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Molecular Evolution, Species Distribution, and Clinical Consequences of an Endemic Aminoglycoside Resistance Plasmid

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During the first 6 years after appearing in one hospital, a 92-kilobase conjugative plasmid, pBWH1, which encoded resistance to chloramphenicol and sulfonamides and determined TEM-1 beta-lactamase and 2″-aminoglycoside nucleotidyltransferase, underwent a variety of molecular changes. It was most prevalent initially in isolates of *Klebsiella pneumoniae*, then in isolates of *Serratia marcescens*, and finally, after nearly disappearing, in isolates of *Enterobacter cloacae*. Evolutionary changes in the plasmid did not account for its shifts in species distribution, since the original molecule was found in isolates of each species. The late resurgence of pBWH1 occurred after a copy of its original molecule entered a distinctive ornithine decarboxylase-negative strain of *E. cloacae*, new to the hospital. The resulting transconjugant strain, chromosomally resistant to topical silver salts and to cephalosporins, and with the addition of pBWH1-encoded aminoglycoside resistance, spread in the hospital by causing an outbreak of sepsis in the burn unit, where these were commonly used antibacterial agents. Thus, an endemic plasmid became prevalent in a new host species because one of its genes supplemented the fitness of an uncommon strain of the species for a particular clinical niche.

The percentages of clinical isolates of different bacterial species resistant to specific antibiotics are frequently calculated to delineate the usefulness of these drugs. Such tabulations will reflect in large part the distribution of resistance plasmids among strains of the species being surveyed, since a majority of resistance genes are carried by plasmids (4). The distribution of the plasmids may be determined by properties of the plasmids themselves such as their transferability, host range, or stability or the selective advantages conferred by their resistance genes or other cryptic genes (4, 5). Also, properties of the host strains such as their fitness to occupy niches in which they come in contact with antibiotics might contribute to the observed distribution of plasmids.

The surveillance of antibiotic resistance plasmids has been aided by plasmid fingerprinting, using restriction endonucleases (6, 9, 10, 15, 24, 27) and computer-assisted analyses (16). However, the difficulty of identifying and following specific plasmids and strains in clinical settings has delayed the understanding of plasmid distribution and hence resistance distribution among species of bacteria. We describe here an unusual opportunity to do this by observing a well-studied plasmid, long endemic in one hospital, which entered a distinctive new strain and caused a new outbreak.

pBWH1, a 92-kilobase IncM plasmid encoding resistance to chloramphenicol and sulfonamide, as well as the 2″-aminoglycoside adenyltransferase (ANT-2″) and TEM-1 beta-lactamase resistance genes, appeared at the Peter Bent Brigham Hospital in 1975 in a previously uncommon urease-negative strain of *Klebsiella pneumoniae* (21). This plasmid, which encoded resistance to gentamicin and tobramycin, later spread to other strains of *K. pneumoniae* and nine other species of *Enterobacteriaceae* in hundreds of clinical isolates. The subsequent decline in the prevalence of pBWH1 and the clinical and genetic events of its later resurgence in a new species to cause an outbreak of burn wound sepsis are described here.

MATERIALS AND METHODS

From 1968 onward, all gram-negative blood isolates at the Peter Bent Brigham Hospital (and, after 1981, the combined Brigham and Women's Hospital) were purified and stocked in a mixture of 50% nutrient agar and 50% nutrient broth. Isolates from other clinical sites which were resistant to aminoglycosides were also obtained and stored. Antibiotic susceptibility testing was performed by a standard disk diffusion method, using commercially prepared disks (BBL Microbiology Systems) and 150-mm Mueller-Hinton plates (GIBCO Diagnostics). Zone diameters (in millimeters) were recorded and computer filed for analyses as described previously (21). MIC determinations were made by using microtiter panels (Micromedia, Boston, Mass.) as well as by the standard agar dilution method utilizing cation supplementation for aminoglycoside MIC determinations (26). Conjugal mating experiments were performed with *Escherichia coli* K-12 SY663 (*his hsdR met* Nal^r Rif^r Δtrp) as the recipient strain. The donor and recipient strains were grown separately overnight in dextrose-phosphate broth and then were grown to early log phase prior to mating. A 0.5-ml portion of each donor and the recipient *Escherichia coli* was suspended in 10 ml of dextrose-phosphate broth and incubated overnight. Selector plates of brucella agar containing 25 μg of chloramphenicol, gentamicin, or carbenicillin per ml plus nalidixic acid and rifampin were streaked with 20 μl of the mating suspension to assay for R-factor transfer to the *Escherichia coli* K-12 strain. Conjugal transfer was corroborated by the preparation of plasmid DNA (8).

pBWH1 was identified in clinical isolates by its characteristic antibiotype consisting of resistance to high concentra-

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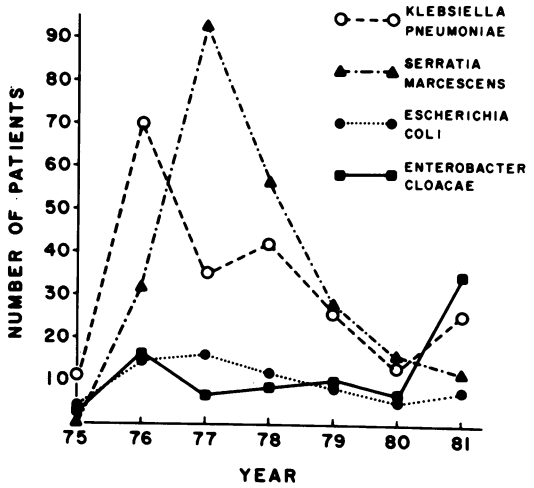

FIG. 1. Annual number of clinical isolates of the four most common species that carried pBWH1, 1975 to 1981. Only one isolate of a species was counted for any patient.

tions of chloramphenicol and sulfonamide and to moderate concentrations of kanamycin, gentamicin, and tobramycin as reflected in inhibition zone diameters of 8 to 14 mm (21). The correlation was confirmed in all of 52 random isolates selected by these criteria at this center over the 6-year period by always finding in transconjugants, transformants, or direct extracts of each the restriction fragments characteristic of pBWH1 (8, 16).

Plasmid DNA was prepared from overnight broth cultures, using a modification of the procedure of Birnboim and Doly (2, 16). Digestions with *Eco*RI endonuclease were performed for 2 h at 37°C, using the buffers recommended by the manufacturer (New England BioLabs, Inc., Beverly, Mass.). DNA fragments were fractionated electrophoretically through 0.7% agarose gel in Tris-acetate buffer (19) and then stained with ethidium bromide and photographed under UV illumination (Ultraviolet Products, Inc., San Gabriel, Calif.).

Resistance to silver nitrate was determined by the agar plate dilution technique in tryptone-yeast extract agar without NaCl supplementation, utilizing a Steers replicator (18). Cross-resistance to silver sulfadiazine was tested by measuring zones of inhibition on brucella agar plates around wells containing silver sulfadiazine. The determination of statistical significance was performed by using Student's *t* test.

RESULTS

Deployment of pBWH1. The number of patients with isolates of *K. pneumoniae* carrying pBWH1 (pBWH1$^+$) halved (from 70 to 34) while the number with pBWH1$^+$ *Serratia marcescens* tripled (from 31 to 92) during the second year of the plasmid's spread in the hospital (Fig. 1). During that year, more than half of the isolates containing pBWH1 were *S. marcescens*. Over the next 3 years the number of patients with pBWH1$^+$ isolates of any species declined steadily. Then, in 1981, *Enterobacter cloacae* became the predominant host species for pBWH1, as reflected in more than fourfold increases in the prevalence of this species' resistance to kanamycin, gentamicin, and tobramycin (Table 1) and a more than threefold increase in patients with isolates of *E. cloacae* with pBWH1 (Fig. 1).

The increase in isolates of pBWH1$^+$ *E. cloacae* was accounted for by 23 patients in the burn unit whose isolates

TABLE 1. *E. cloacae* isolates resistant to aminoglycoside antibiotics at the Brigham and Women's Hospital, 1978–1981

Yr	% Isolates resistant to:		
	Kanamycin	Gentamicin	Tobramycin
1978	5	13	8
1980	8	2	5
1981	32	32	35

were of one *E. cloacae* biotype (API 3205773) distinguished by its lack of ornithine decarboxylase activity (ODC$^-$) (Table 2). In 1980, there had been only five patients in the whole hospital with ODC$^-$ *E. cloacae*, none pBWH1$^+$ (Table 2). In 1981, nearly 70% of the ODC$^-$ *E. cloacae* isolates, but only 5% of other *E. cloacae* isolates, were pBWH1$^+$. Although the number of patients with at least one *E. cloacae* isolate increased by <25% between 1980 and 1981 (237 versus 283), the number of patients with ODC$^-$ *E. cloacae* increased sevenfold, and 80% of them were in the burn unit. There was no comparable increase in the burn unit isolation of other species or in the carriage of pBWH1 by other strains.

Molecular evolution of pBWH1. Figure 2 compares *Eco*RI digestion fragments of plasmids extracted from pBWH1$^+$ isolates in 1976 and 1981, and Fig. 3 compares those of 13 of the 14 burn unit blood isolates of pBWH1$^+$ ODC$^-$ *E. cloacae* in 1981. Plasmids from ODC$^-$ *E. cloacae* blood isolates of 5 of 13 patients from the burn unit outbreak in 1981 had all four resistance genes, transferability (Tra$^+$), and restriction endonuclease fragments identical to those of the prototype of pBWH1 that first appeared in urease-negative *K. pneumoniae* in 1975 (21) (Fig. 2, lanes 3, 6, 8, 13, 14, 16; Fig. 3, lanes a to e). ODC$^-$ *E. cloacae* isolates from three other burn unit patients had variations in the size of the two largest *Eco*RI fragments and retained the four resistance genes but were Tra$^-$ (Fig. 3, lanes f to h). Five other burn unit patients had ODC$^-$ *E. cloacae* with a variant plasmid which lacked the largest fragment, transferability (Tra$^-$), and the TEM-1 gene (TEM-1$^-$) (Fig. 2, lanes 12, 15; Fig. 3, lanes j to m). A plasmid from another 1976 isolate also lacked the largest

TABLE 2. Patients with different isolates of *E. cloacae*, compared by the absence or presence of ODC activity or plasmid pBWH1 or both

Patient location	Type of *E. cloacae* isolate[a]	No. of patients with *E. cloacae* first isolated from:			
		Blood		Other source[b]	
		1980	1981	1980	1981
Burn unit	ODC$^+$, pBWH1$^-$	7	4	36	25
	ODC$^-$, pBWH1$^-$	0	4	1	1
	ODC$^+$, pBWH1$^+$	2	1	6	9
	ODC$^-$, pBWH1$^+$	0	14	0	9
Other hospital wards	ODC$^+$, pBWH1$^-$	6	4	171	203
	ODC$^-$, pBWH1$^-$	0	0	4	6
	ODC$^+$, pBWH1$^+$	1	1	3	1
	ODC$^-$, pBWH1$^+$	0	0	0	1

[a] ODC$^-$ represents a lack of ornithine decarboxylase activity, pBWH1$^-$ represents the absence of the plasmid, and ODC$^+$ and pBWH1$^+$ represent the presence of these properties.

[b] Other sources include sputum, urine, cerebrospinal fluid, and wound cultures.

fragment but was Tra$^+$ and TEM-1$^-$ (Fig. 3, lane i). Other variants from 1976 isolates lacked one or more smaller fragments but were Tra$^+$ and had all four resistance genes. One had an additional large fragment and tetracycline resistance (Fig. 2, lane 9). Plasmid DNA was not isolated from aminoglycoside-susceptible ODC$^-$ *E. cloacae*.

Silver-resistant ODC$^-$ *E. cloacae*. Among 92 isolates of six species of gram-negative bacilli surveyed, including 39 isolates from the burn unit, only ODC$^-$ *E. cloacae* isolates (10 of 19 isolates, or 53%) were resistant to silver nitrate. All of the resistant isolates were also resistant to silver sulfadiazine, which was routinely applied to burn surfaces of patients in the unit. *Escherichia coli* K-12 derivatives in which pBWH1 was transferred or transformed from five of the silver-resistant ODC$^-$ *E. cloacae* isolates were not silver resistant, and plasmid extracts from one of the donors and its transconjugant had identical restriction endonuclease fragments. No silver-resistant transconjugants were obtained by silver nitrate selection of matings between *Escherichia coli* K-12 and these silver-resistant strains. pBWH1 was lost after serial passage of ODC$^-$ *E. cloacae* through media free of antimicrobial agents, but the silver resistance of these strains and their biotypes were unaffected.

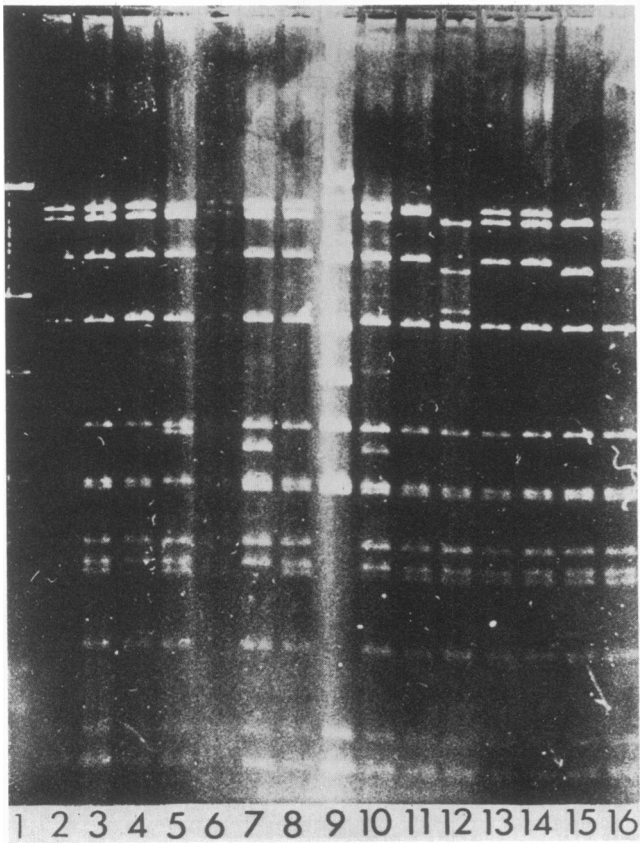

FIG. 2. Agarose gel electrophoresis of DNA fragments from *Eco*RI digests of plasmids extracted from clinical isolates and their transconjugants, 1976 to 1981. Fragments in lanes 2 to 16 are of plasmids extracted from *Escherichia coli* K-12 transconjugants of clinical isolates of *Escherichia, Klebsiella, Serratia, Enterobacter, Citrobacter*, or *Morganella* spp. with the antibiotypes characteristic of pBWH1, except for those in lanes 11, 12, 14, and 15, which are plasmids extracted directly from the isolates. Plasmids in lanes 2 to 10 were from strains isolated in 1976; those in lanes 11 to 16 were from strains isolated in 1981. Plasmids in lanes 12 and 15 lacked beta-lactamase activity, and the one in lane 9 also had tetracycline resistance. Lane 1 contains *Hin*dIII fragments of lambda phage with sizes of 23.5, 9.6, 6.77, 4.44, 2.28, and 1.95 kilobases.

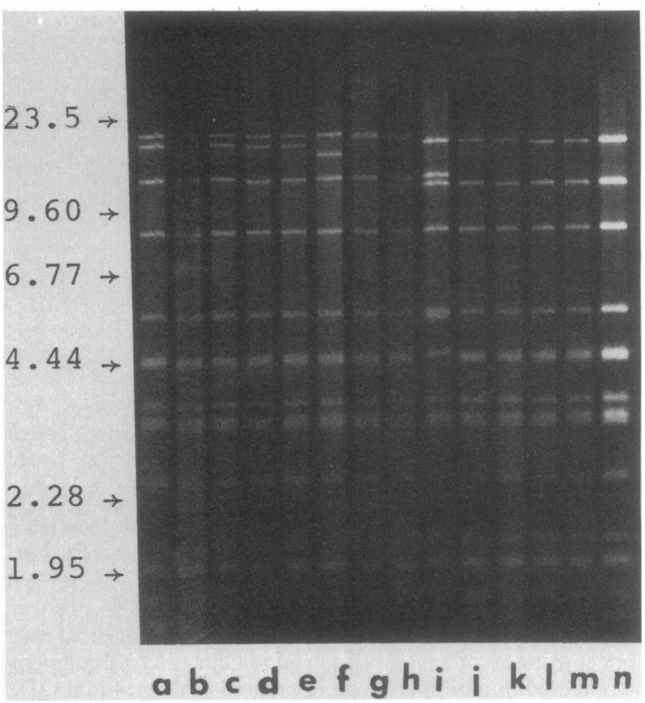

FIG. 3. Agarose gel electrophoresis of DNA fragments from *Eco*RI digests of plasmids extracted from 13 blood isolates of ODC$^-$ *E. cloacae* containing pBWH1. All preparations were digested with *Eco*RI restriction endonuclease. All lanes except lane i contain plasmid fragments derived from ODC$^-$ *E. cloacae* isolates from different burn unit patients at the Brigham and Women's Hospital, 1981. Plasmids in lanes i to n lack the TEM-1 beta-lactamase; those in lanes f to h and j to n are Tra$^-$. Lane b contains plasmid DNA derived from an *Escherichia coli* K-12 transconjugant of an ODC$^-$ *E. cloacae* isolate. Lane i contains plasmid fragments of an *Escherichia coli* transconjugant carrying pBWH1 from 1976. Kilobase size standards derived from a *Hin*dIII digest of lambda phage are displayed at the left. Clinical details are given in Table 4.

Clinical correlations. Burn unit patients who had clinical isolates from any site with pBWH1$^+$ ODC$^-$ *E. cloacae* were more often bacteremic (14 of 23, 61%) than patients with pBWH1$^-$ ODC$^+$ *E. cloacae* (11 of 72, 15%; $P < 0.05$, Student's *t* test) (Table 2). ODC$^-$ *E. cloacae* isolates carrying any of the pBWH1 variants were resistant to all of the antimicrobial agents used at that time in the burn unit, except for amikacin (Table 3). The patients who had pBWH1$^+$ ODC$^-$ *E. cloacae* at any site became bacteremic more often (11 of 12 versus 3 of 11; $P < 0.05$) if they had been treated earlier with an antibiotic inactivated by ANT-2″, such as tobramycin or gentamicin, and bacteremic patients survived more often (5 of 8 versus 0 of 6) if treated with one not inactivated by ANT-2″, such as amikacin (Table 4). Isolation of pBWH1$^+$ ODC$^-$ *E. cloacae* ceased after its distinctiveness was recognized and coincident with a fall in the patient census of the burn unit (Fig. 4).

DISCUSSION

pBWH1 entered the Peter Bent Brigham Hospital in late 1975 and was initially spread in a distinctive urease-negative strain of *K. pneumoniae* (21). Over the next 2 years, pBWH1 was found in 49 different biotypes of *Enterobacteriaceae*

TABLE 3. Contribution of pBWH1 to antibiotic resistance in ODC⁻ *E. cloacae*

Antibiotic	Mean MIC (μg/ml)			
	E. cloacae		*E. coli* K-12	
	pBWH1⁻[a]	pBWH1⁺[a]	pBWH1⁻[b]	pBWH1⁺[c]
Ampicillin	≥16	≥16	1	≥16
Cephalothin	≥64	≥64	2	8
Gentamicin	≤0.5	16	≤0.5	8
Tetracycline	4	4	1	0.5
Carbenicillin	≤8	≥512	≤8	≥512
Chloramphenicol	8	≥32	1	≥32
Tobramycin	≤0.5	16	≤0.5	8
Amikacin	≤1	1	≤1	2
Cefamandole	2	≥64	≤1	≤1
Cefoxitin	64	≥64	≤1	≤1
Cefotaxime	≤2	≤2	≤1	≤2

[a] pBWH1⁻ strains do not contain the plasmid; pBWH1⁺ strains do.
[b] *Escherichia coli* K-12 pBWH1⁻ is the standard SY663 recipient strain.
[c] *Escherichia coli* K-12 pBWH1⁺ is a typical transconjugant.

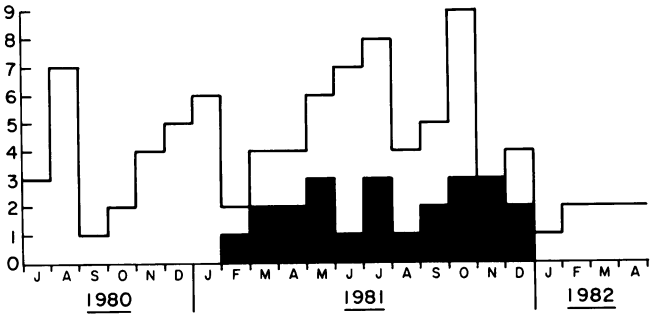

FIG. 4. Burn unit outbreak. Symbols: □, number of patients admitted each month; ■, number of patients with first isolate of ODC⁻ pBWH1⁺ *E. cloacae*.

(21). However, the eightfold increase in gentamicin resistance for *E. cloacae* in 1981 once again represented the spread of a single strain carrying pBWH1, illustrating how tabulation of aggregate species resistance may conceal different epidemiological events (13). This pattern of serial plasmid spread resulting in successive outbreaks in new strains and species raises the question of why certain plasmids become associated with different host strains at different times.

Evolutionary changes in pBWH1 as seen here in its molecular variants did not determine its successive shifts in species, since the prototypical molecule was found in each of the species over the 6-year period. Moreover, this original plasmid appeared to be the one which entered ODC⁻ *E. cloacae* in 1981 since the four other variants of pBWH1 observed in that strain were Tra⁻ and the first of these was not seen until 3 months after the outbreak began; also, aminoglycoside-susceptible isolates of ODC⁻ *E. cloacae* did not contain plasmid DNA. The four Tra⁻ variants of pBWH1 seen in ODC⁻ *E. cloacae* isolates from burn unit patients over the last 7 months of the outbreak may exemplify a tendency of plasmids to lose transferability after becoming established in a strain (22). That one particular variety of pBWH1 among the many Tra⁺ variants observed was the first to enter the hospital and then the first to enter this new strain 6 years later suggests that it encodes advantageous properties.

This plasmid has been found in thousands of isolates since entering this center in 1975 but was not seen in a survey of ANT-2″-encoding plasmids from 20 other U.S. centers (20). ODC⁻ *E. cloacae* had been previously rare in this hospital,

TABLE 4. Clinical characteristics of burn unit patients with ODC⁻ *E. cloacae* containing pBWH1⁺ (ODC⁻ pBWH1⁺)

Patient no.	Days from burn unit admission to first ODC⁻ pBWH1⁺ isolate	Beta-lactam or tobramycin used prior to first ODC⁻ pBWH1⁺ isolate[a]	Amikacin used after first ODC⁻ pBWH1⁺ isolate[b]	Patient with at least one blood isolate (+)	Patient outcome, lived (+) or died (−)	Letter corresponding to lane of plasmid digest in Fig. 3
1	53	β, T	+	+	+	a
2	6	β	−	−	+	
3	3	β	+	+	+	b
4	9	β, T	−	+	−	f
5	8	β, T	+	+	+	c
6	6	β, T	+	+	+	d
7	4	β	−	−	+	
8	4	β, T	+	+	−	j
9	6	β, T	−	+	−	k
10	3	β, T	+	+	−	e
11	3	β, T	−	+	−	l
12	7	−	−	−	+	
13	38	β, T	−	−	+	
14	76	β	−	−	+	
15	20	β	−	−	+	
16	7	β, T	+	+	−	g
17	4	β, T	−	+	−	m
18	3	β	−	+	−	n
19	6	β	+	+	+	n
20	16	β	−	−	+	
21	3	β	−	−	+	
22	21	β	−	−	+	
23	5	β, T	−	+	−	h

[a] β, Beta-lactam used; T, tobramycin used.
[b] +, Amikacin used.

not found in the burn unit and not a host for pBWH1. Thus, a new strain caused a new epidemic after the acquisition of an endemic plasmid.

E. cloacae strains have been reported increasingly to cause burn unit outbreaks with high rates of bacteremia (11, 14, 17). Their chromosomal beta-lactamase genes (25) make them resistant to the cephalosporins used widely in this burn unit (Tables 3 and 4). Chromosomal resistance to silver has been reported in other burn unit strains of *E. cloacae* (1, 7, 14, 23). To outgrow competitors in burn wounds and spread to other burned patients mostly treated with topical silver salts or with broad-spectrum beta-lactam antibiotics or tobramycin or both, the ODC$^-$ strain of *E. cloacae* described here lacked only aminoglycoside resistance, which pBWH1 brought to it. Thus, pBWH1$^+$ ODC$^-$ *E. cloacae* became prevalent and had the highest rate of bacteremia of *E. cloacae* strains because one of the plasmid genes, ANT-2″, supplemented the fitness of the strain for the particular niche of burn wounds treated with a specific antibacterial regimen. This strain lost the plasmid after serial passage in unsupplemented media, which is consistent with the possibility that the burn unit environment helped to select for pBWH1 carriage by ODC$^-$ *E. cloacae*.

pBWH1 could be traced by its distinctive resistance phenotype (antibiotype), and the ODC$^-$ *E. cloacae* strain could be traced by its enzymatic phenotype (biotype), both routinely recorded in the hospital laboratory, which was analogous to the association of pBWH1 and urease-negative *K. pneumoniae* (API 5205773) 5 years earlier (21). Antibiotypes have rarely been used to monitor plasmid spread, and biotypes have been considered not reproducible enough to identify strains (3). Although certain extrachromosomal genes may not notably alter the antibiotype of a particular species (e.g., a TEM-1 beta-lactamase in *K. pneumoniae*), our experience here suggests that the potential of these routinely available phenotypes for surveillance could be further explored, perhaps with improved systems for biotyping.

An on-line computer system programmed to discriminate distinctive antibiotypes and biotypes in current isolates could have recognized pBWH1 as new to the hospital in the first pBWH1$^+$ isolate in 1975 and new to the strain in the first pBWH1$^+$ ODC$^-$ *E. cloacae* isolate in the burn unit in 1981 (12), instead of in retrospect as we did here. The populations of those pBWH1$^+$ strains would have been smallest at those times, presumably, and thus most amenable to containment by the isolation of index cases and by selection of alternative antimicrobial agents. Future surveillance of nosocomial infections should integrate knowledge about specific resistance plasmids as well as bacterial species to control the spread of antibiotic resistance.

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LITERATURE CITED

1. **Annear, D. I., B. J. Mee, and M. Bailey.** 1976. Instability and linkage of silver resistance, lactose fermentation, and colony structure in *Enterobacter cloacae* from burn wounds. J. Clin. Pathol. **29**:441–443.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Butler, D. A., C. M. Lobregat, and T. L. Gavan.** 1975. Reproducibility of the Analytab (API 20E) system. J. Clin. Microbiol. **2**:322–326.
4. **Davies, J., and D. I. Smith.** 1978. Plasmid-determined resistance to antimicrobial agents. Annu. Rev. Microbiol. **32**:469–518.
5. **Falkow, S.** 1981. Bacterial pathogenicity, an overview, p. 91–100. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Publishing Corp., New York.
6. **Farrar, W. E., Jr.** 1983. Molecular analysis of plasmids in epidemiologic investigation. J. Infect. Dis. **148**:1–11.
7. **Gayle, W. E., C. G. Mayhall, V. A. Lamb, E. Apollo, and B. W. Haynes.** 1978. Resistant *Enterobacter cloacae* in a burn center: the ineffectiveness of silver sulfadiazine. J. Trauma **18**:317–323.
8. **Hopkins, J. D., K. H. Mayer, E. S. Gilleece, T. F. O'Brien, and M. Syvanen.** 1986. Genetic and physical characterization of IncM plasmid pBWH1 and its variance among natural isolates. J. Bacteriol. **165**:47–52.
9. **John, J. F., Jr., K. T. McKee, Jr., J. A. Twitty, and W. Schaffner.** 1983. Molecular epidemiology of sequential nursery epidemics caused by multiresistant *Klebsiella pneumoniae*. J. Pediatr. **102**:825–830.
10. **John, J. F., Jr., and W. F. McNeill.** 1981. Characterization of *Serratia marcescens* containing a plasmid coding for gentamicin resistance in nosocomial infections. J. Infect. Dis. **143**:810–817.
11. **John, J. F., Jr., R. J. Sharbaugh, and E. R. Bannister.** 1982. *Enterobacter cloacae*: bacteremia, epidemiology, and antibiotic resistance. Rev. Infect. Dis. **4**:13–28.
12. **Kishi, H., D. Evans, J. D. Hopkins, A. A. Medeiros, and T. F. O'Brien.** 1984. Diagnostic microbiology laboratory susceptibility test results discriminate distinctive antibiotic resistance plasmids. Diagn. Microbiol. Infect. Dis. **2**:309–316.
13. **Lorian, V., and B. A. Atkinson.** 1984. Antimicrobial agent susceptibility patterns of bacteria in hospitals from 1971 to 1982. J. Clin. Microbiol. **20**:791–796.
14. **Markowitz, S. M., S. M. Smith, and D. S. Williams.** 1983. Retrospective analysis of plasmid patterns in a study of burn unit outbreaks of infection due to *Enterobacter cloacae*. J. Infect. Dis. **148**:18–23.
15. **Markowitz, S. M., M. J. Veazey, Jr., F. L. Macrina, C. G. Mayhall, and V. A. Lamb.** 1980. Sequential outbreaks of infection due to *Klebsiella pneumoniae* in a neonatal intensive care unit: implication of a conjugative R plasmid. J. Infect. Dis. **142**:106–112.
16. **Mayer, K. H., J. D. Hopkins, E. S. Gilleece, L. Chao, and T. F. O'Brien.** 1984. Computer assisted correlations between antibiotypes of clinical isolates and the endonuclease restriction fragment types of their plasmids, p. 163–169. *In* S. Mitsuhashi, L. Rosival, and V. Krcmery (ed.), Transferrable antibiotic resistance: plasmids and gene manipulation. Czechoslovak Medical Press, Prague.
17. **Mayhall, C. G., V. A. Lamb, W. E. Gayle, Jr., and B. W. Haynes, Jr.** 1979. *Enterobacter cloacae* septicemia in a burn center: epidemiology and control of an outbreak. J. Infect. Dis. **139**:166–171.
18. **McHugh, G. L., R. C. Moellering, C. C. Hopkins, and M. Swartz.** 1975. *Salmonella typhimurium* resistant to silver nitrate, chloramphenicol, and ampicillin. Lancet **i**:235–240.
19. **Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. **127**:1529–1537.
20. **O'Brien, T. F., M. Pla, K. H. Mayer, H. Kishi, E. Gilleece, M. Syvanen, and J. D. Hopkins.** 1985. Intercontinental spread of a new antibiotic resistance gene on an epidemic plasmid. Science **230**:87–88.
21. **O'Brien, T. F., D. G. Ross, M. A. Guzman, A. A. Medeiros,**

R. W. Hedges, and D. Botstein. 1980. Dissemination of an antibiotic resistance plasmid in hospital patient flora. Antimicrob. Agents Chemother. **17**:537–543.

22. **Rennie, R. P., and I. B. R. Duncan.** 1977. Emergence of gentamicin-resistant *Klebsiella* in a general hospital. Antimicrob. Agents Chemother. **11**:179–184.
23. **Rosenkranz, H. S., J. E. Coward, T. J. Wlodkowski, and H. S. Carr.** 1974. Properties of silver sulfadiazine-resistant *Enterobacter cloacae*. Antimicrob. Agents Chemother. **5**:199–201.
24. **Sadowski, P. L., B. C. Peterson, D. N. Gerding, and P. P. Cleary.** 1979. Physical characterization of ten R plasmids obtained from an outbreak of nosocomial *Klebsiella pneumoniae* infections. Antimicrob. Agents Chemother. **15**:616–624.
25. **Sanders, C. C.** 1983. Novel resistance selected by the new expanded-spectrum cephalosporins. J. Infect. Dis. **147**:585–589.
26. **Thrupp, L. D.** 1985. Susceptibility testing of antibiotics in liquid media, p. 93–150. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
27. **Tompkins, L. S., J. K. Plorde, and S. Falkow.** 1980. Molecular analysis of R-factors from multiresistant nosocomial isolates. J. Infect. Dis. **171**:625–636.