INFECTION AND IMMUNITY, July 1984, p. 139–142
0019-9567/84/070139-04$02.00/0

Vol. 45, No. 1

Protection Against Fatal *Klebsiella pneumoniae* Burn Wound Sepsis by Passive Transfer of Anticapsular Polysaccharide

S. J. CRYZ, JR.,* E. FÜRER, AND R. GERMANIER
Swiss Serum and Vaccine Institute, Berne, Switzerland

Received 21 November 1983/Accepted 28 March 1984

Klebsiella pneumoniae **KP1-0 capsular polysaccharide (PS) was isolated from culture supernatants by coprecipitation with *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide. PS was composed primarily of carbohydrate and contained <1% (wt/wt) protein and nucleic acids. The protective capacity of passively transferred anti-PS was evaluated in a murine burn wound sepsis model. Anti-PS antibody was found to offer high levels of protection ($P \leq 0.02$) against a low challenge dose of *K. pneumoniae* KP1-0. The 50% lethal dose for mice which received anti-PS antibody was increased from 10- to 40-fold over that for mice which received normal rabbit serum. Anti-PS antibody was found to be most effective at reducing mortality when administered before the challenge. In experiments designed to monitor the course of the infection, anti-PS was found to both delay the onset of bacteremia and reduce bacterial counts in the blood. Optimal protection against fatal burn wound sepsis was obtained by the use of a combined antibiotic and passive antibody therapy regimen.**

Gram-negative aerobic bacilli currently account for a substantial percentage of nosocomial infections (2, 6a). Of this bacterial grouping, *Klebsiella* spp. are a leading cause of morbidity and mortality (13, 17). Several hospitalized patient populations are at increased risks to infections with *Klebsiella* spp., including neonates (14, 24) in which frequent epidemics have been reported, patients receiving respiratory therapy (15, 19), and urological (4) and burn wound patients (16, 21). The occurrence of *Klebsiella* strains displaying multiple-drug resistance (3, 28) has greatly complicated therapy. Mortality rates, even in the face of intensive chemotherapy, may approach or exceed 50% (13, 17).

Several *Klebsiella* somatic antigens, including capsular polysaccharide (PS) (6, 8, 12, 26), pili (11), and a heat-labile cell surface antigen of an unknown nature (6), have been implicated as important virulence factors. Of these, PS has been most intensely studied. Possession of a large capsule appears to be essential for virulence of *Klebsiella pneumoniae* in several animal models (8, 10). Anti-PS antibody, elicited in response to immunization either with whole-cell or ribosomal vaccines, confers good levels of protection against a live challenge (6, 12, 26, 27). To date, the efficacy of systemically administered anti-PS antibody has not been evaluated.

In the present report, the efficacy of intravenously administered antiserum raised against purified PS was evaluated. In light of the importance of *Klebsiella* spp. as a human burn wound pathogen (16, 21, 24), protection afforded by anti-PS antibody was studied by using a murine burn wound sepsis model.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *K. pneumoniae* KP1-0 was obtained from Dr. Straus, Texas Tech University Health Science Center, Lubbock, Tex. Cultures were grown at 37°C with shaking in Trypticase soy broth with dextrose (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 1% (vol/vol) glycerol.

Purification of PS. Stationary-phase cultures (16 h) were centrifuged at 12,000 × *g* for 30 min. The semiclear supernatant was decanted off and recentrifuged twice. *N*-Cetyl-*N*,*N*,*N*-trimethylammonium bromide (E. Merck AG, Darmstadt, Federal Republic of Germany) was added as a 10% stock to equal 0.5% (wt/vol). The mixture was stirred for 1 h at room temperature and then centrifuged at 5,000 × *g* for 10 min. The pellet was dissolved in 500 ml of 1 M NaCl by gentle stirring. An equal volume of water was added, followed by the addition of ethanol to yield 25% (vol/vol). This was stirred overnight at 4°C and centrifuged for 30 min at 12,000 × *g*. The pellet was discarded, and the ethanol concentration was increased to 80% (vol/vol). The precipitate was collected by centrifugation, resuspended in water, and ultracentrifuged at 100,000 × *g* for 16 h to remove lipopolysaccharide. PS was precipitated from the clear supernatant with ethanol (80% [vol/vol]), collected by centrifugation, dissolved in water, and lyophilized.

Chemical analysis. Protein was determined by the method of Lowry et al. (18), using bovine serum albumin as a standard. Nucleic acids were quantitated by measuring the absorbance at 260 nm of a 1-mg/ml PS solution in water by using the conversion formula, 50 μg of nucleic acid per ml yields an absorbance of 1.0 in a 1-cm cuvette (29). Residual water content was determined by Karl Fischer titration. 2-Keto-3-deoxyoctonate was quantitated by the thiobarbituric acid method (22). Carbohydrate content was determined by the phenol-sulfuric acid method (9).

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assays for the quantitation of anti-PS antibody were performed as follows. Microtiter plates (Linbro; Flow Laboratories, Inc., Hamden, Conn.) were coated with PS by the addition of 200 μl of a 50-μg/ml solution in 0.1 M sodium carbonate (pH 9.6) per well. Plates were incubated at 37°C for 3 h and then stored at 4°C. Antisera were diluted in phosphate-buffered saline (PBS) containing 0.02% Tween 20 (PBS-Tween 20). Coated microtiter plates were washed three times with PBS-Tween 20, and 200 μl of appropriately diluted antiserum was added per well. Plates were incubated at 22°C for 6 h and then washed three times with PBS-Tween 20. A 200-μl volume of a 1:2,500 dilution of peroxidase-labeled protein A (20) (for the detection of immunoglobulin G) was added per well, and the plates were incubated overnight at 4°C. Plates were washed with PBS-Tween 20,

* Corresponding author.

and 200 μl of substrate solution [10 mg of 2,2'-azino-di(3-ethyl-benzylthiozoline) sulfonic acid 6 (Boehringer Mannheim, Mannheim, Federal Republic of Germany) in 50 ml of 0.1 M $NaPO_4$ (pH 4) to which 125 μl of 10% H_2O_2 was added just before use] was placed into each well.

The reaction was allowed to proceed for 30 min. The absorbance was then measured at 405 nm in a Titertek Multiscan (Flow Laboratories, Inc.). Titers are expressed as the serum dilution which gave an absorbance at 405 nm of 0.4, a value which is at least two standard deviations above the readings obtained when normal rabbit serum (negative control) was tested.

Production of antisera. Antisera to purified PS and to live *K. pneumoniae* were raised in rabbits (2.5 to 3 kg, New Zealand White) as follows. Rabbits were immunized intramuscularly on days 0, 14, 28, and 42 either with 1 mg of PS (first dose in Freund complete adjuvant, second dose in Freund incomplete adjuvant, and the third and fourth doses in 0.5% AlOH) or with 10^{10} washed KP1-0 cells (without adjuvant). Animals were exsanguinated 14 days after the final immunization. Rabbit antisera had an immunoglobulin G enzyme-linked immunosorbent assay titer of 150.

Burn wound model. The murine burn model was used as previously described (7). Mice (18 to 20 g, Swiss Webster White) were anesthetized in an atmosphere of fluoromethyl ether (Ethrane; Abbott Laboratories, North Chicago, Ill.) and subjected to a 10-s ethanol burn over a 2-cm^2 area of the back. The challenge organisms (diluted in PBS) were given subcutaneously at the burn site in 0.5 ml of PBS. The bacterial challenge was quantitated for each experiment by viable plate counts.

Quantitation of bacteria in blood and tissues. Bacterial numbers in the blood, skin (at the challenge site), and liver were quantitated as previously described (7).

Statistical analysis. The mean lethal dose was calculated by the method of Reed and Muench (25). Statistical significance was determined by the chi-square method.

RESULTS

PS was isolated from culture supernatants of *K. pneumoniae* KP1-0 by coprecipitation with *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide. Further purification of PS entailed the removal of nucleic acids by ethanol precipitation and the removal of lipopolysaccharide by ultracentrifugation. PS was composed primarily of polysaccharide with trace quantities of protein, nucleic acids, and lipopolysaccharide present (Table 1). Yields exceeded 400 mg of PS per liter of initial culture supernatant.

The protective capacity of passively transferred anti-PS antibody against fatal *K. pneumoniae* burn wound sepsis was first evaluated against a low challenge dose (Table 2). Three independent experiments were performed with control mice receiving normal rabbit serum. In all three determinations, anti-PS provided high levels of protection against lethal infection ($P < 0.01$ to $P = 0.02$). Mortality was reduced from 70 to 95% in the control groups to 10 to 35% in groups which received specific antibody. In additional experiments, an equivalent degree of protection (86%) was provided by the passive transfer of either anti-PS antibody or antiserum produced against intact *K. pneumoniae* KP1-0 cells.

TABLE 1. Composition of *K. pneumoniae* KP1-0 polysaccharide

Component	% Dry wt
Carbohydrate	70
Protein	0.75
Nucleic acids	0.65
Amino sugars	ND[a]
KDO[b]	0.15
Residual moisture	4.9

[a] ND, None detected.
[b] KDO, 2-keto-3-deoxyoctonate.

TABLE 2. Protection against fatal *K. pneumoniae* sepsis by passive transfer of anti-PS[a]

Experiment no.	Antiserum transferred[b]	Mortality[c]	*P*
1	Normal rabbit	13/15	<0.01
	Anti-PS	2/15	
2	Normal rabbit	7/10	0.02
	Anti-PS	1/10	
3	Normal rabbit	19/20	<0.01
	Anti-PS	7/20	

[a] Mice were challenged with ca. 10 *K. pneumoniae* KP1-0.
[b] A 0.2-ml volume of serum per mouse was transferred intravenously 24 h before the challenge.
[c] Number dead/total number of mice.

Next, the degree of protection provided against fatal infection by passive therapy with anti-PS antibody was more precisely defined by determining the 50% lethal dose (LD_{50}) for treated and control mice (Table 3). More than 50% of the animals in the control group died at the lowest challenge dose in both experiments (<20 organisms). Anti-PS antibody raised the LD_{50} between 10- and 40-fold. Attempts to further increase the LD_{50} by the additional administration of specific antisera at various times postchallenge were hampered by the adverse reactions noted in mice which received multiple doses of anti-PS.

All previous experiments were carried out with the antisera administered before the challenge under conditions in which anti-PS antibody was found to be highly protective. Next, the protective capacity of anti-PS antibody was compared when given pre- and postchallenge (Table 4). Statistically significant protection ($P < 0.01$) was obtained only when specific antiserum was administered 24 h before the challenge. However, the reduced mortality rates seen when anti-PS antibody was given at +24 h and +48 h (71 and 78%, respectively) as compared with that of the control group (93%) suggested that some protection was provided by specific antibody. Antiserum administered at times later than 48 h did not substantially reduce mortality.

The mechanisms by which anti-PS antibody mediates its protective effect was investigated by monitoring the course of the infection. Preliminary experiments showed that although treatment with specific antiserum (given at −24 h relative to the challenge) only slightly reduced the numbers of bacteria in the skin at the challenge site as compared with

TABLE 3. Mean lethal dose for mice receiving normal rabbit serum or anti-PS after challenge with *K. pneumoniae* KP1-0

Serum transferred[a]	LD_{50}[b] in expt:	
	1	2
Normal rabbit	$<1.65 \times 10$	$<1.6 \times 10$
Anti-PS	6.7×10^2	1.6×10^2

[a] Each mouse received 0.2 ml of serum by intravenous route 24 h before challenge.
[b] Groups of six mice were used.

TABLE 4. Protective effect of anti-PS administered pre- and postchallenge against *K. pneumoniae* KP1-0 infection[a]

Serum administered	Administration time (h)[b]	No. dead/total (%)	P[c]
Normal rabbit	−24	14/15 (93)	
Anti-PS	−24	5/15 (33)	<0.01
Anti-PS	+24	10/14 (71)	NS
Anti-PS	+48	11/15 (78)	NS
Anti-PS	+72	14/15 (93)	NS
Anti-PS	+96	12/14 (86)	NS

[a] The challenge dose was ca. 2 × 10 *K. pneumoniae* KP1-0.
[b] Time of antibody administration relative to the time of challenge (0). Each mouse received 0.2 ml of serum intravenously.
[c] NS, Not significant ($P > 0.05$).

that in control mice, bacteremia and liver colonization were markedly reduced (data not shown). Therefore, the development and severity of bacteremia was studied in control and specific antiserum-treated mice (Table 5). Bacteremia was first noted at 2 days postchallenge in control mice and persisted throughout the course of the experiment. By day 5, 80% of control mice were bacteremic, with numbers approaching 10^4/ml of blood. In contrast, the onset of bacteremia was delayed to day 4 in antiserum-treated mice (20% culture positive). Blood cultures were negative on days 5 and 6, with bacteremia again detected on days 7 and 8. However, although between 40 and 60% of mice which received antiserum were bacteremic on these later days, the number of organisms was ca. 1,000-fold less than that concurrently seen in control mice.

Finally, the effect of combined antibiotic and passive therapy on fatal KP1-0 sepsis was evaluated. Gentamicin, administered 24 h postchallenge was ineffective at reducing mortality (Table 6). Whereas anti-PS antibody increased the LD_{50} at least 7-fold, the inclusion of gentamicin into the therapeutic regimen resulted in a further 15-fold increase in the LD_{50} (to yield a total 105-fold increase in the LD_{50}).

DISCUSSION

The importance of PS in the pathogenesis of *K. pneumoniae* experimental infections has been recognized for some time (10). Although the role of capsule in human infections has not been extensively investigated for *Klebsiella* species, a direct correlation between the presence of capsular antigen in the blood and a fatal outcome has been demonstrated (23). The consistently high mortality rates for *Klebsiella* infections (especially pneumonia) (16) provide an impetus for the development of immunotherapeutic-immunoprophylactic agents. However, relatively few studies have been performed in an attempt to investigate the potential of immunological therapy based on anticapsular antibody (5, 6, 12, 26). Furthermore, there have been no previous reports on attempts to provide protection against experimental *Klebsiella* burn infections, even though burn patients are at high risk for *Klebsiella* infection (16, 21).

In the present study, anti-PS was produced against a highly purified antigen. This is in contrast to previous reports of experiments in which whole bacterial cells (5, 6), ribosomal vaccines (12, 26), or polysaccharide preparations containing appreciable amounts of protein (12) were used to elicit an anti-PS response. Although all of the above studies were able to demonstrate protection mediated by anti-PS antibody, recent findings, which have shown that antibody against an outer membrane protein is also protective (6), emphasize the need for highly purified antigens in the performance of such studies.

Anti-PS administered 24 h before the challenge provided high levels of protectin against fatal *K. pneumoniae* burn wound sepsis. An equivalent amount of antibody given postchallenge had a greatly diminished protective capacity. This is most likely due to the fact that in the intervening time between the challenge and antibody therapy the bacterial load had increased to levels such that the quantity of antibody given was insufficient. In addition, the release of soluble cell-free PS by replicating bacteria would also act to complex antibody before its coming in contact with the bacterial cell (8).

In the animal model used in the present study, death was due to overwhelming bacterial sepsis. Anti-PS antibody appeared to mediate its protective effect primarily by preventing or reducing the severity of bacteremia, since bacterial numbers in the skin at the site of infection were comparable in specific antiserum-treated mice and in control mice. Mice which received anti-PS antibody were able to survive infection with low numbers of *K. pneumoniae* in the blood. Bacteremia in this group was substantially reduced in severity and occurred at a much later time than in control animals. These findings probably stem from a depletion of the transferred antibody which allows for the continued seeding of the blood with bacteria from an extracellular site, most likely from the skin at the site of challenge. Anti-PS antibody would therefore function as opsonic antibody, facilitating the clearance of bacteria from the blood. This hypothesis is supported by the finding that anti-PS (secretory immunoglobulin A) has been shown to promote the killing of *K. pneumoniae* in the presence of polymorphonuclear leukocytes (5).

We found that the administration of gentamicin alone was ineffective in treating an established localized *K. pneumoniae* KP1-0 infection. However, when gentamicin was used in combination with passive therapy, the protection afforded was substantially greater than that observed only with immunotherapy. Increased protection in this case was most likely due to the action of both agents to prevent sepsis. The synergistic effect noted could well prove to be an important aspect in the future evaluation of passive therapy for the treatment of *K. pneumoniae* infections.

In two recent reports, infections with *Klebsiella* species were second only to *Pseudomonas aeruginosa* as the cause of fatal burn wound sepsis (16, 21). Passive immunotherapy

TABLE 5. Reduction of bacteremia by passive transfer of anti-PS[a]

Serum transferred[b]	% Bacteremic mice (mean counts per ml of blood) on postchallenge day:							
	1	2	3	4	5	6	7	8
Normal rabbit	<10	20 (2.68 × 10^2)	40 (2.64 × 10^2)	60 (5.8 × 10)	80 (9.2 × 10^3)	80 (2.2 × 10^4)	60 (3.9 × 10^4)	ND[c]
Anti-PS	<10	<10	<10	20 (7.2 × 10^2)	<10	<10	60 (3.8 × 10)	40 (1.6 × 10)

[a] Mice were challenged (day 0) with ca. 10 *K. pneumoniae* KP1-0. Groups of five mice were sacrificed at the times indicated, and the number of bacteria in the blood was determined. The limit of detection was 10 bacteria per ml of blood.
[b] Serum (0.2 ml) was transferred intravenously per mouse 24 h before challenge.
[c] ND, Not determined.

TABLE 6. Effect of combined antibiotic and passive immunotherapy on *K. pneumoniae* KP1-0 burn wound sepsis

Antiserum transferred[a]	Gentamicin administered[b]	LD_{50}[c]
Normal rabbit	No	$<1.55 \times 10$
	Yes	$<1.55 \times 10$
Anti-PS	No	9.94×10
	Yes	1.55×10^3

[a] Serum (0.2 ml) was transferred intravenously 24 h before challenge.
[b] Each mouse received 2 mg of gentamicin intraperitoneally 24 h postchallenge.
[c] Groups of six mice were used per challenge dose.

for the prevention and treatment of *P. aeruginosa* infections in burn patients has shown promising results (1). Using an experimental model relevant to human disease, we have demonstrated that passive therapy with anti-PS is a viable approach for the prevention of *K. pneumoniae* sepsis. Although the present study was limited to a single strain of *K. pneumoniae*, the ease whereby PS could be isolated and purified, together with the high level of protection obtained with anti-PS, warrants further investigation as to the use of *K. pneumoniae* PS as a human vaccine candidate.

ACKNOWLEDGMENTS

We wish to thank E. Kohler and D. Pavlovic for excellent technical assistance and V. Rupp for help in the preparation of this manuscript.

LITERATURE CITED

1. **Alexander, J. W., and M. W. Fisher.** 1974. Immunization against pseudomonas infection after thermal injury. J. Infect. Dis. **130**(Suppl.):152–158.
2. **Allen, J. R., A. W. Hightower, S. M. Martin, and R. E. Dixon.** 1981. Secular trends in nosocomial infections: 1970–1979. Am. J. Med. **70**:389–392.
3. **Casewell, M. W., and I. Phillips.** 1981. Aspects of the plasmid-mediated antibiotic resistance and epidemiology of klebsiella species. Am. J. Med. **70**:459–462.
4. **Christensen, S. C., and B. Korner.** 1972. An endemic caused by multiresistant *Klebsiella* in an urological unit. Scand. J. Urol. Nephrol. **6**:232–238.
5. **Cooper, J. McA., J. J. Finlay-Jones, N. L. Hill, and D. Rowley.** 1983. Local immunity to *Klebsiella pneumoniae* in the lungs of mice. J. Infect. Dis. **147**:312–317.
6. **Cooper, J. McA., and D. Rowley.** 1982. Resistance to *Klebsiella pneumoniae* and the importance of two bacterial antigens. Aust. J. Exp. Biol. Med. Sci. **60**:629–641.

6a. **Cross, A., J. R. Allen, J. Burke, G. Ducel, A. Harris, J. John, D. Johnson, M. Lew, B. MacMillan, P. Meers, R. Skalova, R. Wenzel, and J. Tenney.** 1983. Nosocomial infections due to *Pseudomonas aeruginosa*: review of recent trends. Rev. Infect. Dis. **5**(Suppl.):S837–S845.

7. **Cryz, S. J., Jr., D. Fürer, and R. Germanier.** 1983. Passive protection against *Pseudomonas aeruginosa* infection in an experimental leukopenic mouse model. Infect. Immun. **40**:659–664.
8. **Domenico, P., W. G. Johanson, Jr., and D. C. Straus.** 1982. Lobar pneumonia in rats produced by clinical isolates of *Klebsiella pneumoniae*. Infect. Immun. **37**:327–335.
9. **Dubois, M., K. A. Giles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
10. **Ehrenworth, L., and H. Baer.** 1956. The pathogenicity of *Klebsiella pneumoniae* for mice: the relationship to the quantity and rate of production of type-specific capsular polysaccharide. J. Bacteriol. **72**:713–717.
11. **Fader, R. C., and C. P. Davis.** 1980. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. Infect. Immun. **30**:554–561.
12. **Fournier, J.-M., C. Jolivet-Raynaud, M.-M. Riottot, and H. Jouin.** 1981. Murine immunoprotective activity of *Klebsiella pneumoniae* cell surface preparations: comparative study with ribosomal preparations. Infect. Immun. **32**:420–426.
13. **Graybill, J. R., L. W. Marshall, P. Charache, C. K. Wallace, and V. B. Melvin.** 1973. Nosocomial pneumonia: a continuing major problem. Am. Rev. Respir. Dis. **108**:1130–1140.
14. **Hill, H. R., C. E. Hunt, and J. M. Matsen.** 1974. Nosocomial colonization with *Klebsiella*, type 26, in a neonatal intensive-care unit associated with an outbreak of sepsis, meningitis, and necrotizing enterocolitis. J. Pediatr. **85**:415–419.
15. **Johanson, W. G., A. K. Pierce, J. P. Sanford, and G. D. Thomas.** 1973. Nosocomial respiratory infections with gram-negative bacilli. The significance of colonization of the respiratory tract. Ann. Intern. Med. **77**:701–706.
16. **Jones, R. J., E. A. Roe, and J. L. Gupta.** 1979. Controlled trials on a polyvalent pseudomonas vaccine in burns. Lancet **ii**:977–983.
17. **Lorian, V., and B. Topf.** 1972. Microbiology of nosocomial infections. Arch. Int. Med. **130**:104–110.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Mertz, J. J., L. Scharer, and J. H. McClement.** 1964. A hospital outbreak of klebsiella pneumonia from inhalation therapy with contaminated aerosol solutions. Am. Rev. Respir. Dis. **95**:454–460.
20. **Nakane, P. K., and A. Kawaoi.** 1974. Peroxidase-labeled antibody. A new method of conjugation. J. Histochem. Cytochem. **22**:1084–1091.
21. **Nathan, P., I. A. Holder, and B. G. MacMillan.** 1973. Burn wounds: microbiology, local host defenses, and current therapy. Crit. Rev. Clin. Lab. Sci. **4**:61–100.
22. **Osborn, M. J.** 1963. Studies on the gram-negative cell wall. I. Evidence for the role of 2-keto-3-deoxyoctonate in the lipopolysaccharide of *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S.A. **50**:499–506.
23. **Pollack, M.** 1975. Significance of circulating capsular antigen in klebsiella infections. Infect. Immun. **13**:1543–1548.
24. **Pruitt, B. A.** 1974. Infections caused by *Pseudomonas* species in patients with burns and in other surgical patients. J. Infect. Dis. **130**(Suppl.):8–13.
25. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. **27**:493–497.
26. **Riottot, M.-M., J.-M. Fournier, and H. Jouin.** 1981. Direct evidence for the involvement of capsular polysaccharide in the immunoprotective activity of *Klebsiella pneumoniae* ribosomal preparations. Infect. Immun. **31**:71–77.
27. **Riottot, M.-M., J.-M. Fournier, and J. Pillot.** 1979. Capsular serotypic specificity of the protection conferred on mice by *Klebsiella pneumonia* ribosomal preparations. Infect. Immun. **24**:476–482.
28. **Thomas, F. E., R. T. Jackson, M. A. Melly, and R. H. Alford.** 1977. Sequential hospitalwide outbreaks of resistant serratia and klebsiella infections. Arch. Intern. Med. **137**:581–584.
29. **Wong, K. H., O. Barrera, A. Sutton, J. May, D. H. Hochstein, J. D. Robbins, J. B. Robbins, P. D. Parkman, and E. B. Seligmann, Jr.** 1977. Standardization and control of meningococcal vaccines, group A and group C polysaccharide. J. Biol. Stand. **5**:197–215.