The patient was a gardener living on a farm that raised livestock and thus came into contact with cows, suggesting a possible contamination of the patient from an animal origin. The patient was initially treated with claxacillin, gentamicin and metronidazole for only 3 days and then switched to vancomycin, gentamicin and fosfomycin. After 2 weeks of this intravenous treatment, he was prescribed oral ofloxacin and rifampicin for 8 weeks. A CT scan of the chest 23 days after the initial hospitalization showed a marked decrease in the retrosternal collection and the patient's parotitis, and the infection was considered cured at the end of treatment.

One year later, in February 2012, the patient was sampled at home to detect the possible persistence of mecC-MRSA carriage. Two different methicillin-susceptible S. aureus strains were isolated from nose and throat swabs. mecC PCR was negative, and the genetic backgrounds of the two isolates differed from that of strain LIM84 on the basis of agr typing and DNA microarray results. This demonstrated the absence of long-term colonization with the mecC-positive strain.

This case illustrates the ongoing evolution of bacteria, requiring microbiologists to investigate atypical results of antibiotic susceptibility tests. It also shows that conventional phenotypic tests are still useful for detecting new or atypical resistance mechanisms, which cannot be detected by genotypic methods. This challenges the view that genotypic methods are now the gold standard for resistance studies of S. aureus. As mecC detection is crucial for optimal patient management, this capability should be added to commercial diagnostic tools.

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Transparency declarations
None to declare.

Author contributions
O. B. isolated the strain and wrote the report, F. L. and M. B. were responsible for genotypic analyses, B. F. and P. V. managed the patient and F. L. and M.-C. P. reviewed the report.

References

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The blaCTX-M-1 IncI1/ST3 plasmid is dominant in chickens and pets in Tunisia

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Keywords: antimicrobial resistance, ESBLs, CTX-M, veterinary, plasmids
Sir,

Extended-spectrum β-lactamases (ESBLs) of the CTX-M group have become widespread enzymes conferring resistance to broad-spectrum cephalosporins in humans and animals, and plasmids play a key role in the horizontal transfer of the corresponding genes. Contrary to the situation in humans, the bla\textsubscript{CTX-M-1} gene is abundant in animals. It is also frequently located on plasmids of specific incompatibility (Inc) groups and subtypes, such as IncI1/ST3 plasmids. Indeed, indistinguishable bla\textsubscript{CTX-M-1} IncI1/ST3 plasmids have been found in Escherichia coli from cattle, goats, poultry, horses and pets and in Salmonella enterica from cattle, poultry and humans. In Tunisia, the bla\textsubscript{CTX-M-1} gene was also reported in pets, foodstuff and food animals, particularly in chickens. Moreover, 7.3% of Tunisian healthy humans proved to carry CTX-M-1-producing E. coli, suggesting that foodstuff of poultry origin may contribute to the transfer of the bla\textsubscript{CTX-M-1} gene from animals to humans. The aims of this study were to characterize bla\textsubscript{CTX-M-1}-carrying plasmids from E. coli isolates from chickens and pets in Tunisia and to compare them with previously reported bla\textsubscript{CTX-M-1}-carrying plasmids.

Between December 2011 and April 2012, faecal swabs from 193 chickens, 41 dogs and 4 cats were collected in Tunisia and plated onto cefotaxime-supplemented (4 mg/L) MacConkey agar. One presumptive E. coli colony was selected per plate and identification was confirmed using API20E galleries. All E. coli isolates were then tested for antimicrobial susceptibility by disc diffusion according to the Antibiogram Committee of the French Society for Microbiology guidelines (www.sfm-microbiologie.fr) and ESBL production was confirmed by the double-disc synergy test. Chickens originated from 12 unrelated farms (each with 1000–5000 animals) in the Sousse (7), Mahdia (1), Kairouan (2) and Monastir (2) governorates, and were either diseased or dead (colibacillosis). Dogs and cats were sampled at a single clinic during routine examination (Sousse), and all but three animals were healthy. Seven of the 12 chicken farms (58.3%) were found positive for ESBL producers (each governorate was represented), and eight unrelated ESBL E. coli isolates (using PFGE, not shown) were recovered from the 193 samples (8/193, 4.1%). Seven additional unrelated ESBL E. coli isolates were recovered from the 45 pets (7/45, 15.6%). No isolate belonged to the pandemic human O25b-ST131 E. coli clone. All but one isolate displayed multiple co-resistances (Table 1).

ESBL-carrying plasmids were transferred into electrocompetent E. coli TOP10 cells, and the bla\textsubscript{CTX-M-1} gene was confirmed by PCR and sequencing in 13/15 (87%) transformants. The two last transformants harboured the bla\textsubscript{CTX-M-9} and the bla\textsubscript{CTX-M-15} genes, respectively. Southern blots on S1-PFGE gels with bla\textsubscript{CTX-M-1} and IncF probes demonstrated that all bla\textsubscript{CTX-M-1} and the bla\textsubscript{CTX-M-9} genes were carried on IncI1 plasmids, whereas the bla\textsubscript{CTX-M-15} gene was located on an IncI1 plasmid (not shown). The IncI1 plasmids sizes ranged from 100 to 120 kb, whereas the size of the bla\textsubscript{CTX-M-15} IncI1 plasmid was 160 kb (Table 1).

In chicken isolates, the bla\textsubscript{CTX-M-1} IncI1 plasmids belonged to the ST3 subtype (allelic profile 2/1/4/1/2) or to the closely related ST87 (8/1/4/1/2) subtype (Table 1), which only differs from ST3 by four base pairs at the end of a single locus (repA gene; http://pubmlst.org/plasmid). The bla\textsubscript{CTX-M-9} gene was also located on an IncI1/ST87 plasmid. In pet isolates, all plasmids but one bla\textsubscript{CTX-M-1} IncI1 plasmid belonged to the ST3 subtype, whereas one isolate belonged to the unrelated ST25 (1/4/5/4/1) subtype (Table 1). Restriction fragment length polymorphism (RFLP) profiles of the IncI1/ST3 and IncI1/ST87 plasmids after digestion with PstI or EcoRI were either indistinguishable or highly similar, whatever the animal origin (Figure S1, available as Supplementary data at JAC Online). Hence, this study shows that the bla\textsubscript{CTX-M-1} IncI1/ST3 plasmid is broadly disseminated in chickens and pets in Tunisia.

Interestingly enough, the bla\textsubscript{CTX-M-1} IncI1/ST3 plasmids described here were identical or highly similar to bla\textsubscript{CTX-M-1} IncI1/ST3 plasmids recently reported in France and Belgium in several

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**Table 1. Characteristics of the E. coli isolates and bla\textsubscript{CTX-M-1}-carrying plasmids in this study**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Animal</th>
<th>CTX-M type</th>
<th>Replicons in donors</th>
<th>Replicons in transformants</th>
<th>pMLST or FAB formula</th>
<th>Plasmid size (kb)</th>
<th>Co-resistances</th>
</tr>
</thead>
<tbody>
<tr>
<td>171</td>
<td>Chicken</td>
<td>CTX-M-1</td>
<td>I1/FIB</td>
<td>I1</td>
<td>ST3</td>
<td>100</td>
<td>STR, KAN, TET, SUL, TMP, NAL, ENR</td>
</tr>
<tr>
<td>142</td>
<td>Chicken</td>
<td>CTX-M-1</td>
<td>I1</td>
<td>I1</td>
<td>ST87</td>
<td>115</td>
<td>STR, TET, SUL, NAL</td>
</tr>
<tr>
<td>1629</td>
<td>Chicken</td>
<td>CTX-M-1</td>
<td>I1/FIB/F/P</td>
<td>I1</td>
<td>ST3</td>
<td>110</td>
<td>STR, TET, SUL, TET, NAL</td>
</tr>
<tr>
<td>325a</td>
<td>Chicken</td>
<td>CTX-M-1</td>
<td>I1/FIB</td>
<td>I1</td>
<td>ST3</td>
<td>110</td>
<td>STR, TET, SUL, TMP, NAL, ENR</td>
</tr>
<tr>
<td>325b</td>
<td>Chicken</td>
<td>CTX-M-1</td>
<td>I1/FIB</td>
<td>I1</td>
<td>ST87</td>
<td>110</td>
<td>STR, TET, SUL, TET, SUL, TMP</td>
</tr>
<tr>
<td>462</td>
<td>Chicken</td>
<td>CTX-M-1</td>
<td>I1/FIB/FIB</td>
<td>I1</td>
<td>ST87</td>
<td>110</td>
<td>STR, TET, SUL, TMP</td>
</tr>
<tr>
<td>625</td>
<td>Chicken</td>
<td>CTX-M-9</td>
<td>I1/FIB</td>
<td>I1</td>
<td>ST87</td>
<td>110</td>
<td>STR, TET, SUL, TMP</td>
</tr>
<tr>
<td>645</td>
<td>Chicken</td>
<td>CTX-M-1</td>
<td>I1/FIB/BO</td>
<td>I1</td>
<td>ST3</td>
<td>110</td>
<td>STR, TET, SUL, TMP</td>
</tr>
<tr>
<td>CN1</td>
<td>Dog</td>
<td>CTX-M-15</td>
<td>I1/FIB/FIB/F</td>
<td>I1</td>
<td>F31:A3:81</td>
<td>160</td>
<td>STR, KAN, TOB, NET, GEN, TET, SUL, TMP, NAL, ENR</td>
</tr>
<tr>
<td>CN2</td>
<td>Dog</td>
<td>CTX-M-1</td>
<td>I1/FIB/FIB</td>
<td>I1</td>
<td>ST3</td>
<td>110</td>
<td>STR, TET, SUL, TET, SUL, TMP, NAL</td>
</tr>
<tr>
<td>CN3</td>
<td>Dog</td>
<td>CTX-M-1</td>
<td>I1/FIB</td>
<td>I1</td>
<td>ST3</td>
<td>110</td>
<td>STR, TET, SUL, TMP</td>
</tr>
<tr>
<td>CN4</td>
<td>Dog</td>
<td>CTX-M-1</td>
<td>I1/FIB</td>
<td>I1</td>
<td>ST25</td>
<td>115</td>
<td>STR, TET, SUL, TMP</td>
</tr>
<tr>
<td>CN5</td>
<td>Dog</td>
<td>CTX-M-1</td>
<td>I1</td>
<td>I1</td>
<td>ST3</td>
<td>110</td>
<td>STR, TET, SUL, TMP</td>
</tr>
<tr>
<td>CN6</td>
<td>Dog</td>
<td>CTX-M-1</td>
<td>I1/FIB</td>
<td>I1</td>
<td>ST3</td>
<td>120</td>
<td>STR, TET, SUL, TMP, NAL</td>
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<tr>
<td>CT1</td>
<td>Cat</td>
<td>CTX-M-1</td>
<td>I1/FIB/F</td>
<td>I1</td>
<td>ST3</td>
<td>120</td>
<td>STR, TET, SUL, TMP, NAL</td>
</tr>
</tbody>
</table>

STR, streptomycin; TOB, tobramycin; KAN, kanamycin; APR, apramycin; NET, netilmicin; GEN, gentamicin; CHL, chloramphenicol; TET, tetracyclines; SUL, sulphonamides; TMP, trimethoprim; NAL, nalidixic acid; ENR, enrofloxacin. Resistance to antibiotics in bold was also found in the CTX-M transformants.
animal species and humans (Figure S1, available as Supplementary data at JAC Online). These plasmids were reported not only in E. coli but also in S. enterica, in particular of serovars Llandoff, London, Newport and Typhimurium of chicken and human origin.  

In conclusion, the blaCTX-M-1 IncI1/ST3 plasmid, which is dominant in various animal species in Europe and has also been reported in humans infected with S. enterica, appears to be dominant in food- and non-food-producing Tunisian animals. These data underline the international role of the blaCTX-M-1 IncI1/ST3 plasmid in ESBL epidemiology in animals. The risk of dissemination of the blaCTX-M-1 IncI1/ST3 plasmid from E. coli to S. enterica in Tunisia, as reported in Europe, is also a major concern. Furthermore, this study suggests that the surprisingly high prevalence of CTX-M-1 producers in the Tunisian community might result from the abundance of the blaCTX-M-1 IncI1/ST3 plasmid in animals. There is an urgent need to set up surveillance systems for antimicrobial resistance and antibiotic usage in animals (particularly in the food chain) in Tunisia.

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Transparency declarations
None to declare.

Supplementary data
Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

Outbreak of metallo-β-lactamase VIM-2-positive strains of Pseudomonas aeruginosa in the Ivory Coast

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Keywords: P. aeruginosa, imipenem resistance, carbapenemases

Sir,

Acquired metallo-β-lactamases (MBLs) are increasingly recognized as important determinants of β-lactam resistance in Pseudomonas aeruginosa. To date, seven types of MBL enzyme (IMP, VIM, NDM, SPM, GIM, AIM and FIM) have been identified in this opportunistic pathogen. 1,2 Since its first description in France in 1996, the epidemiologically successful enzyme VIM-2 has been traced in resistant P. aeruginosa strains from various European countries (e.g. Spain, Italy, Croatia, Greece, the Netherlands and Turkey) and more recently in strains from northern (Tunisia and Algeria) and southern (South Africa) Africa. 3,4–6 One study also mentioned its occurrence in Kenya. 7 Illustrating the spread of the VIM-2-encoding gene, blaVIM-2, in sub-Saharan Africa countries, we found here that P. aeruginosa strains from the Ivory Coast harboured an atypical class 1 integron previously described in sporadic isolates from other continents.

Twelve P. aeruginosa isolates that were non-susceptible to imipenem according to the EUCAST interpretation rules (imipenem MIC >4 mg/L) were recovered from separate patients hospitalized