The emergence of nalidixic acid resistant, multiresistant S. Typhimurium DT104 in Denmark. An outbreak in humans traced back to pork.

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Abstract
Infection with the zoonotic Salmonella enterica serovar Typhimurium DT104 has been recognised since the beginning of the 90's as a health problem in several industrialised countries. The present investigation demonstrates that results of surveillance of Salmonella infection in food animals, food production and among humans enabled identification of an outbreak of human salmonellosis caused by a nalidixic acid resistant strain of S. Typhimurium DT104. The source of infection was traced back to a single slaughterhouse and two pig herds.

By combination of epidemiological and microbiological investigations the spread of the infection was documented. Furthermore, results from the investigation demonstrated treatment failures, probably related to reduced susceptibility to quinolones.

Introduction
During the last 10 to 20 years the incidence of foodborne zoonotic salmonella infection has increased in most industrialised countries. In the 80's a worldwide endemic of Salmonella Enteritidis was established mostly as a result of salmonella infection in layer hens (1), but in the 90's spread of multiresistant S. Typhimurium definitive phase type (DT) 104 has emerged and caused concern worldwide (2, 3).

To address the emerging Salmonella problem various control programmes have been adapted in various countries. In Denmark, surveillance and control programmes have been established through the whole production chain "from stable to table" including control of Salmonella in food animal herds, during food processing as well as surveillance at the whole sale and retail level (4). Data from these programmes, together with data of surveillance among humans, are collected and analysed at the Danish Zoonosis Centre. This centre is a network between all major actors in the field of control with foodborne zoonotic infections, including the Danish Veterinary Laboratory, the Food and Veterinary Administration and Statens Serum Institut.

The Danish surveillance programmes provides information on the prevalence and type of distributions of Salmonella enterica at different levels of the food production chain, which allow identification of the major sources of human salmonellosis. The present investigation demonstrates the opportunity to follow the spread of a specific pathogen through the food chain. Furthermore, the results from the investigation connected failures of treatment of human disease with the spread of a strain of S. Typhimurium with reduced susceptibility to quinolones.

Materials and Methods
Surveillance
Human salmonellosis is diagnosed at eight clinical microbiology laboratories and at Statens Serum Institut, which also serves as a national reference laboratory. Antibiotic susceptibility testing of all strains of S. Typhimurium as well as phage typing is performed. Statens Serum Institute conducts telephone interviews with patients prospectively infected with S. Typhimurium resistant to amoxicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (penta-resistance).

Surveillance of food animals and food of animal origin includes testing of every commercial flock of layers and broilers and every pig herd producing more than 100 pigs for slaughter per year. The surveillance is performed by a combination of serological and microbiological methods. Cattle herds are only tested on clinical indication. All national slaughterhouses take part in Salmonella monitoring. Every flock of broilers is tested before and after slaughter, and furthermore, approximately 30,000 food product samples of pork and 3,000 end products of beef are tested yearly. From retail outlets approximately 12-15,000 are tested per year. All Salmonella suspect isolates (approximately 20,000 in 1998) are forwarded to the Danish Veterinary Laboratory for serotyping. Antibiotic susceptibility and phage typing of a selected part of S. Typhimurium isolates is performed. Molecular typing is carried out in situations where further characterisation is needed.
Outbreak investigation

Isolates of S. Typhimurium DT104 with pentaresistance plus nalidixic acid resistance were identified as potential outbreak related. These isolates were tested for susceptibility to fluoroquinolones (ciprofloxacin/loxofloxacin) by either MIC-testing (Sensititre, Trek Diagnostic, UK) or E-test (Biodisk AB, Solna, Sweden) and furthermore characterised by pulsed field gel electrophoresis (PFGE) by use of the restriction enzymes Xba1 and Bsi1. The genetic background for the nalidixic acid resistance was analysed by amplification and sequencing of a 342 base-pair fragment of the gyrA gene using the primers P1 (5'-TACCGTCATAGTTATCCACGA) and P2 (5'-GTACTTTAGCCACATGACGT). Nalidixic acid resistant DT104 isolates from food originating from Germany and United Kingdom and from a Danish swine herd not epidemiologically related to the outbreak were included as controls.

Results

The outbreak was identified on June 18, 1998, when an unusual resistance pattern appeared in S. Typhimurium isolates from five patients (Figure 1). At the same day, it appeared that a sample of pork collected May 26 at a slaughterhouse was positive for S. Typhimurium with the same resistance pattern as the human strains. During the following days, the strains of S. Typhimurium were isolated from two more samples of pork meat samples collected by routine inspection sampling, and in both cases, the wholesalers had received deliveries of pork from the slaughterhouse. The S. Typhimurium strains with pentaresistance plus nalidixic acid resistance was an uncommon pattern in Denmark. Because of the unusual resistance pattern, a working hypothesis was established, that the occurrence of human disease was associated with the findings of multiresistant S. Typhimurium in pork originating from a single slaughterhouse.

Investigation at the slaughterhouse in question identified herds which had delivered pigs to the slaughterhouse the days before the contaminated sample was collected, and these herds, in total 37, were bacteriologically investigated by examination of pen samples. A finishing herd was found to have infected with the outbreak strains, and by further investigation of the trade contact to the infected herd, another infected herd was identified. All in all, nearly 90 herds were examined in connection with the trace-back of the present outbreak, and only these two herds were tested positive. In addition, the outbreak strain was not identified during any of the control programmes, which was performed routinely during the time of the outbreak investigation.

The epidemiological characterisation of the outbreak strains from pig herds, slaughterhouse, pork meat and patients showed that all strains were S. Typhimurium DT104 with identical resistance pattern. The MIC's of the isolates varied between 0.064 and 0.124 mg/L for ciprofloxacin, a decrease in susceptibility of a factor 10 compared with nalidixic acid susceptible isolates. The majority of strains
were inseparable by PFGE, as only two isolates differed by a single band in either the \textit{XbaI} or the \textit{BlnI}-profile. Sequence analysis of the \textit{gyrA} gene identified a base substitution at codon aspartate-87 in all outbreak related strains. This substitution (GAC @ AAC) caused an amino acid change from aspartic acid to asparagine. The strains not related to the outbreak, originating from food from Germany and United Kingdom and from a Danish swine herd, showed base-pair substitution at codon 83 from TCC (serine) to either TCC (phenylalanine) or TAC (tyrosine).

**Discussion**

Infection with the zoonotic \textit{Salmonella enterica} serovar Typhimurium DT104 has been recognised since the beginning of the 90's as a health problem in several industrialised countries (2, 3). \textit{S. Typhimurium} DT104 has a broad host spectrum giving a large reservoir, which makes the control of the infection difficult in the food animal production.Basically, most strains are resistant to five antibiotics (ampicillin, chloramphenicol, streptomycin, sulphonamide, tetracycline), but the organism can also be resistant to other antibiotics including quinolones. As a fluoroquinolone is the drug of first choice for treating extra-intestinal and serious intestinal complications of human salmonellosis, this has a potential of causing therapeutic problems.

The present investigation describes the first major outbreak of human salmonellosis in Denmark caused by a nalidixic acid resistant strain of multiresistant \textit{S. Typhimurium} DT104. In total, the outbreak included 25 culture-confirmed cases, among whom two died due to the infection. All epidemiological and microbiological evidence from the outbreak investigation suggested that the source of the outbreak was pork meat from the identified slaughterhouse, and the source was furthermore traced back to two pig herds supplying slaughter pigs to the suspected slaughterhouse.

Multiresistant \textit{S. Typhimurium} DT104 has shown to be closely related (5), and only limited possibilities of differentiation within this phage type occurs. The epidemic strain identified in the present outbreak had a PFGE profile inseparable from the most frequently identified type in a study of more than 50 unrelated strains from five different countries (5). Sequence analysis of the \textit{gyrA} gene enables differentiation of the outbreak strains from unrelated control isolates. The observed base pair substitution in codon 87 were not observed in any of the control isolates which confirm the relationship between outbreak related isolates.

Telephone interviews with patients made several clinically and epidemiological observations clear (6) including the fact that fluoroquinolone treatment was reported to lack clinical effect in at least four cases. It was not possible to identify how the strain was introduced into the herds and thereby to the food producing chain, neither to document excessive use of fluoroquinolones at herd level. The increased level of quinolone-resistance in \textit{Salmonella} is, however, related to the use of fluoroquinolones, and therefore these drugs should not be used in food animals, unless all other options for antibacterial therapy have been ruled out.

**References**


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