

## Linkage of the Kanamycin Resistance Gene with the Streptothricin Resistance Gene in *Staphylococcus aureus* SA2

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The pKH2 isolated from the multidrug-resistant *Staphylococcus aureus* SA2 is a 40.98-kb plasmid and mediates resistance to ampicillin, clindamycin, erythromycin, kanamycin, and streptomycin. The 3.4-kb *Hind*III fragment conferring kanamycin resistance was cloned from the pKH2 into pBluescript II KS<sup>+</sup> and partial sequence determination of that fragment was carried out. Sequence analysis revealed that the kanamycin resistance gene which encoded aminoglycoside 3'-phosphotransferase was linked to the streptothricin resistance gene. But a nonsense mutation was found in the streptothricin resistance gene and this mutation resulted in a truncated protein of streptothricin acetyltransferase. Homology comparison with nucleotide sequence databases revealed that the 3.4-kb *Hind*III fragment of pKH2 had been derived not from *S. aureus* but from Gram-negative *Campylobacter coli*.

The clinical isolate *Staphylococcus aureus* SA2 was resistant to ampicillin (Am), chloramphenicol, clindamycin (Cl), erythromycin (Em), gentamicin, kanamycin (Km), methicillin, streptomycin (Sm), tetracycline, and tobramycin and harboured four kinds of plasmids (4). Moreover, it was found that this strain was resistant to ethidium bromide and quaternary ammonium compounds (2). The 40.98-kb plasmid, pKH2, encoding resistance to Am, Cl, Em, Km, and Sm was isolated from *S. aureus* SA2 and characterized by protoplast transformation and restriction enzyme mapping techniques (5).

To investigate the Km resistance mechanism in pKH2, the 3.4-kb *Hind*III fragment conferring kanamycin resistance was cloned into pBluescript II KS<sup>+</sup> and partial nucleotide sequence of that fragment was determined by the dideoxychain termination method (6).

Fig. 1 showed the nucleotide sequence of the 1779-bp fragment and the two ORFs. One ORF encoded aminoglycoside 3'-phosphotransferase{APH(3')III} which conferred Km resistance in pKH2. APH(3')III of the pKH2 consisted of 264 amino acids and had 100% identity with APH(3') of the pJH1 and pIP1433 isolated from Gram-positive *Streptococcus faecalis* (7) and Gram-negative *Campylobacter coli* (8), respectively. However, APH(3')III of pKH2 had 98% identities and 99% positives with APH(3')III of the pSH2 of *S. aureus* which

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EcoRV
GATATCATGGAAAGGTCCGCATGGAAACAGCGCTTAAATTAAGTGTAGGCAAGCACTATAAGTTTATTGAAAGGTATATATCCGAGGATTT 90
GTGGGAGAACCTTTGTCCACCTCCGCGATGGATTCCTATGAAACATATGGGAAGCATATTTCTATGCCATCAATGTTTCAGGGCGGT 180
ATCCGCTGAGGTGGGGAAAGGCTTCATTATGCTATCCGAGATGATAGGAATATACAAANATATCCGAGGACATGTATAAAAAATA 270
CACTGTAAACCGGCTCCCTGGATAGCACAATGCGGCTGATATAGAAGAGGGGGGACGAGTATTACAGAAATGAAGCAGCGCAC 360
SAT-START M I T T E M K A G H
CTGAAGATATGATAACCCAGCGAACCATTGGAGGTATAGGTAAAGTATTATACCGAGGTATGAAACAGAGAATTCGACCTTACAGAA 450
L K D I D K P S E P P E V I G K I I P R Y E N E N W T F T E
TTACTCTATGAAAGGCCATATTAAAGCTACCAAGACGAGGAGTGAAGGAGGATGAGAGGGCAGATTGCTTGAATATATGACAAAT 540
L L Y E A P Y L K S Y Q D E E D E E D E E A D C L E Y I D N
ACTGATAGGCTAATATATCTTTAAATCCAGACGATAAATGCGTGGGAAAGCTTAACTCGGAAAAATGGAACCGGTACGCTTATATA 630
T D K V I Y L End
GAAGATATCGGCTATGTAAGGATTCAGGGGGCAAGGCATAGCGAGGGCTTATCATATATCTATAGAAATGGGCAAGCATAAAAAC 720
TTGCTGGACTAATGCTTGAACCCAGGCAATAACTTATAGCTTGTAAATCTCATCATATTTGTGGTTCAAAATGGCTCGGCTGAT 810
ACTATGTTATAGCGAAGCTTTGAAAGGCTGTTTCTGGTATTAAGGTTTAAAGTGAAGCAAGCATGTAATGGGATTCGCTTCTGTA 900
End
TAATAGCTTCTGGGTAATCTTAAATCTGTAGAAGAGGAGGAATAATAAAGGCTTAAATGAGATATCCAGGAAATGAAAA 990
APH(3')I11-START M A K M R I S P E L K K
AAGTATGAAATAACCGCTGCTAAAGATACGGAGGATGCTCTGCTAGGTTATATAGCTGGTGGAGAAATGAAAGCTAT 1080
L I E K Y R C V K D T E G M S P A K V Y K I L V G E N E N L Y
ATTAAAAATGACGGACAGCGGCTATAAAGCGCACACCTATGATGCTGGAACGGGAAAGGACATGCTGCTGGAAGGAAAGCTCC 1170
L K M T D S R Y K G T T Y D V E R E K D M M L W L E G K L P
CTGTCCAAAGGCTCCACCTTTGAACGGCAGATGGCTGGAGCAATCTGCTCATGATGAGGCGATGGGCTCTTTCCTCGGAAAGT 1260
V P K V L H F E R H D G W S N L L M S E A D G V L C S E E Y
ATGAAGATGAAACAGCCCTGAAGAGATTAAGAGCTGTATGCGAGTGCATCAGGCTCTTTCACCTCATGACATATGCGATTGCTCT 1350
E D E Q S P E K I I J E L Y A E C I R L F H S I D I S D C P Y
ATAGCAATAGCTTACAGACGGCCCTTACCGCAATGGATTAATCTACTGATATAAGCATCTGGCGATGCGATGCGAAGAGGAG 1440
T N S L D S R L A E L D Y L L N D L A D V D C E N W E E D
ACACTCCATTAAAGATCCGGGAGGCTGTATGATTTTTAAAGAGGGAAAGCGCGGAGAGGAACTTGTCTTTCCACGGGACCTGG 1530
T P P K D P R E L Y D F L K T E K P P E E L V F S H G D L G
GAGACGACACATCTTTGAAAGATGGCAAGTAAAGTGGCTTATTGATCTTGAAGAGAGGCGAGCGGCGGACAGTGGATGACATG 1620
D S N I F V K D G K V S G F I D L G R S G R A D K W Y D I A
CCTTCTCGTGGGCTGATCAGGGAGGATATGGGGAAACAGTATGTCAGCTATTTTTTACTACTGGGATCTGGGATGGGATGGGATGGG 1710
F C V R S I R E D I G E E Q Y V E L F F D L L G I K P D W E
AGAAAATAAATATATTTTTACTGGATGAATTTTGTAGTACTAGATTAGATGCTATAAAGGCTT 1779
HindIII
K I K Y I L L D E L F End
    
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Fig. 1. Nucleotide and putative amino acid sequences of the 1779-bp *EcoRV-Hind*III fragment of pKH2.

c shown above the sequence of pKH2 at nucleotide 564 is the nucleotide sequence of the pIP1433 isolated from *C. coli*. The sequence of 1779-bp *EcoRV-Hind*III fragment of pKH2 have been registered in GenBank (NCBI) with the accession number U51474.

consists of 263 amino acids (1). The other ORF encoded a truncated protein of streptothricin acetyltransferase (SAT) owing to the nonsense mutation in pKH2. A at nucleotide 564 in pKH2 was substituted to C in the *sat* gene of *C. coli* BE/G4(3). Without this nonsense mutation, the ORF of SAT in pKH2 would end at nucleotide 864 as shown in Fig. 1. Despite encoding a truncated protein, *sat* in pKH2 is the first reported gene in *S. aureus*.

From the above results it may be safely concluded that the 1779-bp DNA fragment containing the *sat* and *aphA* of pKH2 has evolved not from *S. aureus* but from *C. coli* in Korea.

## REFERENCES

1. Gray, G. S. and W. M. Fitch. 1983. Evolution of antibiotic resistance genes: the DNA sequence of a kanamycin resistance gene from *Staphylococcus aureus*. *Mol. Biol. Evol.* **1**: 57-66.
2. Im, S. H., S. J. Yoon, W. K. Kim, C. K. Shin, D. W. Lee, and K. H. Moon. 1996. Characterization of cryptic plasmid of multidrug-resistant *Staphylococcus aureus* SA2. *J. Microbiol. Biotechnol.* **6**: 145-146.
3. Jacob, J., S. Evers, K. Bischoff, C. Carlier, and P. Courvalin. 1994. Characterization of the *sat4* gene encoding a streptothricin acetyltransferase in *Campylobacter coli* BE/G4. *FEMS Microbiol. Lett.* **120**: 13-17.
4. Kang, J. S. and K. H. Moon. 1990. Antibiotic resistance in *Staphylococcus aureus* isolated in Pusan. *Yakhak Hoeji* **34**: 122-125.
5. Kim, K. H., D. W. Lee, J. M. Kim, and K. H. Moon. 1992. Characterization of multidrug resistant plasmid of *Staphylococcus aureus*. *Yakhak Hoeji* **36**: 486-490.
6. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467.
7. Trieu-Cuot, P. and P. Courvalin. 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3'5"-aminoglycoside phosphotransferase type III. *Gene* **23**: 331-341.
8. Trieu-Cuot, P., G. Gerbaud, T. Lambert, and P. Courvalin. 1985. *In vivo* transfer of genetic information between Gram-positive and Gram-negative bacteria. *The EMBO J.* **4**: 3583-3587.

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