Dissemination of the \textit{tetM} Tetracycline Resistance Determinant to \textit{Ureaplasma urealyticum}

MARILYN C. ROBERTS* AND GEORGE E. KENNY

Department of Pathobiology, University of Washington, Seattle, Washington 98195

Received 12 August 1985/Accepted 5 November 1985

\textit{Ureaplasma urealyticum} is an organism considered susceptible to tetracycline. Ten tetracycline-resistant (Tc\textsuperscript{r}) clinical isolates and Tc\textsuperscript{r} serotype 9 were examined. All contained DNA sequences homologous to the streptococcal determinant \textit{tetM}. They differed from each other on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Southern blots, and immunoblots and appeared to represent different strains.

\textit{Ureaplasma urealyticum} is a common inhabitant of the genital tract, having been isolated from the vagina of 70% of normal women and from the urethra of some 20% of normal males (14). The organism, which has also been isolated from the blood of women with mild postpartum fever, from amniotic fluid, from lungs of infants, and from the upper urinary tract (14), seems to invade amniotic fluid, urethral tract (14), seems to be an effective opportunist when it invades beyond its normal habitat. Further, \textit{U. urealyticum} has been shown to cause experimental urethritis when inoculated intraurethrally in humans with negative urethral cultures for potentially pathogenic microorganisms before inoculation. This suggests that ureaplasmas are likely to be pathogenic under natural conditions (14). Tetracycline is the drug commonly used to treat infections caused by \textit{U. urealyticum} (20). A high-level tetracycline-resistant (Tc\textsuperscript{r}) strain was first isolated in 1974 (6). Now 6 to 10% of the Seattle isolates are resistant (24).

Sixty strains of \textit{U. urealyticum} were screened for tetracycline resistance by using agar plate dilution without pregrowth in the presence of tetracycline to determine MICs (19). The study included 7 Seattle isolates from 1970 to 1972 (7), 43 Seattle isolates from 1976 to 1984, 1 Los Angeles isolate (kindly supplied by G. Cassell), and nine serovars of \textit{U. urealyticum} (types 1 to 9) (20). The endpoint was scored as the lowest concentration of antibiotic which prevented colony formation on morpholineethanesulfonic acid agar at 96 h (colonies were visualized by the calcium chloride urea stain [11] at 72 and 96 h). Ten of the clinical strains and serotype 9 were resistant to tetracycline, with MICs of \(\geq 64\) \(\mu g/ml\) (Tc\textsuperscript{r}), while the other strains were inhibited by \(\leq 3.2\) \(\mu g/ml\) and considered tetracycline susceptible (Tc\textsuperscript{s}). The MICs for the nine serotypes reported by Robertson et al. (20), using the broth dilution method, were compatible with our values determined with the agar dilution method.

Representative Tc\textsuperscript{r} and Tc\textsuperscript{s} strains were grown in soy peptone-fresh yeast dilsate broth supplemented with 2 to 10% "agamma" horse serum, 25 mM urea, 50 mM morpholineethanesulfonic acid buffer at an initial pH of 6.2, and 1 mM sodium sulfate (11). Cells were harvested by centrifugation when the pH had reached 7.0 and were concentrated 500-fold. Cleared lysates were prepared by using a modification of the Hansen and Olsen procedure (10) and examined by agarose gel electrophoresis. No bands corresponding to plasmids were evident on the gels. In contrast, when \textit{Mycoplasma hominis} strains were lysed with this method, bands corresponding to \(>2\) megadaltons were often visualized (19).

Recently, we have shown that Tc\textsuperscript{r} \textit{M. hominis} strains contain the \textit{tetM} determinant (19) first described in streptococci (1–3). \textit{tetM} confers high-level resistance, is often located on a conjugative transposon (8, 9), has been found in both oral and enteric streptococci (1–3, 8, 9), and is usually associated with the chromosome (3, 8). Dot blots (18) were prepared from concentrates of \textit{U. urealyticum} strains (about 0.5 mg of cell protein per ml) and boiled for 3 min before spotting 50 to 100 \(\mu l\) onto nitrocellulose paper. A radiolabeled probe prepared by nick translation (19) was made from pJ13, a plasmid composed of the \textit{Escherichia coli} vector pACYC177 (4) and a 5-kilobase \textit{HincII} fragment from \textit{Streptococcus agalactiae} that encodes \textit{tetM} (3). The entire plasmid pJ13 was used as the source of the probe. An extra filter was probe by using whole-chromosomal DNA isolated from \textit{U. urealyticum} serovar 8. Previous work in our laboratory has shown that chromosomal DNA probes from serotypes 1, 4, or 8 hybridized equally well with dot blots prepared from types 1 to 9 (unpublished data). The 11 strains with MICs of \(\geq 64\) hybridized with the probe pJH. This included the Los Angeles strain, sent to us because it was known to be resistant (G. Cassell, personal communication). The 49 susceptible strains did not hybridize with the pJH probe. All the \textit{U. urealyticum} strains, regardless of antibiotic susceptibility, hybridized with whole-chromosomal DNA prepared from type 8 DNA. None of the strains reacted when the radiolabeled cloning vector pACYC177 was used. DNA restriction endonuclease profiles (16) of the Tc\textsuperscript{r} strains showed significant differences in banding patterns. Southern blots were prepared and hybridized with pJ13. No hybridization was observed in the Tc\textsuperscript{r} strains, but hybridization occurred in all of the Tc\textsuperscript{s} strains. The number and size of the hybridizing bands varied with the strain examined, suggesting that they represent different strains rather than a single clone (Fig. 1).

Comparisons of four Tc\textsuperscript{r} isolates with types 1, 3, 4, 8, and 9 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (21) showed that the staining patterns of the various isolates differed as much from each other as one serotype did from another. The strains appeared no more similar to type 9 (a Vancouver, British Columbia, isolate) (6) than to the other serotypes, and no unique band could be related to tetracycline resistance. Immunoblotting (13) of these strains against antisera to types 1, 3, 4, 5, and 8 showed consider-
We thank F. Cartwright, C. Tompkins, and J. Hale for technical support; V. Burdett for the pJ13-carrying E. coli strain; and G. Cassell, K. K. Holmes, and J. Robertson for tetracycline-resistant strains.

LITERATURE CITED


This work was supported by Public Health Service grants AI-12192 and AI-06720 from the National Institutes of Health.


