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Deciphering the growth phase-dependent degradation kintics of antibiotic resistance gene of MRSA during chlorination

Yegyun Choi and Yunho Lee

Gwangju Institute of Science and Technology (GIST),
School of Earth Science and Environmental Engineering,

chldprbs@gm.gist.ac.kr

yhlee42@gist.a.kr

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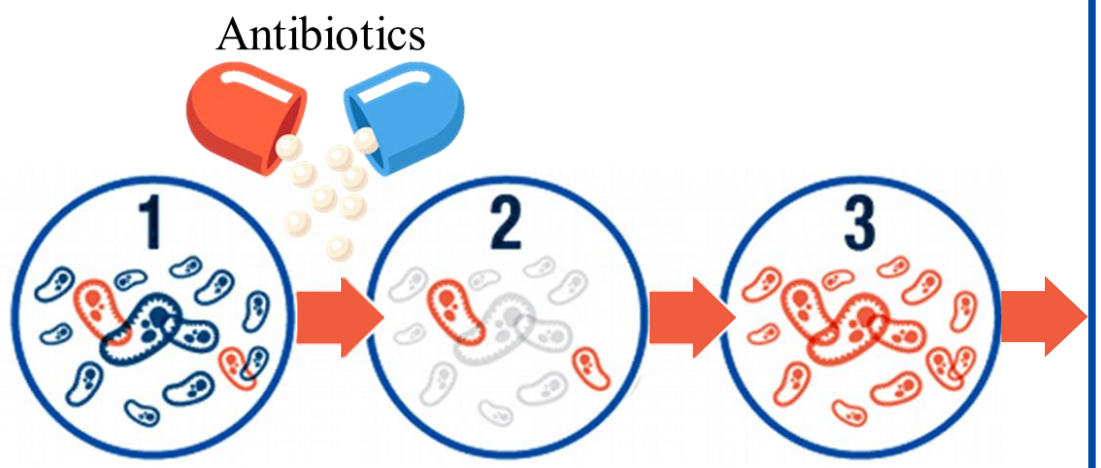
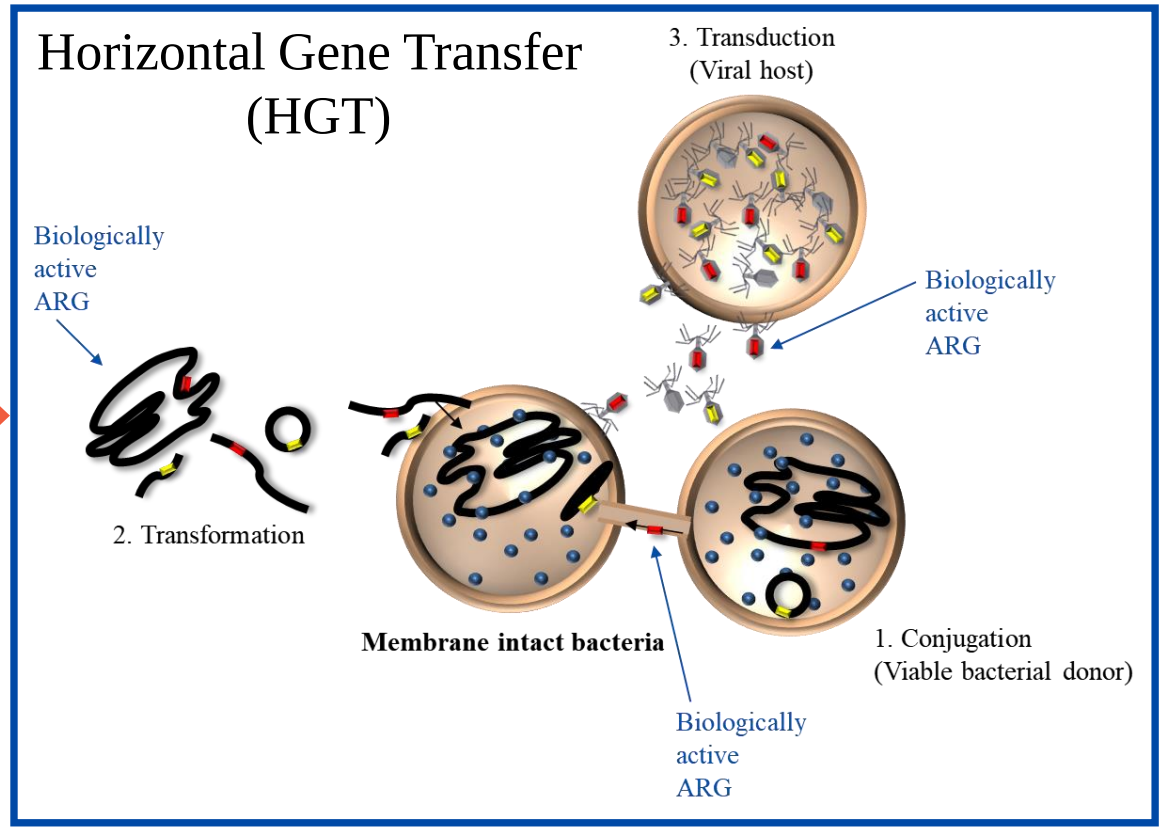
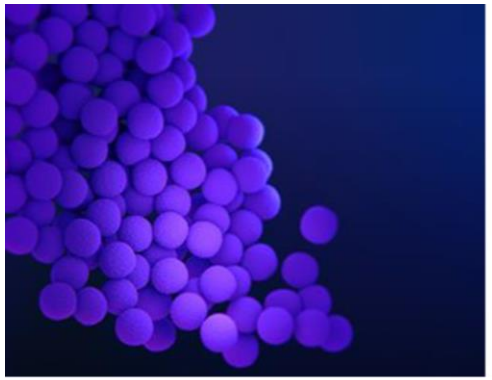
Introduction





- Long-term use of antibiotics ➔ The emergence of **antibiotic resistance (AR)**.
  (Can be a threat to public health by making antibiotics less effective)
- Can be disseminated through HGTs ➔ Known to transfers **antibiotic resistant genes (ARGs)**.

Introduction



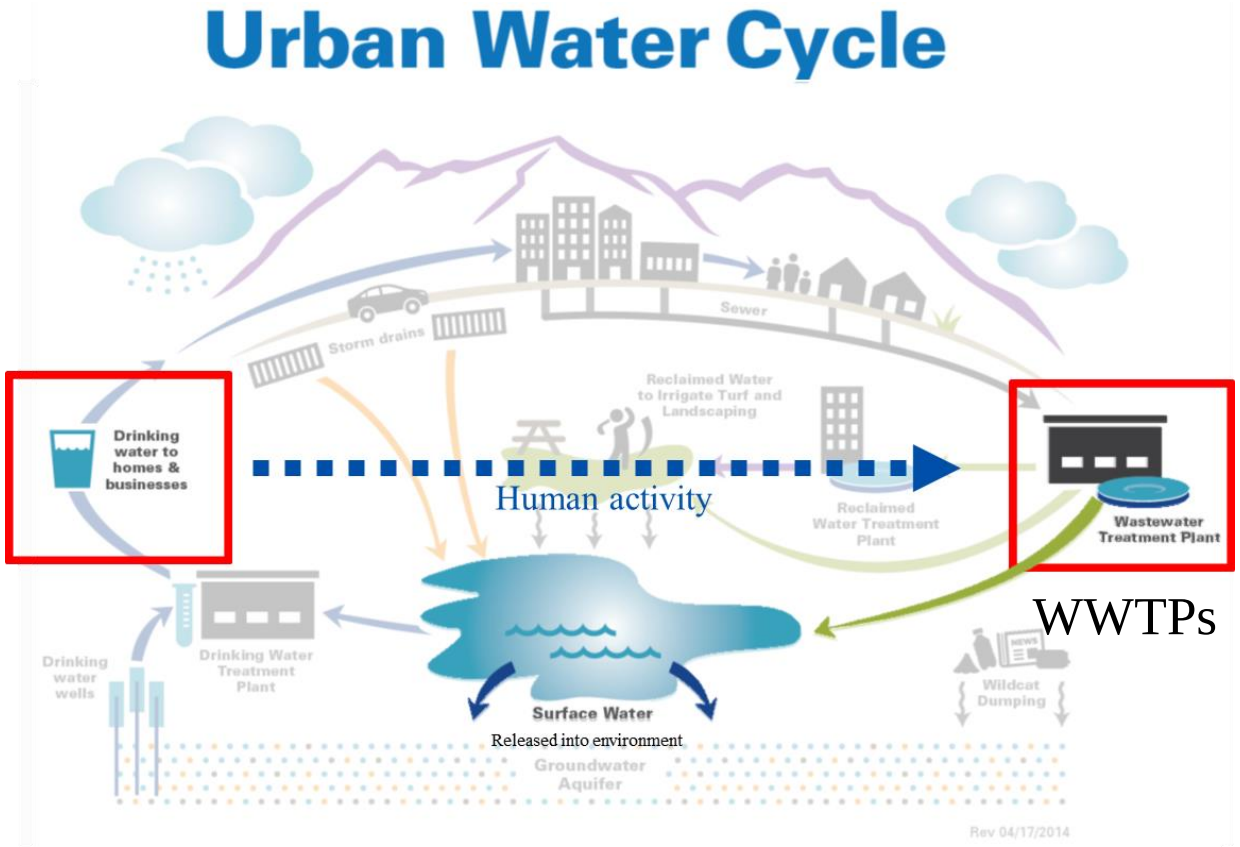
S.*aureus, mecA* gene

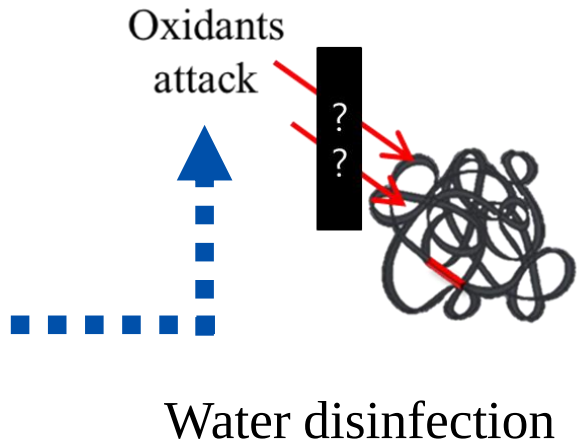
Enterococci, *vanA* gene

P.*araginosa*

- Methicillin resistant *Staphylococcus aureus*, **MRSA** ➔ One of representative antibiotic resistant bacteria (ARB).
- Contain chromosomally encoded ARG, ***mecA* gene**.
- The ***mecA* gene** considered as indicators to assess the AR status in environmental settings
  ➔ Used as representative ARB and ARG.

Introduction

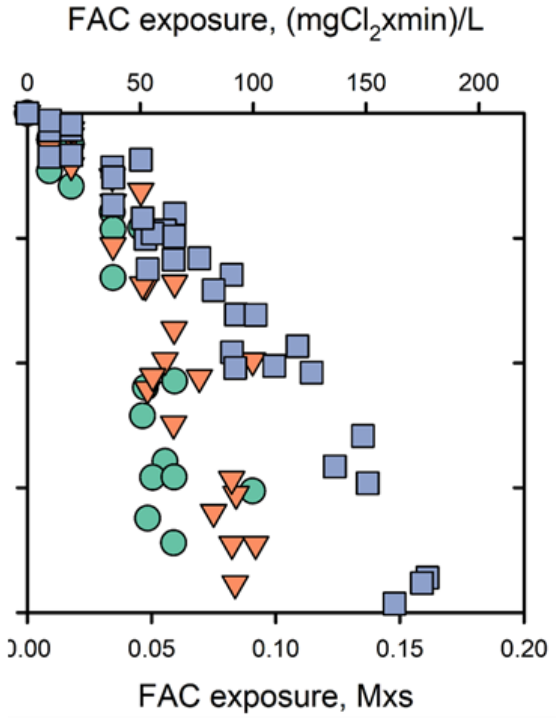




- **Wastewater treatment plants (WWTPs)** ➔ Hotspots for the dissemination of AR.
- **Water disinfection in WWTPs** could act as a barrier to the dissemination of AR.
- Some previous studies have investigated the degradation of ARGs during water disinfection.
- **Chlorine** is well known effective oxidants for ARG degradation.

Introduction

- **Extracellular-ARG (e-ARG)** degradation during chlorination ➔ **FAC two-hit model** (Huan et al.,2020).



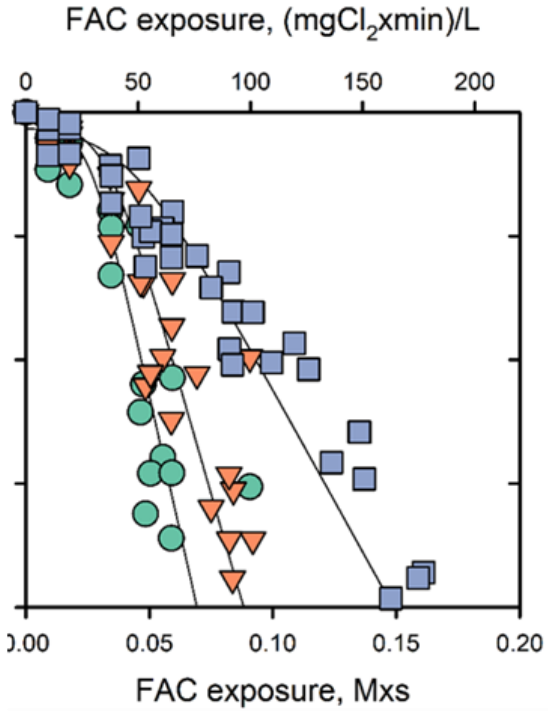
FAC two-hit model.

1. Reversible N-chlorination.

$$Amp + FAC \underset{Na_2S_2O_3}{\overset{k_{FAC,Amp}}{\rightleftharpoons}} Amp_{N\text{-}Cl}$$

2. Irreversible C-chlorination.

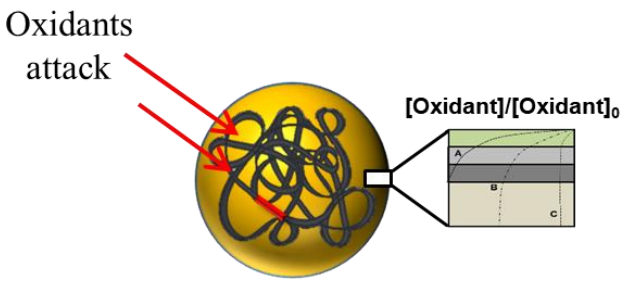
$$Amp_{N\text{-}Cl} + FAC \xrightarrow{k_{FAC,Amp_{N\text{-}Cl}}} Product$$



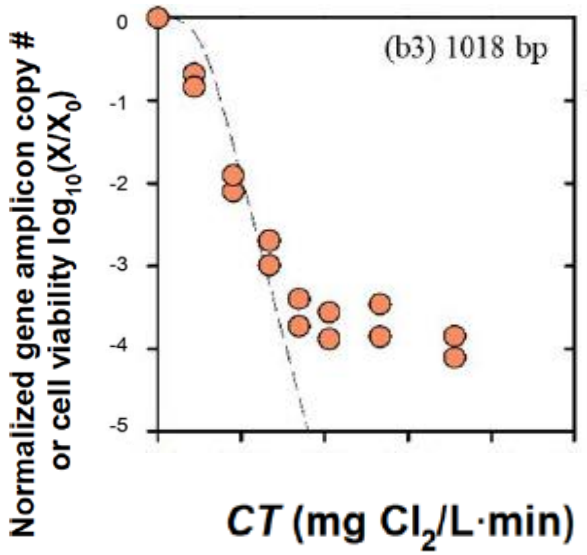
2020, EST, Huan et al

Used to explain the e-*mecA* degradation kinetics (Choi et al., 2021).

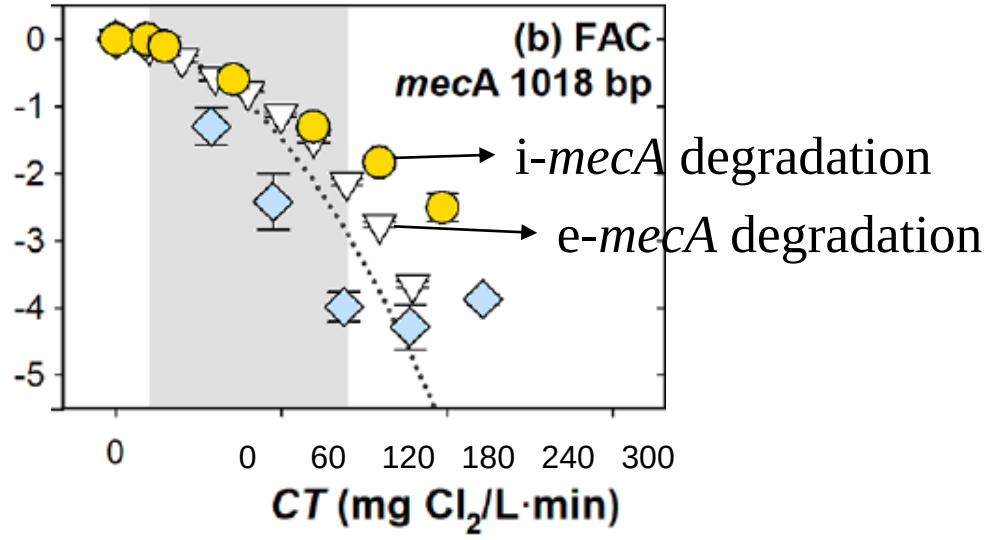
Introduction



- Intracellular-ARG (i-ARG) degradation during chlorination could be more complex ➔ Chlorine should be **diffused** into bacteria to react with i-ARGs.
- **Initial rapid loss of i-ARGs** was observed in **log-phase MRSA** chlorination. Not been consistently observed across cases in **stationary-phase MRSA**.



VS.

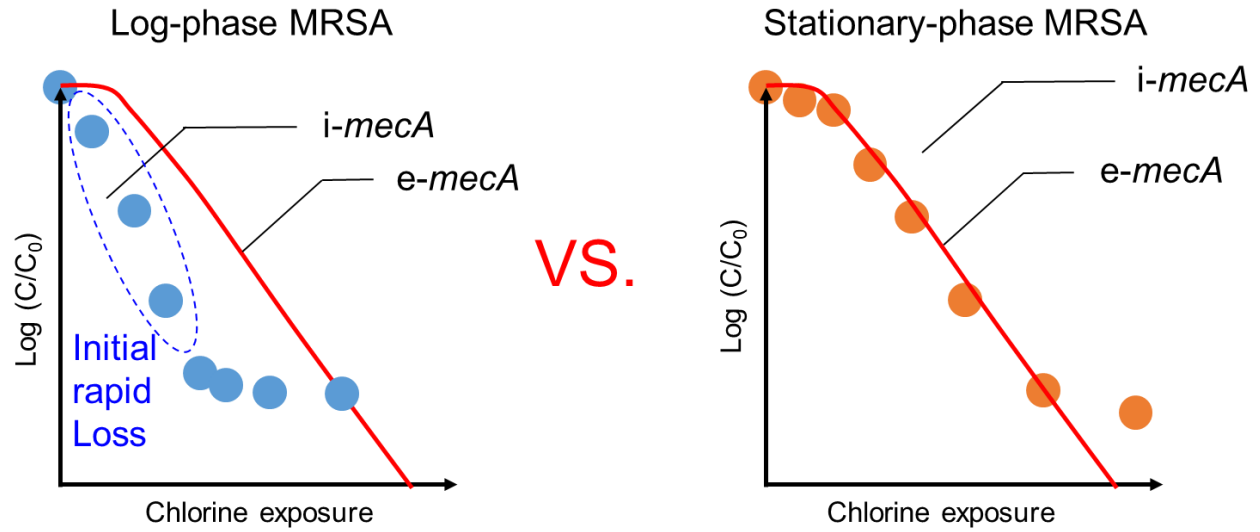


***S.Aureus* – *mecA* (Choi et al., 2021)**

***S.Aureus* – *mecA* (Huan et al., 2022)**

It remains unclear whether the bacterial growth stage influences the kinetics of i-ARG degradation.

Research objectives and scopes



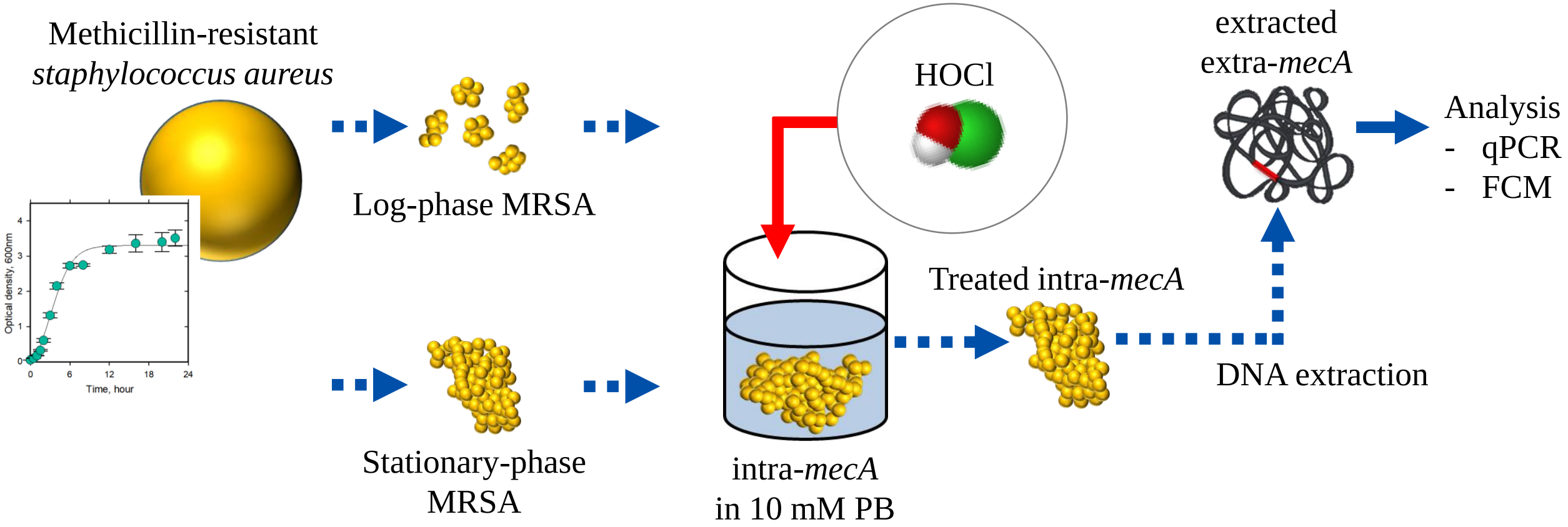
Research objectives

1. Investigating and comparing the degradation kinetics of i-*mecA* in log- and stationary-phase MRSA during the chlorination.

Research scopes

1. The *mecA* is housed on the staphylococcal cassette chromosome *mec* (SCC*mec*) and originated from a clinically relevant MRSA.
2. This study involved processing MRSA cells before and after chlorination for DNA extraction and quantification to determine the concentration of i-*mecA*.

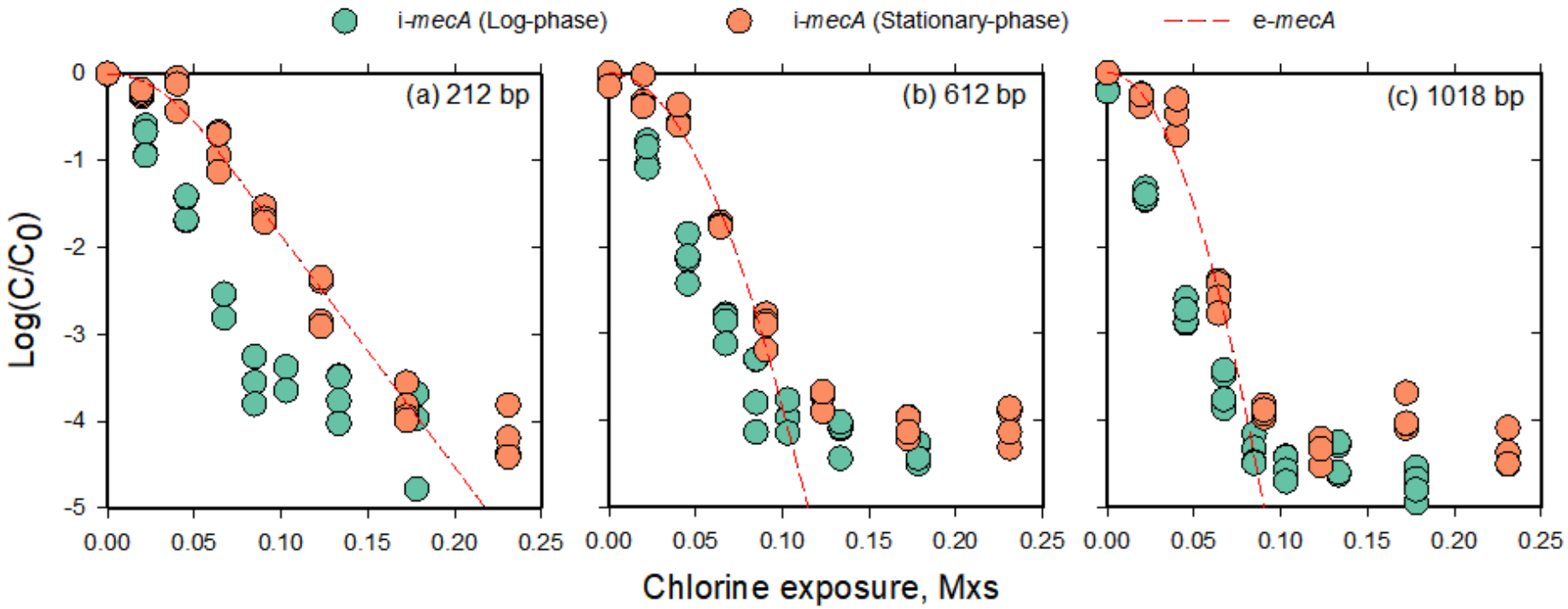
Materials & Methods



- Log- and stationary-phase MRSA were prepared by controlling growth times (5 vs. 16 hours).
- ~10^7 cell/ml MRSA / 10 and 20 mg Cl_2/L in 10 mM phosphate buffer (PB).
- Flowcytometry (FCM) / DNA extraction (Spin kit and PCI method) ➔ Quantitative-PCR (qPCR).

Results. Apparent i-*mecA* degradation kinetics before considering DNA recovery

Figure 1. Logarithmic relative concentration of i-*mecA* as a function of chlorine exposure.

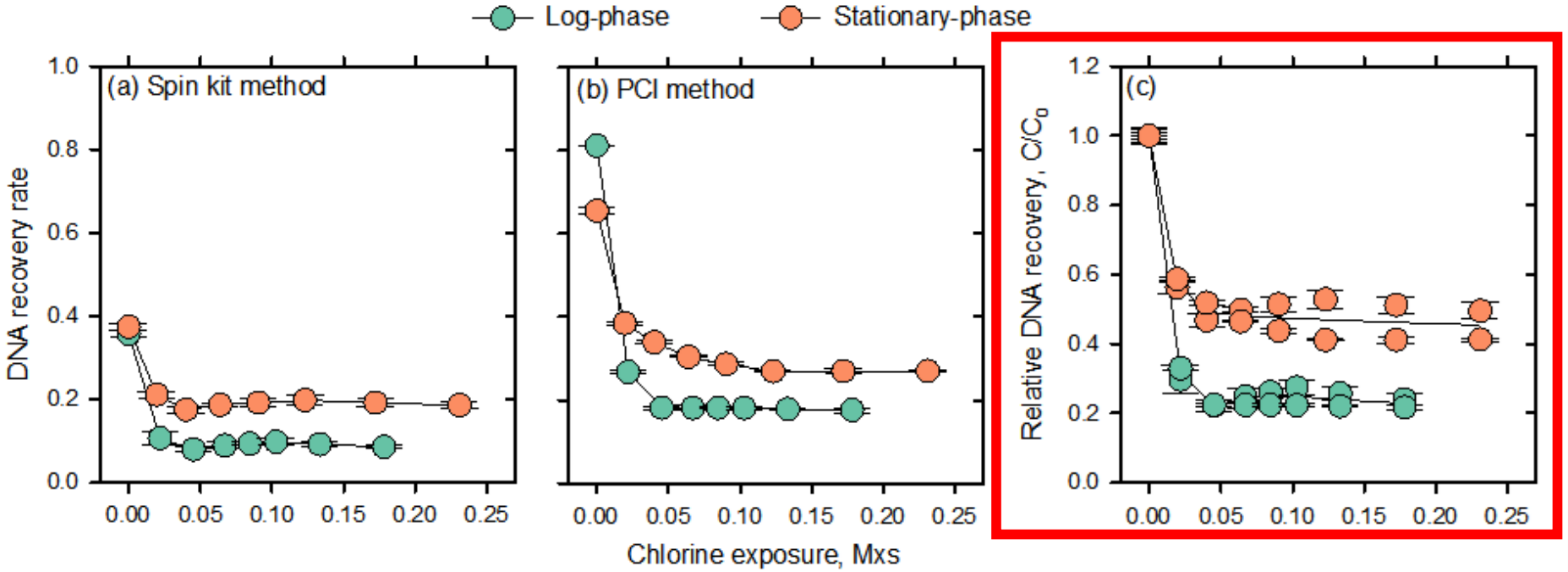


- Log-phase : Faster degradation of i-*mecA* compared with that of e-*mecA* + Tailing kinetics (ceased at ~4 log).
  Not significantly different for qPCR amplicon length ➔ Contradicts the general trend.
- Stationary-phase : The degradation of i-*mecA* was similar to that of e-*mecA*, except for the tailing kinetics.
  Significantly different for qPCR amplicon length.

➔ DNA recovery during DNA extraction might be the reason of this differences in degradation kinetics of i-*mecA*.

Results. Effect of chlorination on the DNA recovery efficiency from MRSA

Figure 2. DNA recovery rate from intact and chlorine-treated MRSA cells in log- and stationary-phase MRSA.



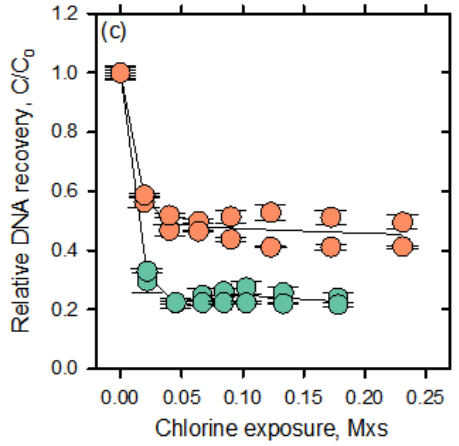
$$\text{DNA recovery} = \frac{C_{sample}}{C_{expected(cell\ numer)}}$$

$$\text{Relative DNA recovery} = \frac{C_{sample}}{C_{intact\ cell}}$$

- Relative DNA recovery : Results from both DNA extraction methods aligned well with each other.
- The DNA recovery significantly dropped right after chlorination : decreased by 77% in the log-phase .
  decreased by 55% in the stationary-phase.

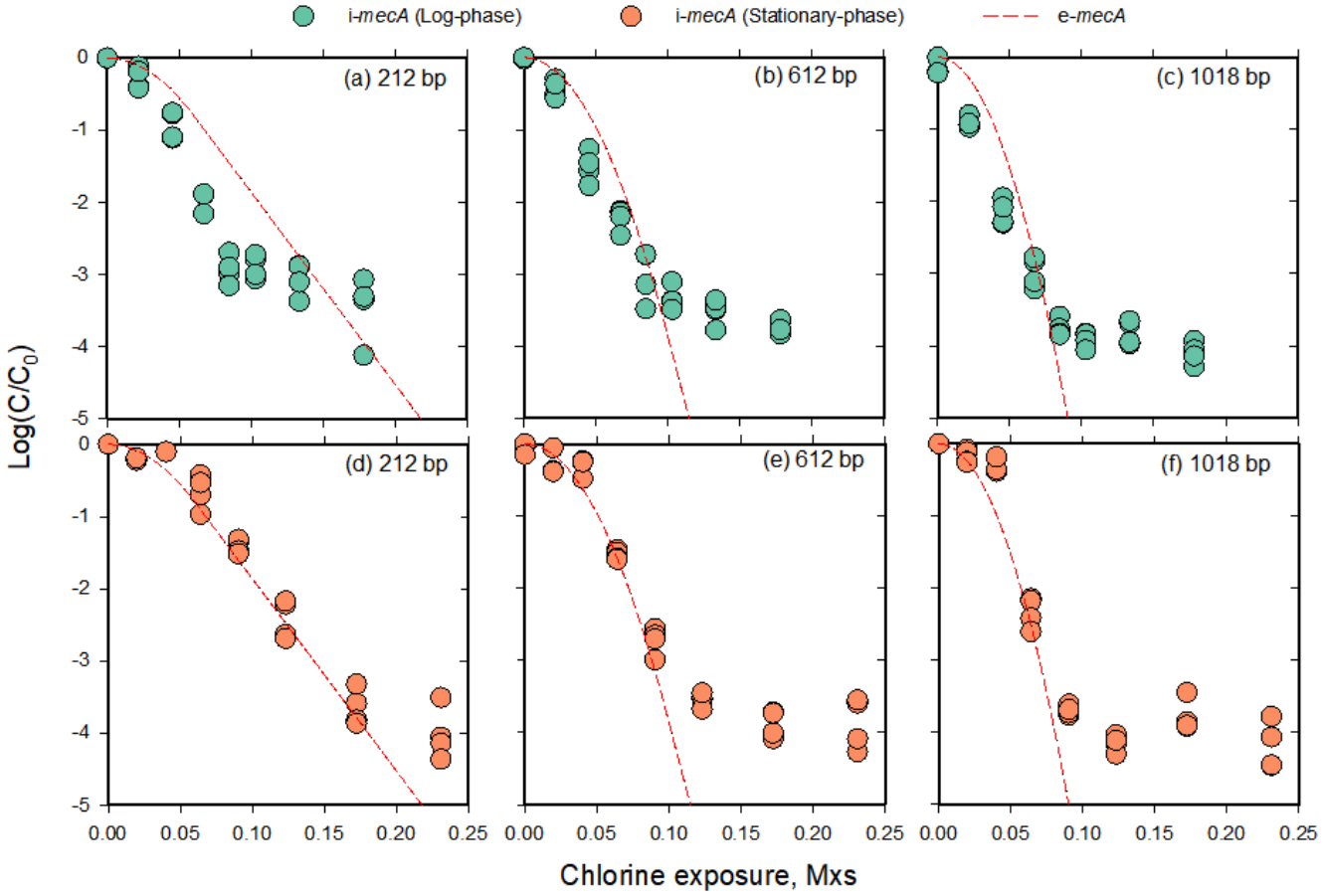
Results. Effect of chlorination on the DNA recovery efficiency from MRSA

- To compensate for the effect of variable DNA recoveries on the quantification of i-*mecA* degradation, i-*mecA* concentrations in each chlorine-treated sample were corrected.



Consideration of DNA recovery issue

$$C_{\text{intra-mecA,corrected}} = \frac{C_{\text{intra-mecA,sample}}}{\left[\frac{C_{\text{dsDNA,sample}}}{C_{\text{dsDNA,0}}}\right]}$$



Results. Degradation kinetics of i-*mecA* after correcting the DNA recovery change.

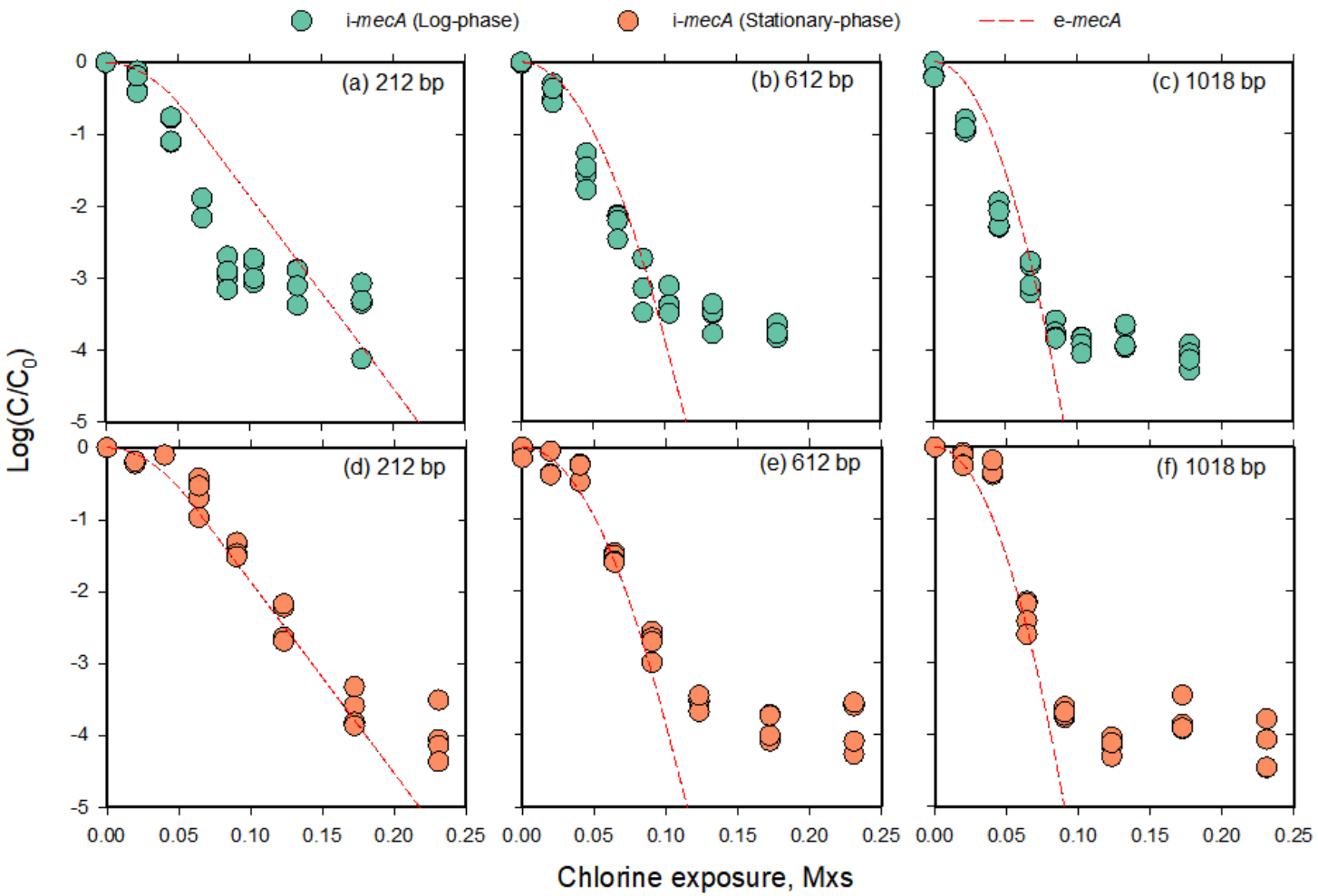


Figure3. Logarithmic relative concentration of i-*mecA* after correcting the DNA recovery.

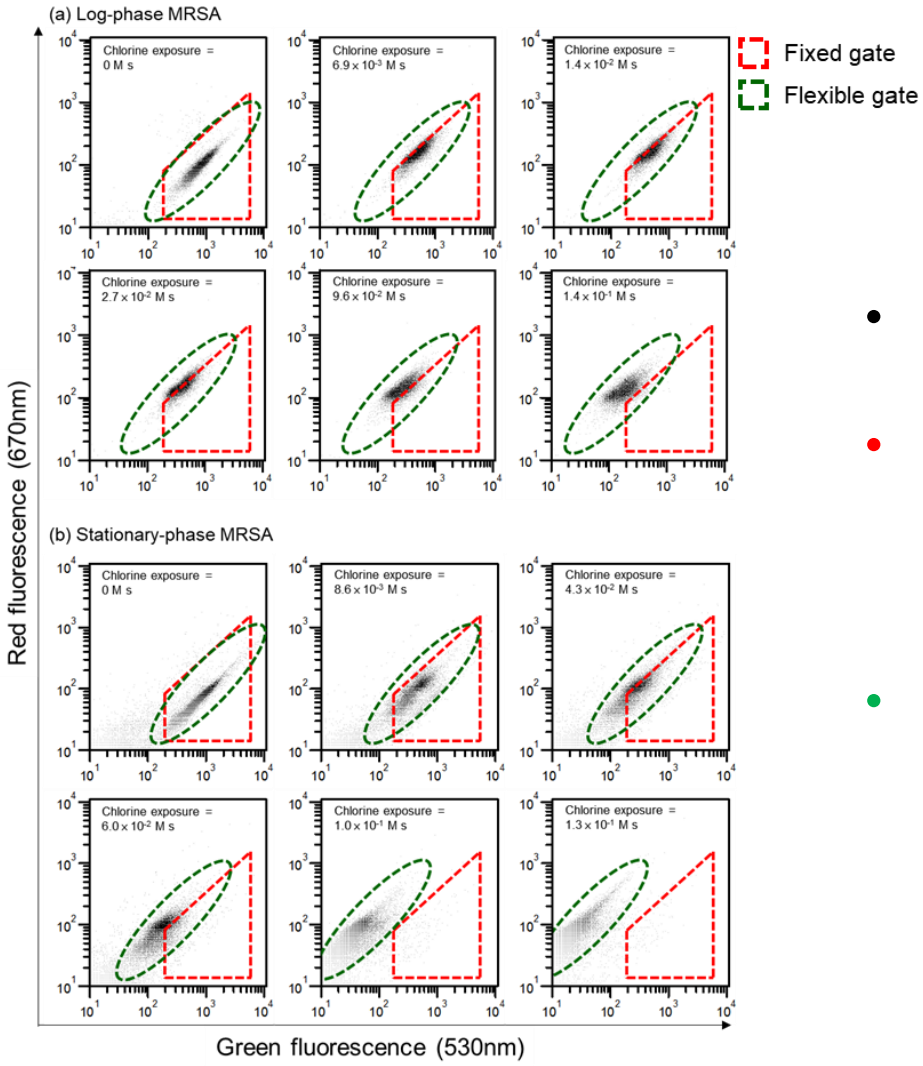
- Log-phase : Degradation kinetics of i-*mecA* more closely aligned with that of e-*mecA*, but remained faster
  ➔ DNA recovery contribute to the degradation of i-*mecA*.

- Stationary-phase : Little change after correction was occurred, but closely aligning with that of e-*mecA*
  ➔ DNA recovery got negligible effect on the degradation kinetics of i-*mecA*.

Overall, these results demonstrate the importance of accounting for variable DNA recovery when determining the degradation kinetics of i-*mecA* during chlorination.

Results. Why does the DNA recovery from MRSA cells decrease upon chlorination.

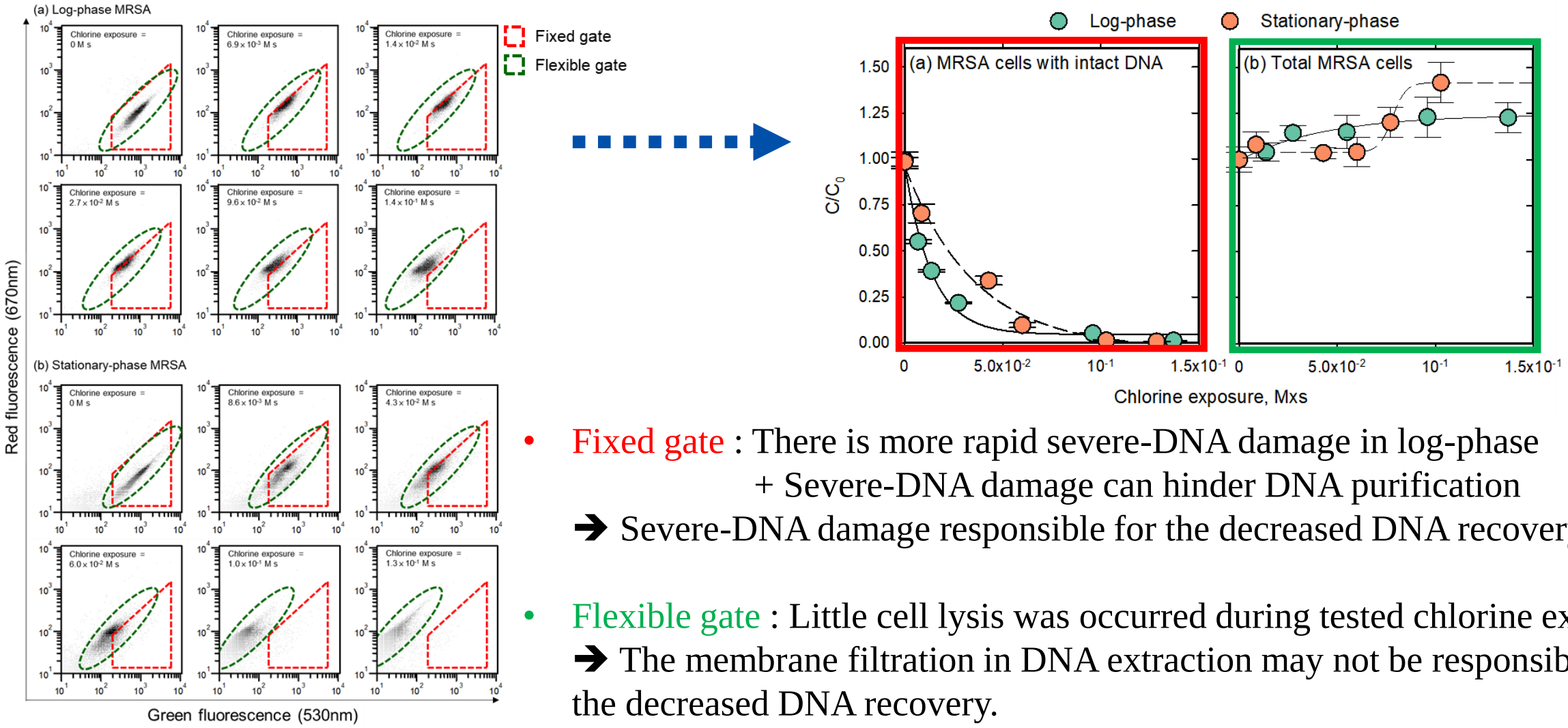
Figure 4. Density plots from flowcytometry (FCM) analysis as a function of chlorine exposure.



- Samples are stained with Syber Green I (SGI).
- The red trapezoids ➔ Indicate the fixed gates
  ➔ Individual cells without severe-DNA damage.
  (single-strand breaks / DNA fragmentation)
- The green circles ➔ Indicate the flexible gates
  ➔ Individual intact MRSA cells.

Results. Why does the DNA recovery from MRSA cells decrease upon chlorination.

Figure 5. Relative concentration of MRSA cells with intact DNA and total MRSA cells.



- Fixed gate : There is more rapid severe-DNA damage in log-phase
  + Severe-DNA damage can hinder DNA purification

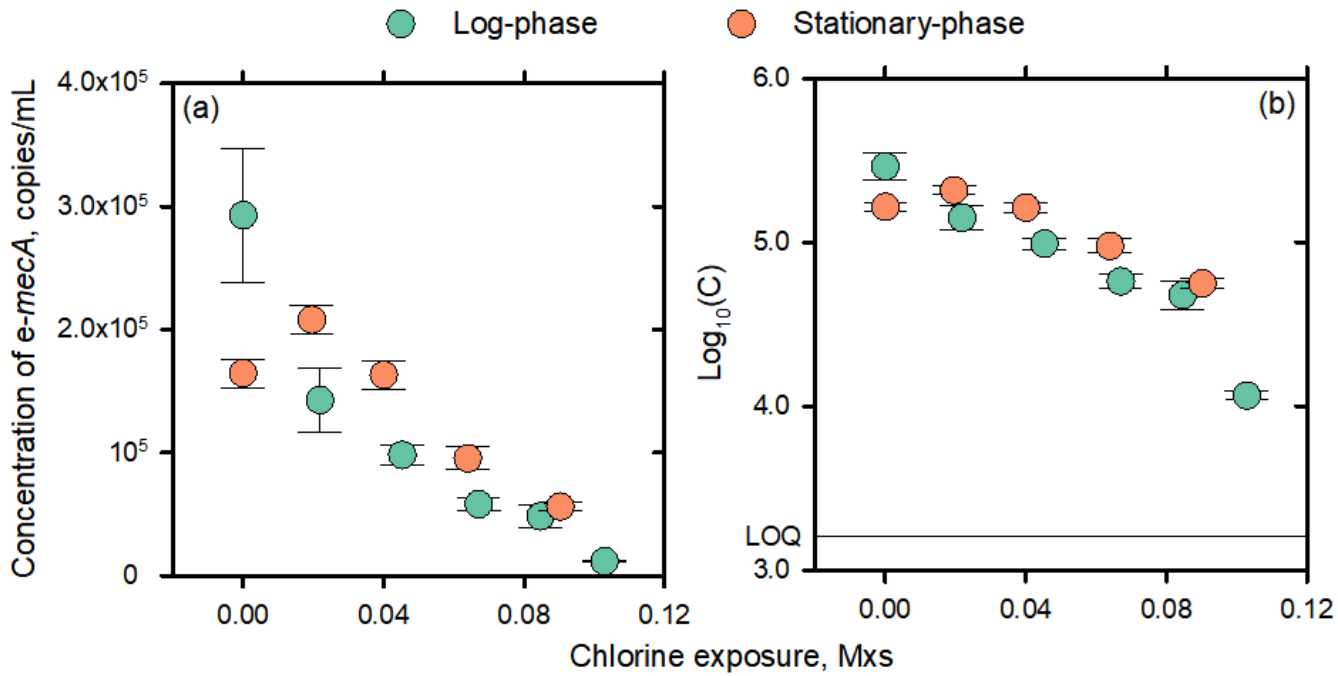
  ➔ Severe-DNA damage responsible for the decreased DNA recovery.

- Flexible gate : Little cell lysis was occurred during tested chlorine exposure

  ➔ The membrane filtration in DNA extraction may not be responsible for the decreased DNA recovery.

Results. Why does the DNA recovery from MRSA cells decrease upon chlorination.

Figure 6. Linear and logarithmic concentrations of e-*mecA* in the filtrates from membrane-filtered MRSA samples before and after chlorination at various chlorine exposures.



- A small fraction of the e-*mecA*, compared to i-*mecA* (~1%), was already present before chlorination.
- The concentration of e-*mecA* in the filtrates did not increase in both log- and stationary-phase during chlorination

  ➔ Leakage of i-*mecA* under the tested chlorine exposures might be insignificant.

Conclusions

- The degradation kinetics of i-*mecA* vary depending on the growth stage of MRSA.
- The rapid decrease in i-*mecA* for log-phase can be attributed to both decreased DNA recovery and actual degradation of i-*mecA*.
- Severe-DNA damage is responsible for the decreased DNA recovery, impacting the efficiency of the DNA extraction.

These conclusions emphasize

1. The importance of considering DNA recovery when assessing i-ARG degradation kinetics during chlorination to prevent overestimating i-ARG removal

2. Selecting the appropriate cell growth stage is crucial when evaluating i-ARG degradation using cultured antibiotic resistant bacteria.

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Article

Deciphering the Growth Phase-Dependent Degradation Kinetics of Antibiotic Resistance Gene of Methicillin-Resistant *Staphylococcus aureus* during Chlorination

Yegyun Choi, Seunggi Lee, and Yunho Lee*

Cite This: https://doi.org/10.1021/acsestwater.4c00171

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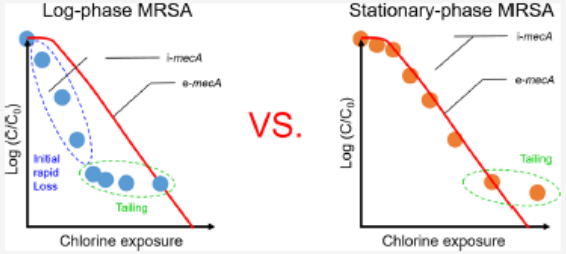
ABSTRACT: Degradation kinetics of the intracellular antibiotic resistance gene (i-ARG) during chlorination, specifically the *mecA* gene (i-*mecA*) in methicillin-resistant *Staphylococcus aureus* (MRSA), exhibit complexities influenced by bacterial growth stages. This study systematically investigated the i-*mecA* degradation kinetics in MRSA cultured in log and stationary phases. Using quantitative polymerase chain reaction, amplicons within i-*mecA* were measured, and markedly different i-*mecA* degradation behaviors under different growth conditions were observed. In log phase MRSA, i-*mecA* rapidly depleted at low chlorine exposures, contrasting with minimal loss in the stationary phase MRSA. This initial rapid loss in log phase could not be entirely accounted for by the decreased DNA recovery rate from MRSA following chlorination. Severe DNA damage, such as strand breaks, occurred more rapidly in log phase MRSA through investigations using flow cytometry, consistent with the initial rapid loss of i-*mecA*. Tailing kinetics were observed for both log and stationary phase MRSA, where i-*mecA* degradation slowed with prolonged chlorine exposure. The formation of MRSA aggregate and the emergence of a chlorine-resistant phenotype were proposed as responsible for the tailing kinetics in log and stationary phase MRSA, respectively. These findings provide valuable insights for evaluating and interpreting the i-ARG degradation kinetics during water chlorination.



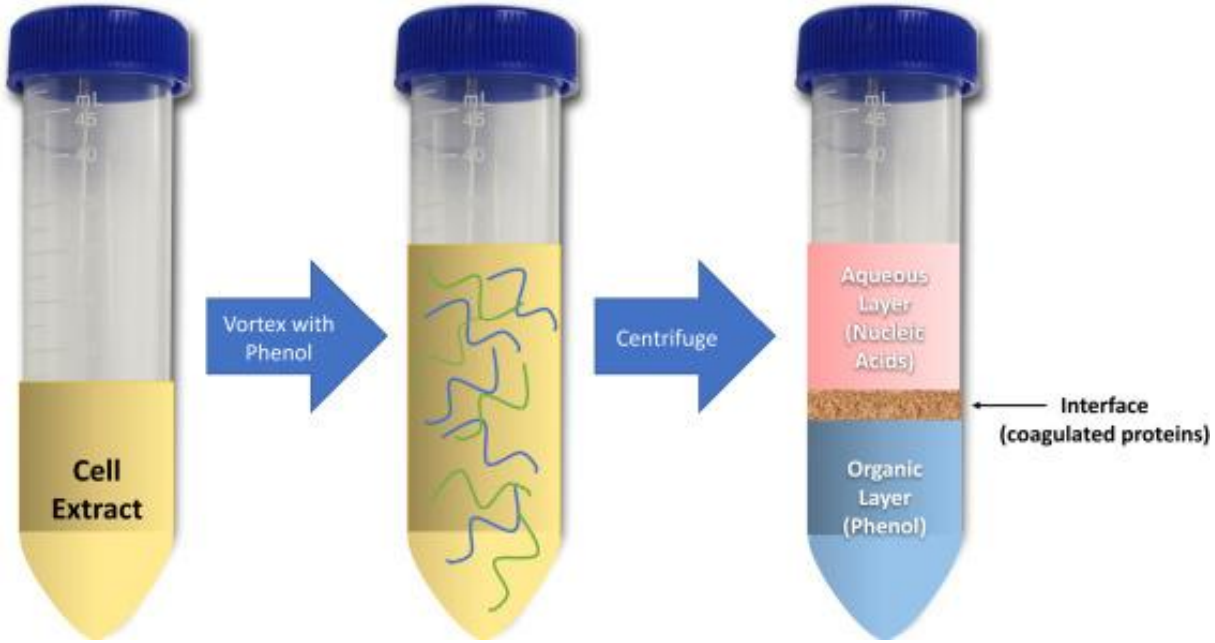
KEYWORDS: *antibiotic resistance gene, Staphylococcus aureus, chlorination, DNA damage, growth phase*

Thanks for listening

Questions?

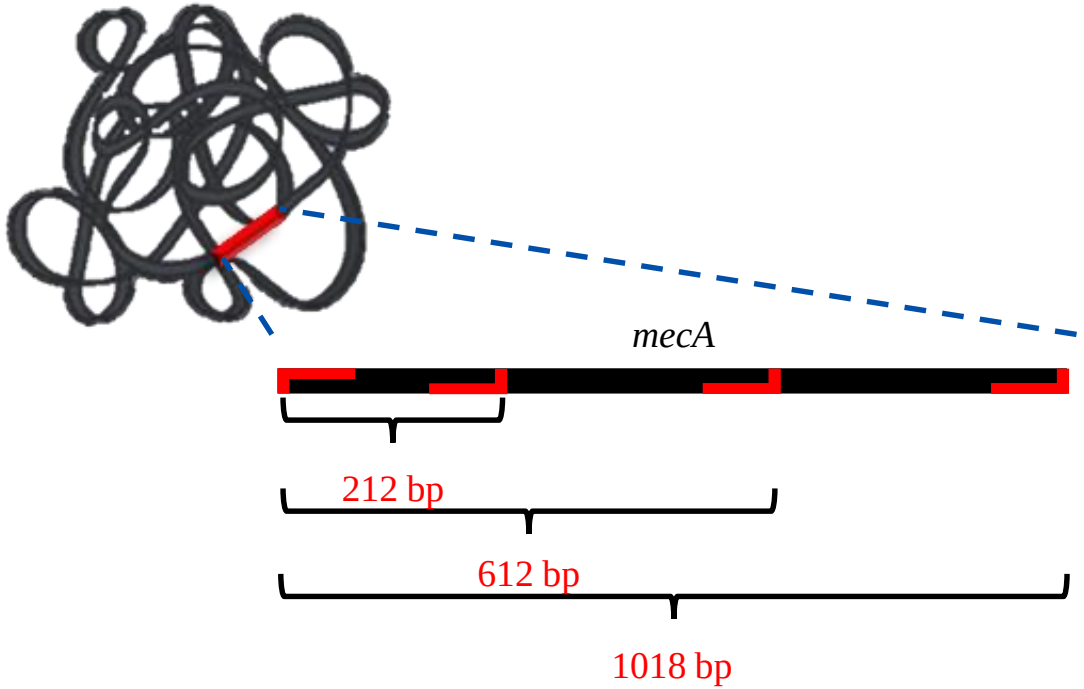
Methods

Fast DNA spin kit for soil (Spin kit) method

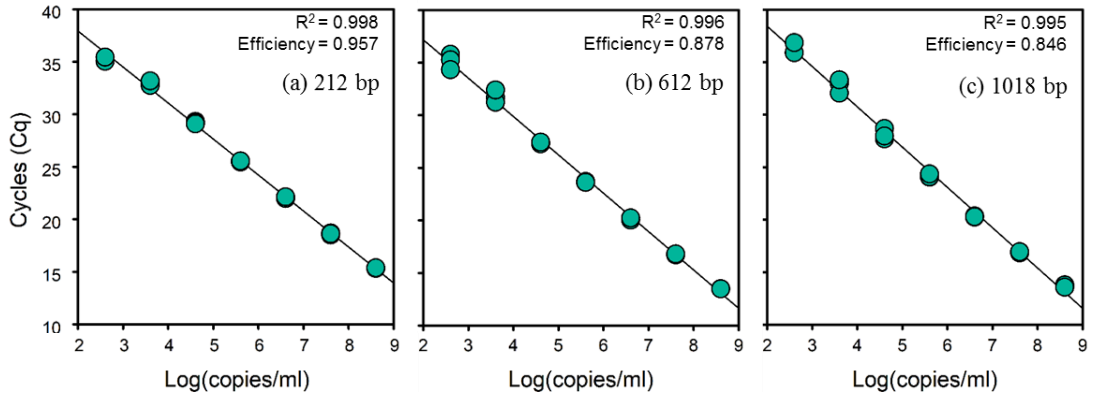


Phenol-Chloroform-Isoamyl alcohol (PCI) method

Methods



Calibration curve



- qPCR is a tool used to analyze the DNA damage.
  ➔ If DNA has damage in amplicon, DNA cannot be amplified and this level is displayed to indicate the damage.
- In here, qPCR assays were performed on 212, 612 and 1018 bp amplicon lengths.
  ➔ Longer amplicon lengths of qPCR assay means increase of the sensitivity for DNA damage
- Calibration curves were made for each amplicon length, and these were used for quantification of experimental results.

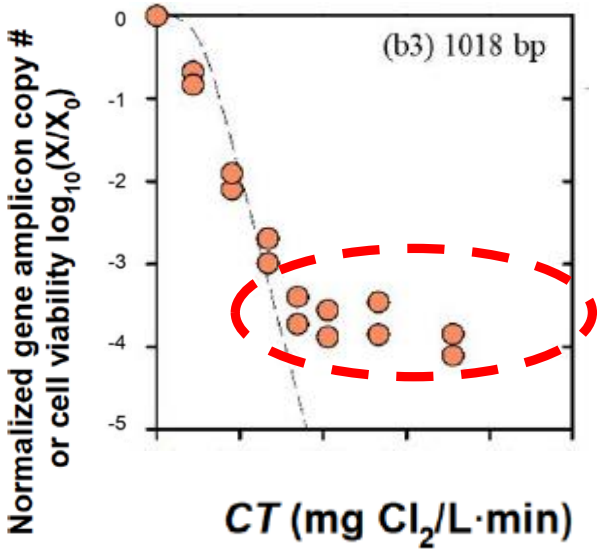
Results

- Chlorine-induced intracellular Fenton-like reactions
  ➔ Intracellular free iron concentrations ranging from 15 to 30 μM for *E.coli*.
  (Woodmansee et al., 2002)
  ➔ This mechanism is similar to that proposed for the inactivation of some bacterial species and their DNA damage caused by exogenous hydrogen peroxide through the intracellular Fenton reaction.
  (Repine et al., 2003; Park et al., 2003; Linley et al., 2012)

- Chlorine-induced impaired DNA repair function (ROS-protection)
  ➔ Chlorine disrupts chemiosmotically coupled energy transduction on the bacterial plasma membrane, resulting in the loss of ATP-dependent DNA repair processes.
  (Suquet et al., 2010)
  ➔ The DNA repair pathways can result in various types of DNA damages, including strand breaks, which are caused by reactive oxygen species (ROS) constantly produced endogenously through metabolic processes.
  (Fasnacht et al., 2021)

Introduction

- The degradation of i-ARGs plateaued at a loss of 3–4 log under high chlorine exposure in both log- and stationary-phase bacteria (tailing kinetics).
  - ➔ Explained by the formation of cell aggregates or biofilms (Choi et al., 2021; Huan et al., 2022).
  - ➔ Remains unclear, whether different status of bacteria depending on bacterial growth stage affect to tailing kinetic of i-ARG degradation.



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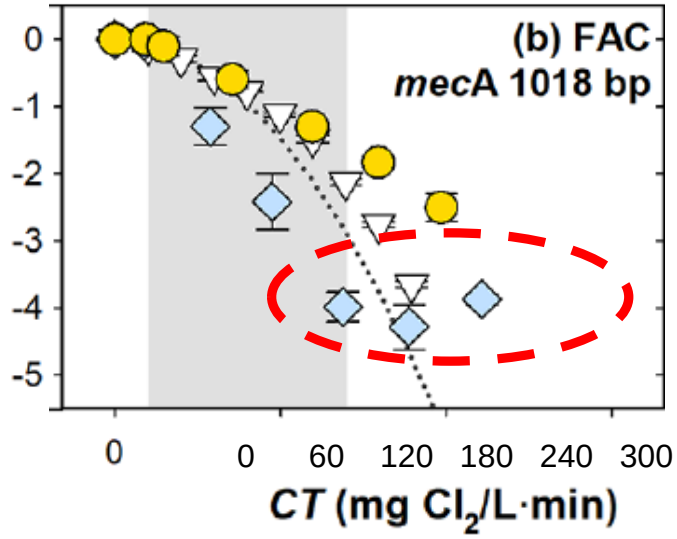
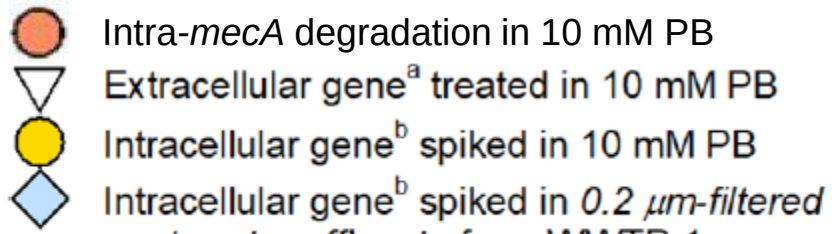
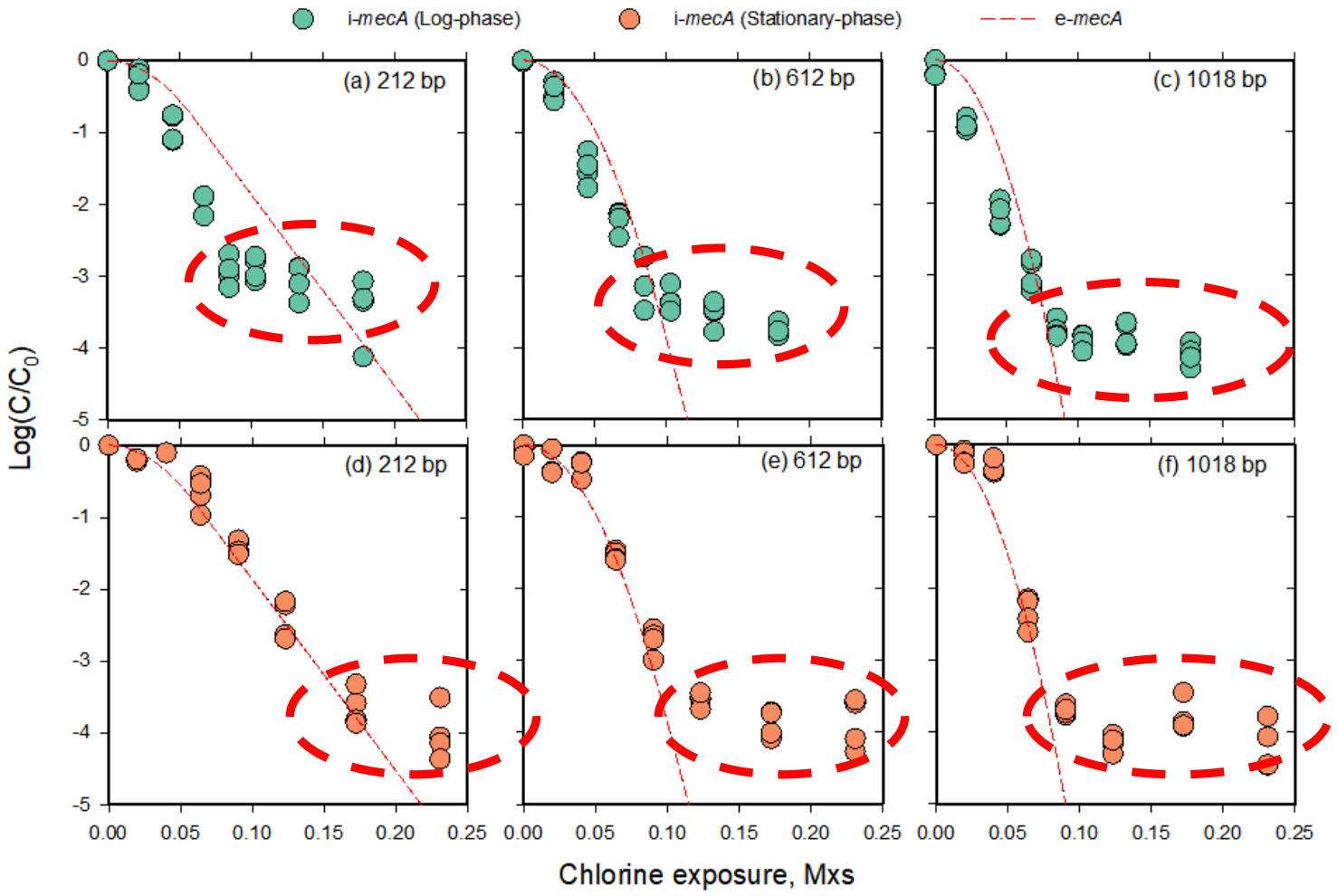




S.Aureus – *mecA* (Choi et al., 2021)

S.Aureus – *mecA* (Huan et al., 2022)

Results



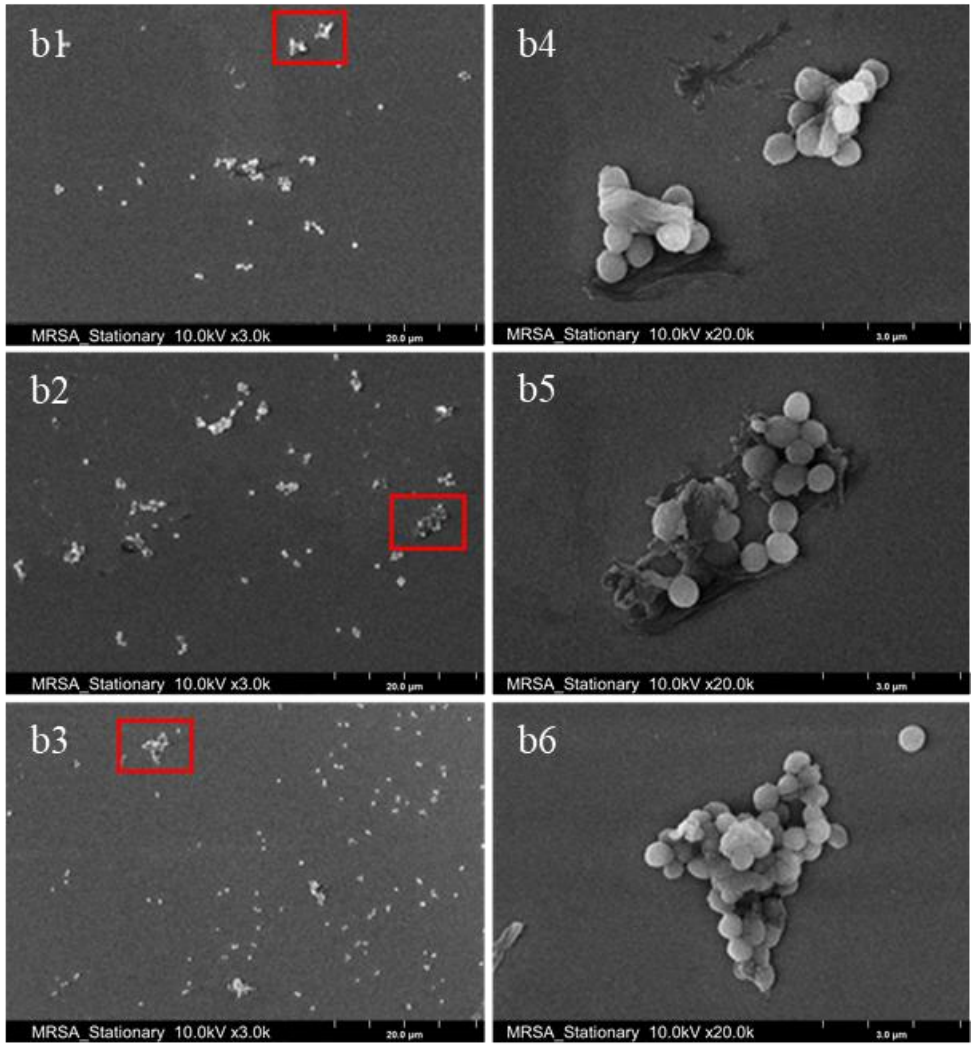
Logarithmic relative concentration of i-*mecA* qPCR amplicons after correcting the DNA recovery change from different growth phase

- Log-phase : More closely with case of e-*mecA*, but remained faster (212 bp).
- Stationary-phase : Changed little after correction and mirrored case of e-*mecA*.
- Tailing kinetics persisted even after correcting DNA recovery for both growth stage.

Overall, results demonstrate the importance of determining and reflecting variable DNA recovery to determine the degradation kinetics of i-*mecA* during chlorination.

Results

(b) Stationary-phase

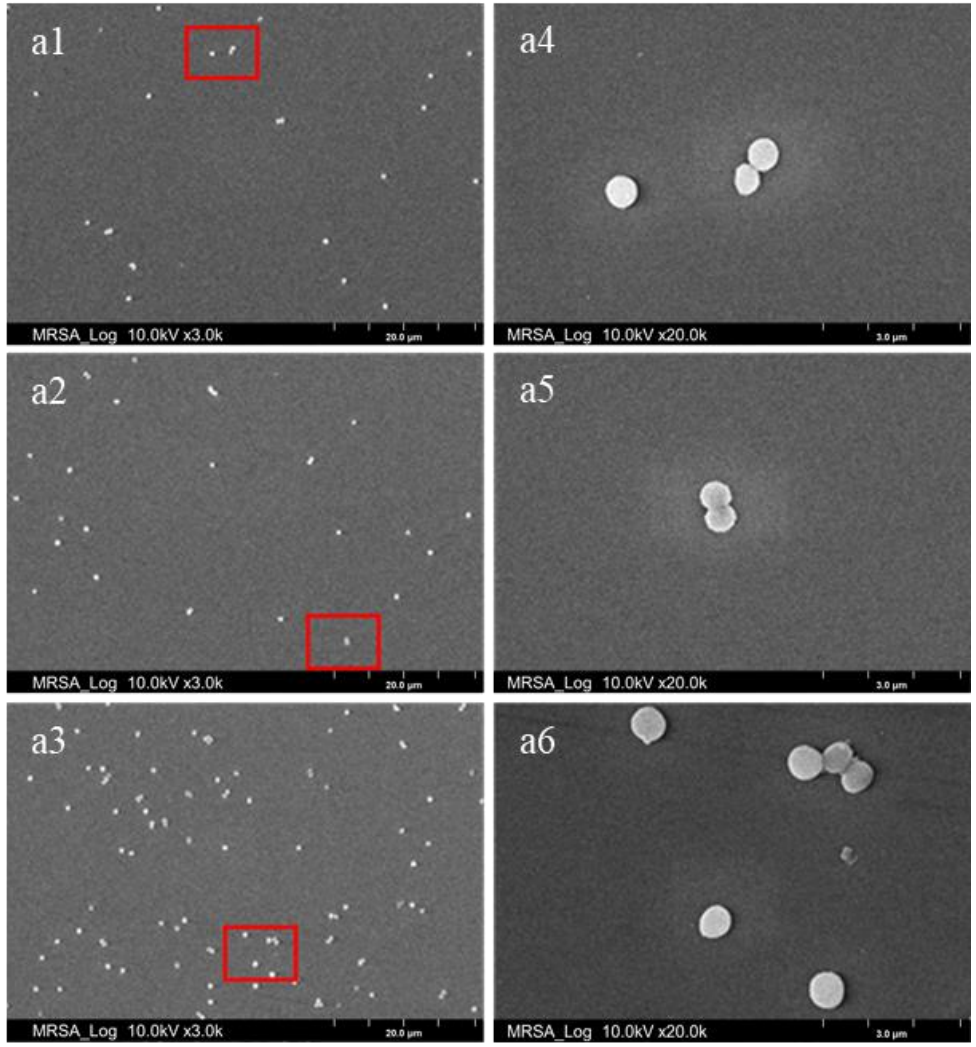


Scanning electron microscopy (SEM) images for the chlorinated log- and stationary-MRSA cell

- Stationary-phase :
  ➔ Some relatively large aggregates and biofilm
  ➔ Might responsible for the observed tailing kinetics.
  (aggregates likely protect i-ARG from chlorine diffusion)

Results

(a) Log-phase



- Log-phase :
  ➔ Single dispersed cells and clusters of double or triple cells
  ➔ No aggregates.

- Alternative explanation was required for tailing kinetics observed in the log-phase
  ➔ *S.aureus* employs redox-sensing regulators for detoxification pathways against redox-active species such as chlorine
  ➔ Presence of chlorine-resistant subpopulations.
  ➔ Might responsible for the observed tailing kinetics.