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# SHORT REPORT

# An outbreak of human salmonellosis caused by ampicillin-resistant *Salmonella enterica* serovar Enteritidis PT13 in the Czech Republic

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## SUMMARY

In summer 2004, an outbreak caused by *Salmonella enterica* serovar Enteritidis phage type 13 (*S*. Enteritidis PT13) was recorded in the Czech Republic. As well being a relatively rare phage type the strain was also ampicillin resistant. Outbreak ($n=39$) and pre-outbreak isolates ($n=13$) were characterized by pulsed-field gel electrophoresis (PFGE), $\beta$-lactamase gene polymerase chain reaction and plasmid profile. The majority of outbreak isolates ($n=37$) were identical in *Xba*I PFGE profile, and two other outbreak isolates each differed from this profile by one or two fragments respectively. The pre-outbreak isolates were uniform in PFGE profile but distinct from the outbreak strain. Ampicillin resistance was confirmed to be encoded by the $bla_{\mathrm{TEM}}$ gene located on the Tn*A* transposon. This gene was readily transferable to a *S*. Enteritidis recipient strain and was associated with the transfer of a 200-kb plasmid. Our results indicate that all *S*. Enteritidis PT13 tested from 2004 belonged to a single outbreak strain which prior to 2004 had not been recognized in the Czech Republic.

Infections due to *Salmonella* spp. represent a major public health problem in many countries. In the Czech Republic, the most common serovar responsible for approximately 96% of human *Salmonella* infections is *S. enterica* serovar Enteritidis followed by serovar Typhimurium (2%) and the remaining cases are caused by other serovars. This distribution of serovars has remained essentially unchanged since the beginning of 1990s when *S*. Enteritidis became predominant.

Phage typing has proved to be a rapid and simple epidemiological typing tool for intra-specific discrimination of *S*. Typhimurium and *S*. Enteritidis isolates. In *S*. Enteritidis, strains of phage type 8 (PT8) are predominant in the Czech Republic, strains of PT4 which are frequent in western Europe [1, 2] are also found in this country [3]. Since 1997, 25 different phage types of *S*. Enteritidis have been identified with the following distribution: PT8 (71%), PT4 (9%), PT1 (7%), PT13 (6%), PT6 (4%) (R. Karpiskova, unpublished observations). Data from the United States and Canada also support a similar predominance of PT8 and a higher frequency of PT13 in comparison with Europe [4, 5]. Antimicrobial resistance is relatively uncommon in *S*. Enteritidis in contrast to *S*. Typhimurium. Nevertheless, cases of drug resistance, mostly to ampicillin as well as multidrug-resistance have been described in this serovar [6–8].

From the end of May to the beginning of December 2004, a new strain of *S*. Enteritidis PT13 which was resistant to ampicillin was detected in human salmonellosis outbreaks in the Czech Republic. Later on, as a result of standard surveillance monitoring of *Salmonella* spp., the same *S*. Enteritidis PT13 strain was isolated on a poultry farm. The aim of this study

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was, therefore, to confirm the clonality of the human outbreak strains of PT13 by molecular analysis and to establish their relationship to pre-outbreak isolates.

A total of 52 *S.* Enteritidis strains were examined. Of these, 38 isolates originated from a possible outbreak from human patients in 2004, one isolate was from a poultry farm and the remaining 13 human strains pre-dated the outbreak and were isolated between 1998 and 2003.

All isolates were phage typed using the *S.* Enteritidis phage-typing scheme [9]. Antimicrobial susceptibility testing was performed by the disc diffusion method according to the standards of the National Committee for Clinical Laboratory Standards [10]. The antibiotic discs tested (Oxoid, Basingstoke, UK) were: ampicillin (AMP, 10 $\mu$g), amoxicillin–clavulanic acid (AMC, 30 $\mu$g), cefotaxime (CTX, 30 $\mu$g), chloramphenicol (C, 30 $\mu$g), streptomycin (S, 10 $\mu$g), kanamycin (K, 30 $\mu$g), gentamicin (CN, 10 $\mu$g), neomycin (N, 30 $\mu$g), apramycin (APR, 15 $\mu$g), sulphonamide (Su, 300 $\mu$g), sulfamethoxazole–trimethoprim (SXT, 25 $\mu$g), trimethoprim (W, 5 $\mu$g), tetracycline (TE, 30 $\mu$g), nalidixic acid (NA, 30 $\mu$g), ciprofloxacin (CIP, 5 $\mu$g), enrofloxacin (ENR, 5 $\mu$g) and colistin (CT, 10 $\mu$g).

DNA purification for macrorestriction analysis, restriction enzyme digestion and pulsed field gel electrophoresis (PFGE) was performed essentially as previously described [11] using the CHEF-DRIII system (Bio-Rad, Hercules, CA, USA). For the plasmid profile analysis, PFGE was also used and non-digested agarose-embedded DNA was directly separated on 1 % agarose gels for 18 h at 5·5 V/cm with switching times of 2–17 s. Following electrophoresis gels were stained with ethidium bromide and DNA bands were visualized under UV light. Lambda concatemers and *Xba*I-digested DNA of *S.* Braenderup H9812 were used as molecular-weight standards [11].

All isolates were tested for the presence of the genes encoding ampicillin resistance. To obtain DNA for polymerase chain reaction (PCR), a loop of bacterial culture was re-suspended in 50 $\mu$l water and boiled for 20 min. The suspension was spun for 1 min and 2 $\mu$l of the supernatant was used as a template DNA in PCR which was carried out in 20-$\mu$l volumes using 10 pmol of each primer and PCR Master Mix (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The primer sequences were TAG CCA TAT TAT GGA GCC TC and TTA ACT TTT CCT TGC TCA GC for detection of the $bla_{\mathrm{PSE1}}$ gene, and GCA CGA GTG GGT TAC ATC G and GGT CCT CCG ATC GTT GTC AG for detection of the $bla_{\mathrm{TEM}}$ gene. PCR cycling consisted of 30 cycles of 40 s at 95 °C, 45 s at 55 °C and 1 min at 72 °C. Under the same PCR conditions, the isolates were also analysed for the presence of *tnpA* transposase of Tn*3* using primers ATC GGA CAC AAA GTT GTA CC and GGA CAA AAG CGA ACT ATC TG. The amplification products were separated by electrophoresis in 1·5% agarose gel, stained with ethidium bromide and visualized under UV light.

Conjugation experiments were performed with five representative strains of *S.* Enteritidis PT13. *S.* Enteritidis 147 $\Delta$*phoP* and *S.* Typhimurium F98 $\Delta$*phoP* strains, both spontaneously resistant to nalidixic acid, were used as recipients. A *phoP* deletion was used as an ultimate marker of the recipient strain and the transconjugants. Ten-microlitre volumes of fresh overnight donor and recipient cultures were mixed and used for inoculation of 4 ml LB broth. After 18 h incubation at either 24 °C or 37 °C, the bacterial cultures were plated on LB agar supplemented with ampicillin and nalidixic acid. *S.* Typhimurium 109/9 strain in which the $bla_{\mathrm{TEM}}$ gene was localized on 210-kb conjugative plasmid (H. Hradecka and I. Rychlik, unpublished observations) was used as a control. The transconjugants were then tested by PCR for the presence of $bla_{\mathrm{TEM}}$, *tnpA* and *phoP* gene deletion. Primers used for detection of the *phoP* gene and its deleted form were TAT TGA TAG TCTGGC CCT GC and TCC AGA TTT TCA GGCAGC TC.

PFGE of *Xba*I DNA digests of all *S.* Enteritidis isolates revealed four different group patterns. The first group consisted of 36 human isolates from the 2004 outbreak and one poultry isolate. Two additional outbreak strains, 2214 and 2215, each differed from the outbreak strain pattern by one or two bands of ~290 kb, or 175 and 60 kb respectively (Fig. 1). The last group was formed by all *S.* Enteritidis PT13 strains isolated randomly before May 2004. These isolates were of a unique *Xba*I macrorestriction profile (Fig. 1).

Although the majority of *S.* Enteritidis strains in the Czech Republic remain susceptible to antibiotics, the outbreak isolates were all resistant to ampicillin. These isolates also contained the $bla_{\mathrm{TEM}}$ gene but were negative for $bla_{\mathrm{PSE1}}$. The $bla_{\mathrm{TEM}}$ gene occurs frequently in various *Salmonella* serovars, including *S.* Enteritidis [6, 7]. The genes coding for TEM-type $\beta$-lactamases are also known to be carried by transposons transferring resistance to ampicillin (Tn*A*), such as Tn*3*. Tn*A* transposons are characterized by

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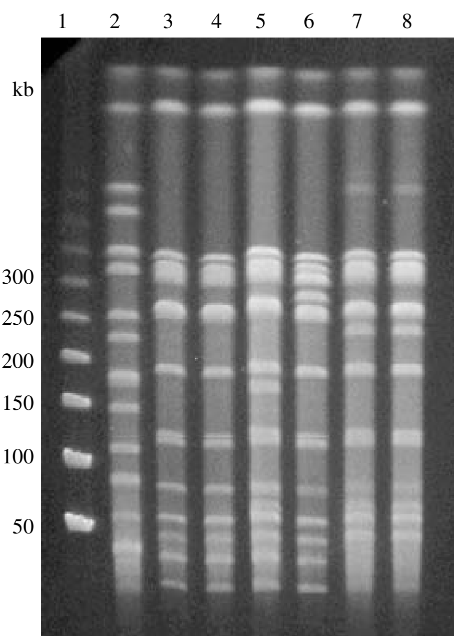


**Fig. 1.** Macrorestriction analysis of *S.* Enteritidis PT13 outbreak strains and strains of the same phage type isolated in the Czech Republic prior to the outbreak. Lane 1, lambda concatemers, 50 kb ladder; lane 2, reference strain *S.* Braenderup H9812; lanes 3 and 4, ampicillin-resistant *S.* Enteritidis PT13 outbreak isolates; lanes 5 and 6, ampicillin-resistant *S.* Enteritidis PT13 outbreak isolates displaying one or two band differences from the outbreak profile; lanes 7 and 8, *S.* Enteritidis PT13 antibiotic sensitive pre-outbreak isolates.

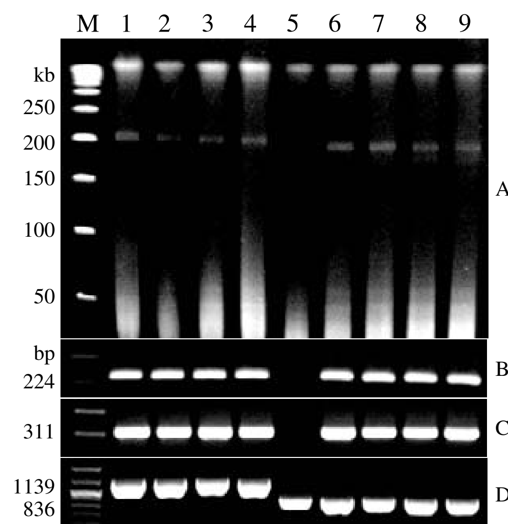


**Fig. 2.** Plasmid profile of ampicillin-resistant *S.* Enteritidis PT13, *S.* Enteritidis 147 $\Delta$*phoP* and resulting transconjugants (panel A). PCR verification of the donor and recipient strains – *tnpA*-specific PCR (panel B); $bla_{TEM}$-specific PCR (panel C); *phoP*-specific PCR (panel D). Lane M in panel A, lambda concatemers; panels B–D, 100-bp ladder. Lanes 1–4, *S.* Enteritidis PT13 donor strains; lane 5, recipient *S.* Enteritidis 147 $\Delta$*phoP*; lanes 6–9, resulting *S.* Enteritidis 147 $\Delta$*phoP* transconjugants.

the *tnpA* gene coding for transposase, the *tnpR* gene encoding the transposase repressor, and short inverted repeat sequences located at both ends as typically represented in the transposon Tn*3*. The presence of *tnpA* was confirmed by PCR which indicates that Tn*1*, Tn*3*, Tn*2601* or Tn*2602* may be present in the recent *S.* Enteritidis PT13 since all of these transposons share considerable homologies and belong to the Tn*A* class of transposons [12].

TEM-type $\beta$-lactamases have been repeatedly reported to be located on high-molecular-weight plasmids in the Enterobacteriaceae [13–15]. We, therefore, sought such plasmids in ampicillin-resistant *S.* Enteritidis PT13 isolates. PFGE of non digested DNA from all the human outbreak and poultry isolates revealed a single high-molecular-weight plasmid of $\sim$200 kb (Fig. 2) which was absent from the strains isolated before May 2004. To determine whether this plasmid encodes the $bla_{TEM}$ and *tnpA* genes and to test whether these genes could be transferred by conjugation simple bacterial mating experiments were performed. When *S.* Typhimurium was used as a recipient strain, no conjugation was observed. This may reflect the presence of different restriction modification systems in this serovar. However, conjugative transfer of the plasmid to *S.* Enteritidis 147 $\Delta$*phoP* was achieved at 37 °C but not at 24 °C. The *phoP* deletion mutants were used to exclude a selection of the donor strain spontaneously resistant to nalidixic acid. The resulting *S.* Enteritidis 147 $\Delta$*phoP* transconjugant was tested by PCR for the simultaneous transfer of $bla_{TEM}$ and *tnpA* genes (Fig. 2). Since both of these genes were present in the transconjugant, it is highly probable that the ampicillin resistance in the original *S.* Enteritidis PT13 strain is encoded by the Tn*A* transposon localized on the conjugative plasmid. This result is in keeping with other studies on *S.* Enteritidis linking the presence of high-molecular-weight plasmids with antibiotic resistance [9, 10]. However, because antibiotic resistance is not widespread in *S.* Enteritidis and most of the strains remain susceptible, these plasmids may not be natural to *S.* Enteritidis and may have been obtained quite recently by conjugative transfer.

In conclusion we have shown that a new strain of *S.* Enteritidis PT13 has emerged in the Czech Republic. This clone can be found both in humans and poultry and is characterized by the same phage

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type and resistance to ampicillin localized on a 200-kb conjugative plasmid.

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## DECLARATION OF INTEREST

None.

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