Evidence for chromosomal and plasmid location of CMY-2 cephalosporinase gene in Salmonella serotype Typhimurium

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Sir,

Resistance to extended-spectrum cephalosporins has emerged among non-typhoid salmonellae (NTS) worldwide due to acquisition of plasmid-mediated β-lactamases including CMY-2.1 The isolation frequency of CMY-2-producing NTS in the USA has gradually increased since 1996 reflecting the widespread transmission of CMY-2-positive NTS among food animal reservoirs. The blaCMY-2 gene has been disseminated by plasmids to various Salmonella serotypes including Typhimurium.2–4 We report here on a human isolate of Salmonella serotype Typhimurium harbouring a CMY-2-encoding plasmid as well as a chromosomally located copy of blaCMY-2.

Salmonella serovar Typhimurium AM19083 was submitted to the National Antimicrobial Resistance Monitoring System in 2003 from Massachusetts. Antimicrobial susceptibility was assessed by broth microdilution (Sensititre, Westlake, OH, USA). The isolate met the phenotypic criteria indicative of class C cephalosporinase production (i.e. resistance or decreased susceptibility to cefoxitin, cefotax and ceftriaxone). It was also resistant to ampicillin, amoxicillin/clavulanate and cefalotin, but susceptible to non-β-lactam drugs including streptomycin, sulphonamides, chloramphenicol, tetracycline, nalidixic acid and ciprofloxacin.

AM19083 was positive in PCR assays with blaCMY-specific primers,3 and the sequence of the amplicon (369 bp) was homologous to an internal fragment of blaCMY-2 (from nt 271 to nt 639, in GenBank accession no. X91840). Isoelectric focusing of sonicated cell extracts in polyacrylamide gels containing ampholines showed production of a β-lactamase species with an isoelectric point of >8.4 corresponding to CMY-2.

β-Lactam resistance was readily transferred by conjugation in broth cultures to a susceptible Escherichia coli host. The plasmid content analysis indicated transfer of an ~90 kb plasmid (designated p19083) that was positive in hybridization with a blaCMY-2 probe prepared as described previously.5 Plasmid p19083 belonged to the incompatibility Group II (IncI1), as found by a PCR-based IncI/rep typing method.6 Furthermore, the

PsI restriction profile of p19083 resembled restriction type B of the IncI1, CMY-2-encoding plasmids previously described in Salmonella Typhimurium and Salmonella Thompson isolates from the USA.5,6

A plasmid DNA preparation from AM19083 was electrophoresed through agarose gels (0.8%). DNA was then transferred to Hybond-N nylon membranes (Amer sham Biosciences, Buckingham, UK) and hybridized with a digoxigenin-labelled blaCMY-2 probe.5 These experiments showed not only the expected hybridization with plasmid p19083, but also a strong signal with the chromosomal material. This was not observed with preparations from two control strains of serotype Typhimurium, one of which (AM18447) also carried two CMY-2-encoding plasmids, p18447-S and p18447-L (~140 and 300 kb, respectively). To clarify this issue, an I-CeuI genomic analysis of isolates AM19083 and AM18447 was performed. The digested fragments were separated by PFGE (6.0 V/cm for 8 h/16 h with a pulsing time linearly ramped from 5 to 20 s/20 to 70 s) (Figure 1a). The blaCMY-2 probe was hybridized with a 16SrRNA-positive I-CeuI fragment of ~500 kb from isolate AM19083. Positive signals probably corresponding to the blaCMY-2-carrying plasmids were also seen (Figure 1b). Subsequent hybridization with IncI/rep probes6 showed that p19083 and p18447-S belonged to IncI1, whereas the blaCMY-2-carrying putative plasmid p18447-L and the 500 kb fragment from AM 19083 were not reactive (Figure 1c). In an attempt to rule out the existence of blaCMY-2-positive plasmids of high molecular weight in AM19083 and, also, to corroborate the plasmidic nature of p18447-L, the plasmid contents of AM19083 and AM18447 were analysed by PFGE after treatment of total DNA with S1 nuclease. The respective electrophoretic profiles indicated the absence of blaCMY-2-carrying ‘megaplasmids’ in AM19083, whereas a band of ~300 kb likely representing the linear form of p18447-L was observed in the preparations from AM18447 (data not shown).

Finally, to further strengthen our hypothesis for the chromosomal acquisition of blaCMY-2 in AM19083, the relevant I-CeuI fragment was purified by electroelution. The resulting preparation contained blaCMY-2 as confirmed by PCR and sequencing. It was also negative in PCR assays designed for replicon typing of plasmids including IncI1 and IncA/C. Nevertheless, the possibility that plasmid segments including rep could have been removed during I-CeuI treatment cannot be excluded.

Production of CMY-2 has been described in a wide variety of NTS serotypes in the USA. The plasmidic location of blaCMY-2 is critical for spread of this resistant determinant. The present findings confirm the prominent role of IncI1 plasmids in this process. Additionally, the likely chromosomal carriage of blaCMY-2 underscores the operation of efficient mobilization mechanisms commonly involving ISEscI and/or IS26.4,7

Multiple copies of a resistance gene may increase the antimicrobial resistance levels and provide more of a selective advantage. Expression of the chromosomal CMY-2 and its possible contribution to β-lactam resistance levels were not studied. β-Lactam MICs for Salmonella Typhimurium AM19083, however, were comparable with those observed for other Salmonella strains carrying only plasmidic blaCMY-2, such as AM18447, but, for many of
with a 16S rDNA-specific probe, (c) a \textit{bla} \textit{Typhimurium} isolates. (a) Isolates AM19083 and AM18447, (b) hybridized

Agents 2004; \textit{Salmonella}

References

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Transparency declarations

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the drugs, exceeded the range of dilutions tested (ampicillin, amoxicillin/clavulanate, ceftiofur, cefoxitin and cephalothin). Additionally, a comparison of total cephalosporinase activities by UV spectrophotometry using cell extracts and cefalotin as a reporter substrate did not indicate any significant influence of the extra chromosomal copy (data not shown). Therefore, the role of the chromosomal \textit{bla}_{\text{CMY-2}} in determining resistance levels in this isolate is unclear. Nevertheless, \textit{Salmonella} strains with chromosomally located \textit{bla}_{\text{CMY-2}} may act as reservoirs for de novo acquisition of resistance by completely different plasmid backbones and other mobile structures. To the best of our knowledge, this is the first study providing indications as to the simultaneous chromosomal and plasmid location of \textit{bla}_{\text{CMY-2}} in \textit{Salmonella}.

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References


\textbf{Research letters}

Figure 1. PFGE of 1-Ceul-digested DNA from \textit{Salmonella} serovar Typhimurium isolates. (a) Isolates AM19083 and AM18447, (b) hybridized with a 16S rDNA-specific probe, (c) a \textit{bla}_{\text{CMY-2}}-specific probe and (d) an \textit{IncI}-specific probe. Molecular weight markers (λ PFGE, New England Biolabs) are in the first lane. Arrow in (c) indicates the 500 kb chromosomal fragment from AM19083, which was positive for \textit{bla}_{\text{CMY-2}}. Hybridization signals in (d) represent the \textit{bla}_{\text{CMY-2}}-positive, \textit{IncI} plasmids carried by both isolates.

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\textbf{Reduced susceptibility to tetracyclines is associated \textit{in vitro} with the presence of 16S rRNA mutations in \textit{Mycoplasma hominis} and \textit{Mycoplasma pneumoniae}}

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Sir,

\textit{Mycoplasma pneumoniae} and \textit{Mycoplasma hominis} are aetiological agents of respiratory and genitourinary tract infections, respectively, for which tetracyclines present potential for empirical treatment.\textsuperscript{1} As mycoplasmas possess a small number of \textit{rrn} operons, one for \textit{M. pneumoniae} and two for \textit{M. hominis}, the target-related mechanism of resistance to tetracyclines caused by 16S rRNA mutations could be expected as previously described for \textit{Helicobacter pylori}.\textsuperscript{2} The purpose of this study was to identify such a mechanism in the reference strains \textit{M. hominis} PG21 and \textit{M. pneumoniae} FH by selecting \textit{in vitro} for tetracycline-resistant mutants and sequencing the 16S rRNA genes of the obtained mutants.