Cloning and Nucleotide Sequence of a Chromosomally Encoded Tetracycline Resistance Determinant, tetA(M), from a Pathogenic, Methicillin-Resistant Strain of Staphylococcus aureus

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This report describes the cloning and sequencing of a chromosomally encoded tetracycline resistance determinant from a clinical isolate of methicillin-resistant Staphylococcus aureus. On the basis of the sequence, the gene is in the tet(M) class, and it was shown that the S. aureus tetA(M) gene is induced at the level of transcription.

We have studied MRSA101, a methicillin-resistant strain of Staphylococcus aureus which was obtained from the peripheral blood of a pediatric patient with acquired immune deficiency syndrome and staphylococcal sepsis in a New York City hospital. We report here the isolation of a tetracycline resistance determinant from MRSA101 and show that this gene belongs to the tet(M) class of tetracycline resistance determinants. We have also demonstrated by Northern (RNA) blot analysis that the tet(M) gene is inducible by subinhibitory concentrations of tetracycline at the level of transcription.

Multiple antibiotic resistance of MRSA101. Drug resistances were assayed by streaking log-phase cultures on GL agar plates (12) with antibiotics added at the indicated concentrations and by observing single-colony formation after 24 h of growth at 37°C (single-colony growth indicating resistance). MRSA101 was resistant to the following antibiotics (concentrations in micrograms per milliliter): ampicillin, 50; carbenicillin, 100; cephalothin, 30; chloramphenicol, 20; erythromycin, 100; gentamicin, 10; nalidixic acid, 30; nitrofurantoin, 30; oxacillin, 1; penicillin, 10; tetracycline, 25; and tobramycin, 10. MRSA101 was also resistant to the heavy metals arsenate (8 x 10^{-4} M) and cadmium (5 x 10^{-5} M).

Plasmid and chromosomal DNAs were isolated as described elsewhere (13), and three plasmid bands of 3.0, 8.0, and >20 kb were visible on agarose gels (results not shown). Protoplast transformation of S. aureus 8325-4 (10) revealed that chloramphenicol resistance was associated with the 3.0-kb plasmid, which had the same restriction enzyme digestion pattern as pCL194 (2). The >20-kb plasmid encoded penicillin, cadmium, and arsenate resistances, phenotypes associated with the S. aureus penicillinase plasmid pI524 (11); the 8.0-kb plasmid was apparently cryptic. The remaining resistances are therefore chromosomally encoded.

Cloning and sequencing the tetracycline gene from MRSA101. Plasmids used in this study are listed in Table 1.

| TABLE 1. Plasmids |
|-------------------|
| Plasmid | Resistance marker* | Description | Size (kb) | Reference |
|---------|-------------------|-------------|----------|-----------|
| pUC8    | Ap'  |  | E. coli cloning vehicle | 2.7 | 20 |
| pMV6N   | Tc' | Ap'  | tet(M) clone | 5.6 | This work |
| pT181   | Tc'  |  | S. aureus tet(K) plasmid | 4.4 | 1 |
| pEI94   | Em'  |  | S. aureus cloning vehicle | 3.7 | 2 |
| pAM120  | Ap'  | Tn916 clone | >20 | 16 |

* Ap', Ampicillin resistance; Em', erythromycin resistance; Tc', tetracycline resistance.

Chromosomal DNA of strain MRSA101 was partially digested with Sau3A1. Restriction enzymes were purchased from Boehringer Mannheim (Indianapolis, Ind.) and used according to the manufacturer's specifications. Size-fract-

![FIG. 1. (A) Agarose gel. Purified DNA was separated by electrophoresis on a 1% agarose gel. The gel was stained with ethidium bromide, transilluminated with UV light at 382 nm, and photographed with a red filter. Lane 1, pMV6N; lane 2, pT181; lane 3, pAM120; lane 4, MRSA101 chromosomal DNA digested with EcoRI; lane 5, MRSA101 chromosomal DNA digested with HindIII; lane 6, MRSA101 chromosomal DNA digested with MboI; lane 7, pMV6N. (B) Southern blot autoradiograph of gel in panel A. Pooled MboI A and B fragments of pMV6N plasmid DNA were labeled with [α-32P]dATP by nick translation and used as a probe.]

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tionated fragments between 2 and 5 kb were obtained from a 5 to 20% neutral sucrose gradient and cloned into the *Escherichia coli* cloning vehicle pUC8 at the BamHI site and transformed into *E. coli* JM107 (21). The pUC9-MRSA101 library was screened for tetracycline-resistant transformants, and one of several resistant colonies was isolated. This strain carried a recombinant plasmid, pMVN6, which carried a 2.9-kb insert. Cleavage with *MboI* yielded insert fragments of 1.6 and 1.3 kb. These two fragments were gel purified (7), pooled, and used as a probe for Southern blot hybridizations (17) of MRSA101 whole-cell DNA to verify that the cloned DNA actually originated from MRSA101. The probe hybridized to a single *EcoRI* fragment, two *HindIII* fragments, and two *MboI* fragments (Fig. 1, lanes 4 through 6, respectively). However, this probe did not hybridize to pT181 (Fig. 1, lanes 2), a staphylococcal plasmid which carries the *tet(K)* gene (nor did the probe hybridize to any of the plasmids from MRSA101 [data not shown]). The probe did hybridize to pAM120, a plasmid carrying Tn916 (Fig. 1, lanes 3) a conjugative transposon from *Enterococcus faecalis* which carries a *tet(M)* tetracycline resistance determinant (16).

The cloned insert from pMVN6 was isolated on a 2.9-kb *SmaI*-*HindIII* fragment (sites in pUC8) and ligated (with T4 ligase from U.S. Biochemical, Cleveland, Ohio) into the *ClaI* site of the *S. aureus* plasmid pE194 by blunt-end ligation (5). Protoplasts of *S. aureus* RN4220 were transformed with selection for tetracycline resistance. As predicted, the tetracycline-resistant transformants recovered contained plasmids that carried the pMVN6 DNA inserted at the *ClaI* site of pE194.

The 1.3- and 1.6-kb *MboI* fragments were cloned into M13mp19 in both orientations (9) and sequenced by the dideoxy-chain termination method (15) with Klenow fragment and/or Sequenase (U.S. Biochemical). Initial sequence data were obtained with an M13mp19 sequencing primer (U.S. Biochemical). The sequence for each strand was completed by using synthetic oligonucleotide primers based on the previously determined sequences. The entire cloned segment consisted of 2,899 nucleotides (Fig. 2). An open reading frame of 1,917 nucleotides is preceded by a gram-positive ribosome-binding site (8). Comparison of the amino acid and nucleotide sequences with those of the *tet(M)* gene from the streptococcal transposon Tn1545 (6) revealed 92.3% similarity at both amino acid and nucleotide sequence levels. Additionally, there was 95% homology to the *tet(M)* gene from *Ureaplasma urealyticum* (14). Therefore, the tetracycline resistance gene from MRSA101 belongs to the *tet(M)* class, and we have designated it *tetA(M)* (4). The sequence of the *tetA(M)* gene is 68.7% AT, which matches the base composition of *S. aureus* DNA (18).

The possibility that *tetA(M)* is associated with a Tn916-like element was tested by Southern blotting. When a recombinant plasmid carrying Tn916 (pAM120) (16) was used to probe digests of MRSA101 DNA, the probe hybridized to fragments of the same size as the probe (described above) from the *S. aureus tetA(M)* gene. Additionally, two small *MboI* fragments of about 800 and 600 bp were observed. This indicates that while the *tetA(M)* gene may be common to MRSA101 and Tn916, at most, 1,400 bp of Tn916 DNA is present on MRSA101 in addition to the *tetA(M)*-specific DNA. This amount of Tn916-specific DNA would not be sufficient to encode transposition functions, which require at least 2.5 kb (16). Therefore, if the *tetA(M)* gene is associated with a transposon, it is probably not similar to Tn916.

**Induction of tetA(M) by tetracycline.** The MIC of tetracycline for MRSA101 was 25 μg/ml. However, when cells grown in broth were first treated with 0.05 μg of tetracycline per ml, the MIC increased to 65 μg/ml. Therefore, MRSA101 tetracycline resistance was inducible by the antibiotic. To test whether induction involved transcription, the level of *tetA(M)* mRNA after induction was determined by Northern blot analysis by the method of Kornblum et al. (3). In Figure 3, lane 1 shows RNA from the culture prior to induction (time zero), lane 2 shows RNA from a culture 1 h after induction with 0.05 μg of tetracycline per ml, and lane 3 shows RNA from a noninduced culture grown in parallel with the lane 2 culture. A strand-specific probe was prepared by using the *tetA(M)* *MboI* fragment from position 1571 to 2997. The greatly increased amount of *tetA(M)*-specific RNA in the induced culture indicates that this gene is induced at the level of the transcript. In the uninduced culture, a single, faint band is visible while the induced culture shows two intense bands, suggesting either two transcriptional starts or two transcripts which share a common start but terminate at different places.

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**FIG. 3.** Northern blot autoradiograph. RNA samples were prepared and separated on a 1.0% agarose-formaldehyde gel by the quantitative method of Kornblum et al. (3). A strand-specific probe corresponding to the *MboI* fragment from position 1571 to 2997 of the *tetA(M)* sequence was used. Lane 1, MRSA101 at time zero; lane 2, MRSA101 grown for 1 h in the presence of 0.05 μg of tetracycline per ml; lane 3, MRSA101 grown for 1 h in the absence of tetracycline.
Nucleotide sequence accession number. The sequence described in this paper has been assigned GenBank accession number M21136.

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