# Differentiation of *Klebsiella-Enterobacter-Serratia* Organisms

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## SUMMARY

**Differentiation of 625 strains of bacteria which fulfilled the requirements laid down for the definition of the tribe *Klebsielleae* was carried out using 6 biochemical tests. Five hundred and forty-six strains were identified as members of the genus *Klebsiella*, 46 of the genus *Enterobacter* and 33 of the genus *Serratia*.**


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Brown and Seidler[1] and Kislak *et al.*,[2] investigating hospital-acquired infections, found that *Klebsiella, Enterobacter* and *Serratia* (KES) organisms were the most frequently isolated Gram-negative bacilli grown in pure culture and were second only to *Proteus* species in mixed cultures. Kayyali *et al.*[3] refer to an outbreak of infant diarrhoea caused by *K. pneumonia* infection, and Steinhauer *et al.*[4] stated that this organism may be found in various abscesses and wound infections. Our experience at the Tygerberg Hospital shows a marked increase in infections caused by KES strains. Sonnenwirth[5] emphasises the importance of accurate identification of KES strains, and Edwards and Ewing[6] and Cowan and Steele[7] described a large number of biochemical tests used to differentiate them. They claimed that some of these methods produce results only after prolonged (4 days) incubation. We have examined KES strains, using various biochemical tests, over a 12-month period in order to find a limited number of tests which would give rapid and reasonably accurate KES differentiation. Such a simplified procedure would be of particular value in small hospital laboratories.

## MATERIAL AND METHODS

The majority of the 625 cultures used were derived from stools, urine, sputum and pus. The 21 *Serratia marcescens* strains isolated from clinical material were supplemented by the inclusion of 12 standard strains of this organism. Primary isolations from clinical material were made on SS agar (Difco) or MacConkey agar (Oxoid). Lactose-fermenting colonies were picked from MacConkey plates only, whereas non-lactose and late lactose-fermenting colonies were picked from MacConkey plates and also from SS plates when isolated from stool specimens. All these colonies were harvested after overnight incubation at 37°C. The colonies were then inoculated into tryptone water (Difco) and incubated for 3 - 4 hours at 37°C. The following biochemical tests were carried out on the lactose-fermenting colonies. Arginine, lysine and ornithine decarboxylase activity was determined, using the methods of Falkow,[8] Fay and Barry[9] and Møller,[10] with suitable controls. Indole production was determined by using 1% tryptone (Difco) containing 0,5% NaCl at pH 7,4. Citrate utilisation was determined with Simmons citrate medium as described by Cowan and Steele,[7] with pH adjusted to 6,1. The Voges-Proskauer test was carried out in glucose-phosphate broth (Difco). Fermentation of arabinose was tested, and motility tests were carried out using sloppy agar medium with Craigie tubes. The non-lactose and late lactose fermenters were inoculated into dextrose, lactose, sucrose and mannitol, and urea,[7] and $H_2S$ production was determined. When organisms showed the biochemical characters of the tribe *Klebsielleae*, the tests described above for the lactose-fermenting organisms were carried out.

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## RESULTS

The 625 strains of bacteria examined fulfilled the requirements laid down by Edwards and Ewing[6] for inclusion in the tribe *Klebsielleae*. Of the strains, 546 belonged to the genus *Klebsiella*, 46 to the genus *Enterobacter* and 33 to the genus *Serratia*. It was found that the separation of these genera could be reasonably accurately accomplished by the use of the 6 biochemical tests shown in Table I.

**TABLE I. BIOCHEMICAL REACTIONS OF *KLEBSIELLA-ENTEROBACTER-SERRATIA* STRAINS**

| | A | L | O | Cit. | Arab. | Indole |
|---|---|---|---|---|---|---|
| *Klebsiella* ... ... ... | − | + | − | + | + | ± |
| *Enterobacter* ... ... | ± | ± | + | ± | + | − |
| *Serratia* ... ... ... | − | + | + | + | − | − |

A = arginine decarboxylase; L = lysine decarboxylase; O = ornithine decarboxylase; Cit. = citrate; Arab. = arabinose.

Both Falkow's[8] method for testing decarboxylase reactions and that of Fay and Barry[9] were found to be unreliable. Møller's[10] method usually gave reliable and consistent results after 24 hours' incubation when controls were satisfactory. Nineteen, or 3%, of cultures required 48 hours' incubation at 37°C before positive results were

obtained. It will be seen from these results that all strains of *Klebsiella* were ornithine and arginine decarboxylase-negative and that some strains of *Klebsiella* were indole-positive only. These strains were differentiated from *Escherichia coli* in that they utilised citrate. On the basis of Edwards and Ewing's[6] summary of biochemical reactions for *Klebsiella*, the results of the ornithine test identified them as either *K. pneumoniae*, *K. ozaenae* or *K. rhinoscleromatis*. However, since these strains were all Voges-Proskauer-positive, they were identifiable as *K. pneumoniae*. Members of the genus *Enterobacter* were separated into two groups by their decarboxylase reactions. The majority of strains of *E. cloacae* were lysine-negative and arginine-positive, while most strains of *E. aerogenes*, *E. hafniae* and *E. liquefaciens* were lysine-positive and arginine-negative.

## DISCUSSION

In the past, the taxonomic relationships of organisms belonging to the tribe *Klebsielleae* have been greatly confused. Edwards and Ewing[6] state that many non-motile cultures that were actually *Klebsiella* were classified as members of the genus *Aerobacter* i.e. *Enterobacter*. A large number of biochemical tests have been developed, which enable the KES genera of the tribe *Klebsielleae* to be accurately identified. In our series the 6 tests used have made possible differentiation of KES strains at the generic level in a high percentage of cases after overnight incubation. With slight extension of these tests, identification at species level is also possible. *E. cloacae* produces arginine decarboxylase, whereas *E. aerogenes*, *E. hafnia*, *E. liquefaciens* and *Serratia marcescens* do not. Of the 4 species of *Enterobacter*, *E. cloacae* does not produce lysine decarboxylase but it is produced by the other 3 species. Differentiation of *E. hafnia* and *E. liquefaciens* would require fermentation of raffinose and rhamnose. It should be emphasised that while the available data show an absolute inability of *Klebsiella* to produce ornithine decarboxylase, such absolute differentiation has not been found in the other species of the tribe *Klebsielleae*.[6] The absence of *K. ozaenae* and *K. rhinoscleromatis* in this series is of interest, since Edwards and Ewing[6] found them to be present in a very small percentage of their isolates.

The tests used here provide a simple, rapid means of differentiating KES strains, which should prove useful in the small clinical pathology laboratory.

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