Rapid Diagnosis of *Pseudomonas aeruginosa* Urinary Tract Infections by Radioimmunoassay

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A solid-phase radioimmunoassay designed to detect serotype 6 *Pseudomonas aeruginosa* antigens was evaluated for its ability to rapidly diagnose urinary tract infections. Twelve *P. aeruginosa* serotypes were easily differentiated in the assay from eight other gram-negative bacterial species. During log-phase growth, the assay detected antigens in culture when approximately $10^6$ or more serotype 6 *P. aeruginosa* organisms were present. Both cell-associated and solubilized antigens were detected. The assay detected antigens in 13 of 17 urine specimens which grew $>10^5$ *P. aeruginosa*, 3 of 38 which grew other gram-negative rods, and none of 83 with no growth. Two of the three positive specimens from the other gram-negative rod group probably also contained *P. aeruginosa*. No preincubation of the urine specimens was required, and results were available within 2.5 h. The assay represents an improvement over other procedures for rapidly diagnosing urinary tract infections in that it allows diagnosis by species and should be adaptable to semiautomation.

**MATERIALS AND METHODS**

**Organisms.** The *P. aeruginosa* serotypes were supplied by Thelma Murasaki, State of New York Department of Health, Division of Laboratories and Research, and George Cole, Parke-Davis and Co., Detroit, Mich. Serotypes were confirmed with commercial typing sera (*Pseudomonas aeruginosa* Serotyping Set, Difco Laboratories, Detroit, Mich.). The *Enterobacteriaceae* were urinary isolates obtained from the microbiology laboratories of the Indianapolis Veterans' Administration and Wishard Memorial Hospitals after identification by the API 20E system (Analytab Products, Inc., Carle Place, N.Y.).

**Solid-phase radioimmunoassay.** The solid-phase radioimmunoassay used was similar to that described for detecting hepatitis B surface antigen (24). Polystyrene tubes were coated with immunoglobulin G (IgG) from a rabbit immunized over a 6-week period with whole serotype 6 *P. aeruginosa* organisms in complete Freund adjuvant followed by intravenous injections with heated organisms every other week for 6 weeks. The IgG was purified on G200 Sephadex (Pharmacia Fine Chemicals, Piscataway, N.J.) (11). Coating involved incubation of 0.2 ml of IgG, 25 μg/ml in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.0), for 1 h at 37°C, followed by aspiration and rinsing three times with 0.15 M NaCl. Next, remaining protein adsorption sites were similarly coated with 5% bovine serum albumin in 0.01 M Tris-hydrochloride (pH 7.0). These tubes were stored dry at 4°C until used in assays. For assays, 0.2 ml of test solution (bacterial culture or urine specimen) was incubated in coated tubes for 1 hour at 37°C, aspirated,
and rinsed with 0.15 M NaCl. Finally, 0.2 ml of radioiodinated IgG in 5% bovine serum albumin-0.1 M Tris-0.15 M NaCl (pH 8.0), was added, incubated for 1 hour, aspirated, and rinsed. The tubes were then counted in a gamma counter. Several radioiodinated IgG preparations were used over the period of the study; the concentrations and specific activities for each preparation were adjusted to maximize sensitivity and are provided with the experimental results. The [125I]IgG was identical to the IgG used to coat the tubes. IgG was iodinated by the chloramine T procedure as modified by McConahey and Dixon (26). Assay results were expressed as the mean counts per minute of triplicate tubes for each urine specimen and quadruplicate tubes for all other specimens. Urine specimens were considered positive for antigen if the counts per minute exceeded the mean of the group of urines with no bacterial growth by three standard deviations.

Handling of urine specimens. Portions of urine submitted to the hospital microbiology laboratories were refrigerated until collected by us within 7 h. These specimens were frozen at −70°C until tested in the radioimmunoassay. Samples from 140 patients were selected to test in the assay. All specimens were tested on a single day after thawing without further incubation.

Colony counts. Colony counts on clinical urine specimens were performed by streaking 0.001 ml of urine from calibrated platinum wire loops (Scientific Products, McGaw Park, Ill.) onto MacConkey agar plates, incubating overnight at 37°C, and counting the resulting colonies. For experiments comparing radioimmunoassay results with culture colony counts, 0.01 ml of appropriate tenfold serial dilutions of culture in phosphate-buffered saline (pH 7.4) were streaked onto Trypticase soy agar, incubated overnight at 37°C, and counted.

RESULTS

Detection of antigens in P. aeruginosa broth cultures. To determine the relationship between numbers of organisms present and antigen detection, six 100-ml portions of heart infusion broth were inoculated with organisms from dilutions of an overnight broth culture of serotype 6 P. aeruginosa to produce initial concentrations ranging from 10^3 to 10^6 organisms per ml. The broths were then incubated at 37°C. Portions were removed at 2.5, 4.5, 6.5, and 8.5 h after inoculation; an additional culture was sampled at 14, 16, 18, and 20 h. Quantitative cultures and radioimmunoassay were performed on each sample. Figure 1 demonstrates that antigens were detected when between 10^5 and 10^6 colony-forming units/ml were present. Similar results were obtained when autoclaved urine was inoculated; antigens were detected at colony counts of 1.7 × 10^5/ml, but not 2.8 × 10^5/ml. Thus, for cultures tested during the log growth phase, antigen detection required approximately 10^6 colony-forming units/ml.

Specificity of the radioimmunoassay for P. aeruginosa. Overnight cultures in broth of 12 P. aeruginosa serotypes and 17 other urinary isolates were tested in the assay to determine whether the assay would detect antigens of other P. aeruginosa serotypes without significant cross-reactions with antigens from other bacterial species. The assay detected higher antigen levels in the P. aeruginosa cultures than in the cultures of Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Proteus morganii, P. mirabilis, Citrobacter freundii, Providencia stuartii, or Streptococcus faecalis (Fig. 2). In two additional similar experiments with 26 other non-Pseudomonas urinary isolates, including P. rettgeri, the 12 P. aeruginosa serotypes could be differentiated easily from the other species. Thus, although the immunoglobulins used in the assay were obtained from a rabbit immunized with serotype 6 P. aeruginosa organisms, cultures of all 12 P. aeruginosa serotypes could be distinguished from cultures of other urinary pathogens.

Cell-associated versus solubilized antigens. Heart infusion broth was inoculated with serotype 6 P. aeruginosa organisms to give approximately 10^6 colony-forming units/ml. Samples were removed at intervals over the subsequent 28 h for colony counts and antigen detec-
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Fig. 2. Detection of antigens by solid-phase radioimmunoassay in 16-h broth cultures. The assay was designed to detect serotype 6 Pseudomonas aeruginosa antigens. The height of each bar represents the mean of four tubes, and the vertical line above each bar represents one standard deviation. The P. aeruginosa serotypes correspond to the Difco serotyping system except F7, which represents Fisher-Devlin-Gnabac immunotype 7 (10). The other species were: EC, Escherichia coli; CF, Citrobacter freundii; PM, Proteus mirabilis; PS, Providencia stuartii; KP, Klebsiella pneumoniae; SM, Serratia marcescens; PMo, Proteus morganii; and SF, Strep-tococcus faecalis. C indicates uninoculated broth. The concentration of [125I]IgG used was 11.0 μg/ml, specific activity 1,025 cpm/ng. Colony counts: P. aeruginosa serotypes, 7.9 x 10^4 to 1.8 x 10^5; other species, 2.5 x 10^4 to 9.4 x 10^4.

Fig. 3. Effect of filtration to remove bacterial cells on antigen detection by radioimmunoassay. Data presented as described in the legend to Fig. 1. Serotype 6 P. aeruginosa cultures were tested with or without filtration, undiluted or diluted 1:16 in buffered saline. At each time tested, more antigen was detected in the unfiltered than the filtered culture. The concentration of [125I]IgG used was 15.0 μg/ml, specific activity 888 cpm/ng.

The samples to be tested in the radioimmunoassay were divided into two parts. One part was passed through filters with pore diameters of 0.45 μm, which removed viable organisms from the filtrate, then tested in the radioimmunoassay; the other part was tested without filtration. Samples were tested at a 1:16 dilution, as well as undiluted, to allow increments of antigen to be detected at high antigen concentrations where the antigen-binding sites may have been completely saturated in the undiluted material. Antigen was detected in both filtered and unfiltered portions (Fig. 3). However, more antigen was present in each unfiltered portion when compared to the appropriate filtered portion, indicating that antigens present on the bacterial cells, as well as antigen free in the medium, were detected in the assay. Similar results were obtained when bacteria were removed by two 30-min centrifugations at 15,000 x g, approximately twice as much antigen was present in the unspun culture as in the supernatant after centrifugation.

Diagnosis of P. aeruginosa urinary tract infection. Urine specimens were tested in the assay without incubation before being added to the antibody-coated tubes. The culture results of the 140 urine specimens tested in the assay were as follows (per milliliter): >10^5 P. aeruginosa (pure or mixed culture), 17 specimens; <10^5 P. aeruginosa, 2; >10^5 E. coli, 7; >10^5 S. marcescens, 7; >10^4 P. rettgeri, 8; >10^5 K. pneumoniae, 4; >10^4 Enterobacter species, 3; >10^4 P. mirabilis, 2; >10^4 mixed Enterobacteriaceae, 7; and no growth, 83. Figure 4 shows radioimmunoassay results for all specimens except the two which grew <10^5 P. aeruginosa per ml; 76% of the P. aeruginosa, 8% of the other gram-negative rods, and none of the no-growth specimens were positive in the assay. The two specimens that grew <10^5 P. aeruginosa per ml, not shown on Fig. 4, were negative in the radioimmunoassay. Thirteen of the 17 P. aeruginosa isolates were available for serotyping. Two of the 13 were isolated from urine specimens which were negative in the assay; the serotypes were 11 and "nontypable." Of the 11 isolates from radioimmunoassay-positive cultures, two were type 11 and one was nontypable. These results suggest that failure to detect antigens in some of the urines infected with P. aeruginosa was not related to serotype.

Of the 38 specimens which grew other gram-
negative rods, 3 were positive in the assay. Two of these three specimens came from the same patient and were obtained 28 days apart. The first grew S. marcescens and the second, P. rettgeri. Interestingly, other urine cultures obtained from this patient 6 days before and 4, 13, and 16 days after the first specimen tested in the radioimmunoassay grew ≥10⁶ P. aeruginosa per ml. The third specimen positive in the radioimmunoassay grew E. coli and P. mirabilis; an earlier specimen from this patient produced similar growth plus C. freundii. These organisms had been discarded before the radioimmunoassay was performed and could not be examined for cross-reacting antigens.

DISCUSSION

Pseudomonas aeruginosa organisms contain at least 55 antigenic substances when analyzed by various quantitative immunoelectrophoretic techniques (15). All but four of these antigens are shared by all P. aeruginosa organisms, regardless of serotype. Thus, IgG from a rabbit immunized with whole organisms of a single P. aeruginosa serotype, used in the solid-phase radioimmunoassay, detected antigens in over-night cultures of all Pseudomonas serotypes which we tested.

Ten of the 55 antigens are also found in many other gram-negative and gram-positive bacterial species (14). Because our P. aeruginosa antitype 6 antiserum contained 14 discernable precipitin arcs when tested by crossed-gel electrophoresis, as described by Hoiby (15), against a sonic extract of type 6 organisms, we felt that interspecies cross-reactions might occur in the assay. However, the assay easily distinguished cultures of such organisms from the cultures of all P. aeruginosa serotypes. This suggests either that the antigens shared by P. aeruginosa with other bacterial species are not released into the culture medium, are not present on the exterior of the cells, or are present in undetectable quantities in the other bacterial species. It is also possible that the rabbit vaccinated with the serotype 6 organisms did not make antibodies against these 10 antigens.

Approximately 10⁸ organisms/ml were necessary for the assay to detect antigens in culture during logarithmic growth. Similar numbers of type 3 pneumococci are required for detection of capsular polysaccharide during logarithmic growth in broth culture (8). Removal of whole organisms by filtration or centrifugation removed part of the detectable antigen indicating that the assay detects both cell-associated and soluble antigens. Similar results have been reported for lipopolysaccharide detection with the limulus lysate assay (29).

Antigens were not detected in 4 of the 17 urine specimens which grew >10⁶ P. aeruginosa/ml. Kass showed that approximately 80% of urines growing more than 10⁸ colonies/ml actually contain more than 10⁶ colonies/ml (18). A similar proportion of the 17 urine specimens which grew more than 10⁶ P. aeruginosa colonies/ml were positive in the radioimmunoassay; the assay-negative specimens may, therefore, have contained between 10⁵ and 10⁶ colonies/ml, which our assay may not have detected.

Three urine specimens which grew organisms other than P. aeruginosa were positive in the assay. Two of these specimens were obtained 28 days apart from a patient who was debilitated secondary to cerebrovascular disease and had an indwelling bladder catheter. His attending physicians attempted unsuccessfully during this period to eliminate his bacteriuria with multiple antibiotic changes. His urine grew P. aeruginosa on four occasions before and between the two specimens tested. These apparently false-positive specimens, therefore, probably contained P. aeruginosa organisms which were overgrown by the other organisms present. The third positive specimen grew E. cloacae and P. mirabilis.
culture 8 days earlier grew these same organisms plus C. freundii. It is possible that the assay detected cross-reacting antigens from these organisms. These organisms, unfortunately, are not available for further studies.

Several techniques have been reported for rapidly diagnosing urinary tract infections. Gram staining (29) and the limulus assay (17) allow differentiation of gram-positive from gram-negative organisms, but identification of the gram-negative bacilli to species level is not possible. Electrochemical and impedimetric analysis (5, 22), quantitation of urine adenosine-5'-triphosphate with the luciferin-luciferase bioluminescent reaction (2) are reasonably rapid, sensitive, and specific for quantitating bacteria in urine, but the bacterial species is not determined. Ajello et al. reported detection of six of eight urine specimens infected with P. aeruginosa by urinary adenosine-5'-triphosphate, pooled sera from rabbits vaccinated with individual P. aeruginosa serotypes (1). Whether the procedure could differentiate contaminated from infected specimens was not stated, however. Solid-phase radioimmunoassay was useful for diagnosing P. aeruginosa urinary tract infections, was rapid, and should be adaptable to semiautomation.

P. aeruginosa accounts for approximately 7% of all hospital-acquired infections and 9% of the urinary tract infections (1). About 28% of secondary nosocomial bacteremias originate from the urinary tract (3). Optimal management of these bacteremias in patients with serious underlying diseases probably includes the use of synergistic antibiotic combinations (20, 23, 30). For P. aeruginosa, the combination of carbenicillin and an aminoglycoside is synergistic for approximately 40 to 70% of isolates (3, 7, 27). This same combination would not be ideal for Klebsiella, however (19). Thus, in seriously ill patients who develop sepsis in the hospital and in whom the urinary tract is the probable source, rapid determination of the bacterial species infecting the urine could influence the initial choice of antibiotics. The solid-phase radioimmunoassay allows this determination to be made in 2.5 h and detects approximately 80% of P. aeruginosa urinary infections. Of the other gram-negative rod infections, only 3 of 38 were positive; of these, two were also probably infected with P. aeruginosa, so that only 1 of the 38 was "falsey" positive.

Thus, the solid-phase radioimmunoassay should be useful for rapidly diagnosing P. aeruginosa urinary tract infections. Urine specimens most rewarding for testing would be from patients with serious nosocomial infections for whom the physician feels that synergistic antibiotic combinations are indicated. The assay should be easily adaptable to an enzyme-linked assay for laboratories unable to perform radioimmunoassays but which have a spectrophotometer. Development of similar assays for other gram-negative rod species would further enhance the value of the assay.

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