SEROTYPING OF *FLAVOBACTERIUM MENINGOSEPTICUM* BY CO-AGGLUTINATION METHOD

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Abstract. The purpose of this investigation was to evaluate the usefulness of a co-agglutination procedure for the typing of *Flavobacterium meningosepticum*. The sensitivity and specificity of the co-agglutination test was compared to the slide agglutination test using reference strains of the bacterial species. Antisera were characterized by both technics to determine their titer and working dilution. The specificity of the sera was assessed by performing tests which include strains of other species and serotypes. A collection of 47 strains of *F. meningosepticum* isolated from clinical specimens were typed by both co-agglutination and slide agglutination methods. Co-agglutination proved to be markedly more specific than the slide procedure although both methods were similar in sensitivity. It was concluded that co-agglutination proved to be an excellent method for the serotyping of *F. meningosepticum*.

INTRODUCTION

Flavobacterium meningosepticum is an infrequently encountered pathogen of man with fewer than 100 infections documented in the literature since the organism was named and its characteristic features defined by King (1959). Most patients were newborn infants with meningitis (Dooley, 1980). Serological classification, which have greatly aided the understanding of the epidemiology of infection with *F. meningosepticum* have been tried by some authos. King (1959) described the use of slide agglutination test for the serotyping of *F. mentingosepticum* and found the method to be rapid and useful for diagnostic and epidemiological studies. The same method was used by other workers (George *et al*, 1961; Owen, 1974; Richard and Laurent, 1981). Co-agglutination technic which was recently applied for serotyping of *Streptococci* (Christensen, 1973), Mycobacteria (Jahlin, 1973), *Neisseria meningitidis* (Per Olcen, 1975) and other bacterial species were used in the present study for serotyping of *F. meningosepticum* strains.

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MATERIALS AND METHODS

Bacterial strains

Sixty-two strains of *Flavobacterium meningosepticum* were kindly provided by Dr B Holmes from the National Collection of type cultures (NCTC) were used in the study. They comprised 47 field strains isolated mainly from clinical materials, 26 were isolates from the United Kingdom and another 21 strains were from other foreign countries, whilst other 15 strains were reference cultures maintained in the NCTC laboratory. The sources of isolation of the field strains are as shown in Table 3.

The reference cultures included in the study were two strains of *Flavobacterium* sp Group 11b, one *Flavobacterium breve,* one strain each of *Moraxella bovis, M. osloensis, M. phenylpyruvica, M. saccharolytica* and *M. urethralis.* All strains were cultured on nutrient agar plates, incubated aerobically at 37°C overnight.

Flavobacterium meningosepticum antisera

Type specific rabbit anti-*Flavobacterium meningosepticum* sera A, B, C, D, E and F (unabsorbed) were kindly provided by Dr Richard of Institut Pasteur, Paris, France. The preparation of antisera was described by Richard *et al* (1979a).

Table 1

Homologous and heterologous titers of anti-*Flavobacterium meningosepticum* antisera with *F. meningosepticum* reference strains and other species by slide agglutination test.

Reference strains	Antisera					
	A	B	C	D	E	F
F.M. serotype A	320	-	Undiluted	-	Undiluted	-
F.M. serotype B	-	160	-	-	-	-
F.M. serotype C	-	-	20	-	-	-
F.M. serotype D	-	-	-	160	-	-
F.M. serotype E	-	-	-	-	160	-
F.M. serotype F	-	-	-	-	-	160
F.M. serotype G	-	-	-	-	Undiluted	-
F.M. serotype H	-	-	-	-	-	-
F.M. serotype I	-	-	-	-	-	-
F.M. serotype J	-	-	-	-	-	-
F.M. serotype K	-	-	Undiluted	-	-	-
F.M. serotype L	-	-	-	-	-	-
F.M. serotype M	-	-	-	-	-	-
F.M. serotype N	-	-	-	-	-	-
F.M. serotype O	-	-	-	-	-	-
F. breve	-	-	-	-	-	-
F. group 11b	-	-	-	-	-	Undiluted
F. group 11b	-	-	-	-	-	Undiluted
Moraxella species[a]	-	-	-	-	-	-

- = negative, F.M. = *Flavobacterium meningoseptcum*
[a]*M. bovis, M. osloensis, M. phenylpyruvica, M. saceharolytiea, M. urethralis*

Titration of antisera by slide agglutination test

Two-fold dilutions of serum in saline were prepared from 1 in 10 to 1 in 2,560 with a final volume of 0.25ml each. A loopfull (5 mm loop) of each dilution was placed onto the surface of glass slides. Using a A wooden toothpick, minute amount of bacteria was taken from a colony on an agar plate and emulsified directly onto the drop of diluted serum. Simultaneously, saline was substituted as a negative control for all test. The slide was tilted for 2 minutes and agglutination was observed with the naked eye as clumping of the milky suspension of the bacteria. The titer of the serum was defined as the highest dilution that gave easily visible agglutination.

Co-agglutination reagent

The co-agglutination reagent was a 10% suspension (w/v) *Staphylococcus aureus* Cowan 1 (CAMR Porton) rich in protein A. For coating of the staphylococci, 0.45ml of a 10% (w/v) suspension was added to 0.05ml of the rabbit antiserum. The mixture was incubated at 37°C in a water bath for 15 minutes and then washed twice with PBS by centrifugation at 6,000*g* for 30 minutes. The coated staphylococci were resuspended to 1% (w/v) in PBS containing 0.1% (w/v) sodium azide. The reagent was stable for the period of the investigation when stored at 4°C.

Performance of co-agglutination test

The co-agglutination test was performed in a similar manner as the slide agglutination test, described previously, except that here bacterial growth was emulsified directly into the antibody-labeled staphylococcal suspension.

Table 2

Homologous and heterologous reactions of *Flavobacterium meningosepticum* sera A to F in co-agglutination test with serotype strains of *F. meningosepticum* and other species.

Reference strains	Co-agglutination reagent					
	A	B	C	D	E	F
F.M. serotype A	3+	-	-	-	-	-
F.M. serotype B	-	3+	-	-	-	-
F.M. serotype C	-	-	3+	-	-	-
F.M. serotype D	-	-	-	3+	-	-
F.M. serotype E	-	-	-	-	3+	-
F.M. serotype F	-	-	-	-	-	3+
F.M. serotype G	-	-	-	-	Undiluted	-
F.M. serotype H	-	-	-	-	-	-
F.M. serotype I	-	-	-	-	-	-
F.M. serotype J	-	-	-	-	-	-
F.M. serotype K	-	-	3+	-	-	-
F.M. serotype L	-	-	-	-	-	-
F.M. serotype M	-	-	-	-	-	-
F.M. serotype N	-	-	-	-	-	-
F.M. serotype O	-	-	-	-	-	-
F. breve	-	-	-	-	-	-
F. group 11b	-	-	-	-	-	Undiluted
F. group 11b	-	-	-	-	-	Umdo;ited
Moraxella species[a]	-	-	-	-	-	-

- = negative, F.M. = *Flavobacterium meningosepticum*
[a]*M. bovis, M. osloensis, M. phenylpyruvica,M. saceharolytiea M. urethralis*

RESULTS

Slide agglutination for titers of sera

The six antisera *F. meningosepticum* A to F were titrated using the slide agglutination technic and the results are shown in Table 2. With the exception of serum C, titers of 160 or greater were obtained. The sera were very specific and few cross reaction were observed. Undiluted serum C, produced agglutination with serotype A and serotype K, and serum E also reacted with serotype A and serotype G. *F. meningosepticum* group 11b reacted with undiluted anti-serum F. None of the *Moraxella* strains tested agglutinated with antisera.

Co-agglutination

The co-agglutination test using antibody labeled staphylococci was tested in a similar manner as the slide agglutination test with the serotype strains described above. The reaction of each serum with the specific bacteria was very strong and clear cut, with the agglutination seen as large clumps of bacteria. Only one cross reaction was found between serum C and serotype K. No other species were agglutinated in these tests. Despite the low titer of agglutination given by serum C, the result suggest type specific IgG was present in the serum. The results are shown in Table 3.

Typing of clinical isolates

The slide agglutination technic was compared with the co-agglutination method for the typing of clinical isolates. The sera for the slide agglutination technic were used undiluted in order to detect

Table 3

Flavobacterium meningosepticum serotype distribution.

Serotype	No.	Source	No.
A	5	Cerebrospinal fluid	(1)
		Eye	(1)
		Cervical swab	(1)
		Respirator	(1)
		Endotracheal tube	(1)
B	1	Wound	(1)
C	2	Cerebrospinal fluid	(1)
		Blood	(1)
D	1	Cerebrospinal fluid	(1)
E	2	Sputum	(1)
		Unknown	(1)
F	15	Blood	(7)
		Pus	(2)
		Cerebrospinal fluid	(1)
		Catheter tip	(1)
		Wound	(1)
		'Hibitane' solution	(1)
		Drinking water	(1)
		Unknown	(1)
Nonspecific strain	8	Sputum	(1)
		Cerebrospinal fluid	(3)
		Pericardium	(1)
		Tracheostomy	(1)
		Pyosalpingitis	(1)
Nontypable	9	Blood	(3)
		Cerebrospinal fluid	(1)
		Wound	(1)
		Gall bladder	(1)
		Ventricular fluid	(1)
		Aspirate	(1)
		Unknown	(1)
Autoagglutination	4	Urine	(1)
		Aspirate	(1)
		Pus	(1)
		Tracheal suction	(1)

weakly reacting strains. Of the 47 isolates of *F. meningosepticum* tested, 26 isolates (55.32%) were typable by both methods. Four isolates were positive only by slide agglutination and another four cultures cross racted with two sera (B and E; C and E). These cross reactions were also found by co-agglutination. There was no association between clinical source and serotype reaction (Table 3). The most frequent serotype encountered was serotype F which comprised 7 isolates from blood. This serotype was also found in other sources including catheter tips and drinking water. The other serotypes were also isolated from a variety of clinical sources, but antisera C and D reacted only with isolates from cerebrospinal fluid or blood and although only 3 strains in total were tested these may be significant markers of pathogenicity.

Table 4

Serotyping of 47 clinical isolates of *F. meningosepticum* by slide and co-agglutination.

Serum	No. and percentage of strain positive	
	No.	%
A	5	10.64
B	1	2.13
C	2	4.26
D	1	2.13
E	2	4.26
F	15	31.92
Nontypable	9	19.15
Others	1[a]	2.13
Others	3[b]	6.38
Others	4[c]	8.51
Others	4[d]	8.51

[a]1 strain gave positive reaction with antsera B and E.
[b]3 strains gave positive reaction with antisera C and E.
[c]Autoagglutination *ie* spontanenously in saline.
[d]Four isolates precipitate by slide and negative by co-agglutinaion.

From the 47 clinical isolates, 15 of them (31.92%) were typed as serotype F, 5 were serotype A, 2 were serotype C and E. The remaining serotypes (B and D) were found only in one culture (Table 4).

DISCUSSION

Serological typing of gram-negative bacteria is widely used for epidemiological purposes and for the identification of certain disease-associated types of strains such as in the case of meningococcal meningitis (Per Olcen, 1975). Conventionally, antisera are raised in rabbits against serotype strains of the species and the sera are evaluated for their titer and cross reactivity. In this study the six antisera originally described by Owen and Lapage (1974) were kindly provided by Dr C Richard, Institut Pasteur, Paris, but his method of typing in glass tubes employing Kahn's technic (Richard, 1979a) seemed too cumbersome and was found not to be economical in terms of the amount of serum needed for each test. It was decided therefore, to investigate alternative methods of serotyping which would require only small volumes of antisera.

Using slide agglutination, few cross reactions were found amongst the serotype strains (Richard, 1981) ever when the sera were used undiluted. Furthermore, the sera were remarkably specific and did not agglutinate with other species except *Flavobacterium* group 11b (King, 1959; Owen and Lapage, 1974) or closely related genera such as *Moraxella*. The titer of serum C was very low but gave strong agglutination when used undiluted in tests with homologous antigen.

The serotype-specific and antibodies in the six sera (A, B, C, D, E, F) were confirmed as IgG because they bound very efficiently to protein A-containing staphylococci. This suggests that the few cross reaction seen with slide agglutination may be due to IgM antibody or that the non-specific antibodies present were too low in concentration to enable it to compete with the homologous antibody to bind with protein A.

The ability to type clinical isolates of *F. meningosepticum* from a variety of sources with the six antisera was fairly good, but a considerable proportion were autoagglutinable in saline or were not typable with the sera. These nontypable strains probably belonged to other serotypes (Richard, 1979a). The autoagglutinable strains may have been antigenically rough as is seen with *Salmonella* R forms, but the cultures of autoagglutinating Flavobacteria did not appear rough from their colony phenotype. Instead these cultures gave stringy viscous suspensions in saline, resulting in a non-homogenous emulsion.

Co-agglutination gave remarkably similar results as slide agglutination but a slightly higher sensitivity is observed in slide agglutination technic. This may be due to the fact that the sera used were undiluted.

Richard *et al* (1979b) found that serotype G was their commoner isolates, most of which come from Strasbourg, France. In this study, serotype F was most frequent which represented 31.92% of the isolates. The collection of clinical cultures in this study came from various geographical sources and thus these results may give a more accurate estimate of the frequency of the serotypes. Unfortunately serotype *G* antisera were not available for testing. There was no abvious association between the serotype and clinical sources, but two serotypes, C and D, may worth investigating for their potential

pathogenicity as in this study, they were isolated either from blood or cerebrospinal fluid. A study done in Malaysia (unpublished data) revealed that out of 70 *Flavabacterium meningosepticum* isolated from CSF, 40% were serotype C.

In conclusion serotyping of *F. meningosepticum* proved to be satisfactory by either the slide (Richard and Laurent 1981) or co-agglutination technic (Kronvall, 1973). Typing of clinical isolates by the co-agglutination method is applicable for epidemiological studies of *F. meningosepticum* and further studies of the distribution of types with a wider range of sera would be worthwhile.

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