CLINICAL MICROBIOLOGY REVIEWS, Apr. 1988, p. 228–243
0893-8512/88/020228-16$02.00/0

Vol. 1, No. 2

Use of Plasmid Profiles in Epidemiologic Surveillance of Disease Outbreaks and in Tracing the Transmission of Antibiotic Resistance

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INTRODUCTION

A number of typing systems have been used to determine whether a set of bacterial isolates represents a single strain or more than one strain. These include serotyping, biochemical typing, auxotyping, isoenzyme typing, antimicrobial susceptibility testing, bacteriophage typing, bacteriocin typing, and plasmid profiles. Newer typing systems are available that use deoxyribonucleic acid (DNA) probes (188) (including probes derived from chromosomal fragments [196] and ribosomal ribonucleic acid as a probe [69]), polyacrylamide gel electrophoresis of bacterial proteins (136), or chromosomal DNA restriction endonuclease digest patterns (17, 20, 21, 104, 107, 200). Some of these techniques are being used on organisms other than bacteria (13, 25). All of these typing systems have been helpful in understanding the natural history of bacterial diseases. Many disease outbreaks were successfully investigated before the methods for plasmid profiles were available (for example, see reference 2). Each method has advantages and disadvantages when applied to a specific situation.

Although this review is limited to a discussion of human pathogens, plasmids have been observed in, and in some cases plasmid profiles have been used to study, pathogens of animals, (142, 146), fish (3), fowl (35), and plants (201). Not every study presented here describes plasmid profiles used in an epidemiological investigation. In some cases the plasmids of a particular species are discussed to emphasize that the potential to do plasmid profile analysis is available. Plasmid sizes are often given by two different terms: megadaltons and kilobases (kb). The conversion factor is 1 megadalton = 1.51 kb. Throughout this review, the term kilobase is used, because it is easy to convert kilobases into gene lengths. An average-sized gene is about 1 kb and encodes a protein of 35,000 daltons.

Recently, some plasmids have been described which are not circular molecules (11, 98). In the case of *Borrelia burgdorferii*, the linear plasmids have been shown to have covalently closed ends (11). Although these linear plasmids are very unusual in their physical form, they are very much like other plasmids in that they encode interesting gene products, including outer surface proteins in *B. burgdorferii* (11) and antibiotic biosynthesis genes in *Streptomyces* spp. (98).

WHAT ARE PLASMIDS AND TRANSPOSONS AND WHY ARE THEY IMPORTANT?

A plasmid is an autonomous self-replicating extrachromosomal DNA element (113). Plasmids are not essential for normal bacterial growth. It has been pointed out that some phage genomes fit within this definition (76). Because of the similarities between plasmids and phage genomes, both can carry antibiotic resistance or toxin genes; for example, some investigators think of bacteriophage as plasmids with protein coats.

Plasmids are infectious. They can be transferred between bacteria of the same or different genera. Usually all functions required for plasmid transfer, including synthesis of pili, are encoded by genes on the plasmid. Thus, after transfer to a second host, these genes may enable a newly formed trans-

conjugant to become a donor in another round of conjugation. This process may be repeated several times. Plasmids were initially discovered because they are infectious (reviewed in reference 56). Some examples given below describe the introduction of a single plasmid into a hospital, followed by its spread to a number of pathogenic bacterial species and genera.

Plasmids can carry genes that code for functions other than transfer and replication, such as antibiotic resistance and toxin, adhesin, metabolic enzyme, and bacteriocin production (56, 76).

A transposable element is a DNA segment that can insert itself into several sites in a genome, independent of the ordinary recombination process (41). A transposon is a complex transposable element that contains genes unrelated to insertion functions and often has insertion sequence elements. The insertion sequence elements usually are at the ends of transposons and are called inverted- or direct-repeat sequences, depending on their orientation. See references 26, 41, 87, and 113 for a general discussion or references 80 and 99 for more detail about two well-characterized transposons, Tn*3* and Tn*10*. Transposons may contain genes encoding antibiotic resistance or toxins. The term "jumping genes" has been used to describe transposons because they can move from one genome to another.

The promiscuity of transposons jumping from DNA to DNA, whether in a plasmid, chromosome, or phage, is a major factor in dissemination of antibiotic resistance. Because plasmids can elaborate their own system for self-transfer between bacteria, it is not unexpected that antibiotic resistance in bacteria has become a major problem. For example, in 1972 trimethoprim resistance (Tp^r) in *Enterobacteriaceae* was associated only with incompatibility group W plasmids, but by 1980 Tp^r was found on incompatibility group W, B, C, D, I, FII, N, P, and X plasmids (28, 49, 185).

HISTORY OF PLASMID PROFILES IN THREE EXAMPLES

In 1975, an outbreak began in Minneapolis when the index case brought gentamicin-resistant *Klebsiella pneumoniae* into a hospital (170). In addition to gentamicin, the resistance (R) plasmid in this strain also encoded resistance to tobramycin, kanamycin, ampicillin, carbenicillin, cephalothin, chloramphenicol, and sulfathiazole. Eventually, plasmids with similar antimicrobial susceptibility patterns were isolated and characterized from 10 clinical isolates that included multiple types of *K. pneumoniae*, as well as *Escherichia coli*, *Enterobacter cloacae*, and *Morganella* (*Proteus*) *morganii*.

Plasmids were isolated from radioisotopically labeled bacteria, using dye-buoyant density centrifugation. The sedimentation values of the plasmids were determined by neutral or alkaline sucrose gradient centrifugation. After digestion with restriction endonuclease, the resulting fragments were separated by agarose gel electrophoresis. The gels were sliced, and the amount of radioactivity in each slice was determined by liquid scintillation counting. The plasmids were shown to be identical by their restriction endonuclease digest patterns and sedimentation values.

The techniques used in this study represented the "state of the art" at that time. They are labor intensive and expensive; the procedures that took several weeks to do at that time could now be done in 2 or 3 days. This study represents the first detailed characterization of R plasmids isolated from multiple bacterial species in the same outbreak, using molecular biology. An unusual antimicrobial susceptibility pattern, first seen in *K. pneumoniae* and then in other species, was the initial indication that an epidemic related to a single plasmid was occurring.

In 1976, Meyers et al. first reported plasmid profile characterization by agarose gel electrophoresis (129). They standardized the method with previously characterized plasmids from *E. coli* K-12, some of which had been transferred from their original hosts into K-12. The plasmids had been isolated by cesium chloride-ethidium bromide gradient centrifugation and characterized by electron microscopy and sucrose gradient sedimentation.

They also examined clinical isolates of *E. coli* from a study of traveller's diarrhea in Mexico. Several other species were surveyed, including *K. pneumoniae*, *Citrobacter freundii*, *Shigella dysenteriae*, *Enterobacter cloacae*, *Salmonella* serotypes, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Bacteroides fragilis*, and *Streptococcus faecalis*. This work introduced a method suitable for surveying several isolates at the same time and one which was simple, relatively inexpensive, and applicable to many bacterial species.

One of the first studies to use plasmid profiles to investigate a salmonellosis epidemic is also one of the most unusual (187). An account of this investigation, written by Berton Roueche, was published in the *New Yorker* (166). *Salmonella* serotype muenchen was isolated from 85 cases in Ohio, Michigan, Georgia, and Alabama. No food source could be implicated as the vehicle of transmission. The rate of exposure to marijuana was higher in patients in Michigan (76%) than in controls (21%). Further epidemiological investigation suggested that exposure to marijuana was a risk factor in the other states. Samples of marijuana from patient households contained as many as 10^7 *Salmonella* serotype muenchen per g. All isolates from both patients and marijuana were uniformly susceptible to all antibiotics tested and were biochemically indistinguishable from control strains. Plasmids of 4.6 and 11 kb were found in every isolate epidemiologically associated with contaminated marijuana. None of the control strains from previous years or from sources unrelated to marijuana had this plasmid profile. Subsequent surveillance of *Salmonella* serotype muenchen isolates suggested that contaminated marijuana had also been distributed in Arizona, West Virginia, Massachusetts, California, and Wisconsin.

PRINCIPLES OF PLASMID PROFILES

The approach to using plasmid profiles as an epidemiological typing system is similar to that of other typing schemes. The ideal situation would be to show that all of the outbreak-associated strains have the same plasmid profile and are the same by any other typing system used. This is still not necessarily sufficient to implicate a common source of infection. There are examples in which most if not all isolates of a bacterial species from one geographic area are identical. In some instances all known isolates have the same plasmid profile. If the plasmid encodes some virulence factor, it may be that only strains with a particular plasmid profile are capable of infecting humans.

For a variety of reasons, when doing plasmid profiles, it must be shown that control isolates from non-outbreak-related patients and environmental isolates (if possible) are different from the outbreak strain. As with any epidemiological study, care must be taken to match the control isolates to case isolates as accurately as possible.

All available information must be used. Strains that have similar plasmid profiles but are grossly different by antimi-

crobial susceptibility testing, phage typing, serotyping, or another typing system are not identical.

One advantage of plasmid profiles over many other typing systems is that a single set of reagents and equipment is applicable to many species of bacteria. Phage typing requires a battery of phage and indicator strains and is standardized for only a few species of bacteria. For example, phage typing systems are not available for *Salmonella* spp. other than *Salmonella* serotype typhimurium and *Salmonella* serotype typhi. Serotyping also requires a battery of antisera, which may be difficult and expensive to prepare and standardize, as well as a large number of known positive control strains. Antimicrobial susceptibility testing has the advantages of being done in a large number of laboratories and being highly standardized. The susceptibility pattern can be related to the plasmid content because of the wide variety of resistance genes that are plasmid encoded. Susceptibility patterns may be influenced by local antibiotic usage, which may make differentiation of outbreak strains from other strains in the same region very difficult. Plasmid profiles often offer a higher level of sensitivity in distinguishing between antibiotic resistance patterns. The same antibiotic resistance pattern can be encoded by unrelated plasmids (146), and even the same antibiotic modification enzymatic activity has been shown to be encoded by two different DNA sequences (109).

The presence or absence of plasmids does not by itself determine whether plasmid profiles can be done. For any typing system to be effective, it must differentiate case from noncase isolates. When plasmid profiles of groups of isolates are compared, there must be sufficient differences in the patterns to allow them to be readily separated. Usually, the lack of plasmids or finding an identical plasmid profile in case strains and control strains known to be unrelated to the disease outbreak makes plasmid analysis of little use. All isolates of *Salmonella* serotype horsham that have been examined (26 strains from the United States, Europe, and Japan) have the same plasmid, as shown by plasmid profile analysis and restriction endonuclease digest patterns (30; K. A. Birkness and L. W. Mayer, unpublished data). This uncommon *Salmonella* serotype may represent an unusual finding, but it illustrates a case in which plasmid profiles are not useful. In some bacterial genera, such as *Salmonella* or *Shigella*, plasmids occur so frequently that multiple isolates of a plasmid-free strain from an outbreak setting would be an epidemiologically significant finding. The lack of plasmid itself may be used as a marker for some isolates in some situations.

Just as antibiotic resistance patterns can be related to the organism's plasmid content, the serotype, phage susceptibility, or other phenotypic characteristics may be plasmid encoded. Metabolic properties, such as the ability to ferment lactose, utilize citrate, and produce urease, are repeatedly seen as plasmid-encoded characteristics. Plasmid-encoded virulence factors may be surface antigens on which other typing systems are based, e.g., serotyping or outer membrane protein profiles. Some restriction-modification systems are plasmid encoded; this may affect phage typing. Thus, different typing systems may not be totally independent of one another.

Plasmid profiles are most useful when combined with other methods for screening or typing. For example, several methods were used in an investigation of the largest outbreak of salmonellosis ever recorded in the United States (168). Over 16,000 culture-confirmed cases were identified in less than 2 months. The number of cases was estimated to be between 150,000 and 201,000. To identify the source of the outbreak, a large number of *Salmonella* serotype typhimurium isolates were first screened by a replica-plating antimicrobial susceptibility testing procedure. Due to the limitations of this method, any strain whose susceptibility pattern differed from that of the outbreak strain by two or fewer individual drug resistances was tested by a standardized disk susceptibility method. Any strain that had an antibiogram similar to that of the outbreak strain (one or no differences) was then screened by plasmid profile. Although this approach was not successful at identifying the contamination source, a large number of isolates could be screened in a short time by the combined methods. Over 1,200 strains were screened by replica plating in less than 1 week. Almost 200 disk antimicrobial susceptibility tests and 107 plasmid profiles were done. Only two strains identical to the outbreak strain were identified, and epidemiological investigation demonstrated that both individuals had been exposed to the contaminated product.

HOST RANGE AND PLASMID EPIDEMICS

Transfer of plasmids is very common between members of the family *Enterobacteriaceae* (47, 57–59, 68, 124, 157, 167, 173, 184), between enteric and other gram-negative bacteria (36, 70, 171, 195), and between or within nonenteric gram-negative organisms (148, 165). The infectious nature of plasmids was a key feature in their discovery (see reference 56 for a review). An early study utilized plasmid profiles to study the probability of R-plasmid transfer between *K. pneumoniae* and *Enterobacter cloacae* in a burn unit (55). Plasmid transfer between staphylococci is also common (40, 88, 114, 191). Plasmid-mediated beta-lactamase production and aminoglycoside resistance have been reported in *Enterococcus* (*Streptococcus*) *faecalis* and other group D enterococci (43, 137, 210). Antibiotic resistance transfer among streptococci can be mediated by plasmids or by other elements called conjugative transposons (38). Plasmid transfer between or within other gram-positive bacteria also occurs (71, 72, 176). Transfer of resistance determinants from gram-positive to gram-negative organisms and *Mycoplasma* spp. is now being seen (134, 164).

Because plasmids can be transferred so readily among bacteria, and some can replicate in various species, a single plasmid has been observed in several bacterial species during a number of outbreaks. These outbreaks are sometimes called plasmid epidemics (32). Transposons carrying antibiotic resistance genes can also be inserted into a number of different replicons. The following examples illustrate these types of events.

A 92-kb conjugative plasmid with several antibiotic resistance genes was first observed in a *K. pneumoniae* isolate in a Boston hospital in 1975 (124). Over the next 2 years, *Serratia marcescens* and then *Enterobacter cloacae* were the predominant organisms isolated with this plasmid, but it was observed in 49 different strains within at least six species of *Enterobacteriaceae*. Restriction endonuclease digests of plasmid DNA from these isolates showed plasmids that were identical or related to that of the index isolate. Some evolution of the plasmid by DNA rearrangement or transposition occurred during this period. The authors concluded that rearrangement was not a direct result of plasmid transfer to other species (i.e., occurring during transformation, etc.) because the original plasmid was also seen in every species.

A similar outbreak that began with a gentamicin-resistant strain of *Serratia marcescens* was observed in Nashville, Tenn. (173). Three types of spread were documented: "(1)

dissemination of individual strains, (2) dissemination of a plasmid among different strains, and (3) movement of a discrete genetic element, or transposon, between plasmids" (173). Over 200 patients in four hospitals were infected with the index strain, as shown by plasmid profiles, phage typing, and serotyping. A large increase in the rate of isolation of gentamicin-resistant *K. pneumoniae* that carried plasmids different from those carried by the *Serratia marcescens* strain was observed. Molecular studies showed that the gentamicin resistance determinant was located on a transposon that had jumped into various plasmids in *K. pneumoniae*. Many other examples have been reported (47, 58, 110, 111, 144, 167, 195), including those occurring in *Staphylococcus aureus* (115, 127).

METHODOLOGY

The plasmid content of most bacterial strains is usually a stable feature, although there are cases in which plasmids are lost during subculture. It may be necessary sometimes to add the appropriate antibiotics to the growth media to maintain plasmids carrying antibiotic resistance genes (147). It is important to remember that changes in plasmid content may also occur in vivo (47, 68, 114, 127, 139, 173). Plasmid content may be very stable even in the absence of selective pressure (34).

Plasmid profiles are relatively easy and inexpensive to perform. A complete system, including a 250-V power supply, electrophoresis cell, 300-nm ultraviolet transilluminator, fixed focal length camera, hood, filters, film, agarose, and instruction manual, is available for $1,600. Assuming one has a typically equipped clinical laboratory (incubator, water bath, centrifuge, etc.), plasmid profiles could be done for a <$2,000 initial investment. A typical system for frequent use would include the following: 2,000-V power supply, $1,600; electrophoresis cell, $300; camera with transilluminator, $6,500; microcentrifuge, $1,800; miscellaneous equipment and supplies, $1,200. More expensive systems are also available. Some biotechnology companies offer small preparations of plasmid DNA as one of their services for as little as $125 for 12 cultures.

As a result of the interest in plasmids and the growth of genetic engineering, a number of procedures for isolating plasmid DNA have been described (15, 16, 44, 46, 60, 65, 75, 85, 96, 158, 175, 183, 193, 204). Because some procedures were designed for laboratory-adapted *E. coli* K-12 strains, some modifications may be required for their use with other species or isolates from clinical material or nature. Fresh isolates may be more difficult to lyse, and some bacteria elaborate deoxyribonuclease (193). Generally, a single nick (breakage of a covalent bond in one of the strands) will cause a plasmid molecule to be lost during the isolation procedure. Care must be taken to prevent this.

The protocols for doing plasmid profiles are readily adaptable to a variety of bacteria by simple modifications. The cell lysis steps usually require the most alterations. In general, if one can lyse the bacteria, one can isolate their nucleic acid. Special steps may be required for gram-positive or acid-fast bacteria. One approach that has been used is to weaken the cell envelope by growing the bacteria with a cell wall synthesis inhibitor such as glycine, cycloserine, or penicillin (44, 128).

Most procedures used to isolate plasmid DNA rely on the physical differences in circular plasmid molecules and linear chromosomal fragments. The chromosomal DNA is mechanically sheared into many fragments during the extraction process. Alkaline lysis and denaturation of the nucleic acid form the basis of many plasmid isolation protocols. After the cells have been weakened by treatment with lysozyme, the cells are lysed and the nucleic acids are denatured, by adding sodium dodecyl sulfate and sodium hydroxide to raise the pH to about 12 to 12.5. Since the individual DNA strands of the plasmid molecules are covalently attached and intertwined, they are prevented from diffusing away from each other during denaturation. The linear fragments of chromosomal DNA are not covalently attached to each other. When denatured, the individual strands of the chromosomal fragments separate and diffuse away from each other. When the sample is neutralized, the plasmid DNA quickly renatures to the double-stranded DNA form (sometimes referred to as snapback). The single strands of the chromosomal fragments cannot renature under these conditions and are precipitated.

The rapid methods used for plasmid profiles enrich for plasmid DNA, but usually produce a sample that still contains some chromosomal DNA as well. The remaining chromosomal DNA usually appears as a band on the gel. The chromosome is sheared into fragments ranging from about 20 to >100 kb. This causes both the leading and trailing edges of the band to be diffuse. A typical plasmid band, or other DNA that is of uniform size, has a sharply delineated leading edge. The trailing edge is often slightly diffuse due to artifacts of electrophoresis.

Excellent complete protocols have been published for plasmid isolation, agarose gel electrophoresis, staining, and photography (44, 48, 95, 120, 158, 159, 194).

In some cases, more than just plasmid profiles may be necessary. Plasmids of identical size but very different sequence and function can exist in many bacteria. When only a single plasmid is present, restriction endonucleases can be used to provide further evidence of the similarities and differences between strains. Restriction endonucleases, or restriction enzymes, cleave DNA at specific sequences (97). Plasmids and other DNA molecules that have identical sequences produce the same set of fragments after digestion with a restriction endonuclease (192). Over a dozen companies supply restriction endonucleases and provide protocols for their use. Restriction endonucleases are sensitive to many of the chemicals used to isolate plasmid DNA, such as phenol, detergent, ethanol, or chelators, so care must be taken to remove these chemicals before digestion.

An example of plasmid profiles is shown in Fig. 1 and restriction endonuclease digestion patterns are shown in Fig. 2. These are taken from the study of a large milk-associated *Salmonella* serotype typhimurium outbreak (168) discussed above. Although the majority of patient or product isolates are identical to the outbreak strain, isolates that have gained or lost a plasmid can also be seen. The outbreak strain plasmid profile pattern was not seen in isolates from unrelated sources or other controls. The isolate from a daycare center where children had consumed contaminated milk is identical to the outbreak prototype strain (third lane) except the band corresponding to a small plasmid is missing. The antimicrobial susceptibility testing pattern and all other typing results indicated that the daycare isolate was identical to the outbreak strain. Some unrelated isolates (Ohio cow) had plasmid profiles identical to that of the daycare center. By comparing the restriction enzyme digest patterns shown in Fig. 2, one can see that the fragments from the daycare isolate are a subset of those from the outbreak strain. This is consistent with the loss of the small plasmid, and we conclude that the daycare center isolate is closely related or identical to the outbreak strain. Some of the fragments from

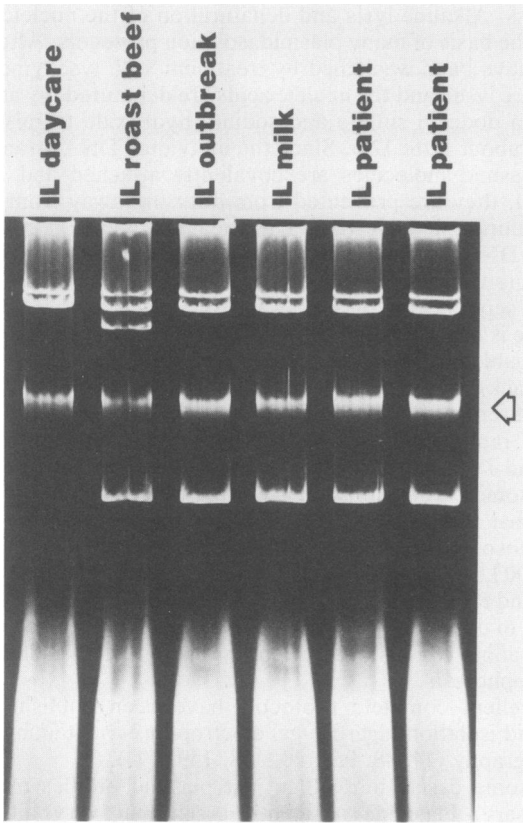

FIG. 1. Photograph of an ethidium bromide-stained agarose gel showing plasmid profiles. Chromosomal DNA bands (arrow), two to four plasmid DNA bands, and RNA smears (near bottom) are seen in each lane. The Illinois (IL) patient and milk isolates are all identical to the outbreak strain, but the daycare isolate is missing a plasmid band and the roast beef isolate has an additional band.

the Ohio cow isolate are similar to those from the outbreak strain, but the overall patterns are different and we conclude that they are not closely related. This similarity of fragment sizes may be coincidence or may represent some distant relationship between these plasmids. Similar results were obtained with the roast beef isolate (Fig. 1, second lane) that was identical to the outbreak strain but also resistant to kanamycin and carrying an additional plasmid. This suggests that kanamycin resistance was encoded by the additional plasmid.

EXAMPLES OF PLASMID PROFILE USE

E. coli

In developing countries, enterotoxigenic *E. coli* infections represent serious health problems. Other important *E. coli* infections include hemorrhagic colitis and hemolytic uremic syndrome (HUS), whose bacterial etiology has only recently been recognized. Plasmid profiles have been used to understand the epidemiology of these diseases.

The bacterial factors required to produce enterotoxigenic *E. coli* disease are toxin(s) and adhesive factor(s) (169). Both factors are usually plasmid encoded, either individually or in combinations with others, including antibiotic resistance determinants (76, 126, 140). One heat-stable toxin (ST) gene is located on a transposon (177). Heat-labile toxin (LT) genes are part of a sequence that structurally resembles a bacterial transposon (209). The toxins show some relationship to the animal species from which the organism was isolated, so that notations such as ST-P for porcine toxin and ST-H for human toxin are common (62). The adhesive factors also display much species specificity. For example, the fimbrial adhesin K88 is associated with porcine disease, K99 is associated with disease in calves or lambs, and human enterotoxigenic *E. coli* strains usually express colonization factor antigen I or II (76). Considering this variety of plasmid-encoded virulence factors, it is not surprising that plasmid profiles are used as markers for specific disease isolates.

Different plasmid profiles, reactivity with DNA probes for LT or ST, serotypes, or antimicrobial susceptibility patterns were seen in isolates from children in the Central African Republic (62), in isolates from Mexico, Bangladesh, or Texas (138, 140), and in isolates from Bangladesh and five other countries (205). Other studies have examined enterotoxin phenotype patterns and their relationship to O:H serotypes (161) or antimicrobial susceptibility patterns (51) without directly examining the plasmid content.

The proportion of enterotoxigenic *E. coli* that carries a hemolysin plasmid has been reported to be about 17% (50). A 90-kb plasmid was associated with one type of hemolysin activity, referred to as HLY1, and plasmids of 30 to 84 kb were seen in strains with a second hemolysin, HLY2.

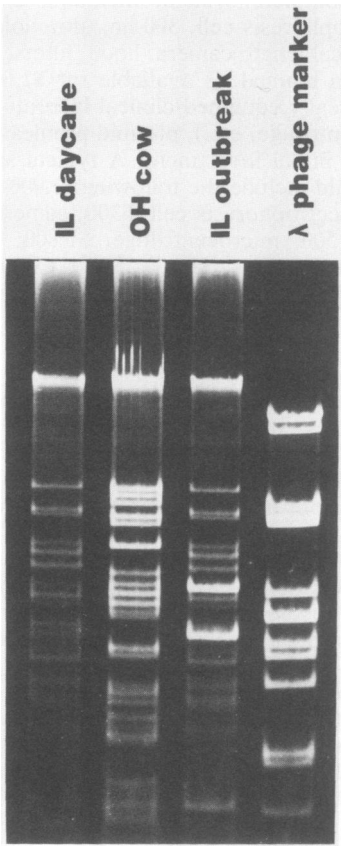

FIG. 2. Restriction endonuclease (*PstI*) digest patterns of plasmid DNA and lambda phage DNA as molecular size marker. The pattern from the Ohio (OH) cow is different from the outbreak strain, but all fragments from the daycare isolate are seen in the outbreak strain. By subtracting the bands shown to be derived from the small plasmid (data not shown), the outbreak strain pattern becomes identical to that of the daycare isolate.

The potential for transferring a plasmid encoding colonization factor antigen I and ST from *E. coli* into other members of the *Enterobacteriaceae* and expression of these virulence factors have been studied (208). The plasmid could be transferred to *Shigella*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Edwardsiella*, *Serratia*, and *Proteus* species. The plasmids were stable in the transconjugants, and both colonization factor antigen fimbriae and ST were expressed. In two animal models, administration of live plasmid-carrying transconjugants of four of the above species, or *E. coli* K-12, resulted in positive tests for ST. Thus, the possibility for increased host range spread of toxin plasmids is high.

A very large plasmid, about 210 kb, has been shown to be associated with enteroinvasiveness of *E. coli* (77). Every Sereny test-positive isolate carried this plasmid, which is similar to the invasiveness plasmid of *Shigella flexneri* (172) and *Shigella sonnei* (102). In this study, only one of the three methods used for isolating plasmid DNA was uniformly effective.

E. coli strains that cause hemorrhagic colitis or HUS also elaborate toxin(s) and adhesin(s), which are probably required to cause disease. At least two toxins are known and they are encoded by phage (145). The adhesin has been shown to be plasmid mediated, and a DNA probe is available to detect the gene for it (112). Earlier studies had shown identical plasmids in a number of O157:H7 isolates from outbreaks in Oregon and Michigan (202). Plasmid profiles of O157 strains that were not H7 were different from the O157:H7 profile. Even though the plasmid profile alone was not sufficient to differentiate the isolates from the two presumed unrelated outbreaks in these two states, the restriction endonuclease digest patterns were shown to be related, but unique and identical for isolates from each state. Another study with the DNA probe showed that O157:H7 and O26:H11 strains harbor related plasmids in the 82- to 112-kb range (112). Restriction endonuclease digest patterns of these plasmids have several fragments of the same size derived from various plasmids, suggesting DNA sequence similarity.

The HUS-causing *E. coli* strains have plasmids related to those from hemorrhagic colitis-causing *E. coli* strains that encode an adhesive factor required for disease. In the HUS strains, enough biological diversity exists to use the restriction endonuclease digest patterns to identify individual HUS strains and differentiate them from closely related strains.

E. coli are frequently seen as recipients during outbreaks involving plasmid transfer (see section, "Host Range and Plasmid Epidemics").

Salmonella Serotypes

Conventional methods of typing salmonellae include serotyping, biochemical typing, phage typing, and antimicrobial susceptibility testing. All of these methods have been useful for studying the epidemiology of *Salmonella* infections.

Salmonellae are relatively easy to grow, can be lysed easily, and have a variety of plasmids, many of which encode antibiotic resistance genes. As a result, plasmid profiles have become an important tool for epidemiologic studies of salmonellosis.

A study of 20 outbreaks of *Salmonella* serotype typhimurium in the United States compared plasmid profile analysis, phage typing, and antimicrobial susceptibility testing to determine the most useful laboratory method for identifying isolates (84). The isolates were shown to be identical by all three methods in 17 of the 20 outbreaks. For nine outbreaks in which control strains were available for comparison, outbreak isolates were differentiated from controls: eight times by plasmid profiles alone, six times by phage typing alone, and four times by antimicrobial susceptibility testing alone. In three outbreaks, all controls were different from the outbreak strains by all three tests. For three outbreaks, the plasmid profiles and phage typing data did not agree. If the data demonstrating a clear epidemiological relationship are correct, plasmid profile was the best for two outbreaks and phage typing was the best for one outbreak. The authors point out that either the phage types or the plasmid profiles may have changed during the investigation or they might have two distinct outbreak strains in these cases.

Another study of 74 *Salmonella* serotype typhimurium isolates during 1981 in Switzerland also concluded that plasmid profiles and phage typing were equally useful and accurate methods (24). For differentiating strains, the antimicrobial susceptibility pattern was less useful, and the authors stated that "biotyping was of no use."

An outbreak of *Salmonella* serotype enteritidis occurred from 1980 to 1981 in a community hospital in Liberia (73). These isolates had a 180-kb conjugative plasmid that carried multiple antibiotic resistance genes. A 60-kb cryptic plasmid was also present. The increased virulence of this strain, causing a high mortality rate (28%), and its multiple antibiotic resistance are unusual for *Salmonella* serotype enteritidis.

Plasmid profiles have also been useful for studying *Salmonella* serotypes drypool (162), heidelberg (117), muenster (14), and others.

Goldstein et al. (64) discuss two interesting *Salmonella* serotype typhi outbreaks that occurred in Mexico during 1972 (149) and in Vietnam during 1973 (23). They compared the isolates to 72 strains obtained in Peru between 1981 and 1983. The isolates from the two outbreaks had the same antibiotic resistance patterns (resistant to chloramphenicol, streptomycin, tetracycline, and sulfonamide), and all harbored a plasmid of the H1 incompatibility group. The isolates from Mexico were all the same phage type, suggesting that a single clone was disseminated in this outbreak. The isolates from Vietnam were 11 different phage types, suggesting the dissemination of a plasmid into several strains. The isolates from Peru were eight different phage types and had four different antibiotic resistance patterns, and 70 of 72 studied had a transmissible plasmid of the H1 incompatibility group. Thus, multiple strains are represented in these sporadic isolates. The incidence of H1 plasmids suggests some affinity between them and *Salmonella* serotype typhi, but other incompatibility groups, including FII, I, and N, have been reported (64). Within the 72 isolates studied, a group of 15 were all the same phage type and had the same antibiotic resistance pattern. This suggests that some of the sporadic isolates really represent an unrecognized small outbreak.

Plasmid profiles have been used to show an association between the presence of either a 24- or a 38-kb plasmid and the likelihood of clinical relapse in patients with typhoid fever (66). These two plasmids did not have any fragments in common after digestion with restriction endonucleases, and no sequence similarity was seen by probing a Southern blot with ^{32}P-labeled 24-kb plasmid. Whether these plasmids are markers for these particular strains or whether they have a direct role in the disease process is not known. More than one strain was isolated from 5 of the 16 patients in this study, indicating that infection with multiple strains is not uncommon in this area of endemic infection.

Two important segments of our knowledge of *Salmonella* epidemiology are directly related to the use of plasmid profiles to investigate outbreaks: (i) the ability to find a previously unrecognized association between isolates from outbreak-related and sporadic cases, and (ii) documentation of the appearance and persistence of antibiotic-resistant strains that represent a serious public health threat. The following examples illustrate these points.

In 1981, an increase in the number of cases of salmonellosis was noted in New Jersey and Pennsylvania (163). *Salmonella* serotype newport and *Salmonella* serotype typhimurium isolated from precooked roast beef were similar to the outbreak isolates. The *Salmonella* serotype newport isolate had a unique plasmid profile. Investigation of both outbreak and sporadic *Salmonella* serotype newport isolates showed that during 1981 45% of strains from reported cases in these areas had the same plasmid profile. Further epidemiologic study showed an association between consumption of precooked roast beef and infection with organisms having this plasmid profile. These additional patients were included as part of the outbreak after further study on the basis of the plasmid profile data. The *Salmonella* serotype typhimurium isolates did not contain any detectable plasmids and therefore the use of plasmid profiles for these organisms was of no value.

Another example of the helpful use of plasmid profiles is in an outbreak of *Salmonella* serotype typhimurium initially associated with raw milk consumption (181). One of the first cases in this study was a fatal infection which did not respond to chloramphenicol therapy. Isolates from this patient and the milk harbored a 158-kb plasmid which encoded multiple antibiotic resistance genes, including chloramphenicol resistance. A survey of *Salmonella* serotype typhimurium isolates during this same time showed that 43% were resistant to chloramphenicol, and 80% of these had the same plasmid. Thus, the chloramphenicol resistance and the plasmid profiles were used to evaluate the spread of this organism in the community.

A third example is included in a survey of animal and human isolates of *Salmonella* serotype typhimurium var. copenhagen, *Salmonella* serotype newport, and *Salmonella* serotype dublin (146). One strain that was endemic in cattle in 20 states was shown by plasmid profiles to be identical to 26 human isolates from two states. The authors discuss the spread of resistance plasmids between animal and human bacterial flora and the significance of retrospective recognition of the related sources of human isolates.

The epidemiology of antibiotic-resistant *Salmonella* serotypes as a public health problem in the United States has recently been reviewed (39). The potential for transmission of antibiotic-resistant organisms from animals raised on antibiotic-supplemented diets to humans is a topic of current controversy (39). Plasmid profiles have been one of the laboratory tests used to study this problem.

In the recent past, chloramphenicol resistance in *Salmonella* serotypes was significant, but rare (37). In 1985, there was about a fivefold increase in the number of *Salmonella* serotype newport strains isolated from patients in California (179). An unusual antibiotic resistance pattern, including resistance to chloramphenicol, was found in 87% of these. The epidemic strain had a characteristic plasmid profile, including restriction endonuclease digest patterns. A case control study implicated ground beef as the vehicle of transmission. Isolates were obtained from hamburger products from case households, slaughterhouses where the meat was processed, dairies which sent cows for slaughter, and sick cows at these dairies. In a survey of farms in the area, it was shown that chloramphenicol-resistant bacteria were more likely to be isolated from animals or waste lagoons on farms where chloramphenicol was used. Plasmid profiles have been used in similar studies of resistant *Salmonella* serotype newport (81), *Salmonella* serotype typhimurium (142, 150), and others.

Sometimes plasmid profiles are included in a study when they do not seem relevant. One study (198) compared the efficacy of cefotaxime and fosfomycin for experimental *Salmonella* serotype typhimurium infections of outbred mice. The occurrence of plasmids in all seven strains used is presented in the results section but not mentioned in the discussion. The potential role of the plasmids in conferring antibiotic resistance or as a strain marker to verify that the infections were caused by the bacteria injected is not discussed.

Shigella spp.

Some of the earliest studies that used plasmid profiles were done with *Shigella dysenteriae* (45), *Shigella flexneri*, and *Shigella sonnei* (90). In addition to R plasmids, a number of small cryptic plasmids were observed in these organisms. The study by Crosa et al. (45) used DNA hybridization to show considerable DNA sequence similarity between several plasmids conferring ampicillin resistance from a variety of *Enterobacteriaceae*. These experiments and others like them were indispensable to our understanding of R-plasmid transmission.

In addition to cryptic and R plasmids, large plasmids in *Shigella flexneri* (172) and *Shigella sonnei* (102) have been shown to encode factors associated with invasiveness.

Plasmid profiles were useful for differentiating isolates from Bangladesh (182). Of the 136 isolates examined, only 1 was plasmid-free. Only 25 different antimicrobial susceptibility patterns, but 99 different plasmid profiles, were seen in this group. Among similar species or serotypes, the mean number of plasmids per strain was between 3.1 and 4.5. Even in this bacterial group, in which plasmids are common, identity of outbreak isolates alone is not sufficient without evidence of differentiation from unrelated case strains or environmental isolates. This is emphasized in a study of *Shigella sonnei* isolates from U.S. students in Guadalajara, Mexico (156). A common plasmid profile was seen in isolates from Mexico City and Houston, Tex., as well as in those from Guadalajara, where it was observed over a 5-year period. Many of these strains carried a plasmid of similar size. The authors point out that "the widespread occurrence of a 3.4-megadalton plasmid cautions against using typing schemes that rely on the presence of a single plasmid" (156).

Two points are important to remember from this discussion. (i) Plasmid profiles are not a magic tool. We do not know if those strains with similar plasmid profiles but from different geographic regions are really unrelated. Isolates from Guadalajara, Mexico City, and Houston, Tex., could be related. Plasmid profiles do not always provide a definitive answer, and (ii) showing that outbreak isolates have identical plasmids is not sufficient. Not showing that such strains differ from unrelated case strains and environmental isolates is analogous to using "numerator data only" or using only one cell of a 2-by-2 square.

K. pneumoniae

Several outbreaks of *K. pneumoniae* infections in a neonatal intensive care unit occurred in Virginia in 1980 (121).

Multiple antibiotic resistance in several serotypes suggested a conjugative R plasmid. Plasmid profiles, including restriction endonuclease digestions, indicated that a plasmid of about 106 kb was present in all of the isolates. Transfer of the plasmid into sensitive recipients suggested that all resistance determinants were carried on this plasmid. A similar series of epidemics at Vanderbilt Medical Center was caused by the introduction of another resistant *K. pneumoniae* strain, which carried an unrelated plasmid of the same size as that isolated from the epidemic strain in Virginia (91). Plasmid analysis is appropriate for demonstrating differences as well as equivalencies. Outbreaks with different but related plasmids in isolates from neonates have also been reported (207).

The genes encoding the virulence factors aerobactin and its receptor are located on a 180-kb plasmid (143). The presence of this plasmid enhanced the virulence of *K. pneumoniae* capsular serotypes K1 and K2 even though all isolates produced enterochelin, which also can be used for iron acquisition. Transfer of a recombinant plasmid containing these genes increased the organism's virulence for mice by 100-fold.

K. pneumoniae are frequently seen as recipients during outbreaks involving plasmid transfer (see section, "Host Range and Plasmid Epidemics").

Citrobacter diversus

Two studies have used plasmid profiles, as well as other typing systems, to study outbreaks of *C. diversus* meningitis in neonatal nurseries (133, 203). Both studies established the possibility of mother-child organism transmission during birth, but point out that carriage by hospital personnel may also be important. Our understanding of this devastating disease is limited. A variety of *C. diversus* strains can be present in the community, but their potential to cause meningitis is not known (133). The use of plasmid profiles has increased our understanding of this disease. A single strain, as shown by plasmid profile, biotype, and serotype, was responsible for cases and asymptomatic colonization of infants in one hospital (133). This suggests that factors other than those elaborated by the bacteria are important.

Other Enteric Bacteria and Miscellaneous Gram-Negative Bacteria

Plasmid profiles have been used to study outbreaks caused by a number of other enteric bacteria, including neonatal meningitis caused by *Enterobacter sakazakii* (141), *Enterobacter cloacae* infections in burn patients (94), nosocomial infections with *Serratia marcescens*, *Providencia rettgeri*, and *Pseudomonas aeruginosa* (174), and gentamicin-resistant *Serratia marcescens* in a neurosurgical close observation unit (92). Plasmids have been reported in *Providencia stuartii* (79, 132), as well as in *Providencia rettgeri* (93). Urease production, sucrose utilization, and lactose fermentation can be plasmid mediated in *Providencia stuartii* (132).

Plasmid-associated virulence determinants have been described in *Yersinia enterocolitica* (155), and plasmid profiles have been used, in conjunction with serotyping and biotyping, to study *Y. enterocolitica* isolated from pediatric cases of diarrhea (131).

The potential use of plasmid profiles has been demonstrated for *Aeromonas* spp. (33, 82) and *Plesiomonas shigelloides* (83). Plasmids have also been reported in *Vibrio cholerae* (160).

A plasmid identical to that found in saprophytic *Neisseria* sp. has been reported in the facultative anaerobe *Eikenella corrodens* (165).

Campylobacter spp.

Since the first report of plasmids in *Campylobacter* spp. (9), several studies have been published that use plasmid profiles as a typing system for these organisms. In these studies, the fraction of strains shown to harbor plasmids has varied from about 20% in one survey (9) and 33% in others (19, 190) to as high as 61% in a survey of 11 outbreaks (18). Among 10 isolates from three small outbreaks, 9 were shown to harbor plasmids; the plasmid profiles were useful in tracing the epidemiological spread of *Campylobacter jejuni* (189).

Plasmids detected in *Campylobacter* range in size from 2 to 162 kb (190). Resistance to tetracycline (186) and kanamycin (103, 105, 151) has been shown to be plasmid mediated.

One study reported that plasmid profiles were not useful in studying a particular outbreak, because plasmids were detected in only 7 of 50 isolates tested (21). Chromosomal restriction endonuclease digests were more useful than either plasmid profiling or serotyping in this study.

Neisseria spp.

N. gonorrhoeae strains that carry plasmids mediating penicillin or tetracycline resistance have been isolated from patients. Different plasmids which encode a TEM beta-lactamase, conferring penicillin resistance, have been described (54, 67, 154, 199). All of the beta-lactamase-producing plasmids are related to one another (67, 178, 199). Plasmid-mediated high-level tetracycline resistance, in the United States, is encoded by the streptococcal *tetM* determinant, which has been inserted into the 37-kb conjugal gonococcal plasmid (134). Another tetracycline-resistant gonococcal isolate, which harbors a 16-kb plasmid, has also been described (89). It is not known whether this is related to the *tetM* conjugal transposon or if the tetracycline resistance determinant is located on the unique 16-kb plasmid. Most gonococci carry a 3.9-kb cryptic plasmid as their only plasmid or in addition to those described above. Only about 2% of gonococci harbor no plasmids when they are isolated (180).

Although plasmid profiles are used to study gonococcal infections, the technique is usually used in concert with other typing methods, including serotyping, antimicrobial susceptibility testing, and auxotyping (86, 100, 101, 134).

Plasmids are very rare in *Neisseria meningitidis* and are probably of limited value in identifying specific strains.

Legionella spp.

A nosocomial outbreak of *Legionella pneumophila* has been investigated, using isoenzyme typing, monoclonal antibody typing, and plasmid profiles (53). Several other studies of plasmid profiles in *Legionellaceae* have been done (10, 22, 118). A variety of plasmids of 30 to 188 kb in size have been seen in these bacteria. In comparisons of typing methods, a high degree of correlation has been shown. No functions encoded by these plasmids or role for plasmids in the pathogenesis of legionellosis has been reported. In one study 12 different plasmid patterns were seen in 95% of the clinical isolates containing plasmids (53). Another study reported that, even though plasmid-bearing strains were more common and seemed to persist in the hospital environment, only plasmid-free strains were associated with disease (22). There are many possible reasons for this apparent disparity, including differences in location, patient popula-

tions, and hospital practices, or the possibility that plasmids do not have a direct role in or any association with disease. Plasmids do serve as excellent markers for some *Legionella* strains.

Using several protocols for plasmid isolation, marked differences have been seen in the susceptibility of strains to detergent lysis (10, 53). Some modifications of these methods were required for routine use, and even then occasional failures to demonstrate plasmids in plasmid-bearing strains were seen. One method was successful for some strains, but not for others. When a different method was used, the converse was observed: strains that yielded plasmids by the first method did not with the second and vice versa.

The transfer of plasmids carrying antibiotic resistance genes from *E. coli* into *L. pneumophila* and other legionellae has been reported (36). These experiments demonstrate the potential for plasmid-mediated antibiotic resistance in clinical isolates of legionellae.

Restriction endonuclease digests of chromosomal DNA are useful for typing *L. pneumophila* serogroup 1 (200).

Haemophilus spp.

Plasmid profiles have been used to study the epidemiology of carriage and infection with *H. influenzae* type b in daycare centers (29). These isolates were resistant to several antibiotics. All isolates produced beta-lactamase and chloramphenicol acetyltransferase. One of two plasmids, of either 68 or 78 kb, was also uniformly present. The strains were identical by isoenzyme typing, biotyping, and capsular typing. Restriction endonuclease digests of plasmid DNA and outer membrane protein profiles were used to differentiate these strains. The authors concluded that more than one strain could be present at the same time in a daycare center with only one index case and no secondary cases.

A study of *Haemophilus ducreyi* isolates from patients with clinical chancroid demonstrated three different sized plasmids (11, 8.6, or 5.4 kb) which encoded a beta-lactamase (74). All six strains examined carried one of these plasmids. The plasmids of epidemiologically linked cases were identical. Plasmids from geographically unrelated isolates were different.

Genetic exchange between *N. gonorrhoeae* and *Haemophilus* spp. may be common in nature. After the recognition of plasmid-mediated beta-lactamase in gonococci, it was speculated that *Haemophilus parainfluenzae* may have been the plasmid source, or at least the host immediately prior to the gonococcus (180). Recently, we have seen what may be transfer in the other direction, with gonococcal-like plasmids reported in *H. parainfluenzae* (122).

Brazilian purpuric fever is a recently described disease with a high case fatality rate (31). Plasmid profiles and other typing systems have been used to analyze *Haemophilus aegyptius* isolates from Brazilian purpuric fever cases, contacts, and controls (D. J. Brenner et al., submitted for publication). More than half of the isolates from Brazil, and some from Egypt and the United States, contain a plasmid of about 35 kb. After digestion of gradient-purified plasmid with restriction endonucleases such as *Hin*dIII, only one isolate from Egypt could be differentiated from the others by differences in fragment patterns. By using a different restriction endonuclease, however, seven different plasmids could be identified within this group of identically sized plasmids. The enzymes useful in separating these plasmids were *Acc*I, *Mbo*II, and *Rsa*I. More commonly used enzymes, such as *Eco*RI, were not useful in this study. Decisions must be made about not only when it is necessary to use restriction endonucleases to confirm the results of plasmid profiles, but also how many restriction endonucleases should be used. Each situation is unique. The questions of how many typing systems to use and how far to pursue each one must be answered by evaluating the data at hand and deciding how important it is to show identity or differences between strains.

Pseudomonas aeruginosa

Plasmids in *Pseudomonas aeruginosa* can encode a number of antibiotic resistance determinants, including unusual mechanisms of resistance (27). In some cases the plasmids appear to be integrated into the chromosome (135) and therefore are not useful for plasmid profiles.

Plasmid profiles, antimicrobial susceptibility testing, biochemical testing, and serotyping have been used to study *Pseudomonas aeruginosa* in contact lens wearers with corneal ulcers (125). In five of six sets of paired isolates from the eye and saline solution used by the patients, all typing systems demonstrated identical strains for each pair. The other pair of isolates differed by a single plasmid and some minor variations in antimicrobial susceptibility testing. By using the combination of typing systems, all strains could be differentiated from unrelated isolates. A similar study has been done on isolates of *Pseudomonas aeruginosa* from dialysis patients with peritonitis associated with contaminated poloxamer-iodine solution (153).

Mycobacterium spp.

Plasmid profiles were used to compare human isolates with environmental isolates of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum* (128). This study showed that aerosol isolates most closely resembled human isolates, supporting the hypothesis that aerosols are an important source of mycobacteria. The potential use of plasmid profiles to study *M. avium* complex isolates from patients with acquired immunodeficiency syndrome has been reported (L. Gaido, L. W. Mayer, and R. C. Good, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, U43, p. 133).

Staphylococcus spp.

Multiresistant staphylococci frequently cause nosocomial infections. Many staphylococcal antibiotic resistance determinants are plasmid encoded (4, 5, 42, 106, 108, 197, 206). A number of other plasmid-encoded functions and cryptic plasmids also occur. The wide variety of plasmids present in staphylococci has made the use of plasmid profiles convenient for studying outbreaks caused by these bacteria.

An outbreak of *Staphylococcus aureus* in a burn unit, which later spread to a surgical intensive care unit, was studied by Locksley et al. (114). This outbreak started with the transfer of a single burn patient into the hospital. Over a 15-month period, 27 patients were infected and an additional 7 patients were colonized with the outbreak strain. Infection with this multiresistant *Staphylococcus aureus* strain was considered the major cause of death in 13 of the 17 deaths that occurred during this study. The surgical intensive care unit was engaged in a study evaluating the use of chloramphenicol to treat bowel sepsis. At two points during the outbreak, the organism acquired distinct R plasmids that conferred resistance to chloramphenicol. Plasmid profiles were used to trace the spread of the outbreak strain through

these wards. The importance of plasmid profiles is emphasized by the authors (114): "Phage typing and antibiograms alone could not distinguish these two chloramphenicol-resistant strains."

Many similar examples of outbreak studies in Australia (63, 116), Ireland (52), Italy (61), and others are in the literature.

A study that compared the use of plasmid profiles as an epidemiologic marker with other typing systems had the benefit of a unique antibiotic resistance marker, rifampin resistance, to verify the results (7). The antimicrobial susceptibility testing pattern and plasmid profiles were of about equal efficiency and speed to differentiate outbreak isolates from those obtained from unrelated patients, contacts, or the hospital environment. The use of plasmid profiles in some cases allowed differentiation of isolates with similar antibiograms. Phage typing was not useful in this outbreak. Because it is an unusual marker, the presence of rifampin resistance was important to test the validity of any comparisons made between plasmid profiles and the other typing systems.

A comparative study has also been done for markers of *Staphylococcus epidermidis*, including plasmid profiles, phage typing, antimicrobial susceptibility testing, and biotypes (152). One example from this study examined 10 cultures that had identical phage and biotypes. The antibiotic resistance patterns differed by only tetracycline resistance or susceptibility. If the "two strong differences" rule of thumb were used, these strains would be considered to be the same. The 10 cultures were shown to be five distinct strains by plasmid profiles. The opposite results were also seen in a different example. Cultures with almost identical plasmid profiles, which were lysed by a single phage and were of the same biotype, had "markedly different antibiograms." This study emphasizes the point that no one typing system will be the best in every case.

Plasmid profiles have been evaluated as an epidemiological marker for *Staphylococcus epidermidis* isolated from patients with prosthetic valve endocarditis (8). Using a rapid lysis technique and agarose gel electrophoresis, the authors found that 93% of prosthetic valve endocarditis isolates harbored plasmids, with 79% having two or more. The majority of the plasmids found were <15 kb in size. When nine isolates from a cluster of prosthetic valve endocarditis cases in Canada were examined, the plasmid profiles of two groups of three isolates each were shown to be identical, suggesting a common source. Examination of sporadic cases was not as clear-cut. There were no identical plasmid profiles among 45 prosthetic valve endocarditis isolates from three institutions. Several isolates differed by a single plasmid. These results are very difficult to interpret, because a single genetic event, such as acquisition of a tetracycline resistance plasmid, could yield isolates similar to those described above. Most of the plasmids in this study were cryptic; no specific function or gene products could be attributed to them. In the absence of any phenotypic changes such as drug resistance, which can be correlated with an alteration in the plasmid content, we can only speculate about the relatedness of strains differing by one plasmid.

A study by Mickelsen et al. (130) demonstrated some instability of R plasmids in *Staphylococcus epidermidis* but also showed that the basic plasmid profile was stable and a useful strain maker. Loss of specific plasmids was associated with the loss of resistance to gentamicin, chloramphenicol, erythromycin, or clindamycin but not methicillin.

Another study of 106 isolates of coagulase-negative staphylococci showed that plasmid profiles were more discriminating, but that in many cases antimicrobial susceptibility testing was sufficient for demonstrating identity or differences (78). Plasmid profiles have been used as a tool to study *Staphylococcus epidermidis* contamination of blood cultures (6).

Some studies have shown that transfer of resistance factors between *Staphylococcus epidermidis* and *Staphylococcus aureus* occurs (40, 88, 191). Thus, a ubiquitous opportunistic pathogen can serve as a reservoir of antibiotic resistance genes that can be spread among staphylococci.

Anaerobes

The anaerobic species reported to harbor plasmids are almost limited to the genera *Bacteroides* and *Clostridium*. The molecular biology, including studies of plasmids, and clinical relevance of antibiotic resistance have been reviewed (12). Other novel resistance patterns, such as chloramphenicol resistance in *Bacteroides uniformis*, are reported to be encoded by plasmids (123). The role of plasmids in conjugal transfer and antibiotic resistance in anaerobic bacteria has also been reviewed (148). The unexpected finding of tetracycline resistance genes in *B. fragilis* that are cryptic in the original host and are expressed only after transfer to *E. coli* has been reported (70).

During an outbreak of diarrhea in hospital patients, plasmid profiles were used to study isolates of *Clostridium perfringens* (119). The isolates were also serotyped and bacteriocin typed. No information was given about antimicrobial susceptibility testing. Three plasmid profiles were found in what was considered to be the outbreak strains; almost all were the same serotype (25 of 26). The three plasmid profiles could be related to one another. When comparing any pair, the difference between them was only a single plasmid gained or lost. It is possible that only one outbreak strain was present, and the gain or loss of a plasmid resulted in the other types. The gain or loss of a single plasmid should be considered a single genetic event just as gain or loss of an antibiotic resistance determinant would be. Such changes are not uncommon. Although a plasmid gain or loss is consistent with the epidemiological data, no conclusion about the actual relatedness of these strains can be made. Digestion of the plasmids with restriction endonucleases may have provided additional information about their origin.

Some plasmids and their close relatives may be widely distributed. Plasmids identical or related to the tetracycline resistance plasmid pCW3 have been observed in *Clostridium perfringens* isolates from Australia, Belgium, France, Japan, and the United States (1). Therefore, epidemiologically unrelated strains may have similar plasmid profiles.

CONCLUSIONS

Plasmid profiles have been used to verify the identity of bacterial isolates or show that they are different. In some cases plasmid profile analysis seems to be the most discriminating typing method, and in other cases plasmid profiles have been of no value. Restriction endonuclease digestion should be done when a single plasmid exists or similar plasmid profiles occur in strains which cannot be differentiated by other methods. The best results are obtained by combining plasmid profiles with other typing data; e.g., a novel antimicrobial susceptibility testing pattern may suggest that a new plasmid has been introduced. The expense,

time, and training required to do plasmid profiles are reasonable. The relevance of plasmid profiles for hospital infection control, antibiotic resistance tracing, and outbreak investigation will continue to increase as more is known about the molecular biology, ecology, and epidemiology of bacterial plasmids.

ACKNOWLEDGMENTS

I thank many of my co-workers for useful discussions about plasmids, especially Don J. Brenner, Mitch L. Cohen, John S. Spika, Rob C. Barnes, Stanley Falkow, and Kris A. Birkness.

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