



Vancomycin resistant *Enterococcus* spp. from crows and their environment in metropolitan Washington State, USA: Is there a correlation between VRE positive crows and the environment?



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ARTICLE INFO

Article history:

Received 26 October 2015

Received in revised form 20 January 2016

Accepted 28 January 2016

Keywords:

VRE

Crows

vanA

vanB

vanC1

Resistance genes

ABSTRACT

Vancomycin-resistant enterococci [VRE] have been isolated from municipal, hospital and agricultural wastewater, recreational beaches, wild animals, birds and food animals around the world. In this study, American crows (*Corvus brachyrhynchos*) from sewage treatment plants (WWTP), dairy farms, and a large roost in a restored wetland with corresponding environmental samples were cultured for VRE. A total of 245 samples [156 crows, 89 environmental] were collected and screened for acquired *vanA*, *vanB* and/or intrinsic *vanC1* genes. Samples were enriched overnight in BHI supplemented with 20 µg/mL aztreonam, 4 µg/mL vancomycin and plated on m-Enterococcus agar media supplemented with 6 µg/mL vancomycin. Selected colonies were grown on BHI media supplemented with 18 µg/mL vancomycin. Of these, 24.5% of the crow and 55% the environmental/cow samples were VRE positive as defined by *Enterococcus* spp. able to grow on media supplemented with 18 µg/mL vancomycin. A total of 122 VRE isolates, 43 crow and 79 environmental isolates were screened, identified to species level using 16S sequencing and further characterized. Four *vanA* *E. faecium* and multiple *vanC1* *E. gallinarum* were identified from crows isolated from three sites. *E. faecium vanA* and *E. gallinarum vanC1* along with other *Enterococcus* spp. carrying *vanA*, *vanB*, *vanC1* were isolated from three environments. All enterococci were multidrug resistant. Crows were more likely to carry *vanA* *E. faecium* than either the cow feces or wetland waters/soils. Comparing *E. gallinarum vanC1* from crows and their environment would be useful in determining whether crows share VRE strains with their environment.

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1. Introduction

Enterococci are facultative anaerobes that are a part of the normal intestinal microbiota of animals and humans. They have the ability to survive in a wide range of environmental conditions including a variety of temperatures (10–45 °C), pH (4.5–10.0) and high sodium chloride (6.5% w/v) concentrations. Because enterococci survive well in many environments they are used as indicator bacteria for marine and fresh water quality (Beaches Act, 2000). Enterococci are important opportunistic pathogens that are able to cause urinary tract infections, bacteremia, endocarditis and meningitis in humans. Enterococci have a high level of intrinsic antibiotic resistance to low levels of penicillin, cephalosporins and aminoglycosides and have acquired high-level resistance to

vancomycin and/or high-level aminoglycosides. In the Center for Disease Control report (CDC, 2013), VRE was labelled as a “serious threat” bacteria for humans.

The vancomycin-resistant *Enterococcus faecium* in clinical isolates were identified in England and France [1988], followed by vancomycin-resistant *E. faecium* in the United States [1987] (O’Driscoll and Crank, 2015) and urban wastewater in England [1993] (McDonald et al., 1997). The rise of vancomycin resistant enterococci [VRE] in the USA and the rest of the world followed different paths. In the USA, vancomycin was a hospital antibiotic and VRE in the USA was first detected in hospital settings. VRE has now spread beyond the hospital into urban and agricultural environments (Chavers et al., 2003; O’Driscoll and Crank, 2015). The antibiotic avoparcin, a glycopeptide related to vancomycin, was never used in agriculture in the USA. In contrast, in other parts of the world avoparcin was used as a growth promoter for livestock (Tacconelli and Cataldo, 2008). Agricultural use promoted the development of VRE in food animals, which facilitated its

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transmission from animals to farm personnel, the general community and later into the hospital (Bager et al., 1997; O'Driscoll and Crank, 2015; van den Boggard et al., 1997).

VRE is thought to persist in the environment and has been isolated from domestic pets, food animals including poultry, fish, wildlife including birds, wastewater treatment plants, drinking water, and both fresh and marine waters (Araujo et al., 2011; Drobni et al., 2009; Getachew et al., 2013; Iversen et al., 2012; Lozano et al., 2015; Oravcova et al., 2013; Poeta et al., 2005; Roberts et al., 2009; Schwartz et al., 2003; Sellin et al., 2000; Torres et al., 2003). In a previous beach study, VRE was isolated from water and sand samples taken at recreational beaches along the West Coast, including one beach site where there was a large population of pelicans. This site had multiple VRE isolates (Roberts et al., 2009) suggesting there may be a linkage between the presence of birds and VRE contamination in the environment.

The reason we and others have chosen crows (*Corvus brachyrhynchos*) is because they live in urban areas, and have generally benefitted from human enterprise and are distributed throughout North America. Crows are very interactive with human impacted environments (Marzluff et al., 2001). They feed at variety of human refuse sites from garbage dumps to waste water treatment plants (WWTP). At WWTP they drink the potentially VRE contaminated exposed effluent. While at wetlands, and farm crows probe cow feces and soil for insects as well as looking for food in urban and suburban communities. In the past few decades, crows have come to dominate urban and agricultural avifaunas worldwide (Marzluff and Angell, 2005). Increases in crow and other corvids populations has occurred rapidly and recently (e.g., over the last four decades in the western US) and now can be found interacting with humans on all major continents. Thus crows make a good model to look at the emergence of VRE in wildlife that thrives in human impacted environments and the potential relationship between crow carriage of VRE and contaminations of their environments (Marzluff et al., 1998, 2001; Withey and Marzluff, 2005). While the current study was underway, Oravcova et al. (2014b) published on VRE isolated from American crows roost

sites in five USA States. They found that *vanA* carrying enterococci ranged between 0.07% and 6.5% of the enterococci isolated.

In the current study, we were interested in determining what relationship there maybe between crow VRE and VRE isolated from the crow environments. We wondered if both the crows and their environment share the same VRE strains. Sample collection started in 2012, with three sites within metropolitan Seattle WA USA. These included the major waste water treatment facility for the city, small dairy farms in the area and a restored wetland on a university campus with an increasing winter roosting population of crows.

2. Material and methods

2.1. Sample collection

American crows (*Corvus brachyrhynchos*) from the major waste water treatment plant in Seattle WA and on four local dairy farms were captured using a Coda Netlauncher (Coda Enterprises, Inc., Mesa, AZ, USA). One farm was sampled twice. To lure crows, the area was baited with bread or other foods. Captured crows were then placed individually into a dog carrier and faecal material collected in sterile 50 mL tubes. The animals were banded and released. All procedures were permitted by University of Washington Institutional Animal Care and Use Committee Protocol 3077-01, Washington Scientific Collection Permit 11-359, and US Scientific Collection Permit MB761139-1. In the UW Bothell/Cascadia College wetlands, which has up to 20,000 roosting crows in the winter months (Wacker personnel communications 2015), plastic sheeting was placed on the ground beneath the roosting trees in the evening and then individual faecal samples were collected in sterile 50 mL tubes the following morning. This technique is similar to what has previously been described (Oravcova et al., 2013, 2014a,b). In addition to faecal sampling, primary and secondary effluent was sampled and wetland surface water samples were collected at sites 1 and 2 (Fig. 1) multiples times using sterile 1 L bottles. Wetland soil samples were collected



Fig. 1. Location of the three sample sites: site 1 is the wastewater treatment plant (WWTP), site 2 is the restored wetland and site 3 is the location of the dairy farms.

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