
【Abstract】
Recently, instances of β-lactamase-negative ampicillin (AMP)-resistant (BLNAR) strains of Haemophilus influenzae have exhibited a marked increase in Japan. Our group determined the MICs of 160 clinical isolates of H. influenzae at a university hospital in Okinawa, the southernmost part of Japan, and found that 27 stains were BLNAR, while 24 strains were β-lactamase-producing. Among the latter, eight strains were resistant to AMP/clavulanic acid. BLNAR strains showed to be more resistant to cephems than non-BLNAR strains. The competitive affinity assay using biotinylated AMP for penicillin-binding protein (PBP) showed that binding of cefotiam to PBP3A/3B was decreased in BLNAR strain C2163, while affinities to other PBPs were not reduced. The sequences of ftsI, the gene encoding transpeptidase domain of PBP 3A and/or PBP 3B, were determined, and it was found that sequences of the ftsI gene of BLNAR strains were heterogeneous mutations. Deduced amino acid sequence analyses of BLNAR strains showed that three residues (Asn–526, Val–547, and Asn–569) were replaced with Lys, Ile, and Ser, respectively. In addition, some BLNAR strains had an additional three residues (Met–376, Ser–384, and Leu–388) in ftsI replaced with Ile, Thr, and Phe, respectively. Furthermore, changes of Asp–350 to Asn–350 and Ser–357 to Asn–357 were also found in most BLNAR strains. These substitutions found in BLNAR strains were located around the penicillin binding sites of PBP3. Multiple substitutions in amino acid sequence seemed to be closely related with extended resistance against β-lactams, including 3rd generation cephems. Randomly amplified polymorphism DNA (RAPD) fingerprinting of clinical isolates of BLNAR strains showed genetic heterogeneity of the strains, suggesting that the prevalence of BLNAR in this region was a result of the emergence of multiple clones of this phenotype.