6. Summary

Microbial contaminations of pharmaceutical products are health threat to patients in worldwide. Proper identification of contaminants profile in the product and production environment are very important in the quality control of pharmaceutical products. When compared to new rapid methods, conventional methods are laborious and take more time to complete the tests. Knowledge of contaminant profile is very important for contamination control and hence, the present work was undertaken to analyze the microbial contaminants in the samples from different areas of pharmaceutical environments, to detect them by applying molecular method, such as PCR amplification technique and to evaluate contamination control using common biocides.

- A total of 252 samples were collected from air, surface and garments, and processed microbiologically during 2009 and 2011. From the samples collected, 6440 bacterial and 185 fungal isolates from air, 511 bacterial and 16 fungal isolates from surfaces and 511 bacterial and 16 fungal isolates from garments samples were detected.

- Species of *Staphylococcus*, *Micrococcus* and *Bacillus* were reported as predominant bacterial contaminants and species of *Penicillium*, *Cladosporium* and *Aspergillus*, as predominant fungal isolates from pharmaceutical environments.

- Of the 61 pharmaceutical samples analyzed for microbial limit tests, which included cardiac, gastrointestinal and chemotherapeutic drugs, no objectional microorganisms were found.

- Two types of multiplex PCR assays were developed to detect objectionable microorganisms (PCR I) and to detect eubacterial, panfungal and *P. aeruginosa* (PCR II).
• Out of 56 possible contaminated and 31 in process samples tested using conventional and multiplex PCR methods, a high percentage of culture positivity was observed in PCR methods over conventional culture method.

• Comparison of multiplex PCR method with the conventional culture method showed that the culture based approach was laborious, time consuming and non specific. By multiplex PCR methods, the contaminants could be detected within 27 hours instead of 5 to 7 days by conventional methods. Moreover, both multiplex PCR I and II assays were not inhibited by the constituents of drugs used.

• The minimum inhibitory concentration of bigunaide and chlorhexidine was lowest (0.5 µg/mL) against the bacterium, Staphylococcus spp and against the fungus, Curvularia spp (2.0 µg/mL), while that of QAC, benzalkonium chloride was lowest (2.0 µg/mL) against the species of Staphylococcus and Micrococcus in the case of bacteria and in the case of fungi, it was lowest (4 µg/mL) against A. flavus and the species of Penicillium and Curvularia.

• Similarly, the MIC of cetrimide was lowest (4 µg/mL) against the species of Bacillus, Micrococcus and Pantea whereas most of the fungi tested showed the lowest MIC value as 8 µg/mL.

Since microbial contamination of pharmaceutical products is one of the major reasons for product recall and manufacturing problems, knowledge of the distribution of survival microorganisms in pharmaceutical environments is critical in the process control of non-sterile and sterile pharmaceutical products. Minimizing or preventing the microbial contamination in pharmaceutical facilities and processes becomes the most important aspect of process control during pharmaceutical manufacturing.

When a microbial contamination is suspected in a production batch, an investigation is rapidly started to determine the contamination source, number and types of microorganisms. For the microbiologist, this task is difficult given the few studies
available which examine common cleanroom bacterial and mould contaminants in pharmaceutical manufacturing industries and rapid detection of contaminants from ophthalmic and chemotherapeutic drugs. Thus, the present investigation would provide data on pharmaceutical environmental microflora and essential information for industrial microbiologists and quality control personnel in understanding clean room environments and for assisting with contamination control.

Standard culture methods followed for the identification and detection of the contaminants for quality evaluation of raw materials and finished products are rather slow, laborious and non-specific. Hence, the available results in the present investigation provide the nucleic acid amplification technology, PCR which could be applied to different types of ophthalmic and chemotherapeutic products and thereby the contaminants could be detected within 24 – 27 hours instead of 7 days by conventional methods.

In contamination control, selection of disinfectant is most vital against pharmaceutical clean room bacterial and fungal isolates. Because of lack of published reports, the surveys of susceptibility pattern would be most vital. The obtained results in the present investigation would provide important information for microbiologists about the effectiveness of in-use disinfectants against the bacteria and fungi present in hospital and pharmaceutical environments.