Correction

Correction: LPS Unmasking of *Shigella flexneri* Reveals Preferential Localisation of Tagged Outer Membrane Protease IcsP to Septa and New Poles

The PLOS ONE Staff

There are errors in Figure 1, “Detection of IcsP/IcsPHA expression and activity on IcsA by Western immunoblotting.” Please see the corrected Figure 1 here.

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Figure 1. Detection of IcsP/IcsP^{HA} expression and activity on IcsA by Western immunoblotting. (A) *S. flexneri* strains 2457T and 2457T *icsP* mutant (*icsP*⁻) were grown in LB and whole cell lysate samples were taken at 0.5, 1, 1.5, 2, 2.5 and 3 h after subculture, followed by electrophoresis on a SDS 15 % polyacrylamide gel and Western immunoblotting with rabbit anti-IcsP antiserum; (B) *S. flexneri* strains 2457T, *icsP*⁻ and *icsP*⁻ harbouring *plcsP*, *plcsP*^{HA} or pBAD30 (as indicated) were grown in LB for 1.5 h to an OD600 reading of ~0.4, washed 3 times, and induced with arabinose for 1 h. Pellet and supernatant protein samples were then prepared and electrophoresed on a SDS 15 % polyacrylamide gel, followed by Western immunoblotting with rabbit anti-IcsA antibodies. The size of the full length IcsA protein (120 kDa) and the cleaved form of IcsA (95 kDa) are indicated; (C) *S. flexneri* strains 2457T and *icsP*⁻ harbouring *plcsP*^{HA} or pBAD30 were grown in LB as described in (B), followed by induction with 0%, 0.003%, 0.006%, 0.0125%, 0.025%, 0.05%, 0.1% or 0.2% (w/v) arabinose for 1 h. Whole cell lysates were prepared and electrophoresed on a SDS 15 % polyacrylamide gel, followed by Western immunoblotting with rabbit anti-IcsP antiserum. The size of the full length IcsP protein (36 kDa) is indicated in (A) and (C). Each lane contains 5 x 10⁷ bacterial cells of each strain.

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There are errors in Figure 4, “Effect of tunicamycin on the LPS of strains expressing IcsP^{HA}.” Please see the corrected Figure 4 here.

Figure 4. Effect of tunicamycin on the LPS of strains expressing IcsP^{HA}. (A) Smooth LPS 2457T *icsP*⁻ strains harbouring *plcsP*^{HA} or *pBAD30* were grown to an OD₆₀₀ reading of ~0.8 in LB, washed 3 times, and treated without TP (U), with PMBN only (P) or with TP treatment for 2 h. Strains were then induced with 0.003% (w/v) arabinose for 1 h, washed 3 times, and grown for an additional 3 h for restoration (R) of LPS Oag; (B) Rough LPS 2457T *icsP*⁻/*rmlD*⁻ strains harbouring *plcsP*^{HA} and either pRMA727 or pACYC184 (as indicated) were grown to an OD₆₀₀ reading of ~0.4 in LB, washed 3 times, and induced with arabinose for 1 h. LPS from strains described in (A) and (B) were isolated and detected by silver staining as described in the *Methods*. The first 15 Oag RUs are indicated on the side of each gel. Each lane contains ~2 × 10⁸ bacterial cells of each strain; (C) Western blots on whole cell lysates obtained from strains in (B) were probed with rabbit anti-IcsP antiserum. The size of the full length IcsP^{HA} protein (~36 kDa) is indicated. Each lane contains 5 × 10⁷ bacterial cells of each strain.
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There are errors in Figure 5, “Effect of LPS Oag-depletion in detection of surface IcsP^{HA}.” Please see the corrected Figure 5 here.

Figure 5. Effect of LPS Oag-depletion in detection of surface IcsP^{HA}. Smooth LPS 2457T *icsP* strains harbouring *plcsP^{HA}* were subcultured in LB broth to an OD₆₀₀ reading of ~0.8, washed 3 times in LB, and then further cultured for 2 h; (A) in the absence of TP, (B) in the presence of PMBN only, or (C) in the presence of TP. Arabinose was included in the final hour of treatment at a concentration of 0.03% (w/v). Samples were then fixed

and subjected to QD IF using antibodies to HA epitope and IcsA. Non-septating and septating cells (upper and lower rows respectively) are shown for each treatment group. Representative bacteria are shown. Scan = Single line-scans measuring the fluorescence intensity of IcsP^{HA} detected along the surface of the bacterium, Bars = 1 μ m, Arrows = direction of line-scan, Arrow heads = septa, Control = grown in absence of both tunicamycin and PMBN, P = PMBN, TP = tunicamycin/PMBN, GL = Grey level, Phase = phase contrast image, IcsP^{HA} = image of fluorescence at 525 nm, IcsA = image of fluorescence at 625 nm, Merge = overlay of IcsP^{HA} and IcsA images.
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Reference

1. Tran ENH, Doyle MT, Morona R (2013) LPS Unmasking of *Shigella flexneri* Reveals Preferential Localisation of Tagged Outer Membrane Protease IcsP to Septa and New Poles. PLoS ONE 8(7): e70508. doi:10.1371/journal.pone.0070508