acetone, and stained with fluorescein isothiocyanate–labeled rabbit anti–*Borrelia burgdorferi* conjugate, which cross-reacted with the *O. coriaceus* spirochete and *B. hermsii*. The antiserum was prepared against whole cells of *B. burgdorferi*, and the gamma globulin fraction was conjugated by the methods of M. Peacock, W. Burgdorfer, and R. A. Ormsbee [*Infect. Immun.* **3**, 355 (1971)]. If fluorescence microscopy revealed spirochetes, then Malpighian tubules, salivary glands, and occasionally coxal organs were examined in the same manner and wet mounts of infected tissues were inspected by dark-field microscopy.
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18. Four Swiss mice were inoculated with 0.2 ml of a suspension containing infected tick tissues triturated in 1.5 ml of phosphate-buffered saline (pH 7.4); seven mice from a second litter were inoculated intraperitoneally with 0.05 ml of a suspension consisting of the infected tissues of two ticks triturated in 2 ml of BSK II medium. They were bled on days 4 through 14 after inoculation, and thick smears were stained with fluorescein isothiocyanate–labeled anti–*B. burgdorferi* conjugate. Each of the BALB/c mice was injected with $\sim 10^7$ organisms and examined similarly for spirochetes. The rabbits were fed upon by 11 to 43 ($\bar{x}$ = 19.3) nymphal or adult ticks, and thick and thin (Giemsa-stained) blood smears were prepared daily for 14 to 21 days. Infected ticks were identified by a direct immunofluorescence study of multiple tissue smears or coxal fluid.
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Intercontinental Spread of a New Antibiotic Resistance Gene on an Epidemic Plasmid

Abstract. *Bacteria of different genera isolated at nine medical centers in different parts of the United States and at one center in Venezuela during the first decade of gentamicin usage carried the gentamicin resistance gene 2″-aminoglycoside nucleotidyltransferase on the same transferable plasmid. Such widespread dissemination of a newly observed resistance gene on one plasmid suggests that a new resistance gene may emerge once on a single plasmid, which then carries it to other centers and other plasmids. The resistance gene might, therefore, be contained if detected early.*

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A gene encoding resistance to an antibiotic may begin to appear in bacteria of many genera at a succession of medical centers only after the antibiotic has been used for several years (*1–8*). Presumably, the antibiotic is eventually used in the presence of an obscure bacterial strain carrying the resistance gene or its ancestor, and the overgrowth of this strain increases the chances of the gene's becoming inserted into a plasmid that can transfer between bacteria (*9–11*). The gentamicin resistance gene, 2″-aminoglycoside nucleotidyltransferase [ANT(2″)], was observed early in its appearance and was found on different plasmids in different centers (*4–6, 8, 12, 13*). These observations were consistent with a separate emergence of the resistance gene at each center. We have now examined isolates from many centers and find that ANT(2″) was on the same plasmid in various genera at nine widely separated centers. This suggests that ANT(2″) had emerged once on this plasmid and had then been widely disseminated by it (*14*).

We studied collections of gentamicin-resistant isolates of enteric bacteria from the Brigham and Women's Hospital, Boston (BW); Hospital Centro Medico, Caracas, Venezuela (CM); and the West Roxbury Veteran's Administration Hospital, Boston (WR); as well as isolates collected from various centers by Schering Laboratories (SL) and a single isolate from the Seattle Veteran's Administration Hospital (SV). The isolates had been stored in agar in sealed tubes at room temperature or at 4°C. Isolates were mated with a strain of *Escherichia coli* K-12 (SY 663; *trpΔ met his nal*^{r} *rif*^{r}). Isolate susceptibility to antibacterials was tested by disk diffusion. Plasmid DNA was extracted by an alkaline method (*15*) and digested by restriction endonucleases Eco RI or Bgl II. Digest fragments, subjected to electrophoresis in 0.7 percent agarose gel, were stained with ethidium bromide and photographed under ultraviolet light.

Eleven isolates from these collections transferred gentamicin resistance on plasmids that shared identical Eco RI fragment sizes (Table 1 and Fig. 1). The similarity of these fragments to those of the previously reported pLST1000 (*6*) led us to adopt that designation for these plasmids. Six of these 11 isolates had been among 38 from the SL collection chosen for mating because they were resistant to the moderate levels of kanamycin, gentamicin, and tobramycin characterizing ANT(2″) (*16*). Another 21 of these 38 isolates also transferred gentamicin resistance, but their plasmid restriction fragments differed, and as far as we could judge no two from different centers were the same. Finding ANT(2″) on a different plasmid in each of a majority of these centers was in agreement with previous reports from individual centers (*4–6, 8, 12, 13*) and contrasts with our finding it on pLST1000 in nine widely separated centers in the United States and one center in Venezuela.

Each of the pLST1000 plasmids also carried resistance to streptomycin and to ampicillin. Enzyme assays performed on cell-free extracts of a pLST1000 transconjugant gave the substrate profile of ANT(2″) (*17*). The pLST1000 plasmid hybridized with a gene probe for ANT(2″) and with another for the 3″-aminoglycoside nucleotidyltransferase [ANT(3″)] (*18*), which produces resistance to streptomycin. Isoelectric focusing studies showed that each of the clini-

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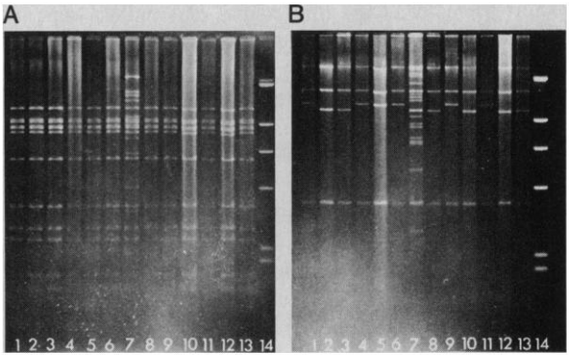

Fig. 1. Photographs of agarose gels in which the plasmids described in Table 1 were subjected to electrophoresis after digestion with Eco RI (A) or Bgl II (B). Sizes of Hind III digest fragments of lambda phage in lane 14 are (in descending order) 23.5, 9.6, 6.77, 4.44, 2.28, and 1.95 kilobases.

Table 1. Clinical isolates carrying pLST1000. Plasmids shown in lanes of Fig. 1 were extracted from *E. coli* transconjugants of the clinical isolates, except those in lanes 5 and 7, which were extracted directly from the isolates.

Lane in Fig. 1	Year	City	Collection	Genus
1	1975	Miami	SL	*Escherichia*
2	1976	Boston	BW	*Serratia*
3	1976	Seattle	SV	*Serratia*
4	1977	Philadelphia	SL	*Klebsiella*
5	1978	Syracuse	SL	*Serratia*
6	1978	Syracuse	SL	*Serratia*
7	1978	Los Angeles	SL	*Citrobacter*
8	1978	Los Angeles	SL	*Citrobacter*
9	1980	Chicago	SL	*Escherichia*
10	1980	Boston	WR	*Klebsiella*
11	1980	Gainesville	SL	*Citrobacter*
12	1982	Caracas	CM	*Klebsiella*
13	1983	Boston	BW	*Enterobacter*

cal isolates in Table 1 and their pLST1000 transconjugants produced both the TEM 1 and OXA 2 beta lactamases (*19*).

The plasmid initially designated pLST1000 has been in an epidemic strain of *Serratia marcescens* and in six other enteric species at one center in Seattle (*6*). The identical plasmid was found in four different genera in two centers in Boston. The *E. coli* isolate from Syracuse came from the cerebrospinal fluid of an infant with meningitis. The pLST1000-carrying *Klebsiella pneumoniae* from Caracas was isolated from the blood of a patient who had never been outside of Venezuela.

Wide geographic dissemination of a single resistance plasmid is not unprecedented, but the earlier examples appear to represent geographic dissemination of host strains bearing particular resistance plasmids. The nontransferring pBP1 has been found in *E. coli* from many countries (*20*). Epidemic strains of *Salmonella, Neisseria gonorrhoeae*, and *Hemophilus influenzae* have carried particular plasmids with them (*21–23*). In contrast, pLST1000 appeared to be epidemic through strains of many genera, and no common carrier strain was found. Furthermore, the genes on those plasmids observed earlier encoded resistance to older antibacterials and had been ubiquitous on other plasmids, so that little could be inferred about their emergence or early spread, whereas pLST1000 appeared in centers where ANT(2″) had not previously been seen.

It is possible that pLST1000 had been at more of the centers, since we examined more than one or two plasmids only from WR, CM, and BW. At BW, where ANT(2″) was usually found on an endemic IncM plasmid, pBWH1 (*5*), pLST1000 was so rare that it was detected only by an extensive survey. This could be an example of a plasmid bringing a resistance gene to a center, transposing it to another plasmid, and then becoming obscured by spread of the second plasmid (*24*). Transposition of ANT(2″) has been reported (*14*). It is also possible for an epidemic plasmid—related or unrelated to pLST1000—to appear dissimilar in different centers if it is less conserved than the exceptionally stable pLST1000. The molecules of pLST1000, as shown in Fig. 1, exhibited only one restriction fragment variation over the decade, which is fewer than has been reported for other plasmids (*8, 22, 25*). More detailed analyses for either of these possibilities might thus extend the list of centers with ANT(2″) genes that could be traced back to one plasmid.

Bacteria had plasmids before antibiotics were used, but plasmids of strains isolated then did not have resistance genes (*9*). Genes encoding resistance to earlier generations of antibiotics emerged and became widely distributed on plasmids before methods for tracing their spread became available (*9–11*). In the example presented here—one of the earliest traceable—the resistance gene spread to many centers from one emergence. Had ANT(2″) been detected and its epidemic potential foreseen where it first emerged on a plasmid, attempts to contain it there or prevent its entry into other alerted centers might have proved more effective than later efforts to curtail patient to patient spread at each of the centers. Such surveillance would be possible now for the emergence of genes encoding resistance to recently introduced antibiotics (*26, 27*).

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