Differentiation of *Klebsiella-Enterobacter* (*Aerobacter*)-*Serratia* by Biochemical Tests and Antibiotic Susceptibility

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Studies were undertaken for the differentiation of members of the *Klebsiella-Enterobacter* (*Aerobacter*)-*Serratia* division by biochemical tests and antibiotic susceptibility. A total of 67 cultures were tested. Strain identification was readily accomplished with the use of motility tests and arabinose fermentation. In addition, a practical schema, based on sensitivity pattern, proved valuable in the classification of the different strains. Most (if not all) *Klebsiella* strains were susceptible to cephalothin, and all were sensitive to colistin. *Enterobacter* strains were resistant to cephalothin but susceptible to colistin. In contrast to those other members of the group, all *Serratia* strains were resistant to both cephalothin and colistin. The combination of a limited number of biochemical reactions and single-disc sensitivity tests appears to be a logical approach for the tentative identification of *Klebsiella-Enterobacter* (*Aerobacter*)-*Serratia* strains.

The importance of the correct identification of bacterial species from clinical specimens cannot be overemphasized. The value of the precise classification of the members of the *Klebsiella-Enterobacter* (*Aerobacter*)-*Serratia* (K-E-S) division has been previously recognized (4, 8-10).

Differentiation of strains of the K-E-S group by biochemical tests and on the basis of susceptibility to ampicillin and the cephalosporins has been studied by other investigators (3, 4, 8-10). In most instances, however, classification has been incomplete, and no separation has been made between *Enterobacter* and *Serratia* strains.

This study was undertaken to establish a sensible approach for the identification of K-E-S strains. The method combines a limited number of biochemical and sensitivity tests, which would be applicable in hospital laboratories.

**Materials and Methods**

*Cultures.* Fifty-seven strains isolated from different clinical materials (previously classified as "Aerobacter") and 10 cultures of *Serratia* were studied.

All cultures were restreaked on MacConkey Agar to exclude the possibility of mixed growth. All isolates were then inoculated into Triple Sugar Iron (TSI; Difco) Agar slants, and after incubation for 18 to 24 hr at 37 C they were transferred to Brain Heart Infusion (BHI; Difco) Agar slants and were incubated under the same conditions. All biochemical reactions and sensitivity studies were performed from the BHI slants.

**Biochemical reactions.** Tests for the preliminary identification of the cultures included reactions in TSI Agar, methyl red (MR-VP Medium; Difco), citrate utilization (Simmons Citrate Agar; Difco), and indole production (tryptone broth; Difco). The final identification of the strains was based on the following tests: motility (Motility Sulfide Medium; Difco) and arabinose fermentation (Difco Phenol Red Broth Base containing 1% arabinose sterilized by filtration). The methyl red, citrate, and indole reactions were read after 48 hr, motility and TSI reactions after 18 to 24 hr, acid production from arabinose in 24 hr.

**Sensitivity studies.** A standardized single-disc method for antibiotic susceptibility testing (2) was employed. For classification purposes, the few strains which fell within the intermediate sensitivity range were regarded as resistant. The antibiotic discs used for the differentiation of the K-E-S strains were cephalothin, 30 μg, and colistin, 10 μg (BBL).

**Results**

The classification of Edwards and Ewing (5) was used. *Klebsiella* was the most prevalent organism isolated. Of the 57 cultures previously classified as "Aerobacter," 32 strains (56%) were identified as *Klebsiella*, 18 (32%) as *Enterobacter*, and 7 (12%) as *Serratia*. Biochemical tests were carried out for final identification of the cultures. Two tests were used, motility and arabinose fermentation.
fermentation. *Klebsiella* showed no motility and arabinose utilization; *Enterobacter* showed motility and arabinose utilization; *Serratia* showed motility but no arabinose utilization.

The incidence of susceptibility of all cultures to the two antimicrobial agents used is given in Table 1.

All strains classified as *Klebsiella* and *Enterobacter* were sensitive to colistin. In contrast, *Serratia* was completely resistant to this drug. All but one of the strains (97%) of *Klebsiella* were sensitive to cephalothin, but *Enterobacter* was highly resistant (94% of the strains). *Serratia* strains showed complete insusceptibility to cephalothin.

**DISCUSSION**

The need for differentiation of bacterial species and the proper use of nomenclature has been emphasized by previous investigators (1, 3, 4, 7, 8). The use of the terms *Klebsiella-Aerobacter* (*Enterobacter*) and *Aerobacter* for members of the K-E-S division, with no attempt to properly classify the organism, is widespread in hospital laboratories in the United States. The differentiation of these organisms is not difficult, but is seldom attempted; *Enterobacter* and *Serratia* species are often classified erroneously (8). In addition to the standard tests used for screening, other tests have been suggested for final identification of the strains. Of these, motility has been more widely used and has been satisfactory in differentiating *Klebsiella* from the typically motile ("Aerobacter") members of the group (3, 4, 9, 10). Motility alone, however, is not sufficient to separate *Enterobacter* from *Serratia*. Since most strains of *Serratia* are nonpigmented, it is evident that the use of additional tests for strain characterization is necessary.

Additional tests to classify an organism are important. When more complete methods of identification were used, only 18 (32%) of the 57 strains previously designated as "Aerobacter" were identified as *Enterobacter* (*Aerobacter*). Furthermore, 7 strains of *Serratia* (12%) were erroneously classified as "Aerobacter" (*Enterobacter*).

The recent increased incidence of isolation of *Serratia* from clinical materials and the recognition of members of the K-E-S group as potential pathogens and as primary etiologic agents in hospital-acquired infections (8) emphasize the value of additional tests for the correct taxonomic characterization of the species.

The predominance of *Klebsiella* (56%) isolated from clinical specimens over *Enterobacter* and *Serratia* (motile strains of the group) is in agreement with other investigations (4, 9).

Motility testing and arabinose fermentation were very satisfactory tests for strain differentiation, and their inclusion, in addition to the conventional tests used for tentative identification, is recommended. All strains tested were readily identified, at least to genus, with the use of this schema.

It is evident that the use of sensitivity studies is an additional tool in the separation of *Klebsiella* from *Enterobacter* and *Serratia* (Table 1).

The data presented show the susceptibility of *Klebsiella* to cephalothin and the resistance of *Enterobacter* strains to the same antibiotic. This confirms the results obtained by several investigators (3, 4, 8, 9, 11). In addition, all *Serratia* strains were uniformly resistant to cephalothin. In the studies by Kock and Rose (8), all cultures of *Serratia* showed similar resistance.

All the strains of *Klebsiella* and *Enterobacter* tested were uniformly sensitive to colistin; in contrast, all *Serratia* strains were invariably resistant. The same results were obtained when polymyxin B was used, thus confirming the findings of Edmondson and Sanford (4) and Gale and Sonnenwirth (6). Rose and Kock (10) reported that colistin was ineffective against 73% of *Enterobacter cloacae* strains. Since no differentiation was made between *Enterobacter* and *Serratia*, it is not unlikely that the resistant "Aerobacter" strains were in fact strains of *Serratia*.

The resistance of *Serratia* to colistin and polymyxin B seems to be a characteristic of *Serratia*, and apparently it is not related to a particular hospital strain or to prior antimicrobial exposure (4).

From the results obtained, it seems that Table 1 could be used as a valuable guide for the tentative differentiation of strains of the *Klebsiella-Enterobacter-Serratia* group.

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**Table 1. Susceptibility patterns of 67 strains of the K-E-S group**

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. tested</th>
<th>Susceptible to a</th>
<th>CF b</th>
<th>CL b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella</td>
<td>32</td>
<td>31 (97)</td>
<td>32 (100)</td>
<td></td>
</tr>
<tr>
<td>Enterobacter</td>
<td>18</td>
<td>1 (6)</td>
<td>18 (100)</td>
<td></td>
</tr>
<tr>
<td>Serratia</td>
<td>17</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

a The first number represents the total number of susceptible strains; the parenthetical number represents the percentage of susceptible strains.

b CF, cephalothin, 30 µg; CL, colistin, 10 µg.
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LITERATURE CITED


