CLASSIFICATION OF THE B. COLI GROUP.*

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The term *B. coli* as an indication of fecal contamination in water and milk has been so often misapplied that the result has been much confusion and frequent misinterpretation of bacterial examinations.

It has been the custom of many bacteriologists to throw out of sanitary consideration all bacteria which do not absolutely conform to the so-called "typical" *B. coli*. There are many known varieties, all of fecal origin and closely related to typical *B. coli*, which will be described in this paper, and there probably exist many more varieties which will be discovered in the future. Any of these varieties, when they occur in water or milk, have a sanitary significance, and because of their close relationship, all should be included in the *B. coli* group.

The fermentative reactions have been chosen as a means of classification, not only because of the ease with which these organisms are thus separated from those of other groups, but because of the facility with which each variety may be separated from the others.

B. coli GROUP.

The general characteristics common to this group are:

Fermentation with gas production with dextrose and lactose, short bacilli with rounded ends, non spore forming, facultative anaerobic, give positive test with esculin, grow at twenty degrees on gelatine and at thirty-seven degrees on agar, non-liquifying in fourteen days on gelatine.

The group consists of four species:

B. communior, (Durham.)
B. communis, (Escherich.)
B. aerogenes, (Escherich.)
B. acidi-lactici, (Hueppe.)

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The relative frequency with which the species of the *B. coli* group have been isolated is shown below:

	MacConkey. No. of Strains from Feces		Graham-Smith. No. of Strains from Flies		Winslow & Walker No. of Strains from Feces	
B. communior	110	23%	15	43%	7	28%
B. communis	178	37%	6	17%	15	60%
B. aerogenes	72	15%	4	11%	1	4%
B. acidi-lactici	120	25%	10	29%	2	8%
	480	100%	35	100%	25	100%

The first two species are separated from the second two by their gas production with dulcite, and the first species of each of these two groups may be separated from the second by its gas production with saccharose.

Each of these species may be separated into four possible varieties in accordance with their gas production with mannite and raffinose. Two varieties of the third, and three of the first and fourth species are now known. All four possible varieties of the second group have been found. These varieties are classified by letters, as, for instance, *B. communis*, A, B, C and D. Under these varieties are classified subvarieties, as in the case of *B. communior*, A_1 and A_2.

Graphically, the separation of the known groups is brought about as follows:

B. COLI GROUP.

Dextrose+.
Lactose+.

Dulcite+.
B. communior.
B. communis.

Saccharose+.
B. communior.

Variety		Mannite	+	Raffinose	+
"	(A)2	"	+	"	+
"	(B)	"	+	"	—
"	(C)	"	—	"	+
"	(D)*	"	—	"	—

Saccharose—.
B. communis.

Variety		Mannite	+	Raffinose	+
"	(B)	"	+	"	—
"	(C)	"	—	"	+
"	(D)	"	—	"	—

Dulcite—.
B. aerogenes.
B. acidi-lactici

Saccharose+.
B. aerogenes.

		Mannite	+	Raffinose	+
(A)1		"	+	"	+
(A)2		"	+	"	+
(A)3		"	+	"	+
(B)1		"	+	"	—
(B)2		"	+	"	—
(C)*		"	—	"	+
(D)*		"	—	"	—

Saccharose—.
B. acidi-lactici.

		Mannite	+	Raffinose	+
(A)1		"	+	"	+
(A)2		"	+	"	+
(B)		"	+	"	—
(C)*		"	—	"	+
(D)		"	—	"	—

\+ Positive reaction.
— Negative reaction.
* Unknown.

In the above table twenty-one varieties of *B. coli* are given, four of which are as yet unknown. Out of the seventeen known varieties the author has cultures of fifteen. The other two, *B. communis* D, and *B. acidi-lactici* D, are described by Winslow & Walker[1].

It will be seen that the classification admits of indefinite expansion as other subvarieties are found. The following is a description of the characteristics of the known members of the *B. coli* group:

B. communior (DURHAM.)

VARIETY A_1. Fermentation with gas production with dextrose, lactose, dulcite, saccharose, mannite and raffinose. Milk coagulated, nitrate reduced, motile, indol positive.

Isolated by West of the Philadelphia Filter Laboratories from contaminated river water and checked by the author; also 7 strains isolated by Winslow from feces; by Dr. Avery of the Hoagland Laboratories, Brooklyn, from two cases of chronic cystitis and from an abscess associated with streptococci in a chronic case of cellulitis; also isolated by the author from contaminated water and from feces.

This appears to be the most common variety of *B. communior*. While raffinose determinations were not made, it is probably the same variety as isolated by MacConkey[2] in 110 strains and by Graham-Smith[3] in 15 strains taken from fecal matter.

VARIETY A_2. Fermentation the same as A_1. Motile, reduces nitrate. Differs from A_1 in not producing indol.

Isolated in three strains by Mr. Thomas W. Melia, of Mt. Prospect Laboratory, from human feces.

VARIETY B. Ferments with gas production with dextrose, lactose, dulcite, saccharose and mannite, but forms no gas with raffinose.

Also distinguished by not coagulating milk, even after heating, and by slow formation of gas in dulcite. In this latter test it usually takes three days for the gas formation to become active. Motile, indol positive, nitrate reduced.

Variety found by Melia, of Mt. Prospect Laboratory, Brooklyn; received from Dr. Avery, of the Hoagland Laboratories, Brooklyn, and isolated from urine from a case of cystitis.

VARIETY C. Fermentation with gas production with dextrose, lactose, dulcite, saccharose, and raffinose. Forms no gas with mannite. Milk coagulated, nitrate reduced, motile, indol positive.

Culture obtained from Dr. J. R. Fraser, McGill University, Montreal.

B. communis. (ESCHERICH.)

VARIETY A. Fermentation with gas production with dextrose, lactose, dulcite, mannite and raffinose. No gas formation with saccharose. Motile, indol slight, nitrate reduced.

Isolated by Currello, at the Bellevue Hospital. Isolated by Avery from a case of cystitis.

VARIETY B. Fermentation with gas production with dextrose, lactose, dulcite and mannite. No gas production with saccharose and raffinose. Milk coagulated, nitrate reduced, motile, indol positive.

This appears to be the most common variety of *B. communis.* Isolated by Avery and checked by the author, from a case of urinary cystitis; by Winslow from feces, and by the author from feces, water and milk.

VARIETY C. Fermentation with gas production with dextrose, lactose, dulcite and raffinose. No gas production with saccharose or mannite. Nitrate reduced, indol positive, motile.

Isolated by Winslow from feces. Isolated by Melia from Brooklyn water supply.

VARIETY D. Fermentation with gas production with dextrose, lactose, and dulcite. No gas production with saccharose, mannite or raffinose. Nitrate reduced, indol positive.

Isolated by Winslow from feces.

B. aerogenes. (ESCHERICH.)

VARIETY A_1. Fermentation with production of gas with dextrose, lactose, saccharose, mannite and raffinose. No gas production with dulcite. Indol positive, nitrate reduced, motility negative. Viscous growth on agar and in lactose bile. In the latter it can be drawn out into a long thin string.

Culture obtained by the author from Bellevue Hospital; also isolated by Dr. White of the Hoagland Laboratories from a urinary fistula, by Winslow from feces, and by the author from the Brooklyn water supply.

VARIETY A_2. Fermentations the same as A_1. Motile, indol negative, nitrate reduction positive. Differs from A_1 in producing growth less viscid or stringy when touched with the needle; in being motile and not producing indol.

Isolated by Avery from a case of chronic urethritis and from a case of cellulitis associated with *B. pyocyaneus* and *Streptococcus pyogenes aureus.*

After the identification of this variety, a vaccine was made which was specific for this particular variety of infection, whereas the vaccine previously made from *B. communis* B apparently had no curative effect.

VARIETY A_3. Fermentations and tests with one exception same as A_2. Isolated from contaminated well water by Phelps and Hammond[4], Differs from A_2 in being slightly liquifying in gelatine stab after about twenty-six days. The total gas and percentage of CO_2 is high when grown in dextrose broth and particularly in liver broth. This species has been at times grouped with *B. cloacae* (Jordan), but the former never fails to produce gas with lactose, while typical *B. cloacae* apparently always gives negative results when dextrose-free lactose solutions are used. Another marked distinction is that true *B. cloacae*, after rejuvenation, is always strongly liquifying, while *B. aerogenes* A_3 never liquifies before twenty days, even after careful rejuvenation over long periods.

VARIETY B_1. Forms gas with dextrose, lactose, saccharose and mannite, but no gas with dulcite and raffinose. Non-motile, indol negative, nitrate reduced. Viscous growth on agar and in lactose bile. May be drawn out into a thin string by using a platinum needle.

Culture obtained from Kral's Laboratory; also isolated by the author from water and milk. This is probably the most common variety of *B. aerogenes*.

VARIETY B_2. Differs from B_1 in being motile, indol positive, and non-viscous in lactose bile.

Isolated by Melia in two strains from feces.

B. acidi-lactici. (HUEPPE.)

VARIETY A_1. Fermentation with gas production with dextrose, lactose, mannite and raffinose. No gas production with dulcite and saccharose. Non-motile, indol positive, nitrate reduction positive.

Isolated by Hueppe from milk.

VARIETY A_2. Fermentation same as A_1. Indol positive, nitrate reduction positive. Differs from A_1 in being motile.

Isolated by West from contaminated river water.

VARIETY B. Fermentation with gas production with dextrose, lactose, and mannite. No gas production with dulcite, saccharose, or raffinose. Milk coagulated, nitrate reduced, motile, indol positive. Isolated by Melia in nine strains from human feces, often exceeding in numbers all other varieties of bacteria in feces.

Culture also obtained from Dr. J. R. Fraser, McGill University, Montreal. This appears to be the most common variety of *B. acidi-lactici*.

VARIETY D. Gas production with dextrose and lactose. No gas production with dulcite, saccharose, mannite or raffinose. Indol positive, nitrate reduced.

Isolated by Winslow from feces.

The accompanying table shows the distinguishing characteristics which separate the individual members of the *B. coli* group. The unknown varieties indicated will probably be discovered later.

B. COLI GROUP.

	Dextrose	Lactose	Dulcite	Saccharose	Mannite	Raffinose	Motility	Indol	Nitrate Reduction	Liquifaction Gelatine in 14 Days	Coagulation of Milk
B. communior A_1	+	+	+	+	+	+	+	+	+	—	+
B. " A_2	+	+	+	+	+	+	+	—	+	—	+
B. " B	+	+	+	+	+	—	+	+	+	—	—
B. " C	+	+	+	+	—	+	+	+	+	—	+
B. " D*	+	+	+	+	—	—	..	..	..	—	..
B. communis A	+	+	+	—	+	+	+	Slight	+	—	+
B. " B	+	+	+	—	+	—	+	+	+	—	+
B. " C	+	+	+	—	—	+	+	+	+	—	+
B. " D	+	+	+	—	—	—		+	+	—	+
B. aerogenes A_1	+	+	—	+	+	+	—	+	+	—	+
B. " A_2	+	+	—	+	+	+	+	—	+	—	+
B. " A_3	+	+	—	+	+	+	+	—	+	positive after 26 days	+
B. " B_1	+	+	—	+	+	—	—	—	+	—	+
B. " B_2	+	+	—	+	+	—	+	+	+	—	+
B. " C*	+	+	—	+	—	+	..	..	..	—	..
B. " D*	+	+	—	+	—	—	..	..	..	—	..
B. acidi-lactici A_1	+	+	—	—	+	+	—	+	+	—	+
B. " A_2	+	+	—	—	+	+	+	+	+	—	+
B. " B	+	+	—	—	+	—	+	+	+	—	+
B. " C*	+	+	—	—	—	+	..	..	..	—	..
B. " D	+	+	—	—	—	—	..	+	+	—	+

\+ Positive reaction.
— Negative reaction.
* Unknown varieties.

SUMMARY.

I. A study of this classification shows that thirteen out of seventeen known varieties of *B. coli* have been isolated from feces or diseased conditions; and that seven of these varieties have been isolated from water. Of the seven varieties isolated from water, four would conform to so-called "typical" *B. coli*, in spite of the fact that they are here grouped under three distinct species, *B. communior*, *B. communis*, and *B. acidi-lactici*. It is evident that the so-called typical *B. coli* does not exist as such, but that the entire group is typical of fecal contamination when water or milk examinations are to be considered.

II. All the known members of this group give positive gas tests with lactose bile, while no other known species gives such a test except *B. Welchii*, a pathogenic bacterium also of fecal origin. This may be readily distinguished from the *B. coli* group by its appearance under the microscope after growing in lactose bile, when long strings of bacteria considerably larger than those of the *B. coli* group are shown. Also unlike all members of the *B. coli* group, *B. Welchii* gives a negative test with esculin solution. It usually gives more rapid and active gas production in lactose bile than does *B. coli*. It is also distinguished by being an obligate anaerobe.

III. The importance of this classification from a medical point of view is shown by the fact that a vaccine made from *B. communis B* was not effective in cases of urethritis and cellulitis when the infection was from *B. aerogenes* A_2. It is evident that different members of the *B. coli* group may not be used indiscriminately for the production of vaccine, but that the variety of the *B. coli* causing the infection should be known and should be the one chosen for this purpose. The above classification readily facilitates the identification of any specific variety.

IV. The classification of bacteria into main groups, according to motility [5], [6] widely separates the most closely allied forms. Winslow[7] has discarded this classification for the coccaceae and called attention to the fact "that this property is not correlated with any other character—arising independently in forms exactly resembling non-motile forms in every other respect." Classification by motility would widely separate three of the varieties of *B. aerogenes* herein given from the other two known varieties whereas their descriptions show an unusually strong natural relationship. A classification based first on form and grouping of cells, second on the relation of their growth to air, third on their fermentive characteristics and, finally, on general cultural and morphological characteristics and biochemical reactions, would bring allied species and

varieties into closely related groups. Carrying out this idea the next group to be classified would be the facultative anaerobic bacilli which ferment dextrose with gas production but do not produce gas in lactose. Then would follow a classification into groups of those facultative anaerobic bacteria which produce acid but no gas when grown in the various sugar media. Just as in qualitative chemistry allied elements are brought together into groups by the reactions which they produce, so in qualitative bacteriology species and varieties having natural relationship may be brought together into groups by a classification based on their fermentative characteristics.

LITERATURE.

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