Identification of Clinically Significant *Mycobacterium fortuitum* Complex Isolates

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Recent outbreaks of nosocomial infections caused by organisms identified as the *Mycobacterium fortuitum* complex suggest that species and subspecies identification is epidemiologically important. In a study of 170 strains, *M. fortuitum* was differentiated from *M. chelonei* by nitrate reduction and iron uptake. *M. fortuitum* was further divided into biovariant *fortuitum*, biovar *peregrinum*, and an unnamed third biovar by inositol and mannitol utilization. *M. chelonei* was further divided into subsp. *chelonei*, subsp. *abscessus*, and an unnamed subspecies by tolerance to 5% sodium chloride, utilization of mannitol and sodium citrate, and uptake of iron.

Numerical taxonomic analysis of rapidly growing mycobacteria by the International Working Group on Mycobacterial Taxonomy has shown clearly defined clusters which have been given species names (9, 15, 17). In some instances, a larger cluster containing several species has been referred to as a complex (16, 17), particularly if distinction of the species is of no medical importance. Examples are *Mycobacterium fortuitum* complex and *M. parafortuitum* complex. Although *M. fortuitum* complex contains two distinct species, *M. fortuitum* and *M. chelonei*, the Centers for Disease Control has reported only the complex until recently because of low numbers of isolates and the failure of differential reactions to correctly classify the species.

Since late 1976, an increase in the number of isolates referred to the Centers for Disease Control and their relationship to clinical disease have given the the background needed to evaluate key tests for the identification of *M. fortuitum* complex.

MATERIALS AND METHODS

Bacterial strains. Reference strains of *M. fortuitum* and *M. chelonei* were obtained from the Trudeau Mycobacterial Culture Collection formerly at the Trudeau Institute, Saranac Lake, N.Y. (presently at the National Jewish Hospital and Research Center, Denver, Colo.). Strains obtained were: *M. chelonei* subsp. *chelonei*, Trudeau Mycobacterial Culture (TMC) 1524, 1537, and 1544 (type strain of the species); *M. chelonei* subsp. *abscessus*, TMC 1542 and 1543; *M. fortuitum* biovar *fortuitum*, TMC 1529 and 1530; and *M. fortuitum* biovar *peregrinum*, TMC 1545 and 1547. A culture of the type strain of *M. chelonei* (subsp.

chelonei) was also obtained as culture 946 from the National Collection of Type Cultures, Colindale, London.

The study also included 170 recently isolated strains which were submitted to our laboratory by state health departments and medical centers or were received as a part of comprehensive epidemiological investigations.

Identification procedures. All cultures were identified as *M. chelonei* or *M. fortuitum* by standard procedures (29) which included observations of pigmentation, growth at 28°C on 5% NaCl and on MacConkey agar prepared without crystal violet, Tween hydrolysis, tellurite reduction, niacin production (TB niacin test strips, Difco Laboratories, Detroit, Mich.), arylsulfatase reactions (3 days and 2 weeks), catalase (semiquantitative and heat stable), and nitrate reductase.

Strains were tested for iron uptake by a modification of the procedure of Szabo and Vandra (23). Briefly, Lowenstein-Jensen medium was prepared with 2.5% (wt/vol) ferric ammonium citrate. Slants of the medium were inoculated with 1 drop of a barely turbid suspension of the culture to be tested and incubated for 10 to 24 days at 28°C in a slanted position with caps loosened. Colonies appeared rusty brown if iron was taken up (+). When colonies became tan and a rusty color developed around the edge of the slant, the reaction was recorded as ±. A negative reaction (−) was recorded when the pigmentation of the colonies remained the same as that observed on Lowenstein-Jensen medium without ferric ammonium citrate.

Utilization of sodium citrate, mannitol, or inositol as a sole source of carbon in the presence of ammoniacal nitrogen was determined by a slight modification of the procedure of Tsukamura (24, 26). The basal medium contained $(NH_4)_2SO_4$, 2.4 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; and distilled water, 950 ml. The salt solution was adjusted to pH 7.0 when sodium

citrate was to be added as the substrate and to pH 7.2 when mannitol or inositol was to be added. The control medium was also adjusted to pH 7.2 with 10% KOH. After the pH adjustment, 20 g of purified agar was added and dissolved by heating. After sterilization at 121°C for 20 min, the basal medium was cooled to 56°C, and 50 ml of one of the filter-sterilized (0.45-μm pore size; Millipore Corp., Bedford, Mass.) substrate solutions was added. Substrate solutions contained 5 g of mannitol or inositol or 5.6 g of sodium citrate per 50 ml. A like volume of sterile distilled water was added to the control medium. Medium with added substrate was dispensed in 8-ml amounts into screw-cap tubes (20 by 150 mm) and solidified in a slanted position. Slants were inoculated with 0.1 ml of a 1:10 dilution of a 7-day culture in Middlebrook-Cohn 7H9 Broth (Difco) and incubated at 28°C for 2 weeks. Growth on the test medium was interpreted as a positive test, and lack of growth, as on the control medium, was interpreted as a negative test.

RESULTS

All *M. fortuitum* complex strains examined in this study grew within 7 days at 28°C or 35°C or both, were nonpigmented, gave a positive 3-day arylsulfatase reaction, and grew on MacConkey agar without crystal violet at 28°C. Reactions for species and subspecies or biovars of the *M. fortuitum* complex are shown in Table 1. *M. fortuitum* strains reduce nitrate and give a positive iron uptake test at 28°C; *M. chelonei* strains do not. The *M. chelonei* strains were identified as to subspecies on the basis of NaCl tolerance and utilization of sodium citrate as a sole carbon source for growth. The *M. fortuitum* strains were divided into biovars by utilization of mannitol as a sole source of carbon for growth.

Reactions of 52 clinical isolates of *M. fortuitum* are compared with those of 4 reference strains in Table 2. Isolates from sternal infections received from five hospitals were identified as *M. fortuitum* biovar *fortuitum* by their failure to utilize mannitol for growth. Four sternal-wound isolates were from the same hospital and

are considered to be associated with a postoperative wound infection outbreak (2). Two of the remaining isolates were also from one hospital, but the infections occurred a year apart. The 11 strains isolated from infections which occurred after mammaplasty augmentation procedures in four hospital centers (5) were also identified as *M. fortuitum* biovar *fortuitum*.

A miscellaneous group of isolates designated group F in Table 2 was identified as *M. fortuitum* biovar *fortuitum*. All of these isolates were implicated in disease, and their sources include sputum (cavitary lung disease), infection at the site of pacemaker insertion, skin lesions or abscesses (some chronic and some disseminating), blood (endocarditis), cerebrospinal fluid (meningitis), osteomyelitis, urine, peritoneal fluid, and a hip wound; three related cultures were associated with joint prostheses and a home whirlpool (1).

Nine miscellaneous isolates designated group P in Table 2 were identified as *M. fortuitum* biovar *peregrinum*. These strains were isolated from sputum (cavitary lung disease), urine, and wounds of the sternum and foot.

The five isolates which are designated as group 3 in Table 2 cannot be assigned to one of the biovars of *M. fortuitum*, since they utilize inositol as a carbon source, although they meet other criteria for identification as *M. fortuitum*. One of the isolates was from a prosthetic porcine heart valve, and the remaining four isolates were from a leg wound, lung tissue, and two separate infections of the foot.

Reactions of 118 clinical isolates identified as *M. chelonei* are compared with those of reference strains in Table 3. Isolates from sternal infections which developed after cardiac surgery in four hospitals were identified as *M. chelonei* subsp. *abscessus*. None of these isolates utilized sodium citrate for growth, and 19 of 21 isolates (90%) grew in the presence of 5% NaCl at 28°C.

TABLE 1. *Key tests for differentiating members of the M. fortuitum complex*

Organism	Test								
							Growth on:		
	Pigment	Arylsul-fatase, 3 days	Mac-Conkey agar without crystal violet	Nitrate reduc-tion	Iron up-take	NaCl toler-ance, 28°C	Sodium citrate	Manni-tol	Inositol
M. fortuitum biovar									
fortuitum	−	+	+	+	+	+	−	−	−
peregrinum	−	+	+	+	+	+	−	+	−
M. chelonei subsp.									
chelonei	−	+	+	−	−	−	+	−	−
abscessus	−	+	+	−	−	+	−	−	−

TABLE 2. *Differential reactions for M. fortuitum strains*

Organism	No. of cultures tested	NaCl tolerance, 28°C	Nitrate reduction	Iron uptake	Utilization of:		
					Sodium citrate	Mannitol	Inositol
TMC reference strain, biovar							
fortuitum	2	+	+	+	−	−	−
peregrinum	2	+	+	+	−	+	−
Clinical isolate							
Sternal infection	9	+	+	+	−	−	−
Mammaplasty	11	+	+	+	−	−	−
Miscellaneous							
Group F	18	+[a]	+	+	−	−	−
Group P	9	+	+	+	−	+	−
Group 3	5	+	+	+	−	+	+

[a] 17 of 18 (94%) cultures.

TABLE 3. *Differential reactions for M. chelonei strains and M. chelonei-like organisms*

Organism	No. of cultures tested	NaCl tolerance, 28°C	Nitrate reduction	Iron uptake		Utilization of:		
				Rust (+)	Tan (±)	Sodium citrate	Mannitol	Inositol
TMC reference strain, subsp.								
chelonei	2	−	−	−	−	+	−	−
abscessus	2	+	−	−	−	−	−	−
"Turtle" strain	2[a]	−	−	−	+	+	−	−
Clinical isolate								
Sternal infections	21	+[b]	−	−	−	−	−	−
Porcine heart valves	19	−	−	−	−	+	−	−
Peritoneal dialysis[c]	29	−	−	−	+	+	+	−
Miscellaneous								
Group A	34	v[d]	−	−	−	−	−	−
Group C	11	v[d]	−	−	−	+	−	−
Group D	4	−	−	−	+	+	+	−

[a] This is not the number of strains but indicates that the strain was obtained from two different sources (see text). Tests were identical for both cultures.
[b] A total of 90% were positive.
[c] *M. chelonei*-like organisms.
[d] v, Variable.

Of the isolates, 14 were from an outbreak of postoperative wound infections at one hospital (2, 14), and 5 were from another unrelated outbreak in Central Europe (22). The two remaining isolates came from single cases occurring at different hospitals.

A total of 19 cultures identified as *M. chelonei* subsp. *chelonei* were associated with porcine heterograft heart valves processed by a single producer, and 18 isolates were recovered by hospital laboratories which cultured tissue remnants removed from the valves at the time of implantation. One isolate was from the pericardial fluid of a patient who received 1 of the 17 porcine valves. All of these isolates were cultured by various hospitals in the United States (3, 4, 10–12, 28) except one culture which was isolated in Spain from a porcine heart valve manufactured by the same company.

The porcine heart valve isolates were difficult to grow on primary culture (10, 11), but they were usually detected in thioglycolate broth after 12 to 14 days of incubation at 37°C. Upon subculture, this group of organisms grew well on Lowenstein-Jensen medium slants within 7 days at 28°C but required 2 to 4 weeks for growth at 35°C. Because of slow growth at the higher temperature, these organisms can be misidentified as unclassified, slowly growing mycobacteria. Some of the other strains of *M. chelonei*

which are included in this study were also found to have the same temperature requirement as the porcine heart valve isolates, and most were identified as *M. chelonei* subsp. *chelonei*. However, not all organisms identified as subsp. *chelonei* have this characteristic.

Cultures from peritoneal dialysis patients and dialysis machines at a kidney center and an associated hospital were identified as members of the *M. fortuitum* complex since they were acid fast (10 to 25% of the organisms in a smear retained the basic fuchsin), gave a 3-day arylsulfatase reaction, and grew on MacConkey agar without crystal violet at 28°C (Table 3). This group is unique in that the isolates show a low semiquantitative catalase test result (column of foam, 10 to 22 mm), are negative for the catalase test at 68°C and pH 7, and utilize mannitol for growth. Since these isolates failed to grow on medium containing 5% NaCl, did not reduce nitrate, gave a ± reaction in the iron uptake test, and utilized citrate as the sole source of carbon, the group was considered an *M. chelonei*-like organism more similar to the type strain (18) of the species (TMC 1544) than to *M. chelonei* subsp. *abscessus*.

Three groups of nonrelated isolates of clinical significance are shown in Table 3. Group A was identified as *M. chelonei* subsp. *abscessus* on the basis of the inability of the isolates to utilize sodium citrate, mannitol, and inositol as a source of carbon. Whereas 62% (21 strains) were associated with lung disease, the other 38% were from various sources, including blood, cornea, urine (disseminating disease), disseminating disease of the leg or thigh, mammaplasty implants, endocarditis, local abscesses, and a surgical wound resulting from removal of a leg vein for cardiac bypass surgery.

Isolates in group C were identified as *M. chelonei* subsp. *chelonei* on the basis of their ability to grow on sodium citrate and their inability to utilize mannitol or inositol as a sole carbon source. Strains in this group were from corneal infection, a kidney resection, sputum (lung disease), and several infections of the leg, thigh, and foot.

The small number of isolates in group D were identified as *M. chelonei*-like strains by their characteristic ± iron uptake reaction, their ability to utilize both sodium citrate and mannitol as a sole source of carbon, and their inability to utilize inositol. The sources of these strains include a sternal infection, a surgical wound resulting from removal of a vein, and sputum. These isolates grew well at both 28°C and 35°C within 7 days.

DISCUSSION

Organisms in the *M. fortuitum* complex are resistant to all commonly used antituberculous drugs except that many strains of *M. fortuitum* are susceptible to capreomycin (James Kilburn, personal communication). Differences in strain susceptibility to other antimicrobial agents, amikacin in particular, indicated that perhaps susceptibility to certain drugs could be associated with species or subspecies of this complex. Tests reported here were selected from a larger set because they are reproducible, the type strains and wild strains can be identified, and further differentiation to the subspecies or biovar level can be made. Preliminary findings of the Antimicrobics Investigation Section of the Bacteriology Division, Centers for Disease Control, confirmed the original findings (21), and indeed, by the scheme reported here, amikacin susceptibility levels were related to species and subspecies of the *M. fortuitum* complex. The strains examined by Swenson et al. (21) are from the group of organisms used in this study.

Swenson et al. (21) found that strains of *M. fortuitum* biovar *fortuitum* generally had amikacin minimal inhibitory concentrations (MICs) of 1 μg/ml or less, and the MIC range for *M. chelonei* was from 4 to 64 μg/ml. *M. chelonei* subsp. *chelonei* isolates generally had a higher amikacin MIC than did *M. chelonei* subsp. *abscessus* isolates. The *M. chelonei*-like organisms were, in general, more susceptible to the drugs tested than those identified as *M. fortuitum* or *M. chelonei*. The *M. chelonei*-like strains had amikacin and kanamycin MICs of 4 μg/ml or less and were usually susceptible to ampicillin in an MIC range of 0.5 to 8 μg/ml (J. Swenson, personal communication). Some *M. chelonei*-like strains (75%) demonstrated susceptibilities to clinically attainable levels of cefoxitan, cefuroxine, sisomicin, doxycycline, minocycline, tetracycline, erythromycin, and sulfamethoxazole-trimethoprim. Most *M. fortuitum* strains tested had an MIC below achievable tissue levels for sulfamethoxazole-trimethoprim and sulfamethoxazole, and some strains showed susceptibility to minocycline and doxycycline. However, tobramycin and erythromycin were the only other drugs to show MICs of achievable levels for some of the *M. chelonei* strains.

A report of 38 cases of infection due to the *M. fortuitum* complex (R. J. Wallace, Jr., E. J. Septimus, V. A. Silcox, and J. Swenson, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 403, 1980) showed that patients infected with *M. fortuitum* generally responded to chemotherapy

with doxycycline, amikacin, or sulfonamides when administered along with surgical debridement; however, patients infected with *M. chelonei* were more difficult to treat. This report agrees with the laboratory finding that *M. fortuitum* is more susceptible to antimicrobial agents than is *M. chelonei*. Since infections due to *M. fortuitum* and *M. chelonei* are more common than previously thought, it is necessary to correctly identify the organisms of this complex as to species so that preliminary drug therapy can be initiated. For a more effective drug regimen, the culture should be tested for susceptibility to nontuberculous antimicrobial agents (21; 20th ICAAC, abstr. no. 403).

Mycobacteria are routinely tested for susceptibility to 10 μg of capreomycin per ml in the Centers for Disease Control Mycobacterial Drug Resistance Laboratory (29), and in the early part of the study it was noted that most of the *M. fortuitum* strains were susceptible to capreomycin at this concentration, whereas *M. chelonei* strains were resistant. When additional strains were added, the percentage of susceptible *M. fortuitum* strains fell to 50%, with no correlation with biovar. An acid-fast organism which meets the criteria for the *M. fortuitum* complex and is susceptible to 10 μg of capreomycin per ml can, therefore, be tentatively identified as *M. fortuitum*. However, an *M. fortuitum* complex organism which is resistant to 10 μg of capreomycin per ml cannot be tentatively identified as *M. chelonei*.

Since most of the published methods used to identify the organisms in the *M. fortuitum* complex as to species or subspecies require special techniques or equipment or a large group of tests not routinely performed in general reference laboratories (6, 8, 9, 13, 17, 24–26), we selected tests which are easy to perform and read that will allow identifications to be achieved. Any organism must be acid fast, be nonpigmented, grow in less than 7 days at its optimum temperature, have arylsulfatase activity at 3 days, and grow at 28°C on MacConkey agar without crystal violet to be placed in the *M. fortuitum* complex. The two species which make up the complex can be differentiated by the reactions shown in Table 1. By determining the ability to utilize inositol, mannitol, and sodium citrate as sole sources of carbon, the two species can be further divided into subgroups or biovars.

The *M. fortuitum* species can be divided into three subgroups. The largest group seen in this laboratory is equivalent to *M. fortuitum* biovar *fortuitum*. The second largest group is *M. fortuitum* biovar *peregrinum*. These two groups have been recognized by several investigators

(6–9, 13, 19, 24–27, 29), but a third group which was seen in this study is different. The number of strains in the latter group was small, but this study confirms the occurrence of a third biotype as suggested by Pattyn et al. (13), Stanford and Grange (20), and Grange and Stanford (7). In addition, some strains assigned to this third group are more resistant to amikacin than are the recognized biovars (J. Swenson, personal communication). The number of infections caused by organisms in this group apparently is very small, and it may take some time to see whether the preliminary observation with the antimicrobial agents is true.

The *M. chelonei* species can be divided into three subgroups. The subspecies seen most often in this laboratory is *M. chelonei* subsp. *abscessus*. It is generally susceptible to a limited number of antimicrobial agents, being the intermediate in the three groups. *M. chelonei* subsp. *chelonei* is the next most commonly encountered group of this species and is the group having the most resistance to antimicrobial agents. A characteristic often found with this subspecies is an optimal growth temperature which is <35°C. Many strains did not grow at 35°C or grew poorly and slowly; however, if grown at 28°C, these strains grew profusely in 2 to 5 days. This observation has been made by Tsukamura (25), Tsukamura et al. (27), and Jenkins et al. (8), as well as by others. Some strains in this subspecies will grow well at 35°C; therefore, this characteristic is not necessary to place an organism into the subspecies.

The *M. chelonei* isolates in the third group, referred to as *M. chelonei*-like organisms, are distinguished by their ability to use mannitol and sodium citrate as a source of carbon. This pattern and the unique characteristic of tanning of the iron uptake medium set the organism apart from the other two subspecies. In addition, this group of organisms is more susceptible to antimicrobial agents than are others of the *M. fortuitum* complex. This characteristic itself would separate the organisms from the recognized subspecies and biovars of the complex. Most of the strains examined for this study were from one major outbreak involving patients receiving peritoneal dialysis (J. D. Band, J. I. Ward, D. W. Fraser, N. J. Peterson, V. A. Silcox, R. C. Good, P. R. Ostroy, J. M. Swenson, and J. Kennedy, J. Infect. Dis., in press).

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