VII.

SUMMARY AND CONCLUSIONS
VII. A. SUMMARY

The enterococci are a complex, diverse, important but neglected organism in clinical laboratories. They are normal flora and are also important nosocomial pathogens. In the present study, the highest prevalence of enterococcal infections was seen in neonates and in the age group of 51-70 years. No significant difference was found between males and females regarding enterococcal infections. The enterococcal urinary tract infection was more common in females (39 cases) in reproductive age group (18-40 years) than the males (15 cases).

Among the 9000 clinical specimens screened, enterococci were isolated from 244 (2.7%) cases. Maximum isolates were obtained from urine samples followed by pus exudates, blood and peritoneal fluid. The enterococci were commonly isolated from UTI followed by wound infection including post surgical wounds and neonatal sepsis. A high incidence of urinary tract infections is seen among patients with amenorrhea, prolonged hospitalized patients with catheterisation and hypertension, cephalosporins therapy and who have structural abnormalities.

Enterococci were more commonly isolated from post operative abscess, pyogenic skin infection (on buttock) or road traffic accident. Common risk factors for enterococcal blood stream and cerebral infection enterococcal are wound or genitourinary infection, biliary portals complications and underlying heart diseases. Either endogenous or exogenously colonised (by contaminated medical equipments) enterococci are resulting in nosocomial infection. E. faecalis causes catheter related blood stream infection as well as it is responsible for biofilm formation.

Enterococcal neonatal sepsis (with/out septicaemia and meningitis) is very common in neonates. More commonly occurs in infants with low-birth weight, premature infants with severe underlying conditions. Furthermore, many of the cases were maternally or hospital acquired infection. Enterococci are also responsible for peritonitis and rarely biliary tract infection and pleural effusion. Intra-abdominal or pelvic wound infections and other wound cultures are frequently polymicrobial but in UTI, blood stream infection and peritonitis enterococci remain mono-microbial.

There is very low index of suspicious observation for enterococcal colonies. A high
incidence of $\beta$-haemolytic strains of *E. faecalis* and *E. faecium* was noted. The enterococci were commonly misidentified as coagulase negative staphylococci, streptococci or
micrococci. Even Vitek 2 automated machine was unable to identify all species (1.2%) of *Enterococcus*. The correct identification of enterococci was possible by just including modified bile esculin test for preliminary identification.

*E. faecalis* (77%) was found to be the predominant isolate from all clinical specimens, followed by *E. faecium* (17%). The high incidences of *E. faecium* was noted in patients with nosocomial infections and were recovered more from burn wounds, surgical wounds, foley’s catheters and umbilical stump.

The incidence of infections with other *Enterococcus* species (6%) such as *E. durans*, *E. avium*, *E. raffinosus*, *E. hirae*, *E. gallinarum*, *E. mundtii* and *E. casseliflavus* has increased especially from patients who are either chronically ill, immunosuppressed or hospitalised patients. Now days, various new sophisticated identification tools (e.g. Vitek 2) are available to identify such unusual species.

Enterococci are intrinsically resistant to many antibiotics. They have a remarkable ability to survive in an environment of heavy antibiotics and become multi drug resistant. They acquire resistance to high level penicillin, aminoglycosides and vancomycin. HLAR is due to release various aminoglycoside modifying enzymes. Treatment of infections caused by multi-drug resistant *E. faecium* (VRE) need careful re-evaluation of in-vitro susceptibility data.

All the enterococcal isolates were subjected for antibiotic susceptibility testing by Kirby Bauer disc diffusion method. *E. faecium* was significantly (statistically) more resistant to penicillin (>88%) than *E. faecalis* (30 to 42%). The penicillin and ampicillin need not be tested separately. We also recommend that β-lactamase inhibitor combinations like amoxicillin with clavulanic acid, ampicillin with sulbactum, piperacillin with tazobactum are more effective than a single antibiotic. Any one of these can be included for testing.

Penicillin resistance has increased in recent years. Penicillin and ampicillin resistance is usually intrinsic, is primarily due to low affinity of the penicillin binding proteins and it results in loss of synergistic effect between β-lactams and aminoglycosides leading to treatment failures. The *Enterococcus faecalis* is expressing β-lactamase enzyme and high level resistance to penicillin (HLPR) and ampicillin (MIC > 256 µ g/ml) have been reported from various locations. However, when the organism is β-lactamase positive, it should be considered resistant to beta-lactam drugs.
In the present study, enterococci were resistant to ciprofloxacin (85%), azithromycin (84%), erythromycin (82%), tetracycline (75%), gatifloxacin (66%), doxycycline (48%) and rifampicin (34%). *E. faecium* was significantly more resistant than *E. faecalis* to all antimicrobial agents except tetracycline and doxycycline. More than 90% *E. faecium* were resistant to erythromycin, pristinomycin, ciprofloxacin and penicillin however; less than 90% isolates of *E. faecalis* were resistant to these antibiotics. Nevertheless, doxycycline resistance was significantly lesser in *E. faecium* (29%) than *E. faecalis* (36%). Glycylcyclines have outstanding activity against enterococci including multidrug-resistant strains. Rifampicin is useful against multidrug resistant enterococci and more significantly active against *E. faecalis* (74%) than *E. faecium* (29%). Gatifloxacin and clinafloxacin are more active than ciprofloxacin and new fluoroquinolones against enterococci.

All the species of enterococci were more sensitive (>94%) to nitrofurantoin, fosfomycin, vancomycin and linezolid. Compared to all other tested antibiotics these antibiotics are more effective and the difference is statistically significant [P<0.005].

Vancomycin resistance is the most recent and disturbing resistance reported in enterococci. It has been progressively reported from all parts of the world (still less in India). Van A phenotype (2 strains) is more widely distributed and thus the predominant type of resistance reported. In addition, vancomycin resistance has seen commonly in *E. faecium*, which is inherently more resistant to multiple drugs. VRE isolates are usually resistant to multiple drugs but may be susceptible to linezolid, nitrofurantoin and fosfomycin. Vancomycin resistant *E. faecium* was susceptible to tetracycline and doxycycline.

Enterococci are becoming gradually more resistant to conventional antibiotic therapy. In addition to high-level aminoglycoside resistance and ampicillin resistance, vigorous spread of vancomycin resistance has resulted in restricted therapeutic options. Treatment of infections caused by VRE, especially *E. faecium*, is really challenging.

Occurrence of VRE is increasing in the United States and Europe. Monitoring is needed in India, since it is an emerging pathogen; not today but in future it may create significant problem in clinical practice. It is crucial to optimize the microbiology laboratories to detect accurately and timely vancomycin resistant enterococci by recommended techniques. We recommend that disc diffusion is not satisfactory method for reporting VRE. The agar screen appears to be the most reliable and easy method for routine screening; other
techniques such as agar dilution, Hi-combi, Vitek 2 or E-test techniques can be used for confirmation of VRE.

Infective endocarditis, UTI and any infection of a sterile site with VRE should be treated aggressively. Linezolid and quinupristin-dalfopristin (against *E. faecium* only) are most likely to be active, but tetracycline and chloramphenicol may be considered for treatment. Linezolid is more versatile drug. Linezolid has been available as intravenous as well as oral preparations. It has been approved by various committees and has shown excellent activity against multi drug resistant enterococci including VRE. It is active against both *E. faecalis* and *E. faecium*. Vancomycin and teicoplanin are reserved drugs, fosfomycin and nitrofurantoin can be recommended only for urinary tract infection while linezolid drug of choice for other infections.

Combination of cell wall active agent plus aminoglycoside will improve the outcome of enterococcal infection, such as meningitis or endocarditis especially with VRE. New approaches of combination therapy (synergy) are under trial for the treatment of VRE infections. The uptake is increased (facilitated) by cell wall active agent (like penicillin), which is effective synergistically. Only streptomycin, gentamicin, and occasionally, kanamycin should be considered for synergy screening with penicillin or other cell wall active agents.

High level gentamicin and streptomycin resistance among *E. faecium* were significantly higher than *E. faecalis*. The resistance to aminoglycosides in enterococci is often associated with multidrug resistance. Association between disc diffusion and agar dilution as well as broth dilution method for detecting HLAR was statistically extremely significant. These methods, therefore, offers a convenient technique by which many laboratories would be able to screen clinically significant *E. faecalis* but not to *E. faecium* isolates for synergy resistance. Some incidence of false resistance and false susceptibility occurred when various media, method or inoculum sizes were used.

High level gentamicin and streptomycin resistance was more in penicillin resistant strains than penicillin susceptible by all the methods. But striking observation was HLAR was found in penicillin susceptible strains up to 20% to 35%. So, there was no good correlation between penicillin susceptibility and HLAR pattern. There was no correlation found between high level aminoglycoside resistance by disc diffusion, agar dilution and broth
dilution methods with antibiotic exact synergy by time kill curve and combined disc diffusion methods.

Two most common methods used for determining exact synergy, are checkerboard and time kill curve test. These are, however, too cumbersome, time consuming and labour intensive for routine use in laboratories. Penicillin and aminoglycoside combined disc and time kill curve technique with routine achievable concentration in human body (antibiotic synergy) have a very good correlation. MIC ranges of penicillin and antibiotic synergism of penicillin with aminoglycoside is negatively correlated with each other. Penicillin and aminoglycosides combined disc technique, therefore, offers an easy, convenient, require less time and cost effective for HLAR and Kirby Bauer disc diffusion method for routine screening test for enterococci in laboratories. We are recommending combined disc method for routine laboratory testing and time kill curve method for its confirmation but yet these methods need standardisation.

VRE is multidrug resistant enterococcal infection emerging in India especially as nosocomial pathogens in urban, tertiary care as well as teaching hospitals. The development of new and rational drug designs, offers the prospect of effective bactericidal monotherapy for enterococci, including VRE. Wiser use of antimicrobial drugs, possibly guided by novel techniques for rapid microbiological diagnosis, and the nascent trend towards the development of narrower-spectrum antimicrobials may diminish some of the selective pressures favouring VRE. Novel therapies, such as vaccine-based immunotherapy, phage therapy, and gene therapies to reverse drug resistance, may offer long-term solutions to the problem of VRE.

We have focused on the emergence of enterococcal antimicrobial resistance by various techniques, which have been less often in E. faecalis and were detected most often in E. faecium. Empiric therapy for enterococcal infections should be guided by local patterns of drug resistance. In addition, clinical investigations are needed to clarify the current strategies to prevent and control the spread of vancomycin resistance, inclusive of cost effectiveness that can substantiate such recommendations. We encourage performing susceptibility testing by accurate techniques for all enterococcal isolates recovered from patients while they are receiving antimicrobial therapy for serious infections like enterococci and staphylococci.
CONCLUSIONS

1. The maximum numbers of enterococci were isolated from neonates and in the age group of 51-70 years.

2. The Females (among reproductive age group) were commonly affected by enterococcal urinary tract infection.

3. Incidence of enterococci in various specimens was 2.7%.

4. The enterococci were most commonly isolated from urinary tract infections followed by wound infection including surgical wounds and neonatal sepsis.

5. Enterococcal UTI was more common in patients with amenorrhea, catheterisation and hypertensions.

6. Enterococcal wound infection was more common in post operative abscess, pyogenic skin infection (especially on buttock) or wound due to road traffic accident.

7. Enterococcus faecalis was the most common species isolated followed by E. faecium, E. durans, E. avium, E. raffinosus, E. hirae and E. gallinarum.

8. E. faecium isolates were significantly more resistant than E. faecalis to most of the antibiotic except doxycycline and tetracycline.

9. Only 2% of penicillin resistant enterococcal isolates were β-lactamase producer.

10. Vancomycin resistance was less (1%) in the present study.

11. E. faecium isolates were significantly more resistant than E. faecalis to HLA.

12. There was statistically significant correlation among high content disc diffusion, agar dilution and broth dilution for detection of HLAR.

13. HLAR was significantly more in penicillin resistance enterococci.

14. There was no correlation between MIC of penicillin and HLAR.

15. Among the penicillin resistance enterococci, 7.26% isolates were susceptible to penicillin and aminoglycoside synergy by TKC and combined disc method.

16. There was negative correlation between penicillin MIC and synergy with aminoglycoside.

17. There was no correlation between HLAR and antibiotic synergy by time kill curve test.