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Molecular Epidemiology of OHIO-1 β-Lactamase

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A total of 31 plasmids, all bearing a gene that encodes a novel, plasmid-mediated Richmond-Sykes class III beta-lactamase designated OHIO-1 and a gene that encodes aminoglycoside 2″-adenyltransferase, have been collected from hospitals in Ohio. By using restriction endonuclease digestion and Southern hybridization, we were able to demonstrate that all these plasmids have a common genetic origin.

In 1986, Shlaes et al. reported the existence of a novel plasmid-mediated beta-lactamase produced by nine strains of organisms in the family *Enterobacteriaceae* (12). All strains were isolated at the Veterans Administration Medical Center, Cleveland, Ohio (CVAMC), and University Hospitals, Columbus, Ohio. Between 1981 and 1986, 31 gram-negative bacilli that produced OHIO-1 beta-lactamase were identified. The additional 22 OHIO-1-producing strains were isolated during a prospective epidemiologic survey of gentamicin-resistant gram-negative bacilli colonizing patients in the CVAMC intermediate-care ward (D. M. Shlaes, C. A. Currie-McCumber, and M. H. Lehman, submitted for publication). All OHIO-1 plasmids also encoded 2″-adenyltransferase, which conferred gentamicin resistance. These observations led us to question whether the gene for OHIO-1 was disseminated in Ohio by several different plasmids or whether a single plasmid spread the gene to multiple bacterial hosts.

Bacterial strains. A total of 22 OHIO-1-bearing plasmids, each approximately 60 kilobases (kb) long, were isolated from *Citrobacter freundii*, *Citrobacter diversus*, *Serratia marcescens*, *Serratia liquefaciens*, *Klebsiella pneumonia*, *Morganella morganii*, *Providencia stuartii*, *Enterobacter aerogenes*, and *Escherichia coli* colonizing patients on the intermediate-care ward of CVAMC (Table 1).

Identification of OHIO-1-bearing plasmids. OHIO-1-producing gram-negative bacilli were identified in the course of nationwide (6) and worldwide (A. A. Medeiros, unpublished observations) surveys of beta-lactamase production in approximately 3,000 isolates and during prospective epidemiologic studies of gentamicin-resistant organisms in the medical intensive-care unit and the intermediate-care ward at the CVAMC (11). Organisms were identified by standard techniques with API 20E strips (Analytab Products, Plainview, N.Y.). OHIO-1 beta-lactamase was verified by substrate profile and isoelectric focusing as described previously (6, 12, 14). Cleared lysates were prepared from each parent strain and visualized on 0.7% agarose gels as described previously (1, 11). At least one plasmid from each parent organism was found to transfer OHIO-1 to *E. coli* C600 or HB101 (4) recipients by transformation or conjugation (11). Plasmids encoding OHIO-1 were maintained as transformants or transconjugants and were stored at −70°C in skim milk media (Difco Laboratories, Detroit, Mich.).

Plasmid DNA purification and restriction endonuclease profiling. Plasmid DNA was extracted from *E. coli* C600 and HB101 transconjugants or transformants and purified with cesium chloride density equilibrium gradients as previously described (11). Each purified plasmid DNA preparation was digested with a variety of restriction endonucleases (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) singly and in combination according to the instructions of the manufacturer. Restriction fragments were visualized after horizontal electrophoresis in 1% agarose gels.

Southern hybridization. Purified pDS075 DNA was digested with the restriction enzymes *Ava*I and *Pst*I used in combination and with *Taq*I used alone. Restriction fragments were transferred to nitrocellulose by the method of Southern (13). ^{32}P labeling of whole plasmid pDS1134 was performed by using [^{32}P]dCTP (Amersham Corp., Arlington Heights, Ill.) with a nick translation kit (Bethesda Research Laboratories). Hybridization was performed overnight, and the filters were washed at 50°C three times for 30 min each time in 0.5% sodium dodecyl sulfate and 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (4). Autoradiography was performed by exposing nitrocellulose filters to Kodak XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) in cassettes containing an intensifying screen.

Common genetic origin of OHIO-1 plasmids. The plasmids studied, their origins, and their assigned restriction endonuclease classes are listed in Table 1. Restriction endonuclease profiles comparing the class I through IV plasmids are shown in Fig. 1 and 2. *Eco*RI-*Pst*I double digestion of a class II plasmid generated numerous DNA fragments identical in molecular weight to those generated by *Eco*RI-*Pst*I digestions of either class III or class IV plasmids (Fig. 1). Minor differences among classes I, II, III, and IV are shown in Fig. 2. Groups I to III shared a maximum DNA fragment size of approximately 7 to 7.5 kb. All four groups contain many low-molecular-weight fragments, including a 0.7-kb *Pst*I internal fragment which contains a portion of the OHIO-1 structural gene (A. Hull, M. A. Kron, and D. M. Shlaes, unpublished observations). Class II contained a doublet at 4.3 to 5.5 kb and an extra 2.4-kb band not present in class III plasmids. Class III contained the doublet at 4.3 to 5.5-kb and a single band at 2.5 kb. Class IV contained a 19-kb fragment, the 4.3 to 5.5-kb doublet, a single band at 2.5 kb, and a single band at 3.5 kb.

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TABLE 1. Origin and classification of OHIO-1-bearing plasmids by restriction endonuclease profile

Plasmid(s)	Date of isolation	Origin	Mol wt (kb)	Restriction endonuclease class
pDS075	1981	CVAMC	12.3	I
pDS076 and pDS142	1981	CVAMC	60	II
p0229, p0220, p2102, and p1020	1984	UHC[a]	60	III
pDS1134 and 21 identical plasmids	1984–1985	CVAMC	60	III
p0218	1984	UHC	60	IV

[a] UHC, University Hospitals, Columbus, Ohio.

12.3-kb nonconjugative OHIO-1 plasmid (class I) derived from 60-kb plasmids. The single 12.3-kb nonconjugative plasmid isolated at CVAMC in 1981 (12) contained no *Eco*RI sites and three *Pst*I sites that generated DNA fragments of 0.7, 4.3, and 7.5 kb (Fig. 2). *Ava*I-*Pst*I double digestion of class I plasmids (data not shown) revealed many DNA fragments identical in molecular weight to those generated by *Ava*I-*Pst*I double digestion of the class III plasmids. An autoradiogram (Fig. 3) was generated by probing a Southern blot of the *Ava*I-*Pst*I double digestion and a *Taq*I digestion of pDS075 (class I) with ^{32}P-labeled pDS1134 (class III). Except for a single DNA fragment of 2.5 kb, all restriction fragments generated from pDS075, ranging in size from 6,500 to 200 base pairs, hybridized to the class III plasmid under stringent conditions. We believe this is consistent with the hypothesis that pDS075 resulted from a large deletion of the 60-kb plasmid with an additional 2.5-kb DNA fragment insertion.

A number of highly stable, widely disseminated R plasmids have been described previously. Some have carried the 2″-adenyltransferase determinant and have been isolated since the 1970s in many parts of the world (7). Other R plasmids, although relatively stable, have been shown to evolve with time under natural conditions within medical centers (2, 3, 10). The recent discovery of a novel beta-lactamase gene, which so far appears to be limited to Ohio, gave us the opportunity to study a geographically well-defined set of R plasmids.

Our goal in this study was to determine whether a single plasmid or multiple plasmids were responsible for the dissemination of OHIO-1. The answer appears to be that all OHIO-1-bearing plasmids have a common origin and differ only by small rearrangements. Even the small class I plasmid appears to be derived from the larger OHIO-1-bearing plasmids. These data may represent the dissemination of a recently derived R plasmid or the discovery of a highly stable, locally endemic plasmid. We favor the former hypothesis, since some plasmid instability is evident from the microheterogeneity observed in restriction endonuclease profiles and from the spontaneous formation of a class I plasmid.

Finally, it is not at all clear why the OHIO-1 gene

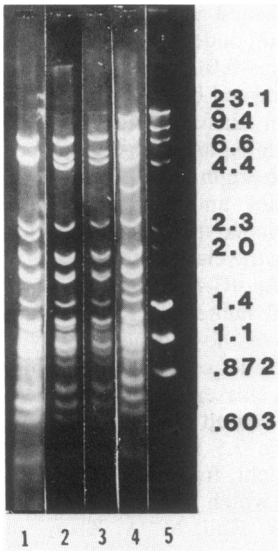

FIG. 1. *Eco*RI and *Pst*I double digestions of OHIO-1 plasmids. Lane 1, pDS142, restriction endonuclease class II; lane 2, pDS1134, class III; lane 3, p2101, class III; lane 4, p0218, class IV; lane 5, *Hin*dIII digestion of bacteriophage lambda DNA plus *Hae*III digestion of bacteriophage ϕX174 DNA for molecular weight standards. Molecular weights are indicated in kilobases on the right.

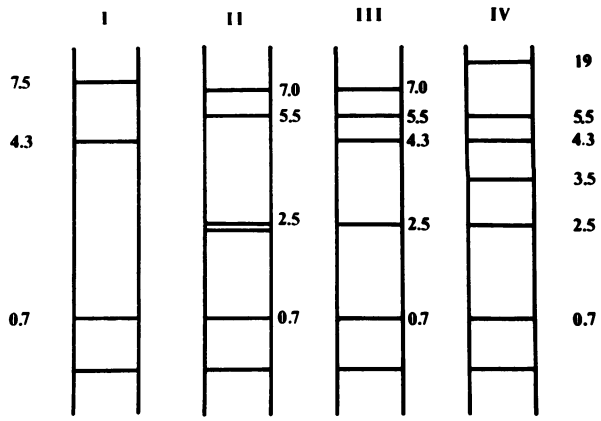

FIG. 2. Important differences and similarities among *Eco*RI-*Pst*I double digestions of OHIO-1 plasmids. Lane 1, class I; lane 2, class II; lane 3, class III; lane 4, class IV. Molecular weight markers in kilobases are at the side of each lane.

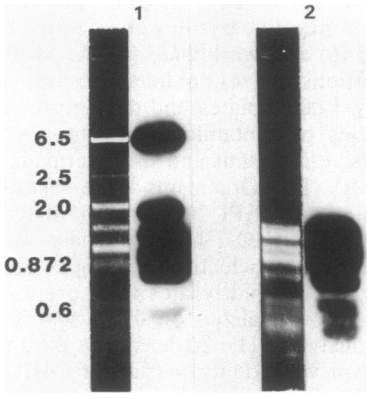

FIG. 3. Homology between 12.3-kb class I and 60-kb class III OHIO-1 plasmids. For each panel, the agarose gel electrophoresis of the 12.3-kb class I OHIO-1 plasmid is shown on the left, with its respective Southern hybridization on the right. Each digestion is hybridized with ^{32}P-labeled pDS1134 (class III) DNA and washed under stringent conditions. (1) *Ava*I-*Pst*I double digestion; (2) *Taq*I digestion. Molecular weights are indicated in kilobases on the left.

disseminated. Does OHIO-1 offer some advantage to the host bacterium over the ubiquitous TEM-1 and TEM-2 beta-lactamase (5, 8, 9)? Perhaps it is the 2″-adenyltransferase gene or some other virulence property of the OHIO-1 plasmids that was selected. Alternatively, it could be that this determinant was disseminated on the hands of hospital employees, allowing survival of the plasmid within the hospital. Experiments to address some of these issues are currently in progress.

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