A STUDY OF THE COLIFORM ORGANISMS INFECTING THE WOUNDS OF WAR.

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(*Report to the Medical Research Committee.*)

(With 1 Chart.)

Already during the present war an extensive literature has grown up on the subject of the bacteriology of septic wounds. It was thought that a detailed study of the coliform organisms met with in these wounds might yield results of interest, and be a useful addition to some of the more general bacteriological contributions.

Cases Investigated.

The material for bacteriological investigation was obtained from the unhealed wounds of soldiers admitted to the East Leeds War Hospital during the second half of 1915 and first half of 1916. In most cases cultures were taken within a few hours of the patient's admission to hospital, but sometimes not for a number of days or even weeks thereafter. The cultures were taken quite indiscriminately from all sorts of wounds, from those which appeared clean as well as from those which were obviously septic, but the great majority belonged to the latter class. Sloughing of bone or of the soft tissues was present in many of them. One wound only was examined in each case, and where more than one wound was present the most septic was selected for investigation. 122 wounds in all were investigated for organisms of the "coliform" group, and of these 70, or 57 %, gave a positive result. Fleming (1915), working at a base hospital in France, found organisms of the coliform group in 74 out of 210 wounds examined, i.e. 35 %. Dean and Mouat (1916), in the investigation of eighteen gangrenous wounds at a home hospital, isolated coliform bacilli in four cases, or 22 %. Dudgeon, Gardner and Bawtree (1915), working in London, found coliform bacilli in not more than 37 wounds out of 100 examined.

https://doi.org/10.1017/S0022172400006665 Published online by Cambridge University Press

In positive cases the length of time intervening between the receipt of the wound and the taking of the culture varied very greatly, the shortest period being five days, the longest 350. It was found, however, that coliform organisms occurred with considerably greater frequency in old wounds than in those which were recently received, and this is particularly true of *B. pyocyaneus*. In the negative cases the average interval between wounding and culturing was 26 days, in the positive cases 49 days, where *B. pyocyaneus* was present 64 days. A more truthful representation of these facts may be made by dividing up the cases into groups corresponding to the stages suggested by Fleming (1915) (Table I).

TABLE I.

To show proportion of wounds, at different stages, infected by coliform bacilli.

Time after infection	No. of cases investigated	No. infected with coliform bacilli	Percentage of positives
Stage I 1–7 days	17	7	41
Stage II 8–20 days	47	20	42
Stage III Over 20 days	58	43	74
Totals	122	70	57 % (average)

If these results are compared with Fleming's, a striking similarity is observable (Table II). The discrepancy in the total percentage is of course accounted for by the higher proportion of cases investigated by Fleming in the earlier stages, when the percentage of positives is lower. The generally higher percentage of positives in my series at all three stages may be partly accounted for by the greater opportunity for contamination afforded by the more prolonged transport.

TABLE II. (Modified from Fleming.)

For comparison with Table I.

Time after infection	No. of cases investigated	No. infected with coliform bacilli	Percentage of positives
Stage I 1–7 days	127	37	29
Stage II 8–20 days	56	18	32
Stage III Over 20 days	27	19	70
Totals	210	74	35 % (average)

METHODS.

The purulent discharge from the wound or a scraping from the surface, if the wound were a clean one, was plated directly on Grünbaum and Hume's (1902) modification of MacConkey's medium (neutral red, crystal violet, bile salt, lactose agar), and incubated for 24 hours at 37·5° C. Discrete colonies of about 1 millimetre diameter and upwards were then picked off and subcultured on bullock's heart agar (Douglas, 1914) for a further 24 hours. The colonies on the bile salt plate were first carefully scrutinised through a hand lens, and as far as possible only one colony of each kind present was subcultured. It was found that by the use of Grünbaum and Hume's medium a great variety of appearances by different coliforms was obtainable, a much greater variety than by the use of the neutral red, bile salt, lactose agar of MacConkey. Often one or more subcultures on bile salt plates were made before finally subculturing on agar, especially if there was any difficulty in obtaining discrete colonies of individual bacilli. After confirmation of the morphological and tinctorial characters of the organism from the agar culture, except in the case of *B. pyocyaneus*, all or most of the following media were inoculated, viz. taurocholate broth containing the following 20 substances, glucose, lactose, saccharose, dulcite, mannite, adonite, inulin, salicin, sorbite, laevulose, galactose, raffinose, maltose, arabinose, dextrin, isodulcite, inosite, glycerin, amygdalin and erythrite, litmus milk, gelatin stabs, gelatin slopes (for motility), peptone water (for indol formation), glucose peptone water (for Voges and Proskauer's reaction), and nutrient broth. In the case of the carbohydrate media and the litmus milk the method of massive inoculation by large loopfuls of culture was practised, and it was found that by using large slopes of bullock's heart agar an abundant growth for this purpose was obtainable within 24 hours. Acid fuchsin (Holman, 1915) was used in the sugar broth tubes as colour indicator and proved most satisfactory. This medium, in the absence of acid, is straw coloured. As soon as acid is formed the colour changes to varying shades of red, from pale rose pink to bright carmine, according to the degree of acidity. A notable advantage of this method is that the results are easily legible by artificial light. The gelatin cultures were grown first at 22° C., then at room temperature, all the others at 37·5° C. In the case of the carbohydrate media the results obtained were noted every day for a week, then again at the end of a fortnight, after which they were discarded. The litmus milk tubes were also observed daily for the first week, but as a rule they

were retained for three or four weeks before being finally discarded. The gelatin stab cultures were kept in the 22° incubator for several weeks and the results noted from time to time. After that they were kept at room temperature for many months, never less than six except where complete liquefaction had occurred.

The Voges and Proskauer reaction was carried out in the usual way by adding an equal quantity of 2 % caustic soda to a three days glucose-peptone-water[1] culture of the microbe, and allowing the test tube to stand on the bench for two or three days. In positive cases a pale orange or pinkish colour with greenish fluorescence makes its appearance in a few hours, and is usually well marked by the following day. The appearance has been aptly likened to that of much diluted alcoholic eosin.

The functions of indol formation and of motility were investigated at some length and may be referred to in greater detail.

Indol Formation.

The indol forming powers of the bacteria were studied by the application of Ehrlich's rosindol reaction. After some preliminary experiments, the following procedure was adopted for routine examinations. The organism to be studied is subcultured from a recent agar slope to a tube containing about 5 c.c. of peptone water[2], and incubated for seven days at 37·5° C. A ring test (Tobey, 1908) is then carried out by floating 1 c.c. of Boehme's reagent[3] on top of the culture by means of a pipette. A positive result is indicated by the gradual development of a rose red or pink ring of varying intensity at the line of junction. Usually one or two minutes suffice for the full development of the reaction. Confirmation of the result is obtained by shaking up the contents of the test tube with 1 c.c. of amyl alcohol, as recommended by MacConkey (1909). In positive cases the alcohol extracts the pigment (rosindol), and forms a pinkish layer on the surface. The test tube (uncorked) is then allowed to stand on the laboratory bench for some days, when the

[1] Peptone (Witte) 10 grm.
Glucose ... ... 20 grm.
Sodium chloride... 5 grm.
Water ... ... to 1000 c.c.

[2] Peptone (Witte) 10 grm.
Sodium chloride 5 grm.
Water ... ... to 1000 c.c.

[3] Paradimethylamidobenzaldehyde 4 grm.
Absolute alcohol (96 %) ... ... 380 c.c.
Concentrated hydrochloric acid ... 80 c.c.

colour of the amyl alcohol slowly deepens to a bright cherry red. In negative cases the colour of the alcohol ranges from grey to varying shades of green and yellow. I have carried out this test in the case of several hundred coliform organisms, and in every instance a definitely positive or definitely negative result has been obtained; there have been no indeterminate results. It has also been shown in the cases examined that the ring test by itself yields thoroughly reliable results; in every case the extraction by amyl alcohol has confirmed the original finding. The addition of an oxidising agent, for example, potassium persulphate, has been found to be unnecessary either for the ring test or for the subsequent extraction. Some interesting observations have been made as a result of keeping the test tubes for some time after amyl alcohol extraction. At first, after the culture plus the benzaldehyde has been shaken up with the alcohol, the latter in positive cases shows a pale pink tint on rising to the surface. This coloration, however, disappears in the course of an hour or two, but after one or two days it returns and gradually increases in intensity. After standing for a week or ten days on the laboratory bench, all the positive cultures show a bright cherry red to deep carmine coloration of the alcohol, which slowly deepens. At the end of a month or six weeks, and still more at the end of two months, the subjacent watery medium assumes, in positive cases, a fairly intense pinkish-violet tint, whereas in negative cases it gradually passes from a greyish or yellowish colour to a pronounced slate blue. Similar phenomena have been observed by MacConkey (1909) and by Seidelin and Lewis (1911). The presence of the blue coloration has been shown by Kligler (1914) to be due to some substance in the peptone and to be entirely independent of the indol test.

MOTILITY.

A large number of experiments were carried out to determine, if possible, the best conditions for the development of motility. The media used were agar (slope, and water of condensation), gelatin, nutrient broth and peptone water, the gelatin being incubated at 22° C., the others at 37·5° C. It was found that on the whole motility could be best demonstrated in an 18 to 24 hours gelatin slope culture, but that a six hours nutrient broth culture was almost as good. Peptone water and the water of condensation in an agar slope were less reliable, the surface of an agar slope notoriously so. The most important point determined was that no one method was infallible. In some cases motility was demonstrated in gelatin cultures when broth cultures failed

to show it, and vice versa. It may therefore be stated that while motility once demonstrated is conclusive, its apparent absence cannot be accepted as final.

Classification.

In deciding which organisms should come within the purview of this investigation the term "coliform" has been interpreted in a very broad and inclusive sense. It has been taken to include all the small, gram-negative, non-sporing bacilli which grow readily on bile salt media and which give a more or less abundant growth on agar. This arbitrary classification has been found to work well in practice, and it is usually quite obvious from the characters of the colony on bile salt agar whether a given organism should be included or not.

Altogether 148 organisms from 70 wounds have been studied, all duplicates from a single wound being excluded. 24 of these are the *B. pyocyaneus*. Of the remaining 124, 86 fall into four well recognised groups, 34 into two atypical but fairly well defined groups, while four only are unclassified. The total number of varieties is 53 (see Table XIII, p. 314). It will be well to give, in the first place, the salient features which have determined the place of each organism in the classification scheme adopted.

(1) *B. coli group.*

Fermentation of glucose and lactose with or without the formation of gas.

(2) *B. proteus group.*

(*a*) Fermentation of glucose and saccharose with formation of acid and gas.

(*b*) Non-fermentation of lactose.

(*c*) Rapid liquefaction of gelatin.

(*d*) Clotting and bleaching of litmus milk and finally more or less digestion of the clot.

(3) *B. Morgan No.* 1 *group.*

(*a*) Fermentation of glucose, laevulose and galactose only with formation of acid and gas.

(*b*) Formation of indol.

(4) *B. faecalis alkaligenes group* (one strain only obtained).

(*a*) Fermentation of none of the carbohydrates tested.

(*b*) Motility present.

(*c*) Litmus milk rendered strongly alkaline.

(*d*) Gelatin not liquefied.

(5) *Group X.*

(*a*) Fermentation of glucose, laevulose, galactose and inosite, with formation of acid but no gas.
(*b*) Non-fermentation of lactose.
(*c*) Litmus milk rendered acid and then strongly alkaline.
(*d*) Motility present.
(*e*) Formation of indol.

(6) *Group Y.*

(*a*) Fermentation of galactose without formation of gas.
(*b*) Non-fermentation of laevulose.
(*c*) Motility absent.

(7) *B. pyocyaneus.*

(8) *Unclassified.*

Table III is a list of the positive cases, showing (1) the situation of the wound, (2) its duration at the time of examination, and (3) the coliform bacilli present arranged in the above-mentioned groups. Compound fractures when present are indicated in column 2. It will be observed that different cases varied greatly as to the number of varieties which they harboured. Thus from 24 cases one variety of coliform only was obtained, from 26 cases two varieties, from 15 three, from 2 four, from 1 five and from 2 seven. The 46 cases from which two or more varieties were isolated include 11 in which a second examination of the wound was made, usually several weeks after the first. As a rule the wounds which contained a number of varieties were highly septic and often stinking, but not necessarily of long standing. It was not found that any particular variety preponderated in the early stages. Thus from a wound five days old an organism belonging to Group Y was isolated, from another five day wound two varieties of *B. coli*, from a six day wound a coli, a proteus, and a member of Group Y, from a seven day wound a member of Group X, etc., etc. The only organism exhibiting a marked preference for any particular stage was *B. pyocyaneus*, which, as already indicated, tended to occur late. The earliest dates at which it was found were 14 and 17 days. (The distribution of the various bacterial groups is further referred to under "General Remarks.")

TABLE III.

List of positive cases with coliform bacilli arranged in groups.

No.	Site of wound, etc.	Duration of wounds (days)	*B. coli* group I	II	III	IV	*B. proteus*	*B. Morgan* No. 1	*B. faecalis alk.*	Group X	Group Y	*B. pyocyaneus*	Unclassified
1	Thigh	83	.	.	2	.	1	.	.	.	.	1	.
		208	.	.	.	.	.	.	.	1	.	.	.
2	Leg, compound fracture of tibia ...	59	.	.	.	.	.	.	.	.	1	1	.
3	Leg, compound fracture of tibia ...	7	.	.	2	1	.	1	.	.	.	.	.
		54	.	1	.	1	.	1	.	.	.	1	.
4	Pelvis, compound fracture	8	.	.	.	.	.	.	1	2	.	.	.
		78	.	.	.	.	.	.	.	1	.	.	.
5	Calf	9	.	.	1	.	.	1	.	.	.	.	.
6	Thigh, compound fracture of femur	13	.	.	.	.	1	.	.	.	1	.	.
		54	.	.	.	.	.	.	.	.	1	.	.
7	Forearm, fracture of ulna	6	1	.	.	.	1	.	.	.	1	.	.
8	Loin	21	1	.	1	.	.	.	.	.	.	.	.
9	Recurrent sepsis of wrist	162	.	.	.	.	1	.	.	.	.	.	.
10	Forearm	35	.	.	.	.	.	1	.	.	1	1	.
11	Chest, compound fracture of rib ...	40	.	.	.	.	1	.	.	.	1	.	.
12	Back	32	.	1	1	.	.	.	.	.	.	.	.
13	Shoulder	40	.	1	.	.	.	1	.	.	.	1	.
14	Ankle, sinus	48	.	.	.	.	.	.	.	.	1	1	.
15	Lumbar region, sinus	43	.	.	.	.	1	1	.	.	.	1	.
16	Buttock	47	1	.	.	.	.	1	.	.	.	.	.
17	Leg	48	.	.	.	.	1	.	.	.	.	1	.
18	Leg	52	.	.	.	1	.	.	.	.	1	1	.
19	Thigh, compound fracture of femur	40	.	.	.	.	.	.	.	.	1	.	.
		50	.	.	.	1	.	.	.	.	1	.	.
20	Elbow, fracture of olecranon ...	15	.	.	.	.	1	.	.	.	.	.	.
21	Arm	38	.	.	.	.	.	.	.	.	1	.	.
		82	.	.	.	.	.	.	.	1	1	1	.
22	Leg	17	.	.	.	.	.	.	.	.	1	1	.
23	Pelvis, compound fracture	67	1	.	1	.	.	.	.	.	.	.	.
24	Thigh, compound fracture of femur	66	.	.	1	.	.	.	.	1	.	1	.
25	Amputation stump of leg	42	.	.	.	.	1	.	.	1	.	.	1
26	Shoulder	70	.	.	.	.	1	.	.	.	.	.	.
		140	.	.	.	.	.	.	.	.	.	1	.
27	Leg	14	.	.	.	.	1	.	.	.	.	1	.
28	Shoulder	7	.	.	.	.	1	.	.	1	.	.	.
29	Toe, amputated	7	1	.	.	.	1	.	.	.	1	.	.
30	Forearm, compound fracture of radius	19	.	.	.	.	1	.	.	.	.	.	.
31	Knee	46	.	1	.	.	.	.	.	.	.	1	.
		66	.	.	.	.	.	.	.	.	.	1	1
32	Buttock	16	.	.	.	.	.	.	.	.	1	.	.
33	Thigh	17	1	.	.	.	.	.	.	.	.	.	.

https://doi.org/10.1017/S0022172400006665 Published online by Cambridge University Press

TABLE III (*cont.*)

No.	Site of wound, etc.	Duration of wounds (days)	*B. coli* group I	II	III	IV	*B. proteus*	*B. Morgan* No. 1	*B. faecalis alk.*	Group X	Group Y	*B. pyocyaneus*	Unclassified
34	Leg	38	.	.	.	.	.	.	.	.	1	1	.
35	Hand	37	.	.	.	.	.	.	.	.	1	1	.
36	Leg, compound fracture of tibia and fibula	108	.	.	.	.	1	.	.	.	.	.	.
37	Leg, compound fracture of tibia and fibula	160	.	1	.	2	.	1	.	.	.	1	1
		181	.	.	.	.	.	.	.	.	1	1	.
38	Arm	8	.	.	1	.	.	.	.	.	.	.	.
39	Forearm	5	.	.	.	2	.	.	.	.	.	.	.
40	Leg	12	.	.	1	.	.	.	.	.	.	.	.
		41	.	.	.	.	.	.	.	.	1	.	.
41	Buttock	7	.	.	.	1	.	.	.	.	.	.	.
42	Elbow, compound fracture-dislocation	5	.	.	.	.	.	.	.	.	1	.	.
		34	.	.	.	1	1	.	.	.	.	.	.
43	Buttock	11	.	.	1	.	1	.	.	.	1	.	.
44	Forearm	9	.	.	.	2	.	.	.	.	.	.	.
45	Wrist	8	.	.	1	1	.	.	.	.	.	.	.
46	Leg	24	.	.	.	.	.	.	.	.	1	.	.
47	Face	70	.	1	.	.	1	.	.	.	1	.	.
48	Shoulder	10	.	.	.	.	.	.	.	.	1	.	.
49	Chest	172	.	.	.	.	1	.	.	.	.	.	.
50	Buttock, sinus	350	.	.	2	1	.	.	.	.	.	.	.
51	Thigh	9	.	.	.	.	.	.	.	.	.	.	1
52	Knee	28	.	.	.	.	1	.	.	.	1	.	.
53	Foot	26	.	.	.	1	1	.	.	.	.	.	.
54	Knee	10	.	.	1	1	.	.	.	.	.	.	.
55	Calf, compound fracture of tibia and fibula	34	.	.	.	.	1	.	.	.	.	1	.
56	Calf, compound fracture of tibia and fibula	80	.	.	.	.	1	.	.	.	.	.	.
57	Hand	23	.	.	.	.	1	.	.	.	.	1	.
58	Leg	18	1	.	.	.	.	.	.	.	.	.	.
59	Shoulder	15	.	.	.	1	.	.	.	.	.	.	.
60	Knee, joint involved	37	.	.	.	.	1	.	.	.	.	.	.
61	Knee	17	.	.	.	1	.	.	.	.	.	.	.
62	Thigh, knee joint involved	25	.	.	.	.	1	.	.	.	.	.	.
63	Leg	32	.	.	.	.	1	.	.	.	.	.	.
64	Buttock	33	.	.	2	.	1	.	.	.	.	.	.
65	Buttock	33	.	.	.	.	1	.	.	.	1	.	.
66	Heel	38	.	.	.	.	.	.	.	.	1	.	.
67	Leg	22	.	.	.	.	.	.	.	.	.	1	.
68	Leg	60	.	.	.	.	.	.	.	.	.	1	.
69	Forearm	48	.	.	.	.	.	.	.	.	.	1	.
70	Knee	84	.	.	.	.	.	.	.	.	.	1	.

https://doi.org/10.1017/S0022172400006665 Published online by Cambridge University Press

B. coli Group.

This group includes all the organisms which ferment glucose and lactose whether they form gas on these media or not, and irrespective of their action on gelatin. It will be shown later that the organisms in this series which ferment lactose and at the same time liquefy gelatin, are more closely related in their other biological reactions to *B. coli* than to *B. proteus*, and they have therefore been included in the *B. coli* group. Altogether 49 lactose fermenters have been isolated from 32 cases, giving a case incidence of *B. coli* infection of 26 %. By utilising the large series of biological reactions already referred to, it is found that this group of 49 organisms can be differentiated into no fewer than 34 distinct varieties (see Table XIII). The group has been divided into four subgroups, as suggested by MacConkey (1905), according to the fermentative reactions with saccharose and dulcite, and the number of strains and number of varieties met with in each are shown hereunder (Table IV).

Table IV.

Subgroup distribution of members of the B. coli *group obtained from wounds.*

	No. of strains	No. of varieties
Subgroup 1 (saccharose – dulcite –)	7	5
„ 2 „ – „ +	6	4
„ 3 „ + „ +	18	12
„ 4 „ + „ –	18	13
Totals	49	34

The common characters of the whole group are these:

(1) Fermentation of glucose, lactose, mannite, laevulose, galactose, maltose and arabinose[1].

(2) Non-fermentation of erythrite[2].

(3) Formation of acid and clot on litmus milk.

All other reactions are variable, as shown in the following table (Table V).

A study of this table brings out a number of interesting points. The large number of common characters possessed by the various members of each of the two first subgroups is striking, as is also the general resemblance, in this respect, between the two. The members of subgroups 3 and 4 show much greater variability amongst themselves, while

[1] Only 20 varieties out of 34 were tested.

[2] Only 22 varieties out of 34 were tested.

TABLE V.

Variable Reactions of the B. coli *group.*

	Saccharose		Dulcite		Adonite		Inulin		Salicin		Sorbite		Raffinose		Dextrin	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Sub-group 1. (5 varieties)	0	5	0	5	0	5	0	5	3	2	5	0	0	2	4	1
Sub-group 2. (4 varieties)	0	4	4	0	0	4	0	4	2	2	4	0	0	1	3	1
Sub-group 3. (12 varieties)	12	0	12	0	2	10	1	11	7	5	9	3	1	0	10	2
Sub-group 4. (13 varieties)	13	0	0	13	5	8	0	13	13	0	11	2	5	2	13	0
Totals	25	9	16	18	7	27	1	33	25	9	29	5	6	5	30	4

	Isodulcite		Inosite		Glycerin		Amygdalin		Gelatin		Motility		Indol		Voges and Proskauer	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Sub-group 1. (5 varieties)	2	0	0	3	5	0	0	2	0	5	2	3	4	1	0	4
Sub-group 2. (4 varieties)	3	0	0	3	4	0	0	1	0	4	2	2	3	1	0	3
Sub-group 3. (12 varieties)	6	0	0	4	11	1	1	0	2	10	7	5	9	3	3	7
Sub-group 4. (13 varieties)	8	3	2	5	8	5	3	3	8	5	8	5	3	10	10	3
Totals	19	3	2	15	28	6	4	6	10	24	19	15	19	15	13	17

the only character common to both subgroups is saccharose fermentation. Subgroups 2 and 3 present certain resemblances, but subgroups 2 and 4 have nothing in common save the group characteristics. Subgroup 3 seems to occupy an intermediate position to 2 and 4, particularly with regard to gelatin liquefaction and the Voges and Proskauer reaction.

The question of the inclusion of gelatin liquefiers in the *B. coli* group is debateable. Perhaps it would be an advantage to recognise a group (lactose +, gelatin +) intermediate between the *B. coli* group (lactose +, gelatin −) on the one hand and the *B. proteus* group (lactose −, gelatin +) on the other. Short of this I am inclined (at least so far as the present series is concerned) rather to include them in the *B. coli* group, with which, in addition to lactose fermentation, they have in common the power of fermenting mannite and arabinose. It will be observed that all the gelatin liquefying lactose fermenters fall into MacConkey's subgroups 3 and 4, i.e. they are all saccharose fermenters, and in this respect they resemble also *B. proteus*. In order therefore to make further comparison of the lactose + gelatin + organisms with the non-liquefying members of the *coli* group it seems reasonable to limit the

survey to subgroups 3 and 4. It is then found that fermentation of salicin and the Voges and Proskauer reaction afford further evidence of the closer affinity of the gelatin liquefying lactose fermenters to the *B. coli* than to the *B. proteus* group (see Table VI). With regard to the action on litmus milk, also, these organisms behave as *B. coli* and not as *B. proteus* (Table XIII).

TABLE VI.

Showing the affinities of the Gelatin + Lactose + coliforms.

	Salicin		Voges and Proskauer	
	+	−	+	−
Gelatin − members of subgroups 3 and 4 of *coli* group	10	5	5	8
Gelatin + „ „ „	10	−	8	2
B. proteus group	−	4	−	4

Indol formation by the B. coli *group.* Of the 34 varieties studied 19 formed indol on peptone water, 15 did not. Indol formers markedly predominate in subgroups 1, 2 and 3; the reverse is the case in subgroup 4 (Table VII).

TABLE VII.

Formation of Indol by the B. coli *group.*

	Total No. of cases	No. of cases indol +	Percentage of indol + cases
Subgroup 1	5	4	80
„ 2	4	3	75
„ 3	12	9	75
„ 4	13	3	23

Voges and Proskauer's reaction. In the present series of coliform organisms a positive Voges and Proskauer's reaction was given only by certain members of subgroups 3 and 4 of the *B. coli* group. All the other bacilli tested were negative (Table VIII).

TABLE VIII.

The Voges and Proskauer reaction in the B. coli *group.*

	No. of cases investigated	No. of cases V. and P. +	Percentage of cases V. and P. +
Subgroup 1	4	0	0
„ 2	3	0	0
„ 3	10	3	30
„ 4	13	10	77

In this series, therefore, it is found that all varieties which give the Voges and Proskauer reaction are saccharose fermenters, while of the 17 varieties which are V. and P. − 7 do not attack this sugar. A somewhat similar relationship exists between the Voges and Proskauer reaction and salicin fermentation. All V. and P. + organisms ferment salicin, but of the 17 V. and P. − varieties 11 only are salicin fermenters. No such correlation exists between the Voges and Proskauer reaction and dulcite fermentation. Of the 13 V. and P. + varieties, 3 or 23 % are dulcite +. These observations are in accordance with the findings of Levine (1916) and of Kligler (1914), both of whom found that salicin fermentation is more closely correlated to the Voges and Proskauer reaction than is dulcite fermentation. A much more interesting observation from the point of view of wound infection is made by Levine (1916). In studying 157 strains of *B. coli* (39 from sewage and 117 from faeces), he found that all those which gave the Voges and Proskauer reaction, nine in number, were derived from sewage, while none of the 117 organisms of faecal origin was positive to the Voges and Proskauer test. From this he concludes that the Voges reaction is characteristic of non-faecal strains of *B. coli*. A scrutiny of MacConkey's tables (1909) reveals figures confirming this view. Out of 497 lactose fermenting bacilli investigated, 91 were V. and P. +, and of these only 19, or 21 %, were of faecal origin. Of the 406 V. and P. − strains 332, or 81 %, were obtained from faeces. The chief sources from which the V. and P. + strains were obtained were soil, roof washings, pond water, rain water, grains, malt and cheese. The large number of V. and P. + organisms in the present series would therefore point to a non-faecal source for at least a considerable proportion of the coliform bacilli infecting these wounds, and the probable nature of such previous habitat is indicated by the authors quoted. A similar conclusion is arrived at by a study of the subgroup distribution of the members of the *B. coli* group. In Table IX the proportion of strains (not varieties) of lactose fermenters from wounds falling into each of MacConkey's subgroups is shown, and a comparison instituted with the organisms isolated by MacConkey from, on the one hand, human, cow and horse faeces, and on the other cesspool sewage, soil, pond water, rain water, and roof washings. From this it would appear that, even as regards lactose fermenters alone, wound coliforms occupy an intermediate position to the other two, but have on the whole a closer resemblance to the non-faecal than to the faecal series.

TABLE IX.

Subgroup distribution of strains of B. coli *from* (1) *wounds*, (2) *faeces*, (3) *non-faecal sources* (*per cent.*).

	Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4
1. Lactose fermenters from wounds (M. J. S.)	14·6	12·2	36·6	36·6
2. Lactose fermenters from human, cow and horse faeces (MacConkey)	17·8	31·9	39·6	10·7
3. Lactose fermenters from sewage, soil and surface waters, etc. (MacConkey)	8·4	4·2	49·3	38·1

Notes on individual members of the B. coli *group* (Table XIII). (The numbers refer to the varieties described in Table XIII.)

2 and 4 (4 strains) give the reactions of *B. vesiculosus*. They differ only in that 2 ferments salicin while 4 does not.

3 (1 strain) gives the reactions of *B. grünthal* except that it is dextrin −.

5 (1 strain) resembles *B. vesiculosus* but does not form indol.

6 (2 strains) gives the reactions of *B. coli communis*.

7 (2 strains) is similar but non-motile (*B. coli immobilis*).

10 (2 strains) resembles *B. pneumoniae* (Friedländer) in every respect except that it gives the Voges and Proskauer reaction.

12 (1 strain) is interesting as being the only inulin fermenter in the whole series.

14 (4 strains) gives the reactions of *B. coli communior* (*B. pseudocoli* Castellani, 1912). One organism was tested on and found to ferment amygdalin, with formation of acid only.

15 (2 strains) = *B. neapolitanus*.

16 (1 strain) differs from *B. coli communior* only in that it does not ferment sorbite.

17 (1 strain) is the same as *B. MacConkey* No. 74, which was originally isolated from human faeces.

19 (2 strains) differs from *B. neapolitanus* only in that it does not ferment salicin.

22 (2 strains) = *B. MacConkey* No. 102. This organism was twice isolated by MacConkey from pond water.

23 (1 strain) = *B. lactis aerogenes* (Lewis, 1916). Indol is formed.

24 (3 strains) = *B. lactis aerogenes* (Castellani, MacConkey, etc.). Indol is not formed. One strain only was tested on amydalin and was found to form acid but not gas.

25 (2 strains) is the same as No. 22 (*B. MacConkey* 102) except that it does not ferment glycerin.

26 (1 strain) is the same as 25 except that it is non-motile.

27 and 29 (2 strains) give the same reactions as *B. cloacae*, but one ferments glycerin the other not. They are salicin +, but Castellani gives *B. cloacae* as salicin −.

31 (2 strains) = *B. coscoroba*, but is salicin +.

https://doi.org/10.1017/S0022172400006665 Published online by Cambridge University Press

B. PROTEUS GROUP.

Under this head 29 organisms, or 19·6 % of the total, are grouped, of which no fewer than 25 strains give identical reactions (No. 37 in Table XIII). These, which may be taken as the reactions of *B. proteus vulgaris*, are as follows: (1) fermentation (with production of gas in some, or more usually in all, of the tubes) of glucose, saccharose, laevulose, galactose and glycerin, (2) non-fermentation of all other substances tested, (3) rapid liquefaction of gelatin, (4) motility, usually very active, (5) non-production of indol, (6) absence of the Voges and Proskauer reaction, (7) on litmus milk variable reactions leading to clotting, bleaching, and finally digestion of the clot. The variations from this typical formula are very slight. Two strains are apparently non-motile (No. 38), and two ferment maltose (Nos. 35 and 36). No. 35 in addition forms indol, the only one out of 29 found to possess this property.

Members of the group were isolated from 29 cases, giving a case incidence of 24 %, almost equal to that of the *B. coli* group itself. As to the significance of these organisms in wounds, it may be asserted confidently that they are evidence of non-faecal contamination. The *Bacillus proteus* is commonly found in connection with putrefactive processes generally, and apart from this is apparently widely distributed in nature. It is, in my experience, distinctly uncommon as an inhabitant of the colon in man. In the course of a recent investigation of several thousand samples of faeces from dysentery convalescents I have isolated a *proteus* bacillus less than a dozen times. As a pathogenic agent this organism is now well recognised.

B. MORGAN No. 1.

Seven organisms give the fermentation reactions of *B. Morgan* No. 1 (Morgan, 1906), and of these four are classical in every respect. These organisms ferment glucose, laevulose and galactose only, with formation of acid and gas, and they form abundant indol on peptone water. Gelatin is not liquefied, and they do not give the Voges and Proskauer reaction. The four classical examples in this series are motile, and render litmus milk strongly alkaline after a variable number of days. The remaining three are apparently non-motile, and they produce no change in litmus milk, even after three weeks. An apparently identical non-motile organism has been isolated by MacConkey (MacConkey, 1909) from horse faeces. *B. Morgan* No. 1 is of course a fairly common faecal organism in man, apart altogether from the diarrhoeal conditions in

which its occurrence was first observed by Morgan, and its presence in these wounds is presumably to be taken as evidence of excretal contamination.

B. faecalis alkaligenes.

This organism was isolated on one occasion only. It is motile, does not ferment any of the substances tested, and neither forms indol nor liquefies gelatin. The Voges and Proskauer reaction is not given. It produces intense late alkalinity on litmus milk. It also is presumably derived from a faecal source.

Group X.

This is a fairly compact group of eight organisms, separable into three varieties, giving rather peculiar reactions. Five strains are apparently identical (No. 43 in Table XIII), and may be described first. Glucose, saccharose, laevulose, galactose and inosite are fermented, with formation of acid only. None of the other 15 substances tested are attacked. Gelatin liquefaction is slowly produced, only commencing in from two to six weeks after inoculation. By the end of two months from $\frac{1}{2}$ in. to 1 in. of a gelatin stab is liquefied, the whole tube only in from three to four months. The organism is actively motile and it forms abundant indol in peptone water. The Voges and Proskauer reaction is not obtained. On litmus milk the medium is first rendered slightly acid, and this lasts for several days. The reaction then slowly changes to alkaline, and this may be accompanied by a certain amount of bleaching. Alkalinity is usually well marked in from seven to fourteen days, but in one case it only developed during the third week, the medium having first been completely decolorised. Clotting does not occur at any stage.

It will be noticed that, while in its fermentation reactions and certain other features this organism closely resembles *B. proteus vulgaris*, it differs from it in certain important characteristics, viz.: (1) in the absence of gas production in its fermentation of the sugars, (2) in the fermentation of inosite, (3) in the absence of glycerin fermentation, (4) in the formation of abundant indol, (5) in its slow liquefaction of gelatin, and (6) in its alkaline reaction in litmus milk, with absence of clotting and digestion of the clot.

The three remaining strains in this group differ from those already described on one or two points only. The chief distinction is that gelatin is not liquefied. Two strains (No. 43) ferment glycerin, and one (No. 44) fails to ferment saccharose.

I have failed to find any reference to organisms giving these reactions, but several of Castellani's (Castellani, 1912) Ceylon strains (of faecal origin) differ on a few points only from the non-liquefying members of this group (*B. negombensis* and *B. talavensis*). The whole group, and especially the non-liquefying members, bear a certain resemblance to *B. Morgan* No. 1. The chief points of distinction are (1) the absence of gas in the fermented sugars, (2) the fermentation of inosite, (3) the liquefaction of gelatin by No. 42, (4) the fermentation of glycerin by No. 43, and (5) the fermentation of saccharose by both 42 and 43. In the case of No. 44, therefore, which is saccharose −, glycerin −, and gelatin −, the resemblance is a fairly close one.

It is an interesting fact that all three varieties were, in one instance (case 4), isolated from a single wound, Nos. 42 and 44 at the first examination, No. 43 at a second examination 70 days later. Probably this is an example of bacterial mutation (*vide infra*).

It may be remarked here that in the present series of organisms fermentation of inosite occurred in 6 out of 31 varieties tested, viz. the three varieties of Group X just described, two varieties of *B. lactis aerogenes*, and the very atypical organism No. 49. Formation of gas occurred only in the case of *B. lactis aerogenes*.

GROUP Y.

26 organisms, or 17·6 % of all those studied, were found to belong to a very well defined and compact group, the essential common characters of which are these: galactose A, laevulose −, motility −, indol −, and Voges and Proskauer −. The only variable characters are the action on gelatin and on glucose. None of the other carbohydrates, etc., tested are fermented. These organisms grow readily on bile salt, neutral red, crystal violet agar, forming in 24 hours medium sized colonies of a light pink colour with greenish fluorescence. On agar plates the individual colonies are also fluorescent, while stroke subcultures on agar slopes give rise to a thick white growth within 24 hours. All the members of the group show, when subcultured on the carbohydrate broths, a striking bluish or greenish iridescent ring on the side of the test tube, immediately above the upper level of the meniscus. According to their action on glucose and on gelatin the group has been divided into four varieties, thus:

							No. of strains
I.	Glucose	A	Gelatin	+	...	...	12
II.	„	A	„	−	...	...	3
III.	„	−	„	+	...	...	10
IV.	„	−	„	−	...	...	2

Growth on gelatin.

A. Non-liquefiers (varieties II and IV).

Gelatin stab cultures grown at 22° C. show, after a day or two, a filiform growth along the needle track. In the course of three or four weeks the stab may become slightly beaded, or villous outgrowths may take place from the upper part, but growth is never very prolific. On the surface a much more abundant growth takes place, forming a thick, greyish white layer.

B. Liquefiers (varieties I and III).

For the first two or three weeks the gelatin stab culture develops in a similar fashion to that described above, being either filiform or very finely beaded, with a thick growth on the surface. Liquefaction usually starts in the third or fourth week, occasionally slighter later, and forms at first a tiny cup beneath the central part of the thick surface layer. Liquefaction progresses very slowly downwards, but reaches the side of the tube in the course of a few days. In two months' time about $\frac{1}{4}$ in. is usually liquefied, and in four months about $\frac{1}{2}$ in. When liquefaction occurs, the thick surface layer of growth remains *in situ* as a pellicle, while an abundant dense deposit falls to the bottom. The liquefied portion of gelatin is usually clear, but may contain flocculi. In a few cases liquefaction commences only after about six weeks, and progresses even more slowly than above described.

Growth on litmus milk.

All the members of the group gave closely similar reactions with litmus milk. The differences between individual strains were slight and unimportant, and referred chiefly to the rate of production of the various changes. The first noticeable change was either slight bleaching or slight acidification, or a combination of the two, and took place as a rule on the third or fourth day. In the case of one organism the milk became slightly bleached within the first 24 hours, in seven strains the first changes were noticed within 48 hours, while in three no change was observable until the fifth day. In 24 out of the 27 strains clotting occurred in from three to ten days. The three exceptions to this showed only complete bleaching at the end of seven days, but all clotted during the course of the second week. In some cases clotting was preceded or accompanied by slight reddening of the litmus, and usually by more or less bleaching as well. In others clotting was preceded or accompanied by bleaching only, usually complete. At the end of a fortnight all milks

were firmly clotted and litmus completely bleached, except that in the great majority the top $\frac{1}{8}$ in. to $\frac{1}{4}$ in. of clot had become strongly acid.

I have never come across a faecal coliform giving these reactions, and it seems probable that these organisms are derived from soil and water. In some respects they resemble Jordan's (1903) "*Fluorescens*" group, but they are quite non-motile and the gelatin liquefiers act only very slowly. Castellani (1912) has described an organism (*B. gintotensis*) having characters identical with variety II in the above table, except that it produces acid with arabinose in addition to its action on glucose and galactose. It was obtained from the faeces of a native of Ceylon.

UNCLASSIFIED COLIFORMS.

No. 49 (Table XIII). This very extraordinary organism ferments glucose, saccharose, mannite, adonite, laevulose, galactose, isodulcite, inosite, and erythrite, with formation of acid but no gas. None of the other substances is attacked. It is the only organism in the whole of this series which ferments erythrite. Litmus milk is rendered alkaline after four or five days, and then clotted and bleached, with finally solution of the clot. It does not liquefy gelatin nor form indol: it is non-motile and the Voges and Proskauer reaction is not given. An organism having somewhat similar characters was obtained by MacConkey from a specimen of human sputum. It did not ferment saccharose, however, and it formed gas on adonite. It may be noted that organism No. 49 formed acid on saccharose only after 14 days. Another closely similar organism (*B. kandiensis*) was obtained by Castellani from the faeces of a healthy native of Ceylon. It differs from No. 49 only in being motile and in fermenting glycerin.

No. 50. This organism closely resembles the third variety of Group Y (No. 47), from which it differs only in that it is actively motile and is a rapid liquefier of gelatin.

Nos. 51 *and* 52. Like *B. faecalis alkaligenes*, these fail to ferment any of the sugars, but both liquefy gelatin, and while No. 51 is motile No. 52 is not. On litmus milk No. 51 produces early clotting and bleaching, No. 52 the same result after a preliminary stage of alkalinity. In other respects they are the same as *B. faecalis alkaligenes*. No. 51 corresponds to the *B. fluorescens liquifaciens* of Jordan (1903), and an apparently identical organism was isolated by MacConkey from faeces and tap water, and by Wilson (1908) from the urine.

GENERAL OBSERVATIONS.

I. *Group distribution.*

Table X shows the group distribution of the 148 strains of coliform bacilli studied, the number of varieties occurring in each group, and the case incidence in the 122 wounds examined.

TABLE X (showing group distribution).

Group	No. of strains	No. of varieties	Case incidence
1. *B. coli*	49	34	26 %
2. *B. proteus*	29	4	24
3. *B. Morgan* No. 1	7	2	5·7
4. *B. faecalis alkaligenes* ...	1	1	·8
5. Group X	8	3	5
6. Group Y	26	4	20
7. *B. pyocyaneus* ...	24	1	20
8. Unclassified	4	4	3

These figures may be compared with the results obtained by Dudgeon, Gardner and Bawtree (1913) in the investigation of 100 wounds.

True "Colon" bacillus	11 %
"Coliform" bacilli ...	15 %
True *B. proteus* ...	5 %
Atypical *proteus* ...	· 2 %
B. pyocyaneus ...	4 %

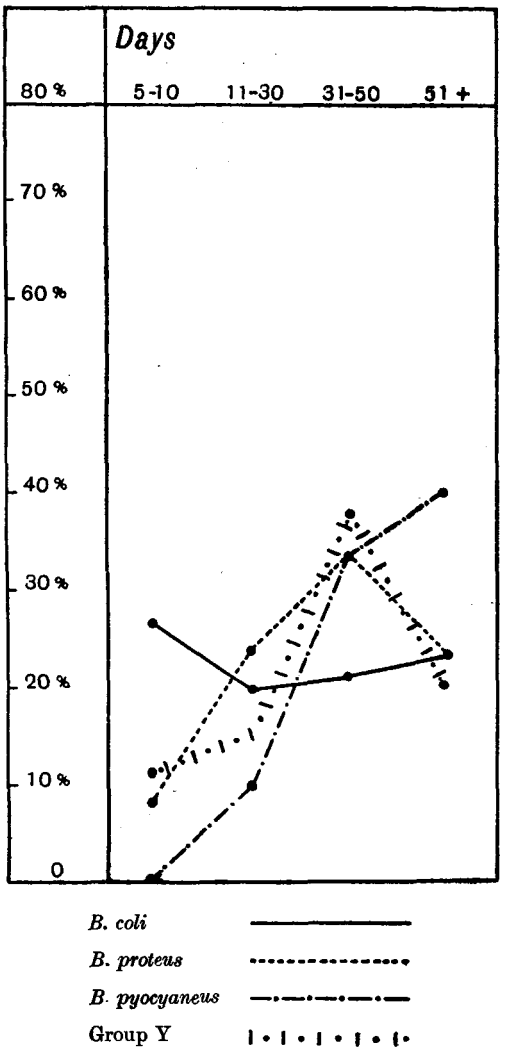
Goadby (1916), in the examination of 200 wounds, found *B. coli* present in 40 % and *B. proteus* in 47 % of cases, while *B. pyocyaneus* "was isolated on many occasions."

II. *Group distribution in relation to the age of the wound.*

It is of interest to observe the frequency of occurrence of these various bacterial groups in wounds of different ages, and for this purpose the wounds have been classified as follows: (A) 5 to 10 days old, (B) 11 to 30 days old, (C) 31 to 50 days old, and (D) over 51 days old. The result is shown in the accompanying Chart and in Table XI.

Members of the *B. coli* group have thus been recovered from a considerable proportion of wounds (20 % to 27 %) at all age periods. The curves of the *proteus* and Y groups show a remarkable parallelism. Starting at a low level (8 % and 11 %) during the first age period, they rise steadily to 34 % and 38 % respectively in the third age period, falling again considerably in the fourth. The *pyocyaneus* curve differs from both the foregoing. This organism, which did not occur once during the first age period, rose steadily thereafter to a maximum of 40 % in the fourth age period. It would be interesting to know the

CHART. *Incidence of various coliform bacilli at different age periods.*



incidence of infection by these organisms during the first five days. Distaso (1916) has expressed the opinion that infection by the *B. coli* group is probably constant in the earliest stages of wounds but has not been described, as "the soldiers come for observation too late and the phase is one which passes quickly." The author recognises a second phase in which "the *B. coliformis* (i.e. the *B. coli* group only) disappears,

https://doi.org/10.1017/S0022172400006665 Published online by Cambridge University Press

TABLE XI.

Incidence of various coliform bacilli in wounds of different ages.

Age period	No. of wounds examined*	Percentage infected with coliform bac.	Percentage infected with these groups: 1 *Coli*	2 *Proteus*	3 Y	4 *Pyocyaneus*
5 to 10 days	37	40	27	8	11	0
11 to 30 days	39	48	20	23	15	10
31 to 50 days	29	83	21	34	38	34
Over 51 days	30	77	23	23	20	40

* In this table, 13 wounds which were examined on a second occasion after a considerable interval are each entered as two.

and the field is occupied by the anaerobic microbes of putrefaction," and a third phase in which cocci and the *B. subtilis* group only are present. He also expresses the opinion that the coliform organisms described in wounds in the late phase are either *B. proteus* or *B. pyocyaneus*. The observations recorded in the present communication show that some of these statements are only partially true. It is found, for example, that members of the *B. coli* group are present in a considerable proportion of wounds at all stages, but in the later phases *B. proteus* and *B. pyocyaneus* certainly occur with greater frequency. The continuous rise in the curve of *B. pyocyaneus* and the absence of this organism from wounds in the earliest stages, clearly indicate that this is of the nature of a "hospital infection."

Goadby (1916) has examined wounds from the earliest stages onwards, and his results as regards the *coli* and *proteus* groups do not by any means support the views advanced by Distaso (Table XII).

TABLE XII.

Incidence of coli *and* proteus *infection of wounds* (*Goadby*).

Age period	Total cases examined	Percentage infected: (1) *B. coli*	(2) *B. proteus*
1–8 days	64	39	50
5–10 days	57	33	42
11–30 days	40	47	47
Over 31 days	39	43	51

Contamination of wounds by fresh human faeces, on which Distaso lays great stress, is certainly indicated in a considerable number of cases by the presence of such definitely faecal organisms as *B. coli communis*, *B. neapolitanus*, *B. faecalis alkaligenes*, *B. Morgan* No. 1 and others. The great majority of the coliforms present, however, are probably derived from non-faecal sources, e.g. the *proteus* group, the Voges and Proskauer-positive members of the *coli* group, Group Y, and *B. pyocyaneus*.

It is worthy of note that no organisms giving the fermentation reactions of the Paratyphoid-Gaertner group have been obtained from any of the wounds.

III. *Observations bearing on the question of bacterial variation.*

In five cases out of 46 in which several varieties of coliform bacilli were isolated from one wound, it has happened that two of the strains present have differed from each other on one or two points only. In the light of recent work on bacterial variation (Twort, Penfold, etc.), it seems reasonable to suppose that we are here dealing with strains derived from a fairly recent common ancestor, and that variation has occurred by the acquisition of new characters or by the loss of old, or both. The instances referred to are these.

(1) In case 1 (Table III) two organisms of the *B. coli* group, subgroup 3 (Nos. 17 and 20 in Table XIII), were isolated. They differ from each other in respect of three characters only. No. 17 is salicin +, sorbite −, and indol −, while No. 20 is salicin −, sorbite +, and indol +.

(2) In case 3 two organisms of the *B. coli* group, subgroup 3 (Nos. 16 and 21 in Table XIII), were isolated, which vary only with regard to salicin fermentation, No. 16 being salicin +, No. 21 salicin −.

(3) In case 4 two organisms of Group X (Nos. 42 and 44) were isolated which differ on two points only. No. 42 forms acid on saccharose and liquefies gelatin, No. 44 does neither.

(4) In case 12 two closely similar organisms of the *B. coli* group (Nos. 6 and 14) were isolated. No. 6 is saccharose −, raffinose −, No. 14 saccharose +, raffinose +; otherwise they are identical.

(5) In case 64 two members of the *B. coli* group, subgroup 3 (Nos. 15 and 19), were isolated. They differ only in that No. 15 ferments salicin, while No. 19 does not.

In 11 cases from which coliform bacilli had already been recovered a second examination of the wound was made after an interval of some days or weeks, and in four of these the same organism was present on both occasions. In case 3 *B. Morgan* No. 1 (No. 39 in Table XIII) was thus obtained again after an interval of 47 days, in case 19 a member of Group Y (No. 47 in Table XIII) after 10 days, and in cases 31 and 37 *B. pyocyaneus* after intervals of 20 and 21 days. In two other instances (cases 6 and 21) a member of Group Y was isolated after intervals of 41 days and 44 days, the strains on each occasion differing only with regard to the fermentation of glucose. In case 4, as already mentioned, two closely similar organisms belonging to Group X (Nos. 42 and 44)

TABLE XIII.

The Biological Reactions of the Coliform Bacilli isolated from the Wounds of War.

Serial Number	Frequency of occurrence	Glucose	Lactose	Saccharose	Dulcite	Mannite	Adonite	Inulin	Salicin	Sorbite	Laevulose	Galactose	Raffinose	Maltose	Arabinose	Dextrin	Isodulcite	Inosite	Glycerin	Amygdalin	Erythrite	Litmus Milk Early		Litmus Milk Late	Gelatin	Motility	Indol	Voges and Proskauer's Reaction
3. COLI GROUP																												
Subgroup I (MacConkey)																												
1	1	+	+	−	−	+	−	−	+	+	+	+	.	+	.	+	.	.	+	.	.	A	.	AC	−	+	+	.
2 *B. vesiculosus*	3	+	+	−	−	+	−	−	+	+	+	+	−	+	+	+	+	−	+	−	−	AC	.	CB	−	−	+	−
3	1	+	+	−	−	+	−	−	+	+	+	+	.	+	+	−	.	−	+	.	.	AC	.	CB	−	+	+	−
4 *B. vesiculosus*	1	+	+	−	−	+	−	−	−	+	+	+	.	+	+	+	.	−	+	.	.	AC	.	ACB	−	−	+	−
5	1	+	+	−	−	+	−	−	−	+	+	+	−	+	+	+	+	.	+	−	−	AC	.	ACB	−	−	−	−
Subgroup II (MacConkey)																												
6 *B. coli communis*	2	+	+	−	+	+	−	−	+	+	+	+	−	+	+	+	+	−	+	−	−	AC	.	ACB	−	+	+	−
7 *B. coli immobilis*	2	+	+	−	+	+	−	−	+	+	+	+	.	.	+	+	+	−	+	.	−	AC	.	ACB	−	−	+	−
8	1	+	+	−	+	+	−	−	−	+	+	+	.	+	.	+	.	−	+	.	.	AC	.	ACB	−	+	−	.
9	1	+	+	−	+	+	−	−	−	+	+	+	.	+	+	−	+	.	+	.	−	AC	.	ACB	−	−	+	−
Subgroup III (MacConkey)																												
10	2	+	+	+	+	+	+	−	+	+	+	+	.	+	.	+	.	.	+	.	.	A	AC	CB	−	−	−	+
11	1	+	+	+	+	+	+	−	−	+	+	+	.	+	.	+	.	.	+	.	.	AC	AC	CB	−	−	±	.
12	1	+	+	+	+	+	−	+	+	+	+	+	.	+	.	+	.	.	−	.	.	A	AC	CB	+	+	−	+
13	1	+	+	+	+	+	−	−	+	+	+	+	.	+	.	+	+	.	+	.	−	CB	.	ACB	+	−	+	+
14 *B. coli communior*†	4	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	+	A*	−	AC	.	CB	−	+	+	−
15 *B. neapolitanus*	2	+	+	+	+	+	−	−	+	+	+	+	.	+	+	+	+	−	+	.	−	AC	.	ACB	−	−	+	−
16	1	+	+	+	+	+	−	−	+	−	+	+	.	+	.	+	.	.	+	.	.	AC	.	ACB	−	+	+	−
17	1	+	+	+	+	+	−	−	+	−	+	+	.	+	.	−	.	−	+	.	.	AC	.	CB	−	+	−	−
18	1	+	+	+	+	+	−	−	−	+	+	+	.	+	.	+	+	.	+	.	−	AC	.	ACB	−	+	+	.
19	2	+	+	+	+	+	−	−	−	+	+	+	.	+	+	+	+	.	+	.	−	AC	.	ACB	−	−	+	−
20	1	+	+	+	+	+	−	−	−	+	+	+	.	+	+	−	.	−	+	.	.	AC	.	CB	−	+	+	−
21	1	+	+	+	+	+	−	−	−	−	+	+	.	+	.	+	.	.	+	.	.	AC	.	AC	−	+	+	−
Subgroup IV (MacConkey)																												
22	2	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	−	+	−	−	A	.	AC	+	+	−	+
23 *B. lactis aerogenes*‡	1	+	+	+	−	+	+	−	+	+	+	+	.	+	.	+	.	+	+	.	.	A	.	ACB	−	−	+	+
24 *B. lactis aerogenes*§	3	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	A*	−	AC	.	CB	−	−	−	+
25	2	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	A	.	AC	+	+	−	+
26	1	+	+	+	−	+	+	−	+	+	+	+	.	+	.	+	+	.	−	.	−	A	.	CB	+	−	−	+

27	*B. cloacae*	1	+	+	+	−	+	−	−	+	+	+	+	+	+	+	+	+	−	+	A	−	A	.	AC	+	+	−	+
28		1	+	+	+	−	+	−	−	+	+	+	+	.	+	+	+	+	.	+	.	−	AC	.	CB	−	−	−	+
29	*B. cloacae*	1	+	+	+	−	+	−	−	+	+	+	+	.	+	+	+	+	.	−	.	−	A	AC	CB	+	+	−	+
30		1	+	+	+	−	+	−	−	+	+	+	+	−	+	+	+	−	.	+	.	−	A	.	AC	+	+	+	−
31		2	+	+	+	−	+	−	−	+	+	+	+	.	+	.	+	−	.	+	.	−	Alk	A	AC	−	−	+	−
32		1	+	+	+	−	+	−	−	+	+	+	+	.	+	+	+	.	−	−	.	−	A	.	ACB	−	+	−	+
33		1	+	+	+	−	+	−	−	+	−	+	+	+	+	‡	+	+	.	−	A	−	A	.	AC	+	+	−	+
34		1	A	A	A	−	A	−	−	A	−	A	A	−	A	A	A	−	−	A	−	−	A	.	AC	+	+	−	−
B. PROTEUS GROUP																													
35		1	+	−	+	−	−	−	−	−	−	+	+	.	+	.	−	.	.	+	.	.	A	AC	CBD	+	+	+	−
36		1	+	−	+	−	−	−	−	−	−	+	+	.	+	−	−	.	−	+	.	.	Alk	.	CBD	+	+	−	−
37	*B. proteus vulgaris*	25	+	−	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	A Alk	.	CBD	+	+	−	−
38		2	+	−	+	−	−	−	−	−	−	+	+	.	−	.	−	.	−	+	.	.	A	AC	CBD	+	−	−	−
B. MORGAN NO. 1																													
39		4	+	−	−	−	−	−	−	−	−	+	+	.	−	−	−	.	−	−	.	.	− Alk	.	Alk	−	+	+	−
40		3	+	−	−	−	−	−	−	−	−	+	+	.	−	−	−	.	−	−	.	.	−	.	−	−	−	+	−
B. FAECALIS ALKALIGENES																													
41		1	−	−	−	−	−	−	−	−	−	−	−	.	−	−	−	.	−	−	.	.	−	.	Alk	−	+	−	−
GROUP X																													
42		5	A	−	A	−	−	−	−	−	−	A	A	−	−	−	−	−	A	−	−	−	A	B	Alk	+	+	+	−
43		2	A	−	A	−	−	−	−	−	−	A	A	.	−	.	−	.	A	A	.	.	A	.	Alk	−	+	+	.
44		1	A	−	−	−	−	−	−	−	−	A	A	−	−	−	−	.	A	−	.	.	A	B	Alk	−	+	+	−
GROUP Y																													
45		12	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	+	−	−	−
46		3	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	−	−	−	−
47		10	−	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	+	−	−	−
48		2	−	−	−	−	−	−	−	−	−	−	A	−	−	−	−	−	−	−	−	−	B	CB	ACB	−	−	−	−
UNCLASSIFIED																													
49		1	A	−	A	−	A	A	−	−	−	A	A	−	−	−	−	A	A	−	−	A	Alk	.	BD	−	−	−	−
50		1	−	−	−	−	−	−	−	−	−	−	A	.	−	.	−	.	.	−	.	.	AC	.	CBD	+	+	−	.
51		1	−	−	−	−	−	−	−	−	−	−	−	.	−	.	−	.	.	−	.	.	CB	.	CB	+	+	−	.
52		1	−	−	−	−	−	−	−	−	−	−	−	.	−	.	−	−	.	−	.	.	Alk	B	CB	+	−	−	.

* One organism only tested. † *B. pseudocoli* (Castellani). ‡ Lewis. § Castellani.

A = Formation of acid. + = Formation of acid and gas, Liquefaction of gelatin, motility, Formation of indol, or a Voges and Proskauer's reaction.

AC = Acid and clot in litmus milk. B = Bleaching. CB = Clot and bleaching in litmus milk.

ACB = Acid, clot and bleaching in litmus milk. BD = Bleaching and digestion of clot. Alk = Alkalinity.

CBD = Clot, bleaching in litmus milk, digestion of clot.

were isolated on the first occasion, while on the second, after an interval of 70 days, an organism of the same group (No. 43) but differing slightly from both the others was obtained. Apart from these instances the organisms isolated on each second occasion appeared to bear no relationship whatever to those obtained at first. For example, in case 37 the first examination showed the presence of *B. Morgan* No. 1, the extremely atypical organism No. 49, *B. pyocyaneus*, and three varieties of *B. coli*. After an interval of 21 days there were present *B. pyocyaneus* and a member of Group Y.

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