Letters to the Editor

Small Plasmids Are Involved in Amoxicillin-Clavulanate Resistance in Escherichia coli

The introduction of β-lactamase inhibitors (clavulanate, sulbactam, or tazobactam) in association with β-lactam antibiotics is one of the current strategies to overcome bacterial resistance. Unfortunately, β-lactamases are also “inhibitors” of these new compounds. In Escherichia coli, the frequency of amoxicillin-clavulanate-resistant strains (MIC equal to or more than 16/8 μg/ml) is around 20 to 30% of that of amoxicillin-resistant isolates in certain Madrid hospitals. We previously reported that bacterial resistance to amoxicillin-clavulanate in clinical strains of E. coli can result from hyperproduction of plasmid-determined TEM-1 β-lactamase (J. L. Martínez, E. Cercenado, M. Rodríguez-Creixems, M. F. Vicente-Pérez, A. Delgado-Iribarren, and F. Baquero, Letter, Lancet ii:1473, 1987). The same conclusion was reached about an amoxicillin-clavulanate-resistant E. coli strain from England (H. Williams, A. King, K. Shannon, and I. Phillips, Letter, Lancet i:304–305, 1988). These preliminary observations were confirmed in the United States in a recent paper by Sanders et al. (2). The genetic basis of β-lactamase hyperproduction remained unknown, but the possibility of interbacterial transfer of this character was worrisome because of concern about an epidemic spread of amoxicillin- (or ticarcillin-) clavulanate resistance. We studied 10 resistant E. coli strains presenting MICs ranging from 16/8 to 64/32 μg of amoxicillin-clavulanate per ml. In all cases, a TEM-1 enzyme was the only β-lactamase detectable on isoelectrofocusing, and the amount of enzyme produced was 3 to 60 times higher than that corresponding to control strain E. coli HB101(pMM4::Tn3) (susceptible to amoxicillin-clavulanate) carrying a single copy of the gene encoding the TEM enzyme (1). The 10 resistant strains were lysed to obtain their plasmid DNA, which served to transform a susceptible E. coli HB101 strain. Transformants with amoxicillin-clavulanate resistance (MIC superior to 16/8 μg/ml) were obtained in all cases. A large conjugative plasmid (55 kilobases), presenting one to two copies per chromosome, was found in only one of these transformants. Only small plasmids were detected in the other nine strains: in two cases they were 2.5 kilobases in size and the rest ranged in size from 5.5 to 7.0 kilobases. These plasmids presented at least 10 copies per bacterial chromosome, as determined by the standard technique of cesium chloride-ethidium bromide equilibrium gradient ultracentrifugation. All of them gave positive hybridization with a probe for TEM β-lactamase consisting of a 420-base-pair BglII-HincII fragment of plasmid pBR322. Theoretically, hyperproduction of TEM-1 β-lactamase could be the result of tandem repetitions of the gene encoding the enzyme, “up” mutations of the promoter, or high copy number of plasmids carrying the gene. In the strains studied by us, the last possibility seems to be the most frequent, such that resistance appears to be the consequence of a gene-dosing effect. Other presumptive conclusions can be deduced from our results. Firstly, resistance to amoxicillin-clavulanate seems not to be clonal in our isolates, since the TEM-1 gene was located in plasmids of different sizes. Secondly, most of the plasmids encoding amoxicillin-clavulanate resistance were nonconjugative. This fact may limit the spread of such resistance, even though we obtained positive mobilization with conjugative IncFII plasmids in five cases. It can be suggested that the proportion of amoxicillin-clavulanate-resistant strains reflects the proportion of strains carrying β-lactamase determinants in small multicyclic plasmids. These strains obviously existed long before the introduction of amoxicillin-clavulanate in clinical therapeutics.

LITERATURE CITED


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Increasing Rates of In Vitro Resistance to Ciprofloxacin and Norfloxacin in Isolates from Urine Specimens

We read with interest the paper by Kresen and Wiedemann on the development of resistance to nalidixic acid and the fluoroquinolones (6) and would like to extend discussion of this emerging problem.

The recent introduction of various fluoroquinolones has improved oral treatment and prevention of urinary tract infections (5, 7, 12). However, resistance to these compounds can be induced easily in vitro (1, 2, 8), and development of cross-resistance among the quinolones is reported more and more frequently during therapy (3, 11).

The agar diffusion method of the National Committee for Clinical Laboratory Standards is an appropriate procedure for susceptibility testing with the newer quinolones (4, 9, 10, 13). We therefore used this method to monitor prospectively the frequency of resistance to ciprofloxacin and norfloxacin of all urine isolates during the first 3 months of 1987 and...