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Analysis of plasmid-mediated ESBL production and colistin resistance in Escherichia coli from pig nostrils: a new reservoir of concern?

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INTRODUCTION AND OBJECTIVES

Pig nostrils are frequent habitats of livestock-associated MRSA, but very few studies describe their role as a potential reservoir of ESBL-producing and/or colistin-resistant *E. coli*. Therefore, we aimed to investigate the presence of resistant *E. coli* in this niche, as well as their ability to spread depending on their chromosomal or plasmidic localization.

RESULTS

- ESBL-producing *E. coli* were recovered from 7 of 40 pigs tested (17.5%) but in none of the farmers, and the nine ESBL-*E. coli* isolates obtained were characterized as: *bla*_{CTX-M-32}/ST48-A (n=1), *bla*_{CTX-M-14}/ST117-F (n=2), *bla*_{SHV-12}/ST847-ST14914-B1 (n=2), *bla*_{CTX-M-1}/ST5440-ST101-B1/ST93-A (n=3), and *bla*_{CTX-M-65}/ST10-B1 (n=1). All *bla*_{ESBL} genes were horizontally transferable by conjugation, through 40-200 kb plasmids of incompatibility group IncI1 (n=3), IncX1 (n=3) and IncHI2 (n=1) (Table 1).
- IncI1 plasmids displayed different genetic environments: i) IS26-*bla*_{SHV-12}-*deoR*-IS26; ii) *wbuC*-*bla*_{CTX-M-32}-*ISKpn26* (IS5); and iii) IS930-*bla*_{CTX-M-14}-IS26. The IncHI2 plasmid presented the IS903-*bla*_{CTX-M-65}-*fipA* genetic environment, with multiple additional resistance genes associated to: i) Tn21-Tn5393-like transposon containing two atypical class 1 integrons; ii) a Tn1721-like transposon displaying an atypical class 1 integron harbouring *aadA2*-*arr3*-*cmlA5*-*bla*_{OXA-10}-*aadA24*-*dfrA14* and IS26-*bla*_{TEM-95}-*tet(A)*-*lysR*-*floR*-*virD2*-IS*Vsa3*-IS3075-IS26-*qnrS1* genes; iii) a tellurite resistance module sharing full coverage-identity with plasmids previously reported in Enterobacterales (Figure 1). Other plasmids harbouring clinically relevant genes were detected, such as a *ColE*-type plasmid carrying the *mcr-4.5* gene.
- Chromosomally-encoded genes (*fosA7*) or integrons (*intI1*-*dfrA1*-*aadA1*-*qacE*-*sul1* or *intI1*-IS15-*dfrA1*-*aadA2*) were also identified. Finally, an IncY plasmid featuring a class 2 integron (*intI2*-*dfrA1*-*sat2*-*aadA1*-*qacL*-IS406-*sul3*) was detected, but not associated with a *bla*_{ESBL} gene.

Table 1. Characteristics of the nine *bla*_{ESBL}-carrying *E. coli* isolates and carrying plasmids.

ID	ST-PhG ¹	<i>bla</i> _{ESBL} -carrying plasmid		
		ESBL genetic environment	Replicon type/ pMLST (Size)	Conjugation frequency
X5084	48-A	<i>wbuC</i> - <i>bla</i> _{CTX-M-32} - <i>ISKpn26</i>	I1/ST36 (CC5) (79 kb)	6.1 × 10 ⁻²
X5726 ^a	10-B1	IS930- <i>bla</i> _{CTX-M-65} - <i>ISEcp1</i>	HI2/ST3 (260 kb)	3.6 × 10 ⁻⁴
X5723	93-A	IS930- <i>bla</i> _{CTX-M-1} -IS26	X1 (50 kb)	5.1 × 10 ⁻³
X5093	117-F	IS930- <i>bla</i> _{CTX-M-14} -IS26	I1/ST80 (CC31) (98 kb)	2.2 × 10 ⁻⁶
X5100	117-F	IS930- <i>bla</i> _{CTX-M-14} -IS26	I1/ST80 (CC31) (100 kb)	2.4 × 10 ⁻⁶
X5159	847-B1	IS26- <i>bla</i> _{SHV-12} - <i>deoR</i>	I1/ST3 (CC3) (100 kb)	2.8 × 10 ⁻⁶
X5107	14914-B1	IS26- <i>bla</i> _{SHV-12} - <i>deoR</i>	I1/ST3 (CC3) (100 kb)	2.5 × 10 ⁻⁶
X5757	101-B1	IS26- <i>bla</i> _{CTX-M-1} - <i>mrX</i>	X1 (50 kb)	3.3 × 10 ⁻⁵
X5388 ^b	5440-B1	IS26- <i>bla</i> _{CTX-M-1} - <i>mrX</i>	X1 (43 Kb)	2.7 × 10 ⁻⁶

^aOther co-located resistance genes: *bla*_{TEM-1B}, *aac(3)-IV*, *aph(3'')-Ib*, *aph(3')-Ia*, *aph(4)-Ia*, *aph(6)-Id*, *tet(A)*, *bla*_{TEM-95}, *arsR*, *floR*, *bla*_{OXA-10}, *aadA24*, *dfrA14*, *qnrS1*, *cmlA5*, *arR3*, *aadA2*

^bOther co-located resistance genes: *bla*_{TEM-1A}, *mrX*.

MATERIALS AND METHODS

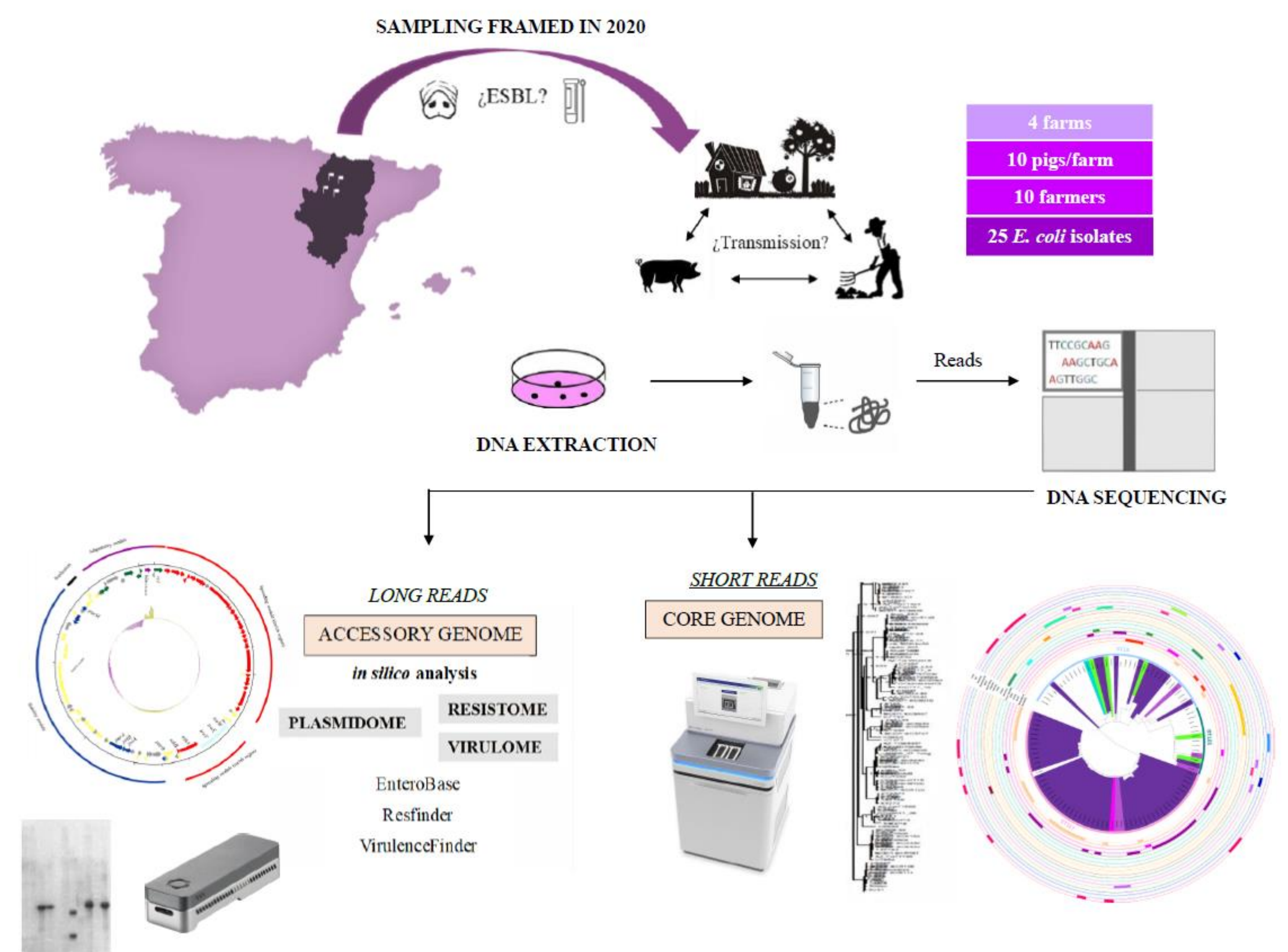


Figure 1. X5726 IncHI2 pST3 (CC3)

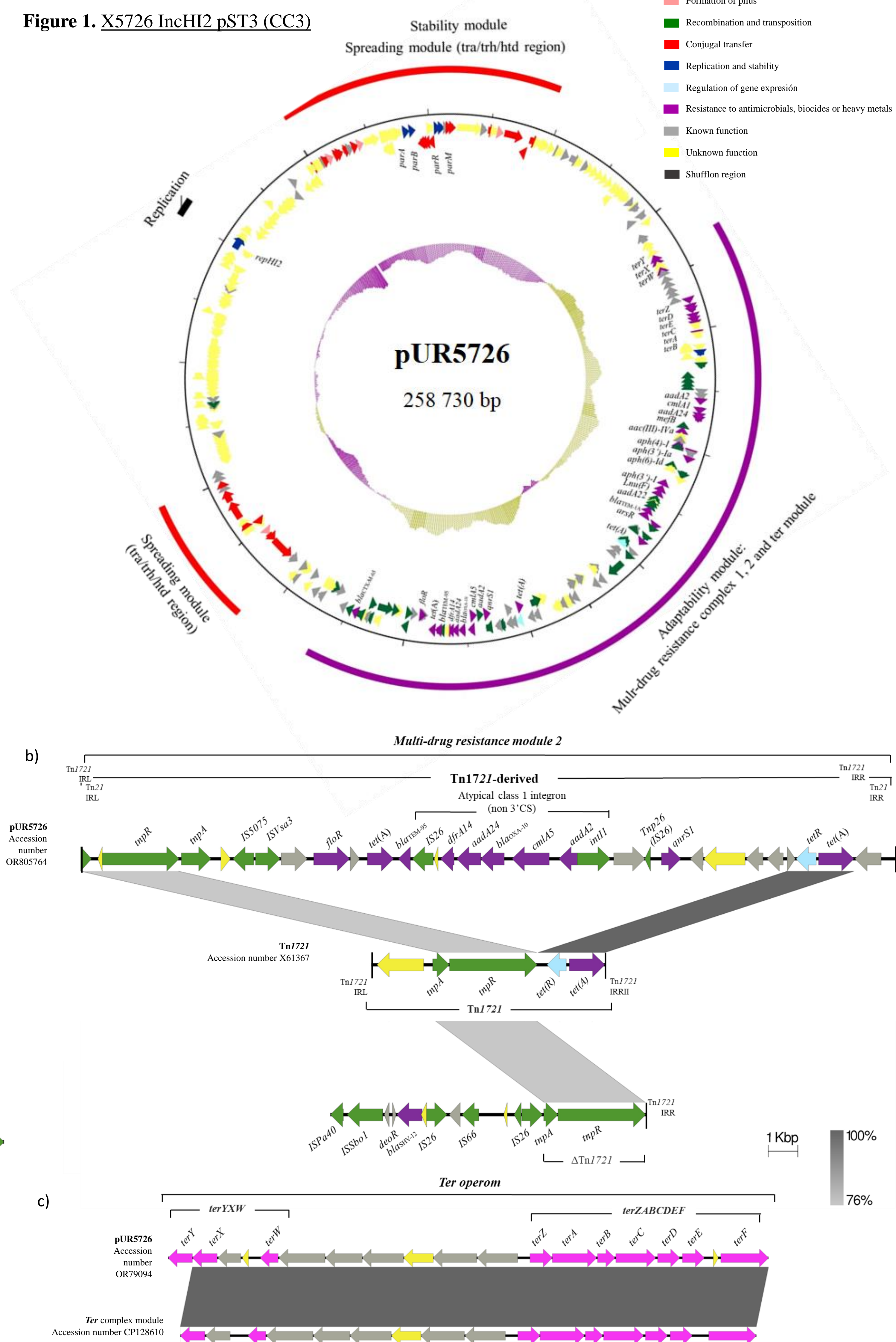


Figure 1. Linear illustration of the resistance complexes of pUR5726 (IncHI2, pST3) and comparative mapping of this region with Tn21 (accession number AF071413), Tn5393 (accession number LT985287) and Tn1721 (accession number X61367) also with pUR5159 (IncI1, pST3). A few important genes have been tagged. The coding reading frames are shown as arrows ("transcription direction" is indicated by the arrowheads). IS are displayed as boxes and the arrows without boxes represent transposon genes. Vertical lines denote the IRs of the ISS, the transposons or the In2 integron.

CONCLUSIONS

✓ Pig nasal cavities are another important niche to consider that might favor the spread of ESBL-*E. coli* and *mcr*-mediated colistin-resistance. This situation would warrant closer monitoring, especially in a sector where close contact between animals in intensive farms increases the risk of antimicrobial resistance dissemination.

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