Corrigendum

The role of the *Serratia marcescens* SdeAB multidrug efflux pump and TolC homologue in fluoroquinolone resistance studied via gene-knockout mutagenesis

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The authors would like to make the following corrections to the above paper.

1. In Table 1, page 456, strains/plasmids HASF300, pKS:hasF:Gm^r, pUCGM, pKNGhasFGm^r are added and the characteristics of pEXSH, pEXH, pEXS, pEXR, pKS:sdeB, and pKS:HasF are amended. The correct version of the table is given below.

2. Methods, Bacterial strains and plasmids, page 455, first column

(a) Line 40, beginning with, ‘The streptomycin-resistant strains...’, replace with:

The streptomycin-resistant strains (with pKNG101, pKNGsdeB, pKNGhasF, pKNGsdeR, pKNGhasFGm^r) were grown on LB plates and in LB broth containing 50 µg streptomycin ml⁻¹ (Sigma-Aldrich).

Continues with ‘The carbenicillin-resistant strains...’

(b) Line 45, additional text:

The gentamicin-resistant strains (with pUCGM, pKS:hasF:Gm^r) were grown on LB plates and in LB broth containing 20 µg gentamicin ml⁻¹ (Sigma-Aldrich).

Continues with ‘Strains SDEAB1, SDEAB2, HASF100...’

(c) Line 45, beginning with ‘Strains SDEAB1, SDEAB2, HASF100...’, replace with:

SDEAB1, SDEAB2, HASF100, HASF200 and SDER1 strains were grown on LB plates and in LB broth containing 100 µg ampicillin ml⁻¹ and 50 µg streptomycin ml⁻¹, HASF300 was grown on media containing 20 µg gentamicin ml⁻¹ and 50 µg streptomycin ml⁻¹, and SDEAB3/HASF300 was grown on media containing 25 µg kanamycin ml⁻¹ and 20 µg gentamicin ml⁻¹.

Continues with ‘UOC-67, MT616, CC118...’

3. Construction of *S. marcescens* hasF-, sdeB- and sdeR-deficient strains, page 455, second column

(a) Line 6, additional text:

For creation of a *hasF* insertion mutant, a 900 bp *Pst*I-flanked Gm^r cassette from pUCGM was inserted into a unique *Pst*I restriction site within the *Eco*RI/*Eco*RI flanked *hasF* fragment of pKS:HasF resulting in pKS:hasF:Gm^r. pKS:hasF:Gm^r was digested with *Eco*RI to remove the 2.4 kb disrupted *hasF* and the ends of the fragment were made flush with T4 DNA polymerase. The disrupted *hasF* gene was then ligated into the pKNG101 replacement vector resulting in pKNGhasFGm^r, which was then transformed into *E. coli* CC118 in similar fashion as above.

Continues with ‘The *sdeB* gene (Kumar & Worobec, 2005a) was recloned as...’

(b) Line 6, beginning with ‘The *sdeB* gene (Kumar & Worobec, 2005a) was recloned...’, replace with:

The *sdeB* gene (Kumar & Worobec 2005a) was cloned as a 1.7 kb *Eco*RI/*Eco*RI fragment into pKS, and transformed into UOC-67 in order to be disrupted by the insertion of a kanamycin resistance cassette into a unique *Pst*I restriction site.

Continues with ‘The *sdeR* gene (GenBank accession...’

(c) Line 15 of text, beginning with 'Conjugation between the *E. coli*...', replace with:

Conjugation between the *E. coli* strain harbouring each of the disrupted *S. marcescens* *sdeB*, *hasF* (both insertional and deletional mutations) and *sdeR* genes and the wild-type *S. marcescens* strain was carried out using *E. coli* helper strain MT616 (Finan *et al.*, 1986) to create SDEAB1, HASF100 (deletional mutation), HASF300 (insertional mutation) and SDER1, respectively.

Continues with 'Conjugation was also carried out...'

(d) Line 23, beginning with 'For the double *sdeB/hasF*-deficient...', replace with:

For the double *sdeB/hasF*-deficient mutant (SDEAB3/HASF300), conjugation was carried out between *E. coli* CC118(pKNGsdeB) and *S. marcescens* (HASF300), the *hasF*-deficient strain, using *E. coli* MT616 as a helper.

Continues with, 'Analysis of transconjugants was based on sucrose...'

4. Complementation of *sdeB*-, *hasF*- and *sdeR*-deficient strains, page 455, second column

(a) Line 34, beginning with 'For complementation purposes...', replace with:

For complementation purposes, the expression vector pEX1.8 was used for cloning a 1.5 kb wild-type *EcoRI/HindIII* *hasF* fragment (pEXH), a 3.1 kb wild-type *EcoRI/HindIII* *sdeB* fragment (pEXS) and a 0.4 kb wild-type *EcoRI/HindIII* *sdeR* fragment (pEXR).

Continues with 'In addition, *hasF* and *sdeB* were introduced...'

(b) Line 39, beginning with 'To complement both *hasF* and *sdeB*...', replace with:

To complement both *hasF* and *sdeB* simultaneously, a 3.1 kb *EcoRI/EcoRI* *sdeB* fragment was cloned into the *EcoRI/EcoRI* site, and a 1.5 kb *hasF* *HindIII/HindIII* fragment was cloned into the *HindIII/HindIII* site of pEX1.8 (pEXSH).

Continues with 'To construct strain SM2000, pEXH was...'

References

- Finan, T. M., Kunkel, B., De Vos, G. F. & Signer, E. R. (1986). Second symbiotic megaplasmid in *Rhizobium meliloti* carrying exopolysaccharide and thiamine synthesis genes. *J Bacteriol* **167**, 66–72.
- Kaniga, K., Delor, I. & Cornelis, G. R. (1991). A wide-host-range suicide vector for improving reverse genetics in Gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*. *Gene* **109**, 137–141.

Kumar, A. & Worobec, E. A. (2005a). Cloning, sequencing and characterization of the SdeAB multidrug efflux pump of *Serratia marcescens*. *Antimicrob Agents Chemother* **49**, 1495–1501.

Pearson, J. P., Pesci, E. C. & Iglewski, B. H. (1997). Roles of *Pseudomonas aeruginosa* *las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J Bacteriol* **179**, 5756–5757.

Schweizer, H. D. (1993). Small broad-host-range gentamycin resistance gene cassettes for site-specific insertion and deletion mutagenesis. *Biotechniques* **15**, 831–834.

Table 1. Bacterial strains and plasmids used in the study

Abbreviations: Kan, kanamycin; Amp, ampicillin; Str, streptomycin; Car, carbenicillin; Gm, gentamicin.

Strain or plasmid	Characteristics	Source/reference
<i>E. coli</i>		
MT616	MT607 (<i>pro-82 thi-I hsdR17 supE44</i>):pRK600	Finan <i>et al.</i> (1986)
CC118	<i>araD139 Δ(ara leu)7697 ΔlacX74 phoA20 galE galK thi rpsE rpoB argEam recA1</i>	Dr C. Manoil, University of Washington, Seattle, USA
<i>S. marcescens</i>		
UOC-67	Wild-type, ATCC 13880	American Type Culture Collection
T-861	Clinical isolate	D. Hoban, Health Science Centre, Winnipeg
SDEAB1	<i>sdeB</i> -deficient UOC-67	This study
SDEAB2	<i>sdeB</i> -deficient T-861	This study
HASF100	<i>hasF</i> -deficient UOC-67 (<i>hasF</i> deletionally mutated)	This study
HASF200	<i>hasF</i> -deficient T-861 (<i>hasF</i> deletionally mutated)	This study
HASF300	<i>hasF</i> -deficient UOC-67 (<i>hasF</i> insertionally mutated)	This study
SDEAB3/HASF300	<i>sdeB/hasF</i> -deficient	This study
SDER1	<i>sdeR</i> -deficient UOC-67	This study
SDER2	SDER1/pEXR	This study
SM3000	SDEAB1 with pEXS	This study
SM2000	HASF300 with pEXH	This study
SM1000	SDEAB3/HASF300 with pEXSH	This study
SDER3	UOC-67/pEXR	This study
SDER4	UOC-67/pEX1.8	This study
Plasmids		
pKS (+)	ColE1 replicon, Amp ^r , <i>lacZ'</i>	Stratagene
pKS:SdeB	pKS with a 1.7 kb <i>EcoRI/EcoRI sdeB</i> fragment	This study
pKS:SdeB:Km ^r	pKS:SdeB with a 1.4 kb Kan ^r insertion in <i>sdeB</i>	This study
pKS:HasF	pKS with a 1.5 kb <i>EcoRI/EcoRI hasF</i> fragment	This study
pKS:ΔHasF	pKS:HasF with 700 bp <i>HincII/KpnI</i> fragment deleted	This study
pKS:hasF:Gm ^r	pKS:HasF with a 900 bp Gm ^r insertion in the <i>EcoRI/EcoRI</i> -flanked <i>hasF</i> fragment	This study
pKS:SdeR	pKS with 400 bp <i>BamHI/EcoRI sdeR</i> fragment	This study
pKS:SdeR:Km ^r	pKS:SdeR with a 1.4 kb Kan ^r insertion in <i>sdeR</i>	This study
pEX1.8	ColE1 replicon, Amp ^r -Car ^r , carries <i>lacI^Q</i> and a P _{tac} promoter	Pearson <i>et al.</i> (1997)
pEXSH	pEX1.8 with 3.1 kb <i>EcoRI/EcoRI sdeB</i> fragment and 1.5 kb <i>HindIII/HindIII hasF</i> fragment	This study
pEXH	pEX1.8 with wild-type 1.5 bp <i>EcoRI/HindIII hasF</i> fragment	This study
pEXS	pEX1.8 with wild-type 3.1 kb <i>EcoRI/HindIII sdeB</i> fragment	This study
pEXR	pEX1.8 with 405 bp <i>EcoRI/HindIII sdeR</i> fragment	This study
pKNG101	Suicide vector, <i>pir⁻ oriR6K mobRK2 sacB Str^r</i>	Kaniga <i>et al.</i> (1991)
pKNGsdeB	pKNG101 with 3.1 kb insertionally disrupted <i>sdeB</i> fragment	This study
pKNGhasF	pKNG101 with 800 bp deleted <i>hasF</i>	This study
pKNGhasFGm ^r	pKNG101 with 2.4 kb <i>hasF</i> insertion fragment	This study
pKNGsdeR	pKNG101 with 1.8 kb insertionally disrupted <i>sdeR</i> fragment	This study
pKIXX	1.4 kb Kan ^r cassette flanked by <i>SmaI</i>	Pharmacia PL Biochemicals
pUCGM	900 bp Gm ^r cassette flanked by <i>PstI</i>	Schweizer (1993)