

[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 ceftipime. 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 an 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 a 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: Val₈₄→Ile, Ala₁₈₄→Val, and Gly₂₃₈→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 Sephadex G-200.

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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 pneumococcal ADH demonstrated no cross-reactivity with ADH analog in *Escherichia 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