

ANTIBIOTIC SUSCEPTIBILITY TEST AGAINST U. UREALYTICUM ISOLATED FROM CLINICAL SPECIMEN.

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비뇨생식기계 감염환자로부터 분리된 *Ureaplasma urealyticum*의 항생물질에 대한 감수성

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= 국문 초록 =

비뇨생식기계 감염환자로부터 분리된 203주의 *Ureaplasma urealyticum*을 대상으로 oxytetracycline, minocycline 및 josamycin에 대한 감수성 정도를 시험관 회석법에 의한 최저발육 저지 농도로 조사하였다.

Oxytetracycline, minocycline 및 josamycin의 최종 발육저지농도의 평균치는 각각 4.0, 8.0 및 0.5 $\mu\text{g/ml}$ 였다.

Oxytetracycline에 대해서는 분리균주의 11.9%만이 감수성이었고 13.8%는 중등도의 내성균이었으며, 74.3%는 고도 내성균이었다.

Minocycline에 대해서는 분리균주의 12.9%만이 감수성이었고, 2.0%는 중등도의 내성균이었으며 85.1%는 고도 내성균이었다.

Josamycin에 대해서는 분리균주의 87.7%가 감수성이었으며 12.3%만이 내성균이었다. 분리균주중 10주는 3가지 약제에 모두 내성이었다.

INTRODUCTION

All mycoplasmas including *U. urealyticum* had been said to be susceptible to tetracyclines^{2,5,11,24)} and macrolide antibiotics^{5,8,24)}. In 1974, however, Ford and Smith³⁾ first isolated a tetracycline-resistant strain of *U. urealyticum* from a patient with persistent nongonococcal urethritis. Recently highly resistant strains of *U. urealyticum* to tetracyclines and erythromycin were reported so that these antibiotics are not always recommended as the first choice of drugs for *U. urealyticum* infections.^{4,8,9,17,20,21)}

The present study was undertaken to determine the minimum inhibitory concentration(MIC) of oxytetracycline, minocycline and josamycin against 203 strains of *U. urealyticum* isolated from patients with genitourinary tract infection during a period from 1981 to 1983 in Korea.

MATERIALS AND METHODS

Organism: The 203 isolates of *U. urealyticum* were identified by the growth characteristics, colonial morphology and positive urease spot test.^{13,15,16)}

Medium: Ureaplasma broth(10-B)^{13,15,16)} and agar(A7)¹⁴⁾ media were used for primary isolation as well as the identification of *U. urealyticum*. The MICs of the drugs tested were determined in the ureaplasma broth.

Antimicrobial agent: Drugs tested were oxytetracycline, Korea Pfizer Inc. Co., Seoul, Korea; minocycline, Yuhan Ltd., Seoul, Korea; josamycin, Yamanouchi Pharm. Co., Tokyo, Japan. All the agents were kindly supplied.

Each stock solution of oxytetracycline and minocycline was prepared in distilled water at the concentration of 1,000 μg per ml, and that of josamycin was made by dissolving 10mg of the compound in 0.2 ml of 1N HCl followed by the

addition of 9.8 ml of distilled water. Each stock solution was sterilized by passage through a membrane filter(0.22 μm pore size, Millipore Corp., Bedford, Mass. U.S.A.).

Susceptibility test: The initial and final MICs of oxytetracycline, minocycline and josamycin to the 203 isolates of *U. urealyticum* were determined by the direct broth dilution technique.^{2,3,18,20,21)} Each tube containing 2 ml of ureaplasma broth with 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.16, or 0.03 μg of each antibiotic per ml was inoculated with 0.05ml of ureaplasma culture fluid containing 10^5 colony forming units(CFU) per ml. All the tubes were incubated at 37°C for 3 days and observed daily for color change caused by a given ureaplasma at the time the control tube containing the same strain changed the color.^{1,20,21)} The lowest concentration of antibiotic that completely inhibited color change after 3 days of incubation was referred to as the final MIC. Incubation beyond 3 days did not result in any further color change.

RESULTS

The initial and final MICs of the antibiotics tested against the 203 clinical isolates of *U. urealyticum* and cumulative percentages of susceptible strains to each antibiotic are shown in Figs. 1 and 2. The initial MICs of oxytetracycline and minocycline were ranged from 0.03 μg to 0.8 μg per ml and those of josamycin were from 0.06 μg to 4.0 μg per ml. The final MICs of oxytetracycline were from 0.06 μg to 16.0 μg per ml and those of josamycin were from 0.25 μg to 8.0 μg per ml, where as those of minocycline were from 0.06 μg to over 16.0 μg per ml and 32.0% of the isolates were not inhibited by 16.0 μg per ml of this antibiotic.

As shown in Table 1, the lowest median values for the both initial and final MICs were 0.12 μg

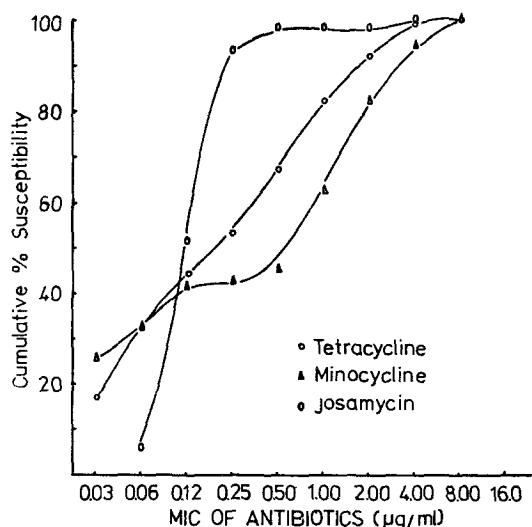


Fig. 1. Initial MICs for the 203 clinical isolates of *Ureaplasma urealyticum* to antibiotics tested.

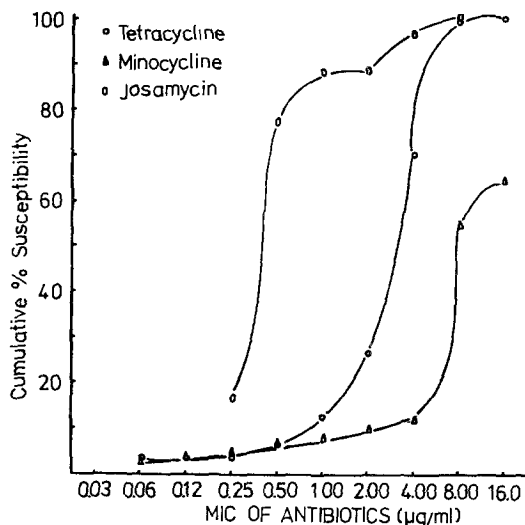


Fig. 2. Final MICs for the clinical isolates of *Ureaplasma urealyticum* to antibiotics tested.

Table 1. Median MIC of Three Antibiotics to *Ureaplasma urealyticum* Isolated from Patients

Antibiotic	No. of strains tested	Median (μg/ml)	
		Initial MIC	Final MIC
Oxytetracycline	203	0.25	4.0
Minocycline	203	1.00	8.0
Josamycin	203	0.12	0.5

Table 2. Susceptibilities of Ten Strains of Isolated *Ureaplasma* to Three Kinds of Antibiotics

Isolated Strains	Final MIC (μg/ml)		
	Oxytetracycline	Minocycline	Josamycin
4	16.0	< 16.0	8.0
7	16.0	16.0	8.0
14	8.0	4.0	8.0
15	16.0	< 16.0	8.0
17	16.0	< 16.0	8.0
19	4.0	< 16.0	8.0
45	8.0	< 16.0	8.0
68	16.0	< 16.0	16.0
75	8.0	< 16.0	8.0
154	< 16.0	< 16.0	0.5

and 0.5 μg per ml of josamycin, the next were 0.25 μg and 4.0 μg per ml of oxytetracycline, and the highest were 1.0 μg and 8.0 μg per ml of minocycline, respectively.

The results showed that josamycin was clearly the most active antibiotic against the ureaplasma isolates. Several isolates required widely deviated high concentrations of final MIC for each drug as shown in Table 2.

DISCUSSION

Earlier reports^{2,11,24)} claimed that all the ureaplasmas were susceptible to tetracyclines. However, Ford and Smith⁷⁾ reported a tetracycline-resistant strain associated with non-specific urethritis.

Spaepen and Kundsins²¹⁾ found that 85.2, 83.3, 72.2, and 33.3% of their clinical specimens were inhibited by 1 μg per ml of minocycline, deoxycycline and tetracycline, respectively. On the other hand, Stimson et al²³⁾ reported that MICs of tetracycline against 7.0% of their isolates were 256 μg per ml or higher. Steigbigel et al²²⁾ reported that ureaplasmas were inhibited by an achievable blood level of 4.0 μg per ml of tetracycline.

Spaepen et al²⁰⁾ found tetracycline-resistant strains from patients with a history of reproductive failure. The same investigators²⁰⁾ considered ureaplasmas requiring tetracycline at a final MIC of 1.0 μg per ml to be susceptible, 2.0 μg per ml to be intermediate, and 4.0 μg per ml to be resistant. In the present study, only 11.9% of our isolates were sensitive, 13.8% intermediate and 74.3% resistant to oxytetracycline as judged from the final MICs by Spaepen et al.²⁰⁾

Our isolation rate of resistant strain of ureaplasma against oxytetracycline was higher than the reports by others.

This results also indicate that the oxytetracycline should not be recommended for the empirical treat-

ment of ureaplasma infections in Korea because of the high frequency of resistant strains.

Steigbigel et al²²⁾ reported that ureaplasmas were inhibited by an achievable blood level of 2 μg per ml of minocycline based on the final MIC. Spaepen and Kundsins²¹⁾ reported that minocycline was the most active antibiotic to ureaplasma. On the other hand, Magalhaes and Veras⁹⁾ stated that 33% of their isolates were resistant to 4 μg per ml of minocycline. In the present study, only 12.9% of the isolates were sensitive to 4.0 μg , 55.1% were resistant to 8.0 to 16.0 μg , and 32% were resistant to over 16.0 μg per ml of this antibiotic.

This result indicate that most of our isolates (85.1%) were highly resistant to minocycline.

Ohsono¹⁰⁾ reported that josamycin inhibited growth of many kinds of bacteria including mycoplasmas by a concentration of 1 μg per ml. In the present study, 87.7% of the isolates were sensitive to 1.0 μg per ml of this antibiotic.

A possible explanation for the high incidence of resistant ureaplasmas in Korea is that these antibiotics have been sold for many years without any medical control.

In conclusion, josamycin is higher antiureaplasma activity than other antibiotics tested, and minocycline and oxytetracycline are not the drug of first choice for ureaplasma infections unless the susceptibility test is carried out.

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