P0597
Poster Session II
Molecular diagnostic methods in bacteriology - miscellaneous

DETECTION OF VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): A RAPID, SIMPLE AND COST-EFFECTIVE ALTERNATIVE TO PCR

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Objectives: A number of chromogenic media have been used to improve turn-around-time to VRE isolation. However, PCR remains to be the 'gold standard' for culture confirmation. The objective of this study was to develop an alternative method to PCR that is comparable in performance but faster, cheaper and can be operated in laboratories without PCR facilities. Loop-mediated amplification (LAMP) has been recognized as a isothermal target amplification method that is highly specific, sensitive and faster than PCR. We developed and validated a multiplex LAMP method to detect vanA and vanB genes in VRE for culture confirmation.
Method: Isolates presumptively identified as VRE on Dalynn Colorex VRE plates (n=356) were analyzed by a conventional multiplex PCR as per standard operating procedure to detect *vanA* and *vanB* genes and by multiplex LAMP. LAMP primers for *vanA* and *vanB* genes were designed by Primer Explorer V4 software (Eiken Chemical Co.) by using gene sequences obtained from the Genbank. This LAMP method simultaneously amplifies and detects a 196bp region of vanA gene and 237bp of vanB gene. LAMP was carried out at 65 ^{0}C for 30 min using a standard LAMP reaction mixture containing Bst 2.0 WarmStart DNA polymerase (New England BioLabs). The DNA template for PCR and LAMP was prepared by making a cell suspension of presumptive VRE colonies on Colorex VRE plates to a density of 1 McFarland. Fifty microliters of the suspensions was mixed with 50µl of lysis buffer and boiled for 10 min. Two microliters of clear supernatant was used for amplification. LAMP amplification was detected using Genie® II (OptiGene, UK) real-time fluorescence detection instrument. In LAMP vanA and vanB genotypes were identified based on the time to amplification reading on Genie® II.
Results: Three-hundred and thirty four specimens (274 vanA and 60 vanB) were confirmed as VRE by PCR. Twenty-two specimens were identified as non-VRE by PCR and LAMP. Three specimens that were positive for both vanA and vanB by PCR were identified as vanA by multiplex LAMP. When vanA and vanB gene targets were amplified separately by LAMP all three specimens showed the presence of both genes. LAMP results were 100% concordant with the PCR results for detection of VRE.
Conclusion: Confirmation of VRE by the detection of *vanA* and *vanB* genes using LAMP is an accurate molecular method as PCR. However, it is significantly faster and cheaper than the PCR. In addition LAMP is an isothermal method and does not require a thermocycler. The amplification specificity is extremely high due to the utilization of six oligonucleotide primers that recognize eight distinct regions on the target DNA. Amplification of *vanA* and *vanB* genes by LAMP can be used as an alternative method for PCR for VRE culture confirmation.