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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-xylose, mannitol, lactose, sucrose, maltose 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 5×10^5 to 1.5×10^6 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

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

Characteristic	Patient no.			
	1	2	3	4
Age (years)	77	74	9	77
Sex	Male	Female	Male	Male
Underlying condition	Prostatic adenoma	Bladder carcinoma	Ectopic kidney	Bladder carcinoma
Urological procedure	Transurethral resection	Cistostomy; radiotherapy	Ascendent pyelography	Cistostomy; radiotherapy
Time since the urological procedure and e.c. symptoms[a]	5 months	19 months	36 months	14 months
Microbiological data				
Urine (CFU of *Corynebacterium* group D2 per ml)	$>10^5$ (3 samples)	$>10^5$ (3 samples)	$>10^5$ (4 samples)	2×10^4 and 6×10^4 (2 samples)
Stone	Heavy growth of *Corynebacterium* group D2	Heavy growth of *Corynebacterium* group D2		
Treatment[b]	Tetracycline; ammonium chloride; citric acid[c]	Tetracycline; acetic acid[c]; citric acid[c]	Erythromycin; rifampin	Tetracycline

[a] All patients suffered symptoms of urinary tract infections after the urological procedure, but encrusted cystitis (e.c.) was diagnosed several months later.
[b] Plus surgery.
[c] Topical treatment.

and without 1.9-mg/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), cephalothin (Lilly), erythromycin (Abbott), rifampin (Lepetit), tetracycline (Pfizer), vancomycin (Dista), novobiocin (Merck Sharp and Dohme), norfloxacin (Liade), 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 acetohydroxamic 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 acetohydroxamic 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

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 nitrocephin 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.

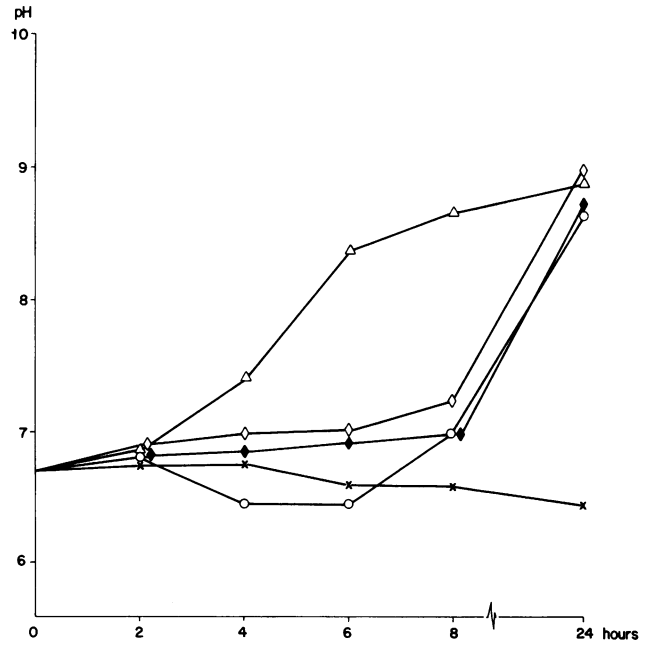

FIG. 1. Kinetic study of the ureolytic activity of five bacterial strains in Christensen urea broth (inoculum of 5×10^5 to 1.5×10^6 CFU/ml). Symbols: ×, *E. coli* ATCC 25922; ○, *K. pneumoniae* ATCC 23357; △, *P. vulgaris* ATCC 6380; ◆, *Corynebacterium* group D2 (strain 1); and ◇, *Corynebacterium* group D2 (strain 2).

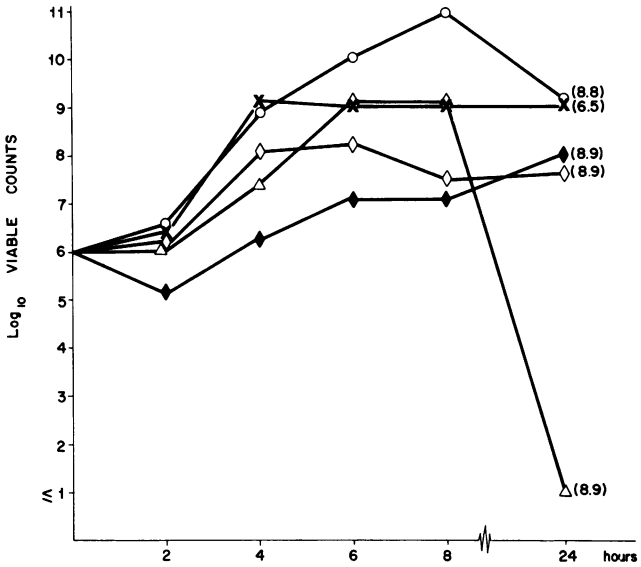

FIG. 2. Kinetic study of five bacterial strains in Christensen urea broth: relationship between bacterial counts and hours of incubation. Initial pH was 6.7; final pH is given in parentheses. Symbols are as defined in the legend to Fig. 1.

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

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

Antimicrobial agent	Disk potency (μg)	Zone of inhibition (mm) in:			
		Strain 1	Strain 2	Strain 3	Strain 4
Vancomycin	30	28	25	34	30
Tetracycline	30	16	20	34	23
Erythromycin	15	36	6	40	6
Clindamycin	2	28	6	30	6
Rifampin	2	10	12	30	6
Novobiocin	30	30	30	16	32
Pipemidic acid	20	12	13	10	6
Norfloxacin	10	24	26	30	32
Nitrofurantoin	300	12	10	15	12

[a] No zone of inhibition was obtained with benzylpenicillin (6 μg), ampicillin (10 μg), ticarcillin (75 μg), azlocillin (75 μg), cephalothin (30 μg), cefoxitin (30 μg), latamoxef (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), amikacin (30 μg), colistin (10 μg), nalidixic acid (30 μg), sulfadiazine (300 μg), co-trimoxazole (25 μg), and metronidazole (5 μg).

TABLE 3. MICs (μg/ml) of nine antimicrobial agents at two pH values (7.4 and 8.5) against four strains of *Corynebacterium* group D2

Sample	MIC (μg/ml) of:																	
	Ampicillin		Cephalothin		Erythromycin		Rifampin		Tetracycline		Vancomycin		Novobiocin		Norfloxacin		Gentamicin	
	7.4	8.5	7.4	8.5	7.4	8.5	7.4	8.5	7.4	8.5	7.4	8.5	7.4	8.5	7.4	8.5	7.4	8.5
Corynebacterium strain no. 1	>1,024	>1,024	512	1,024	0.06	≤0.03	4	64	16	128	0.5	2	0.5	8	0.5	1	>1,024	512
Corynebacterium strain no. 2	1,024	1,024	1,024	>1,024	1,024	1,024	4	64	4	64	0.5	4	0.5	8	1	1	>1,024	512
Corynebacterium strain no. 3	>1,024	>1,024	256	256	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≤0.25	1	32	512	1	2	>1,024	>1,024
Corynebacterium strain no. 4	256	512	128	512	1,024	512	8	64	16	64	0.5	2	≤0.25	8	1	2	>1,024	1,024
Escherichia coli ATCC 25922	4	8	8	16	—[a]	—	—	—	—	—	—	—	—	—	—	—	0.5	0.5
Staphylococcus aureus ATCC 25923	—	—	—	—	0.25	≤0.25	≤0.25	≤0.25	0.25	1	1	4	≤0.25	1	2	2	—	—

[a] —, Not tested.

urease activity, at least as strong as the *P. vulgaris* studied. Furthermore, *Corynebacterium* group D2 seems to be more alkali resistant than *P. vulgaris* and *K. pneumoniae* because a higher viability was obtained with corynebacteria at pH 10.0 than with the gram-negative bacilli (data not shown).

Acetohydroxamic acid showed an effective and interesting activity against the urease of *Corynebacterium* group D2. Griffith et al. (2, 3) showed that this drug was an effective inhibitor of some bacterial ureases in vitro and in vivo, had a bacteriostatic effect against many pathogenic gram-negative bacteria (2), and may be synergistic with some antibiotics (8). Our data show that acetohydroxamic acid inhibits urease of *Corynebacterium* group D2, thus lowering the pH of the urine and possibly allowing better environmental conditions for the antibiotics to act.

Acetohydroxamic acid at a 1.9-mg/ml concentration did not inhibit a high inoculum of *Corynebacterium* group D2 but showed some inhibitory activity with lighter inocula (unpublished data). Only vancomycin and norfloxacin were uniform and very active against the four corynebacteria studied, followed by rifampin, novobiocin, erythromycin, and tetracycline. All of the strains were resistant to ampicillin, cephalothin, and gentamicin and did not produce beta-lactamase. Most of these results agree with those obtained by Kelly et al. (6), except for the erythromycin-clindamycin susceptibility. Because we were very interested in the treatment of urinary tract infections and encrusted cystitis, we studied norfloxacin, which showed excellent antimicrobial activity against these multiply and highly resistant corynebacteria. Most of the antimicrobial agents tested, including vancomycin and norfloxacin, were less active at high pH, which is a great inconvenience due to the fact that all of the urinary tract infections produced by these microorganisms occurred with alkaline urines. Nevertheless, norfloxacin showed a marked activity against these corynebacteria. Treatment of encrusted cystitis often requires a cystoscopic resection of the encrusted stones. Antimicrobial agents must be used according to the susceptibility studies of the offending microorganisms and must take into account the high pH in which these drugs have to work. The use of acidifiers and inhibitors of the bacterial enzyme urease, such as acetohydroxamic acid, may be necessary to achieve better clinical results. Clinical assay of the efficacy of norfloxacin combined with acetohydroxamic acid in these patients is now in progress.

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ADDENDUM

After this manuscript was submitted, a report by Takebe et al. (J. Clin. Microbiol. **20**:869–873, 1984) was published regarding stone formation by *Ureaplasma urealyticum* in human urine. Although we did not investigate *Ureaplasma* in the clinical specimens obtained from our patients, we have observed that *Corynebacterium* group D2 produces struvite crystals when inoculated into sterile normal human urine (*Ureaplasma*-free), with an increase in pH of the infected urine (manuscript in preparation).

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