported the house crow (*Corvus splendens*) and brown-headed gull (*Chroicocephalus brunnicephalus*) living close to human activities and hospital areas as potential carriers of clinically associated extended spectrum beta-lactamases (ESBL)-producing bacteria in Bangladesh (18, 19). There is no information regarding carriage of VRE among these wild birds that were reported to be carriers of ESBL producers.

The aim of this study was to determine the prevalence of VRE in crows foraging in hospital areas of Bangladesh



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and to investigate whether this bird species constitutes a potential reservoir for VRE.

## Materials and methods

In total, 238 fresh fecal samples from house crows (*C. frugilegus*) were collected during February 2010 from the ground areas of Rajshahi Medical College Hospital ($n = 200$) and Chittagong Medical College Hospital of Bangladesh ($n = 38$). Fresh fecal droppings were collected by sterile cotton swabs, which after collection were immediately stored at $-80^{\circ}$C in bacterial freeze media containing Luria–Bertani broth (Becton, Dickinson and Company, Sparks, MD), phosphate-buffered saline, and 4.4% glycerol. Samples were shipped to Sweden for analysis and cold chain logistics were used for shipment. Fecal samples in bacterial freeze media were inoculated by sterile swabs. The swabs were immediately inoculated with the sample into 1 mL bile azide esculin broth and stored at $-70^{\circ}$C for analysis.

Screening for VRE was performed in a selective bile azide esculin broth supplemented with 4 mg/L vancomycin and 60 mg/L aztreonam (ICN Biomedicals, Inc., Aurora, OH), and PCR for *vanA* and *vanB*, according to the methods described previously (20, 21). Briefly, this real-time PCR protocol was used to detect the *vanA* and *vanB* gene. Amplification was conducted in a total volume of 20 μL containing 12.1 μL of PCR water, 4 μL of LC480 M-Mix (2 ×), 0.4 μL of *vanA* F (10 μM) (5′-CGGCAAGA-CAATATGACAGCAA-3′), 0.4 μL of *vanA* R (10 μM) (5′-TCAGTACAATGCGGCCGTTA-3′), 0.4 μL of *vanB* F (10 μM) (5′-GGGAGGATGGTGCGATACA-3′), 0.4 μL of *vanB* R (10 μM) (5′-CCGAAATCGCTTGCTCAA-3′), 0.15 μL of *vanA* prob (10 μM) (5′-HEX-CAGTTA-TAACCGTTCCCGCAGACCTT-BHQ1-3′), 0.15 μL of *vanB* prob (10 μM) (5′-FAM-CTTTGTGAAGCCGG-CACGGTCAGGTT-BHQ1-3′), and 2 μL of the samples. The following cycling parameters were used in the PCR run: 95°C for 5 min (95°C for 10 s, 60°C for 30 s) for 45 cycles. Finally, the amplified PCR product was analyzed.

## Results, discussion, and conclusion

All together 238 fecal samples were analyzed. Surprisingly, none of them demonstrated a color change after testing in the bile azide esculin broth meaning no growth of enterococci was demonstrated. Neither *vanA* nor *vanB* were confirmed through PCR screening. These results indicate that there was no VRE carriage by the house crows in Bangladesh. As the testing in bile azide esculin broth did not demonstrate growth of any enterococci, the findings may be interpreted as a low propensity of crows to carry enterococci in general, and thus also to carry VRE. However, previous findings of VRE among birds of the genus *Corvus* (15, 17) indicate that carriage of VRE is indeed possible, and that crows therefore can be used as sentinels. VRE also reported in different wild bird species like European robins (*Erithacus rubecula*), quail (*Coturnix coturnix conturbans*), Common chaffinch (*Fringilla coelebs*), Blackcap (*Sylvia atricapilla*) and buzzards (*Buteo buteo*) (16, 22). More interestingly, wild birds very close to contact with human activities, for example crows and gulls, are potential carriers of clinically important VRE (14, 17). A study from Bangladeshi hospitals showed a low prevalence (3%) of *E. faecalis* among patients with urinary tract infections (23). Another study from Bangladesh showed that *E. faecalis* is the dominant species among patients with Enterococcus infection and all *E. faecalis* and *E. facium* strains isolated from patients were resistant to vancomycin (24). Thus, VRE is a serious challenge for hospitals; however, there are no reports about the prevalence of VRE from live-stock, poultry, or wild birds in Bangladesh.

The house crow (*C. splendens*) has a widespread distribution in South Asian countries like Bangladesh and India, and it lives close to humans. It occupies different ecological niches, including household areas, city dumps, hospital dumps, and water sources like lakes, ponds, and rivers. The house crow has been shown to carry several human pathogenic bacteria in its intestines (25, 26). A study on these samples demonstrated that 59% of the crows living on hospital waste were potential carriers of clinically relevant ESBL-producing *E. coli* and *Klebsiella* (19), and hospital waste has been reported as a potential source of antibiotic-resistant bacteria (19, 27). These crows were reported as potential carriers of clinical relevant human-associated ESBL-producing *E. coli* ST13-O25b clones, likely because of their foraging behaviors in hospital waste dumps (28). It has also been documented that wild birds can carry VRE and ESBL-producing bacteria simultaneously (16). Thus, the high prevalence of ESBL-producing bacteria among crows living in hospital areas has increased our suspicion about the possible carriage and spread of VRE as well, like in Azores, Portugal (16).

This study indicates the absence of VRE harboring *vanA* and *vanB* genes among crows foraging on hospital wastes even though they were potential carriers of ESBL-producing Enterobacteriaceae (28). Thus, spread through hospital waste via house crows does not seem to be a major mechanism for dissemination of VRE in Bangladesh. However, proper steps should be taken to stop the possible future environmental spread of VRE, as well as other resistant microorganisms, through hospital waste and from hospitals settings in Bangladesh.

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## References


1. Lebreton F, Willems RJL, Gilmore MS. Enterococcus diversity, origins in nature, and gut colonization. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, eds. Enterococci: from commensals to leading causes of drug resistant infection. Boston, MA: Massachusetts Eye and Ear Infirmary; 2014, pp. 1–45.
2. DiazGranados CA, Zimmer SM, Klein M, Jernigan JA. Comparison of mortality associated with vancomycin-resistant and vancomycin-susceptible enterococcal bloodstream infections: a meta-analysis. Clin Infect Dis 2005; 41: 327–33.
3. Graham M, Ballard SA, Grabsch EA, Johnson PD, Grayson ML. High rates of fecal carriage of nonenterococcal vanB in both children and adults. Antimicrob Agents Chemother 2008; 52: 1195–7.
4. Courvalin P. Vancomycin resistance in Gram-positive cocci. Clin Infect Dis 2006; 42(Suppl 1): S25–34.
5. Withey JC, Marzluff JM. Dispersal by juvenile American crows affects population dynamics across a gradient of urbanization. Auk 2005; 122: 205–21.
6. Xu X, Lin D, Yan G, Ye X, Wu S, Guo Y, et al. vanM, a new glycopeptide resistance gene cluster found in *Enterococcus faecium*. Antimicrob Agents Chemother 2010; 54: 4643–7.
7. Nomura T, Tanimoto K, Shibayama K, Arakawa Y, Fujimoto S, Ike Y, et al. Identification of VanN-type vancomycin resistance in an *Enterococcus faecium* isolate from chicken meat in Japan. Antimicrob Agents Chemother 2012; 56: 6389–92.
8. Willems RJ, Top J, van Santen M, Robinson DA, Coque TM, Baquero F, et al. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. Emerg Infect Dis 2005; 11: 821–8.
9. Dahl KH, Mater DD, Flores MJ, Johnsen PJ, Midtvedt T, Corthier G, et al. Transfer of plasmid and chromosomal glycopeptide resistance determinants occurs more readily in the digestive tract of mice than in vitro and exconjugants can persist stably in vivo in the absence of glycopeptide selection. J Antimicrob Chemother 2007; 59: 478–86.
10. Rice LB, Carias LL, Donskey CL, Rudin SD. Transferable, plasmid-mediated vanB-type glycopeptide resistance in *Enterococcus faecium*. Antimicrob Agents Chemother 1998; 42: 963–4.
11. Armeanu E, Bonten MJ. Control of vancomycin-resistant enterococci: one size fits all? Clin Infect Dis 2005; 41: 210–16.
12. Kruse H, Johansen BK, Rorvik LM, Schaller G. 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 1999; 5: 135–9.
13. Ramos S, Igrejas G, Rodrigues J, Capelo-Martinez JL, Poeta P. 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. Vet J 2012; 193: 301–3.
14. Drobni M, Bonnedahl J, Hernandez J, Haemig P, Olsen B. Vancomycin-resistant enterococci, Point Barrow, Alaska, USA. Emerg Infect Dis 2009; 15: 838–9.
15. Oravcova V, Ghosh A, Zurek L, Bardon J, Guenther S, Cizek A, et al. Vancomycin-resistant enterococci in rooks (*Corvus frugilegus*) wintering throughout Europe. Environ Microbiol 2013; 15: 548–56.
16. Silva N, Igrejas G, Rodrigues P, Rodrigues T, Goncalves A, Felgar AC, et al. Molecular characterization of vancomycin-resistant enterococci and extended-spectrum beta-lactamase-containing *Escherichia coli* isolates in wild birds from the Azores Archipelago. Avian Pathol 2011; 40: 473–9.
17. Oravcova V, Zurek L, Townsend A, Clark AB, Ellis JC, Cizek A, et al. American crows as carriers of vancomycin-resistant enterococci with vanA gene. Environ Microbiol 2014; 16: 939–49.
18. Hasan B, Melhus A, Sandegren L, Alam M, Olsen B. The gull (*Chroicocephalus brunnicephalus*) as an environmental bioindicator and reservoir for antibiotic resistance on the coastlines of the Bay of Bengal. Microb Drug Resist 2014; 20: 466–71.
19. Hasan B, Drobni P, Drobni M, Alam M, Olsen B. Dissemination of NDM-1. Lancet Infect Dis 2012; 12: 99.
20. Palladino S, Kay ID, Costa AM, Lambert EJ, Flexman JP. Real-time PCR for the rapid detection of vanA and vanB genes. Diagn Microbiol Infect Dis 2003; 45: 81–4.
21. Kaarme J, Hasan B, Rashid M, Olsen B. Zero prevalence of vancomycin-resistant enterococci among Swedish preschool children. Microb Drug Resist 2015; 21: 65–8.
22. Radhouani H, Pinto L, Coelho C, Sargo R, Araujo C, Lopez M, et al. MLST and a genetic study of antibiotic resistance and virulence factors in vanA-containing Enterococcus from buzzards (*Buteo buteo*). Lett Appl Microbiol 2010; 50: 537–41.
23. Rahman SR, Ahmed MF, Begum A. Occurrence of urinary tract infection in adolescent and adult women of shanty town in Dhaka City, Bangladesh. Ethiop J Health Sci 2014; 24: 145–52.
24. Akhter S, Asna ZH, Rahman MM. Prevalence and antimicrobial susceptibility of enterococcus species isolated from clinical specimens. Mymensingh Med J 2011; 20: 694–9.
25. Aruji Y, Tamura K, Sugita S, Adachi Y. Intestinal microflora in 45 crows in Ueno Zoo and the in vitro susceptibilities of 29 *Escherichia coli* isolates to 14 antimicrobial agents. J Vet Med Sci 2004; 66: 1283–6.
26. Lee HY, Stephen A, Sushela D, Mala M. Detection of protozoan and bacterial pathogens of public health importance in faeces of *Corvus* spp. (large-billed crow). Trop Biomed 2008; 25: 134–9.
27. Sharpe M. High on pollution: drugs as environmental contaminants. J Environ Monit 2003; 5: 42n–6n.
28. Hasan B, Olsen B, Alam A, Akter L, Melhus A. Dissemination of multidrug resistant ESBL-producing *E. coli* O25b-ST131 clone and role of house crow (*Corvus splendens*) foraging on hospital waste in Bangladesh. Clin Microbiol Infect 2015; 21: 1000.e1–4.