Journal of Microbiology and Biotechnology
Vol. 6. No. 2, 145 - 146, 1996

NOTE

Characterization of Cryptic Plasmid of Multidrug-resistant *Staphylococcus aureus* SA2

IM, SUNG HWAN, SUNG JOON YOON, WOO KOO KIM, CHUL KYO SHIN, DAE WOON LEE, AND KYUNG HO MOON*

College of Pharmacy, Kyungsung University, 110-1, Daeyon-Dong, Nam-Gu, Pusan 608-736, Korea

The 2.4-kb cryptic plasmid (pKH8) of multidrug-resistant *Staphylococcus aureus* SA2 was characterized by complete nucleotide sequencing and homology comparison. pKH8 was found to contain three open reading frames. Protein analysis of pKH8 showed that pKH8 was a multidrug resistance plasmid and mediated resistance to ethidium bromide and quaternary ammonium compounds.

The clinical isolate *Staphylococcus aureus* SA2 was resistant to ampicillin (Am), chloramphenicol (Cm), clindamycin (Cl), erythromycin (Em), gentamicin (Gm), kanamycin (Km), methicillin (Mc), streptomycin (Sm), tetracycline (Tc), and tobramycin (Tm) and harboured four kinds of plasmids (3). Transformation experiment demonstrated that the 40.98-kb plasmid, pKH2, encoded resistance to Am, Cl, Em, Km, and Sm (4). Transformation experiments and nucleotide sequence determinations also revealed that the 4.44-kb pKH6 and the 4.14-kb pKH7 mediated resistance to Tc and Cm, respectively (5-7, 14). But the 2.4-kb cryptic plasmid pKH8 was found not to mediate any resistance to the above mentioned antibiotics (5). To investigate the function of the pKH8, a complete nucleotide sequence determination was carried out.

Sequence determination by the dideoxy chain termination method (12) after cloning of the pKH8 into pBluescript II KS$^+$ and sequence analysis with the nucleic acid databases (1) revealed that the pKH8 was a multidrug resistant plasmid conferring resistance to ethidium bromide and quaternary ammonium compounds (eg, benzalkonium chloride and cetyltrimethylammonium bromide) by QacC protein (Fig. 1). As shown in Table 1, MICs of ethidium bromide, benzalkonium chloride, and cetyltrimethylammonium bromide for the *S. aureus* SA2 confirmed this result. MICs of three compounds for *S. aureus* SA2 were four-fold higher than those for *S. aureus* RN4220 which did not have any plasmids and resistances.

The *qacC* gene (10), also known as *smr* (2) or *ebr* (13), mediates resistance to antiseptics and disinfectants via active efflux (11), and has been found on small and large plasmids of *S. aureus* such as the 2.4-kb plasmid, pSK89, and the 47.8-kb conjugative plasmid, pSK41 (10), respec-

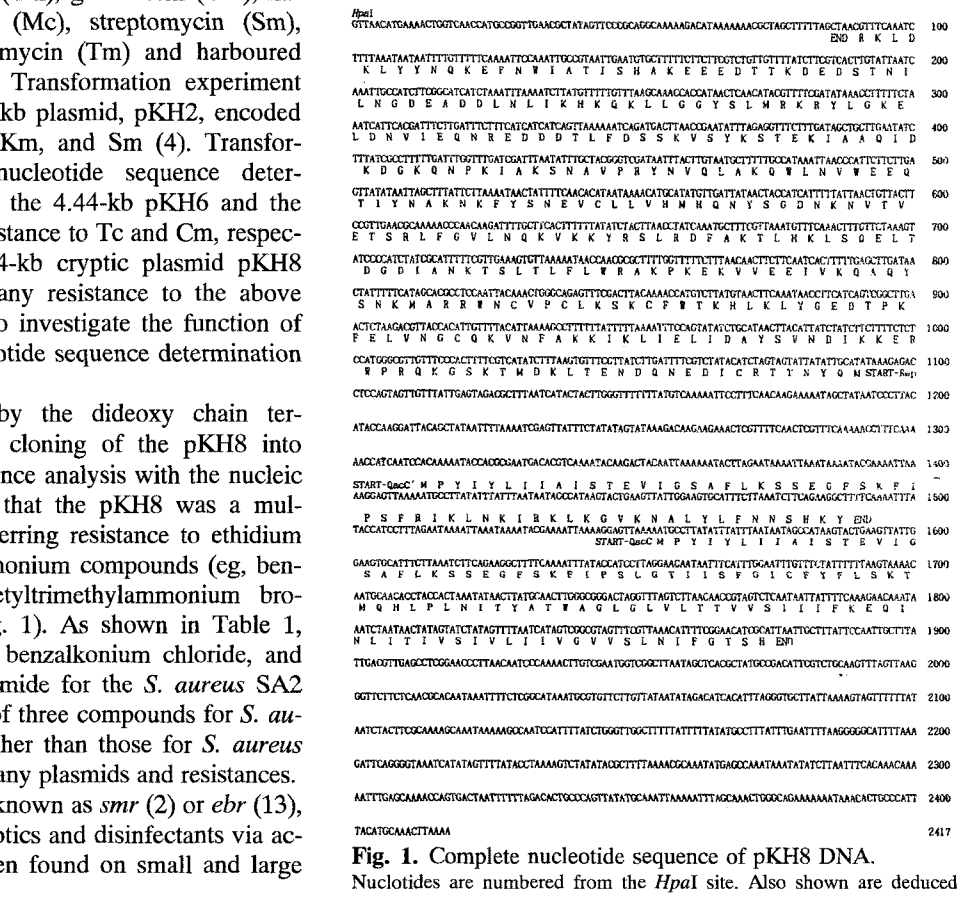

Fig. 1. Complete nucleotide sequence of pKH8 DNA.
Nuclotides are numbered from the *HpaI* site. Also shown are deduced potential amino acid sequences of the three putative open reading frames. The sequence of the pKH8 have been registered in GenBank (NCBI) with the accession number, U50077.

*Corresponding author
Key words: *Staphylococcus aureus* SA2, multidrug resistance plasmid, nucleotide sequence, QacC protein

Table 1. MICs of dye and disinfectants for the *S. aureus* strains.

Strains	MIC (μg/ml)[a]		
	Bc	Cb	Eb
S. aureus SA2	8	8	32
S. aureus RN4220	2	2	8

[a]Abbreviations for dye and disinfectants: Eb, ethidium bromide; Bc, Benzalkonium chloride; Cb, cetyltrimethylammonium bromide.

tively. The *qacC* has also been detected on plasmids in coagulase-negative staphylococci, although physical mapping revealed no apparent structural similarity between representative *qacC* plasmids from *S. aureus* and coagulase-negative staphylococci (8). The complete nucleotide sequence of the 2.4-kb pSK108, the representative of a small *qacC* plasmid from the coagulase-negative staphylococci, *S. epidermidis*, was determined (9).

In contrast to the above fact, comparisons between the complete nucleotide sequence of the pKH8 of *S. aureus* SA2 and those of the pSK89 of *S. aureus* and the pSK 108 of *S. epidermidis* showed that the pKH8 was not a family member of pSK89 but of pSK108. There was 99. 75% homology between pKH8 isolated in Korea and pSK108 isolated in Australia.

It is considered that great care should be taken to prevent nosocomial infections caused by this kind of strain such as *S. aureus* SA2, because this strain is resistant to not only commonly used antibiotics but also antiseptics and disinfectants often used for keeping patients from the infections in hospital.

REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**: 403-410.
2. Grinius, L., G. Dreguniene, E. B. Goldberg, C. J. Liao, and S. J. Projan. 1992. A staphylococcal multidrug resistance gene product is a member of a new protein family. *Plasmid* **27**: 119-129.
3. Kang, J. S. and K. H. Moon. 1990. Antibiotic resistance in *Staphylococcus aureus* isolated in Pusan. *Yakhak Hoeji* **34**: 122-125.
4. 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.
5. Lee, D. W. 1996. Characterization of plasmids from multidrug-resistant *Staphylococcus aureus* SA2. Ph. D. Thesis, Kyungsung University.
6. Lee, D. W. and K. H. Moon. 1993. Characterization of chloramphenicol resistant plasmid of multidrug resistant *Staphylococcus aureus*. *Yakhak Hoeji* **37**: 621-624.
7. Lee, D. W. and K. H. Moon. 1995. Characterization of tetracycline resistance plasmid of multidrug-resistant *Staphylococcus aureus*. *Yakhak Hoeji* **39**: 6-9.
8. Leelaporn, A., I. T. Paulsen, J. M. Tennent, T. G. Littlejohn, and R. A. Skurray. 1994. Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. *J. Med. Microbiol.* **40**: 214-220.
9. Leelaporn, A., N. Firth, I. T. Paulsen, A. J. Hettiaratchi, and R. A. Skurray. 1995. Multidrug resistance plasmid pSK108 from coagulase-negative staphylococci; relationship to *Staphylococcus aureus qacC* plasmids. *Plasmid* **34**: 62-67.
10. Littlejohn, T. G., D. DiBerardino, L. J. Messerotti, S. J. Spiers, and R. A. Skurray. 1991. Structure and evolution of a family of genes encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *Gene* **101**: 59-66.
11. Littlejohn, T. G., I. T. Paulsen, M. T. Gillespie, J. M. Tennent, M. Midgley, I. G. Jones, A. S. Purewal, and R. A. Skurray. 1992. Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. *FEMS Microbiol. Lett.* **95**: 259-266.
12. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463-5467.
13. Sasatsu, M., K. Shima, Y. Shibata, and M. Kono. 1989. Nucleotide sequence of a gene that encodes resistance to ethidium bromide from a transferable plasmid in *Staphylococcus aureus*. *Nucleic Acids Res.* **17**: 10103.
14. Yoon, S. J., D. W. Lee, W. K. Kim, C. K. Shin, S. H. Im, and K. H. Moon. 1995. Nucleotide sequences of Rep and CAT proteins encoded by chloramphenicol resistance plasmid pKH7. *Yakhak Hoeji* **39**: 676-680.

(Received March 4, 1996)