Introduction

Salmonella spp. are recognized as some of the most common pathogens causing enteritis worldwide. Diarrhoea is an important problem in developing countries and is normally related to unhealthy sanitary conditions. Furthermore, the development of antibiotic resistance in enteropathogens, including Salmonella spp., has increased the problem. Resistance to some β-lactam antibiotics, tetracyclines, chloramphenicol, or trimethoprim is reported with increasing frequency. Quinolones are an alternative therapy. However, the development of quinolone resistance in Salmonella spp. during therapy of patients with enterocolitis has been reported. Isolation of drug-resistant micro-organisms from food is an increasing public health problem. Being easily spread among the population, they can cause an epidemic outbreak, especially if sanitary conditions are not optimum.

This report concerns the various mechanisms of antibiotic resistance of a multiresistant clone of Salmonella typhimurium isolated from fish.

Materials and methods

Bacterial strains

Five strains of S. typhimurium were isolated from finfish in Coimbatore, India.

Determination of MICs

Susceptibility to antimicrobial agents was assessed by the Etest method (AB Biodisk, Solna, Sweden).

Mechanisms of antibiotic resistance and epidemiological relationships were investigated for five multiresistant strains of Salmonella typhimurium isolated from fish in India. Four strains showed resistance to nalidixic acid, chloramphenicol, tetracycline, co-trimoxazole, gentamicin and β-lactam antibiotics. The remaining strain was susceptible to all β-lactam antibiotics tested and to co-trimoxazole but resistant to the other antibiotics tested. Epidemiological analysis performed by REP-PCR showed that the five isolates belonged to the same clone. Resistance to nalidixic acid was related to a single mutation in the gyrA gene. Chloramphenicol resistance was related to the production of chloramphenicol acetyl-transferase. An OXA-1 β-lactamase, located in an integron, was responsible for resistance to ampicillin. These results indicate the health hazard posed by the fact that S. typhimurium may acquire or develop several mechanisms of resistance to a variety of antibiotics, including quinolones, and can thus cause disease in humans which may be difficult to treat.
Epidemiological analysis

Epidemiological relationships among the five strains were studied by plasmid analysis and REP-PCR. Plasmids were isolated using Wizard Plus Maxipreps (Promega, Madison, WI, USA), following the procedure described by the manufacturers. Recovered products were resolved by electrophoresis in 0.7% agarose gels. REP-PCR was performed as previously described.⁴

Detection of mutations in the gyrA and parC genes

Amplification and sequencing of the fragments of gyrA and parC genes containing the respective quinolone-resistance-determining regions were performed as previously described.⁵

Detection of chloramphenicol acetyl-transferase activity

The presence of chloramphenicol acetyl-transferase activity was determined using the spectrophotometric method described by Azemun et al.⁶

Detection of β-lactamases

Extraction of β-lactamases was performed by ultrasonication of exponentially growing cultures. Their presence was determined by isoelectrofocusing (IEF) gel electrophoresis in a PhastSystem (Pharmacia AB, Uppsala, Sweden) and detection with nitrocefin. TEM-1, PSE-2, OXA-2 and OXA-3 were used as controls of pI.

Detection of the β-lactamase gene in an integron

The presence of the β-lactamase gene in an integron was investigated by two different PCRs. The first was performed with the upper primer for blaOXA,² and the lower primer for the integron described by Levesque & Roy.⁷ The second was performed with the lower primer for blaOXA,² and the upper primer for the integron.⁷ Both PCR amplifications were performed following the procedure described previously.⁵ The PCR products were then sequenced.

Results

The epidemiological relationships of the five S. typhimurium strains were established by plasmid profiles and REP-PCR. Three of the strains (S-6, S-9 and S-10) had a plasmid of 3.0 kb that was not present in the other two. REP-PCR results showed that all strains belonged to the same clone (Figure).

The resistance pattern of these strains is shown in the Table. All the strains were resistant to nalidixic acid, tobramycin, gentamicin, chloramphenicol, tetracycline and nitrofurantoin. Only the S-9 strain was susceptible to all the β-lactam antibiotics tested and to co-trimoxazole.

In order to explain the reduced susceptibility to quinolones, a 343 bp fragment of the gyrA gene was amplified and sequenced, showing that all the strains presented an Asp-87→Gly substitution. A 291 bp fragment of the parC gene was amplified and sequenced, but no mutations were found.

The IEF showed the presence of a β-lactamase with a pI of c. 7.3 in the four isolates resistant to β-lactam antibiotics. PCR results showed the presence of a blaOXA gene located in an integron element with a molecular weight of c. 1900 bp. The integron was sequenced, showing the presence of a blaOXA, and an aadA gene.

Resistance to chloramphenicol in the five isolates was associated with the presence of chloramphenicol acetyltransferase activity.

Discussion

Five multiresistant strains of S. typhimurium isolated from food were analysed. Plasmid profile analysis showed that three of the strains contained a plasmid of 3.0 kb (S-6, S-9 and S-10), whereas in the other two strains (S-7 and S-8) no plasmid was detected. However, the gain or loss of plasmids is well established, therefore the analysis of the
plasmid profile is not a definitive typing method. Epidemiological studies performed by REP-PCR showed that these five strains were the same clone. A similar power of discrimination was observed between REP-PCR and low-frequency restriction analysis of chromosomal DNA and pulsed-field gel electrophoresis.²

In previous studies on the molecular basis of quinolone resistance in S. typhimurium the presence of mutations in the gyrA gene has been associated with the development of quinolone resistance.³⁵⁸ These mutations are located in the positions equivalent to the Ser-83 and/or Asp-87 of Escherichia coli. Sequencing studies showed a mutation in position 87, resulting in a substitution of Asp by Gly. This mutation has been previously described in Indian clinical isolates of S. typhimurium.⁹ No mutations were observed in the parC gene of our strains.

Chloramphenicol resistance was associated in all the strains with the presence of chloramphenicol acetyl-transferase activity, as previously reported in S. typhimurium.² In Gram-negative bacteria, resistance to β-lactam antibiotics is mainly associated with the production of β-lactamases. In our study this resistance was explained by the presence of a β-lactamase of pI c. 7.3, identified by PCR as an OXA-1 type β-lactamase. The susceptibility of the S-9 strain, which could be considered the parenteral strain, to co-trimoxazole and β-lactam antibiotics suggests the possibility that the genes encoding enzymes responsible for these resistances are included in mobile genetic elements, such as integrons or transposons. Genes codifying both OXA-type β-lactamases and trimethoprim resistance have been described in these elements.⁷ In our study the ampicillin-resistant S. typhimurium strains had an integron containing a blaOXA-1 and an aadA gene. An integron containing a blaOXA-1 and an aadA gene has been described by Colonna et al.⁹ in Salmonella wien, suggesting that this could be a frequent mechanism of resistance transference in these micro-organisms.

Our results indicate the potential human health hazard of multiresistant S. typhimurium isolated from food and corroborate the increasing levels of antibiotic resistance in this micro-organism.

### Acknowledgements

This work was supported in part by grant SAF97/0091. We thank Servicios Científico Técnicos of the University of Barcelona for their help in DNA sequencing.
References


Received 15 July 1998; returned 15 September 1998; revised 24 November 1998; accepted 5 January 1999