

Quantitative Microbial Risk Assessment from Vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* from a specific neighbourhood in Dhaka City, Bangladesh

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With the rise of rapid evolution of therapeutic antibiotic resistance among human pathogens, low-middle income countries are recommended to evaluate the burden of antimicrobial resistance (AMR) among critical pathogens to prioritize disinfection and prescription of effective antibiotics. In this study, we present the level of risk factors among vancomycin-resistant Enterococci (VRE) from urine and blood samples from one neighborhood in Dhaka South area in order to quantify the risk to public health from this particular pathogen. Clinical samples were cultured on bile esculin agar to identify enterococci and then molecular identification was done targeting *ddl<sub>e</sub>* gene using conventional PCR. The PCR-confirmed isolates of enterococci were quantified for major virulence genes (*asa*, *gelE*, *esp*, *hlyA*, *cyt*) in qPCR and were categorically classified into risk levels (hyper-virulent > virulent > quasi-virulent > non-virulent) depending on the number and degree of virulence factors present. The PCR-confirmed isolates were also analyzed with qPCR for presence of AMR associated genetic elements (*vanA*, *vanB*, *vanC/vanR*) and categorically classified into AMR risk levels (high-risk AMR > moderate-risk AMR > low-risk AMR > intermediate-responsive) on the degree of antimicrobial resistance genetic elements expressed. A risk matrix was developed to calculate the cumulative risk score for each isolate. This risk matrix consists of a summation of virulence factors, antimicrobial resistance genes and clonality scores (burden of community-associated vs hospital associated strains). Out of 132 isolates presumed to be pathogenic enterococci on Bile esculin agar, only 15 were PCR-confirmed (positive for *ddl<sub>e</sub>* gene) with *Enterococcus faecalis* ATCC 51299 with control strain. 13% of the isolates were hyper-virulent (positive for all 5 virulence genes *asa*, *gelE*, *esp*, *hlyA*, *cyt*) and moderate-risk AMR (vancomycin-resistant in disc-diffusion and positive for *vanB* and *vanC/vanR*), 40% were virulent (positive for *asa*, *gelE*, *hlyA*) and moderate-risk AMR (vancomycin-resistant in disc-diffusion and positive for *vanB* only) and 47% were quasi-

virulent (positive for at least 2 virulence genes) and low-risk AMR (vancomycin-intermediate resistance in disc-diffusion test and low-level expression of *vanB* gene). 54% of the isolates were hospital-associated and the rest showed inconclusive results in clonality analysis (RFLP of *adk*, *gydI* and *pst* gene cluster). Taken together, the densely-populated neighborhood-of-interest in Dhaka South shows mild-to-moderate risk of hospital-associated infection from vancomycin-resistant Enterococcal pathogens.