Technical method

A simple and rapid method for the differentiation of the members of the genus *Klebsiella*

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One of the recent trends in clinical microbiology has been the increasing frequency with which *klebsiella* are isolated. These bacteria are now quite commonly isolated from urine, pus, sputum, and blood. It may be that this increased incidence is related to the widespread use of certain antibiotics (Price and Sleight, 1970).

The rapid differentiation of the members of the genus *Klebsiella* presents a problem in the clinical laboratory. The most widely accepted scheme of classification is that of Cowan, Steel, Shaw, and Duguid (1960), in which the distinctions are made on biochemical grounds. However, this can be a time-consuming method, involving methyl red and Voges-Proskauer reactions which may take up to three days to complete. Other schemes, based on capsular typing, are rapid but less specific, since the capsule serotypes are widely distributed within the genus (Edwards and Fife, 1955) and *Klebsiella aerogenes* may possess the capsular antigen of one of the more pathogenic members. A more recent approach to the problem used a modification of the biochemical classification (Darrell and Hurdle, 1964), allowing tentative identification to be accomplished 'late on the day after the isolation of the organism'.

As the latter report (Darrell and Hurdle, 1964) stresses, there is a pressing need to distinguish *Klebsiella aerogenes* rapidly from the other members of the genus, especially in sputum culture where the former organism has little significance but the other members of the genus may signify the presence of Friedlander's pneumonia.

The method to be described is based on the observation that *Klebsiella aerogenes* will produce a mucoid colony with a dark centre and a metallic sheen when growing on eosin-methylene blue agar.

Materials and Methods

Gram-negative rods suspected of being *Klebsiella*
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were collected and screened for motility, fermentation of inositol, and production of hydrogen sulphide by the composite medium of Donovan (Donovan, 1966). Organisms which were non-motile, fermented inositol within 48 hours, and did not produce hydrogen sulphide were accepted as *Klebsiella*, and were then identified using the criteria of Cowan *et al* (1960). The results are set out in table I.

Organism	Number of Strains	Colonial Appearance on Eosin-methylene Blue Agar ¹		
		Type A	Type B	Type C
<i>Kl. aerogenes</i>	34	34	0	0
<i>Kl. pneumoniae</i>	13	0	13	0
<i>Kl. edwardii</i>				
(two varieties)	7	0	0	7
<i>Kl. ozaenae</i>	3	0	0	3
<i>Kl. rhinoscleromatis</i>	1	0	0	1
Totals	58	34	13	11

Table I *Comparison of biochemical classification and colonial appearance on eosin-methylene blue agar in 58 strains of Klebsiella*

¹Type A, dark centre to colony + metallic sheen; type B, dark centre to colony, no sheen; type C, translucent colony, no dark centre, no sheen.

All the strains were inoculated onto eosin-methylene blue agar (EMB), and incubated for 18 to 24 hours at 37°C. Eosin-methylene blue agar was made up according to the following formula:

Peptone (Oxoid)	..	..	..	..	..	..	10.0	g/litre
Lactose	..	..	..	..	..	..	10.0	g/litre
Dipotassium hydrogen phosphate	..	..	..	..	..	..	2.0	g/litre
Eosin Y	..	..	..	..	..	..	0.4	g/litre
Methylene blue	..	..	..	..	..	..	0.065	g/litre
Agar	..	..	..	..	..	..	15.0	g/litre
pH	..	..	..	..	..	..	6.8 (approx.)	

Initially the medium was made up from the basic constituents; however, subsequent testing showed that commercial dehydrated eosin methylene blue (Oxoid) gave identical reactions and this was then adopted as standard. Each batch was tested by inoculating the medium with a strain of *Escherichia coli* and *Enterobacter aerogenes* and confirming that these test organisms produced their characteristic appearances on the medium.

Finally, the reference strains of the genus *Klebsiella*, designated by Cowan *et al*, were obtained and processed in the same manner. These strains were:

NCTC Number	Strain	NCTC Number	Strain
8172	<i>Kl. aerogenes</i>	5050	<i>Kl. ozaenae</i>
9633	<i>Kl. pneumoniae</i>	5051	<i>Kl. ozaenae</i>
5046	<i>Kl. rhinoscleromatis</i>	5052	<i>Kl. ozaenae</i>
9496	<i>Kl. edwardii</i> var. <i>atlantae</i>	5054	<i>Kl. edwardii</i> var. <i>edwardii</i>

The total number of strains tested was 58.

APPEARANCES OF COLONIES ON EOSIN-METHYLENE BLUE

After overnight (18-24 hour) incubation, each plate was examined by direct and transmitted illumination, and the colonial appearances were classified as follows:

Dark centre to the colony + metallic sheen	..	..Type A
Dark centre to the colony (no sheen)	..	..Type B
Translucent colony (no centre, no sheen)	..	..Type C

These results are also found in table I.

Discussion

It can be seen from table I that the appearances on eosin-methylene blue agar correlate with the biochemical classification. Thus, *Klebsiella aerogenes* was rapidly distinguished from the other members of the genus. It is also seen that *Klebsiella pneumoniae* can be rapidly identified, but that the remaining members of the genus all have the same colonial appearance on eosin-methylene blue agar. Its use is

thus limited to screening *Klebsiella* and rapidly eliminating *Klebsiella aerogenes* from consideration in possible cases of Friedlander's pneumonia.

It is suggested that eosin-methylene blue could be used in the routine media inoculated for cultural diagnosis of chest infections, in which case any *Klebsiella* encountered would be quickly found to be *Klebsiella aerogenes* or not. A presumptive report could then be issued after only 18 to 24 hours. The inoculation of Donovan's medium from one of the colonies would confirm the presence of a *Klebsiella* over the next 18-24 hours, thus allowing a final report to be issued. The only organism likely to produce a similar appearance to *Klebsiella aerogenes* on eosin-methylene blue agar is *Escherichia coli*; this would be detected by the Donovan's medium, but the significance of the presumptive report would remain unaltered.

Final identification of a pathogenic member of the genus, ie, Friedlander's bacillus, would still be done by biochemical means, but the screening procedure should reduce this to a minimum.

References

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Book review

IARC Monographs: The Evaluation of Carcinogenic Risk of Chemicals to Man. Volume 4: Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents. IARC Working Group, meeting in Lyon, June 1973. (Pp. 286. Sw.Fr. 18.-) **Volume 5: Some Organochlorine Pesticides.** Views of the IARC Working Group, meeting in Lyon, October, 1973. (Pp. 241; formulae. Sw.Fr. 18.-). **Assessment of the Carcinogenicity and Mutagenicity of Chemicals: Report of a WHO Scientific Group.** *Wld Hlth Org. techn. Rep. Ser.*, 1974, No. 546. (Pp. 19. Sw.Fr. 4.-). Geneva: World Health Organization. 1974. Available through HMSO, London.

The two latest monographs from IARC on the evaluation of carcinogenic risks of chemicals to man follows the format of their predecessors. They provide excellent summaries of current knowledge and the volume which includes the aromatic amines will be of particular value. Some of the contemporary problems in assessing carcinogenic and mutagenic mechanisms (and their interrelationship) are clearly laid out in the WHO booklet.

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