European clinical isolate of Proteus mirabilis harbouring the Salmonella genomic island 1 variant SGI1-O

Benoît Doublet¹*, Laurent Poirel², Karine Praud¹, Patrice Nordmann² and Axel Cloeckaert¹

¹INRA, UR1282, Infectiologie Animale Santé Publique, IASP, Nouzilly F-37380, France; ²Service de Bactériologie–Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K-Bicêtre, France

*Corresponding author. Tel: +33-2-47-42-72-95; Fax: +33-2-47-42-77-74; E-mail: Benoit.Doublet@tours.inra.fr

Keywords: multidrug resistance, Proteus, hipAB, toxin–antitoxin module

Sir,

The 43 kb Salmonella genomic island 1 (SGI1) is a Salmonella-derived site-specific integrative mobilizable element that was initially characterized in multidrug-resistant (MDR) Salmonella enterica serovar Typhimurium DT104 (where DT stands for definitive phage type) strains.¹,² SGI1 is integrated into the last 18 bp of the chromosomal trmE gene (also called thdF).¹,² SGI1 has been shown to be mobilized in trans using a conjugative helper plasmid that can be transferred to Salmonella and Escherichia coli recipient strains.² SGI1 contains a complex class 1 integron, named In104, responsible for the MDR phenotype and located in the 3’ part of the island.¹ The complex integron In104 possesses two attachment sites (attJ1) for resistance gene cassettes, and numerous variants named SGI1-A to SGI1-T have been described in several S. enterica serovars.¹-⁵ In 2007–08, two studies reported the identification of SGI1-like variants in Proteus mirabilis clinical and food isolates from China and Palestine.¹,⁶ Chromosomal insertion of the SGI1-like element has been shown in those P. mirabilis strains. It was subsequently speculated that the target site for SGI1 integration in P. mirabilis could be also, as observed in S. enterica, the trmE gene, since an homologous gene was identified in the chromosome of P. mirabilis.⁷ The presence of SGI1 in clinical isolates of P. mirabilis is of interest, since it may further contribute to multidrug resistance in P. mirabilis.

Here, we have analysed an MDR P. mirabilis clinical strain, NKU, recovered from rectal and nasal swabs of a patient hospitalized at the Bicêtre hospital in 2007. This patient did not report any recent history of travel abroad. This strain displayed a broad-spectrum β-lactam resistance profile, and in addition was resistant to chloramphenicol, streptomycin, tetracycline, sulphonamides, trimethoprim and fluoroquinolones. Phenotypic detection of extended-spectrum β-lactamase (ESBL) production performed as described previously was positive.⁶

PCRs to detect ESBL genes (blaTEM, blaSHV and blaCTX-M) performed using previously described primers followed by sequencing identified the blaTEM-52 ESBL gene.⁵ Conjugation experiments to transfer the ESBL resistance phenotype were unsuccessful. However, electroporation of the P. mirabilis plasmid DNA to E. coli strain XL1-Blue permitted transfer of the blaTEM-52 resistance gene without any additional resistance marker. Thus, the blaTEM-52 gene was located as a single resistance gene on a non-conjugative plasmid. The lack of identification of other plasmid-borne resistance determinants prompted us to investigate the possible presence of an SGI1-like element in P. mirabilis NKU.

Detection of SGI1 was performed by PCR using primers listed in Table S1 (available as Supplementary data at JAC Online; http://jac.oxfordjournals.org/), as previously described.¹,⁴ The PCR result was positive for the left junction, indicating that SGI1 was inserted in the chromosome at the 3’ end of the chromosomally located trmE gene (Figure 1). However, fragment 2, corresponding to the right junction between SGI1 and the downstream chromosomal hipAB gene, was not obtained. This suggested a genetic variation, possibly involving deletions at the SGI1 3’ end and/or in the adjacent chromosomal region.

PCR mapping (PCRs 4–6) and Southern blot hybridization of XbaI-digested genomic DNA, performed as described previously (see also Table S1, Figure 1), confirmed the presence of the entire SGI1.⁵,⁷ To identify the genetic content of the SGI1 complex integron, PCR mapping was performed as described previously, but gave negative results, indicating that P. mirabilis NKU probably harboured a variant of SGI1.⁷ The integron cassette array was detected by PCR using primers CS1 and CS2, yielding a single 1.3 kb fragment, and sequencing identified the dfrA1-orfC gene cassettes previously described in the SGI1-O variant from P. mirabilis.⁸ Further PCR mapping confirmed the genetic organization of SGI1-O (Table S1, Figure 1). The SGI1-O variant harboured the dfrA1 and sul1 genes, conferring resistance to trimethoprim and sulphonamides, respectively, and has been previously identified in P. mirabilis in China.⁹

The 3’ end of SGI1-O was conserved in P. mirabilis NKU, but the absence of detection of the right junction suggested a likely genetic rearrangement in the chromosome sequence of P. mirabilis strain NKU. To better analyse this rearrangement in the downstream chromosomal region of SGI1, another reverse primer was designed 2 kb downstream of the direct repeat right of SGI1 in the chromosome sequence of P. mirabilis strain HI4320, according to GenBank accession number NC_010554 (Table S1, Figure 1). PCR results revealed a 1714 bp deletion in the chromosome of P. mirabilis strain NKU compared with the genome sequence of P. mirabilis strain HI4320 (Figure 1). The nucleotide sequence obtained (deposited in GenBank under accession number HM560958) indicated that this deletion was likely to be the result of a precise recombinational event between 49 bp perfect direct repeats bracketing the hipAB gene. In P. mirabilis strain NKU, the variant SGI1-O was thus inserted in its specific attachment site (the last 18 bp of the trmE gene) and followed by a single 49 bp perfect direct repeat, which resulted from the loss of the hipBA module (Figure 1). Moreover, this
49 bp element presented a repetitive palindromic structure that is known to represent a preferential site for recombinational events. Homologues of the hipBA module are largely identified in Enterobacteriaceae and have been described as a toxin–antitoxin (TA) module in which HipA, the toxin, is neutralized by the antitoxin HipB. Recently, the implication of this TA module was demonstrated in multidrug tolerance that is related to the presence of dormant bacterial cells also called persisters.

This study has identified what is, to our knowledge, the first SGI1-positive _P. mirabilis_ in Europe, highlighting the increased dissemination of SGI1, especially by horizontal transfer to other human pathogenic bacteria.

**Figure 1.** Schematic view of the variant SGI1-O, as integrated in _P. mirabilis_ strain NKU. The _P. mirabilis_ HI4320 trmE gene, the 49 bp direct repeats bracketing the hipBA module and ORF PMI3124 are shown at the top, and the complex class 1 integron of SGI1-O (not to scale) is shown at the bottom. Base pair coordinates are from the sequence with GenBank accession number AF261825. PCRs carried out to map the SGI1s are indicated by thick black bars and are numbered (see Table S1). DR-L and DR-R are the 18 bp left and right direct repeats, respectively, bracketing SGI1. IRi and IRt are 25 bp imperfect inverted repeats defining the left and right end of complex class 1 integrons. The p1-9 probe and XbaI restriction sites (X) are indicated.
Acknowledgements
We would like to acknowledge C. Mouline for expert technical assistance.

Funding
This work was supported by public funds from the French National Institute of Agronomic Research, the INSERM (U914), the Ministère de l’Éducation Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, and grants from the European Community (TROCAR, HEALTH-F3-2008-223031 and TEMPOtest-QC, HEALTH-2009-241742).

Transparency declarations
None to declare.

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

References

J Antimicrob Chemother 2010
doi:10.1093/jac/dkq288
Advance Access publication 4 August 2010

Zone breakpoints, by the CLSI disc method, for 15 µg tigecycline discs corresponding to EUCAST MIC breakpoints

Russell Hope*, Teresa Pilana, Dorothy James, Marina Warner and David M. Livermore

Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency, 61 Colindale Avenue, London NW9 5HT, UK

*Corresponding author. Tel: +44-20-8327-6493; Fax: +44-20-8327-6264; E-mail: russell.hope@hpa.org.uk

Keywords: susceptibility testing, agar dilution, error minimization

Sir,
Tigecycline is a broad-spectrum glycylcycline antibiotic, derived from the tetracycline minocycline. It was licensed in the USA in 2005 and in the European Union in 2006 for use in the treatment of complicated skin and soft tissue infections and in complicated intra-abdominal infections. Acquired ribosomal protection [e.g. tet(M)] and efflux [tet(A–E)] tetracycline resistance mechanisms do not compromise tigecycline,1–3 which may be useful in infections due to multiresistant Enterobacteriaceae.

The European Committee on Antimicrobial Susceptibility Testing/European Medicines Agency (EUCAST/EMA) have defined tigecycline MIC breakpoints as follows: Staphylococcus spp., Enterococcus spp., susceptible ≤0.5 mg/L and resistant >0.5 mg/L; Staphylococcus spp., susceptible ≤0.5 mg/L and resistant >0.5 mg/L; Enterobacteriaceae, susceptible ≤1 mg/L and resistant >2 mg/L. The US FDA has set somewhat higher breakpoints for the Enterobacteriaceae at susceptible ≤2 mg/L and resistant >4 mg/L. The EUCAST/EMA values have been accepted by the BSAC, whilst the CLSI has yet to set values.

A 15 µg tigecycline disc has been proposed for universal use in susceptibility testing and zone breakpoints for its use with the BSAC method using IsoSensitest agar have been published.4 The BSAC disc method is, however, little used outside the UK. Many European laboratories use the CLSI disc method, with heavier inocula on Mueller–Hinton agar, but now, owing to the legal status of the EMA, must seek to have this agree with EUCAST MIC breakpoints rather than those of the CLSI.5 The present project therefore aimed to identify the zone breakpoints for the CLSI method that best correspond to the EUCAST MIC breakpoints. Whilst the work was underway, EUCAST themselves published provisional zone breakpoints for 15 µg tigecycline discs, and the present analysis compares the performance of their criteria and those we have derived.

Isolates (n=357) were selected from the BSAC bacteraemia survey collection,6 which collects 10–20 isolates of each of 12 species groups per annum from 25 hospital laboratories in the