A multidrug-resistance region containing \( \text{bla}_{\text{CTX-M-65}} \), \( \text{fosA3} \) and \( \text{rmtB} \) on conjugative IncFII plasmids in \( \text{Escherichia coli} \) ST117 isolates from chicken

The \( \text{bla}_{\text{CTX-M}} \) gene has become the most common gene encoding extended-spectrum \( \beta \)-lactamase (ESBL) in multidrug-resistant Gram-negative bacteria worldwide. To date, 147 subtypes of the CTX-M enzyme have been reported (http://www.lahey.org/Studies/other. asp#table1). Since 2007, \( \text{bla}_{\text{CTX-M-65}} \) has become one of the predominant \( \text{bla}_{\text{CTX-M}} \) genes in ESBL-producing bacterial isolates from animals in China (Yuan et al., 2009; Deng et al., 2011). The \( \text{bla}_{\text{CTX-M}} \) genes often coexist with genes encoding 16S rRNA methylases such as \( \text{armA} \) and \( \text{rmtB} \), or with \( \text{fosA3} \) that confers resistance to fosfomycin (Hou et al., 2012; Lee et al., 2012; Ho et al., 2013). In China, \( \text{rmtB} \) is the most prevalent 16S rRNA methylase gene among the \( \text{Enterobacteriaceae} \).

Plasmids in which the four resistance genes \( \text{bla}_{\text{TEM-1}}, \text{bla}_{\text{CTX-M-65}}, \text{fosA3} \) and \( \text{rmtB} \) coexist were recently found in \( \text{Escherichia coli} \) isolates from ducks and dogs in China (Sun et al., 2012; He et al., 2013). Mobile elements mobilize resistance genes. The \( \text{ISEcp1} \)-\( \text{bla}_{\text{CTX-M-9}} \)-\( \text{IS903-iroN} \) structure is a typical transposition unit in the \( \text{bla}_{\text{CTX-M-9}} \) cluster, to which \( \text{bla}_{\text{CTX-M-65}} \) belongs (He et al., 2013). The \( \text{rmtB} \) gene is commonly carried by the \( \text{Tn3} \) transposon (Doi et al., 2004) and \( \text{fosA3} \) has been found in the \( \text{IS26} \)-formed composite transposon (Lee et al., 2012). Since chicken was considered as a reservoir for extraintestinal pathogenic \( \text{E. coli} \) in humans (Bergeron et al., 2012), the detection of \( \text{bla}_{\text{TEM-1}}, \text{bla}_{\text{CTX-M-65}}, \text{rmtB} \) and \( \text{fosA3} \) in an isolate of animal origin may represent an emerging threat to public health. In our present study, we characterized a multidrug-resistance region (MRR) of an \( \text{F33A-B} \)-plasmid carrying \( \text{bla}_{\text{TEM-1}}, \text{bla}_{\text{CTX-M-65}}, \text{rmtB} \) and \( \text{fosA3} \) that was isolated from an \( \text{E. coli} \) avian strain of the sequence type 117 (ST117).

In a survey on antimicrobial-resistant bacterial strains in China in 2009, an \( \text{E. coli} \) strain, EC011, was isolated from chicken in Changchun Province, North-east China. The species identification was performed using the VITEK 32 automated identification system (bioMérieux). The \( \text{MIC} \) of fosfomycin was determined using the agar dilution method and was interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2010). The MIC of fosfomycin was determined using the agar dilution method on Mueller–Hinton agar containing 25 \( \mu \text{g} \) glucose 6-phosphate ml\(^{-1} \) as described previously (Hou et al., 2012). \( \text{E. coli} \) strain ATCC 25922 was used as the control. The EC011 strain was resistant to ampicillin (MIC \( > 256 \mu \text{g} \text{ml}^{-1} \)), ceftoxime (MIC=64 \( \mu \text{g} \text{ml}^{-1} \)), ciprofloxacin (MIC=16 \( \mu \text{g} \text{ml}^{-1} \)), amikacin (MIC \( > 256 \mu \text{g} \text{ml}^{-1} \)), gentamicin (MIC \( > 256 \mu \text{g} \text{ml}^{-1} \)), doxycycline (MIC=64 \( \mu \text{g} \text{ml}^{-1} \)), florfenicol (MIC=256 \( \mu \text{g} \text{ml}^{-1} \)) and fosfomycin (MIC \( > 256 \mu \text{g} \text{ml}^{-1} \)). The presence of genes encoding ESBLs, 16S rRNA methylases and the plasmid-encoded fosfomycin resistance determinants were detected using PCR as described previously (Hou et al., 2012; Pan et al., 2013). Amplicons were sequenced using \( \text{3730xl} \) DNA Analyser (Applied Biosystems). The EC011 strain had the \( \text{bla}_{\text{TEM-1}}, \text{bla}_{\text{CTX-M-65}}, \text{rmtB} \) and \( \text{fosA3} \) genes. This isolate belonged to the \( \text{K537631} \) (GenBank accession nos JN232517 and KF537631). The MRR was deposited in GenBank (accession no. KF537631). The EC011 MRR was in a complex structure and was bounded by \( \text{IS1} \) at both ends as seen in \( \text{pV517} \) (GenBank accession nos JN232517 and JPF27996, respectively; Fig. 1). The MRR consisted of the \( \text{IS26-ASEcp1} \)-\( \text{bla}_{\text{CTX-M-65}} \)-\( \text{IS903-iroN} \)-\( \text{IS26} \)-\( \text{fosA3-orf1A} \)-\( \text{IS1294} \) and \( \text{AntrP-bla}_{\text{TEM-1}} \)-\( \text{rmtB-IS26} \) modules that might have been adjoined by three \( \text{IS26} \) elements in the same direction and one \( \text{IS1294} \) element (Fig. 1).
A similar structure was recently found in the pHN7A8 (F33:A-B-) and pXZ (F2:A-B-) plasmids in *E. coli* isolated from dogs (He et al., 2013) and ducks (Sun et al., 2012), respectively, in China. The MRR of the pHN7A8 and pXZ plasmids were almost identical (Fig. 1), suggesting that a common MRR has been mobilized into different plasmids. The IS\(^{26}\)-fosA3-orf1D-IS\(^{1294}\)

![Diagram](https://example.com/diagram.png)

**Table 1.** Primers used for the PCR amplification of the MRR DNA sequences

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Primer name</th>
<th>Nucleotide sequence (5′→3′)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upstream flanking region of <em>pemK</em></td>
<td>CA1</td>
<td>ATGTCGCASACHGAAAATGC*</td>
<td>3559</td>
<td>Osborn et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>pemK-R</td>
<td>TATGCCAACCACATCCACCAAGA</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>pemK-F</td>
<td>CAATATCACTGCGCTAGCTAC</td>
<td>3191</td>
<td>This study</td>
</tr>
<tr>
<td>Upstream flanking region of <em>bla</em>(_{CTX-M-65})</td>
<td>IS(^{26})-R</td>
<td>TATGTTCAGATAATGCCCGAT</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td><em>S</em>=G or C; H=A or C or T.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A similar structure was recently found in the pHN7A8 (F33:A-B-) and pXZ (F2:A-B-) plasmids in *E. coli* isolated from dogs (He et al., 2013) and ducks (Sun et al., 2012), respectively, in China. The MRR of the pHN7A8 and pXZ plasmids were almost identical (Fig. 1), suggesting that a common MRR has been mobilized into different plasmids. The IS\(^{26}\)-fosA3-orf1A-IS\(^{1294}\)

**Fig. 1.** Structural features of plasmid pEC011 MRR compared to those of IncFII-type plasmids EC096TF (GenBank accession number JQ343850), pHK23a (GenBank accession number JQ432559), pXZ (GenBank accession number JF927996) and pHN7A8 (GenBank accession number JN232517). Arrows indicate open reading frames and the arrowheads indicate the direction of transcription. The similar regions are indicated by grey shading, except for the segments of IS\(^{26}\)-\(\Delta\)IS\(^{Ecp1}\)-*bla*\(_{CTX-M-3}\*orf477\)-IS\(^{26}\) in pHK23a and IS\(^{26}\)-\(\Delta\)IS\(^{Ecp1}\)-*bla*\(_{CTX-M-65}\*IS903D*iroN*-IS\(^{26}\) in pXZ.
element in the MRR in our study exhibited greater variability than the IS26-fosA3-orf1-orf2A-IS26-IS1294 element. As a member of the IS91 family, the IS1294 element is an atypical insertion sequence that lacks terminal inverted repeats, transposes using rolling-circle replication, and does not duplicate the target nucleotide sequence (Tavakoli et al., 2000). The insertion-site (CAAG) footprint left by IS1294 reflects the deletion of orf1A-orf2A-IS26. Moreover, the clustering of these components of the IS26-IS1294-IS1294-IS26-IS1294-IS1294-IS26 modules were likely to be the result of homologous recombination between two copies of IS26 that generated the different MRR (Fig. 1) (Lee et al., 2012; Sun et al., 2012; He et al., 2013; Ho et al., 2013).

**E. coli** ST117 is not only an avian pathogenic *E. coli* but it can also cause clinical infection such as septicaemia and urinary tract infections in humans (Bergeron et al., 2012; Mora et al., 2012). Furthermore, chicken was considered as a reservoir of extraintestinal pathogenic *E. coli* in humans (Bergeron et al., 2012). In the present study, the strain EC011 from chicken carrying pEC011 (F33:A-:B-) belonged to ST117 and phylogenetic group D. As ST117 is a lineage associated with avian diseases and clinical infections, the introduction of a plasmid such as pEC011 carrying four resistant genes is significant and may represent a challenge for treatment and public health. It is worth noting that the strain HN7A8 from dog carrying pHN7A8 (F33:A-:B-) belonged to phylogenetic group B1, suggesting that the horizontal transmission of a multidrug-resistance plasmid may have occurred between commensal *E. coli* and pathogenic *E. coli*. Therefore, this conjugative plasmid from avian *E. coli* belonging to ST117, carrying four resistant genes conferring resistance to different antimicrobial agents, may be worrying.

In conclusion, we characterized a MRR on a conjugative plasmid that contained the blaTEM-1, blaCTX-M-65, fosA3 and rmtB antimicrobial resistance genes, which was isolated from an ST117 *E. coli* of avian strain. The mobile genetic elements IS26, IS1294 and Tn3 may have contributed to the evolution of the MRR through recombination. However, it is unclear whether the structure of the MRR was generated through classical transposition, one-ended transposition, homologous recombination or IS26-mediated co-integration. Further investigations of the mechanism responsible for generating this MRR are needed. The spread of IncFII plasmids with MRRs harbouring blaTEM-1, blaCTX-M-65, rmtB and fosA3 among *E. coli* strains of animal origin may represent an emerging threat to public health through contact with or the consumption of infected animals.

**Acknowledgements**

This work was supported by a grant from the National Natural Science Foundation of China (grant numbers 31201956 and 31372481). We wish to thank Yun-song Yu (State Key Laboratory for Diagnosis and Treatment of Infectious Disease, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, PR China) for kindly providing the *E. coli* V517 plasmid DNA.

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