CHAPTER 7

CONCLUSIONS
Four hundred forty three enterococci were isolated from over 10,765 clinical samples (stools and urine) from patients attending PGIMER, Chandigarh. Majority of the enterococcal isolates were found to *E. faecalis* followed by *E. faecium, E. gallinarum* and *E. casseliflavus*. Though *E. faecalis* and *E. faecium* were isolated from urine as well as stool samples, but *E. gallinarum* and *E. casseliflavus* were isolated only from stool samples.

Vancomycin resistance enterococci was found in 36 isolates belonging to *E. faecium, E. faecalis, E. gallinarum* and *E. casseliflavus*.

Vancomycin resistance varied from 6 to ≥ 254 μg/ml amongst VRE. High vancomycin resistance (present in *E. faecium*) was associated with high resistance to teicoplanin.

Four genotypes of vancomycin resistance were present in enterococci and these were: *vanA, vanB, vanC1* and *vanC2*. *vanA* genotype was associated with high vancomycin resistance. DNA sequencing of *vanA* genes showed homology with other *vanA* genes reported in literature e.g. *E. faecium* transposon Tn1546 (M97297) as well as *E. faecium* plasmid PUW786 (AF516335) and *E. faecium* plasmid pIP816 (X56895). *vanB* gene showed 99% homology with *E. faecium* strain MLG856-2 D-alanine:D-lactate ligase (*vanB*) gene (AY655711), and 97% identity with *E. faecalis* vancomycin resistance protein (*vanB*) gene (EFU72704) and 96% identity with *E. faecalis vanB* gene (AY665551). *vanC1* gene showed one significant match of 99% homology with *E. gallinarum vanC* vancomycin-resistance gene cluster (AF162694), as well as *E. gallinarum* strain NO4-0414 vancomycin resistance gene cluster (DQ022190). *vanC2* sequence exhibited 97% homology with *E. casseliflavus vanC2* genes (AB070704), (AB070698) and 96% homology with *E. casseliflavus vanC2* gene (AB070703).

Very limited data is available from India on VRE. The results of the present study also reflect that transposon or plasmid mediated phenotypes like VanA and VanB, conferring moderate to high level resistance to vancomycin are not frequently encountered in rectal colonization of Indian patient samples when compared to studies carried out in Europe and USA where such strains are rampant. Most of our rectal colonization isolates had intrinsically resistant phenotypes like VanC1 and VanC2. Hence till now there
are very few patients in our hospital who carry transferable drug resistant enterococci in the intestine whereas 4% of urine isolates were VRE. In majority of these patients, UTI was nosocomial in origin. Prolonged hospital stay, renal failure, dialysis, third generation cephalosporins and aminoglycosides use emerged as important risk factors for UTI. In contrast to studies from West where colonization is much more common than infection, in our study it was the opposite. It is a known fact that VRE may be transferred through contaminated hands of health care workers and fomites. We also suspect this in our patients. Molecular epidemiologic studies like PFGE would have shed more light on this aspect. Since the problem is just beginning, it is important to institute control measures so that it can be tackled in the beginning only. Once VRE are established in the hospital environment, their frequent resistance to multiple antibiotics makes it difficult to avoid further selective pressure in their favor.

Therefore following control measures are recommended:

(i) Early detection of VRE colonization in patients admitted in high risk areas like transplant units, ICUs and oncology units.

(ii) Since vancomycin is used in gastroenterology ward for treating antibiotic associated colitis, there is a need to watch this reservoir of infection.

(iii) Isolation of a patient who has VRE colonization and develops diarrhea to restrict further spread of infection.

(iv) Strict barrier precautions and improved hand hygiene practices for a VRE colonized/ infected patient.

(v) Improved cleaning and disinfection of high-risk areas to decrease the spread of VRE.

(vi) Excessive usage of broad-spectrum antibiotics to be discouraged.

The present study showed for the first time van genes sequencing of clinical isolates in India.