[PC2-8] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Novel Plasmid-Mediated β-lactamase That Hydrolyzes Broad-Spectrum Cephalosporins in a Clinical Isolate of Klebsiella pneumoniae

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A new extended-spectrum β-lactamase with an isoelectric point (pI) of 6.2 was detected in *Klebsiella pneumoniae* F161 that was isolated from a patient with infection. This strain was highly resistant to the third or fourth generation cephalosporins such as ceftazidime, ceftriaxone, cefoperazone, and cefpirome. Analysis of this strain by the double disk diffusion test showed synergies between amoxicillin-clavulanate (AMX-CA) and cefotaxime, and AMX-CA and aztreonam, which suggested that this strain produced a extended-spectrum β-lactamase (ESBL). Genetic analysis revealed that the resistance was due to the presence of a 9.4-kb plasmid, designated as pKP161, encoding for new β-lactamase gene (bla). Sequence analysis showed that a new *bla* gene of pKP161 differed from bla_{TEM-1} by three mutations leading to the following amino acid substitutions: Val_{84}→Ile, Ala_{184}→Val, and Gly_{238}→Ser. These mutations have not been reported previously in the TEM type β-lactamases produced by clinical strains. The novel β-lactamase was overexpressed in *E. coli* and purified by ion exchange chromatography on Q-Sepharose and CM-Sepharose, and then further purified by gel filtration on Sehadex G-200.

[PC2-9] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Immunological characterization and localization of the alcohol-dehydrogenase in Streptococcus pneumoniae

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Heat shock proteins serve as chaperone by preventing the aggregation of denatured proteins and promote survival of pathogens in harsh environments. In bacteria, ethanol shock induced the major chaperone GroEL and DnaK, but in Streptococcus pneumoniae, it induced neither GroEL nor DnaK but alcohol dehydrogenase (ADH). In this study, ADH gene encoding a 104-kDa (p104) protein was identified and characterized. The deduced amino acid sequence of pneumococcal ADH shows homology with other members of the ADH family, and particularly with Entamoeba histolytica ADH2 and E. coli ADH. S. pneumoniae adh is composed of 883 amino acids and its estimated isoelectric point is 6.09. Although ADH is conserved between S. pneumoniae and E. coli, immunoblot analysis employing antisera raised against pneumococcus ADH demonstrated no cross-reactivity with ADH analog in Eschericha coli, Staphylococcus aureus and human HeLa cells. Also secretion of ADH was demonstrated by subcellular fractionation and immunoblot analysis of proteins. These results suggest that S. pneumoniae ADH could be a highly feasible candidate for diagnostic marker.

[PC2-10] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Aloe vera gel enhanced in vitro antimicrobial activity of propolis against oral pathogens

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Aloe and propolis have been used for thousand of years in folk medicine for several purposes. They possess several biological activities such as anti-inflammatory, antifungal, antiviral and regenerative. Although the antibacterial activity of propolis has already been demonstrated, very few studies have been done on clinical relevance in dentistry. We investigated antimicrobial activity of ethanol extract of aloe and propolis and water soluble fraction of propolis against oral pathogens. We also investigated the synergistic effect of aloe and propolis. Three microorganisms were used as follows: Streptococcus mutans, Enterococcus faecalis, Enterococcus hirae. The antimicrobial activity was tested by serial broth dilution method, and the antimicrobial activity was expressed by minimal inhibitory concentration (MIC). To investigate the synergistic effect of aloe and propolis, the extract of propolis diluted serially was used for each strain. The ethanol extract of aloe showed weak antimicrobial activity, while both of ethanol extract and water-soluble fraction of propolis inhibited greatly all microorganisms tested. However, the ethanol extract of aloe enhanced significantly antimicrobial activity of propolis against oral pathogens.

[PC2-11] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A NAT for reliable HCV RNA screening of blood

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Current methods to detect hepatitis C virus (HCV) are based on its antibody detection. Viral contamination of a donated blood can potentially escape such detection during the window phase of infection, when no antibody is present or the level of antibody is too low to detect. Application of nucleic acid amplification technology (NAT) for the direct detection of HCV can actually reduce the window period and contribute to the safeguard of blood and blood products. The objective of this study was to develop a highly sensitive in-house method for the HCV RNA screening via NAT. The superiority of the protocol being developed was compared with commercial methods as control, and was applied to blood samples. In this study, five sets of primers were designed and then two sets of primers were selected for RT-PCR. We have found that QIAamp viral RNA isolation kit is the most efficient extraction kit for these systems after several PCR conditions such as annealing temperature, reverse transcription temperature, MgCl2 concentration, etc. were optimized. In order to determine the positive cut-off point, a diluted series of the WHO HCV International Standard (96/790) were tested under these conditions, and the detection limit was calculated to be 5 IU . In order to validate the specificity of this analytical procedure, we are performing the test using HCV RNA negative plasma pools which has already been confirmed by European Medicine Evaluation Agency (EMEA).

[PC2-12] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Immunological characterization and localization of the alcohol- dehydrogenase in Streptococcus pneumoniae

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Heat shock proteins serve as chaperone by preventing the aggregation of denatured proteins and promote survival of pathogens in harsh environments. In bacteria, ethanol shock induced the major chaperone GroEL and DnaK, but in Streptococcus pneumoniae, it induced neither GroEL nor DnaK but