Corynebacterium Group D2 as a Cause of Alkaline-Encrusted Cystitis: Report of Four Cases and Characterization of the Organisms

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In four patients with alkaline-encrusted cystitis, Corynebacterium group D2 was isolated from consecutive urine cultures and stones. Encrusted cystitis occurred in bladders harboring inflammatory or tumorous lesions in patients with chronic or recurrent urinary tract infections appearing after surgery or instrumentation. The urease activity of Corynebacterium group D2 and the neutralization of this enzyme by acetohydroxamic acid are shown. Clinical improvement, disappearance of struvite crystals, and decrease of the urine pH were obtained when these bacteria were eliminated from urine samples. Corynebacterium group D2 strains were highly resistant to many antimicrobial agents but were highly susceptible to norfloxacin and vancomycin when tested at two pHs (7.4 and 8.5).

Alkaline-encrusted cystitis is a chronic inflammatory condition of the bladder, described by Francois in 1914 (1) as a more or less localized ulcerative inflammation with deposits of ammonium magnesium phosphate on the surface and on the walls of the ulcer. Hager and Magath (5) related this disease to the implantation of urea-splitting gram-negative bacilli in a bladder which already harbored some form of inflammatory or tumorous lesion. Various microorganisms have been involved in this chronic disease, including species of Streptococcus (5, 10), Staphylococcus (5), and mainly Proteus (5). This report describes four cases of encrusted cystitis, with the isolation of a urea-splitting gram-positive bacillus identified as Corynebacterium group D2.

CASE REPORTS

From June 1983 to March 1984, we studied four patients diagnosed with alkaline-encrusted cystitis by cystoscopy and biopsy, whose main clinical and microbiological data are summarized in Table 1. All patients had chronic or recurrent urinary tract infections which appeared after surgery or instrumentation and in two cases were associated with megalovoltage therapy of the bladder. Symptoms of encrusted cystitis appeared 5 to 36 months after the urological procedures. Three of the four patients were referred to our hospital because of symptoms of chronic cystitis and negative routine urine cultures. All patients had dysuria, urgency, frequency, suprapubic pain, and hematuria. Three of them eliminated small stones of struvite (ammonium magnesium phosphate) which produced, in the case of a child, an acute urethral obstruction. The urines were strongly alkaline (pH higher than 8.0), with a definite odor of ammonia. Twelve clean-catch urine samples were obtained (two to four specimens per patient), and all of them showed a pure culture of coryneform bacteria which was highly resistant to most antimicrobial agents. Two stones obtained by cystoscopy from two different patients were studied. Crystal analysis disclosed that they consisted of struvite, and microbiological studies showed heavy growth of the same corynebacteria.

After several failures to achieve sterilization of the urine samples, a cystoscopic resection of the encrusted stones was made in three of the four patients by administering various oral antimicrobial agents or ammonium chloride or instilling in the bladder weak solutions of acetic or citric acids. All patients improved their clinical conditions with these double or triple measures, and urine samples were cleared of bacteria.

MATERIALS AND METHODS

Twelve clean-catch urine samples from the four patients and two stones obtained by cystoscopy from two of them were cultured aerobically on blood agar and Cled agar at 37°C, and 14 apparently identical cultures were obtained. Their ability to grow on blood agar at 25, 37, and 42°C and on MacConkey agar at 37°C, motility, and biochemical tests (urease, nitratase, indole, gelatin hydrolysis, oxidase, catalase, activity on triple sugar iron, and acidification of glucose, D-xyllose, mannitol, lactose, sucrose, malate, and starch) were studied (13). The ureolytic activity was quantified in two strains (isolates from patients no. 1 and 2) by using Christensen urea broth (Difco) and a bacterial inoculum of 5 × 10^7 to 1.5 × 10^8 CFU/ml incubated with agitation at 37°C. The pH values were determined in a Beckman 3500 digital pH meter at time zero and 2-, 4-, 6-, 8- and 24-h intervals. For comparison, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 23357, and Proteus vulgaris ATCC 6380 were also studied. Viable counts were determined by diluting bacteria growing in Christensen urea broth and subculturing on blood agar with a calibrated loop at the same intervals at which pH values were determined.

Resistance to various pHs of the two corynebacteria and the three enterobacteria mentioned was determined in Mueller-Hinton broth (Difco) adjusted to pHs of 7.0, 8.0, 9.0, and 10.0. Viability of bacteria in these media was studied by subculturing after 8 and 24 h of incubation at 37°C.

Neutralization of bacterial urease by acetohydroxamic acid was studied with one representative strain isolated from each patient on plates of Christensen urea agar (Difco) with
and without 1.9-ng/ml concentrations of this compound and an inoculum of ca. 10^7 CFU per spot. The experiment was carried out at 37°C, and urealytic activity and growth were recorded at 30 min and then hourly until 7 h and after 24 h. The antimicrobial susceptibility of these bacteria was studied by the disk diffusion test in Mueller-Hinton agar. The inoculum was prepared from a 24-h culture in Mueller-Hinton broth containing 20% sterile rabbit serum and 1% Tween 80 and adjusted to the turbidity of a McFarland 0.5 standard. The inoculum was spread with a sterile cotton swab. The plates were incubated at 37°C for 24 h and then examined, and the diameters of the zone of complete inhibition were measured.

MICs were determined with an overnight culture identical to the one used in the disk diffusion test but standardized by dilution in tryptic soy broth (Difco) at 1:10 and inoculated with a Steers replicator (11) onto two sets of Mueller-Hinton agar (pH 7.4 and 8.5) containing twofold increasing concentrations of antimicrobial agents. The antimicrobial agents tested were ampicillin (Beecham), cephalexin (Lilly), erythromycin (Abbott), rifampin (Lepetit), tetracycline (Pfizer), vancomycin (Dista), novobiocin (Merck Sharp and Dohme), norfloxacin (Liale), and gentamicin (Schering). The plates were incubated for 24 h at 37°C and examined for growth. Beta-lactamase activity was studied by using the nitrocephin method (9).

RESULTS

Microorganisms were isolated after 24 to 48 h of incubation at 37°C on blood agar and Cled agar. All 14 strains isolated were identical from morphological, cultural, and biochemical viewpoints. Bacteria grew on blood agar as pinpoint colonies after 48 h of incubation at 25, 37, and 42°C. Colonies were whitish, opaque, smooth, convex, circular, entire, and nonhemolytic. Microorganisms had the appearance of gram-positive bacilli typical of diphtheroids, although they were often coccobacillary and nonmotile. They were catalase positive, oxidase negative, indole negative, unable to reduce nitrates to nitrites, and rapidly urease positive. Of seven carbohydrates examined, none was acidified after 7 days of incubation. Bacteria did not grow on MacConkey agar, were neutral on triple sugar iron agar, and did not hydrolyze gelatin after 15 days of incubation. From all of these characteristics the bacteria were identified as Corynebacterium group D2.

Figure 1 shows the pH values obtained for two corynebacteria and control strains in Christensen urea broth at defined intervals. All urea-splitting bacteria reached pH values of ca. 9.0 after 24 h of incubation.

Figure 2 shows the relationship between bacterial counts and hours of incubation. Corynebacteria, although having grown less than the enterobacteria, retained high viability, whereas viability of P. vulgaris dropped dramatically by 24 h.

All bacteria grew in Mueller-Hinton broth at pHs from 7.0 to 9.0, but not at pH 10.0. The viability of gram-negative bacilli decreased at pH 10.0 after 8 and 24 h, whereas corynebacteria remained unaffected. Ureolytic activity and growth of four corynebacteria on Christensen urea agar with and without 1.9 mg of acetoxyhydroxamic acid per ml showed no bacterial growth at 7 h, but all microorganisms grew after 24 h of incubation on every plate. Ureolytic activity of the four corynebacteria appeared on Christensen urea agar between 30 and 60 min of incubation, but no urea-splitting bacteria showed this activity on Christensen urea agar with acetoxyhydroxamic acid added, even after 24 h of incubation.

Table 2 shows the antimicrobial agents, disk potency, and zones of inhibition obtained with the four corynebacteria studied by the disk diffusion test. These previous results allowed us to select six possibly useful antimicrobial agents (vancomycin, tetracycline, erythromycin, novobiocin, rifampin, and norfloxacin) and three others which showed no zone.

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**TABLE 1.** Clinical and microbiological data of four patients with alkaline-encrusted cystitis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>77</td>
<td>74</td>
<td>9</td>
<td>77</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Underlying condition</td>
<td>Prostatic adenoma</td>
<td>Bladder carcinoma</td>
<td>Ectopic kidney</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>Urological procedure</td>
<td>Transurethral resection</td>
<td>Cistostomy; radiotherapy</td>
<td>Ascendent pyelography</td>
<td>Cistostomy; radiotherapy</td>
</tr>
<tr>
<td>Time since the</td>
<td>5 months</td>
<td>19 months</td>
<td>36 months</td>
<td>14 months</td>
</tr>
<tr>
<td>urological procedure and e.c. symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiological data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine (CFU of</td>
<td>&gt;10^5 (3 samples)</td>
<td>&gt;10^5 (3 samples)</td>
<td>&gt;10^5 (4 samples)</td>
<td>2 × 10^4 and 6 × 10^4 (2 samples)</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>Heavy growth of</td>
<td>Heavy growth of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>group D2 per ml</td>
<td>Corynebacterium</td>
<td>Corynebacterium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stone</td>
<td>group D2</td>
<td>group D2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Tetracycline;</td>
<td>Tetracycline;</td>
<td>Erythromycin;</td>
<td>Tetracycline</td>
</tr>
<tr>
<td></td>
<td>ammonium</td>
<td>acetic acid</td>
<td>rifampin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>chloride; citric</td>
<td>; citric acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>acid</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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* All patients suffered symptoms of urinary tract infections after the urological procedure, but encrusted cystitis (e.c.) was diagnosed several months later.

* Plus surgery.

* Topical treatment.
of inhibition (ampicillin, cephalothin, and gentamicin) for comparison in the agar dilution test.

Table 3 shows the MICs of the nine selected antimicrobial agents against the four corynebacteria and control strains. The most active compounds were vancomycin, norfloxacin, and novobiocin, followed by tetracycline and rifampin. The higher pH decreased the activity of most antimicrobial agents except gentamicin and erythromycin; however, all strains were resistant to gentamicin, and only two were sensitive to erythromycin. Beta-lactamase activity was negative in all corynebacteria by the nitrocefin test.

**DISCUSSION**

Papers dealing with human infections caused by *Corynebacterium* group D2 are very rare, and we have no knowledge of reports involving these microorganisms as a cause of alkaline-encrusted cystitis. This disease is a serious condition that could be produced by various urea-splitting microorganisms (5, 10), but we believe this is the first report involving gram-positive bacilli, in this case, *Corynebacterium* group D2. All of our patients suffered from a very severe encrusted cystitis, and only these microorganisms were isolated from repeated urine cultures and stones obtained by cystoscopy. No other microorganisms were isolated from these specimens, and all available information (4, 12) suggests that infection by urea-splitting bacteria is the most likely explanation for the presence of ammonium magnesium phosphate crystals in alkaline urines. No other reasons seem to explain the high pH of the specimens, clinical improvement, disappearance of struvite crystals, and normalization of the urine pH when urine samples were cleared of these bacteria. For all of these reasons, we think that, in our patients, *Corynebacterium* group D2 was a very important factor causing or maintaining alkaline-encrusted cystitis in a previously damaged bladder. We do not know the actual circumstances in which infection by *Corynebacterium* group D2 took place. All patients had underlying diseases for which they had been instrumented and suffered subsequently from chronic or recurrent urinary tract infections.

*Corynebacterium* group D2 was as previously described by King (7); the morphological, cultural, biochemical, and antimicrobial susceptibility characteristics resemble those of *Corynebacterium* group JK. The urease activity and inability to acidify glucose of the former are the main differential characteristics from the better known group JK. All of these microorganisms are fastidious, so we lengthened the observation of the urine cultures up to 48 to 72 h, especially in patients with alkaline urines and struvite crystals in a freshly observed sample since infection with urea-splitting microorganisms was the most likely explanation. The microorganisms involved in all of our patients had strong and rapid

![FIG. 1. Kinetic study of the ureolytic activity of five bacterial strains in Christensen urea broth (inoculum of 5 × 10⁶ to 1.5 × 10⁹ CFU/ml). Symbols: ×, *E. coli* ATCC 25922; ○, *K. pneumoniae* ATCC 23357; △, *P. vulgaris* ATCC 6380; ♦, *Corynebacterium* group D2 (strain 1); and ⊕, *Corynebacterium* group D2 (strain 2).](image1)

![FIG. 2. Kinetic study of five bacterial strains in Christensen urea broth: relationship between bacterial counts and hours of incubation. Initial pH was 6.7; final pH is given in parentheses. Symbols are as defined in the legend to Fig. 1.](image2)

**TABLE 2. Antimicrobial susceptibility of four strains of *Corynebacterium* group D2 by the disk diffusion test**

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Disk potency (µg)</th>
<th>Zone of inhibition (mm) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain 1</td>
<td>Strain 2</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Rifampin</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Pipemidic acid</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>300</td>
<td>12</td>
</tr>
</tbody>
</table>

*No zone of inhibition was obtained with benzylpenicillin (6 µg), ampicillin (10 µg), ticarcillin (75 µg), azlocillin (75 µg), cephalexin (30 µg), cefotaxime (50 µg), cefotaxime (30 µg), cefazidime (30 µg), cephalosporin (50 µg), gentamicin (10 µg), amikacin (30 µg), colistin (10 µg), nalidixic acid (30 µg), sulfadiazine (300 µg), cotrimoxazole (25 µg), and metronidazole (5 µg).*
ADDENDUM

We sincerely thank Robert E. Weaver, Special Bacteriology Section, Centers for Disease Control, Atlanta, Ga., who identified the first three strains isolated from our patients as Corynebacterium vulgari.

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LITERATURE CITED