ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2001, p. 2299–2303 Vol. 45, No. 8
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.8.2299–2303.2001

Large Drug Resistance Virulence Plasmids of Clinical Isolates of *Salmonella enterica* Serovar Choleraesuis

CHISHIH CHU,[1] CHENG-HSUN CHIU,[2] WAN-YU WU,[1] CHI-HONG CHU,[3] TSUI-PING LIU,[1] AND JONATHAN T. OU[1]*

Department of Microbiology and Immunology, Chang Gung University College of Medicine,[1] and Department of Pediatrics, Chang Gung Children's Hospital,[2] Kweishan 333, Taoyuan, and Department of Surgery, Tri-Service General Hospital, Taipei 100,[3] Taiwan

Received 11 December 2000/Returned for modification 9 April 2001/Accepted 26 May 2001

Salmonella enterica **serovar Choleraesuis generally causes systemic human salmonellosis without diarrhea, and therefore, antimicrobial treatment is essential for such patients. The drug resistance information on this organism is thus of high value. Serovar Choleraesuis usually harbors a virulence plasmid (pSCV) of 50 kb in size. Of the 16 clinical isolates identified to be serovar Choleraesuis, all except one harbored a pSCV and seven of them carried a pSCV of more than 125 kb in size. A pSCV was defined as a plasmid carrying *spvC* and characteristic deletions detected by PCR and by DNA-DNA hybridization (for the former criterion). The results of PCR, restriction fragment profiles, and Southern DNA-DNA hybridizations of the profiles all indicated that such larger pSCVs were derived from the 50-kb plasmid recombined with non-pSCVs found in some clinical isolates. Fifteen of the 17 strains, including a laboratory strain, were then tested for drug resistance against 16 antibiotics with E-test and the dilution method. The laboratory strain, which harbored a 50-kb pSCV and a 6-kb non-pSCV, was resistant only to sulfonamides (SUL), and its resistance gene, *sulII*, checked with PCR and DNA-DNA hybridization, was located on the 6-kb non-pSCV. All 14 clinical strains were resistant to multiple drugs. Of the 14, 7 were resistant to SUL, and the resistance gene was located on a plasmid. The *sulII* gene, but not $bla_{\text{TEM-1}}$, was carried only on the 6-kb non-pSCV. Of the remaining six large plasmids, three of 90 kb, two of 136 kb, and one of 140 kb, the last three were pSCVs and carried the other SUL gene (*sulI*) and the $bla_{\text{TEM-1}}$ gene. The six strains were also resistant to trimethoprim-sulfamethoxazole. None of the 50-kb pSCVs carried resistance genes. These drug resistance genes on the large pSCVs were apparently also acquired through recombination.**

Human nontyphoidal salmonellosis is usually self-limiting and does not require antimicrobial treatment. There are consistent observations that antimicrobial therapy for uncomplicated gastroenteritis does not reduce the duration or severity of symptoms, but in fact, it prolongs the excretion of the bacteria in feces in convalescence and results in the emergence of resistant organisms (1, 5). When the pathogen infects beyond the intestines, causing systemic diseases, however, antimicrobial therapy is required and quite essential. Consequently, the knowledge of the likelihood of resistance to antimicrobial agents is of considerable value to clinicians.

Among more than 2,000 *Salmonella enterica* serovars, serovar Choleraesuis shows a high predilection for invasive infections in humans, frequently causing systemic infection without diarrhea (6, 9), and therefore parenteral antimicrobial therapy is the mainstay of treatment for such patients. Furthermore, serovar Choleraesuis is one of the seven *Salmonella* serovars that are known to contain a virulence plasmid (2, 7, 8, 11, 16). The virulence plasmid is involved in the expression of the virulence of these serovars in their respective specific natural hosts (11). A number of regions on the plasmid that are important for virulence have been identified (8; see reference 11 and references therein). On the other hand, the virulence plasmid of *Salmonella* has so far been thought to be unrelated to drug resistance as there are few reports on the association of the virulence plasmids with antibiotic resistance (14).

The incidence of serovar Choleraesuis infection is rather high in Taiwan (6). In a large medical center located in southern Taiwan, the frequency of detecting salmonellae is ranked 6th every year, and the bacteria with frequency rankings above that of salmonellae are all opportunistic pathogens (unpublished observation). Among the salmonellae, the frequency of serovar Choleraesuis infection is second only to serovar Typhimurium and serovar Schwarzengrund in Taiwan (6). Almost all clinical isolates of serovar Choleraesuis derived from Taiwanese patients, and 100% of those isolated from blood (7), contained the virulence plasmid (pSCV). Unlike the virulence plasmid of the other serovars, however, the size of the indigenous pSCV in these isolates varies greatly, although all carry a common virulence operon, *spv* (8, 11). Most of these clinical isolates were also resistant to a number of antimicrobial agents. Therefore, the current study was undertaken to describe the possible sources of the larger pSCV and to evaluate whether or not there are any pSCVs that are also drug resistance factors. We report here that the larger pSCV was probably formed via recombination with non-pSCV plasmids, which might also be the reason for some pSCVs carrying drug resistance genes.

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Chang Gung University College of Medicine, 259 Wenhua 1 Rd., Kweishan 333, Taoyuan, Taiwan. Phone: 886-3-3286455. Fax: 886-3-3286455 and 886-3-3283031. E-mail: jontou@mail.cgu.edu.tw.

MATERIALS AND METHODS

Bacterial strains and plasmid profiles. The *Escherichia coli* strain used was strain 9726, and the *S. enterica* strains used were serovar Typhimurium OU5045 (strain C5), a laboratory strain which contains a virulence plasmid (pSTV), and

TABLE 1. Primers used to detect genes *spvC, sulI, sulII*, and *bla* and deletion regions within the *pef* operon and between genes *samA* and *traT*

Primer	DNA sequence	Size of DNA fragment (bp)	Accession no. or source
Esam	CCCGATAGCCCTGACGAAGAAT		D90202
TraT4	TGTGCTCATCGAACCACACCCT	1,800	This study
H4-pefF	CGAACAGGGTGATGAATGAGAT		L08613
RepB3	CGGCGTGAAAGAGCGCATGATGAT	859	U64797
SpvC1	CTTGCACAACCAAATGCGGAAGAT		
SpvC2	CTCTGCATTTCACCACCATCACG	570	X56727
Sull-F	TGGTGACGGTGTTCGGCAT		
Sull-R	GCTAGGCATGATCTAACCCT	841	X15014
SulI-F	TCAACATAACCTCGGACAGT		
SulII-R	GATGAAGTCAGCTCCACCT	707	X57730
AP-F	ATGAGTATTCAACATTTCCGTGT		
AP-R	TTACCAATGCTTAATCAGTGAGG	876	J01749

serovar Choleraesuis OU7085, which contains a 50-kb pSCV and a 6-kb plasmid. Clinical isolates were derived from the patients who came to Chang Gung Memorial Hospital and Chang Gung Children's Hospital for treatment between 1996 and 1997. Strains of group C1 were isolated from the blood and feces of patients and serovar Choleraesuis was identified with anti-*Salmonella* H-antigen serum by the tube agglutination method. All isolates were routinely cultured at 37°C on Luria-Bertani plates or broth for experiments. Plasmid profiles were determined by the Kado-Liu method (12). The 90-kb pSTV of serovar Typhimurium OU5045 and the 50-kb pSCV of serovar Choleraesuis OU7085 served as controls. Plasmid DNA was extracted by the alkaline lysis procedure and further purified with a CsCl gradient formed by centrifugation with a Ti70.1 rotor (Beckman model LM8) at 55,000 rpm (8). When there were two plasmids, individual plasmids were purified further by gel elution. Plasmid DNA was then digested with restriction enzyme *Hin*dIII or *Bam*HI for the restriction fragment plasmid profile.

Antimicrobial susceptibility. The MICs of antibiotics against serovar Choleraesuis isolates were determined by either E-test (AB BIODISK) or the broth dilution method in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (15). The following antibiotics were tested by E-test: amoxicillin (AMX), ampicillin (AMP), ceftriaxone (CRO), gentamicin, cephalothin, chloramphenicol, erythromycin, and tetracycline. The following antibiotics were tested by the broth dilution method: sulfonamides (SUL) and trimethoprim (TMP). Susceptibility of the isolates to trimethoprim-sulfamethoxazole (SXT) was determined by the disk diffusion method. Susceptibility to the newer agents ceftazidime, CRO, cefepime, ofloxacin, and ciprofloxacin was also determined by the broth dilution method.

Isoelectric focusing and β-lactamase assay. AMP-resistant serovar Choleraesuis isolates and *E. coli* strain 9726 (Amp^r) were grown overnight at 37°C in 5 ml of Luria-Bertani broth containing 100 μg of AMP/ml. The test sample was prepared as described by the manufacturer (Pharmacia). One milliliter of each overnight culture was centrifuged and the pellet was suspended in 100 μl of lysis buffer (0.54 g of urea, 2% Triton X-100, 2% 2-mercaptoethanol, 2% Pharmalyte 3–10, 1.4 mg of phenylmethylsulfonyl fluoride, and 0.2 mg of Pefabloc [Merck] per ml). The solution was then mixed with 100 μl of loading dye (0.54 g of urea, 2% 2-mercaptoethanol, 2% Pharmalyte 3–10, and 0.54% Triton X-100 per ml), and 20 μl was used for determination of the isoelectric focusing (IEF) point of β-lactamase. The three β-lactamases of *E. coli* strain 9726, with IEF pIs of 5.4, 7.6, and 8.2, were used as standards. The IEF point of β-lactamase was measured in a polyacrylamide gel (Ampholine PAG plate, pH 3.5 to 9.5; Amersham Pharmacia Biotech) in an LKB Multiphor 2117 apparatus (Pharmacia). β-Lactamase activity was examined by spreading 2 ml of a 0.05% (wt/vol) solution of nitrocefin (Glaxo-Wellcome), which would produce a pink color when reacted with β-lactamase, onto the gel.

PCR amplification and sequencing. Primers were designed from the following genes and regions: *spvC*, the β-lactamase gene (TEM type), *sulI, sulII*, and the regions flanking the two deletions, in the *pef* operon and the *samA-traT* region, respectively (8). The sequences of the primers and the lengths of the PCR fragments amplified are listed in Table 1. The PCR buffer was obtained from Ab Peptides, Inc., and the amplification was carried out by a standard procedure. The annealing temperature and DNA extension time varied, however, depending on the primers used and the length of amplified DNA fragment, at a rate of ca. 1 kb/min. The amplified PCR product was purified by using the Wizard PCR Preps kit (Promega) and sequenced by an ABI 373A automatic sequencer (Perkin-Elmer, Applied Biosystems). The search for homologous sequences was done in the GenBank database by using the FASTA software through the Internet.

DNA-DNA hybridization. DNA-DNA hybridization was carried out by the standard procedure. Purified DNA was first digested with restriction enzyme *Hin*dIII or *Bam*HI and electrophoresed in an agarose gel (0.6% GTG; FMC). The DNA bands were transferred onto a Zeta-probe membrane (Bio-Rad), and DNA-DNA hybridization and washing were performed according to the membrane manufacturer's recommendation, except that 0.5% (instead of 1%) sodium dodecyl sulfate was used in the final wash. Each probe was labeled with [^{32}P] dCTP by the randomly primed labeling method (Random Primed labeling kit; Gibco-BRL). Hybridized DNA was detected with an X-ray film with an intensifying screen.

Identification of a pSCV. The plasmids of strains identified as serovar Choleraesuis were extracted and identified as pSCVs by checking for the presence of the *spvC* gene and the deletions specific to pSCV. For the presence of the *spvC* gene, PCR and DNA-DNA hybridization were used. For PCR, amplification was carried out with the pair of *spvC* primers (Table 1) and the extracted plasmid as template. The result of PCR was confirmed by DNA-DNA hybridization with the PCR-amplified *spvC* gene fragment derived from OU7085 as the probe. PCR was also used to determine the presence or absence of pSCV-specific deletions (8). The two deletions, with an area of more than 6 and 25 kb, were located in the *pef* operon and the *samA-traT* region, respectively (8), as mentioned above. Therefore, PCR products would be produced from a pSCV, because the area between the pair of primers would be shortened and amplified, whereas no PCR products would be observed when a virulence plasmid was not a pSCV. A plasmid with a positive reaction to these tests was identified as a pSCV.

RESULTS

Characterization of indigenous plasmids of serovar Choleraesuis. Of the 25 *Salmonella* group C1 strains isolated from the blood and feces, 16 were identified as serovar Choleraesuis. All 16 strains, listed in Table 2 with laboratory strain OU7085, contained at least one plasmid, the size of which ranged from 6 to 140 kb. The results of the tests for the presence of *spvC* indicated that all isolates contained a virulence plasmid (pSCV), except strain OU7533, the only strain isolated from feces, which was without a pSCV (Table 2). The pSCVs from various strains were 50, 125, 136, and 140 kb in size. There were five strains that contained, in addition to a 50-kb pSCV,

TABLE 2. Bacterial strains and plasmid profiles

Serovar Cholaraesuis strain	Plasmid size (kb)		Source
	pSCV	Non-pSCV	
OU7085	50	6	This lab
OU7516	136		Blood
OU7517	136		Blood
OU7518	136		Blood
OU7519	125		Blood
OU7520	140		Blood
OU7521	50	90	Blood
OU7522	50		Blood
OU7523	50		Blood
OU7524	136		Blood
OU7525	50	75	Blood
OU7526	50	90	Blood
OU7527	50	6	Blood
OU7528	125		Blood
OU7529	50	90	Blood
OU7531	50	90	Blood
OU7533		130	Feces

TABLE 3. Characteristics of large pSCVs

Strain	Plasmid size (kb)	Result of probing with plasmid:			Presence of deletion in:	
		OU7085[a]	OU7519	OU7533	*pef*	*tra*
OU7085	50	+	+	−	+	+
OU7518	135	+	+	+	+	+
OU7519	125	+	+	+	+	+
OU7520	140	+	+	+	+	+
OU7524	135	+	+	+	+	+
OU7528	125	+	+	+	+	+
OU7533	130	−	+	+	−	−

[a] Only the 50-kb pSCV was used as the probe.

a larger nonvirulence plasmid (75 or 90 kb). The *Hin*dIII and *Bam*HI restriction fragment profiles of the plasmids in strains OU7516, OU7517, and OU7518 were identical. Strain OU7518, therefore, was chosen as a representative of the three and was used in the following experiments.

There was a likelihood that these large pSCVs (size, >125 kb) might have derived from genetic recombination between the 50-kb pSCV and another nonvirulence (containing no *spvC*) plasmid (75, 90, or 130 kb). All pSCVs of various sizes were *spvC* positive and contained the two deletion regions, specific markers of a pSCV, located within the *pef* operon and the *samA-traT* region (8). The DNA fragments in the deletion regions amplified should be 890 bp for the *pef* region and 1,800 bp for the *samA-traT* region, and correctly sized products were obtained from all putative pSCVs. The area of the *spv* operon and the two deletion regions span most of the 50-kb pSCV. These observations suggested that pSCVs of >125 kb contained the standard 50-kb pSCV DNA (Table 3). On the other hand, the 130-kb non-pSCV of OU7533 was *spvC* negative and did not contain the deletion. To check the sequence homology of these plasmids, DNA-DNA hybridization with a whole 50-kb plasmid of OU7085 and the plasmids of OU7519 and OU7533 as probes was performed. The plasmids, including controls, a 50-kb pSCV and the 6-kb non-pSCV, were digested with a restriction endonuclease, electrophoresed, transferred to a nitrocellulose membrane, and hybridized. The 50-kb pSCV of OU7085 hybridized to all fragments corresponding to those derived from the 50-kb pSCV and presumably derived from the 50-kb pSCV section of clinical pSCVs of various sizes (Table 3). There was virtually no sequence homology between the 50-kb pSCV and the non-pSCVs of various sizes, including the 6-kb plasmid, listed in Table 2. When the 125-kb pSCV of OU7519 was used as the probe, it hybridized to the fragments derived from the non-pSCVs of OU7525, OU7531, and OU7533. On the other hand, the 130-kb non-pSCV of OU7533 hybridized to the fragments derived from the non-pSCV section of all larger pSCVs (Table 3). It also hybridized to the fragments derived from the non-pSCVs (but not to the 6-kb plasmid) of the clinical isolates, but not to the 50-kb pSCV of all strains. These observations indicated the existence of a homologous sequence between all larger non-pSCVs. These results also suggested that the larger pSCVs contained the sequences of both the common 50-kb pSCV and the non-pSCVs described here. This means that the larger pSCVs were likely derived from a recombination between the 50-kb common pSCV and larger non-pSCVs, since large sections of both the 50-kb pSCV and a non-pSCV were involved.

Antibiotic susceptibility. As shown in Table 4, except for the laboratory strain OU7085 which showed resistance to only SUL, all clinical isolates were resistant to two or more antibiotics, including the pSCV-less strain (OU7533) isolated from feces. However, all isolates remained susceptible to recent cephalosporins (ceftazidime, CRO, and cefepime) and fluoroquinolones (ofloxacin and ciprofloxacin) tested (data not shown).

Virulence plasmids carrying β-lactamase and SUL resistance genes. There were eight pSCV-containing strains that were resistant to SUL (Table 4). Two genes, *sulI* and *sulII*, were known to control SUL resistance. The presence or absence of the two genes was therefore checked with PCR, and the amplified DNA fragments derived from *E. coli* were labeled and used for hybridization to locate the site of the genes. The results shown in Table 5 indicate that in the laboratory strain OU7085 and clinical isolate OU7527, the *sulII*, but not the *sulI* gene, is located on the 6-kb plasmid and not on the pSCV. The remaining six SUL-resistant strains (OU7518 [representing OU7516 and OU7517, whose plasmid profiles were identical], OU7520, OU7524, OU7526, OU7529, and OU7531), of which three were pSCVs, all contained *sulI* on the larger plasmid, and in addition, all six were SXT resistant (Table 4). The nucleotide sequences of the amplified DNA fragments of *sulI* and *sulII* were identical to those shown in GenBank (accession no. X15014 and X57730).

The above six SUL-resistant strains and a clinical isolate, OU7521, were resistant to AMX and AMP (MICs >256 μg/ml), and therefore, PCR was performed to detect and amplify the TEM-type β-lactamase (*bla*) gene. The results of PCR and DNA-DNA hybridization were in agreement and showed that the *bla* gene was of the TEM type and was located on the pSCV. The IEF point of the β-lactamase was 5.4 for all AMP-resistant isolates. Thus, all these observations, together with the determined sequence, indicated that the *bla* gene was

TABLE 4. Resistance of clinical serovar Choleraesuis isolates to 10 antibiotics[a]

Strain	Resistance to antibiotic[b]									
	AMX	AMP	GEN	CEF	CHL	ERY	TET	SXT[c]	SUL	TMP
OU7085									R	
OU7518	R	R	R		R	R	R	R	R	R
OU7519			R		R	R	R			
OU7520	R	R	R		R	R	R	R	R	R
OU7521	R	R	R		R	R	R			
OU7522			R		R	R	R			
OU7523			R		R	R				
OU7524	R	R	R		R	R	R	R	R	R
OU7525			R		R	R	R			
OU7526	R	R	R	R	R	R	R	R	R	R
OU7527			R			R			R	
OU7528			R		R	R				
OU7529	R	R	R		R	R	R	R	R	R
OU7531	R	R	R		R	R		R	R	R
OU7533			R		R	R	R	R	R	R

[a] Only resistance (R), assigned according to the criteria of NCCLS (15), is indicated.

[b] GEN, gentamicin; CEF, cephalothin; CHL, chloramphenicol; ERY, erythromycin; TET, tetracycline; TMP, trimethoprim. All strains were sensitive to CRO.

[c] Tested by the disk diffusion method.

TABLE 5. Detection of genes *sulI, sulII*, and *bla* by PCR and DNA-DNA hybridization[a]

Strain	*sulI*			*sulII*			*bla*		
	PCR detection	Plasmid size (kb)	Presence of pSCV	PCR detection	Plasmid size (kb)	Presence of pSCV	PCR detection	Plasmid size (kb)	Presence of pSCV
OU7085	−			+	6	No	−		
OU7518	+	136	Yes	−			+	136	Yes
OU7519	−			−			−		
OU7520	+	140	Yes	−			+	140	Yes
OU7521	−			−			+	90	No
OU7522	−			−			−		
OU7523	−			−			−		
OU7524	+	136	Yes	−			+	136	Yes
OU7525	−			−			−		
OU7526	+	90	No	−			+	90	No
OU7527	−			+	6	No	−		
OU7528	−			−			−		
OU7529	+	90	No	−			+	90	No
OU7531	+	90	No	−			+	90	No

[a] PCR results are the same as those for DNA-DNA hybridization. The results of PCR and DNA-DNA hybridization were consistently in agreement.

bla_{TEM-1}. The sequence showed that there were three point mutations in the amplified region, T→C at nucleotide (nt) 228, G→T at nt 396, and C→T at nt 602, in contrast to that of pBR322; otherwise, the amino acid sequence of the enzyme was unchanged. All three pSCVs with *sulI* carried bla_{TEM-1}, while all 90-kb non-pSCVs, three with the *sulI* gene, carried bla_{TEM-1}.

DISCUSSION

Sixteen serovar Choleraesuis clinical isolates, but not OU7533 isolated from feces, harbored a pSCV, the size of which was either 50 kb, the most common size, or more than 125 kb, and none of the plasmids with a size between 75 and 100 kb were pSCVs (Table 2). There are at least two mechanisms with which to form the larger pSCVs (>125 kb): genetic recombination, including cointegration, or transposition. Since all large pSCVs contained a large portion of both the 50-kb pSCV and a non-pSCV, it appeared that they were the products of a recombination (Table 3). The homology shown among the larger nonvirulence plasmids suggested that these plasmids might have evolved similarly from the same origin.

All 15 serovar Choleraesuis isolates tested, including laboratory strain OU7085, showed resistance to at least one antibiotic (Table 4), though all were sensitive to newer antibiotics. Fifty percent (7 of 14) of the isolates were AMP resistant and AMX resistant, and all seven were found to contain the TEM-1-type β-lactamase gene. This is in accordance with data indicating that the majority of the serovars, other than serovar Typhimurium, produce TEM-1-type β-lactamases (14). Direct sequencing of the bla_{TEM-1} PCR products yielded the result that the gene sequence shared nearly 100% identity with the TEM-1 gene carried by plasmid pBR322 (GenBank).

The resistance to SXT detected was rather high, appearing in 50% (7 of 14) of the isolates. On the other hand, of the 14, 7 were resistant to TMP, and 9 were resistant to SUL. Clinically occurring SUL resistance in gram-negative enteric bacteria is largely plasmid mediated and is due to the presence of alternative drug resistance variants of dihydropteroate synthases (18, 21). Two such plasmid-carried enzymes have been characterized, and these enzymes (encoded by *sulI* and *sulII*) show a high degree of amino acid identity (19). The *sulII* gene is usually found on small plasmids belonging to the IncQ family (RSF1010) and also on plasmids of another type represented by pBP1 (20). The *sulI* gene, on the other hand, is normally found linked to other resistance genes and located on the Tn*21* family (17). Consistent with the above reports (18, 19), the *sulII* gene was found on a 6-kb small plasmid in two strains of serovar Choleraesuis, and the *sulI* gene, which was linked to bla_{TEM-1}, was carried on the six large plasmids. Three (including the 136-kb plasmid of OU7518) of these six were pSCVs (Table 5). The 136-kb plasmids of OU7516 and OU7517, identical to that of OU7518, may also be such drug resistance pSCVs.

We have shown here (Tables 4 and 5) that some non-pSCVs and pSCVs are drug resistance factors and that the pSCVs of clinical isolates even carry multiple drug resistance genes. Of the pSCVs checked, the drug resistance genes were carried only by the larger pSCVs (Table 5), and no drug resistance genes were carried by the 50-kb ones. In view of the above observations on the possible formation of larger pSCVs and since only larger pSCVs contain drug resistance genes, it is likely that the larger pSCV acquired the drug resistance gene via recombination. There is a report that some serovar Typhimurium strains also carry the β-lactamase gene on a 90-kb non-pSTV (14). It is suggested that such resistance genes are carried by integrons, transposons able to jump to and from the chromosome, and/or acquired by transfer of an R factor (14). Such an acquisition mechanism seems to be different from ours, which, as mentioned, appears to be via recombination.

So far, no virulence plasmids of the other serovars are found to vary in size or contain a drug resistance locus. We have so far checked more than 200 strains of serovar Typhimurium, but unlike for serovar Choleraesuis none of the pSTVs showed deviation from the common size of 90 kb or had the presence of a drug resistance gene. Tens of clinical serovar Enteritidis isolates also showed the regular pSEV size of 60 kb, without deviation and without drug resistance. Serovar Choleraesuis is a highly virulent and invasive serovar that readily causes systemic infections without diarrhea in humans, and therefore, salmonellosis caused by serovar Choleraesuis generally re-

quires antimicrobial therapy. Resistance to antibiotics in serovar Choleraesuis, therefore, constitutes a problem in the choice of treatment for infections caused by this organism. Furthermore, as seen in Table 2, the clinical strains isolated from blood invariably harbored a pSCV, and thus, a pSCV may play an important role for a serovar Choleraesuis strain to cause bacteremia (7). The emergence of drug-resistant pSCVs would thus require a careful therapeutic approach to salmonellosis caused by serovar Choleraesuis.

What would be the advantage for a pSCV to be large as well as to carry drug resistance genes? The larger size may not have any advantage except that the process of its formation is likely the means for the virulence plasmid to acquire drug resistance, an advantage in an unfavorable drug environment.

ACKNOWLEDGMENTS

This work was supported in part by grants NSC87-2314-B-182-067, from the National Research Council; DOH87-HR-606, from the National Health Research Institute, Department of Health, Executive Yuan, Taiwan; and CMRP876 from Chang Gung Memorial Hospital, Taoyuan, Taiwan.

REFERENCES

1. **Aserkoff, B., and J. V. Bennett.** 1969. Effect of antibiotic therapy in acute salmonellosis on the fecal excretion of salmonellae. N. Engl. J. Med. **281:** 636–640.
2. **Chiu, C. H., and J. T. Ou.** 1996. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, *invA* and *spvC*, by an enrichment broth culture-multiplex PCR combination assay. J. Clin. Microbiol. **34:**2619–2622.
3. **Chiu, C. H., C. Chu, and J. T. Ou.** 2000. Lack of evidence of an association between the carriage of virulence plasmid and the bacteremia of *Salmonella typhimurium* in humans. Microbiol. Immunol. **44:**741–748.
4. **Chiu, C. H., T. Y. Lin, and J. T. Ou.** 2000. Age-related differences in nontyphoid *Salmonella* bacteremia in clinical presentation and outcome: association with specific serovars but not necessarily with the virulence plasmids. Clin. Infect. Dis. **30:**239–240.
5. **Chiu, C. H., T. Y. Lin, and J. T. Ou.** 1999. A clinical trial comparing oral azithromycin, cefixime and no antibiotics in the treatment of acute uncomplicated *Salmonella* enteritis in children. J. Paediatr. Child Health **35:**372–374.
6. **Chiu, C. H., T. Y. Lin, and J. T. Ou.** 1999. Predictors for extraintestinal infections of non-typhoidal *Salmonella* in patients without AIDS. Int. J. Clin. Pract. **53:**161–164.
7. **Chiu, C. H., T. Y. Lin, and J. T. Ou.** 1999. Prevalence of the virulence plasmids of nontyphoid *Salmonella* in the serovars isolated from humans and their association with bacteremia. Microbiol. Immunol. **43:**899–903.
8. **Chu, C., S. F. Hong, C. Tsai, W. S. Lin, T. P. Liu, and J. T. Ou.** 1999. Comparative physical and genetic maps of the virulence plasmids of *Salmonella enterica* serovars Typhimurium, Enteritidis, Choleraesuis, and Dublin. Infect. Immun. **67:**2611–2614.
9. **Cohen, J. I., J. A. Bartlett, and R. Corey.** 1987. Extra-intestinal manifestations of *Salmonella* infections. Medicine (Baltimore) **66:**349–388.
10. **Fierer, J., M. Krause, R. Tauxe, and D. Guiney.** 1992. *Salmonella typhimurium* bacteremia: association with the virulence plasmid. J. Infect. Dis. **166:**639–642.
11. **Gulig, P. A., H. Danbara, D. G. Guiney, A. J. Lax, F. Norel, and M. Rhen.** 1993. Molecular analysis of *spv* virulence genes of the Salmonella virulence plasmid. Mol. Microbiol. **6:**825–830.
12. **Kado, C., and S. T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
13. **Kawahara, K., Y. Haraguchi, M. Tsuchimoto, N. Terakado, and H. Danbara.** 1988. Evidence of correlation between 50-kilobase plasmid of *Salmonella choleraesuis* and its virulence. Microb. Pathog. **4:**155–163.
14. **Llanes, C., V. Kirchgesner, and P. Plesiat.** 1999. Propagation of TEM- and PSE-type β-lactamases among amoxicillin-resistant *Salmonella* spp. isolated in France. Antimicrob. Agents Chemother. **43:**2430–2436.
15. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial susceptibility testing, fifth international supplement. Document M100–S5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **Ou, J. T., L. S. Baron, X. Y. Dai, and C. A. Life.** 1990. The virulence plasmids of *Salmonella* serovars *typhimurium, choleraesuis, dublin*, and *enteritidis* and the cryptic plasmids of *Salmonella* serovars *copenhagen* and *sendai* belong to the same incompatibility group, but not those of *Salmonella* serovars *durban, gallinarum, give, infantis* and *pullorum*. Microb. Pathog. **8:**101–107.
17. **Sundstrom, L., P. Radstrom, G. Swedbery, and O. Skold.** 1988. Site-specific recombination promotes linkage between trimethoprim and sulfonamide resistance genes. Sequence characterization of *dhfrV* and *sulI* and a recombination active locus of *Tn*21. Mol. Gen. Genet. **213:**191–201.
18. **Swedberg, G., and O. Skold.** 1980. Characterization of different plasmid-borne dihydropteroate synthases mediating bacterial resistance to sulfonamides. J. Bacteriol. **142:**1–7.
19. **Swedberg, G., and O. Skold.** 1983. Plasmid-borne sulfonamide resistance determinants studied by restriction enzyme analysis. J. Bacteriol. **153:**1228–1237.
20. **Van Treeck, U. F., F. Schmidt, and B. Wiedemann.** 1981. Molecular nature of a streptomycin and sulfonamide resistance plasmid (pBP1) prevalent in clinical *Escherichia coli* strains and integration of an ampicillin resistance transposon (Tn*4*). Antimicrob. Agents Chemother. **19:**371–380.
21. **Wise, E. M., Jr., and M. M. Abou-Donia.** 1975. Sulfonamide resistance mechanism in *Escherichia coli*: R-plasmids can determine sulfonamide-resistant dihydropteroate synthases. Proc. Natl. Acad. Sci. USA **72:**2621–2625.