**Escherichia coli** of animal origin in Norway contains a **bla**\textsubscript{TEM-20}-carrying plasmid closely related to **bla**\textsubscript{TEM-20} and **bla**\textsubscript{TEM-52} plasmids from other European countries

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Sir,

The situation regarding antimicrobial resistance in bacteria from food-producing animals in Norway is, in an international perspective, favourable. The resistance frequencies are moderate and the situation has been stable since the start of the Norwegian monitoring programme in the veterinary sector (NORM-VET) (www.vetinst.no) in the year 2000. We report the first bacterial isolate of animal origin detected in Norway with reduced susceptibility to generation cephalosporins, showed a 135 bp deletion in the promoter and a G→T mutation at position 162. These mutations were not identified in the sequence from *E. coli* 1248, and this may explain the lower MICs exhibited.

The **bla**\textsubscript{TEM-20} sequence has previously been detected in *Salmonella* Paratyphi B dT\textsuperscript{+} from poultry in the Netherlands.\textsuperscript{2} The strain *Salmonella* Paratyphi B dT\textsuperscript{+} 63.48, kindly donated to us for further investigation, was compared with *E. coli* 1248. The **bla**\textsubscript{TEM-20} gene was also carried by a conjugative plasmid in the *Salmonella* Paratyphi B dT\textsuperscript{+} strain, and the two **bla**\textsubscript{TEM-20} sequences were 100% identical. The transconjugant was resistant to \textbeta-lactams only with the same MICs as the *E. coli* 1248 transconjugant. Both **bla**\textsubscript{TEM-20} plasmids were assigned to the incompatibility (Inc) group I1 by PCR-based replicon typing.\textsuperscript{3}

Plasmid multilocus sequence typing (pMLST) for subtyping IncI1 plasmids was applied to the **bla**\textsubscript{TEM-20} plasmids.\textsuperscript{5} The following alleles (accession numbers) were obtained: repFL-1 (EU370458), ardaA-2 (EU370453), trbA-pndC-2 (EU40466), sogS-3 (EU70463) and pilL-3 (EU370457). These alleles corresponded to sequence type 5, previously assigned to an IncI1 plasmid carrying **bla**\textsubscript{TEM-52} identified in a *Salmonella* Infantis strain isolated in Belgium in 2005\textsuperscript{5} (the alleles showed 100% nucleotide identity except for one nucleotide in the ardaA locus). This **bla**\textsubscript{TEM-52} plasmid is reported to be widely disseminated among different *Salmonella* serovars from poultry and humans in Belgium and France.\textsuperscript{6} The **bla**\textsubscript{TEM-20} plasmids were large (>100 kb) and showed profiles that seemed to be similar when comparing the *PsrI* and *EcoRI* restriction patterns with published restrictions patterns of the **bla**\textsubscript{TEM-52} plasmid.\textsuperscript{5,7}

The **bla**\textsubscript{TEM-52} gene has been identified within a Tn\textsuperscript{3} transposon derivative on the plasmid described earlier (accession number EF141186).\textsuperscript{8} Primers were designed in order to amplify and sequence a similar region (6.6 kb) in *E. coli* 1248 and in *Salmonella* Paratyphi B dT\textsuperscript{+}. A Tn\textsuperscript{3}-related transposon, with 100% identical nucleotide sequence, was detected in both strains. Only one amino acid, E104K, distinguishes TEM-20 from TEM-52. Comparison between the **bla**\textsubscript{TEM-20} sequence and the **bla**\textsubscript{TEM-52} sequence revealed only one nucleotide difference (producing amino acid alteration E104K). Five additional nucleotide differences were found when the remaining parts of the DNA region were aligned, indicating a close relationship between the **bla**\textsubscript{TEM-20} and **bla**\textsubscript{TEM-52} transposons. In conclusion, two closely related plasmid scaffolds carrying identical transposons were identified, associated with **bla**\textsubscript{TEM-20} in *E. coli* from Norway and in *Salmonella* Paratyphi B dT\textsuperscript{+} from the Netherlands, and with **bla**\textsubscript{TEM-52} in *Salmonella* from Belgium and France.
Detection of an extended-spectrum β-lactamase (ESBL)-positive *E. coli* from livestock in Norway was unexpected as there are no commercial preparations containing cephalosporins licensed for veterinary use. An interview with the farmer confirmed that there had been no use of cephalosporins for animals or humans at the farm. The strain, or plasmid, might have been part of bacterial flora of animals taken to Norway for breeding purposes.

The bla*TEM-20* plasmid conferring low-level resistance to cephalosporins may represent a challenge with regard to detection of ESBL-positive isolates. The plasmids might also have the potential to develop into variants conferring higher levels of resistance as only one mutation may contribute to a change in the phenotype.

**Accession number**
The nucleotide sequence determined is available under accession number EU527189.

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**Transparency declarations**
None to declare.

**References**


**Potential evolution of hydrolysis spectrum for AmpC β-lactamase of *Escherichia coli* **

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Sir, *Escherichia coli* harbours a chromosome-encoded *ampC* gene that is expressed naturally at a very low level due to a weak promoter. Spontaneous mutations in the promoter region may induce constitutive overproduction of the AmpC β-lactamase, thus conferring resistance to narrow-spectrum cephalosporins.<sup>1</sup>

Recently, a novel mechanism of resistance to β-lactams has been identified, cephalosporinases with broadened substrate activity in several clinical isolates of Enterobacteriaceae. These extended-spectrum AmpC (ESAC) β-lactamases are derived from wild-type cephalosporinases by structural modifications in the R1 or the R2 binding sites that contribute to the binding of the C-3 and C-7 side chains of cephalosporins, respectively.<sup>2</sup> The R1 binding site is mainly formed by the Ω loop, whereas the R2 binding site is formed by the N-terminal extremity of the helix H-11 (Asn-346), the C-terminal extremity of the helix H-9 (Asn-289) and the R2 loop that contains the H-10 helix. The resulting ESAC β-lactamases exhibit increased catalytic efficiencies towards extended-spectrum cephalosporins (ESCs), including cefepime and cefpirome, and also slightly against imipenem.<sup>2,3</sup> These variants significantly reduce the susceptibility to ESCs, and also the susceptibility to imipenem and ertapenem in strains lacking membrane permeability.<sup>2,4</sup> A recent epidemiological survey revealed that it is an emerging mechanism of resistance in *E. coli*.<sup>1</sup>

All the ESAC β-lactamases characterized so far have presented only a single structural alteration responsible for the broadened hydrolysis spectrum.<sup>1,2</sup> The objective of the present work was to predict the potential evolution of the hydrolysis spectrum displayed by the AmpC β-lactamase of *E. coli* by combining several amino acid replacements in the R1 and the