Comparative physical map analysis of plasmids carrying ceftriaxone resistance gene in Salmonella enterica serotypes Chol

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ABSTRACT

More and more drug-resistant clinical isolates have emerged in recent years, especially significant numbers of Enterobacteriaceae showed resistance to the third generation cephalosporins. Through screening over hundreds of clinical isolates from Chang Gung Memorial Hospital, we found two isolates, Salmonella enterica serotype Choleraesuis, SC72, and Salmonella enterica serotype Anatum, SA323, both of which carried ceftriaxone-resistant plasmids, designated as pSC72-1 and pSA323-1, respectively. It is unusual for Salmonella clinical isolates from Taiwan to exhibit ceftriaxone-resistance. Further PCR and sequencing analysis revealed that both plasmids harbored blaCTX-M-3 gene conferring the resistance phenotype. Both plasmids, pSC72-1 and pSA323-1, were proved to be conjugative through conjugation experiments, which were carried out using Salmonella enterica serotype Typhimurium (LBNP4417) and Escherichia coli (HB101) as recipients. The differences between two plasmids were investigated further. The sizes of pSC72-1 and pSA323-1 was 74-kb and 82-kb, respectively. The physical maps of both plasmids were also constructed from overlapping the restriction fragments digested with various combinations of restriction enzymes. Results from restriction fragment profiles and Southern hybridization suggested that these two plasmids are not closely related, although both carried the same drug resistance gene blaCTX-M-3. Since most clinical isolates which carried blaCTX-M-3 were either E. coli or Klebsiella pneumoniae, four E. coli isolates and five K. pneumoniae isolates, all ceftriaxone-resistant, were selected from Linkou Chang Gung Memorial Hospital. In order to see if these isolates possess the same drug resistance constructs, PCR assays, DNA sequencing, and Southern blot experiments were performed. The results showed that the plasmids of these E. coli and K. pneumoniae isolates harbored identical blaCTX-M-3 downstream of tnpA. Two of the conjugative plasmids pKP104-1 and pKP116-1, were further digested with various restriction enzymes and revealed the same pattern of restriction fragment profiles. The results suggested that the dissemination of ceftriaxone-resistant plasmids carrying blaCTX-M-3 among clinical isolates of K. pneumoniae. Primers specific for nikA were designed to characterize these plasmids. All plasmids except pSA323-1 contained the same conjugation system. To our knowledge, this is the first physical map of ceftriaxone-resistant plasmid from clinical isolates of S. Choleraesuis or S. Anatum in Taiwan. This pioneer research work would help to the understanding of epidemiology and the control of infections caused by S. Choleraesuis and S. Anatum.

Keywords : Salmonella enterica serotype Choleraesuis ; Salmonella enterica serotype Anatum ; drug resistance gene ; ceftriaxone ; conjugation ; physical map

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