5.0 Conclusions

- In the present study, *E. coli* was found to be the most dominant pathogen, showing its presence in 577 samples of the total 1790 analyzed. It was found to be highly resistant to β-lactams / Penicillins, Tetracycline and Folate pathway inhibitors.

- MSSA was the second most prevalent organism detected in 152 pus samples and showed high resistance to Folate pathway inhibitors as compared to other group of antibiotics.

- *Pseudomonas*, *Klebsiella* and *Proteus* have also contributed to the infections and were highly resistant to β-lactams / Penicillins, Tetracycline and Folate pathway inhibitors.

- Aminoglycoside class of antibiotics demonstrated the highest antimicrobial activity (78 – 100%) against all the clinical isolates followed by Cephalosporins (70 – 100%), Quinolones (62 – 100%), Macrolids (58 – 100%) and Phenicol (51 – 100%). Comparatively, β-lactams / Penicillins, Folate pathway inhibitors and Tetracycline showed lower antimicrobial activity.

- In case of UTI causing pathogens, *Enterobacter spp.* was found to be 100% sensitive to Nitrofuran while the other isolates viz. *E. coli*, *Klebsiella*, *Pseudomonas* and *Proteus* have showed sensitivity in the range of 25 – 60%.

- Interestingly, in the region under study, *Acinetobacter spp.*, which is one of the most dangerous pathogen creating havoc in hospital environment was found to be 100% sensitive to Aminoglycosides, β-lactamase
inhibitors, Carbapenems, Quinolones and Cephalosporin class of antibiotics.

- *Salmonella* isolates were found to be 100% sensitive to all antibiotic classes except Tetracyclines (50% sensitive).
- The MRSA isolates were also 100% sensitive to β-lactamase inhibitors, Quinolones, Carbapenems and Glycopeptides while β-lactams / Penicillins, Folate pathway inhibitors along with Cephalosporins showed no effect on the same.

- Of the 1790 clinical samples analyzed, one of the multidrug resistant (MDR) isolate was selected for curing studies. The isolate was identified as *Klebsiella pneumoniae* based on morphological, cultural, biochemical and molecular testing.
- It showed resistance to 16 out of 19 antibiotics, belonging to β-lactams, Cephalosporins, Aminoglycosides, Quinolones, Phenicol and tetracycline groups. The MIC’s for these antibiotics was found to be 800 µg/ml or more.
- Such type of MDR is indicative of presence of high copy number of single or multiple plasmids. The plasmid profile of *Klebsiella pneumoniae* showed presence of 6 distinct plasmid bands in the range of 3 to 35 kb.
- Reversal of plasmid encoded resistance to multiple antibiotics was attempted using a naphthoquinone of herbal origin.
- Plumbagin, 5-hydroxyl-2-methyl 1, 4-naphthoquinone, present in the roots of a herb namely, *Plumbago zeylanica* (Chitrak) was used as a curing agent.
• The MIC and SIC of plumbagin against actively growing cells of *Klebsiella pneumoniae* was calculated to 50 µg/ml and 25 µg/ml, respectively indicating its significant antibacterial activity.

• Exposure of actively growing cells to subinhibitory concentrations of plumbagin for several cycles resulted in reversal of antibiotic resistance to Levofloxacin, Tetracyclin, Trimethoprim/Sulphamethoxazole and Chloramphenicol. The efficiency of curing ranged between 14 to 38%.

• Curing of *Klebsiella pneumoniae* by plumbagin resulted in the loss of a high molecular weight plasmid (~10 kb) in the cured derivative.

• The curing efficiency obtained is higher by several log cycles than what is expected in spontaneous loss of plasmids.

• Results of RT-PCR of ‘ori’ genes of *Klebsiella pneumoniae* and its cured derivative showed 73% reduction in copy number in exposed bacterial population, further substantiating that observed reversal of antibiotic resistance is because of curing and not because of mutation.

• Three types of ESBL genes were detected on the plasmid of *Klebsiella pneumoniae*. These include TEM, OXA and SHVS types of β-lactamases.

• The gene specific PCR of cured derivative of *Klebsiella pneumoniae* did not yield amplification showing that the resistance to extended spectrum of β-lactams as well as Quinolone group of antibiotics in *Klebsiella pneumoniae* is plasmid encoded.

• Incompatibility group specific PCR, with 18 primer pairs, demonstrated the plasmids of *Klebsiella pneumoniae* under study belonged to FIA and FIB incompatibility group.
- Curing of pKPI plasmid (harboring ESBL genes) by plumbagin is a significant observation of the present study as it is an important constituent of number Ayurvedic formulations. Its meaningful utilization will help to reverse the antibiotic resistance in clinical isolates so commonly observed these days.

To the best of our knowledge, this is the first ever report of plasmid carrying genes encoding resistance for both Quinolones and Cephalosporins. Resistance to the latest generation of antibiotics, that too plasmid encoded, is of special significance as they are frequently used to treat infections caused by Gram negative MDR bacteria.

The study resulted in generation of representative status of the antibiotic sensitivity pattern of commonly found organisms in nosocomial infections of the city of Jalgaon and surrounding area. This could be useful for the clinicians in general and of the region in particular to help make them choose correct antibiotic and ensure the judicious use of the same for their patients.

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