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Stability of Plasmid Content in *Salmonella wien* in Late Phases of the Epidemic History

M. CASALINO,[1] A. COMANDUCCI,[2] M. NICOLETTI,[2] AND F. MAIMONE[1]*

Dipartimento di Biologia Cellulare e dello Sviluppo, Sezione di Scienze Microbiologiche,[1] and Istituto di Microbiologia,[2] Università di Roma La Sapienza, Città Universitaria, 00185 Rome, Italy

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Prevalence, genetic characteristics, and *Eco*RI cleavage analysis of plasmids identified in clinical strains of *Salmonella wien* isolated in recent years showed that the plasmid content in this serotype has remained uniform and stable over more than a decade and also late in the epidemic history. No correlation between decrease in *S. wien* isolations and naturally occurring systematic changes in the DNA of its most common FI*me* plasmid was structurally detectable.

Multiply antibiotic-resistant strains of *Salmonella wien* have been isolated in outbreaks of human enteritis, usually in pediatric departments, nurseries, or maternity wards. They were first observed in Algeria in 1969 (8, 11); during the 1970s, this serotype was widespread in countries of the Mediterranean and Middle East: predominantly in France, Italy, Yugoslavia, Tunisia, and Iraq. Cases associated with *S. wien* have also occurred in the Republic of Ireland, Great Britain, Belgium, the Federal Republic of Germany, Austria, and India (7–10, 12, 13). Epidemiological data indicate that the *S. wien* strain involved in epidemics was drug resistant and generally resistant to ampicillin (Ap^r), chloramphenicol (Cm^r), kanamycin (Km^r), streptomycin (Sm^r), sulfonamide (Su^r), and tetracycline (Tc^r) (2, 8–13). Furthermore, although thousands of cases have occurred in Western Europe since 1969, from 1950 to 1969 only a few dozen sporadic cases due to *S. wien* had been reported, and all isolates tested were susceptible to the main drugs (8).

Many *S. wien* strains isolated in different countries between 1970 and 1976 have been examined for their plasmid content. Most of the drug-resistant strains carried at least two plasmids: an IncFI*me* conjugative or nonconjugative R plasmid of large size and a small nonconjugative R plasmid of 9 megadaltons (MDa) (1, 2, 9, 10, 14). This uniformity of plasmid content and the epidemiological indications strongly supported the view that the distribution of this serotype had a clonal origin and suggested the hypothesis that the FI*me* plasmid might contribute to communicability and virulence.

To investigate whether the remarkably decreasing prevalence of *S. wien* in Italy in recent years (1978 to 1980) could be correlated with naturally occurring systematic changes in its plasmid content or in any structurally detectable portions of its FI*me* plasmids, we decided to analyze the plasmid complement of *S. wien* strains isolated in these late phases of the epidemic history.

A total of 1,200 clinical isolates (480 in 1978, 400 in 1979, and 320 in 1980) out of more than 3,000 *S. wien* strains isolated in Italy between 1978 and 1980 were examined for drug resistance in the Enteric Pathogens Reference Centers. Of the isolates, 70% (75% in 1978, 65% in 1979, and 75% in 1980) were Ap^r Cm^r Km^r Sm^r Su^r Tc^r; a few of them were also resistant to nalidixic acid (Nal^r). About 3 to 5% were resistant to gentamicin (Gm^r) instead of or in addition to Tc^r, and some strains had fewer than six resistance markers. Only 2 to 10% of the isolates were susceptible to all main drugs tested.

The resistance pattern and colicinogeny of 19 Italian *S. wien* strains representative of the main groups are shown in Table 1. All 19 *S. wien* strains carried an R plasmid with a molecular weight ranging from 70×10^6 to 110×10^6 (determined by gel electrophoresis). The most frequent resistance pattern coded for by this plasmid was Ap^r Cm^r Km^r Tc^r and resistance to mercuric ion (Hg^r) (Table 1). All plasmids, including the two nonconjugative (Tra^-) plasmids, were incompatible with F′ *lac pro* and FI*me* plasmids and compatible with plasmids of groups FII, H1, H2, Iα, M, W, and P. Nine kanamycin-susceptible (Km^s) mutant derivatives of the IncFI plasmids from *S. wien* WZM8, WZM11, WZM14, WZM15, WZM17, WZM19, WZM21, WZM22, and WZM29 were tested for compatibility with the K-MP10 plasmid. Seven plasmids displayed incompatibility with the K-MP10 plasmid and on this basis were assigned to the FI*me* subgroup. The compatible ones were the two 70- to 80-MDa plasmids isolated from the Gm^r strains WZM8 and WZM11 (Table 1). Preliminary results from genetic and physical analyses of miniplasmids obtained by partial restriction enzyme digests of different FI*me* plasmids from *S. wien* strains (M. Nicoletti, M. Casalino, B. Colonna, and F. Maimone, manuscript in preparation) suggest that these smaller Gm^r plasmids are natural derivatives of FI*me* plasmids which lost the genetic region involved in the K-MP10 plasmid incompatibility. They were therefore classified as FI*me* in spite of their compatibility phenotype. (Techniques for conjugation, transformation, incompatibility testing, and plasmid DNA purification and digestion were the same as those used previously, see reference 9).

The FI*me* plasmids pZM141, pZM151, and pZM171 (from *S. wien* WZM14, WZM15, and WZM17, respectively) were analyzed by digestion with the restriction endonuclease *Eco*RI. Their restriction patterns were compared with those of FI*me* reference plasmids isolated in Italy in 1974 (year of the first *S. wien* isolations) and 1976 (year of the highest number of isolations).

Figure 1 shows *Eco*RI digests of pZM141, pZM151, pZM171, and reference plasmid pZM33. This plasmid (Ap^r Cm^r Hg^r Km^r Tc^r, FI*me*, Tra^+) (100 MDa) was identified in *S. wien* WZM3 (9) which belonged to a group of seven *S. wien* strains with the same plasmid content isolated during a

* Corresponding author.

TABLE 1. Plasmid content of *S. wien* strains

Strain	Yr of isolation	Resistance pattern and colicinogeny[a]	Phenotype for plasmid (approx. mol. mass in MDa): IncFI*me* (70–110)[b]	IncIα (50–60)	Ap^r Sm^r Su^r plasmid (9)	Cryptic plasmid (1.4)
WZM21, WZM22, WZM23, WZM24	1978	Ap^r Cm^r Hg^r Km^r Sm^r Su^r Tc^r	Ap^r Cm^r Hg^r Km^r Tc^r, Tra$^+$	−	+	+
WZM26		Ap^r Cm^r Hg^r Km^r Sm^r Su^r Tc^r	Ap^r Cm^r Hg^r Km^r Tc^r, Tra$^-$	−	+	+
WZM20		Ap^r Cm^r Hg^r Km^r Sm^r Su^r Tc^r Cia	Ap^r Cm^r Hg^r Km^r Tc^r, Tra$^+$	Cia, Tra$^+$	+	+
WZM8		Ap^r Cm^r Gm^r Hg^r Km^r Sm^r Su^r Cia	Ap^r Cm^r Gm^r Hg^r, Tra$^+$	−	+	+
WZM9		Ap^r Cm^r Gm^r Hg^r Km^r Sm^r Su^r	Ap^r Cm^r Gm^r Hg^r Km^r, Tra$^+$	Cia, Tra$^+$	+	+
WZM10		Ap^r Cm^r Gm^r Hg^r Km^r Sm^r Su^r Cia	Ap^r Cm^r Gm^r Hg^r Km^r, Tra$^+$	Ap^r Cia, Tra$^+$	+	+
WZM11		Ap^r Cm^r Gm^r Hg^r Km^r Sm^r Su^r	Ap^r Cm^r Gm^r Hg^r Km^r, Tra$^+$	−	+	+
WZM14, WZM19, WZM25, WZM28	1979	Ap^r Cm^r Hg^r Km^r Sm^r Su^r Tc^r	Ap^r Cm^r Hg^r Km^r Tc^r, Tra$^+$	−	+	+
WZM15		Ap^r Cm^r Hg^r Km^r Sm^r Su^r Tc^r	Ap^r Cm^r Hg^r Km^r Tc^r, Tra$^+$	−	+	−
WZM18		Ap^r Cm^r Hg^r Km^r Sm^r Su^r Tc^r Cia	Ap^r Cm^r Hg^r Km^r Tc^r, Tra$^+$	Cia, Tra$^+$	+	+
WZM29	1980	Ap^r Cm^r Hg^r Km^r Sm^r Su^r Tc^r	Ap^r Cm^r Hg^r Km^r Tc^r, Tra$^+$	−	+	+
WZM16		Ap^r Cm^r Tc^r	Ap^r Cm^r Tc^r, Tra$^-$	−	−	+
WZM17		Ap^r Cm^r Hg^r Km^r Nal^r	Ap^r Cm^r Hg^r Km^r, Tra$^+$	−	−	+

[a] All 19 strains were susceptible to rifampin, trimethoprim, and spectinomycin. Cia, Production of colicin Ia.

[b] The Ap^r Cm^r Hg^r Km^r Tc^r plasmids and the Ap^r Cm^r Hg^r Km^r plasmid (from strain WZM17) had molecular masses of 90 to 110 MDa. The Ap^r Cm^r Gm^r Hg^r Km^r plasmids, the Ap^r Cm^r Gm^r Hg^r plasmid (from strain WZM8), and the Ap^r Cm^r Tc^r plasmid (from strain WZM16) had molecular masses of 70 to 80 MDa.

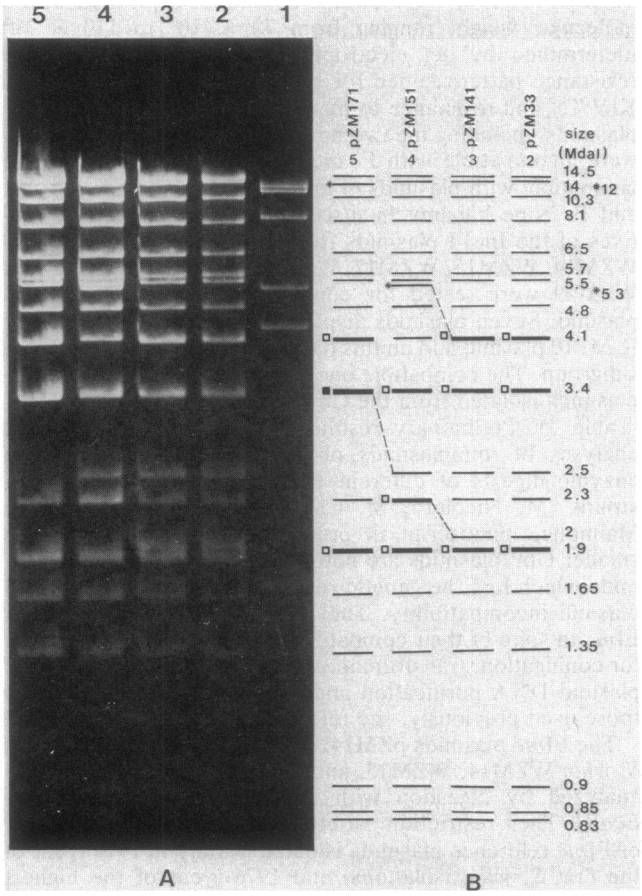

FIG. 1. (A) Agarose gel electrophoresis of *Eco*RI restriction endonuclease digests of R1*drd-19*.K1 (reference fragments of known molecular size) (lane 1) (3, 4, 6), pZM33 (lane 2), pZM141 (lane 3), pZM151 (lane 4), and pZM171 (lane 5). (B) Schematic representation of the gel shown in A. Doublets (□) and triplets (■) were detected by microphotodensitometry analysis and confirmed by double digestions with *Eco*RI and *Hin*dIII. Dashed lines indicate corresponding *Eco*RI fragments representative of characteristic structural variations naturally occurring in FI*me* plasmids in *S. wien* (see text).

ward outbreak in a general hospital in Rome in 1976. The Ap^r Cm^r Hg^r Km^r Tc^r plasmids pZM33, pZM141, and pZM151 had 22 cleavage sites for *Eco*RI, generating 22 fragments from 0.83 to 14.5 MDa. All fragments obtained from pZM141 were identical with those from pZM33, whereas the digestion of pZM151 produced 20 fragments identical with those from pZM33 and one more 2.3-MDa fragment and a 5.3-MDa fragment instead of the 2.0-MDa fragment and one of the two 4.1-MDa fragments of pZM33 and pZM141. The Ap^r Cm^r Hg^r Km^r plasmid pZM171 lacked one *Eco*RI site; its digest contained a new 3.4-MDa fragment (in addition to the usual 3.4-MDa doublet) (Fig. 1) and a 12-MDa fragment but not the 2.0-, 2.5-, and 14-MDa fragments. *Eco*RI digestion patterns of spontaneous tetracycline-susceptible (Tc^s) mutants of FI*me* Ap^r Cm^r Hg^r Km^r Tc^r plasmids isolated in the laboratory (data not shown) suggested that the loss of 2.0- and 14-MDa fragments and the appearance of only one 12-MDa fragment in the digest of pZM171 could be attributed to a deletion of the Tc^r region. Moreover, this larger difference and the three variations represented by the presence of *Eco*RI fragments of 2.0 or 2.3 MDa, 2.5 or 3.4 MDa, and 4.1 or 5.3 MDa (dashed lines in Fig. 1B) were the same as those observed to occur, together or separately, between pZM33 and four other FI*me* reference plasmids isolated in Italy in 1974 and 1976 (not shown in Fig. 1).

A conjugative plasmid encoding for production of colicin Ia was found in four *S. wien* strains (WZM9, WZM10, WZM18, and WZM20; Table 1). It belonged to IncIα, and its molecular weight, measured by gel electrophoresis, was 50 $\times$ 10^6 to 60 $\times$ 10^6. Of the 19 *S. wien* strains, 17 carried an Ap^r

Sm^r Su^r plasmid of 9 MDa, similar to those described in previous years (9, 10); a small cryptic 1.4-MDa plasmid was also present in 18 strains (Table 1). No other plasmid was detected in the strains tested.

In summary, an extensive survey for drug resistance of more than 1,000 *S. wien* strains isolated in Italy in the years of decreasing prevalence of this serotype showed that multiply antibiotic-resistant strains remained by far the predominant variety, and the most common resistance pattern was still Ap^r Cm^r Km^r Sm^r Su^r Tc^r.

The prevalence and general features of the plasmids identified in 19 resistant strains of *S. wien* isolated during this period displayed little change from those of plasmids characterized in Italian strains isolated in 1974 and 1976 (9) and in numerous *S. wien* strains of various origins isolated between 1970 and 1976 (2, 10; unpublished data). This finding reveals a considerable uniformity and stability of plasmids in clinical isolates of *S. wien* in a large geographical area (from North Africa and the Middle East to Western Europe) over more than a decade and even late in the epidemic history. Unlike *S. wien*, genetic instability of the FI*me* R plasmid has been observed in strains of *Salmonella johannesburg* isolated in Hong Kong from 1973 to 1979 (5).

The differences in *Eco*RI cleavage analysis between the reference plasmid pZM33 and the three plasmids isolated in 1979 and 1980 were not specific for FI*me* plasmids isolated at a time when the prevalence of *S. wien* was rapidly declining. Analogous variations in the size of *Eco*RI fragments have been found in digests of FI*me* plasmids isolated early and in the middle of the epidemic history. These results do not exclude the possibility that DNA sequences independent of drug resistance regions on FI*me* plasmids in *S. wien* may code for virulence properties. However, no epidemiological correlation could be found between the strong decrease in *S. wien* isolations and naturally occurring, structurally detectable, systematic changes (eventually involving those possible virulence sequences) in the DNA of its most widely distributed FI*me* R plasmid.

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