SUSCEPTIBILITY OF *UREAPLASMA UREALYTICUM* FROM SEMINAL FLUID TO ANTIMICROBIAL AGENTS

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Semen specimens from 678 oligozoospermic men who attended our male infertility clinic were checked for the presence of *Ureaplasma urealyticum*, and 239 out of 678 strains proved positive. The susceptibility of these strains to eight antimicrobial agents was determined by a broth dilution system. The median MIC of each drug was as follows: tetracycline 0.78 μg/ml; doxycycline 0.10 μg/ml; minocycline 0.10 μg/ml; lincomycin 3.13 μg/ml; erythromycin 1.56 μg/ml; rokitamycin 0.10 μg/ml; enoxacin 12.5 μg/ml and ofloxacin 3.13 μg/ml. This result confirmed the effectiveness, *in vitro*, of tetracyclines and rokitamycin against genital *U. urealyticum* infections.

Key words: *Ureaplasma urealyticum*, MIC

Introduction

Ureaplasma urealyticum (*U. urealyticum*) was first reported by Shepard[1] in 1954 and characterized by its small colonies on agar, urease activity and fastidious growth requirements.

U. urealyticum has been suggested as one of the causative organisms of non-gonococcal urethritis like *Chlamydia trachomatis*[2] In 1972 Gnarpe and Friberg[3] reported that *U. urealyticum* had been isolated from the semen and cervical mucus of infertile couples more frequently than in fertile control couples. Since then, the possible relationship between genital *U. urealyticum* infections and human reproductive failure has been widely discussed, but there are only a few reports[4~7] available concerning *U. urealyticum* susceptibility to antimicrobial agents. In the preliminary experiment, all the penicillins and cephalosporins were found ineffective against *U. urealyticum*. We therefore adopted the following three groups of drugs: macrolides, tetracyclines and new quinolone antimicrobial agents. Minimum inhibitory concentrations (MICs) of these drugs against *U. urealyticum* separated from the semen of oligozoospermic men were determined by the broth dilution system using microtiter plates.

Materials and Methods

1. Clinical specimens

All patients were required to wash their glans penis with soap and water and urinate before collecting semen. These urine samples were checked for the presense of *U. urealyticum*. The semen was collected in a sterile plastic tube by masturbation from patients attending the male infertility clinic of Kobe University School of Medicine after a 4-day abstinence. The semen was fully liquefied at room temperature. After semen examinations (sperm density, motility, swimming speed and morphology), 100 μl of each specimen was used to detect *U. urealyticum*.

2. Detection and cloning of *U. urealyticum*

A T-broth was used for the primary isolation of *U. urealyticum*. It consisted of 3.0 g of Difro PPLO broth without crystal violet, 40 ml of unheated horse serum, 20 ml of 23 % yeast extract, 2 ml of 10 % urea, 0.8 ml of 0.5 % phenol red, 20×10^4 units of penicillin-G, 2.9 ml of 2.54 % tallium acetate (not used for the determination of MIC) and 140 ml of distilled water, and the pH was adjusted to 8.0 with 2-3 drops of conc. hydrochloric acid.

The growth of *U. urealyticum* in T-broth is accompanied by hydrolysis of urea and accumulation of ammonia. Since T-broth is weakly buffered, the reaction becomes progressively alkaline, and the color of the incorporated phenol red indicator changes from yellow to red. The specimen, 10 μl was introduced into 2 ml of T-broth and incubated for 48 h at 30°C in room air. When the semen specimen culture was positive for *U. urealyticum*, cloning on T-agar (twice) was conducted by using the push block technique to get *U. urealyticum* strains. These separated strains were stored below −80°C until use.

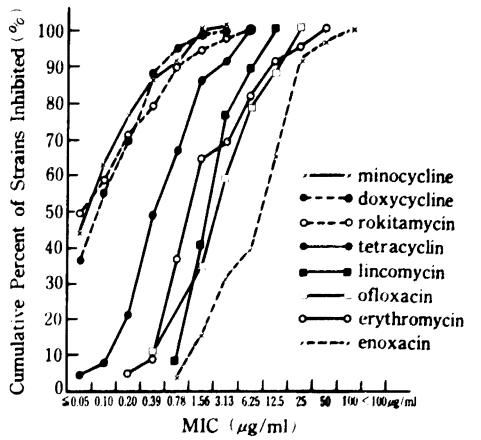
3. Determination of MIC

Serial dilutions of the antimicrobial agents were prepared on the day when each agent was tested. Tests were performed in the wells of microtiter plates (Falcon). The initial MIC was designated as the lowest concentration of the drug to inhibit a color change of the broth with *U. urealyticum*. The 200 strains stored were reincubated for 48 h at 30°C in the T-broth to test their viability. If the broth color changed, that strain was harvested to the microtiter plates at a concentration of 10^4 colony forming units/ml. Serial dilution of an antimicrobial agent was made in wells from 2 to 2^{10} times. The initial MIC was measured when the control well containing *U. urealyticum* without antimicrobial agent first showed a color change. In most cases, 48-hour incubation was enough for a color change.

Results

A total of 678 semen specimens were tested for the presence of *U. urealyticum*, and 239 (35.3 %) proved to be positive. All the specimens were cloned three times on differential T-agar, and their viability was carefully checked. From the 239 semen specimens, 200 strains were finally cloned and stored below −80°C until use. For these 239 patients 81 % of urine specimens were negative for *U. urealyticum*.

Since *U. urealyticum* is a rapidly growing microorganism, we adopted the initial MIC, which we believed to represent clinical susceptibility better than the final MIC. Using these 200 strains, the initial MICs were measured. The antimicrobials



Antibiotic \ MIC (μg/ml)	≤0.05	0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	<100	Total Strains
Tetracycline	9	6	27	54	**36**	41	9	18						200
Doxycycline	72	**36**	31	36	13	9	2	1						200
Minocycline	90	**35**	27	20	9	18	1							200
Lincomycin					18	63	**72**	27	20					200
Erythromycin			9	9	56	**54**	9	27	18	7	11			200
Rokitamycin	98	**17**	26	18	21	7	7	6						200
Enoxacin					8	23	31	17	**50**	54	11	6		200
Ofloxacin				21	22	26	**49**	40	19	23				200

Fig. 1. Top: cumulative percentage of MICs of each drug. Bottom: distribution of MICs (double squares indicate the median MICs).

used fell into three general groups: tetracyclines (tetracycline, minocycline, doxycycline), macrolides (lincomycin, erythromycin, rokitamycin), and new quinolone antimicrobial agents (enoxacin, ofloxacin). The distribution of MICs and cumulative percent of MICs for each antimicrobial agent are summarized in Fig.1. To each drug, *U. urealyticum* was susceptible within a relatively narrow range of concentration. The median MIC was 0.78 μg/ml (range 0.05—6.25) for tetracycline, 0.10 (0.05—6.25) for doxycycline, 0.10 (0.05—3.13) for minocycline, 3.13 (0.78—12.5) for lincomycin, 1.56 (0.20—50) for erythromycin, 0.10 (0.05—6.25) for rokitamycin, 12.5 (0.78—100) for enoxacin and 3.13 (0.39—25) for ofloxacin.

Discussion

Gnarpe and Friberg[3] have documented that in couples with unexpected infertility of more than five years' duration, *U. urealyticum* was found in 85 %

of the men and 91 % of the women. In the control group, 23 % of pregnant women and 22 % of their spouses were found to have *U. urealyticum*. But other groups have failed to demonstrate any difference in the isolation rate between fertile and long-standing infertile couples[8,9]. In our study, 35.8 % of the infertile men proved positive for *U. urealyticum* in their semen. This is equal to the rate reported by deLouvois[8]. Over 80 % of the urine specimens were negative for *U. urealyticum*. This suggests that *U. urealyticum* isolated from semen is not a simple contamination of the urethral flora but that the focus of *U. urealyticum* infection is located in the seminal vesicles and prostate. In our preliminary experiment, the MICs of penicillins and cephalosporins against *U. urealyticum* were more than 100 μg/ml (data not shown). This is because *U. urealyticum* lacks cell walls. To eradicate genital *U. urealyticum*, macrolides and tetracyclines have been used. Our data for tetracyclines and macrolides support this. No strains have exceeded an MIC of 12.5 μg/ml using tetracycline, minocycline or doxycycline. Among macrolides, lincomycin showed fairly good and rokitamycin very good MICs, compatible with those of minocycline and doxycycline. The distribution of MICs of tetracycline appeared bimodal, and of minocycline, but not doxycycline, and likewise for erythromycin but not the other macrolides. However, there is no good explanation for these results. Other new quinolone antimicrobial agents were not so effective against *U. urealyticum in vitro*[7]. From our results, the MICs for 14 % of tetracycline, for 32 % of erythromycin and for most of lincomycin, enoxacin and for 21 % of ofloxacin are all higher than the blood levels on standard dosage, suggesting that these are not suitable therapeutic agents against *U. urealyticum*. Finally, we recommend minocycline, doxycycline and rokitamycin for the treatment of genital Ureaplasma infections. In our clinical experience, when 21 male patients whose semen specimens were positive for *U. urealyticum* were treated with doxycycline 100 mg/day for 28 days and their sexual partners with the same regimen simultaneously, in eighteen cases *U. urealyticum* was eradicated (unpublished data) from the semen.

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不妊患者精漿より分離された *Ureaplasma urealyticum* の抗菌剤に対する MIC

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男子不妊患者678名の精漿のうち239で *Ureaplasma urealyticum* が陽性であった。これをクローニングして得た200株を対象としてそのペニシリン系，セフェム系，テトラサイクリン系，マクロライド系，ニューキノロンに対する MIC を *in vitro* で測定した。ペニシリン系，セフェム系はいずれも100 μg/ml 以上であった。他の薬剤に関しては MIC の中央値は tetracycline 0.78 μg/ml，doxycycline 0.10 μg/ml，minocycline 0.10 μg/ml，lincomycin 0.10 μg/ml，erythromycin 1.56 μg/ml，rokitamycin 0.10 μg/ml，enoxacin 12.5 μg/ml，ofloxacin 3.13 μg/ml であった。この結果より常用量で充分な効果を挙げると考えられたのは doxycycline，minocycline と rokitamycin であった。

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