# Does *Staphylococcus aureus* use copper resistance to evade host immune responses?

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## Introduction:

Copper is an essential trace element used in the cellular metabolism of Prokaryotes and Eukaryotes due to it's redox potential ($Cu^{+} \leftrightarrow Cu^{2+}$). This transition metal is also toxic if present beyond cellular needs resulting in copper toxicity & ooxidative stress caused by Fenton chemistry.

Macrophage phagocytosis involve killing of intracellular bacteria with toxic levels of copper, [1] and several bacterial pathogens including S*treptococcus pneumoniae* and *Mycobacterium tuberculosis*, require transition metal efflux and detoxification systems to thrive inside hosts cells [2]. These pathogens have been shown to utilize copper resistance genes for full virulence in pulmonary, intraperitoneal and intravenous models of infections [3-5].

In *S. aureus* copper resistance is mediated by P-type efflux transporters; conserved CopA and unique CopB, which is found in pathogenic strains either as part of a replicating plasmid or incorporated into the chromosome (Table 1). The *copB* gene is part of the same operon as the gene encoding multicopper oxidase (mco).

Table 1. CsoR – controlled expression of copper resistence genes in *S. aureus*.

| Strain/ copper avaiablity | Transcriptional regulation of copper resistance by CsoR | Resistance |
|---|---|---|
| MRSA wt/ - $Cu^{2+}$ | REPRESSION | LOW |
| MRSA wt/ + $Cu^{2+}$ | DEREPRESSION | HIGH |
| MRSA CsoR CHC/ + $Cu^{2+}$ | REPRESSION | LOW |

Expression of *copA* and *copB/mco* is regulated by the CsoR repressor controlled by the availability of $Cu^{2+}$ [6] (Table 1). In the absence of copper CsoR represses copper resistance genes. Upon copper availability CsoR-coordinates $Cu^{2+}$ derepressing transcription of copper resistance genes, leading to an increase in metal tolerance of the bacteria. A CsoR C41A/H66A/C70A (CHC) variant defective in $Cu^{2+}$ co-ordination represses copper resistance genes despite metal availability (Table 1).

## Aim:

To investigate the role of copper resistance in promoting MRSA survival against phagocytic killing by macrophages & in human blood.

## Determinants of hyper-resistance to Copper

MRSA isolates used in the study carry copper resistance genes, either as part of their chromosome (ch) or a replicating plasmid (p) (Table 2). A mutant of MRSA252 strain defective in CsoR-coordination of $Cu^{2+}$ (CsoR C41A/H66A/C70A) was made by allelic exchange using a temperature sensitive plasmid pIMAY.

Table 2. Copper resistance genes & resulting levels of $Cu^{2+}$ tolerance by MRSA strains.

| Strains | *copA* | *copB* | *mco* | MIC $Cu_2S0_4$ (mM) |
|---|---|---|---|---|
| USA300 (CA-MRSA) | Yes (ch) | No | No | 4 |
| USA300 *copA::Tn* | No | No | No | 4 |
| 14-2533T | Yes (ch) | No | No | 5 |
| 14-2533T *csoR* CHC | Yes (ch) | No | No | 5 |
| 14-2533T pSCBU | Yes (ch) | Yes (p) | Yes (p) | 10 |
| 14-2533T pSCBUΔ*mco* | Yes (ch) | Yes (p) | No | 10 |
| 14-2533T pSCBUΔ*copB* | Yes (ch) | No | Yes (p) | 10 |
| MRSA252 | Yes (ch) | Yes (ch) | Yes (ch) | 8 |
| MRSA252 *csoR* CHC | Yes (ch) | Yes (ch) | Yes (ch) | 5 |
| MRSA252Δ*mco* | Yes (ch) | Yes (ch) | No | 5 |
| MRSA252Δ*copB* | Yes (ch) | No | Yes (ch) | 5 |

## CsoR Cys41 His66A & Cys70 are essential for $Cu^{2+}$dependent derepression of copper resistance, both *in vitro* & *in vivo*

Fig. 1.

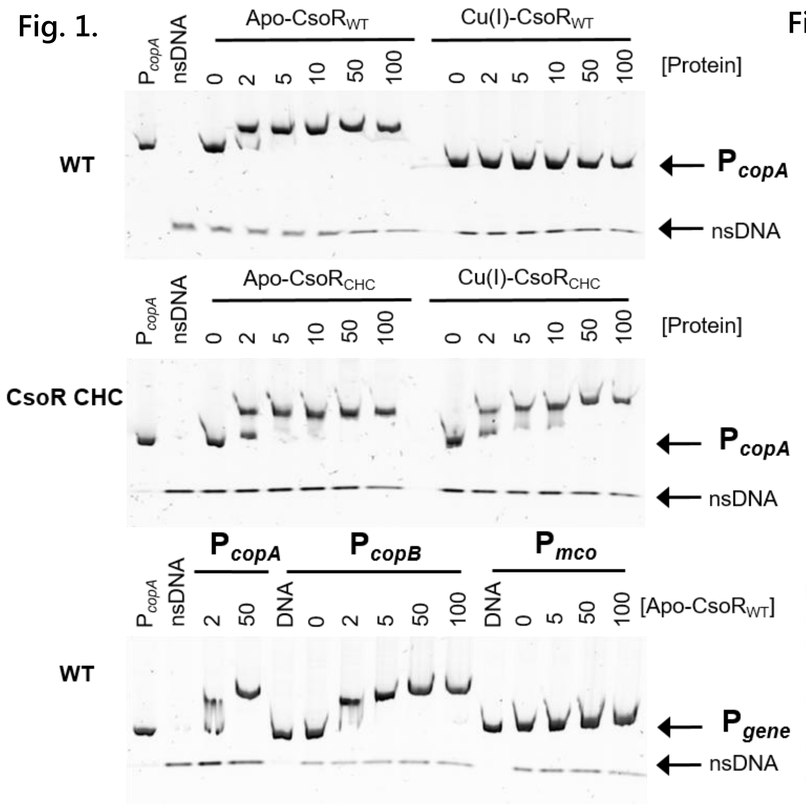


**Fig. 1. CsoR & CsoR CHC binds to the *copA & copB* but not the *mco* promoter sequence *in vitro*.** EMSA analysis was performed to test for Cu2+ dependent binding of purified recombinant CsoR and CsoR C41A/H66A/C70A (CHC) variant protein to the promoter sequences of the *copBmco* locus. PCR products (~200 bp) containing the sequences upstream of either promoter were mixed with a PCR product of non-specific DNA sequence (nsDNA), and probed for binding of purified recombinant CsoR variants.

Fig. 2.

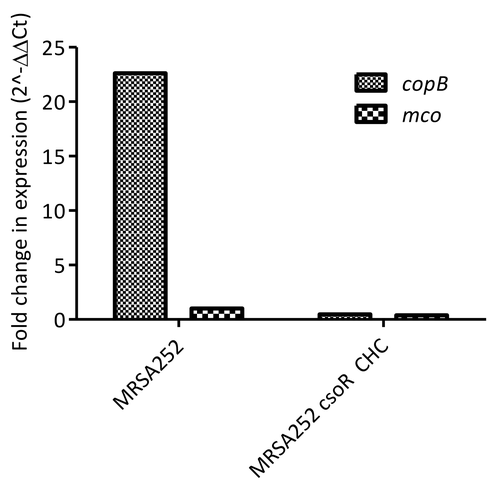


**Fig. 2. CsoR Cys41 His66A & Cys70 are required for *copB* upregulation during phagocytic killing in INFγ-activated macrophages in the presence of Cu2+.** qRT-PCR was used to determine the transcription of *copB and mco* during phagocytic killing or intracellular MRSA252 or MRSA252 CHC. ΔΔCT method was used to determine the relative change of expresion to applied houskeeping gene (*saeR*). Presented is the mean of n=2.

## CsoR-mediated hyper-resistance to copper promotes MRSA survival inside the macrophages & in whole huamn blood

Fig. 3.

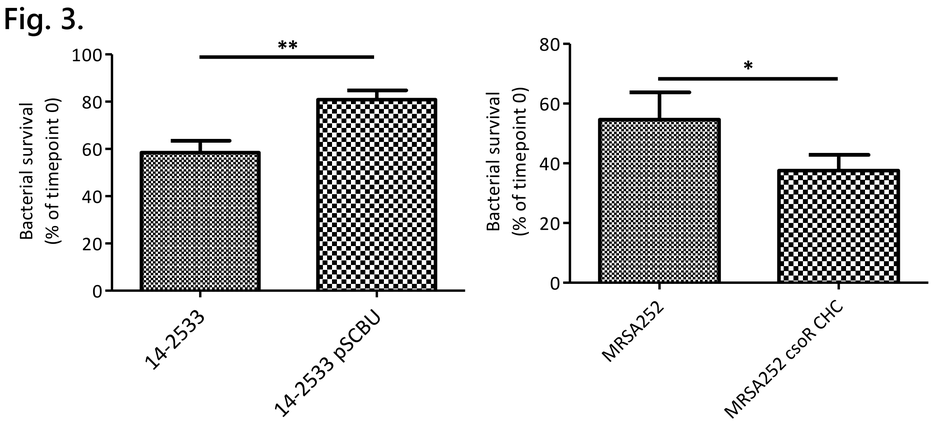


**Fig. 3. Increased survival of macrophage killing by copper resistant *S. aureus* variants.** Mouse macrophage RAW264.7 were seeded at $2\times10^6$ per mL in DMEM into 24-well plates and activated with mouse INF γ (40ng/mL) and $Cu_2SO_4$ (40μM) for 18h. S. aureus variants (as indicated were inoculated into the wells at an MOI of 10 in DMEM only allowing phagocytosis for 30min following killing of extracellular bacteria with gentamycin/lysostaphin for 30 min. Macrophage was lysed at this time (T0) and after 3h incubation (T3h) and intracellular bacteria were subjected to viable count to determine the levels of survival. The CsoR C41A/H66A/C70A expressing variant of the MRSA252 isolate is indicated as csoR CHC. Presented are means ± SD of three independent experiments. Statistical significance is indicated, ***P<0.005 * P<0.05*.

Fig. 4.

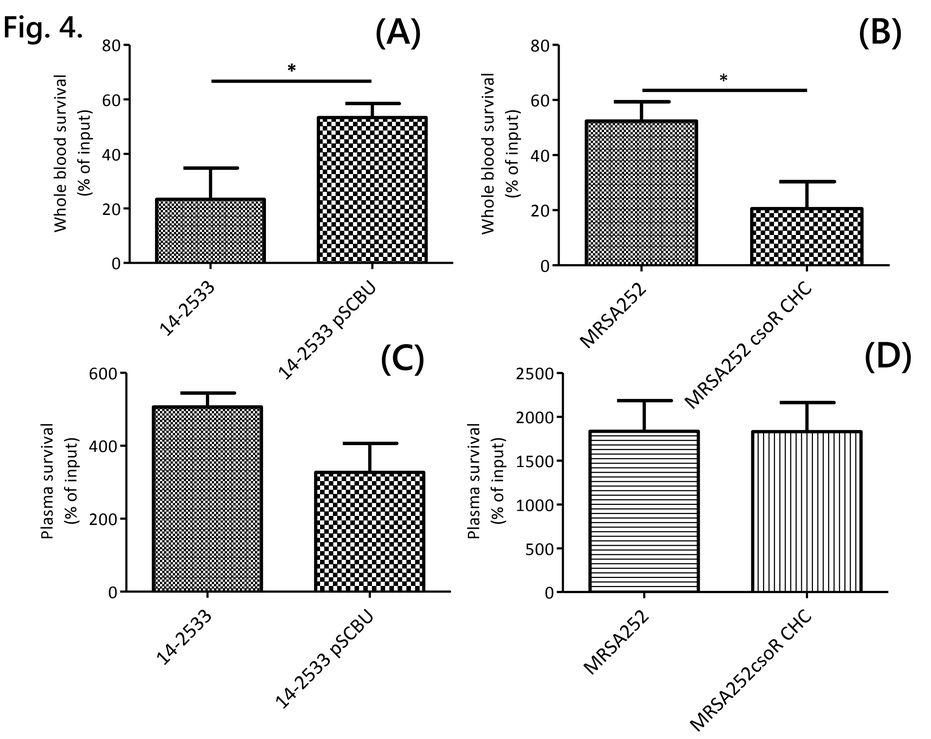


**Fig. 4. Increased survival of copper-resistant *S. aureus* in human blood.** *S. aureus* variants (ca. $1\times10^{-4}$ CFU/mL) were inoculated into freshly drawn human blood (A-B) or plasma fraction of the blood (CD). Following 3h incubation at 37°C with shaking blood and plasma were subjected to viable count to determine the levels of bacteria. Bacteria were rown in plasma as a control to determine that observed differences in whole blood survival are due to phagocytic killing. The CsoR C41A/H66A/C70A expressing variant of the MRSA252 isolate is indicated as csoR* (BD). Presented data are means ± SD of three independent experiments. Statistical significance is indicated, ** P<0.05*.

## Plasmid-expressed mco promotes growth of *S. aureus* in sub-inhibitory concentrations of $Cu^{2+}$

Fig. 5.

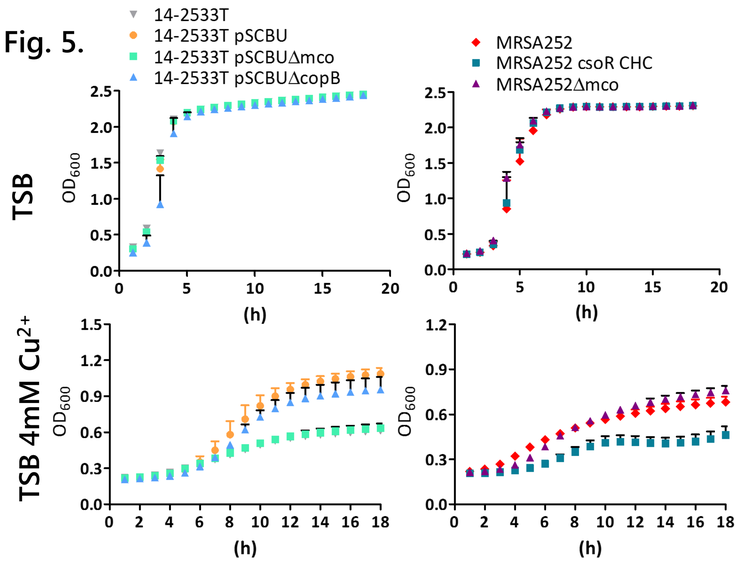


**Fig. 5. *S. aureus* growth in tryptic soy broth (TSA).** *S. aureus* strains (as indicated) were inoculated into 96-well plates containing either TSA alone (top) or supplemented with 4mM of $Cu_2S04$ (bottom). Presented data are representative of three independent experiments

## References:

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