Use of Agarose Gel Electrophoresis of Plasmid Deoxyribonucleic Acid to Fingerprint Gram-Negative Bacilli

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Agarose gel electrophoresis of the plasmid deoxyribonucleic acids from 60 gram-negative bacilli recovered during investigations of nosocomial epidemics was used to fingerprint the strains. This method was as specific at differentiating bacterial strains as more conventional phenotyping methods. In all cases, plasmid band fingerprints of epidemic strain isolates were identical whereas coisolate plasmid deoxyribonucleic acid patterns were different. Agarose gel electrophoresis of plasmid deoxyribonucleic acid is proposed as a method which can be used in conventional microbiology laboratories as an adjunct to, or possibly, replacement for other methods of identifying bacterial strains.

Gram-negative bacilli commonly cause nosocomial infections and can cause large outbreaks which are often difficult to control (2, 10). The design of effective measures to halt nosocomial epidemics requires the identification of reservoirs of infection and the mode of pathogen transmission in hospitals. Thus, the identification and differentiation of bacterial strains isolated during epidemiological studies is often required. Methods of species identification which are readily available in most hospital laboratories include biotyping and determination of the antimicrobial susceptibility patterns of the isolates. However, methods to determine additional markers which are used to fingerprint isolates, such as serotyping, bacteriocin production, bacteriocin susceptibility, and phage typing, are not performed by most hospital laboratories. An additional problem is that no typing schemes have been developed for some species of gram-negative bacilli.

While investigating the spread of an R-plasmid into several species of Enterobacteriaceae (11), we found that the total plasmid contents of nosocomial gram-negative rods as detected by agarose gel electrophoresis (AGE) seemed to be excellent markers of individual strains (12). All isolates of a particular strain (later verified by other typing methods) showed identical AGE plasmid band patterns. AGE of different strains produced different banding patterns, even if the strains had similar antimicrobial susceptibility patterns. To further this association explore, we examined the AGE plasmid band patterns of isolates which were collected in the course of investigating seven large outbreaks of nosocomial infections in several cities. These results were then compared with the data derived from biotyping, serotyping, bacteriocin typing, and antibiograms. Our study suggested that AGE patterns can be used as markers of bacterial strains and that AGE may be useful as a tool for investigating the epidemiology of nosocomial infections.

MATERIALS AND METHODS

Bacterial strains. We examined 60 gram-negative isolates obtained during epidemiological investigations by the Hospital Infections Branch, Centers for Disease Control, (10) or from nosocomial outbreaks investigated by us (9, 11). The designation epidemic strain was applied to organisms which were isolated from infected patients who were linked epidemiologically and which had been shown previously by serotyping (3), bacteriocin typing (1, 4), or a combination of these methods to be the same strain. Coisolates were organisms of the same genus and species which had similar antimicrobial susceptibility patterns and were usually from the same hospital, but were not linked epidemiologically to patients infected with the epidemic strain. These coisolates also had different serotypes or bacteriocin types. The phenotypic markers of isolates from two epidemics are shown in Table 1 to illustrate our use of the term coisolate.

Phenotyping. Isolates were phenotyped by the Hospital Infections Laboratory, Centers for Disease Control, using serotyping (3), bacteriocin typing (1, 4), and biotyping.

Preparation of plasmid DNA. Deoxyribonucleic acid (DNA) for AGE was prepared by slightly modifying the method of Hansen and Olsen (5). Polyethylene
glycol-precipitated DNA was suspended in a solution containing 0.25 M NaCl, 50 mM tris(hydroxymethyl)aminomethane, and 10 mM ethylenediaminetetraacetic acid (pH 8.0) and then precipitated in 2 volumes of 95% ethanol overnight at −20°C. Samples of partially purified DNA were resolved on 0.7% agarose gels as described by Meyers et al. (7). DNA bands were visualized by staining with ethidium bromide (0.4 μg/ml in water) for 20 min and photographed during exposure to long-wave ultraviolet light.

RESULTS
The AGE plasmid band patterns of multiply resistant enteric epidemic strains and coisolates from seven nosocomial outbreaks were examined, and in every case we found a distinctive AGE pattern for the epidemic strain. Figure 1 shows the band patterns of six different coisolates (wells A through C and E through H) and the epidemic strain (well D) from a Serratia marcescens outbreak in Atlanta, Ga. All isolates of the epidemic strain were serotype ONT:H8 (Table 1), contained a single plasmid of approximately 25 megadaltons, and had AGE patterns identical to the pattern shown in Fig. 1, well D. Each coisolate had a different serotype (Table 1) and a distinct plasmid band pattern. Even though the antibiograms of three of the coisolates were identical (Table 1), their AGE patterns confirmed that they represented three different strains.

Figure 2 shows the AGE patterns for a second S. marcescens epidemic, which occurred in Seattle, Wash. Lysates of three isolates of the bacteriocin type 16 epidemic strain (Fig. 2, wells B through D) contained a single, large, 45-megadalton plasmid. The single coisolate obtained during this outbreak (well A) was bacteriocin type 45 and contained two types of plasmids, including a large 50-megadalton plasmid and a smaller 12-megadalton plasmid which was present in multiple copies. The two different AGE patterns confirmed that these Serratia isolates had different phenotypic markers (Table 2) and thus were two different strains.

AGE of Pseudomonas aeruginosa isolates

TABLE 1. Phenotypic characteristics of isolates from a nosocomial epidemic of S. marcescens infections in Atlanta, Ga.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotypic markers</th>
<th>Antibiotic susceptibilities*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemic</td>
<td>ONT:H8</td>
<td>Ak, Tm</td>
</tr>
<tr>
<td>Coisolate 1</td>
<td>O13:H8</td>
<td>Ak, Tm</td>
</tr>
<tr>
<td>Coisolate 2</td>
<td>O14:H4</td>
<td>Ak, Tm</td>
</tr>
<tr>
<td>Coisolate 3</td>
<td>ONT:HNT</td>
<td>Ak, Tm, Gm, Tmx</td>
</tr>
<tr>
<td>Coisolate 4</td>
<td>O14:H12</td>
<td>Ak, Tm, Gm, Tmx, Km, Sm</td>
</tr>
</tbody>
</table>

*Antibiotics to which the isolates were susceptible as determined by the disk diffusion method. Abbreviations: Ak, amikacin; Tm, tobramycin; Gm, gentamicin; Km, kanamycin; Tmx, trimethoprim-sulfamethoxazole. These isolates were also resistant to chloramphenicol, tetracycline, sulfonamide, ampicillin, carbenicillin, cephalothin, furadantin, and polymyxin.
from an epidemic in Atlanta, Ga., showed that the epidemic strain (Fig. 2, wells E, G, and H) had a different AGE pattern than the coisolate (well F). The antibiograms of all of the *Pseudomonas* isolates examined were essentially identical (resistant to all antimicrobial agents tested except tobramycin), showing that isolates with identical antibiotic susceptibility patterns were not always derived from a single strain.

Figure 3 and Table 3 show data from two nosocomial epidemics due to *Providencia rettgeri* in St. Louis, Mo., and Memphis, Tenn. In both instances, coisolates had AGE patterns which differed from the pattern of the epidemic strain. Although the epidemic strains from the two different geographic locations had one phenotypic marker in common (serotype 042), their distinct AGE band patterns correlated with their different bacteriocin types and biotypes. In this case, several phenotyping schemes were needed to determine that these isolates were actually different strains, whereas this was readily apparent from an examination of their plasmid patterns.

Similar correlations between phenotypes and plasmid band patterns were observed for *Klebsiella pneumoniae* isolates obtained from hospitals in Oklahoma and Wisconsin.

**DISCUSSION**

Frequently, the investigation of an outbreak of nosocomial infections is aided by the ability to fingerprint strains of the same genus. In this study of seven epidemics of infections caused by four different genera of multiply resistant gram-negative bacilli, we found that AGE banding patterns were distinctive for different strains and thus served as specific fingerprints. In each case, gram-negative bacterial isolates which were recovered from a single geographic site over a short period of investigation and had identical phenotypes as determined by conventional typing schemes also had identical plasmid patterns as determined by AGE. Isolates which were phenotypically distinct had different AGE patterns, so that the same plasmid pattern was not observed in isolates of different strains. AGE plasmid banding appears to be a highly specific

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**TABLE 2. Characteristics of isolates from an epidemic of *S. marcescens* infections and an epidemic of *P. aeruginosa* infections**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Location</th>
<th>Species</th>
<th>Phenotypic marker(s)</th>
<th>Antibiotic susceptibilities*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemic</td>
<td>Seattle</td>
<td><em>S. marcescens</em></td>
<td>Bacteriocin type 16</td>
<td>Gm', Tn', Km', Ak*</td>
</tr>
<tr>
<td>Coisolate</td>
<td>Seattle</td>
<td><em>S. marcescens</em></td>
<td>Bacteriocin type 50</td>
<td>Gm', Tn', Km', Ak*</td>
</tr>
<tr>
<td>Epidemic</td>
<td>Atlanta</td>
<td><em>P. aeruginosa</em></td>
<td>Serotype 2, pyocin type 4</td>
<td>Tn'</td>
</tr>
<tr>
<td>Coisolate</td>
<td>Atlanta</td>
<td><em>P. aeruginosa</em></td>
<td>Serotype 1, pyocin types 1 through 8</td>
<td>Tn*</td>
</tr>
</tbody>
</table>

*Abbreviations: Gm', gentamicin intermediate susceptible; Tn', tobramycin intermediate susceptible; Km', kanamycin intermediate susceptible; Ak*, amikacin susceptible; Gm', gentamicin resistant; Km*, kanamycin resistant; Tn*, tobramycin susceptible.

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**TABLE 3. Phenotypic characteristics of isolates from two epidemics due to *P. rettgeri***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Location</th>
<th>Phenotypic markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemic</td>
<td>St. Louis</td>
<td>Biotype 10, serotype 042, bacteriocin type 4</td>
</tr>
<tr>
<td>Coisolate 1</td>
<td>St. Louis</td>
<td>Biotype 9, bacteriocin type 8</td>
</tr>
<tr>
<td>Coisolate 2</td>
<td>St. Louis</td>
<td>Biotype 9, bacteriocin type 1</td>
</tr>
<tr>
<td>Epidemic</td>
<td>Memphis</td>
<td>Biotype 9, serotype 042, bacteriocin type 2</td>
</tr>
<tr>
<td>Coisolate</td>
<td>Memphis</td>
<td>Biotype 9, bacteriocin type 1</td>
</tr>
<tr>
<td>Coisolate 2</td>
<td>Memphis</td>
<td>NT*</td>
</tr>
</tbody>
</table>

* All isolates were susceptible to amikacin.
* NT, Not typed.

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**Fig. 3. AGE of *P. rettgeri* isolates from Memphis, Tenn. (wells E through I), and St. Louis, Mo. (wells A through D). Wells A and B contained epidemic strain DNA, whereas wells C and D contained DNAs from two different coisolates (St. Louis). The Memphis epidemic strain (wells E through G) plasmid band pattern is clearly different from the patterns of the two coisolates (wells H and I).**
tool for distinguishing different hospital strains of enteric organisms. Recent studies of staphylococcal plasmids in nosocomial isolates confirm our observations concerning the utility of this method (8). The phenotypes of the isolates in this study do not appear to be colinked to any particular plasmid complement, as evidenced by the observation that two P. rettgeri strains recovered from different cities (Table 3 and Fig. 3) had the same serotype but distinctly different plasmid profiles.

There were some potential drawbacks to AGE fingerprinting, the most obvious of which is that it is not useful when isolates are free of plasmids. In our experience this is unusual for most nosocomial Enterobacteriaceae and staphylococci. Theoretically, spontaneous plasmid loss during subculture or storage could alter the profiles, but this did not occur in our study. In fact, we found that it was difficult to cure several of these nosocomial gram-negative rods of their R-plasmids by using a wide variety of in vitro methods. An additional potential problem arises from the ability of enteric bacteria to undergo interstrain transfer of plasmids. Strains may acquire new plasmids, which usually does not affect the phenotype but can alter the plasmid band profile. On the other hand, this might present a problem in the use of AGE to fingerprint strains collected over a long period of time. However, Parisi and Hecht (8) have suggested that plasmid band fingerprints of Staphylococcus epidemidis strains might be more specific in distinguishing strains than phage typing or biotyping. On the other hand, acquisition of a plasmid may change the phenotype (6), but this would be reflected in the plasmid band analysis.

Plasmid band fingerprinting by AGE requires no special reagents or biological materials, is relatively rapid and reproducible, and, therefore, could be used by large clinical laboratories as well as by central reference laboratories. Recent modifications of the method used in this study (B. Portnoy, personal communication) have increased the speed of the test so that plasmid DNA patterns can be examined within 5 to 6 h. AGE should be especially helpful in studying certain genera, including Citrobacter and Enterobacter, since methods of differentiating strains beyond determinations of biotypes or antimicrobial susceptibilities are not available at present.

ACKNOWLEDGMENTS

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LITERATURE CITED


