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In Vitro Susceptibility of *Mycobacterium fortuitum* Complex to Cephem Antibiotics

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ABSTRACT

The *in vitro* susceptibility of *Mycobacterium fortuitum* complex (30 strains of *M. fortuitum*, 30 strains of *M. chelonei* subsp. *abscessus* and 30 strains of *M. chelonei* subsp. *chelonei*) to 15 cephem antibiotics was studied on Kirchner's agar medium (containing 10% bovine serum). MIC_{80} (MIC at which 90% of strains were inhibited) of drugs against these organisms was 100 μg/ml or higher, however, cefoxitin and cefotetan were more active than the other compounds tested, against *M. fortuitum* strains.

Mycobacterium fortuitum complex is widely distributed in the environment[7,14] and is sometimes pathogenic for man[12,14]. This mycobacterial complex is resistant *in vitro* to all of the anti-tuberculosis drugs in use[6,9], and development of more effective drugs has to be considered. Recent studies demonstrated that cephem antibiotics have good *in vitro* activity against these organisms[1-3,5,8,11]. The current study was aimed at determining the *in vitro* susceptibility of *M. fortuitum* complex to 15 cephem antibiotics.

Ninety strains of *M. fortuitum* complex cultured on 1% Ogawa medium and stocked in our laboratory were used: 30 strains of *M. fortuitum* (origin; 17 from human, 13 from others), 30 strains of *M. chelonei* subsp. *abscessus* (origin; 29 from human, 1 from others) and 30 strains of *M. chelonei* subsp. *chelonei* (origin; 19 from human, 11 from others). Antimicrobial powders were obtained from the following manufactures: Cephapirin from Bristol Myers Co., Tokyo; cephacetrile, cefotiam, cefsulodin, and cefmenoxime from Takeda Chemical Ind., Osaka; cephaloridine and cefamandole from Shionogi Pharmaceutical Co., Osaka; ceftezole from Chugai Pharmaceutical Co., Tokyo; cefoxitin from Daiichi Seiyaku Co., Tokyo; cefazolin and ceftizoxime from Fujisawa Pharmaceutical Co., Tokyo; cefuroxime from Japan Glaxo Co., Tokyo; cefotaxime from Hoechst Japan Co., Tokyo; cefoperazone from Toyama Chemical Co., Tokyo; and cefotetan from Yamanouchi Pharmaceutical Co., Tokyo. Stock solution of each antimicrobial agent was prepared immediately before use by hydrating a known weight of the drug in distilled water, except for cefmenoxime which was dissolved in $NaHCO_3$.

For susceptibility test, the bacterial suspension was prepared by diluting organisms grown in Dubos Tween-albumin medium at 33°C for 5 to 7 days with saline containing 0.1% Tween 80 so as to give the value of $OD_{540\ nm}=0.1$ (approximately 10^7 CFU/ml) using the Shimadzu Spectronic 20. Susceptibility testing was carried out using the method endorsed by the Japan Society of Chemotherapy[10] and Kirchner's agar medium (supplemented with 10% bovine serum) containing 100–0.2 μg of drugs per ml. The minimal inhibitory concentrations (MICs) of the drugs were determined 7 days after incubation at 33°C. The MICs were read as minimum concentrations completely inhibiting the growth of organisms or allowing no more than five colonies to grow.

Table 1 shows the MICs of 15 cephem antibiotics for the organisms tested. The susceptibility of all strains of the two species of organisms

Table 1. MICs of 15 cephem antibiotics for *M. fortuitum* complex, after 7 days of incubation

Agent	MICs (μg/ml)					
	M. fortuitum (30 strains)		*M. chelonei* subsp. *abscessus* (30 strains)		*M. chelonei* subsp. *chelonei* (30 strains)	
	Range	MIC_{90}[a]	Range	MIC_{90}	Range	MIC_{90}
Cephapirin	>100	>100	>100	>100	>100	>100
Cephacetrile	>100	>100	>100	>100	>100	>100
Cephaloridine	50–>100	>100	>100	>100	≧100	>100
Ceftezole	>100	>100	>100	>100	>100	>100
Cefazolin	>100	>100	>100	>100	>100	>100
Cefoxitin	25–100	100	25–>100	>100	25–>100	>100
Cefotiam	>100	>100	>100	>100	>100	>100
Cefsulodin	>100	>100	>100	>100	>100	>100
Cefuroxime	>100	>100	>100	>100	>100	>100
Cefamandole	>100	>100	>100	>100	>100	>100
Ceftizoxime	>100	>100	≧100	>100	≧100	>100
Cefotaxime	50–>100	>100	>100	>100	>100	>100
Cefmenoxime	50–>100	>100	>100	>100	25–>100	>100
Cefoperazone	>100	>100	>100	>100	>100	>100
Cefotetan	25–100	100	≧100	>100	≧100	>100

[a] MIC at which 90% of strains were inhibited (μg/ml).

was low, the MIC_{90} being ≧100 μg/ml. However, there was a susceptibility of *M. fortuitum* strains to cefoxitin (range, 25–100 μg/ml) and cefotetan (range, 25–100 μg/ml), of *M. chelonei* subsp. *abscessus* strains to cefoxitin (range, 25–>100 μg/ml), and of *M. chelonei* subsp. *chelonei* strains to cefoxitin (range, 25–>100 μg/ml) and cefmenoxime (range, 25–>100 μg/ml). Fig. 1 shows the distribution of strains, the MICs of cefotetan and cefoxitin of which were 25–50 μg/ml and ≧100 μg/ml, respectively. The MICs of cefotetan for *M. chelonei* (subsp. *chelonei* and *abscessus*) were ≧100 μg/ml in all the strains, but for *M. fortuitum*, 84% of the strains were susceptible to concentrations of 25–50 μg/ml. On the other hand, the distribution of susceptibility of *M. fortuitum* strains to cefoxitin did not differ greatly from that to cefotetan, but a larger number of strains of *M. chelonei* (subsp. *chelonei* and *abscessus*) showed a susceptibility to 25–50 μg/ml of this drug than to the same concentrations of cefotetan.

Cynamon and Patapow[1] and Cynamon and Palmer[2,3] studies the susceptibility of 13 strains of *M. fortuitum* to cefoxitin, cefotetan, cefmetazole and cephalothin using the agar dilution test and report that cefoxitin inhibited 12 strains at the concentration of 25 μg/ml; cefotetan, 11 strains at 50 μg/ml; cefmetazole, 12 strains at 12.5 μg/ml; and cephalothin, 12 strains at 256 μg/ml. Swenson et al[11] reported that agar dilution tests demonstrated the MICs of cefoxitin and cefuroxime against *M. fortuitum* (18 strains) to be 16–≧64 μg/ml and ≧64 μg/ml, respectively, and the MICs of cefoxitin, cefamandole and cefuroxime against *M. chelonei* (15 strains) to be 32–≧64 μg/ml, 32 μg/ml and ≧64 μg/ml, respectively. When compared with their findings, our test results showed higher MICs of these drugs against *M. fortuitum* complex (see Table 1). This may be due to the difference in medium, because they used Müller-Hinton agar and we used Kirchner's agar medium supplemented with 10% bovine serum. Because some strains of *M. chelonei* grow poorly or do not grow at all on Müller-Hinton agar[11] or on heart infusion agar supplemented with 4% glycerol (data not

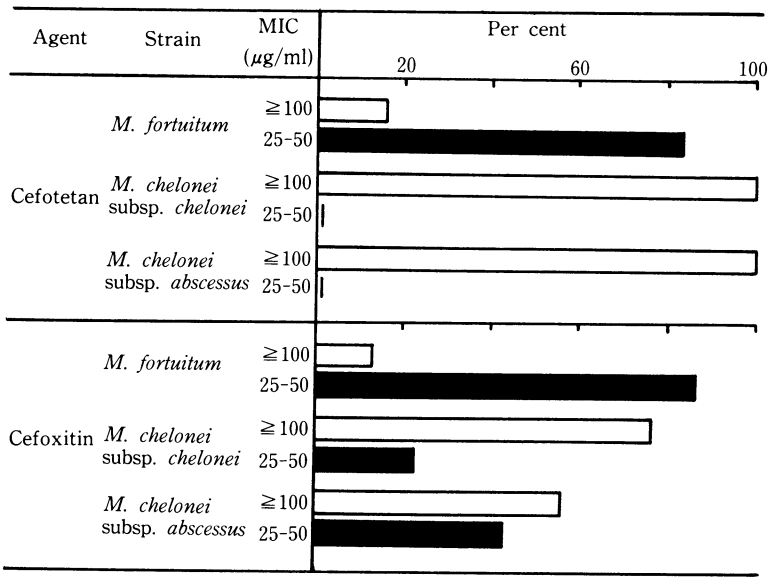

Fig. 1. Susceptibility distribution of *M. fortuitum* complex to cefotetan and cefoxitin

shown), we used Kirchner's agar (supplemented with 10% bovine serum). MICs of cephem antibiotics against *M. fortuitum* complex are from 2 to 4 times higher in Kirchner's agar than 4% glycerol-heart infusion agar (data not shown). Concerning differences in antimicrobial ability of agents, Wallace et al[18] reported that the MICs of amikacin, gentamicin and doxycycline against *M. fortuitum* and *M. chelonei* were from 2 to 8 times higher in case of 7H10 agar than in Müller-Hinton agar. Gangadharam and Gonzales[4] reported that ethambutol inhibits *M. tuberculosis* consistently at higher concentrations in 7H10 medium than it does in Löwenstein-Jensen medium.

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