

BIOCHEMICAL CLASSIFICATION OF COLIFORM BACILLI IN SPUTUM

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COLIFORM bacilli are frequently found in routine examination of sputum. They are usually regarded as belonging either to the typical and atypical *B. coli*, or to the *Friedländer* group; but surprisingly little work has been done in the investigation of these organisms, apart from that on the *Friedländer* group. It was therefore decided to obtain coliform bacilli from routine specimens of sputum and to make an attempt at classifying these strains.

Classification of organisms of the *B. coli* group is by no means an easy matter. Various workers have tried to group them by serological methods, but have found this impracticable owing to their heterogeneity—that is, a serum produced against a certain strain agglutinates that strain, but has usually little or no agglutinating power against other strains. Pathogenicity towards animals also is of no help in this question. Many strains of the *Friedländer* group kill mice in high dilutions, as might be expected of a capsule-bearing organism, but many other coliform strains can also be proved to be highly pathogenic to mice.

A great deal of work on the classification of coliform bacilli has been done by public health workers, mainly with the object of determining which can be regarded as of fæcal origin, and thus by their presence affording evidence of dangerous contamination of water supplies. The fermentation of various carbohydrates is used as a basis of classification along with some other tests, including what is known as the "Imvic" reactions—that is, the Indol test, the methyl-red test, the Voges-Proskauer reaction, and Koser's citrate utilisation test.

It was decided to adopt the following working scheme, which divides the organisms into seven groups according to their biochemical properties.

1. *B. coli* Group.—Only strains which gave as Imvic reactions + + — were regarded as belonging to this group.
2. *B. anaerogenes* Group.—Fermentation of two or more sugars with production of acid only.
3. *B. proteus-Morgani* Group.—Only sugar fermented being glucose with acid and gas (AG), or with acid only (A).
4. *B. pyocyaneus*.—Characterised by its colour.
5. *B. fæcalis alkaligenes* Group.—Only characteristic being the absence of fermentation of any sugars.

6. *B. Friedländer-Aerogenes Group*.—These two types were grouped together as most research workers agree that we do not possess criteria on which to base a separation. Fermentation of either adonite and/or inosite with AG or A, or strains with as Imvic reactions — — + +.
7. *B. coli intermedius*.—Every strain which did not comply with the criteria of one of the above groups.

Method.—The specimens of sputum were plated out on MacConkey plates as well as inoculated on the usual media. On an average one out of five sputa yielded some coliform colonies. In only a very few instances the blood agar plate yielded coliform bacilli on primary culture, while the MacConkey plate showed no growth. The plates were incubated for forty-eight hours, as it was noticed that during the first twenty-four hours the growth was frequently completely or partially inhibited.

The sugars employed were glucose, lactose, dulcitol, saccharose, mannitol, maltose, adonite and inosite, while a tube with litmus milk and all media for the Imvic reactions were also inoculated, and a gelatin stab culture was made. With the exception of the last, which was kept for two months at room temperature, all other media were incubated at 37° C. for a standard time of forty-eight hours.

The Voges-Proskauer reaction was done both as originally described and according to O'Meara's modification. As stressed repeatedly by other workers, the latter was more often positive than the former, the proportion being 21 : 13. A positive reaction with O'Meara's modification only was regarded as a positive Voges-Proskauer reaction.

GENERAL OBSERVATIONS

Coliform bacilli were obtained in all kinds of morbid conditions, ranging from acute illnesses like diphtheria, lobar and bronchopneumonia to chronic conditions like asthma, tuberculosis and bronchiectasis. No case of a *Friedländer* pneumonia was included in the present series.

One hundred different strains were obtained from 80 patients, fourteen specimens of sputum yielding two, and three specimens even yielding three different strains of coliform bacilli. Here also there was an even distribution of acute and chronic conditions.

In thirty-three specimens of sputum the coliform bacilli were fairly numerous, but only in four cases was an almost pure growth obtained. Two of these were obtained from patients who were suspected of tuberculosis, and both the strains were ranged with the *Friedländer-Aerogenes* group. The two others were obtained from patients suffering from acute bronchitis, and asthma with acute bronchitis. The first strain was classified as leptothrix and the second one as belonging to the *B. proteus-Morgani* group.

Twelve different combinations of Imvic reactions were observed. In order to facilitate their being mentioned later these will be enumerated here :—

(1) + + - -	(7) + - + +
(2) - - + +	(8) - + - -
(3) - - - -	(9) - + - +
(4) + + + +	(10) - + + -
(5) + + - +	(11) - + + +
(6) + - - +	(12) - - - +

DESCRIPTIONS OF GROUPS

1. *B. coli* Group.—Thirteen real *B. coli* strains were isolated. Nine were motile, all fermented glucose, lactose, mannite, maltose with AG, three saccharose with AG, and none liquefied gelatin. Five formed acid and clot, and five acid only in litmus milk.

2. *B. anaerogenes* Group.—Seven strains were isolated, five being motile. Three liquefied gelatin. Two strains fermented milk, with acid only. The Imvic reactions were as follows: one each of combinations 3, 4 and 8, and two each of combinations 9 and 11.

3. *B. proteus-Morgani* Group.—Eleven strains were isolated, seven being motile. Three fermented glucose with AG, the remainder with A only. Six liquefied gelatin. None fermented litmus milk. Five different Imvic reactions were obtained: one of combination 3, two each of combinations 9 and 7, and three of combinations 1 and 12, the Voges-Proskauer reaction always being negative.

4. *B. pyocyaneus*.—Five strains were isolated. All were actively motile, fermented glucose with A only, liquefied gelatin and had the same Imvic reactions: - - - + (combination 12).

5. *B. faecalis alkaligenes* Group.—Eight strains were isolated, only two being motile. The three different Imvic reactions were distributed as follows: one of combination 9, two of combination 12, and five of combination 3.

6. *B. Friedländer-Aerogenes* Group.—Thirty strains were isolated, of which seventeen were mucoid. Seven strains were motile, of which four showed combination 2 Imvic reactions, which is characteristic of real *B. aerogenes* strains. Two of these four fermented neither adonite nor inosite. Four strains of this group fermented the sugars with A only. These, of course, fermented adonite and inosite also with A only. The remainder showed the following fermentation types of adonite and inosite.

Adonite.	Inosite.	Number of Strains.
AG	AG	11
AG	A	3
AG	—	7
—	A	2
—	—	3

Gelatin was liquefied seven times, maltose was always fermented, mannite by all but one strain. Ten different Imvic reactions were observed.

Combination.	Number of Strains.
1	1
2	5
4	2
5	1
6	1
7	1
8	4
9	10
10	4
12	1

Litmus milk was fermented 11 times with A only, and twice with acid and clot.

7. *B. coli intermedius* Group.—Twenty-five strains were classified as such, fourteen showing motility. Ten liquefied gelatin. Litmus milk was fermented twelve times with A, and five times with acid and clot. Five strains were non-lactose fermenters. Six Imvic combinations were found.

Combination.	Number of Strains.
4	2
5	6
8	5
9	8
10	1
11	3

It should be mentioned here that Parr (1938, 1939) describes as *B. intermedius* Imvic combinations 4, 5, 7, 9, 10, 11 and, in addition, the combinations + + + - and + - + -, which were not observed at all among all the hundred strains of this series, while combination 7 was not found among the *B. intermedius* examined.

8. *Leptothrix*.—One strain was obtained. It was included among the coliform bacilli as on first culture (MacConkey plate) it was Gram-negative and indistinguishable from coliform bacilli. Only on subsequent subculture it became partially Gram-positive and showed characteristic bulbous formations. It was non-motile and fermented no sugars. Its Imvic reactions were of combination 3 (- - - -). Gelatin was not liquefied.

DISCUSSION

It is, of course, fully realised that the aforementioned division of coliform bacilli into seven different groups is a very debatable one indeed. Thus, for example, a strain which fermented glucose and saccharose with A only was placed with *B. intermedius*, while a strain which fermented glucose alone with A was regarded as a member of

the *B. proteus-Morgani* group. Yet in the great majority of strains there was no doubt as to the classification of a particular bacillus.

The *intermedius* group contains exclusively organisms which would be classified as *B. coli* as to their biochemical properties, were it not that their Imvic reactions differed from the classical + + - -. The only constant reaction was methyl red+. If one regards them all as atypical *B. coli*, this group together with the real faecal *B. coli* type would number 38, thus outnumbering the *Friedländer Aerogenes* group, while all three groups together account for two-thirds of all strains isolated in the present series.

SUMMARY

One hundred strains of coliform bacilli were cultivated from non-selected specimens of sputum. These were isolated from 80 patients, some of whom yielded two or three different strains. In four cases the coliform bacilli were obtained in almost pure culture. A great variety of these organisms was obtained. Twelve different Imvic reactions were found. The strains were divided into eight different groups, the percentage for these being: *B. coli*, 13 per cent.; *B. anaerogenes*, 7 per cent.; *B. proteus-Morgani*, 11 per cent.; *B. pyocyaneus*, 5 per cent.; *B. faecalis alkaligenes*, 8 per cent.; leptothrix—which was included for its being Gram-negative and growing well on MacConkey's medium—1 per cent.; *B. Friedländer-Aerogenes*, 30 per cent.; and *B. coli intermedius*, 25 per cent. The *B. coli* and *B. coli intermedius* groups taken together constituted 38 per cent. of the total.

REFERENCES

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