Title of dissertation				
Drug Resistance Determinants in Clinical Isolates of Enterococcus faecalis in Bangladesh: Identification of Oxazolidinone Resistance Gene optrA in ST59 and ST902 Lineages				
RONPAKU Fellow				
Name	**Sangjukta ROY**			
Position	Lecturer		ID No.	R11817
Department	Microbiology			
Institution	Mymensingh Medical College		Nationality	Bangladesh
Japanese Advisor				
Name	Nobumichi KOBAYASHI			
Position	Professor	Institution	Sapporo Medical University	

Enterococcus is ubiquitously distributed in the environment and constitutes the normal flora of the intestinal tract in humans and animals. However, among this genus, particularly *Enterococcus faecalis* has been recognized as one of the common opportunistic pathogens implicated in urinary tract infection, wound/surgical site infection, and bloodstream infection. *E. faecalis* has an ability to acquire resistance to several antimicrobials such as glycopeptides, while it exhibits intrinsically reduced susceptibility to various antibiotics including cephalosporins. However, in Bangladesh, only limited information is available for the drug resistance of *Enterococcus*. The objective of this study was to determine the prevalence of drug resistance and its genetic determinants for *E. faecalis* clinical isolates in north-central Bangladesh.

In this study, a total of 210 *E. faecalis* clinical isolates from the urine specimens of patients with urinary tract infections were analyzed. These isolates were collected in Mymensingh Medical College (MMC) hospital and Swadesh private hospital in Mymensingh, Bangladesh, consecutively, for a 15-month period starting from January 2018. Chromogenic agar plate was used for bacterial culture and species was confirmed genetically by the analysis of PBP5 gene or 16S rRNA gene. Susceptibility to antimicrobials and minimum inhibitory concentration (MIC) was measured by broth microdilution test. The presence of drug resistance genes was examined by uniplex or multiplex PCR. IS*256*-flanking pattern of aminoglycoside modifying enzyme (AME) gene *aac(6')-Ie-aph(2")-Ia* genes was assigned by PCR assay. Isolates exhibiting non-susceptibility to linezolid (LZD) (MIC, ≥ 4 μg/mL) were analyzed for the presences of *cfr*, *fexA*, *optrA* by PCR, followed by further gene sequencing. Sequence type (ST) of *E. faecalis* based on the MLST scheme was identified by sequencing of seven loci of housekeeping genes, with the use of web-based genotyping tool PubMLST.

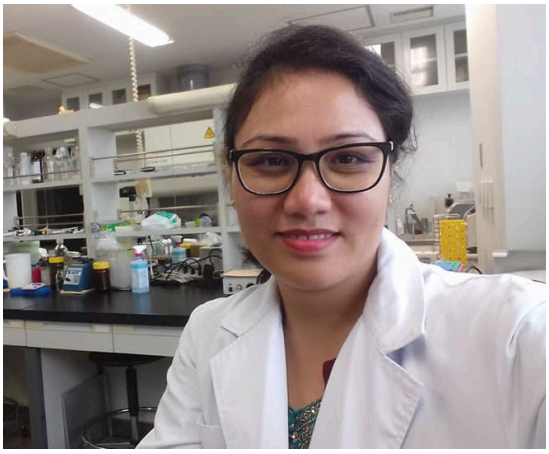
Among the 210 *E. faecalis* isolates, the resistance rates to erythromycin, levofloxacin, and gentamicin (high level) were 85.2%, 45.7%, and 11.4%, respectively, while no isolates were resistant to ampicillin, vancomycin and teicoplanin. The most prevalent resistance gene was *erm(B)* (97%), and any of the four AME genes were detected in 99 isolates (47%). The AME gene *aac(6')-Ie-aph(2")-Ia* was detected in 46 isolates (21.9%), which were classified into four IS*256*-flanking patterns A, B, C, and D (9, 7, 7, and 23 isolates, respectively) of this gene. The pattern A having IS*256* at both sides, and pattern B having IS*256* at only upstream of *aac(6')-Ie-aph(2")-Ia* showed high-level resistance to gentamicin, while

patterns C and D were more associated with low-level resistance. Tetracycline resistance was ascribable to *tet(M)* (61%) and *tet(L)* (38%), and mutations in the quinolone resistance-determining region of both GyrA and ParC were identified in 44% of isolates. Five isolates (2.4%) exhibited non-susceptibility to LZD, and harbored the oxazolidinone resistance gene *optrA*, as well as *fexA*, phenicol exporter gene located closely to *optrA*. The *optrA*-positive isolates belonged to ST59, ST902, and ST917 (CC59), while common lineages of other multiple drug-resistant *E. faecalis* isolates were ST6, ST28, CC16, and CC116.

Nucleotide sequences of the *fexA–optrA* cluster from the five isolates were identical to that reported for *E. faecalis* strains in Taiwan. Compared with the OptrA prototype, deduced amino acid sequences of *optrA* (655 amino acids) in our study had three divergent amino acids (K3E, Y176D, G393D) (EDD variant) which was reported in China. Among the five *optrA*-positive isolates, strain SJ82 was further analyzed for the broader region containing *fexA–optrA* cluster and compared with published sequences in GenBank database. Whole region of strain SJ82 *fexA–optrA* cluster was found to have novel genetic organization, while it was most similar to a clinical strain in Taiwan, and similarlity in upstream or downstream of *fexA–optrA* cluster was found with those of *E. faecalis* strains from human or pigs in China, USA, Norway, Denmark, etc.

The present study first revealed the prevalence of drug resistance determinants of *E. faecalis* and their genetic profiles in Bangladesh. It was remarkable that oxazolidinone resistance-determinant *optrA* was identified in *E. faecalis* isolates showing non-susceptibility to LZD, although these isolates were derived from urinary tract infections without the use of this antimicrobial for treatment. Although the non-susceptibility rate to LZD of enterococci was reported as < 1% at the global level, detection rate in the present study in Bangladesh (2.4%) represents higher prevalence and was comparable to that in China (~3.9%). It was confirmed that *optrA*-cluster of *E. faecalis* in Bangladesh contains resistance genes to other antimicrobials, e.g., chloramphenicol and macrolides. Therefore, by the use of these antimicrobials in human or animals, *E. faecalis* harboring *optrA* is suggested to be selectively spread. Because LZD is one of the limited choices for threatment of severe infections with MRSA and vancomycin-resistant *Enterococcus*, *optrA*-positive isolates may increase the potential risk in healthcare settings. Present study indicated the need for further epidemiological investigation of antimicrobial resistance in *E. faecalis*. This research of thesis was published in Microorganisms (2020, 8:1240).

Photos



In the laboratory, department of Hygiene, Sapporo Medical University

Discussion with supervisors after remote thesis defense (Feb.16, 2022)