CHAPTER 5

SUMMARY

Antimicrobial resistance has snowballed to a serious public health concern with economic, social and political implication. The emergence of multi-drug resistant strains of gram negative bacteria (Pseudomonas, Escherichia coli, Klebsiella.) and gram positive bacteria (Staphylococcus species) is the more worrisome in the present therapeutic scenario. The problem of changing resistance pattern will remain an ongoing threat for both developed and developing countries.

This study was designed to isolate and characterize the different types of bacteria and their plasmid from several types of clinical specimens, the clinical specimens were collected from the patients attending the Government General Hospital Anantapur.

Further more, it was observed that the isolates were tentatively identified as, Escherichia Coli, Klebsiella species, Proteus species. Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidies, Streptococcus pyogens, Streptococcus faecalis, Streptococcus pneumoniae.
With regard to the sex wise incidences, females are slightly more susceptible, than the males to the infections. Its shows significant differences between the male and female. The number of Multi-drug resistant (MDR) bacteria was separated from the clinical isolates, this MDR strains shows resistance to commonly used antibiotics.

The majority of multi-drug resistant bacteria were isolated from Pyogenic and Wound infections. Most frequently observed organisms are Escherichia Coli, Klebsiella coli, Pseudomonas, Proteus species, Staphylococcus epidermidies. High resistance rate to all antibiotics were observed. The multi-drug resistant bacteria was also isolated from the Urinary Tract Infection (UTI).

Antibiotic susceptibility pattern of the isolates revealed that first generation cephalo-sporins, ciprofloxacin, norfloxacin, netrofurantoin and third generation cephalosporins, cefotaxim, ceftriaxone, ceftazidime, exhibit a high degree of drug resistance to Enterobacteria and Staphylococcus species.

Co-Trimoxazole, Gentamycin, Nalidixic acid, Tobranycin and Carbencillin also showed high resistant rate, Sparfloxacitin, and Amikacin were the most effective antibiotics against gram negative. Cefotaxime, Azithromycin and Methicillin were the most affective antibiotics against gram positive bacteria.

Conjugative and non-conjugative plasmids were isolated from Multi-drug resistant gram negative bacteria (Escherichia Coli, Klebsiella Pneumonia and Pseudomonas aeruginosa). Few isolates were found to harbour a single and similar plasmids especially in Escherichia Coli, Klebsiella Pneumonia and
Pseudomonas aeruginosa. Staphylococcus aureus and Staphylococcus epidermidies also harbour conjugative and non-conjugative plasmid, further more it was observed that among the Staphylococcus aureus and Staphylococcus epidermidies where found to mediate a single and similar plasmids.

In this study, the investigation of the clinical strain Kp4 harbouring resistance gene to Ampicillin and the third generation Cephalosporin. There multiple resistance genes are probably on the 80 Kbp plasmid. Kp5 present in strain Ec1, kp4 is a transferable plasmid and is stable in Escherichia Coli DH5α. A single and similar plasmid were found to harbour a strain Kp2 and Kp3 also this large plasmid may be responsible for the dissemination of Antibiotic resistance in hospital setting (High and CK Wim 2000) Ec4, Ec5, strains harbour small 7-9 kb plasmid these shows non-conjugative nature. Kp5 strain harbour 50 kb. Shows Aminoglycoside resistance. Trans conjugates occur in Escherichia Coli (Helena Bujdakova et al., 1998). The spread of Clinical isolates with such cross resistance is believed to represent a critical factor for nosocomial out breaks in hospitals.

Multi-drug resistant (MDR) isolates of Pseudomonas aeruginosa, obtained from hospitalized patients in the present study we have isolated a plasmid from PA1, PA2 & PA3, PA1 & PA2 harbour single and similar plasmid, 48 kb which was found responsible for Amikacin and Gentamicin resistance (Nagoba BS, et al., 1997; veenu, Sikka R, and Arora DR, 1998; Shahid M, 2004). Transformation experiments were attempted on these isolates to
determine changes in plasmid content associated with antibiotic resistance pattern. It was noted that the Transformants Escherichia Coli HB101 resistant to Amikacin after the transformation experiment while Escherichia Coli HB101 was sensitive to all the drugs prior to the experiments.

In this study Methicillin resistance strains were correlated with the presence of plasmids, between the Staphylococcus aureus and Staphylococcus epidermidies. The strain SA$_2$ and SA$_3$ harbour plasmids of 35 kb. Both the strain showed single and similar plasmid. SE$_1$ strains also harbours the same molecular weigh of 35 kb single and similar plasmid this strain SE$_1$ shows the transfer of conjugal resistance plasmid from SE$_1$ to SA$_1$ strains (Betty A et al., 1983; Goering and Ruft 1983; Gordon. L. et al., 1983; Bastos MCF, et al., 1999) hypothesis that these strains might serve as a potential reservoir for resistance genes in Staphylococcus, the process of genetic transfer of resistance marker from Staphylococcus epidermidies to Staphylococcus aureus was examined.

The present investigation is, therefore, particularly concerned with the following objectives

- To Isolate the common hospital tract infection micro organisms.
- To Determine the susceptible pattern of antibiotics to all bacteria by Kirb-burg-disc diffusion method
- Isolation & Characterization of the multi-drug resistant bacteria
- To Isolate & Characterize the plasmids from the multi-drug resistant bacteria
To Determine the molecular weight of plasmids and correlate between the plasmid

To Transfer the isolated plasmid to the resistant strain

To Conjugate the isolate (donor) to recipient cell

To Determine the relationship between the gram positive and gram negative plasmid

The salient findings of the present investigation:

Both gram negative, and gram positive bacteria were isolated from different specimens.

Most of the strains both gram positive and gram-negative showed highly resistance to commonly used antibiotics especially against, Ciprofloxacin, Nalidiric acid, Cefotaxime, Gentamicin and Ampicillin.

Few of the strains both gram positive and gram negative showed susceptibility to commonly used antibiotics such as, Sparfloxacin and Amikacin.

Most of the bacteria showed Multi-drug resistance to antibiotics such as, Cefotaxime, Ampicillin, Gentamycin, Amikacin, Methicillin, Tobramycin and Carbencillin.

Escherichia Coli, Klebsiella, Pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus and Staphylococcus epidermidies are MDR strain harbouring plasmids.
The plasmids isolated from gram negative MDR bacteria show a molecular weight of 89 kbp. This large single plasmid was isolated from the Escherichia Coli and Klebsiella Pneumonia.

The plasmid isolated from Pseudomonas aeruginosa showed a molecular weight of 48 kb. The same molecular weight plasmid was observed in the Pseudomonas species isolated from different patient samples from a hospital.

Gramm positive MDR Staphylococcus epidermidies & Staphylococcus aureus harbour a 35 kb plasmid indicating that the plasmid may be disseminating among the Staphylococcus species.

Transformation experiments were attempted on these isolated plasmids to determine changes in plasmid content associated with antibiotic resistance pattern.

Transformants Escherichia Coli DH5α, the transferable plasmid shows stable and exhibit all the drug resistant properties in recipient.

Conjugative and non-conjugative plasmids were isolated from both gram negative and gram positive bacteria.

Trans conjugative experiments were attempted on the Escherichia Coli, The strain kp5 harbouring 50 kb plasmids shows conjugative nature recipient to Escherichia Coli strain.

This plasmids may be responsible for the dissemination of antibiotic resistance in hospital strains either in conjugative or non-conjugative nature.

To conclude, our investigation shows that not only emerging multi-drug resistant strains, but also R-plasmids may be disseminated in hospitals.
Spread of conjugative and Non-conjugative plasmid among gram negative and gram positive strains.

These organisms provide a reservoir of plasmid that may ultimately spread to more pathogenic bacteria.

We recommend that physician seeks updated knowledge of the common antibiotic sensitivity pattern when starting empirical antibiotic therapy to patient with any type of infections.