Typing Methods for *Proteus rettgeri*: Comparison of Biotype, Antibigrams, Serotype, and Bacteriocin Production

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Two hundred five strains of *Proteus rettgeri* from epidemic and nonepidemic sources were differentiated by a new biotyping scheme, agglutination in O antisera, antimicrobial resistance patterns, and a new scheme based on bacteriocin production. The *P. rettgeri* were divided into 10 groups by their fermentation of lactose, sucrose, D-mannitol, and salicin. These groups were then subdivided into 19 biotypes by other biochemical reactions. Bacteriocin production was tested by the cross-streak method. Thirty-four bacteriocin-sensitive indicator strains were evaluated, and 16 were selected for the final scheme and used to type the 205 *P. rettgeri*, which were divided into 15 bacteriocin types. Serologically, 43% of the *P. rettgeri* were O42, 13% were untypable, 4% were O15, and 3% each were O33, O64, and O84 in addition to 31 remaining serotypes. Strains of *P. rettgeri* from known outbreaks contained fewer biotypes, O groups, and bacteriocin types and were more resistant to antimicrobial agents than endemic strains. Strains with common patterns with all four marker systems were frequently associated with outbreaks. A strong correlation between multiple antibiotic resistance and bacteriocin production was shown.

Nosocomial infections with *Proteus* are now well documented. Prevalence surveys of *Proteus* infections have documented the nosocomial nature of these infections and their relationship to urinary tract instrumentation, the compromised host, and antimicrobial drug usage (2, 4, 5, 8, 43). The most frequently recovered species in most hospitals is *Proteus mirabilis*; thus, most epidemiological investigations of nosocomial infection problems have been made with this species. All *Proteus* species can infect the urinary tract (4, 16-18, 69), and serious infections have been associated with other sites (15).

In the past, methods of typing have been developed almost exclusively for *P. mirabilis* (6, 14, 22-24, 34, 36, 38, 63, 64, 66) and to a lesser extent for *Proteus vulgaris*, *Proteus morganii*, and *Proteus rettgeri* (7, 31, 47, 52-54, 63, 66). Investigators have attempted to demonstrate production of bacteriocins for possible use in typing other *Proteus* spp. (20-22, 25, 34).

Recent nosocomial infection problems involving antibiotic-resistant *P. rettgeri* have led to medical complications in patients and increased interest in this organism (16, 26, 37, 39, 45, 67, 68, 73). In some hospitals isolates are resistant to all antimicrobial agents tested (16, 26, 37, 39, 62, 67, 68), and the genetic mechanisms involved in this high level of resistance have been inves-

tigated (19, 49, 70). *P. rettgeri* mainly cause infections of the urinary tract, but wound and burn infections and septicemia have been noted (3, 26, 44, 50, 71). These organisms infect compromised hosts, particularly patients with spinal cord injuries who need long hospitalization and catheterization. In addition, previous urinary tract instrumentation and drug therapy appear to be significant factors associated with these nosocomial infection problems (16, 26, 45, 68, 73).

P. rettgeri was isolated by Rettger in 1904 while he was studying cholera-like diseases of fowl (56, 57). Several years later, Rettger's bacillus was described in detail by Hadley et al. and named *Bacterium rettgeri* (35). This organism was studied by a number of investigators over the next 40 years (10, 11, 13, 30, 32, 33, 48, 58-61, 65, 74) and finally placed into the genus *Proteus*, i.e., *P. rettgeri*. More recently, Kauffmann (40-42) and Namioka and Sakazaki (46) have proposed that the genus *Proteus* be split, with Rettger's bacillus being placed in a new genus, *Rettgerella*. Based on DNA hybridization studies, Brenner et al. (12) recently proposed that a group of urea-positive strains of *P. rettgeri* (biogroup 5) are, in fact, members of *Providencia stuartii*.

The only ways to type isolates from *P. rettgeri*

infections are by the biochemical characteristics and any unusual antibiotic susceptibility patterns (16, 18, 27-29, 46, 54); there is also a serotyping system based on somatic O antigens, but it is not in general use (52, 53). Since many *P. rettgeri* have similar biochemical and susceptibility patterns, the source and mode of transfer of infecting organisms often remain unknown because of lack of appropriate typing schemes.

The purpose of this study was to develop new and better typing procedures to differentiate strains of *P. rettgeri* and to aid in tracing the transmission of these organisms within the hospital. With additional marker systems, the mechanism of infection, i.e., from patient to patient, from patient to hospital personnel to patient, or from environmental sources to patient, would be more clearly defined. A comparison of these typing procedures, using epidemic and endemic strains of *P. rettgeri*, would be vital in determining the efficacy of each marker system.

MATERIALS AND METHODS

Sources of bacterial strains. A total of 205 *P. rettgeri* isolates were obtained from state health departments from the Enterobacteriology Branch, Bureau of Laboratories, Center for Disease Control (CDC) (104 strains); the American Type Culture Collection (ATCC) (6 strains); epidemics in St. Louis, Mo. (18 isolates) and in Memphis, Tenn. (35 isolates); and a university hospital in Missouri that had had recurring problems with *P. rettgeri* for several years (42 strains). For simplification, the following abbreviations will be used in parts of the text and tables: MISC for CDC and ATCC strains combined (miscellaneous), SL for St. Louis isolates, M for Memphis isolates, and U for university strains.

All *P. rettgeri* used in this study were recovered from different specimen sources, i.e., a patient, an animal, or the environment. Multiple isolates from the same source were not used. Most *P. rettgeri* isolates were isolated from urine specimens, but strains were also recovered from feces, wounds, sputum, and blood from humans and a few from animal and environmental sources (Table 1).

Primary isolation. Stock cultures of known *P. rettgeri* and isolates tentatively identified as *P. rettgeri* were washed with brain heart infusion broth with 0.5% beef extract (BHI) or inoculated directly into BHI broth and then incubated at 35 to 37°C for 24 h. After incubation, a loopful (0.01 ml) of broth was streaked on primary isolation medium (MacConkey agar) and incubated overnight at 35 to 37°C. Representative types of nonlactose-fermenting colonies were picked and inoculated to triple sugar iron agar slants. Triple sugar iron reactions were recorded, and cultures giving reactions typical of *P. rettgeri* (acid/acid or alkaline/acid) were inoculated into a series of biochemicals for confirmation.

Biochemical characterization. All *P. rettgeri* isolates were inoculated into fermentation tubes (Difco enteric fermentation base) containing 1% carbohy-

TABLE 1. Specimen source of 205 *P. rettgeri* isolates

Specimen source	No. of cultures	% of total
Human		
Urine	140	68
Feces	16	8
Wound	13	6
Sputum	6	3
Blood	7	3
Animal	6	3
Unknown	10	5
ATCC	6	3
Environmental	1	1

drates or polyhydric alcohols with inverted Durham tubes to demonstrate gas production (Table 2). Andrade solution was used as the pH indicator. All fermentation tubes were incubated at 35 to 37°C, examined at 48 h, 72 h, and 5 days, and then removed and left at room temperature for 7- and 14-day readings before a negative reaction was recorded.

Other tests were completed, incubated at 35 to 37°C, and read at different time periods: methyl red, nitrate to nitrite, Voges-Proskauer, and indole at 48 h; phenylalanine deaminase, growth on cetrimide agar, and KCN at 24 h; lysine decarboxylase, arginine dihydrolase, and ornithine decarboxylase each day up to 5 days; and citrate, urea, motility, malonate, peptone iron agar, mucate, acetate, esculin, and gelatin each day up to 14 days.

Stock cultures were kept in motility media and on blood agar base slants, sealed, and stored at room temperature for future use in the comparison of typing procedures.

Determination of antimicrobial agent susceptibility patterns. The Kirby-Bauer method (9, 17) was used to determine susceptibility patterns of all *P. rettgeri* isolated in this study. The following susceptibility disks in high content were used: nitrofurantoin, nalidixic acid, chloramphenicol, ampicillin, streptomycin, kanamycin, sulfathiazole, carbenicillin, cephalothin, colistin, tetracycline, trimethoprim-sulfamethoxazole, and gentamicin.

Demonstration of bacteriocin production. The first 96 organisms (50 SL and M, 46 U and MISC) identified as *P. rettgeri* were used as primary streaks and tested against each other in batches of 10 to 20 isolates, using the cross-streak method to find bacteriocin-sensitive indicators. All 205 *P. rettgeri* were cross-streaked to these initial 96 strains. The method was similar to Abbott and Shannon's (1) except for the following: cultures for primary streaks and potential indicators were grown in BHI broth; broth for primary streaks was inoculated to the middle of a glass petri plate (15 by 100 mm) containing 20 ml of Trypticase soy agar with 5% sheep blood (TSBA); plates were placed under a chemical fume hood and exposed to chloroform (1.5 ml) for 15 min and aired for 5 min; eight broth cultures, visually compared to a 0.5 barium sulfate standard (0.5 ml of 1% BaCl₂ to 99.5 ml of 0.36 N H₂SO₄), were inoculated across the primary streak; primary streaks in broth, on TSBA plates, and after cross-streaking were incubated at 35 to 37°C for 18 to

24 h; areas of inhibition were classified as: +++ = >2 cm; ++ = 1-2 cm; + = <1 cm; and $\pm$ = any thinning of growth. Those strains showing any areas of inhibition were considered potential indicator strains.

Determination of bacteriocin types. Sixteen selected indicators (all from MISC) were chosen and used to type the 205 *P. rettgeri*. The 16 indicator strains are available as lyophilized cultures from Roger L. Anderson. Indicators used to demonstrate production of bacteriocins were ++ or +++ and had sharp, well-defined edges of growth when inhibited. Indicators were transferred each day for the next day's use and made up every 2 weeks from stock culture slants.

All 205 *P. rettgeri* isolates were used as primary streaks in the cross-streak method and tested against these 16 selected indicator organisms to determine distinct bacteriocin types or groups. If differences in the reaction of indicators varied by one indicator, the test strain was given a new designated bacteriocin type.

Studies with broth filtrates of known bacteriocin producer strains. Strains of *P. rettgeri* were grown in 125-ml Erlenmeyer flasks containing 50 ml of BHI broth. After incubation on a mechanical shaker (120 cycles/min) at 35 to 37°C for 24 h, growth was decanted into 50-ml centrifuge tubes, centrifuged for 20 min at 25,000 $\times g$ in a Sorvall (Ivan Sorvall Inc., Newtown, Conn.) SS-3 high-speed centrifuge, and decanted into a Nalge (Nalge Corp., Rochester, N.Y.) 100-ml filter unit (0.45- μ m grid membrane), and negative pressure was applied to obtain sterile filtrates. Sterile filtrates were aseptically decanted into sterile screw-cap tubes (16 by 125 mm) and stored at 4°C in the refrigerator.

The following indicator organisms were grown in BHI broth, visually compared to a 0.5 barium sulfate standard, and inoculated to blood agar plates: strains 95, 114, 129, 149, 153, 154, 158, 177, 183, 188, and 190. After drying, 0.05 ml of undiluted bacteriocin-producing filtrates and 0.05 ml of twofold dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32 of these same filtrates were added to each indicator plate and allowed to dry. Concurrently, the pH was checked on all broth filtrates of known bacteriocin producer strains and an uninoculated BHI broth control.

Presence or absence of bacteriophage activity was determined qualitatively by macroscopic observation and quantitatively by production of individual plaques of phage lysis when the more dilute filtrates were spotted on indicator plates. In addition, a small segment of agar within the clear zone of inhibition was transferred to 3 ml of Trypticase soy broth and thoroughly agitated to insure complete mixing. Four 10-fold dilutions were made in Trypticase soy broth, and 0.05 ml of the undiluted suspension and 0.05 ml from each dilution were added to plates seeded with appropriate *P. rettgeri* indicator strains.

Filtrates showing bacteriocin activity were heated to ascertain heat stability. Suspensions were heated to 70°C, boiled, and autoclaved at 121°C for 10 min, and 0.05 ml of each treated filtrate was spotted to TSBA plates which had been inoculated with indicator organisms. Also, 0.9 ml of each broth filtrate was treated with 0.1 ml of concentrated trypsin (Difco Laboratories, Detroit, Mich.), mixed, and placed in a 37°C

water bath for 10, 20, 45, and 60 min. After the allotted time, 0.05 ml of the filtrate-trypsin mixture was added to indicator strains on TSBA.

All inoculated indicator strains on TSBA plates containing either treated or nontreated filtrates were incubated at 35 to 37°C for 18 to 24 h to determine persistence of inhibitory activity.

O antigen determination. Serogrouping of the 205 *P. rettgeri* isolates on the basis of O antigens was done by John L. Penner, Department of Microbiology, University of Toronto, Ontario, Canada, using slide agglutination procedures developed in his laboratory and previously reported (52, 53).

Computer analyses. The resistance pattern, biotype, bacteriocin type, serological group, and source of each *P. rettgeri* strain used were entered into a computer. Data were broken down by original source, and frequencies were determined for each type within the source. Analysis of multiple variables was accomplished by cross-tabulation of types to detect common patterns associated with each source.

RESULTS

Biochemical properties of *P. rettgeri*. All 205 *P. rettgeri* isolates (53 epidemic and 152 nonepidemic) produced acid from glucose, 10% produced gas (several bubbles to 10 to 15%) from glucose, and all were urea positive. Important negative reactions were obtained from dulcitol fermentation, Voges-Proskauer, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, and hydrogen sulfide production on triple sugar iron agar. A detailed listing of the biochemical properties of *P. rettgeri* strains used in this investigation is shown Table 2.

Determination of *P. rettgeri* biotypes. Fermentation of lactose, sucrose, D-mannitol, and salicin in any combination was considered a cardinal reaction in this provisional typing system. The 205 isolates of *P. rettgeri* were divided into 10 major groups based initially on the fermentation of these four compounds and subdivided into 19 distinct biotypes (Table 3). Of these biotypes, type 9 contained 83 of 205, or 41% of the strains under study.

Five biotypes comprising 18 *P. rettgeri* isolates were isolated from patients and the environment during an epidemic in St. Louis (Table 6). Of these 18 isolates, 12 (66%), 2 (11%), 2 (11%), 1 (6%), and 1 (6%) were of biotypes 10, 1, 7, 5, and 9, respectively. Three biotypes were represented in the 35 *P. rettgeri* isolates recovered from patients in Memphis. Thirty-three (94%) of the isolates were biotype 9, 1 (3%) was biotype 7, and 1 (3%) was biotype 11. The 42 university *P. rettgeri* organisms represented six biotypes: 23 (55%) were biotype 9, 10 (24%) were biotype 10, 3 (7%) were biotype 2, 3 (7%) were biotype 6, 2 (5%) were biotype 17, and 1 (2%) was biotype 1. One hundred ten strains from

TABLE 2. *Biochemical reactions of 205 strains of P. rettgeri from various sources*

Test or substrate	% of strains positive	
	Within 48 h	Between 3-14 days
Hydrogen sulfide (TSI) ^a	0	
Urease	100	
Indole	87	
Methyl red	99	
Voges-Proskauer	0	
Citrate (Simmons)	91	
KCN	97	
Motility	92	
Gelatin (22°C)	0	1
Lysine decarboxylase	0	
Arginine dihydrolase	0	
Ornithine decarboxylase	0	
Phenylalanine deaminase	99	
Glucose Acid	100	
Gas ^b	6	
Lactose	9	0.5
Sucrose	52	40
D-Mannitol	38	0.5
Salicin	19	2
Dulcitol	0	
Maltose	2	0.5
Raffinose	3	
D-Sorbitol	1	13
Adonitol	37	0.5
Trehalose	62	0.5
D-Xylose	13	2
L-Rhamnose	28	3
Inositol	94	3
L-Arabinose	0	5
Glycerol	86	14
Melibiose	9	3
Cellobiose	4	22
meso-Erythritol	30	9
D-Mannose	98	
Nitrate → nitrite	99	
Mucate	0	
Malonate	0	
Sodium acetate	90	
Cetrimide	87	
Esculin	25	

^a TSI, Triple sugar iron.^b Gas volumes are small (several bubbles to 10 to 15%).

miscellaneous sources were classified into 19 different biotypes. Biotypes 9, 1, 7, 15, 8, and 14 were most frequently observed and contained 26 (24%), 24 (22%), 10 (9%), 10 (9%), 9 (8%), and 9 (8%) *P. rettgeri* organisms, respectively. The remaining 22 (20%) *P. rettgeri* from miscellaneous sources were of 13 different biotypes.

Antibiotic susceptibility patterns of *P. rettgeri*. The susceptibility patterns of *P. rettgeri* isolated from different sources are shown in Table 4. Differences in susceptibility to gentamicin, nalidixic acid, kanamycin, and carbenicillin

were apparent when epidemic and endemic *P. rettgeri* strains were compared. In comparison with the epidemic strains, the *P. rettgeri* from miscellaneous sources had a greater sensitivity to the antimicrobial agents tested. Also, these miscellaneous isolates had higher frequencies of intermediate zones of inhibition by antimicrobial agents.

Five patterns of susceptibility were observed with organisms from two known outbreaks of infection. Of 18 isolates isolated from an infection problem in St. Louis, 9 (50%) isolates were completely resistant to all antimicrobial agents tested, 3 were susceptible only to nalidixic acid, and 6 showed other susceptibility patterns. Of 35 isolates recovered from a Memphis outbreak, 30 (86%) were completely resistant, 2 were susceptible only to kanamycin, and 3 had other susceptibility patterns. Thirteen patterns of susceptibility were determined for 42 *P. rettgeri* isolates from a university-affiliated hospital. The 110 miscellaneous strains were grouped into 56 different patterns of susceptibility.

Development of a typing system based on bacteriocin production. Thirty-four (17%) of the 205 *P. rettgeri* were inhibited by certain primary streaks and could be considered potential indicators; 29 of the 34 (85%) were discovered by the inhibitory activity produced by the initial 32 primary streaks examined. The majority, 26 of 32 (81%) of these primary streaks, were *P. rettgeri* isolated from epidemics. Not all 96 primary streaks inhibited the same indicators, and in no case was the designated potential indicator strain inhibited by its own primary streak.

Fifteen bacteriocin types resulted from inhibition patterns produced by primary streaks on 16 indicator strains (Table 5). Of the 15 bacteriocin types, 2 were from St. Louis, 5 were from Memphis, 12 were from a university, and 11 were from miscellaneous sources. Of 18 *P. rettgeri* isolates recovered from St. Louis, 13 (72%) were classified as type 4, and 27 of 35 (77%) from Memphis were type 2. Frequency of bacteriocin types was more diverse with university isolates, whereas types 1 and 3 accounted for 94 of 110 (85%) of the patterns associated with miscellaneous organisms.

P. rettgeri types 2 and 4 produced higher titers of bacteriocins in broth and inhibited indicator strains at a dilution of 1:32, the highest dilution used. The pH of all broth filtrates was checked and found to be within a range of 7.1 to 7.5 and comparable to an uninoculated BHI control with a pH of 7.15. Individual plaques of phage lysis were not observed on seeded agar plates at any dilution.

Inhibitory activity of undiluted broth filtrates

TABLE 3. Descriptive biotypes of *P. rettgeri* based on specific fermentations and other biochemical tests^a

Major fermentation groups				Biotype	Other biochemical reactions for type differentiation	No. of <i>P. rettgeri</i> per type
Lac	Suc	Man	Sal			
— ^b	+ ^c	+	—	1	Acid Ado, Rha, Ino, Ery, Mann	27
				2	Acid Ado, Xyl, Rha, Ino, Ery, Mann	5
				3	Acid Tre, Ino, Mann; negative Rha, Ery	3
—	—	+	—	4	Acid Ado, Rha, Ino, Ery, Mann	6
—	—	+	+	5	Acid Ado, Rha, Ino, Mel, Cel, Ery, Mann	1
				6	Acid Ado, Ino, Ery, Mann; negative Rha; positive esculin	6
—	+	+	+	7	Acid Ado, Rha, Ino, Ery, Mann; positive esculin	13
				8	Acid Ado, Ino, Ery, Mann; negative Rha; positive esculin	9
—	+	—	—	9	Acid Tre, Ino, Mann; positive indole	83
				10	Acid Tre, Ino, Mann; negative indole	23
				11	Acid Tre, Xyl, Ino, Cel, Mann	3
				12	Acid Mal, Tre, Xyl; negative Ino, Mann, indole	2
				12A	Acid Sor, Ado, Tre, Xyl, Ino, Gly, Cel, Mann	1
—	+	—	+	13	Acid Sor, Tre, Ino, Mann	1
				13A	Acid Mal, Xyl, Gyl, Mann; negative Ino, citrate; positive esculin	1
+	+	—	—	14	Acid Tre, Ino, Mann	9
+	+	+	+	15	Acid Ado, Xyl, Ino, Ery, Mann; positive esculin	10
+	+	+	—	16	Acid Raf, Ado, Rha, Ino, Ery; negative Mann	1
—	—	—	—	17	Acid Sor, Tre, Ino, Gly, Cel, Mann	1

^a Abbreviations: Ado, adonitol; Ara, arabinose; Cel, cellobiose; Dul, dulcitol; Ery, erythritol; Glu, glucose; Gly, glycerol; Ino, inositol; Lac, lactose; Mal, maltose; Man, mannitol; Mann, mannose; Mel, melibiose; Raf, raffinose; Rha, rhamnose; Sal, salicin; Sor, sorbitol; Suc, sucrose; Tre, trehalose; and Xyl, xylose.

^b No acid production after 14 days of incubation.

^c Acid production within 14 days.

TABLE 4. Antimicrobial agent susceptibility patterns of *P. rettgeri* from different sources

Interpretive zones of inhibition and source of strains	% of <i>P. rettgeri</i> susceptible to drug tested ^a												
	F/M	C	S	ST	CF	TE	GM	NA	AM	K	CB	CL	S×T
Resistant													
St. Louis	100	83	72	72	100	89	72	50	72	72	72	88	67
Memphis	100	100	94	97	100	97	94	94	94	91	94	100	69
University	98	83	79	88	88	100	45	14	86	64	69	100	81
Miscellaneous	91	63	63	66	75	94	24	29	51	20	19	90	42
Intermediate													
St. Louis	0	0	0	0	0	0	0	0	0	0	0	6	5
Memphis	0	0	3	0	0	0	3	3	0	0	0	0	28
University	0	0	5	0	7	0	19	0	0	0	0	0	2
Miscellaneous	5	7	8	4	7	0	11	1	3	1	7	4	16
Sensitive													
St. Louis	0	17	28	28	0	11	28	50	28	28	28	6	28
Memphis	0	0	3	3	0	3	3	3	6	9	6	0	3
University	2	17	17	12	5	0	36	86	14	36	31	0	17
Miscellaneous	4	30	29	30	18	6	65	70	46	79	74	6	42

^a F/M, Nitrofurantoin; C, chloramphenicol; S, streptomycin; ST, sulfathiazole; CF, cephalothin; TE, tetracycline; GM, gentamicin; NA, nalidixic acid; AM, ampicillin; K, kanamycin; CB, carbenicillin; CL, colistin; and SxT, trimethoprim-sulfamethoxazole.

was removed by heating at 70°C for 10 min but was not affected by the addition of trypsin to the filtrate after 60 min of incubation.

Serological typing of *P. rettgeri*. Isolates of *P. rettgeri* were serotyped on the basis of somatic O antigens. The majority of isolates from St. Louis (72%) and Memphis (94%) were O42, whereas 69 and 13% of the strains from a

university and miscellaneous sources, respectively, were of this same serotype (Table 6). Thirty-seven different serotypes were associated with 110 miscellaneous *P. rettgeri* strains. Those organisms from a university source were of 10 serotypes. Epidemic isolates from Memphis and St. Louis were of only three and four different serotypes, respectively. The highest percentage

TABLE 5. Bacteriocin production patterns of *P. rettgeri* on 16 selected indicator strains

Bacteriocin type	No. of strains	Inhibition pattern ^a against <i>P. rettgeri</i> indicator strains															
		94	95	97	114	129	132	139	149	153	154	158	166	177	183	188	190
1	85	—	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—
2	33	—	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+
3	28	—	—	—	—	—	+	+	—	—	—	—	T ^b	—	—	—	—
4	24	—	—	—	+	+	+	+	+	+	+	—	+	+	—	+	+
5	9	—	—	—	+	+	+	+	—	+	—	—	—	+	—	+	—
6	6	—	+	—	+	+	+	+	+	+	—	—	+	+	+	+	—
7	3	—	+	—	+	+	—	—	—	+	—	+	—	+	—	+	—
8	2	—	—	—	+	+	+	—	—	+	+	—	—	+	—	+	—
9	4	—	—	—	—	—	+	+	—	—	—	—	—	—	—	+	—
10	3	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—
11	3	—	—	—	—	+	+	+	—	+	—	—	—	—	—	—	—
12	2	—	—	+	—	—	+	+	—	—	—	—	—	—	—	—	—
13	1	—	—	—	—	+	+	—	—	+	—	—	+	+	—	+	—
14	1	—	—	+	—	—	+	+	—	—	—	—	—	—	—	+	—
15	1	—	—	+	+	+	+	+	—	+	—	—	+	+	—	+	+

^a +, Inhibition of indicator (+++, ++, or + as defined in text); —, no inhibition.^b T, Thinning of growth.TABLE 6. Most frequent patterns observed when comparing bacteriocin production, serotype, biotype, and susceptibility patterns of *P. rettgeri* isolated from epidemic and nonepidemic sources

Source of <i>P. rettgeri</i> and pattern breakdown	Isolates		Typing procedures				
	No.	%	Bacteriocin type	Serotype	Biotype	Resistance pattern	
						<11 ^a	≥11 ^a
St. Louis							
Most frequent	12	66	4	O42	10		12
Other patterns	2	11	3	UT ^b	7	2	
	2	11	3	O17	1	2	
	1	6	4	O42	9		1
	1	6	3	O81	5	1	
Memphis							
Most frequent	26	74	2	O42	9		26
Other patterns	4	11	6	O42	9		4
	1	3	2	O26	9		1
	1	3	5	O42	9		1
	1	3	4	O42	9		1
	1	3	3	O11	7	1	
	1	3	5	O42	11		1
University							
Most frequent	7	17	4	O42	10	3	4
Other patterns	25 (35) ^c	83					
Miscellaneous							
Most frequent	6	5	1	O15	15	1	5
Other patterns	81 (104)	95					

^a Resistant to fewer than or 11 or more antibiotics.^b UT, Untypable.^c Number in parentheses refers to number of organisms in those patterns.

of nontypable *P. rettgeri* was from miscellaneous sources.

Comparison of typing procedures. When bacteriocin activity, biochemical properties, antigenic types, and antibiograms of organisms by original source were analyzed, common patterns of similarity resulted (Table 6). More identical

patterns with these four typing procedures were observed for *P. rettgeri* isolates from epidemics than for endemic strains, as the following percentages suggest: St. Louis, 66% (12 of 18); Memphis, 74% (26 of 35); a university, 17% (7 of 42); and miscellaneous sources, 5% (6 of 110).

More than 70% of all isolates isolated from

outbreaks of infection in St. Louis and Memphis were typable when using bacteriocin production, serotype, and biotype methods. Of the university strains, 69, 93, and 100% were typable using bacteriocin production, specific antigen groups, and biochemical characteristics, respectively. Of the 110 miscellaneous *P. rettgeri* strains, only 15% were typed by means of bacteriocins, 81% by serology, and 100% by biotype.

The association of bacteriocin activity with resistance patterns is illustrated in Table 6. All 13 and 34 *P. rettgeri* isolates demonstrating inhibitory action from St. Louis and Memphis, respectively, show resistance to at least 11 of the 13 antimicrobial agents tested. Twenty-nine university *P. rettgeri* isolates inhibited indicator strains, and 25 (86%) of these were resistant to more than 11 antimicrobial agents. Seven of 15 (47%) bacteriocinogenic *P. rettgeri* from miscellaneous sources were resistant to this same number of drugs.

DISCUSSION

A provisional typing system is proposed for *P. rettgeri* based on bacteriocin production. Inhibition patterns of indicator strains proved quite useful in the differentiation of epidemic and endemic *P. rettgeri* strains. Epidemic-associated isolates of *P. rettgeri* had fewer bacteriocin types and higher percentages of specific types than university and miscellaneous strains. Induction of *P. rettgeri* bacteriocins has been recently demonstrated (21, 69). The application of these findings was to find a new typing system capable of distinguishing epidemic strains of *P. rettgeri* from endemic strains and to aid in determining the transmission of these organisms within the hospital.

Undiluted and diluted BHI broth filtrates of bacteriocin producer strains did retain inhibitory activity when seeded on bacteriocin-sensitive indicator plates, which indicated that a typing system based on bacteriocin sensitivity was possible. The inhibitory substance present in broth filtrates was not due to low pH or bacteriophage activity but was found to be heat labile and not affected by treatment with trypsin. Hamon and Peron in 1962 found that some of the bacteriocins of *P. aeruginosa* were resistant and others were sensitive to the action of trypsin (72). In our experience, trypsin did not inactivate the inhibitory substance which may indicate non-proteinase activity.

Results from this investigation suggest a close association of *P. rettgeri* that were resistant to 11 or more antimicrobial agents and involved in patient infection with the production of bacteriocins. Organisms which were less resistant and

not associated with infection appeared to be non-bacteriocin producers when tested on agar. Possibly, a plasmid (or plasmids) which codes for bacteriocin production and antibiotic resistance was acquired by these epidemic strains.

Penner et al., in 1975, examined 674 *P. rettgeri* isolates to determine the biochemical properties of those cultures, biogroupings within the species, and frequencies of biochemical types (54). These investigators modified the scheme proposed by Namioka and Sakazaki (46) by adding a fifth biotype.

A biotyping system was established by using the fermentation reactions of lactose, sucrose, D-mannitol, and salicin. In this investigation, 205 *P. rettgeri* isolates from epidemic and nonepidemic sources were placed into 10 major groups and subsequently divided into 19 biotypes. A good correlation was shown for biotypes to the epidemic source of organisms.

Recent taxonomic changes have been proposed for organisms within the *Proteus-Providencia* group. In 1975, Penner et al. raised questions as to the efficacy of the urease test when used to differentiate *P. rettgeri* and *Providencia* (54). Certain characteristics, i.e., positive reactions with adonitol, D-arabitol, and erythritol to urease-negative organisms, were more characteristic of *P. rettgeri* than of *Providencia*. Variation in urease activity of *P. rettgeri* and *Providencia stuartii* hospital strains was investigated by Penner et al. in 1976. *P. rettgeri* organisms that fermented trehalose and were urease variable demonstrated antigenic characteristics of O-type *Providencia* strains. Apparently, these strains represent intermediates and biochemically behave more like *Providencia stuartii* than *P. rettgeri* (55). More recently, Penner and Hennessy in 1977 proposed the reassignment of *P. rettgeri* biogroup 5 strains to *Providencia stuartii* on the basis of somatic O antigens (51).

One hundred twenty-three *P. rettgeri* assigned to biotypes 9 and 10, serogroup O42, and other biotypes in our investigation were urea positive and negative in salicin, L-rhamnose, adonitol, and meso-erythritol and thus would be included in Penner's biogroup 5; 121 (98%) produced acid in trehalose. Of these 123 isolates, 13 were from St. Louis, 34 were from Memphis, 35 were from a university, and 41 were from miscellaneous sources. These 123 strains would be called *Providencia stuartii* urea positive by both Penner and the CDC Enteric Laboratory.

Serological group O42 was the type most commonly associated with St. Louis, Memphis, and university *P. rettgeri* strains. The majority (67 of 75) of these O42 *P. rettgeri* strains were resistant to 11 or more antimicrobial agents,

associated with disease, and inhibitory to a large number of indicator strains. Because of the isolation of O42 *P. rettgeri* from close geographic locations, one might hypothesize that this particular serotype was originally localized and then spread to surrounding areas. Many of the patients having *P. rettgeri* urinary tract infections had had spinal cord injuries, which resulted in these patients being moved from one health care center to another within the same geographical area. This may explain the localization of this specific serogroup.

All four typing methods were shown to be quite useful in differentiating epidemic and endemic *P. rettgeri*. Biotyping and antimicrobial agent susceptibility testing would be practical for use in the clinical laboratory during epidemiological investigations of infections caused by *P. rettgeri*. The serotyping schema developed by Penner is an excellent system, but antisera for the most common O groups are not commercially available. Bacteriocin typing would be cumbersome for most laboratories, and until the association of resistance and bacteriocin production is more fully understood, this system may have limited use with the more sensitive *P. rettgeri*.

Some variation was observed with biotyping and bacteriocin typing with epidemic isolates recovered from two single-strain outbreaks (Table 6). One of 13 isolates from an epidemic in St. Louis was indole positive, whereas 12 were indole negative. One could hypothesize that the patient from which the indole-positive strain was recovered was infected but was not part of the infection problem, as was observed with 12 patients infected with indole-negative *P. rettgeri*. Alternatively, the one biotype 9 isolate could have been part of the outbreak but regained the indole-positive characteristic. Since the only biochemical difference between *P. rettgeri* biotypes 9 and 10 is production of indole, one could postulate that indole production is a result of reversion of a single nucleotide change and that these strains are indeed the same. One isolate from Memphis was biotype 11. Biotypes 9 and 11 are closely related biochemically, with acid production with xylose and melibiose shown for biotype 11 organisms.

Three different bacteriocin types were noted with 6 *P. rettgeri* from Memphis that were otherwise identical to 26 organisms with the same marker patterns. Lack of stability with bacteriocin typing was observed for these six isolates since they were part of a single strain outbreak and should be bacteriocin type 2. A change in the sensitivity of indicator strains most likely explains differences in the bacteriocin types.

Each marker system appears to be reproduc-

ible with time by repeated testing. Fifty selected strains of *P. rettgeri* from epidemic and nonepidemic sources have been routinely checked with these four typing methods and found to be of the same type as previously described. Indicator strains remained active after testing with known bacteriocin producer strains.

Identical patterns were shown for *P. rettgeri* organisms from epidemic sources by cross-tabulations of types. Dissimilarity of *P. rettgeri* recovered from university and miscellaneous sources was shown by the lack of specific matches. Twelve strains from three different outbreaks were inadvertently included in the MISC isolates. These organisms demonstrated common patterns and were easily differentiated from the other endemic strains.

The number of types of *P. rettgeri* isolates from a source seemed related to its role in disease etiology. Consistently smaller numbers of specific serological, bacteriocin, and biochemical types were associated with organisms isolated from outbreaks of infection, whereas larger numbers of types were demonstrative of endemic strains. Isolates from known outbreaks were shown to be more resistant to drugs, whereas endemic strains were more sensitive. Strains of *P. rettgeri* from miscellaneous or nonepidemic sources illustrate best this diversity of types.

The use of multiple typing systems as defined in this study will more clearly characterize *P. rettgeri* strains and epidemiologically help by indicating the organism's similarity and dissimilarity to the infecting strains. Increasing the number of marking systems available for distinguishing an epidemic strain of *P. rettgeri* will aid in defining the epidemiology involved with intrahospital spread of organisms.

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