

Diversification in *Salmonella* Typhimurium DT104

To the Editor: Multidrug-resistant (MDR) *Salmonella* Typhimurium definitive phage type (DT) 104 with chromosomally encoded resistance to ampicillin, chloramphenicol, streptomycin/spectinomycin, sulfonamides, and tetracyclines (ACSSpSuT) was first identified and characterized in the United Kingdom in the early 1990s (1). MDR DT104 has subsequently caused numerous outbreaks throughout the world (1). The organism is characterized by a distinctive *Xba* I-generated pulsed-field profile (PFP), designated Xtm 1 (2), carriage of the 90-kb *S. Typhimurium* serovar-specific plasmid (SSP), and presence of the 43-kb *Salmonella* genomic island 1 (SGI 1), which is composed of integrons containing, respectively, the ASu (*bla*_{CARB-2} and *sul1*) and SSP (*aadA2*) genes, with intervening plasmid-derived genes coding for chloramphenicol/florfenicol (*cmlA*) and tetracycline resistance (*tetG*) (1,3,4). The same genetic characteristics have been observed in MDR strains of the closely related DTs 12 and 104b (5) and in some strains of phage type U302 (4). All isolates of MDR DT104 ACSSpSuT contain the same gene cassettes irrespective of source or country of origin. Although MDR DT104 has declined during the last 5 years, the organism remains the most common MDR *Salmonella* in the United Kingdom and many other European countries (6).

Since 1998, MDR DT104 has undergone changes in both resistance spectrum and genetic structure. In the United Kingdom, outbreaks of MDR DT104 have been caused by new subclones with additional resistance to trimethoprim (Tm) (R-type ACSSpSuTTm) (7), by clones with decreased susceptibility to ciprofloxacin (Cp_L) (R-type ACSSpSuTCp_L) (1), and by

strains of R-type SSpSu. In 2002, an outbreak of MDR DT104 ACSSpSuTTm with >200 cases was recognized (7). The outbreak strain was characterized by 3 plasmids of 6.8, 3.0, and 1.5 kb. The 6.8-kb plasmid coded for resistance to sulfonamides and trimethoprim, with trimethoprim resistance being mediated by *dhfr1b*. The outbreak strain lacked the *S. Typhimurium* SSP and was negative by polymerase chain reaction (PCR) for a 437-bp internal fragment of the *Salmonella* plasmid virulence (*spv*)C gene. The absence of the SSP was reflected in the PFP, which was identical to Xtm 1 but lacked a fragment of ≈90 kb that corresponds to the presence of the SSP (4). A strain of R-type ACSSpSuT that also lacked the SSP and with a PFP indistinguishable from that of the ACSSpSuTTm strain caused a simultaneous outbreak with >40 cases (7). This strain was also characterized by 2 plasmids of 3.0 and 1.5 kb but did not possess the 6.8-kb sulfonamide-trimethoprim resistance plasmid.

Decreased susceptibility to ciprofloxacin coupled with resistance to nalidixic acid (Nx) was first reported in MDR DT104 in 1996 in the United Kingdom (1). Four mutations in *gyrA*, each giving rise to resistance to Nx/Cp_L, were subsequently identified in MDR DT104. The most common mutation was aspartate (Asp)-87 to asparagine (AAC) and involved a change from aspartate (GAC) to AAC. An identical mutation was identified in a MDR DT104 strain responsible for an outbreak in Denmark in 1998 (8). In 1999, an Asp-87 to glycine mutation was identified in a Cp_L MDR DT104 strain responsible for a major outbreak in northwestern England (1). Subsequently, >500 sporadic MDR DT104 infections in the United Kingdom have involved strains with Cp_L.

In 2004, 2 simultaneous outbreaks of DT104 were recorded in the United Kingdom in which the strains were of

R-type SSpSu, 1 involving >100 cases and 1 involving >50 cases. Isolates were characterized by pulsed-field gel electrophoresis coupled with plasmid profile analysis. Resistance genes were identified by using PCR with primers specific for *bla*_{TEM} (A), *bla*_{CARB-2}, *cmlA*, *catI* (C), *catIII* (C), *aadA2*, *sul1*, and *tetG* (4). Testing for SGI1 was conducted by using primers U7-L12 and LJ-R1 for the left junction and primers 104-RJ and 104-D for the right junction (9). All isolates exhibited the Xtm 1 PFP, but strains from the 2 outbreaks could be differentiated by the presence or absence of an additional plasmid of ≈3 kb. By using PCR, all isolates possessed *aadA2* and *sul1* but were negative for *bla*_{TEM}, *bla*_{CARB-2}, *cmlA*, *catI*, *catIII*, and *tetG*. Similarly, all strains were positive for the left junction of SGI1. These results indicate that the SGI1 was in the same chromosomal location as for DT104 ACSSpSuT but was lacking the right junction.

These findings demonstrate that evolutionary changes have occurred involving both loss and acquisition of drug resistance genes, the development of chromosomal mutations conferring resistance to Nx/Cp_L, and in certain subclones, the loss of the SSP. None of these changes has altered the virulence of the organism, with bloodstream invasion in only 1.6% of 408 cases in the United Kingdom. These findings contrast with those in the United States, where MDR nontyphoidal *Salmonella* have been associated with excessive numbers of bloodstream infections (10).

All facets of the organism should be explored for epidemiologic investigations. These should include phage type, antibiogram, plasmid profile, and PFP, coupled with the identification of resistance genes and integrons, and, when appropriate, the characterization of mutations conferring resistance to quinolone antimicrobial agents.

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Extended-spectrum β -Lactamase-producing Flora in Healthy Persons

To the Editor: Extended-spectrum β -lactamase (ESBL)-producing gram-negative bacilli are endemic in hospitals. In intensive care units, 2% prevalence of ESBL-producing organisms has been reported (1). Exceedingly high rates of ESBL-producing bacteria in Indian hospitals prompted us to look at the fecal carriage of ESBL in the community (2).

One hundred healthy executives received a comprehensive health check at our tertiary care center in central Mumbai from August to September 2004. The predominant isolates from stool samples obtained for routine examination were cultured, and initial screening for ESBL production was conducted by using the disk diffusion method according to NCCLS guidelines (3). For these isolates, the ESBL phenotypic confirmation was performed with ceftazidime-clavulanate for an increase in zone diameter by 5 mm (disk potentiation). In addition, the ATB BLSE strip (bioMérieux, Lyon, France) was used to confirm the presence of inhibitor (sulbactam)-suscep-

tible enzymes and to differentiate the strains from those that were either inhibitor resistant or harboring other β -lactamases, such as those of AmpC derivation. The ATB BLSE strip consists of a varying concentration of ceftazidime, 0.5–32 mg/L, and aztreonam, 0.5–8 mg/L, with varying combinations of these agents with a β -lactamase inhibitor, i.e., + sulbactam, 0.06–1 mg/L. Cefotetan (4 and 32 mg/L) and imipenem (4 and 8 mg/L) were also included in the strip. The test was considered positive when a variation of ≥ 4 dilutions was observed between the antimicrobial agent tested alone and the agent combined with the inhibitor. Eleven of the 100 samples screened were positive for ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*. Seven of the 11 were confirmed by using the ATB BLSE strip. The MIC of ceftazidime and aztreonam in all 7 isolates was 8 μ g/mL. We might be underreporting ESBL producers in these cases by not including the cefotaxime-clavulanate combination in addition to the ceftazidime-clavulanate concentration. The percentage resistance to ciprofloxacin was 45%. All isolates were susceptible to amikacin and the carbapenems. None of the executives gave a history of hospitalization in the last year or history of antimicrobial drug consumption in the last 6 months.

This trend in patients with no apparent risk factors for ESBL carriage calls for urgent attention. Unknown environmental factors are likely playing a key role in maintaining this selective pressure. Larger studies are required to substantiate these findings.

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