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The distribution of *Bacteroides fragilis* serotypes amongst clinical strains

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SUMMARY

Three hundred and twenty-two strains of *Bacteroides fragilis* isolated from infected patients at three different hospitals were tested against 20 type specific *B. fragilis* antisera using the tube agglutination technique. Of these strains 41·3% were assigned to a single O-serotype, a further 20·5% were agglutinated by several antisera and could not be classified and the remainder showed no reactions. Three different serotypes were prevalent in the three hospitals and minor geographical variation was observed.

No correlation was found between serotypes and the origin of infection, but those from the blood were the most readily typable strains. No correlation was found between serotypes and biotypes of *B. fragilis*.

INTRODUCTION

Bacteroides fragilis is the anaerobic micro-organism most commonly associated with clinical infections (Finegold, 1974, 1977; Gorbach & Bartlett, 1974). It has been subdivided on the basis of its biochemical reactions into five subspecies (Holdeman & Moore, 1974). These subspecies vary in their ability to promote infection. *B. fragilis fragilis*, the least common of the subspecies in human faeces (Moore & Holdeman, 1974) is the most common cause of clinical infections, whereas the subspecies predominantly found in the faeces are less frequently isolated from infected sites (Finegold, Attebury & Sutter, 1974; Jones & Fuchs, 1976; Polk & Kasper, 1977). This suggests that *B. fragilis fragilis* may possess a virulence factor which is not present in other subspecies. This virulence factor may be related to its encapsulation (Polk & Kasper, 1977); and more specifically to its capsular polysaccharide (Onderdonk *et al.* 1977). Antisera raised against this capsular polysaccharide reacted with almost all clinical strains of *B. fragilis fragilis* but not with other subspecies (Polk & Kasper, 1977).

Other workers, however, have found that *B. fragilis fragilis* forms an antigenically heterogeneous group. A variable number of distinct serogroups based on the O-antigen have been found within the subspecies (Beerens *et al.* 1971; Lambe & Moroz, 1976; Hofstad, 1975; Elhag, Bettelheim & Tabaqchali, 1977), and

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O-antisera were used to serogroup *B. fragilis* strains isolated from clinical infections (Romond, Beerens & Wattre, 1972; Lambe & Moroz, 1976). Different serogroups were found to vary in their pathogenicity; certain types were more common amongst the strains causing infections (Romond *et al.* 1972). However, the number of strain-specific antisera used so far have been very few. For this reason, using our recently described method (Elhag & Tabaqchali, 1978) we have tested 322 strains of *Bacteroides fragilis* isolated from clinical infections from three different hospitals against 20 specific antisera, in order to define the number of strains which are typable, to investigate the association of the different serotypes with infection and to find out if any geographical variation exists.

MATERIALS AND METHODS

Bacterial strains

Three hundred and twenty-two strains of *B. fragilis* isolated from clinical laboratory specimens derived from infected sites were studied. Of these, 180 were obtained at St Bartholomew's Hospital, London (hospital 1). The remainder of the strains were kindly provided by Professor I. Phillips from St Thomas's Hospital, London (hospital 2), and Dr O. A. Okubadejo from St Mary's Hospital, Portsmouth (hospital 3); the numbers were 81 and 61 strains respectively.

All the strains of *B. fragilis* were cultured and identified as previously described (Elhag & Tabaqchali, 1978).

Raising of antisera

Pure antisera against live cultures of 20 serotypes of *B. fragilis* were prepared as described by Elhag & Tabaqchali (1978).

Preparation of antigens

O-antigens were prepared by steaming broth cultures of all 322 *B. fragilis* strains at 100 °C for 30 min and further treating as previously described (Elhag *et al.* 1977).

Testing of antigens

Each one of the O-antigens was tested against each of the 20 antisera, diluted 1/5 (v/v) in buffered physiological saline (BPS) at pH 7·2 as described by Elhag & Tabaqchali (1978).

The suspensions showing agglutination reactions at such dilutions were tested against the reacting antiserum at doubling dilutions from 1/20 to 1/1280. A standard homologous antigen suspension was similarly tested with each serum as a positive control. A saline negative control was also included with each test. A positive reaction was considered when a suspension was agglutinated at a titre equal to or higher than that of the positive control. Agglutination reactions at lower titres were disregarded and considered as negative.

Table 1. *Serotyping of* B. fragilis *clinical strains from three hospitals*

Hospital	No. of bacteroides strains	Sertotypes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Typable: Single O-antigen (%)	Typable: Multiple O-antigens (%)	Typable: Total (%)	Non-typable
1	180	4	9	3	—	4	3	3	1	—	1	1	2	3	—	—	10	12	6	9	2	73 (40·6)	40 (22·2)	113 (62·8)	67 (37·2)
2	81	3	3	2	—	—	3	1	1	—	1	1	2	1	—	—	—	2	4	8	2	34 (42·0)	15 (18·5)	49 (60·5)	32 (39·5)
3	61	1	1	—	—	2	1	3	—	—	1	1	4	—	—	—	1	4	1	6	—	26 (42·6)	11 (18·0)	37 (60·7)	24 (39·3)
Total	322	8	13	5	—	6	7	7	2	—	3	3	8	4	—	—	11	18	11	23	4	133 (41·3)	66 (20·5)	199 (61·8)	123 (38·2)

Table 2. *The relation between* Bacteroides fragilis *serotypes and the type of infection*

Type of infection	No. of bacteroides strains	Serotypes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Typable: Single O-antigen (%)	Typable: Multiple O-antigens (%)	Typable: Total (%)	Non-typable
Appendicitis and infections following bowel surgery	135	1	5	3	—	2	3	2	1	—	2	3	5	4	—	—	6	3	3	7	—	50 (37·0)	33 (24·4)	83 (61·5)	52 (38·5)
Infections of the female genital tract	30	1	1	—	—	—	—	2	—	—	—	—	—	1	—	—	—	2	3	4	1	15 (50·0)	1 (3·3)	16 (53·3)	14 (46·7)
Bacteraemia	15	—	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	3	1	1	1	10 (66·7)	1 (6·7)	11 (73·3)	4 (26·7)
Others	45	2	2	1	—	1	—	1	—	—	1	—	1	1	—	—	—	1	2	4	1	18 (40·0)	8 (17·8)	26 (57·8)	19 (42·2)

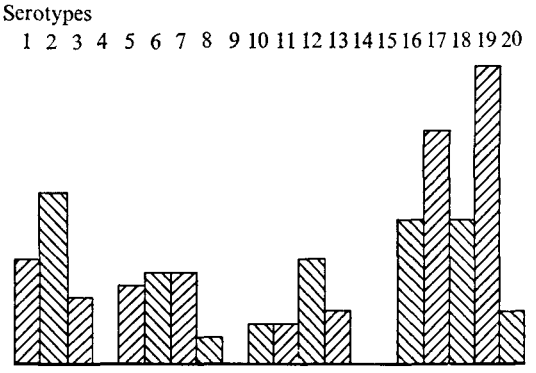

Fig. 1. Distribution of *Bacteroides fragilis* serotypes amongst clinical strains.

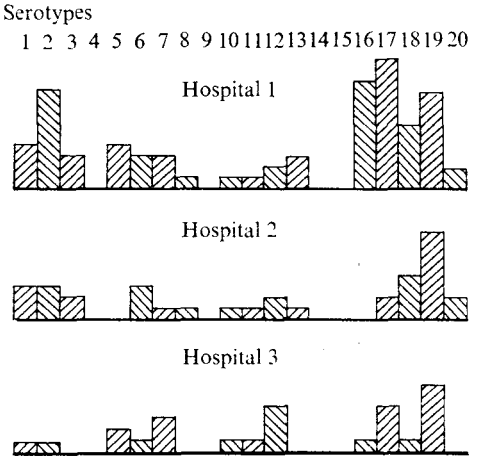

Fig. 2. Distribution of *Bacteroides fragilis* serotypes amongst clinical strains from three different hospitals.

RESULTS

The results of the serological typing of all the strains are shown on Table 1. A total of 133 strains (41·3%) were agglutinated by only one of the antisera. A further 66 strains (20·5%) were agglutinated by more than one antiserum, and the remainder 123 (38·2%) showed no reactions. The distribution of the strains which were agglutinated by only one of the antisera is illustrated in Fig. 1. This showed that the majority of the strains belonged to serotypes 2, 17 and 19, and none belonged to serotypes 4, 9, 14 and 15.

The distribution of the serotypes in the individual hospitals (Fig. 2) was similar to the general pattern as demonstrated in (Fig. 1), but some differences were observed. Serotype 17 was isolated most frequently at hospital 1, whereas serotype 19 was the commonest in hospitals 2 and 3. Serotype 16, one of the commonest in hospital 1, was not found in hospital 2 and was rarely found amongst

Table 3. *The relationship between serotypes and biotypes of* B. fragilis

B. fragilis subspecies	No. *B. fragilis* subspecies	*B. fragilis* serotypes																			Typable			Non-typable
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Single O-antigen (%)	Multiple O-antigens (%)	Total (%)	
		fragilis NCTC 9343	*fragilis* NCTC 10584	*thetaiotaomicron* 10582	*vulgatus* 10583	*fragilis* 9344	*fragilis* 8560	*fragilis* 10581	*ovatus* ATCC 8483	*distasonis* ATCC 8503	*fragilis*	*fragilis*	*fragilis*	*distasonis*	*distasonis*	*distasonis*	*fragilis*	*fragilis*	*fragilis*	*fragilis*	*fragilis*				
fragilis	269	6	11	1	—	4	6	6	—	—	1	2	8	2	—	—	11	18	11	23	4	114 (42·4)	61 (22·7)	175 (65·1)	94 (34·9)
thetaiotaomicron	23	1	—	3	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	6 (26·1)	2 (8·7)	8 (34·8)	15 (65·2)
vulgatus	15	1	—	1	—	1	—	—	1	—	—	—	—	2	—	—	—	—	—	—	—	6 (40·0)	2 (13·3)	8 (53·3)	7 (46·7)
distasonis	10	—	2	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	3 (30·0)	1 (10·0)	4 (40·0)	6 (60·0)
ovatus	5	—	—	—	—	1	1	—	—	—	2	—	—	—	—	—	—	—	—	—	—	4 (80·0)		4 (80·0)	1 (20·0)

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the strains of hospital 3. There was no difference in the percentages of strains which reacted with a single or multiple antisera or showed no reactions amongst the strains derived from the three hospitals.

Table 2 shows the relation between the serotypes of *B. fragilis* and the type of infection. Of the strains isolated from infections following appendicitis and bowel surgery 37·0 % reacted with one of the antisera and 24·4 % had multiple reactions. However, 50 % of the strains derived from infections of the female genital tract and 66·7 % of those isolated from the blood cultures were agglutinated by only one of the antisera. Of the strains obtained from other sites (liver abscess, brain abscess, infected skin, bones and urinary tract), 40 % reacted with only one of the antisera. The number of strains which showed no reactions with any of the 20 antisera was lowest in the bacteraemia group, 26 % only, compared with 38–46 % in the other groups.

The relations between the different serotypes and the biotypes of *B. fragilis* are shown in Table 3. Of the 322 strains reported in this study, 269 (83·6 %) were identified as *B. fragilis* subsp. *fragilis*, 23 (7·1 %) as subsp. *thetaiotaomicron*, 15 (4·7 %) as subsp. *vulgatus*, 10 (3·1 %) as subsp. *distasonis*, and 5 (1·6 %) as subsp. *ovatus* (Table 3).

The majority of the *B. fragilis fragilis* reacted with antisera raised against the same subspecies, but nonetheless some cross-reactions did occur amongst the various serotypes and biotypes. Strains of the same biotype, e.g. *thetaiotaomicron* were agglutinated by antisera raised against not only the same subspecies, no. 3, but also against *B. fragilis fragilis* nos. 1 and 11 and *ovatus* no. 8. Similarly subsp. *ovatus* agglutinated with antisera raised against *B. fragilis fragilis* 5, 6 and 10 (Table 3).

Relatively fewer *B. fragilis fragilis* cross-reacted with antisera raised against other subspecies. Furthermore the percentage of *B. fragilis fragilis* strains which were not agglutinated by any of the 20 antisera was only 35 % as compared with the remainder (46–65 %), except for *ovatus* where only five strains were tested (Table 3).

DISCUSSION

This study demonstrates that the serological scheme reported previously by us, based on the agglutination test (Elhag & Tabaqchali, 1978) can be used to serotype *B. fragilis* strains isolated from various clinical specimens. It provides a greater number of absorbed type-specific antisera than previously described (Beerens *et al.* 1971; Lambe & Moroz, 1976), 13 different types within subspecies *fragilis*, four *distasonis* and one each of *thetaiotaomicron*, *ovatus* and *vulgatus*.

Using these 20 absorbed type specific *B. fragilis* antisera, it was possible to assign 41·3 % of the 322 strains of *Bacteroides fragilis* to a single O-serotype. A further 20·5 % could not be assigned to a single O-serotype as they were agglutinated by several antisera. These reactions were so variable that it was not possible to classify the latter group into a serological scheme. Lambe & Moroz (1976) on the other hand, using seven absorbed type specific antisera against *B. fragilis fragilis* strains were able to classify 32 out of 98 strains into a single O-serogroup

and the remainder which gave more than a single reaction into a further 14 serogroups consisting of multiple components. In our study however, the 66 strains (20·5%) which gave multiple reactions showed such a complex variety of O-antigens that it was prohibitive to attempt to serogroup them on a pattern similar to that described by Lambe & Moroz (1976).

This multiplicity of reactions observed against the absorbed antisera illustrates the diversity of the antigenic factors within the lipopolysaccharide moiety as was shown by Hofstad (1977). Nonetheless, O-antigens derived from 124 strains (38·2%) did not react with any of the 20 antisera, suggesting that more strains could be used to raise further antisera, and that even this extended scheme falls short of being adequate for serotyping all clinical strains.

Of those reacting with a single antiserum types 2, 17 and 19 were the prevalent strains in the three hospitals (Table 1, Fig. 1). These strains were *B. fragilis fragilis* originally isolated from clinical infections and used to raise the antisera (Elhag & Tabaqchali, 1978). This may possibly demonstrate the association of certain serotypes of *B. fragilis* with virulence. Romond *et al.* (1972) showed that 74% of 58 presumably pathogenic strains of *B. fragilis* were agglutinated by two of their six specific O-antisera (E_1, E_2 or E_1 E_2) raised against two strains of *B. fragilis fragilis*. Kasper *et al.* (1977), on the other hand demonstrated that most strains of *B. fragilis fragilis* were encapsulated and that the capsular polysaccharide derived from these strains constituted the virulence factor (Onderdonk *et al.* 1977). Furthermore, strains lacking this capsule are less capable of promoting infection (Onderdonk *et al.* 1977). It would be of interest therefore, to study our more prevalent serotypes further in order to find out if there is a variation in the properties of these strains which could explain the association of certain serotypes with infection.

There was a slight variation of the prevalence of certain types in the three hospitals (Fig. 2). This might be due to geographical variation of the normal flora of people resident in the different areas. Nevertheless, one cannot exclude the possibility of hospital infection caused by strains of *B. fragilis* prevalent in the various hospitals. Patients could acquire new hospital strains, similar to *Escherichia coli* (Cooke, Ewins & Shooter, 1969) and to *Pseudomonas aeruginosa* (Al-Dujaili & Harris, 1975). These prevalent serotypes of *B. fragilis* might colonize the gastrointestinal tract of patients and under favourable conditions could initiate an infection. Perhaps further studies on the distribution of *B. fragilis* serotypes in the faeces of normal subjects and patients in hospital and their relation to those isolated from infected sites may shed some light on the epidemiology of these microorganisms.

We tried to correlate the O-serotypes with the origin of infection, using 225 of the clinical strains on which sufficient clinical data were obtained. No direct correlation was found, but the typability of the strains varied according to the isolation site (Table 2). More strains isolated from the blood were typable, often with a single O-antiserum, whereas only a few of those derived from infections following bowel surgery were so. This variation is probably due to the contamination of surgical wounds by commensal strains of *Bacteroides*, where the problems of sampling may play a role, whereas such contamination is unlikely to occur in normally sterile

sites as the blood. If so, then this might suggest that virulent strains of *B. fragilis* are more readily typable whereas normal commensals are less so. This will become clearer when our further studies on the faecal flora are completed.

No distinct correlation was found between *B. fragilis* biotypes and serotypes. A number of different subspecies belonged to a single serotype and different serotypes were assigned to a single subspecies (Table 3). Similar findings were also reported by Beerens *et al.* (1971). However, the typability of strains of different subspecies varied. Strains of subsp. *fragilis* were more frequently typable and with fewer cross reactions with other antisera than the other subspecies. This may represent the higher number (13) of *B. fragilis* antisera used, and the predominance of subsp. *fragilis* (83%) amongst the clinical strains studied.

The serology of *B. fragilis* may prove to be useful as a diagnostic and epidemiological tool. This may eventually help in understanding more fully the role of these bacteria in infection.

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