CHAPTER – 7

SUMMARY

➢ Of the 111 Enterobacteriaceae study isolates 60.4% (67/111) carried at least one the carbapenemase encoding gene. The most common species was *Klebsiella pneumoniae* - 29.7% (33); *Escherichia coli* - 11.7% (13), *Citrobacter freundii* - 9% (10); *Enterobacter cloacae* - 9% (10); *Providencia rettgeri* - 0.9% (1)

➢ The carbapenemase producers were mostly recovered from patients in the Intensive care units – 92.5% (62/67)

➢ They are associated with a multitude of infections such as urinary tract, bloodstream, respiratory and skin and soft tissue infections

➢ Of the 179 Non fermenting gram negative bacilli (NFGNB), carbapenemase encoding genes were detected in 85% (It included *Acinetobacter baumannii* - 62% (111); *Pseudomonas aeruginosa* - 21.8% (39); 0.6% (1) each of *Acinetobacter lwoffii* and *Pseudomonas stutzeri* (1).

➢ Most isolates were recovered from critically ill patients with lower respiratory tract infections

➢ Antimicrobial susceptibility to other classes of antimicrobial agents

  - Enterobacteriaceae: All the study isolates were resistant to the III generation cephalosporins, aztreonam, cefepime, piperacillin-tazobactam and ciprofloxacin. Resistance to amikacin was 76.6%. Resistance to imipenem and meropenem was encountered only in 45%
of the study isolates applying the CLSI –M100-S21 guidelines. Susceptibility to colistin and tigecycline was universal.

- **NFGNB**: All the 179 study isolates were resistant to ceftazidime, pipercillin-tazobactam, imipenem, meropenem, amikacin and ciprofloxacin. All *P. aeruginosa* and the lone *P. stutzeri* were resistant to aztreonam. 91.8% of *P. aeruginosa* and 97.4% of *A. baumannii* were susceptible to colistin. Among *A. baumannii* 93.1% remained susceptible to tigecycline. The lone *A. lwofii* was susceptible to both tigecycline and colistin.

- **Phenotypic methods**

  - **Enterobacteriaceae**: All the 111 Enterobacteriaceae were modified Hodge test (MHT) positive as this was a criteria for inclusion in the study. Of them screen tests for Metallo betalactamas (MBL) was positive in 48.6% and screen test for Klebsiella pneumoniae carbapenemase (KPC) was positive in 32.4%.

  - **NFGNB**: The MHT was positive in 94.4% of the study isolates and the MBL screen test in 80.4%.

- **Distribution of carbapenemase encoding genes in Enterobacteriaceae**

  - The most common gene was *bla*$_{NDM}$ (57.7%), followed by *bla*$_{VIM}$ (6.3%) and *bla*$_{OXA-181}$ (1.8%). Notably KPC and other types of MBLs namely IMP, SIM, SPM, GIM were not detected in any of the study isolates.
Of concern is the fact that 27 NDM producers remained susceptible to carbapenems as per CLSI-M100-S21 criteria. Carbapenem resistance determination cannot be reliably predicted based on the MIC breakpoint criteria. Screening and/or confirmatory test including molecular testing is required for adequate detection of carbapenemase production. This implies that all III generation cephalosporin resistant Enterobacteriaceae should be tested for carbapenemase production.

The MBL screen test was negative in a substantial proportion (18/27) of NDM producers (66%). All The VIM producers were MBL screen test positive. Hence the overall sensitivity and specificity of MBL screen test is 67.7% and 78.3% respectively.

In a proportion of the MHT positive isolates (44/111), none of the carbapenemase encoding genes looked for were detected which may be attributed to false positivity. The possible mechanisms could be CTX-M production and/or hyperproduction of Amp C together with porin loss as all these isolates exhibited positive ESBL and Amp C screen test.

Distribution of carbapenemase encoding genes in P. aeruginosa

Of the carbapenem resistant P. aeruginosa, 63.9% carried one or more of the MBL encoding genes. The most common gene was bla\textsubscript{VIM} (55.7%), followed by bla\textsubscript{NDM} (6.5%) and bla\textsubscript{IMP} (1.6%). Coexistence of multiple MBL genes was seldom encountered. The other MBLs namely, SIM, GIM and SPM were not detected in this study.
- MHT had good correlation in the presence of MBL gene with 100% sensitivity. The MBL screen test however was negative in one isolate thus reducing its sensitivity to 97.4%

- In 36.1% of carbapenem resistant *P. aeruginosa*, none of the genes looked for were detected indicating the presence of novel carbapenemase encoding genes and/or non-carbapenemase mediated mechanisms such as porin defects and upregulation of efflux pumps.

- Distribution of carbapenemase encoding genes in *A. baumannii*

- Almost all the carbapenem resistant *A. baumannii* study isolates carried one or more carbapenemase encoding gene (95.7%). Majority of them carried the OXA gene (91.3%) with OXA-23 like and OXA-51 like being the most common types. MBL encoding gene was detected in 58.6%. The MBL genes detected were *bla* <sub>VIM</sub> (46.5%), *bla* <sub>NDM</sub> (11.2%) and *bla* <sub>IMP</sub> (0.9%).

- The sensitivity of MHT and MBL screen test was 100%.

- In a minor proportion of the *A. baumannii* study isolates (4.3%), none of the genes were detected. The possible mediating mechanism in these may be upregulated efflux pumps or loss of porins.

- Overall the most common type of carbapenemase in Enterobacteriaceae is the NDM; in *P. aeruginosa* it is VIM while in *A. baumannii* it is the OXA.

- In contrast to Enterobacteriaceae, among NFGNB resistance to carbapenems as determined by disc diffusion testing or MIC reliably predict the presence of carbapenemase encoding gene.
PCR remains the gold standard for the accurate detection of carbapenemase producers.

Lack of universal susceptibility to tigecycline and colistin among NFGNB is a cause for concern. These drugs also have their own limitations and are not indicated as the drug of choice in a variety of clinical conditions.

The carbapenemase producing strains in this study are clonally diverse. However a few of the MBL producing A. baumannii and NDM producing K. pneumoniae had evidence of clonal relatedness, thus indicating the possibility of horizontal transfer between patients or a common environmental source. The extensive clonal diversity implies a strong selection pressure and substantially representative of divergent acquisition of multiple clones with limited dissemination between patients.