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Differential Susceptibilities of *Mycobacterium avium* and *Mycobacterium intracellulare* to Sodium Nitrite

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Sixty-seven of 72 strains of *Mycobacterium avium* (93.1%) were resistant to sodium nitrite at a concentration of 3 mg/ml in 7H11 agar medium, while 57 of 59 strains of *Mycobacterium intracellulare* (96.6%) were susceptible to the agent. The difference in the susceptibilities of *M. avium* and *M. intracellulare* to sodium nitrite is therefore useful for the differentiation of the two species.

In the course of the experiments for nitrite reduction (5, 7), we found that most *Mycobacterium intracellulare* strains could grow in 7H9 medium containing sodium nitrite, but most *Mycobacterium avium* strains could not. We therefore examined the susceptibilities of *M. avium* and *M. intracellulare* to sodium nitrite in a 7H11 agar medium.

Lung disease-associated *M. avium* (72 strains) and *M. intracellulare* (59 strains) isolated from individuals in national sanatoria in various areas of Japan were used. All strains produced smooth and transparent colonies after growth in 7H11 agar medium (Difco Laboratories, Detroit, Mich.). These organisms were identified by using the Gen-Probe Rapid Diagnostic System for the *M. avium* complex (Gen-Probe Inc., San Diego, Calif.) (1–3). The organisms were cultured in 7H9 broth medium (Difco) at 37°C until the optical density at 540 nm reached 0.1 (approximately 10^7 CFU/ml), which was determined by using the Novaspec 4049 spectrophotometer (LKB Biochrom Ltd., Cambridge, England). The bacteria in the medium were subjected to serial 10-fold dilutions with physiological saline containing 0.1% Tween 80.

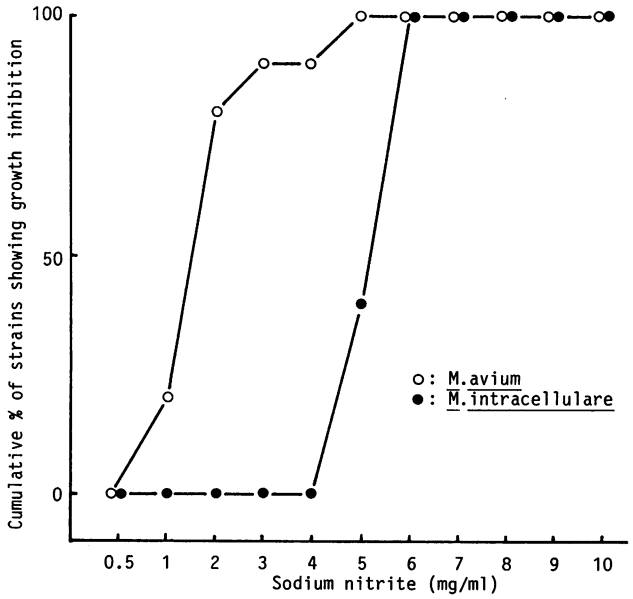

FIG. 1. Susceptibilities of *M. avium* and *M. intracellulare* strains to sodium nitrite.

Susceptibility tests were carried out by the agar dilution method by using 7H11 agar plates. Five microliters of the test bacterial suspension mentioned above was spotted with a microplanter (Sakuma Co., Tokyo, Japan) onto 7H11 agar plates containing 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg of sodium nitrite (Wako Pure Chemical Ind., Ltd., Osaka, Japan) per ml. The growth inhibitory concentrations were read as the concentrations of the agent that completely inhibited growth of the test organisms.

Figure 1 shows the inhibitory efficacy of sodium nitrite at various concentrations against the growth of both *M. avium* (10 strains) and *M. intracellulare* (10 strains). The growth of 80% of *M. avium* strains was inhibited by 2 mg of sodium nitrite per ml, and growth of 90% of *M. avium* strains was inhibited by both 3 and 4 mg/ml. However, all the *M. intracellulare* strains grew on medium containing even 4 mg of sodium nitrite per ml.

Table 1 shows the susceptibilities of the *M. avium* (72 strains) and *M. intracellulare* (59 strains) to sodium nitrite at a concentration of 3 mg/ml. Bacterial growth was observed in 5 strains (6.9%) of *M. avium* and 57 strains (96.6%) of *M. intracellulare*, indicating a much higher resistance of *M. intracellulare* to sodium nitrite compared with that of *M. avium*.

Results of the present study indicate the differences in the susceptibilities of *M. avium* and *M. intracellulare* to sodium nitrite; i.e., in most cases, *M. avium* is more susceptible than *M. intracellulare*. As shown in Table 1, the differentiation of *M. avium* and *M. intracellulare* can be done with 93 to 96% accuracy by using sodium nitrite susceptibility testing at 3 mg/ml. *M. avium* and *M. intracellulare* are difficult to distinguish from each other by conventional cultural and biochemical tests. Therefore, the susceptibility testing of the *M. avium* complex organisms to 3 mg of sodium nitrite per ml described here is useful in the clinical diagnosis of *M.*

TABLE 1. Growth of *M. avium* and *M. intracellulare* on 7H11 agar medium containing sodium nitrite at a concentration of 3 mg/ml

Species	No. of strains	No. (%) with:	
		Positive growth	Negative growth
M. avium	72	5 (6.9)	67 (93.1)
M. intracellulare	59	57 (96.6)	2 (3.4)

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avium and *M. intracellulare*. Recently, some DNA probe tests were developed to identify the two species (3, 4, 6). Although the method described here is useful and is cheaper than the DNA probe tests, greater accuracy in distinguishing between the two species is achieved by the more expensive probes. The probes offer 100% accuracy, whereas the accuracy of distinguishing between the organisms by determining their susceptibility or resistance to sodium nitrite ranges from 93 to 96%. The probe method should be the method of choice if differentiation is important.

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