

NOSOCOMIAL PATHOGENS IN THE ICU: STRATEGIES IN OVERCOMING INFECTION AND COLONIZATION

Marzida binti Mansor¹ and Shamala Devi Sekaran²

¹Department of Anaesthesiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

²Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

ABSTRACT

The increasing evidence that antibiotic therapy is becoming less effective in the treatment of infections, including nosocomial pathogens, with the widespread prevalence of antibiotic resistant pathogens has led to the burgeoning interest in the development of alternative therapeutic agents. This is aided by the readily available genomic databases which, to date, include the whole genome sequences of several important nosocomial pathogens such as *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The discovery of quorum sensing (QS) systems regulating bacterial virulence has opened a new avenue for the control of nosocomial infections. QS is a cell-density dependent signaling system used by bacteria to coordinate gene expression within a population with the most well studied QS systems being the LuxR-LuxI homologous system which include the autoinducer molecules (AI) and the N-acyl-homoserine lactones (AHLs). The AHL system is involved in the regulation of many host-associated phenotypes including the production of virulence factors which suggests that the silencing of this system could affect the pathogenicity of these bacteria. Various studies have already explored the potential use of QS-inhibitory compounds.

The main aims of this project are (1) to develop a rapid multiplex assay for the detection of nosocomial pathogens (*Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*), (2) to evaluate the quorum sensing genes in the above mentioned nosocomial pathogens as potential targets in antimicrobial therapy and (3) to assess quorum inhibiting properties of natural compounds in order to identify potential candidates for antimicrobial therapy as alternatives to antibiotics.

498 strains were collected from University Malaya Medical Centre and identified using routine standard laboratory diagnostic tests. Six commonly occurring nosocomial pathogens namely *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter spp* were used for this study. The conventional multiplex PCR assay was carried out in two sets of three pathogens each, because the amplicon size of 6 genes chosen would not allow their detection in single multiplex reaction. The first set consisted of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The second set consisted of *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter spp*.

The iQ SYBR Green Supermix was used for the multiplex real time PCR Assay. The reaction was optimised and was carried out in 2 sets of 3 pathogens each depending on melting temperature of primers. This was done as the T_m values of primers were too close for a clear differentiation. The first set consisted of *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Acinetobacter spp.* While the second set consisted of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

300 clinical samples were obtained from samples sent for blood culture from the University Malaya Medical Centre. Identification of bacteria was done by standard tests which include Gram stain followed by specific biochemical tests for each organism. DNA was extracted using the Epicentre Masterpure DNA purification kit according to the manufacturer's instructions. With the extracted DNA samples eubacterial PCR was performed which identifies if the samples contained any bacterial DNA. The multiplex real time PCR was performed on the samples positive for eubacterial PCR to identify the exact bacterial pathogen within the scope of the above developed multiplex assay.

With the conventional and SYBR Green Real time multiplex PCR assays bacteria were detectable based on their PCR positive results. This assay was evaluated using clinical specimens and was found to be quite sensitive and specific. In the clinical isolates that were blind tested 46 samples showed positive results in eubacterial PCR and further analysis of these samples in multiplex real time PCR detected the identity of 43 bacterial pathogens that fall within the scope of this assay which is around 93.7% efficiency. Their PCR results matched with the conventional culture identifications.

In this study, we also sought to find out if the biofilm formation among clinical isolates of *Acinetobacter spp.* is under the control of autoinducing quorum sensing molecules. Biofilm formation among clinical isolates of *Acinetobacter spp.* was assessed and the production of signal molecules were detected with *Chromobacterium violaceum* CV026 biosensor system. Using the microtitre plate assay biofilm formation was quantified among the 50 isolates of *Acinetobacter spp.* in microtiter plates. About 60% of the isolates formed biofilms when incubated for prolonged hours. Partial characterisation of autoinducers was carried out by thin layer chromatography bioassay and the culture supernatants further subjected to quantification. Further detection showed that some of these biofilm forming strains produced long chain signal molecules. Thin layer chromatography bioassay confirmed that five of these isolates produced N-decanoyl homoserine lactone and two isolates produced acyl-homoserine lactone with a chain length exceeding C12. The data are consistent with the presence of quorum sensing signal molecules among the biofilm forming clinical isolates of *Acinetobacter spp.* This is of great significance as the signal molecules aid in biofilm formation which in turn confer various properties of pathogenicity to the clinical isolates including drug resistance.

The quorum quenching properties of plant and soil sources were determined and the extracts of *Phyllanthus* species, garlic, lemon and soil isolates were found to exhibit strong quorum quenching capabilities.

The use of quorum sensing signal blockers to attenuate bacterial pathogenicity is therefore highly attractive, particularly with respect to the emergence of multi antibiotic resistant bacteria.