CHAPTER 7
CONCLUSIONS
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In the present study higher resistance rates were observed in our collection of Enterobacterial isolates. Among third-generation cephalosporins, 100% isolates were resistant to cefotaxime, followed by resistance to fourth-generation cephalosporins. In our collection fluoroquinolones also showed higher resistance rates such as 88.4% to ofloxacin but comparatively lower resistance rates to aminoglycosides was noticed. Resistance rates and patterns to third- as well as fourth-generation cephalosporins denotes the presence of class A and class C type β-lactamases in this collection of North Indian Enterobacteriaceae.

All cefotaxime resistant isolates were found as ESBL producers on the basis of any of the three detection methods such as double disk synergy test (DDST), combination disk test (CDT) and modified three-dimensional extract test (MTDET). In the present study we found CDT as the best detection method for identification of ESBL producers.

In our Enterobacterial collection, on molecular basis we found 93% (121/130) occurrence for blAESBLs which included blacTX-M 92.5% (112/121) as the predominant type of ESBL gene followed by blatEM 54.5% (66/121) and blashv 45.4% (55/121). 62.8% isolates of this collection of Indian Enterobacteria showed co-occurrence of blacTX-M with blatEM or blashv or with both of these genes. Out of total blAESBLs harbouring isolates, 29.7%
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(36/121) were harbouring single gene of \( \text{bla}_{\text{CTX-M}} \) while only 3.3% (4/121) and 2.4% (3/121) isolates harboured single gene of each \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{SHV}} \), respectively. This shows that in our collection higher occurrence of multiple genes has occurred due to the selection pressure developed by increasing usage of cephalosporins.

- In the present study, by multiplex PCR, we found CTX-M-genogroup-1 as the predominant genogroup, but for the first time we also observed CTX-M-genogroup-9 in one \( \text{E. coli} \) isolate that was confirmed by sequencing. This is showing that other CTX-M types are also emerging in Indian Enterobacteria.

- Due to the presence of resistance to third- as well as fourth- generation cephalosporins in our isolates, of the ESBL positive isolates, 71.9% (87) also showed co-existence of \( \text{bla}_{\text{ampC}} \) with \( \text{bla}_{\text{ESBLs}} \). Moreover, in 112 isolates that harboured \( \text{bla}_{\text{CTX-M}} \) genes, 75% showed co-existence of \( \text{bla}_{\text{ampC}} \). This shows that in our collection of Indian Enterobacteriaceae class C type beta-lactamases is concurrently present with class A type beta-lactamases on the same plasmid.

- On comparative studies of class A and class C type \( \beta \)-lactamases in the Enterobacterial collections during 2009 and 2010, we found nearly similar occurrence of \( \text{bla}_{\text{ESBLs}} \) in 92.1% and 94.4% isolates in the respective years, 2009 and 2010. On characterization of co-existence of class A and class C type \( \beta \)-lactamases, we found that co-existence of these classes decreased from 81.4% to 58.8% in the respective years, 2009 and 2010. This is showing
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improvement of the prescription policies of antibiotics as well as the implementation of straight infection control policies in our Institution.

- On determination of various mobile genetic elements (sul-1, CS region, ISEcp1, IS26 and ORF513), we found 89.2% (116/130) isolates carrying these mobile genetic elements with various bla genes and passively help in their mobilization. 92.8% isolates that harboured bla\textsubscript{CTX-M} genes were found to be associated with these mobile genetic elements. We noticed higher number of isolates harbouring combination of CTX-M-TEM-SHV that were associated with these mobile genetic elements for mobilization.

- We also found 4.6% isolates which harboured only gene of bla\textsubscript{CTX-M} but not associated with any mobile genetic elements, and passively in these cases bla genes were mobilized through plasmids.

- 76.1% (99/130) isolates were found to harbour integrons (sul-1 and CS regions) and showed higher association of bla genes with sul-1 type class-1 integrons than CS region. This denotes higher occurrence of classic class-1 integrons in our collection as previously reported by Partridge \textit{et al.} (2009) where it is being reported that presence of sul-1 gene in 3'CS region of class 1 integron is the characteristic of classic class 1 integron and absence of this normal 3'CS region denotes the presence of non-classic class 1 integron. We also found that 17 isolates that harboured CS regions but not associated with sul-1 gene which is showing the presence of non-classic class 1 integrons for mobilization of various bla\textsubscript{ESBLs} in this Enterobacterial collection. We also
found higher number of isolates harbouring combination of $\text{bla}_{\text{CTX-M}}+\text{bla}_{\text{TEM}}+\text{bla}_{\text{SHV}}$ associated with class 1 integrons.

- We observed that all isolates that harboured SHV/TEM or combination of these genes were associated with sul-1 type integrons while none of the isolate was found associated with the CS region of integrons, only one isolate that harboured SHV gene was found associated with CS region and IS26 like insertion sequence. So we can hypothesized that in our Enterobacterial collection those isolates that harboured only SHV or TEM genes are mobilized more frequently by sul-1 type class 1 integrons.

- On determination of various insertion sequences (ISEcp1, ORF513 and IS26) we found 83.8% (109/130) Enterobacteriaceae isolates that harboured insertion sequences such as ISEcp1, ORF513 (ISCR1) and IS26. Like integrons we also found these insertion sequences in most of the isolates that harboured combination of $\text{bla}_{\text{CTX-M}}+\text{bla}_{\text{TEM}}+\text{bla}_{\text{SHV}}$. High frequency (56.9%) of isolates harbouring $\text{bla}_{\text{ESBLs}}$ was found associated with ISEcp1, while least (33%) were found with IS26.

- We also found that in the present collection the isolates that harboured TEM or TEM+SHV genes were not associated with ISEcp1, while the isolates harbouring only SHV-gene were associated with ISEcp1. This shows probably $\text{bla}_{\text{TEM}}$ and combination of $\text{bla}_{\text{TEM}}+\text{bla}_{\text{SHV}}$ is not mobilized by these mobile elements, while $\text{bla}_{\text{SHV}}$ gene is mobilized by this insertion sequence.

- In the present study we observed that IS26 is most commonly found in those isolates that harboured $\text{bla}_{\text{SHV}}$ genes (with other ESBLs) and this shows that
the mobilization of SHV type of ESBLs is by IS26 elements. We also found various molecular sizes of IS26 elements inserted with \( \text{bla}_{\text{ESBLs}} \), but amplicon size of \(~850\text{bp}\) was the most frequently observed. We found four isolates that harboured only \( \text{bla}_{\text{CTX-M}} \) genes associated with only IS26 elements, and thus suggests that this gene can even be mobilized only by the help of this insertion sequence.

- We also found CTX-M genogroup-9 like gene associated with ISEcpI and IS26, while this gene was not associated with ORF513 (also known as ISCR1).

- Of the 69 isolates that harboured sul-1 type class-1 integrons, 73.9\% (51) were found to harbour ORF513 which shows the higher occurrence of complex class-1 type integrons with \( \text{bla}_{\text{ESBLs}} \) including \( \text{bla}_{\text{CTX-M}} \) in our North Indian collection of \text{Enterobacteriaceae}.

- We found all isolates to harbour \(~23\) kb plasmid, and on analysis for the presence of various CTX-M genogroups on plasmids, we found presence of CTX-M-genogroup-1 and -9 on the plasmid.

- By RAPD typing to determine the clonal relationship of our bacterial isolates, it can be concluded that probably the very same clone is circulating in the gynaecology, surgery and orthopaedics wards as these wards are sharing the same building block in our hospital and hence the chances of cross contamination are high. As some isolate from different wards were also found matching, so it can be concluded that still stringent sterilization conditions are not maintained in our hospital wards/operation theatres; however, variability
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among isolates is still maintained. Therefore, we suggest further work-out steps to minimize the spread of bla genes in our hospital environment.