Biochemical and Clinical Characteristics and Antibiotic Susceptibility of Atypical 
*Enterobacter cloacae*

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The characteristics of an atypical group of the family *Enterobacteriaceae* resembling *Enterobacter cloacae* were studied. The urinary tract was the most common source of these organisms, and most strains represented infections of secondary clinical significance. In contrast to typical *Enterobacter* strains, the atypical strains were highly susceptible to the cephaplorins; otherwise, there was a high degree of susceptibility to five other antibiotics and resistance to ampicillin except in very high concentration.

The importance of precise microbiological differentiation of members of the genera *Klebsiella*, *Enterobacter*, and *Serratia* isolated from clinical specimens was stressed by Steinhauer et al. (12), Fields et al. (7), and Edmondson and Sanford (3). The biochemical reactions of the tribe *Klebsiellae* were described by Ewing (6), and antibiotic susceptibility patterns, particularly with respect to the striking differences in susceptibility to the cephaplorins, have been studied by several investigators (1, 3, 13).

This report describes the biochemical and clinical characteristics and antibiotic susceptibility of an aberrant or atypical group of organisms of the family *Enterobacteriaceae* which, although they do not completely fulfill any of the criteria for generic assignment, resemble *Enterobacter cloacae* sufficiently to be referred to as "atypical *E. cloacae*.*"

**MATERIALS AND METHODS**

Organisms of the family *Enterobacteriaceae* were identified as atypical *E. cloacae* by reactions with triple sugar-iron-agar (TSI), Simmons citrate-agar, Christensen urea-agar, indole test broth, and ornithine decarboxylase (Moeller base) in 0.3% agar. All strains underwent further study with tests described by Edwards and Ewing (4). The decarboxylase tests were modified by the addition of 0.3% agar (10), which avoids the necessity of the oil overlay. The indole, methyl red, and Voges-Proskauer tests were modified according to Douglas (2). The nomenclature and biochemical reactions used in this study are based on the taxonomic system of Ewing (5).

Quantitative antibiotic susceptibility tests were performed on all isolates by the agar-dilution technique by use of the Steers, Foltz, and Graves inoculating apparatus (11). Inoculum size ranged from $10^6$ to $10^8$ viable units. Minimal inhibitory concentrations (MIC) were determined after 18 to 24 hr of incubation. Ranges of concentration tested for all antibiotics represented those concentrations routinely used in this laboratory on clinical isolates. Cephalothin and cephalaxin were carried to a concentration of 100 $\mu$g/ml to compare the activities of the newer cephaplorin given orally to the older cephalaxin given systemically. Ampicillin was also tested at higher concentrations because of the high degree of in vitro resistance encountered at 20 $\mu$g/ml or less. The antibiotics used were: cephalothin, cephalaxin, streptomycin, ampicillin, kanamycin, tetracycline, chloramphenicol, and polymyxin B.

Clinical data were obtained by review of each patient's clinical records, and each case was then designated as representing primary, secondary, or indeterminate infection. The criteria utilized for assignment to each category of clinical evaluation were similar to those used by Edmondson and Sanford (3), with the exception that organisms assigned to the commensal category by them were placed in the indeterminate category in this study.

**RESULTS**

**Bacteriologic.** Biochemical reactions of the atypical *E. cloacae* strains isolated are listed in Table 1. With respect to the TSI reaction, 15 strains produced an acid slant and acid butt with variable amounts of gas; the remainder produced an alkaline slant and acid butt with variable amounts of gas. All strains were indole-positive, methyl red-positive, and Voges-Proskauer-negative. All strains utilized citrate and all but nine hydrolyzed urea within 48 hr. All strains but
one were motile. The decarboxylase test pattern was quite characteristic: negative, positive, and positive for lysine, arginine, and ornithine, respectively. None of the isolates deaminated phenylalanine, liquified gelatin, or produced hydrogen sulfide. All but three strains utilized malonate. All of the strains fermented arabinose and rhamnose with the production of gas; none fermented raffinose. Growth in KCN broth was positive in 23% of the strains tested.

**Antibiotic susceptibility.** MIC values for the 52 strains are listed in Table 2 according to the cumulative percentage of strains inhibited by each antibiotic at each given level. The two cephalosporins were quite similar in their effect, inhibiting more than 90% of the strains at levels which are attainable in serum with the usually recommended dose. With ampicillin, only 5% of the strains were inhibited by 50 μg/ml and 78% by 100 μg/ml. More than 90% of the 52 strains were inhibited by the remainder of the antibiotics at levels which are attainable in serum with the usually recommended doses.

**Clinical data.** In the interval between May and October 1968, cultures from 52 patients contained atypical *E. cloacae*. Since October 1968, cultures from an additional 48 patients have yielded atypical *E. cloacae* with biochemical and antibiotic susceptibility characteristics identical to those reported in this paper. The specimens from which they were isolated came from the Mayo Clinic, the wards and emergency rooms of the three hospitals served by this laboratory, and the Section of Experimental and Anatomic Pathology which serves these institutions. There was no single predominant institutional source of isolates. The greatest number of isolates came from urine. The patients' ages ranged from 2 weeks to 95 years (mean, 55 years). In two cases, the organism was isolated from two sources (urine and postoperative wound; sputum and urine), and in one case the organism was isolated from three sources (urine, postoperative wound, and tracheal stoma). Clinical evaluation of the isolates and their presence as pure or mixed culture are shown in Table 3.

No particular organism was predominant in mixed cultures with atypical *E. cloacae*. The following organisms were isolated from mixed...
DISCUSSION

Our attention was first directed to the atypical E. cloacae for two reasons. First, they gave the combination of TSI, citrate, urea, ornithine decarboxylase, and motility reactions characteristic of the genus Enterobacter, but with a positive indole reaction. Second, but all a few of the atypical E. cloacae strains were found to be susceptible to the cephalosporins at 20 μg/ml or less.

Fife et al. (8) reported the biochemical reactions of strains of the tribe Klebsiellae studied at the National Communicable Disease Center. Among the E. cloacae, 0.5% yielded a positive indole test, 0.3% were methyl red-positive, and 99.5% were Voges-Proskauer positive. In contrast, the atypical strains have been uniformly indole-positive, methyl red-positive, and Voges-Proskauer-negative. The percentages of atypical E. cloacae demonstrating (i) citrate utilization, urea hydrolysis, and motility, (ii) decarboxylation of arginine and ornithine and lack of decarboxylation of lysine, (iii) fermentation of arabinose and rhamnose, and (iv) malonate utilization were comparable to the results of these tests carried out on E. cloacae by Fife et al. (8). In contrast again to typical E. cloacae, none of the atypical strains fermented raffinose or liquefied gelatin. On the basis of these differences, Young et al. (Bacteriol. Proc., p. 106, 1968) proposed that these organisms be assigned separate generic status within the family Enterobacteriaceae. Because of the number of characteristics shared in common with E. cloacae, it may be more appropriate to consider a separate species assignment to these organisms. Studies are currently in progress at the National Communicable Disease Center to determine the taxonomic status of these organisms. In the meantime, these are being called "atypical E. cloacae."

Of clinical interest was the susceptibility of the atypical E. cloacae to the cephalosporins. Edmondson and Sanford (3) and Washington and Bourgeois (13) pointed out that cephalothin in concentrations of 20 μg/ml or less is quite effective against strains of Klebsiella but not against Enterobacter. Griffith and Black (9) demonstrated similar activity for cephalaxin. The atypical strains in this study were highly susceptible to the cephalosporins at 20 μg/ml or less. In this laboratory, during the same interval, a comparable number of typical Enterobacter strains showed no susceptibility to cephalothin at 20 μg/ml or less. In the atypical group the most effective agents at levels which are readily attainable in serum with the usually recommended doses were tetracycline, polymyxin B, and kanamycin, in which respect they resembled the typical strains (3). However, all 52 test strains appeared to be susceptible to all other antibiotics tested with the exception of ampicillin. In this respect these strains differed from the typical strains studied by Edmondson and Sanford (3) and by our own laboratory, for which streptomycin, tetracycline, and chloramphenicol were of intermediate effectiveness. The results with ampicillin were comparable to those of Edmondson and Sanford (3) and our own data for typical Enterobacter strains.

Although the greatest number of atypical E. cloacae strains isolated were considered to be of secondary clinical importance, 10 isolates were of primary significance. In all instances in which it was considered to be of primary or secondary importance, the atypical strain was isolated either in pure culture or as the predominant organism in a mixed culture. The remainder of the isolates, present either in insignificant numbers in the urine or as a few colonies in a mixed culture, were placed in the indeterminate category. The most important underlying factors responsible for assignment to the secondary category were prior antibiotic therapy, obstructive uropathy or congenital vesical disorder, and prior urethral catheterization. There was no obvious epidemiological source or pattern of origin of the atypical strains; they were isolated with nearly equal frequency from the inpatient and outpatient services of the Mayo Clinic and allied hospitals.

LITERATURE CITED


