Supplementary text

Supplementary materials and methods

Bacterial culture

Bacterial strains were grown in tryptic soy broth. For nafcillin challenge, the strains were grown in the presence of 0.25µg/ml nafcillin for 4h and then plated on tryptic soy agar (TSA) plates (antibiotics added as per requirement) and incubated at 37C for CFUs.

List of Strains

Strains	Description	Reference
SF8300ex	Derivative of SF8300 lacking mezA and blaZ	(1)
Δ*stk1*	SF8300ex lacking *stk1*	This study
Δ*stp1*	SF8300ex lacking *stp1*	This study
Δ*stp1*+E	Δ*stp1* containing pTX_{Δ} vector	This study
Δ*stp1*+wt	Δ*stp1* expressing wt *stp1*	This study
Δ*stp1*+SRB	Δ*stp1* expressing SRB *stp1* (G169S)	This study
Δ*stp1*+SRT	Δ*stp1* expressing SRT *stp1* (Q31X)	This study
SF8300ex [E]	SF8300ex with empty pAmilux vector	This study
SF8300ex [*Ppbp4*]	SF8300ex with pAmilux containing *pbp4* promoter of SF8300ex	This study
Δ*stk1* [E]	Δ*stk1* with empty pAmilux vector	This study
Δ*stk1* [*Ppbp4*]	Δ*stk1* with pAmilux containing *pbp4* promoter of SF8300ex	This study
Δ*stp1* [E]	Δ*stp1* with empty pAmilux vector	This study
Δ*stp1* [*Ppbp4*]	Δ*stp1* with pAmilux containing *pbp4* promoter of SF8300ex	This study

List of Primers

Primer	Sequence	Description
Stp1-for	AAAGGATCCACAAGTAGAAACGAGGTAAAGACAAATGC	*stp1* cloning in pTX_{Δ}
Stp1-rev	ATTACGCGTATCATACTTTATCACCTTCAATAGCCGC	*stp1* cloning in pTX_{Δ}
stp1-stk1-P1	ACAAGAGCTCCAAAAATTTCGAGCGAAACAGATTTTTGAATGG	*stp1* & *stk1* deletion
stp1-P2	TTTATTATTTTACCTATCATTTGTCTTTACCTCGTTTCTACTTGTCGTTCCTTTGC	*stp1* deletion
stp1-P3	ATGATAGGTAAAATAATAAATGAACGATATAAAATTGTAGATAAGC	*stp1* deletion
stp1-P4	TACACCCCCGGGTCTGGCTTTTGGAATTGCTGATGATGAGCAGGCCC	*stp1* deletion
stk1-P2	TACATTTACTTCAATTATATTCATACTTTATCACCTTCAATAGCCGCGAGTATGAAAGTAACG	*stk1* deletion
stk1-P3	ATATAATTGAAGTAAATGTACCGAGGTTTCTATTTGGAAGTC	*stk1* deletion
stk1-P4	TATTCCCGGGTATCTCTAATTGATGCTTAACATTACAATTAGG	*stk1* deletion

List of plasmids

Plasmid	Description	Reference
pTX_{Δ}	Used as vector control	(1)
pTX_{Δ}+*stp1*_wt	Used to constitutively express wt *stp1*	This study
pTX_{Δ}+*stp1*_SRB	Used to constitutively express SRB *stp1*	This study
pTX_{Δ}+*stp1*_SRT	Used to constitutively express SRT *stp1*	This study
pJB38	Used for creation of Δ*stk1* and Δ*stp1* mutants	(2)
pAmilux	Used as vector control for reporter assay	(1)
pAmilux+*Ppbp4*	Used for reporter assay for *pbp4* promoter	(1)

Fig S1

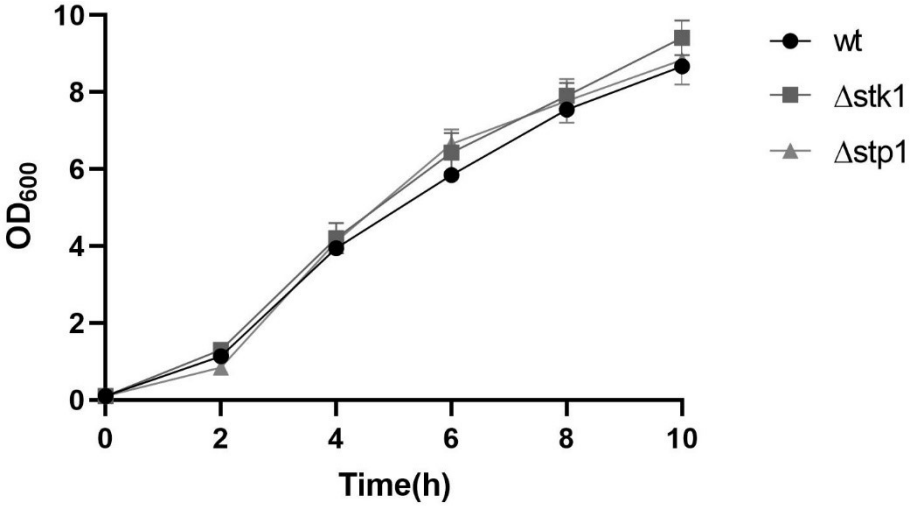

Fig. S1. Growth profiles of SF8300ex (wt), Δ*stk1* and Δ*stp1* in TSB. Experiments were repeated at least twice.

Fig. S2

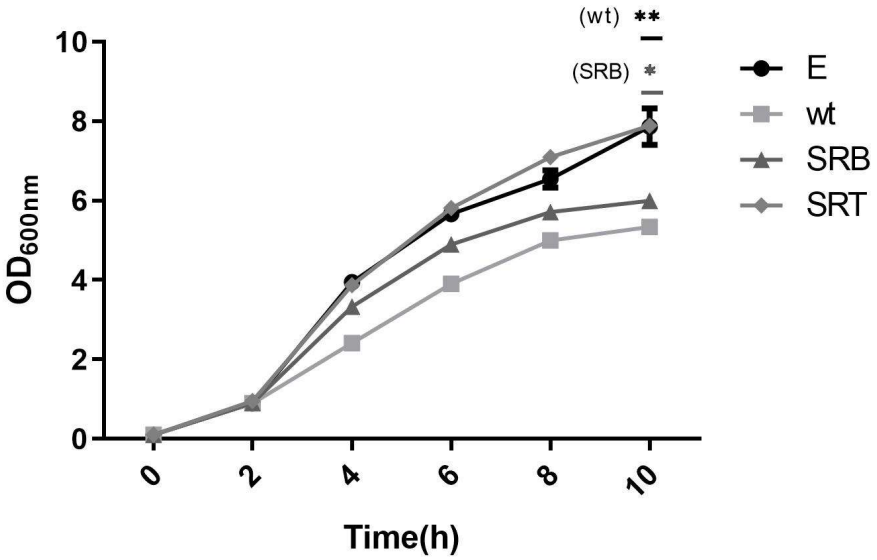

Fig. S2. Growth profiles of Δ*stp1* complemented strains in media in TSB. Δstp1 complemented with empty vector pTX_{Δ} (E), wild type *stp1* (wt) and mutant *stp1s*

G169S (SRB) and Q31X (SRT). *; P=0.0157, **; P=0.0055. Student's t test used to analyze the *P* values using GraphPad prism software. Experiments were repeated at least twice.

Fig. S3

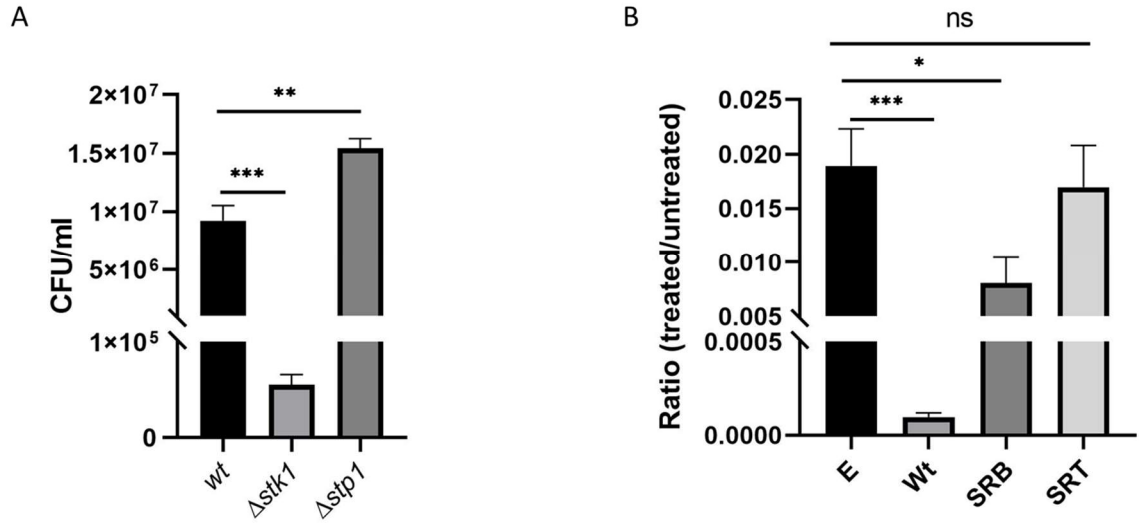

Fig. S3 Deletion of *stp1* facilitates resistance in SF8300ex. (A) CFU/ml of knock out strains (Δ*stk1* and Δ*stp1*) and wild type SF8300ex after 4h of 2µg/ml cefoxitin treatment. (B) Ratio of survival of Δ*stp1* complemented strains after 4h of 2µg/ml cefoxitin treatment. Δ*stp1* complemented with empty vector pTX_{Δ} (E), wild type *stp1* (wt) and mutant *stp1*s G169S (SRB) and Q31X (SRT). *; P=0.01, **;P= 0.0022, ***; P ≤ 0.0007. Student's t test used to analyze the *P* values using GraphPad prism software. All the experiments were repeated at least twice.

Fig. S4

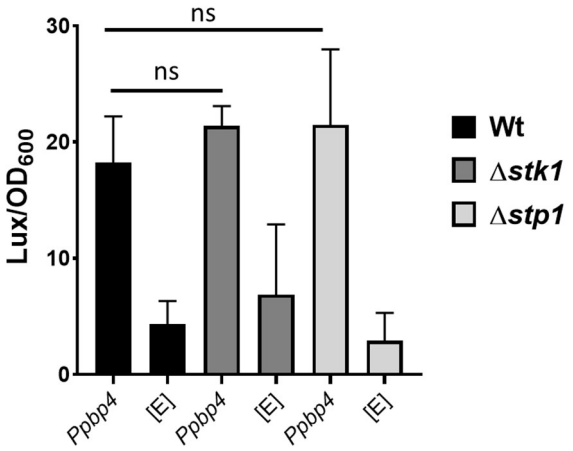

Fig. S4 *pbp4* expression in Δ*stk1* and Δ*stp1* strains of SF8300ex strain. *pbp4* promoter from wild type were cloned in to lux reporter plasmid *pAmilux*. The resultant plasmid and its empty vector [E] were transformed into SF8300ex and its Δ*stk1* and Δ*stp1* strains. Lux signals and bacterial OD_{600} were measured at 2h post culture and data are presented as lux signal divided by OD_{600}. Student's t test used to analyze the *P* values between Wt *Ppbp4* and Δ*stk1 Ppbp4* and Δ*stp1 Ppbp4* using GraphPad prism software. Experiments were repeated at least twice.

References

1. Chatterjee SS, Chen L, Gilbert A, da Costa TM, Nair V, Datta SK, Kreiswirth BN, Chambers HF. 2017. PBP4 Mediates beta-Lactam Resistance by Altered Function. Antimicrob Agents Chemother 61.
2. Basuino L, Jousselin A, Alexander JAN, Strynadka NCJ, Pinho MG, Chambers HF, Chatterjee SS. 2018. PBP4 activity and its overexpression are necessary for PBP4-mediated high-level beta-lactam resistance. J Antimicrob Chemother 73:1177-1180.