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Carriage of Mobilizable Plasmid-Mediated β -Lactamase Gene in Ampicillin-Resistant Escherichia coli Strains with Origin of Normal Fecal Flora

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Abstract: Aim: The aim of this study was to investigate the carriage of b-lactamase genes in ampicillinresistant (Amp^r) Escherichia coli (E. coli) isolates from human normal fecal flora. Methods: Ten Amp^r E. coli strains isolated from the stool samples of 21 healthy persons with no antibiotic use during at least three months were screened for TEM-, SHV-, or OXA-type β-lactamase genes by polymerase chain reaction (PCR). The susceptibility of the strains to antibiotics was determined by disk diffusion method, and minimum inhibitory concentration (MIC) of ampicillin to the strains was determined by agar dilution method. Plasmid transfer assays were performed by broth mating technique. Plasmid DNA was isolated by alkaline lysis method. Digoxigenin-labeled TEM-1 probe was used in hybridization assays. Results: Two of 10 strains were found to be carrier for only TEM-type β-lactamase gene (bla_{TEM}) by PCR, and

their resistances to ampicillin were conjugatively transferred to a recombinant E. coli K-12 strain C600. MIC of ampicillin to two representative strains and their transconjugants was detected as >512 µg/ml. Moreover, b-lactamase inhibitor resistance was also observed in these two strains and their transconjugants. Digoxigenin-labeled TEM-1 DNA probe was hybridized to some non-conjugative but mobilizable plasmid DNAs purified from two of the TEM-gene-carrying organisms. Conclusions: These results indicate that commensal E. coli strains carrying β -lactamase gene in the bowel environment could retain resistance determinants on small-sized resistance plasmids (R plasmids) and become a potential reservoir for resistance genes in the community, even in the absence of recent antibiotic consumption.

Key Words: Fecal flora, ampicillin-resistant Escherichia coli, mobilizable β-lactamase gene

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