Characterization of an Unusual Strain of Proteus rettgeri Associated with an Outbreak of Nosocomial Urinary-Tract Infection

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An outbreak of nosocomial urinary-tract infection was caused by a strain of Proteus rettgeri that fermented lactose overnight and was resistant to all antimicrobial drugs tested. The nonmotile isolates shared an O (somatic) antigen that differed from those of wild-type P. rettgeri. The organisms proved markedly serum-sensitive. In rats, the isolates elicited an acute interstitial nephritis with associated transient bacteriuria. Attempts to transfer the lac\textsuperscript{+} trait and drug-resistance markers to recipient strains of Escherichia coli K-12 failed; exposure of the isolates to acridine orange yielded small numbers of non-lactose-fermenting variants which, however, were still as drug-resistant as before. Epidemiological studies failed to uncover the source of this unique strain and appeared to indicate exogenous spread of infection.

Between April 1969 and September 1970, a total of 51 patients, all but 6 of whom were of the same surgical ward, contracted nosocomial urinary-tract infection due to an unusual, that is, promptly lactose-fermenting and multiple-drug-resistant, strain of Proteus rettgeri (31). The organism appeared to colonize the urinary tract of roughly two-thirds of the patients; the remainder of the patients revealed moderate fever as the only sign other than bacteriuria indicative of urinary-tract infection. The majority of patients had been hospitalized for extended periods of time; many of them had indwelling catheters, and most of the patients had received various antimicrobial drugs prior to acquisition of infection with the organism. In this report, we describe several features of this unique strain of P. rettgeri.

MATERIALS AND METHODS

Bacteria. Seven isolates of "wild-type"* P. rettgeri from various clinical sources (designated P. rettgeri I to VII) and 15 isolates of the unusual strain of P. rettgeri were studied. Among the latter isolates, all of which were from urinary-tract specimens, 12 fermented lactose overnight (designated LF P. rettgeri 1 to 12) and 3 failed to ferment lactose (coded NLF P. rettgeri 13 to 15). The organisms were identified according to the usual criteria (9, 33) and were maintained on Brain Heart Infusion agar slants at 4 C.

Media. Nutrient broth, Brain Heart Infusion broth, Mueller Hinton broth, and tryptic soy broth were purchased from Difco, as were Mueller Hinton agar, MacConkey agar with added crystal violet, eosin-methylene blue agar, Casman's base (in 5% sheep blood-agar), and Brain Heart Infusion agar. MacConkey agar without crystal violet was procured from Colab Laboratories, Inc., Chicago Heights, Ill.

Antibiotics. Stock solutions of the antimicrobial drugs sodium ampicillin (Bristol Laboratories, Syracuse, N.Y.), carbenicillin (Beecham Pharmaceuticals, Clifton, N.J.), cephalothin (Eli Lilly & Co., Indianapolis, Ind.), chloramphenicol (Parke, Davis & Co., Detroit, Mich.), gentamicin sulfate (Schering Corp., Bloomfield, N.J.), kanamycin sulfate (Bristol Laboratories), nalidixic acid (Sterling-Winthrop Research Institute, Rensselaer, N.Y.), polymyxin B, and tetracycline hydrochloride (Pfizer Laboratories, New York, N.Y.) were prepared in distilled water (nalidixic acid was dissolved in 0.1 N NaOH), ultramembrane-filtered (0.2-\mu m membrane filters; Nalge Sybrion Corp., Rochester, N.Y.) for sterilization, and frozen and kept stored at \(-65\) C.

Antimicrobial drug susceptibility tests. The standardized method of Bauer et al. (3) was employed for disc susceptibility tests. For quantitative drug-sensitivity determinations, tube broth dilution tests (30) and the microtiter procedure (32) were performed in Mueller Hinton broth with bacterial inocula adjusted to yield approximately 1.5 \times 10^6 organisms/ml at zero time. The minimal inhibitory concentration (MIC) of a drug was defined as the lowest concentration of drug that completely inhibited growth at 35 C for 16 to 18 hr, as judged by visual inspection. The minimal bactericidal concentration (MBC) of an antimicrobial drug represented the lowest concentration of drug that yielded no colonies from 3-mm
loopful samples streaked from clear tubes or wells to quarter sectors of 5% sheep blood-agar plates, which were incubated at 35 C for 18 hr. A strain of Escherichia coli of known antibiotic susceptibility (E. coli ATCC 25922) served as a control for all drug-sensitivity tests performed.

To screen for possible additive or synergistic effects of various pairs of antimicrobial drugs, strips of sterile Whatman no. 1 filter paper (5 by 0.5 cm) were soaked in respective stock solutions and placed at right angles to one another on Mueller Hinton agar plates (100 by 15 mm) that had been streaked with the isolates under study (26).

Serological tests. New Zealand White rabbits (two rabbits per isolate) were hyperimmunized with LF P. rettgeri isolates 2, 4, 6, 8, and 10, according to the technique of Roschka (20). The bacteria were grown on Brain Heart Infusion agar slants for 24 hr at 35 C and removed with sterile 0.15 M saline. The suspensions were boiled for 2 hr and centrifuged at 2,000 x g for 15 min. The sediments were suspended in 95% ethanol and incubated at 35 C for 4 hr. The suspensions were centrifuged, washed twice with acetone, and dried overnight at 35 C. The dry powders were transferred to screw-capped test tubes and kept at room temperature. For injection, the powders were suspended in sterile 0.15 M saline at 4-day intervals. Blood was obtained through cardiac puncture on the 5th day after the last injection. The collected rabbit blood specimens as well as blood specimens from patients were processed as described previously (16). The sera were heat-inactivated at 56 C for 30 min, after which the respective pairs of sera were pooled, frozen, and kept stored at -65 C. Immediately prior to use, the sera were exposed to 56 C for 10 min.

For control purposes, rabbit hyperimmune sera were absorbed with homologous and heterologous LF P. rettgeri O antigen preparations according to the technique of Kopeloff and Kopeloff (14).

For screening purposes, slide O agglutination tests were performed (9); tube O agglutination tests were carried out and interpreted in accordance with the technique of Felix and Bensted (11). For indirect (passive) hemagglutination (IHA) tests, sheep red cells were sensitized according to the method of Neter et al. (19); the microtiter modification of Lee et al. (15) served for titration of the rabbit hyperimmune sera.

Tests for susceptibility to the bactericidal activity of human serum. Tests to determine the susceptibility of the organisms to the bactericidal activity of human serum were performed and interpreted according to previously published procedures (16, 34).

Attempts to characterize the lac+ trait and multiple-drug resistance of LF P. rettgeri isolates as episomally mediated. The method of Anderson and Lewis (2) was used in attempts to demonstrate bacterial conjugation and transfer of resistance to carbenicillin, chloramphenicol, gentamicin, kanamycin, polymyxin B, or tetracycline from LF P. rettgeri isolates 1 and 7 to recipient E. coli K-12 strains 1485 lac+ F− and CS100 lac− F−.

The technique of Watanabe and Fukasawa (35) was used in attempts to “cure” LF P. rettgeri isolates. The organisms were pregrown and diluted in Nutrient Broth, pH 7.6, to yield 104, 103, and 102 organisms/ml at zero time; these were exposed to 100, 50, 25, 12.5, and 6.25 µg of acridine orange (Fisher Scientific Co., Raleigh, N.C.) per ml. The growth obtained was subcultured to MacConkey agar, and the plates were examined for the appearance of NLF colonies. These colonies were subcultured, examined biochemically and serologically, and tested for antibiotic disc susceptibility. Randomly chosen LF colonies likewise were tested for antibiotic sensitivity.

Animal, pathogenicity studies. Female Holtzman (Sprague-Dawley) rats, which weighed 200 to 250 g, were inoculated in their left kidney (5). Groups of four rats each were infected with approximately 7.5 x 104 organisms of LF P. rettgeri isolates 7 and 9, and NLF isolates 13 and 15. Two rats received P. rettgeri III (positive controls), and two rats were inoculated with sterile 0.15 M saline (negative controls).

Urine was aseptically aspirated from the exposed urinary bladders of the animals immediately after lethal anesthesia (methoxyurane). Urine samples were plated semiquantitatively on 5% sheep blood-agar and MacConkey agar (undiluted, one 3-mm loopful; 1:1,000 dilution, one 1:1,000 calibrated loopful). The kidneys of each animal were removed aseptically and processed as follows: one (longitudinal) half of each kidney was ground in 0.15 M saline in glass mortars. The homogenates were plated semiquantitatively as above; all organisms isolated were fully identified. The remaining halves of the kidneys were placed into buffered neutral Formalin for fixation; after routine hematoxylin and eosin staining (17), sections were examined for microscopic evidence of nephritis.

RESULTS

Identification. The LF and NLF P. rettgeri isolates under study grew readily on all media employed. The isolates were characterized by an unmistakable odor, not unlike that of cheese-crackers. The isolates produced a metallic sheen on eosin-methylene blue agar. The isolates gave the following biochemical reactions that were consistent with P. rettgeri: deamination of phenylalanine, hydrolysis of urea, utilization of citrate, late fermentation of inositol, lack of lysine and ornithine decarboxylase, and lack of gas formation during fermentation of glucose, with the exception of NLF isolates 13 to 15, which produced less than 10% gas in Durham's tubes in glucose broth (Table 1). All isolates were nonmotile. The NLF isolates thus differed from their LF counterpart in that they lacked the lac+ trait and produced small amounts of gas in glucose. The wild-type P. rettgeri isolates yielded typical reactions. The identity of LF P. rettgeri isolates 1, 3, 5, 7, and 8 was confirmed as that of promptly lactose-fermenting P. rettgeri by W. H. Ewing of
the Center for Disease Control, Atlanta, Ga. (personal communication).

**Serological studies.** All LF and NLF isolates were agglutinated by the five rabbit anti-O hyperimmune sera when tested by the slide agglutination technique. The titers of the hyperimmune sera ranged from 1:2,048 to 1:4,096 against homologous as well as against heterologous isolates as revealed by the tube O agglutination test. With the IHA procedure, the titers of the five antisera ranged from 1:5,120 to 1:40,960; again, the titers of the antisera against homologous isolates compared favorably with those against heterologous isolates. Absorption of the rabbit hyperimmune sera with heat-killed, ethanol- and acetone-extracted cells from homologous and heterologous isolates reduced the IHA titers to less than 1:80. None of the five rabbit hyperimmune sera agglutinated any of the seven wild-type P. rettgeri isolates examined. None of the sera obtained from five proven bacteriuric patients yielded titers greater than 1:32, as determined with the IHA procedure.

Several of the LF P. rettgeri isolates were exposed to fresh serum from homologous and heterologous patients as well as to fresh serum from a presumably healthy adult donor. As shown in Table 2, the isolates proved markedly sensitive to the bactericidal activity of fresh human serum.

**Antibiotic susceptibility tests.** It was found that LF P. rettgeri isolates 1 to 12 were resistant to all antimicrobial drugs examined (Table 3). However, NLF P. rettgeri isolates 13 and 14 proved susceptible to carbenicillin, gentamicin sulfate, kanamycin sulfate, nalidixic acid, and triple sulphonamide. In contrast, NLF isolate 15 was as resistant as the LF isolates.

The LF isolates and NLF isolate 15 tolerated greater than 100 µg of ampicillin, cephalothin, chloramphenicol, kanamycin, nalidixic acid, and tetracycline per ml; the isolates were inhibited by 100 µg of polymyxin B per ml, but were not inhibited by carbenicillin at concentrations up to 500 µg/ml. All LF isolates were resistant to gentamicin, the concentrations of the drug required for inhibition ranging from 25 to 50 µg/ml. An exception was LF isolate no. 3, which proved borderline-resistant, in that this isolate was inhibited by 12 µg of gentamicin per ml. This particular isolate yielded a zone of inhibition 11 mm in diameter around gentamicin discs; the other LF isolates yielded zones of inhibition ranging from 10 to 13 mm in diameter. J. A. Waitz of the Schering Corp., Bloomfield, N.J., likewise found that the gentamicin concentrations required to inhibit the LF isolates ranged from 30 to greater than 50 µg/ml (personal communication). All drug combinations examined with the filter-strip method proved indifferent in activity.

**Bacterial conjugation: exposure to acridine**

| TABLE 1. Biochemical identification of LF, NLF, and wild-type P. rettgeri isolates |
|----------------------------------|------------------|------------------|------------------|
| Test employed                    | LF P. rettgeri   | NLF P. rettgeri  | Wild-type P. rettgeri |
| Motility                         | -                | -                | +                |
| 35 C                             | -                | -                | +                |
| 25 C                             | -                | -                | +                |
| Indole                           | +                | +                | +                |
| Methyl red reaction              | +                | +                | +                |
| Acetoin (Voges-Proskauer reaction)| -                | -                | -                |
| Citrate utilization              | +                | +                | +                |
| Urease                           | +                | +                | +                |
| Phenylalanine deaminase          | +                | +                | +                |
| Lysine decarboxylase             | -                | -                | -                |
| Ornithine decarboxylase          | -                | -                | -                |
| Glucose                          | Acid only        | Acid, less than 10% gas | Acid only |
| Lactose                          | +                | -                | -                |
| ONPG hydrolysis                  | +                | -                | -                |
| Mannitol                         | +                | +                | +                |
| Maltose                          | -                | -                | -                |
| Arabinose                        | -                | -                | -                |
| Inositol                         | + (48 hr)        | + (48 hr)        | + (24 hr)        |
| Gelatin liquefaction             | -                | -                | -                |
| Nitrate reduction                | +                | +                | +                |
| H₂S (Kligler iron agar)          | -                | -                | -                |
| Cytochrome oxidase               | -                | -                | -                |
| O-F test (Hugh-Leifson)          | F(fermentation)  | F                | F                |
Table 2. Susceptibility of LF P. rettgeri isolates to the bactericidal activity of fresh human serum

| Time after exposure to serum (min) | LF P. rettgeri 6 exposed to fresh serum of patient no. 6 | LF P. rettgeri 5 exposed to fresh serum of patient no. 4 | LF P. rettgeri 5 exposed to T-serum |
|-----------------------------------|-----------------------------------------------------|-----------------------------------------------------|---------------------------------|
|                                   | MIC (μg/ml)                                          | MIC (μg/ml)                                          | Heat-inactivated                |
|                                   | Zone size (mm)                                       | Zone size (mm)                                       |                                 |
| 0                                 | 2.6 × 10^3a                                         | 2.8 × 10^3                                          | 3.1 × 10^3                      |
| 45                                | 5.0 × 10^3                                          | 5.0 × 10^3                                          | 1.0 × 10^4                      |

* Numbers listed indicate number of colony-forming units per milliliter (survivors).

Table 3. Results of antibiotic-susceptibility tests

| Antimicrobial drug | LF isolates 1-12; NLF isolate 15 | NLF isolates 13 and 14 | Control E. coli ATCC 25922 |
|--------------------|-----------------------------------|------------------------|---------------------------|
|                    | Zone size (mm)        | MIC (μg/ml) | Zone size (mm)        | MIC (μg/ml) | Zone size (mm)        | MIC (μg/ml) |
| Amoxicillin (10)*  | 6^                    | > 100        | 6                       | > 100       | 18                      | 6           |
| Carbenicillin (30) | 6                     | > 500        | 26                       | < 15        | 25                      | 8           |
| Cephalothin (30)   | 6                     | > 100        | 6                       | > 100       | 19^                    | 3           |
| Chloramphenicol (30) | 10-13^                 |             | 15                       | < 6         | 22                      | 0.8         |
| Gentamicin (10)    | 6                     | > 100        | 23                       | < 12        | 22                      | 6           |
| Kanamycin (30)     | 6                     | > 100        | 26                       | < 3         | 24                      | 3           |
| Neomycin (30)      | 6                     |             | 12                       | < 20        | 22                      | < 3         |
| Nitrofurantoin (30)| 6                     |             | 6                        | < 22        | 22                      | < 2         |
| Polymyxin B (30)   | 6                     | 100          | 6                        | 100         | 15                      | 0.6         |
| Streptomycin (10)  | 6                     | > 100        | 6                        | > 100       | 18                      | 3           |
| Tetracycline (30)  | 6                     | > 100        | 6                        | > 100       | 21                      | 0.8         |
| Triple sulfonamide (300) | 6               |             | 28                       |             | 21                      |             |

* Numbers in parentheses indicate drug content of discs.
^ The disc measured 6 mm in diameter; therefore, readings of 6 mm indicate no zone of inhibition.
+ The isolates yielded zones of inhibition of varying diameter around gentamicin discs.
= LF isolate no. 3 required 12 μg of gentamicin/ml for inhibition; the remainder of the isolates were characterized by minimal inhibitory concentrations ranging from 25 to 50 μg/ml.
# Control Staphylococcus aureus ATCC 25923 yielded a zone of inhibition of 34 mm in diameter and was inhibited by 0.2 μg of cephalothin/ml.
\* Denotes not determined.

orange. Attempts to transfer multiple-drug resistance from LF P. rettgeri isolates to recipient cells of E. coli K-12 failed invariably. A selected number of LF P. rettgeri isolates were exposed to acridine orange at concentrations ranging from 100 to 6.25 μg/ml (Table 4). Exceedingly small numbers of NLF colonies were obtained from LF isolate 1 after exposure to 50 μg of acridine orange per ml; these proved o-nitrophenyl-β-D-galactoside (ONPG)-negative, yet still had the characteristic odor, were positive for urease and phenylalanine deaminase, were O-agglutinable, and produced less than 10% gas in glucose; they revealed the same disc antibiogram as the parent strain. However, the sole NLF variant obtained from LF isolate 11 after exposure to 25 μg of acridine orange per ml was ONPG-positive, but failed to ferment lactose overnight; in all other respects, it was identical to the previous NLF variants. All LF colonies examined after exposure to acridine orange proved as multiple-drug resistant as before.

Animal pathogenicity studies. Experiments with left intrarenally inoculated rats were performed to learn more about the predilection of this particular strain for the mammalian urinary tract. The control rats inoculated with 0.15 m saline, which were sacrificed on the 15th and 29th days after inoculation, yielded sterile and morphologically unremarkable kidneys. Of the two positive control rats that had been inoculated with wild-type P. rettgeri III, one animal, which was killed on day 15, revealed no renal lesions; however, the homogenates of both kidneys yielded 5 × 10^4 colonies of P. rettgeri III. The other control animal, which was examined on day 29 after injection, had a morphologically normal right kidney. The left kidney, however, appeared
extensively inflamed. Several irregularly shaped areas of the renal parenchyma, which were not confined to the path of the inoculating needle, showed evidence of acute interstitial nephritis (13); the arteries, arterioles, and glomeruli were not involved, nor was the renal pelvis affected. Those rats which had been inoculated with LF \( P. \text{rettgeri} \) isolates 7 and 9 revealed left renal morphological changes, which did not appear as severe; however, tubular white cell casts were prominent. The wedge-shaped areas of inflammation had developed along the path of the inoculating needle and were sharply demarcated from the surrounding healthy parenchyma. The left kidney of these animals remained bacteriologically positive for 8 days; subsequent cultures were negative. Those rats that had been injected with NLF \( P. \text{rettgeri} \) isolates 13 and 15 had positive urine cultures on the 15th day after inoculation. One rat that had received NLF \( P. \text{rettgeri} \) 13 yielded greater than \( 10^0 \) organisms/ml of urine, and the homogenates of both kidneys gave colony counts in excess of \( 10^6 \) organisms/ml. The left kidney showed evidence of acute interstitial nephritis; in addition, the right kidney of this particular animal revealed inflammatory involvement of the renal pelvis suggestive of contralateral ascending urinary-tract infection. Urine and kidney homogenate cultures were negative on days 22 and 29 after injection.

**Epidemiological studies.** Epidemiological studies failed to detect the source of this particular strain of \( P. \text{rettgeri} \). As to the reservoirs of this organism, it was found that the utility room, used for emptying and cleansing of bedpans, was grossly contaminated (sink, floor, bedpans, bedpan hopper, and air). Ward air samples were negative, as were the unused catheter kits tested. None of the ward personnel, including physicians, carried this organism; all stool, perineal, and hand skin cultures examined were negative. Experimentally, the organism was transferred to the hands of the epidemiology surveillance nurse after brief manipulation of the catheter tubing and collection bag of a bacteriuric patient; however, simple soap-washing (Dial soap) rendered the hands free from this organism. Only 4 of 12 examined patients with bacteriuria due to this organism yielded positive stool cultures.

**DISCUSSION**

This unique strain of \( P. \text{rettgeri} \) had a number of traits compatible with \( E. \text{coli} \), such as prompt fermentation of lactose, production of a metallic sheen on eosin-methylene blue agar, and, in the case of the three NFL variants, production of gas in glucose. However, the majority of the biochemical reactions of this organism were found to be consistent with those of \( P. \text{rettgeri} \), e.g., hydrolysis of urea, deamination of phenylalanine, utilization of citrate, fermentation of inositol, and lack of lysine and ornithine decarboxylases. Furthermore, several of the isolates of this particular strain proved susceptible to the species-specific activity of bacteriocins elaborated by wild-type \( P. \text{rettgeri} \) isolates III and VI, after induction with mitomycin C (M. E. Craddock and W. H. Traub, Experientia, in press). Thus, we choose to designate this organism a promptly lactose-fermenting strain of \( P. \text{rettgeri} \) (21, 24, 25, 28, 29).

Unfortunately, our attempts aimed at demonstration of transfer of the lac\(^+\) trait and antibiotic-resistance markers from the isolates to recipient \( E. \text{coli} \) failed; the small numbers of NFL variants obtained after exposure of LF isolates to acridine orange are not indicative of "cure" of this trait, since these variants might have arisen through spontaneous or acridine orange-induced mutation. Thus, the data obtained do not allow one to state with any degree of certainty whether these traits are chromosomal or episomal in nature (25). The isolates of this unusual strain proved pathogenic for the urinary tract of rats, as revealed by the resultant interstitial nephritis with associated transient bacteriuria. Only in one animal was there evidence of right renal pelvic

### Table 4. Non-lactose-fermenting variants obtained through exposure of LF \( P. \text{rettgeri} \) isolates to acridine orange

| LF \( P. \text{rettgeri} \) isolate | No. of organisms/ml at zero time | Acridine orange (µg/ml) |
|----------------------------------|---------------------------------|------------------------|
| 1                                |                                 | 0 (control)            |
|                                   |                                 | 12.5                   |
|                                   |                                 | 25                     |
|                                   |                                 | 50                     |
|                                   |                                 | 100                    |
| 10\(^4\)                          |                                |                        |
| No growth                        | 32 LF                           | >200 LF                |
| 10\(^3\)                          |                                |                        |
| No growth                        | No growth                       | >200 LF                |
| 11                                |                                 | 0 (control)            |
|                                   |                                 | 12.5                   |
|                                   |                                 | 25                     |
|                                   |                                 | 50                     |
|                                   |                                 | 100                    |
| 10\(^4\)                          |                                |                        |
| 11 LF                            | 11 LF                           | >200 LF                |
| 10\(^3\)                          |                                |                        |
| 2 LF                             | >200 LF                         | >200 LF                |
| 10\(^3\)                          | No growth                       | >200 LF                |
|                                   |                                 | 1 NLF, >200 LF         |
|                                   |                                 | >200 LF                |

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involved, a finding suggestive of ascending urinary-tract infection.

Although this unique strain carried epidemiological markers that facilitated its isolation, our studies failed to disclose the source of this organism. Nor were we able to define precisely the mode of acquisition of urinary-tract infection during this outbreak. The epidemiological data suggested exogenous spread of infection, that is, transmission through contaminated bed utensils, and, possibly, through contaminated hands of ward personnel (1, 4, 6–8, 10, 12, 18, 22, 23, 27).

On the basis of indirect evidence, this strain of *P. rettgeri* was interpreted to colonize the urinary tract of most of the patients involved. First, the isolates gave rise to asymptomatic bacteriuria in the majority of patients; only a third of the patients had low-grade fever as the only additional sign of urinary-tract infection. Second, none of the patient sera tested had titers greater than 1:32 as determined with the IHA technique.

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**LITERATURE CITED**

1. Adler, J. L., J. P. Burke, and M. Finland. 1971. Infection and antibiotic usage at Boston City Hospital, January 1970. Arch. Intern. Med. 127:460–465.
2. Anderson, E. S., and M. J. Lewis. 1965. Characterization of a transfer factor associated with drug resistance in *Salmonella typhimurium*. Nature (London) 206:843–849.
3. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 45:493–496.
4. Burke, J. P., D. Inagaki, J. O. Klein, H. M. Gezon, and M. Finland. 1971. *Proteus mirabilis* infections in a hospital nursery traced to a human carrier. N. Engl. J. Med. 284:115–121.
5. Burrous, S. E., and J. B. Cawein. 1969. Rat pyelonephritis model suitable for primary or secondary screening. Appl. Microbiol. 16:448–451.
6. Desautel, B. E. 1969. The causes of catheter-induced urinary infections and their prevention. J. Urol. 101:757–760.
7. Dutton, A. A. C. and M. Ralston. 1957. Urinary tract infection in a male urological ward. With special reference to the mode of infection. Lancet 1:115–119.
8. Ebedo, L., and G. Laurel. 1958. Hospital infection of the urinary tract with *Proteus*. A clinical-bacteriologic study with special reference to modes of infection. Acta Pathol. Microbiol. Scand. 43(Suppl. 126):93–105.
9. Edwards, P. R., and W. H. Ewing. 1962. Identification of Enterobacteriaceae, 2nd ed. Burgess Publishing Co., Minneapolis.
10. Eichhoff, T. C., P. S. Brachman, J. V. Bennett, and J. F. Brown. 1969. Surveillance of nosocomial infections in community hospitals. I. Surveillance methods, effectiveness, and initial results. J. Infec. Dis. 129:305–317.
11. Felix, A., and H. Bensted. 1954. Proposed standard agglutinating sera for typhoid and paratyphoid A and B fevers. Bull. World Health Organ. 14:919–926.
12. Hardy, P. C., G. M. Ederer, and J. M. Matsu. 1970. Contamination of commercially packaged urinary catheter kits with the Pseudomonad EO-1. N. Engl. J. Med. 282:33–35.