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Comparison of the Chinese Schema and the International Antigenic Typing System for Serotyping *Pseudomonas aeruginosa*

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Twelve strains of *Pseudomonas aeruginosa* representing 12 serogroups in the serogrouping schema used in the People's Republic of China were compared with serogroups in the International Antigenic Typing System (IATS). The first eight groups originated in the People's Republic of China, and group II appears to have a new major antigen that is not found in the IATS. Groups I, III, IV, V, VI, VII, and VIII correspond to groups 11, 6, 9, 4, 8, 3, and 1, respectively, of the IATS. Groups IX, X, XI, and XII are immunotypes 3, 4, 5, and 1, respectively, of Fisher et al. (M. W. Fisher, H. B. Devlin, and F. J. Gnabasik, J. Bacteriol., 98:835–836, 1969); they exhibited a wide range of serological cross-reactions but correspond mainly to IATS groups 2, 3, 10, and 6, respectively.

Serotyping for *Pseudomonas aeruginosa* has a long and confusing history because microbiologists from different nations tended to use different serotyping schemata for this organism and the results obtained and published were often not directly comparable. An attempt has been made by the Subcommittee on *Pseudomonadaceae* of the International Committee on Systematic Bacteriology to establish an international schema for serotyping of this species. The resultant schema (5) has 17 serogroups which contain practically all of the known major antigens of the schemata that have been used in Europe, the USSR, the United States, and Japan. The serotyping schema used for this species by microbiologists in the People's Republic of China has remained unknown to outsiders because of a lack of communication between this group and the Western world in the last 30 years.

The serotyping schema that has been used in China is based on a schema devised by J. Yuan and Z. Zhao of the Shanghai Biological Products Institute (BPI) in 1963 (8). They collected 474 strains of *P. aeruginosa* from all parts of China and recognized 11 serogroups among these strains, which came mostly from burn wounds. In the 1970s, standard strains from many foreign schemata, including those of Verder and Evans (7), Lanyi (4), Fisher et al. (2), and Homma (3), were obtained for comparative studies. The latest schema, which was developed in 1984, consists of eight serogroups from the original schema of Yuan and Zhao (8) and four serogroups from the schema of Fisher et al. (2). This schema is the basis for the production of vaccines and antisera for treatment of *P. aeruginosa* infections; these materials are produced at the BPI in Chengdu, Sichuan Province. The 12 strains of *P. aeruginosa* that form the basis of the Chinese serotyping system have become available recently for comparative study, and the present communication describes such a study.

MATERIALS AND METHODS

Bacterial strains. The 12 strains of *P. aeruginosa* that form the basis of the Chinese serotyping schema were obtained from S. P. Wang of the Chengdu BPI (Table 1). The first eight strains originated at the Shanghai BPI, where the original work of Yuan and Zhao (8) was done, and they carried the designations of the Shanghai BPI along with a CMCC number. I deposited these organisms in the American Type Culture Collection, Rockville, Md. These strains form the basis of their groups I, II, III, IV, V, VI, VII, and VIII, respectively.

The strains that have been used as the basis of groups IX, X, XI, and XII in the Chinese schema are immunotypes 3, 4, 5, and 1 of Fisher et al. (2), respectively, and they correspond roughly, but not completely, to International Antigenic Typing System (IATS) groups 2, 1, 10, and 6, respectively. They were received with both CMCC and American Type Culture Collection numbers.

Serotyping. The original eight strains of Yuan and Zhao (8) were serotyped by the usual procedure in this laboratory (5). Slide agglutination of a live cell suspension was done first with a 1/10 dilution of the standard antisera. Whenever positive reactions were obtained, twofold serial dilutions of the sera were made to test titers with autoclaved cells. Control series using homologous autoclaved cells were included in each test to compare the titers of unknown cells and the homologous cells. Whenever the titers of the unknown cells were 1/8 or less than that of the homologous cells and no other antisera had agglutinated the cells, antisera were prepared with the unknown cells. Cross absorption of these antisera was done to confirm the specificities of the new antigens.

RESULTS

The strains of groups I to VIII in the Chinese schema were agglutinated on a slide by antisera to IATS groups 11, 5, 6, 9, 4, 7 and 8, 3, and 1, respectively. In tube agglutination with serial dilutions of the sera, group I, III, IV, V, VI, VII, and VIII cells were agglutinated to full titers by the antisera to the corresponding IATS groups (Table 2). However, group II cells were agglutinated to only a 1/80 dilution by IATS group 5 serum, whereas the homologous cells were agglutinated to a 1/640 dilution. Antiserum was prepared with this strain by standard techniques; it agglutinated homologous cells to a 1/640 dilution but agglutinated IATS group 5 and 16 cells to titers of only 1/80 and 1/20, respectively. These cross-reacting antibodies could be absorbed easily with group 5 and 16 cells.

About 200 strains of *P. aeruginosa* are kept in stock cultures in this laboratory for various purposes. About 20 strains belong to either group 5 or 16 of the IATS system. All of these strains were tested with absorbed serum of Chinese

TABLE 1. Twelve strains of *P. aeruginosa* that form the basis of serogrouping in the Chinese schema

Chinese serogroup	CMCC no.	Origin	ATCC no.
I	(B) 10115	Shanghai BPI	43389
II	(B) 10117	Shanghai BPI	43390
III	(B) 10118	Shanghai BPI	43391
IV	(B) 10119	Shanghai BPI	43392
V	(B) 10120	Shanghai BPI	43393
VI	(B) 10121	Shanghai BPI	43394
VII	(B) 10123	Shanghai BPI	43395
VIII	(B) 10124	Shanghai BPI	43396
IX	(B) 10202	Fisher PD-05074 immunotype 3	27314
X	(B) 10203	Fisher PD-05141 immunotype 4	27315
XI	(B) 10204	Fisher PD-05140 immunotype 5	27316
XII	(B) 10210	Fiaher PD-05139 immunotype 1	27312

group II. None reacted. Another 80 strains from various other serogroups were randomly selected and tested. None reacted. It appeared, therefore, that the group II antigen is very rare among strains isolated in this locality. It should be noted that in the People's Republic of China, groups I, II, III, and VII are the most common serogroups and these four groups are included in the vaccine and antisera manufactured there.

Groups IX, X, XI, and XII in the Chinese schema are immunotypes 3, 4, 5, and 1, respectively, of Fisher et al. (2). Their major antigens correspond roughly to those of IATS groups 2, 3, 10, and 6, respectively.

DISCUSSION

There are at least two reasons for trying to compare the serogrouping schemata used in different countries. One is to establish the corresponding serogroups from different schemata. The second is the possibility that some serogroups in the unknown schemata may turn out to contain new antigens that are not included in the IATS system. One example of a new antigen is that of group 17 of the IATS system, which came from the schema of Meitert et al. (6) in Romania.

Another reason for interest in the schema used in the People's Republic of China is that China and the USSR are probably the only two major countries in which vaccines and antisera can be used in clinical practices without fear of litigation when some adverse effects occur. Such fear has inhibited most attempts to introduce new vaccines in the United States. The Chengdu BPI is producing not only vaccines but antisera for use in the treatment of burn wound infections. These sera are produced by immunization of healthy volunteers with a vaccine that contains heat-killed cells of Chinese groups I, II, III, and VII, the most common groups in China. Groups I, III, and VII correspond to IATS groups 11, 6, and 3, respectively. Group II has no corresponding serogroup in the IATS, although antisera produced with this group exhibited minor cross-reactions with IATS groups 5 and 16. Most vaccines made in Western countries are based on the IATS (5), and therefore the major difference between the Chinese vaccine and those made in Western countries is the group II antigen in the Chinese vaccine, which is not present in Western vaccines.

Groups IX, X, XI, and XII in the Chinese schema are immunotypes 3, 4, 5, and 1, respectively, of Fisher et al. (2), which had been selected for the purpose of vaccine production. They are apparently selected for a wide range of antigenicities so that only seven strains would cover most of the antigenic range of *P. aeruginosa*. For example, immunotype 1 of Fisher et al. (2) has a major antigen that corresponds with that of IATS group 6, but antiserum produced with this strain, when used undiluted, agglutinates on slides cells of IATS groups 1, 3, 4, 6, 9, 14, 15, and 17. It is not desirable to use this group of organisms for grouping or typing, in which specificity should be the major criterion.

Recognition of the Chinese group II antigen as a new major antigen indicates that this antigen can be included in the IATS as group 18. The addition of more and more serogroups will make the practice of serogrouping more expensive and time consuming and tends to discourage the use of this procedure in clinical laboratories. On the other hand, failure to recognize these rare groups will result in so-called nontypable strains (1) and make serogrouping incomplete. In fact, the apparent rarity of certain serogroups in some countries and areas may be due to the absence of antisera to recognize them. A reasonable compromise to deal with this dilemma may be to have a typing center which has all of the antisera for rare groups while most of the clinical laboratories perform routine serogroupings using only antisera of groups that are common in that area.

It will become desirable to express antigens of *P. aeruginosa* and the antibody content of antisera by a multiple number instead of a single number. For example, the Chinese group II strain can be described as having antigens 18:5:16, the number underlined indicating the major antigen

TABLE 2. Titers of agglutination of cells in groups I to VIII of the Chinese schema by antisera to IATS groups compared with those of homologous cells

Antiserum group	Agglutination titer[a] with:									
	Chinese schema group:								IATS group:	
	I	II	III	IV	V	VI	VII	VIII	5	16
IATS										
1	0	0	0	0	0	0	0	320/640	0	0
3	0	0	0	0	0	0	1,280/1,280	0	0	0
4	0	0	0	0	1,280/1,280	0	0	0	0	0
5	0	80/640	0	0	0	0	0	0	640	40/640
6	0	0	1,280/1,280	0	0	0	0	0	0	0
8	0	0	0	0	0	640/1,280	0	0	0	0
9	0	0	0	320/640	0	0	0	0	0	0
11	1,280/1,280	0	0	0	0	0	0	0	0	0
Chinese II	0	640	0	0	0	0	0	0	80/640	20/640

[a] Titer of agglutination of unknown cells by serum/titer of agglutination of homologous cells. 0, No agglutination or titer of less than 20.

which determines that this strain belongs to group 18. It will also be obvious that this strain will be agglutinated by antisera to groups 5 and 16 and antisera produced with this strain will agglutinate cells of groups 5 and 16. This type of practice will result in a better understanding of the effectiveness, or the lack of it, of vaccines. Most of the confusion that exists today regarding the serogrouping of *P. aeruginosa* is due to the use of a single number to express the antigens of a group of organisms that in most cases are multiple.

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