The Differentiation of Escherichia and Klebsiella Types

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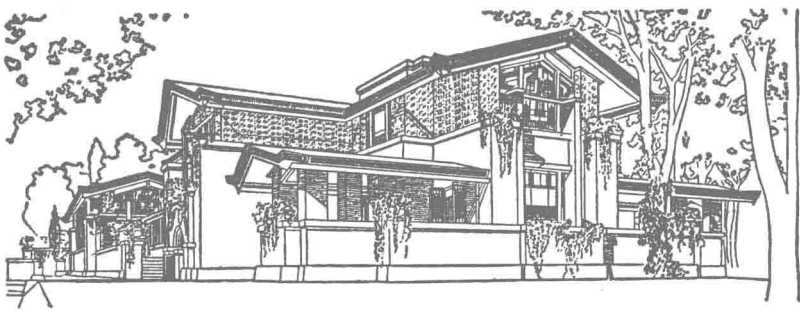

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Preface

THE SCOPE OF THIS WORK IS LIMITED TO A DISCUSSION OF the natural phenomena and methods used in the practical classification of *Escherichia* and *Klebsiella* strains by biochemical and serologic methods. The classification is based upon the antigenic analysis of cultures with due regard to the several forms of variation known to affect antigenic structure.

In order to classify the organisms successfully and, in fact, to prepare the reagents necessary for serologic study, it is imperative that the worker understand the variational changes which occur in these groups. These include the O-H variation of Weil and Felix, the S-R variation of Arkwright, and the K-O variation of Kauffmann. If these phenomena are not understood and their effects kept constantly in mind, confusion and failure to arrive at a correct diagnosis can only result.

If the above variational changes are recognized and if the recommended methods are strictly adhered to, little difficulty should be experienced in the classification of *Escherichia* and *Klebsiella.*

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BIOCHEMICAL DIFFERENTIATION

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Differences Between Escherichia and Klebsiella Types

IF IN KEEPING WITH BERGEY'S MANUAL WE WANT TO DIVIDE the tribe Eschericheae into genera, only two genera "Escherichia" and "Klebsiella" should be set up, not three: "Escherichia," "Aerobacter," and "Klebsiella."[1] The establishment of genera being based on biochemical methods, it is necessary to enter Aerobacter and Klebsiella types into one common group. Owing to the rules of nomenclature, it is not permissible to designate this group as "Aerobacter" because the term "Klebsiella" has priority.

To the *Escherichia group* (Coli group) belong Gram-negative, non-sporing rods which usually are motile and form indole, ferment adonitol and inositol but rarely, and give mostly a negative Voges-Proskauer reaction and a positive methyl-red reaction. They do not decompose urea, and usually they do not grow on Simmons' sodium-citrate agar. Apart from the A forms and one L form, they possess no capsule, and as a rule they form no mucus.[2]

In contrast hereto the *Klebsiella group* is made up by non-motile, Gram-negative, non-sporing rods that do not form indole and which ferment adonitol, inositol, and other carbohydrates or alcohols, often breaking down urea and frequently giving a positive Voges-Proskauer reaction and a negative

methyl-red reaction. As a rule these bacteria grow on Simmons' sodium-citrate agar; normally they possess a capsule, and most of them form mucus.

As in all other groups of Enterobacteriaceae, there is no

TABLE I.

Biochemical Behaviour of Typical Escherichia and Klebsiella Strains
(According to F. Kauffmann)

	Escherichia					Klebsiella				
	1	2	3	4	5	1	2	3	4	5
Adonitol	−	−	−	−	−	+	+	+	+	+
Dulcitol	−	+[3]	+[3]	+	+	−	−	−	+	−
Sorbitol	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	−	+[6]	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Salicin	+[4]	+[3]	−	−	+[3]	+	+	+	+	+
Inositol	−	−	−	−	−	+	+	+[3]	+	+
Lactose	+	+	+	+	+	×	+	×	+	+
Sucrose	−	−	−	+	+	+	+	+[3]	+	+
Mannitol	++	++	++	++	++	++	++	+−	++	++
Glucose	++	++	++	++	++	++	++	+−	++	++
Indole	+	+	+	+	+	−	−	−	−	−
H_2S	−	−	−	−	−	−	−	−	−	−
Gelatin	−	−	−	−	−	−	−	−	−	−
Simmons' Glucose Agar	+	+	+	+	+	+	+	−	+	+
Simmons' Citrate Agar	−	−	−	−	−	−	+	−	+	+
KNO_3	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	−	−	−	−	−	+	+	−	+	+
Methyl-red	+	+	+	+	+	−	−	+	−	−
Urea Agar	−	−	−	−	−	+	+	−	+	+
Urea liquid	−	−	−	−	−	+	+	−	+	+

Key: Escherichia 1 = U 5/41, 2 = U 9/41, 3 = U 14/41, 4 = Bi 7509/41, 5 = Bi 316/42.
Klebsiella 1 = Friedländer 916, 2 = Friedländer B 5055, 3 = Rhinoscleroma C 5046, 4 = No. 919, 5 = No. 119.
Adonitol - Glucose: − = negative after 30 days, + = positive after 1 day, × = late and irregular.
Gelatin: − = negative after 60. days.
Simmons' Agar, Voges-Proskauer, Methyl-red, Urea: − = negative after 4 days.

single criterion that is characteristic of the Escherichia group exclusively or of the Klebsiella group alone. Still, a combination of some reactions will generally permit a decision as to the grouping of a given culture. There are cultures, however, that occupy an intermediate position, illustrating again that in nature no sharply defined group is to be found. Therefore, where it is not possible sharply to define the individual biochemical groups, the demarcation each from the other will be more or less arbitrary. There will always remain some strains which cannot readily be entered in one of the groups. This applies not only to Escherichia and Klebsiella groups, but also to all other groups within Enterobacteriaceae, e.g., the Salmonella and Arizona groups.

As already stated repeatedly, only the individual types can be defined sharply, not the various groups, among which transitions are to be found.

In order to demonstrate the difference between the two groups (Escherichia and Klebsiella) 10 typical cultures of Escherichia and Klebsiella are entered in Table I.

Considering first the upper part of Table I, it is a striking feature that—in contrast to Escherichia strains—the Klebsiella strains promptly attack nearly all the substances added to the media. It is possible only to differentiate the Klebsiella strains in dulcitol-positive and dulcitol-negative cultures. It must be emphasized in particular that adonitol and inositol are fermented by all the Klebsiella strains. In contrast hereto, adonitol is attacked only by about 10 per cent of the Escherichia strains, and inositol even less frequently. None of the Klebsiella strains forms indole. While a majority of the Klebsiella strains grow on Simmons' citrate agar, a majority of the Escherichia strains give a negative reaction on this medium.

The Voges-Proskauer reaction is nearly always negative for Escherichia strains, usually positive for Klebsiella strains. Conversely, the methyl-red reaction is usually positive for Escherichia strains—in contrast to Klebsiella strains. Hitherto no Escherichia strain has been found to give a positive urea reaction, whereas most Klebsiella strains proved capable of decomposing urea.

On recording the more important differences between typical Escherichia and Klebsiella cultures we have the following schema:

	Typical Behaviour of Strains of the Escherichia group	*Klebsiella group*
Adonitol	−	+
Inositol	−	+
Indole	+	−
Simmons' sodium-citrate agar	−	+
Voges-Proskauer	−	+
Methyl-red	+	−
Urea agar	−	+
Motility	+	−

In the following a comparison will be made between cultural aspects of 100 Escherichia cultures and 100 Klebsiella strains freshly isolated from urine:

While most of the cultures examined by the author belong to the Escherichia group or to the Klebsiella group, various strains occupy an intermediary position. The latter category includes, for instance, an original "B. lactis aerogenes" from Escherich belonging to the Escherichia O group 9 forming indole and slowly liquefying gelatin. Just as the Salmonella group also includes indole-forming and gelatin-liquefying strains, cultures with these properties occur also in the Kleb-

siella group. Some cultures designated as *B. cloacae,* form no indole but liquefy gelatin.

So far we have been dealing with differentiation of the Klebsiella group from the Escherichia group, and now we will turn to the cultural differential diagnosis within these groups and refer in Table II to some biochemical Klebsiella types.

From Table II it is evident that dulcitol is fermented by two types, 1:3 and 1:10, whereas the other types are unable to

	Cultural Behaviour of	
	100 Escherichia Strains (According to F. Kauffmann)	*100 Klebsiella Strains*
Adonitol	11+, 1–+, 88–	100+
Dulcitol	40+, 28–+, 32–	33+, 67–
Sorbitol	96+, 3–+, 1–	100+
Arabinose	100+	100+
Xylose	94+, 5–+, 1–	100+
Rhamnose	85+, 11–+, 4–	93+, 7–+
Maltose	100+	100+
Salicin	12+, 67–+, 21–	100+
Inositol	1+, 2–+, 97–	98+, 2–+
Lactose	100+	99+, 1–+
Sucrose	22+, 15–+, 63–	99+, 1–+
Mannitol	100+	100+
Glucose	100+	100+
Gas production	99+, 1–	100+
Indole	92+, 8–	100–
H_2S	4+, 96–	100–
Gelatin	100–	100–
Simmons' Glucose agar	100+	99+, 1–
Simmons' Citrate agar	3+, 97–	98+, 2–
KNO_3	100+	100+
Voges-Proskauer	100–	91+, 9–
Methyl-red	100+	7+, 93–
Urea agar	100–	99+, 1–

Key: For simplification we have designated in the above schema a positive reaction after 24 hours with +, a delayed positive reaction with –+, and a negative reaction with –. Thus 11 +, 1 –+, 88 – mean that 11 strains were promptly positive, one strain fermented the medium late, and 88 strains gave a negative reaction. Failing gas formation, which was tested only in glucose and mannitol, was decided after four days' observation, as also the absence of growth on Simmons' glucose and citrate agar. Failure to form indole was observed in two tubes tested after one and two days. The KNO_3, Voges-Proskauer, methyl-red, and urea reactions were read after four days. Gelatin was observed 60 days.

TABLE II

Biochemical Differentiation in the Klebsiella Group
(According to F. Kauffmann)

Strains	Formula	Dulcitol	Urea	Organic acids			V.P.	Number of strains
				d	Ci.	Mu.		
A 4208	1 : 1	−	+	+2	+2	+1	−	3
A 916	1 : 1	−	+	+2	+2	+1	+	1
B 5055	1 : 2	−	+	+1	+3	+3	+	1
B 243	1 : 2	−	+	−	+1	+1	+	2
C 5056	1 : 3	+	+	−	+1	+1	−	3
B 7380	2 : 2	−	+	−	−	+1	−	2
B 4631	2 : 2	−	+	−	+1	+1	+	1
Rhin. C. 5046	2 : 3	−	−	−	−	−	−	6
Oz. D 5050	2 : 4	−	+	+2	−	+1	−	1
Oz. E 5051	2 : 5	−	−	+2	+2	+1	−	1
Oz. F 5052	2 : 6	−	−	+3	−	+7	−	1
Aer. 4140	1 : 7	−	+	+1	+1	+1	+	1
No. 1015	1 : 8	−	+	−	+1	+1	+	19
" 56	• : 9	−	+	−	−	+1	+	27
" 919	1 : 10	+	+	−	−	+1	+	26
" 390	3 : 11	−	+	−	−	+1	+	2
" 313	1 : 12	−	+	+1	+1	+1	+	1
" 1470	• : 13	−	+	+1	−	+1	+	1
" 1193	• : 14	−	+	−	−	+1	+	1
Total								100

Key: Organic acids in Bacto-Peptone-solution:
d = d-tartrate, Ci. = sodium-citrate, Mu. = mucate.
V.P. = Voges-Proskauer.
Rhin. = Rhinoscleroma,
Oz. = Ozaena,
Aer. = Aerogenes,
No. = number.

attack this alcohol. The more frequently occurring capsular types 8, 9, and 10 may also be differentiated culturally, by means of dulcitol and sodium-citrate (tested in fluid Bacto-Peptone medium).

	Dulcitol	*Sodium-citrate*
Type 8	−	+
" 9	−	−
" 10	+	−

The rhinoscleroma bacteria occupy a special position, as they are unable to decompose urea and organic acids, and they fail to grow on Simmons' glucose and sodium-citrate agar.

Just as in the Klebsiella group, it is possible in the Escherichia group too to set up biochemical types, some of the most frequent of which are presented in Table I. Here only a few deviating types will be mentioned briefly. Some strains, biochemically and serologically belonging to the Escherichia group, ferment lactose but late and irregularly, or not at all. Other strains grow on Simmons' citrate-agar, and form H_2S but not indole.

By employment of adonitol, dulcitol, rhamnose, salicin, inositol and sucrose, numerous biochemical types may be set up even though they are not all perfectly constant. The practical significance of these biochemical types lies in the circumstance that it is possible further to subdivide the serotypes of the individual O groups.

The groups (genera) are defined by means of biochemical methods, whereas the subdivision of the individual groups primarily is carried out after serologic methods, so that the further subdivision of these serotypes in biochemical types is a secondary measure of classification.