

Effects of NaCl Concentration on Antimicrobial Susceptibility Testing

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Abstract: Antimicrobial resistance is a major One Health issue, and marine environments are increasingly recognized as important reservoirs of antibiotics and resistance genes. However, antimicrobial susceptibility testing for marine-derived microorganisms still relies largely on clinical standard protocols, and the effects of NaCl concentration on test results have not been sufficiently evaluated. In this study, we examined the effects of NaCl concentration on antimicrobial susceptibility testing using standard reference strains, including *Escherichia coli* and *Enterococcus faecalis*. Disk diffusion and minimum inhibitory concentration (MIC) assays were performed using Mueller–Hinton agar, Marine agar, and Mueller–Hinton media supplemented with 0.0–2.0% NaCl. Significant differences in inhibition zone diameters were observed between Mueller–Hinton agar and Marine agar, particularly for erythromycin, tetracycline, and trimethoprim/sulfamethoxazole. In both *E. coli* and *E. faecalis*, inhibition zones for streptomycin and erythromycin decreased as NaCl concentration increased. In MIC assays, most antibiotics remained relatively stable at NaCl concentrations up to 2.0%, whereas gentamicin showed greater sensitivity to strain, NaCl concentration, and incubation temperature. Additional validation with other standard reference strains also revealed condition-dependent variation in several strain–antibiotic combinations. These findings indicate that NaCl concentration can affect antimicrobial susceptibility testing and should be carefully considered when evaluating marine-derived microorganisms.

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Keywords: Antimicrobial resistance, CLSI, Disk diffusion, MIC, NaCl

Introduction

Antimicrobial resistance (AMR) is a global public health issue that threatens humans, animals, and the environment, and the importance of the One Health approach has been increasingly emphasized in recent years as a way to understand and manage this problem at the level of an integrated ecosystem rather than in separate sectors [1, 2]. Aquatic environments are considered important environmental reservoirs where antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes introduced from land-based sources, livestock production, aquaculture, domestic wastewater, and industrial activities can accumulate, and where horizontal gene transfer may occur within and between microbial communities [3, 4]. Among these environments, marine systems are especially important for understanding the emergence and spread of AMR because they are characterized by broad dispersal,

diverse salt conditions, complex mineral composition, and unique microbial communities.

Despite this importance, several limitations remain in the evaluation of antimicrobial susceptibility or resistance in microorganisms derived from marine environments and marine organisms. At present, the most widely used standards for antimicrobial susceptibility testing are based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI), and disk diffusion and broth dilution using Mueller-Hinton (MH) media are among the most common methods [5]. These standardized methods provide a major advantage because they make antimicrobial susceptibility results comparable across clinical microorganisms. However, when they are directly applied to microorganisms originating from marine environments or to bacteria with salt-dependent physiological traits, they may not fully reflect actual growth characteristics or antibiotic responses [6]. In other words, although clinical standard methods are highly useful in terms of reproducibility and comparability, they may not always provide test conditions that are fully appropriate for evaluating antimicrobial susceptibility in marine-derived microorganisms.

Marine-derived microorganisms often require higher salt concentrations or specific ionic conditions than those used in conventional clinical media, and these requirements may affect not only microbial growth but also the results of antimicrobial susceptibility testing itself. In some studies and laboratory applications, Marine Agar (MA) has been used, or NaCl has been added to conventional MH media at levels of 1–2% to support the growth of marine microorganisms [6]. MA, which is widely used for culturing marine microorganisms, contains various mineral components and approximately 1.84% NaCl, whereas NaCl supplementation of MH media can serve as a practical way to partially mimic marine conditions. However, such adjustments may influence not only microbial growth but also the physicochemical behavior of antibiotic molecules and the physiological responses of bacteria. For example, changes in salt or cation concentration can affect the diffusion rate, solubility, and stability of antibiotics in media, as well as their interactions with the bacterial surface or outer membrane. In addition, these changes may influence porin function, membrane potential, ion homeostasis, and intracellular drug uptake pathways, potentially producing a phenotypic resistance-like response in susceptibility tests even in the absence of true genetic resistance [7].

Changes in NaCl concentration in the medium may support microbial growth, but they may also act as confounding factors that reduce the accuracy of antimicrobial susceptibility testing. As NaCl concentration increases, the activity of some antibiotics may decrease antagonistically, and osmotic changes together with altered membrane permeability may also affect the efficiency of antibiotic uptake into bacterial cells [8,9]. Therefore, it is important to identify experimental conditions that reflect the growth requirements of marine-derived microorganisms while remaining as compatible as possible with existing standard susceptibility testing methods. In particular, to avoid either applying clinical standard methods to marine environments without validation or overemphasizing marine-specific conditions to the point of losing comparability with established standards, it is necessary to quantitatively evaluate the range over which changes in NaCl concentration influence susceptibility test results [8].

The present study aimed to provide fundamental data for identifying antimicrobial susceptibility testing conditions that are more suitable for marine environments. First, using the standard reference strains *Escherichia coli* ATCC 11775 and *Enterococcus faecalis* ATCC 19433, we compared disk diffusion results between the CLSI standard MH medium and MA to evaluate how medium conditions influence inhibition zone formation. We then analyzed changes in antimicrobial susceptibility and minimum inhibitory concentration (MIC) under stepwise NaCl supplementation of MH media using seven standard reference strains. This approach may help establish more reliable criteria for antimicrobial susceptibility testing of marine-derived microorganisms.

Results and Discussion

Comparison of antimicrobial susceptibility according to culture medium

A comparison of antimicrobial susceptibility between Mueller-Hinton Agar (MHA), the CLSI standard medium, and Marine Agar (MA), which is commonly used for culturing marine microorganisms, showed that inhibition zone size differed according to the culture medium even when the same standard reference strains were used. In the disk diffusion assay with *E. coli* (ATCC

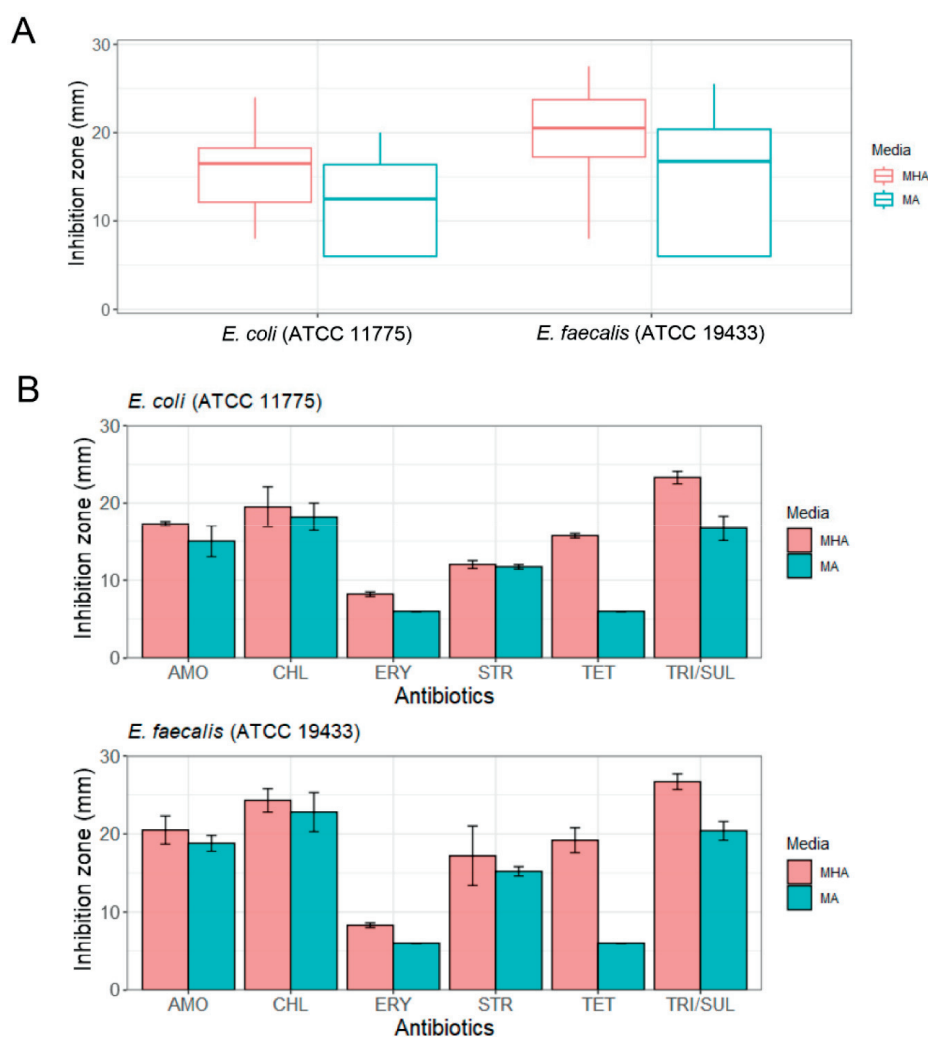


Fig. 1. Comparison of antimicrobial susceptibility between Muller-Hinton Agar (MHA) and Marine Agar (MA) for *Escherichia coli* (ATCC 11775) and *Enterococcus faecalis* (ATCC 19433). (A) Box plots showing the overall distribution of inhibition zone diameters across different media. (B) Bar graphs representing the zone of inhibition (mm) for six specific antibiotics (AMO: Amoxicillin; CHL: Chloramphenicol; ERY: Erythromycin; STR: Streptomycin; TET: Tetracycline; TRI/SUL: Trimethoprim/Sulfamethoxazole).

11775) and *E. faecalis* (ATCC 19433), measurements obtained from the two media were generally reproducible, and the variance structure was also similar between media (p -value=0.96, F =0.98). However, the mean inhibition zone diameter was approximately 3.75 mm larger on MHA than on MA, and this difference was statistically significant (p <0.05) (Fig. 1A). These results indicate that susceptibility test results for the same antibiotic can vary under culture conditions relevant to marine environments.

When individual antibiotics were examined, relatively large differences in inhibition zone diameter between the two media were observed for erythromycin (ERY), tetracycline (TET), and trimethoprim/sulfamethoxazole (TRI/SUL), and these differences were greater than would be expected from simple experimental error (Fig. 1B). These results indicate that the effects of culture medium on disk diffusion results are not uniform across antibiotics. The significant shift in inhibition zone diameter observed even in standard reference strains suggests that inhibition zone criteria established under clinical standard methods may not always be directly applicable to marine-related culture conditions. Therefore, when antimicrobial susceptibility is evaluated in marine-derived microorganisms or gut-associated microorganisms from marine organisms, the medium used in the assay should be considered together with the clinical reference criteria rather than applying those criteria without adjustment. This interpretation is also supported by a recent study showing that differences in pH and cation composition of Mueller–Hinton media were associated with variation in inhibition zone diameters [10].

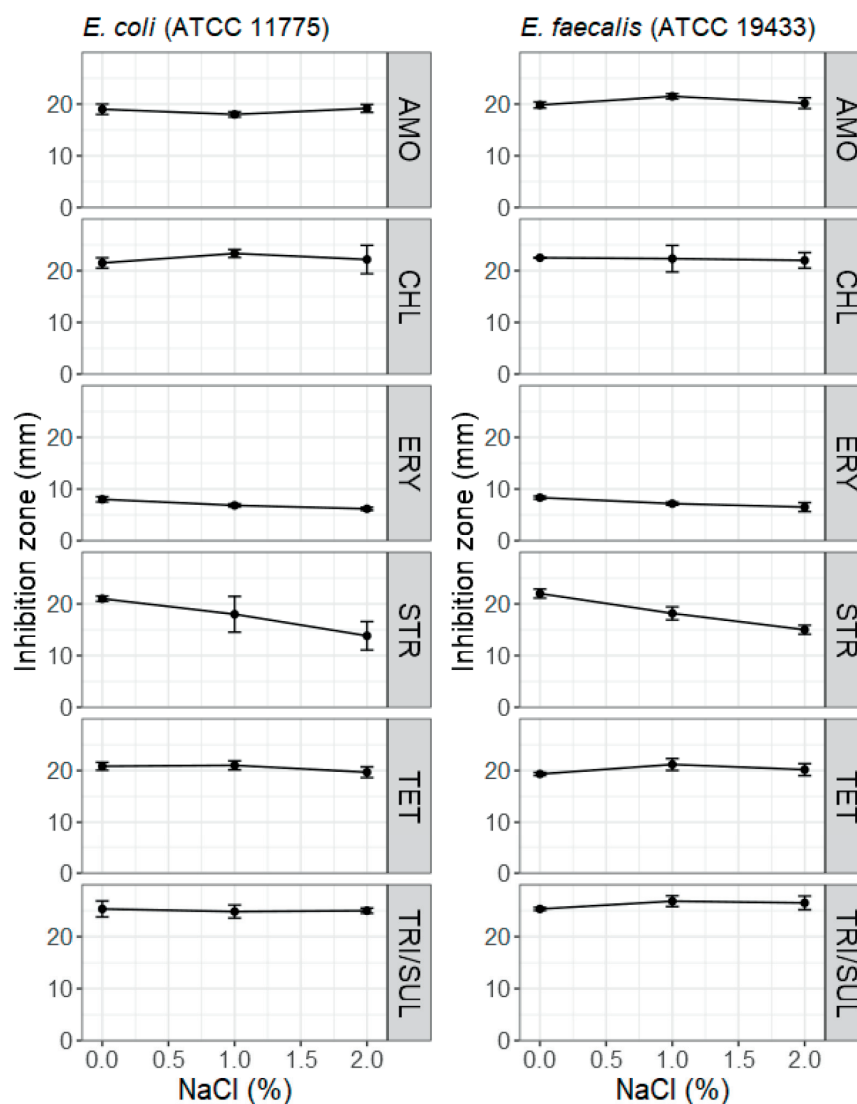


Fig. 2. Effect of NaCl concentration (0.0%, 1.0%, and 2.0%) on the inhibition zone diameters of *E. coli* and *E. faecalis*.

Effects of NaCl concentration on disk diffusion assay

Correlation analysis of the effects of NaCl concentration added to MHA (0.0%, 1.0%, and 2.0%) on inhibition zone diameter showed that increasing NaCl concentration was associated with clear changes in susceptibility for some antibiotics (Fig. 2). In *E. coli* (ATCC 11775), the inhibition zones for streptomycin (STR) and erythromycin (ERY) gradually decreased as NaCl concentration increased ($\rho=-1$, $p<0.05$), indicating that even within the same strain, the response to NaCl may differ depending on the antibiotic tested. A similar pattern was observed in *E. faecalis* (ATCC 19433), in which the inhibition zone diameters of streptomycin (STR), erythromycin (ERY), and chloramphenicol (CHL) decreased with increasing NaCl concentration ($\rho=-1$, $p<0.05$).

In contrast, both strains maintained relatively stable inhibition zone sizes for amoxicillin (AMX), tetracycline (TET), and trimethoprim/sulfamethoxazole (TRI/SUL) despite the increase in NaCl concentration. These findings suggest that the effects of NaCl on antimicrobial susceptibility testing do not appear uniformly across all antibiotics, but rather occur selectively depending on the characteristics of each antibiotic and its sensitivity to ionic conditions. In particular, the decrease observed for STR and ERY suggests that these antibiotics may be more sensitive to increased NaCl concentration than others.

These observations indicate that a reduced inhibition zone under increased NaCl conditions should not be interpreted immediately as an increase in resistance. Instead, increased NaCl concentration may affect susceptibility test results through changes in

antibiotic diffusion and bacterial response. Under the conditions tested in this study, some antibiotics, such as STR and ERY, appeared to be more sensitive to NaCl concentration, suggesting that antibiotic-specific caution is needed when interpreting susceptibility results for microorganisms associated with marine environments.

Evaluation of basal growth and MIC under different temperature and salinity conditions

Although the disk diffusion assay was performed at 35°C according to CLSI criteria, the effects of incubation temperature (25°C vs. 35°C) and NaCl concentration (0–2.0%) on basal bacterial growth were first evaluated before broth dilution-based MIC measurements were conducted (Fig. S1). In both *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29212), OD₆₀₀ values were higher at 35°C than at 25°C, indicating greater overall growth at the higher temperature. In addition, when NaCl concentration increased from 1% to 2%, OD₆₀₀ values showed only slight increases or remained stable without a marked change. These results suggest that, within the range tested in this study, increased NaCl concentration alone did not strongly inhibit basal growth in either strain.

In this study, MIC measurements were first performed at 25°C to examine how changes in NaCl concentration affect antimicrobial susceptibility under lower-temperature culture conditions that partially reflect marine environments. In *E. coli* (ATCC 25922), relatively stable MIC measurements were obtained for most antibiotics as NaCl concentration increased, except for gentamicin. In contrast, gentamicin did not show a consistent relationship with NaCl concentration but showed irregular values relative to the CLSI criteria (Fig. S2). This suggests that gentamicin may respond more sensitively to changes in test conditions than the other antibiotics examined.

A similar pattern was observed in *E. faecalis* (ATCC 29212). For antibiotics other than gentamicin, MIC evaluation across the NaCl gradient was relatively stable, and the measured values were generally consistent with the CLSI reference values for this strain (Fig. S3). However, gentamicin showed disturbed MIC values from 1% NaCl and above, indicating that gentamicin in *E. faecalis* may also be more sensitive to NaCl conditions than other antibiotics.

When antimicrobial susceptibility was later evaluated at 35°C in *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29212) according to CLSI criteria, most antibiotic combinations remained generally consistent with CLSI reference values, even though some variation in OD₆₀₀ was observed as NaCl concentration increased from 1% to 2% (Fig. 3). However, unlike the results at 25°C, the gentamicin MIC of *E. coli* (ATCC 25922) at 35°C was closer to the CLSI reference range and showed an increasing trend as NaCl concentration increased. This result indicates that, for specific antibiotic-strain combinations, not only NaCl concentration but also incubation temperature can significantly influence antimicrobial susceptibility test results.

Taken together, most antibiotics showed relatively stable MIC measurements within the tested range of 0–2% NaCl, whereas gentamicin appeared to be sensitive to both temperature and NaCl changes. This suggests that applying a single testing condition to all antibiotics may not be appropriate in antimicrobial resistance assessment under marine-related or low-temperature conditions. Gentamicin, which belongs to the aminoglycoside class, may show MIC variation even with small changes in ionic conditions in the test medium. Increased ionic strength or higher divalent cation concentrations may affect aminoglycoside binding to the bacterial surface and its uptake into bacterial cells, resulting in apparent changes in susceptibility. A recent study also showed that variation in calcium and magnesium concentrations can affect aminoglycoside disk diffusion results [10]. Therefore, the gentamicin variation observed in this study may reflect condition-dependent test interference rather than genetic resistance alone, and special caution is needed when gentamicin susceptibility is evaluated in marine-derived microorganisms.

Validation of NaCl-dependent susceptibility patterns using multiple reference strains

We further analyzed antimicrobial susceptibility across different NaCl concentrations using seven additional standard reference strains (Fig. S4–S7). The antibiotics tested were chloramphenicol, tetracycline, penicillin, kanamycin, and vancomycin, and both OD₆₀₀-based growth inhibition patterns and MIC values were examined for each strain as NaCl concentration increased. Overall,

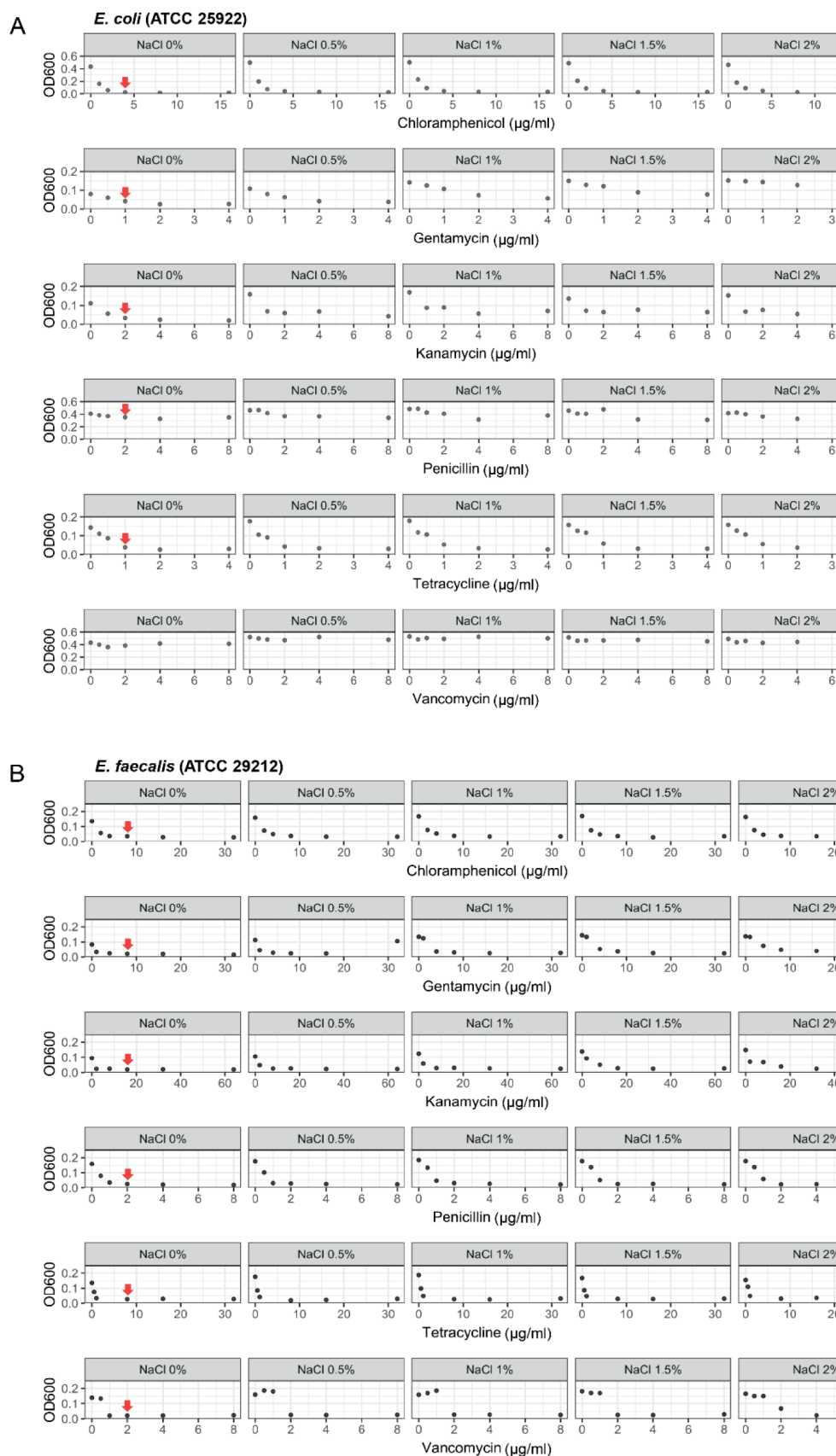


Fig. 3. Minimum Inhibitory Concentration (MIC) assessment of (A) *E. coli* (ATCC 25922) and (B) *E. faecalis* (ATCC 29212) under different NaCl concentrations (0% to 2%) at 35 °C. Growth was monitored via OD₆₀₀ against increasing concentrations of antimicrobial agent. Red arrows indicate the observed MIC values for each tested drug.

except for penicillin, most antibiotics showed trends that were not markedly different from previously reported MIC values within the NaCl range of up to 2%.

Penicillin showed a somewhat different pattern. In several strains other than *E. faecalis* (ATCC 19433) and *S. enterica* (ATCC 14028), MIC values differed from previously reported values regardless of NaCl concentration. In addition, for vancomycin, somewhat higher values than previously reported MIC values were observed in *E. coli* (ATCC 11775) and *S. enterica* (ATCC 14028). These findings suggest that, for some strain-antibiotic combinations, MIC evaluation under marine-related conditions or under the conditions used in this study may differ from existing reference values.

These findings suggest that some antibiotic-strain combinations are more sensitive to testing conditions than others, and that their reference ranges may require additional validation under modified culture conditions. The MIC values obtained for each standard reference strain in this study were summarized in Table 1 and compared with the reference ranges. Such comparisons may provide useful baseline information for determining which antibiotic combinations are relatively stable and which are more vulnerable to condition-dependent variation in future antimicrobial susceptibility testing of marine-associated microorganisms.

In general, the NaCl content of seawater is known to be approximately below 3% [10], and several previous studies, including the present study, have suggested that NaCl concentrations of up to 2% can be applied relatively stably in minimum inhibitory concentration (MIC) testing for antibiotics [6]. In the present study, antimicrobial susceptibility was evaluated across multiple standard reference strains and antibiotic combinations under different NaCl concentrations, and the results showed that, overall, MIC measurements for many antibiotics remained relatively stable within the NaCl range of up to 2%. These findings suggest that a certain degree of NaCl adjustment may be experimentally acceptable when antimicrobial susceptibility is assessed in marine-derived microorganisms or marine-related samples.

However, this general pattern was not consistent across all antibiotics or strains. In particular, some antibiotic classes appeared to remain vulnerable to test interference even under NaCl conditions below 2%. Ion-sensitive antibiotics such as aminoglycosides may respond even to relatively small increases in NaCl concentration, and previous studies have suggested that increasing NaCl in the medium can markedly elevate the MIC values of gentamicin or amikacin, leading to an apparent increase in resistance even in the absence of true genetic resistance [11]. In our study, gentamicin also showed a more sensitive response than the other antibiotics to changes in NaCl concentration and incubation temperature, and the stability of its MIC values differed depending on the standard strain, NaCl concentration, and incubation temperature. These results suggest that antimicrobial susceptibility testing under marine-related conditions should not rely on a single uniform testing condition for all antibiotics or strains. In particular, condition-sensitive antibiotics such as gentamicin require more careful interpretation under modified NaCl and temperature conditions.

In conclusion, this study systematically examined the effects of NaCl conditions on antimicrobial susceptibility testing using

Table 1. Summary of observed MIC values for seven bacterial type strains under 2% NaCl conditions compared with established reference ranges. Reference MIC ranges from CLSI and EUCAST represent available genus-level information that includes the tested type strains

Type strains	MIC break point (µg/ml) / Reference ^{a,b,c}				
	Chloramphenicol	Tetracycline	Penicillin	Kanamycin	Vancomycin
<i>Enterococcus faecalis</i> (ATCC 19433)	16 / 8–32a	4 / 4–16a	8 / 8–16a	128	2 / 4–32a
<i>Escherichia coli</i> (ATCC 11775)	16 / 8–32a	16 / 8–16a	32	32 / 16–64a	64
<i>Staphylococcus aureus</i> (ATCC 6538)	32 / 8–32a	16 / 4–16a	ND / 0.12–0.25a	8	2 / 2–16a
<i>Pseudomonas aeruginosa</i> (ATCC 10145)	32–64	8–128	ND	<256	ND
<i>Salmonella enterica</i> (ATCC 14028)	16 / 8–32a	16 / 8–16a	16	32 / 16–64a	64 / 4–32a
<i>Bacillus clausii</i> (ATCC 700160)	16 / 8–32b	16 / 4–16b	ND / 0.12–0.25b	64	2 / ≤4b
<i>Bacillus cereus</i> (ATCC 14579)	8 / 8–32b	4 / 4–16b	ND / 0.12–0.25b	64	2 / ≤4b

a: CLSI M100 [12], b: CLSI M45 [14], c: EUCAST [15], ND: no data; MIC could not be determined within the concentration range tested in this study.

standard reference strains under marine-related culture conditions. While most antibiotics showed relatively stable results within a certain NaCl range, some antibiotics were more sensitive to changes in NaCl concentration and temperature. These findings can serve as baseline data for more accurate tracking of the emergence and spread of antimicrobial resistance originating from aquaculture and marine environments, and may ultimately provide experimental support for the development of an integrated One Health-based surveillance system that considers both human and ecosystem health.

Materials and Methods

Bacterial strains and culture conditions

In this study, changes in antimicrobial susceptibility and MIC under different NaCl conditions were evaluated using standard reference strains relevant to antimicrobial susceptibility testing in marine-related conditions. A total of nine standard reference strains were used: *E. faecalis* (ATCC 19433/KCTC 3206 and ATCC 29212/KCTC 2011), *E. coli* (ATCC 11775/KCTC 2441 and ATCC 25922/KCTC 1682), *Staphylococcus aureus* (ATCC 6538/KCTC 3881), *Pseudomonas aeruginosa* (ATCC 10145/KCTC 1750), *Salmonella enterica* (ATCC 14028/KCTC 2515), *Bacillus clausii* (ATCC 700160/KCTC 3825), and *Bacillus cereus* (ATCC 14579/KCTC 3624). All strains were obtained from the Korean Collection for Type Cultures (KCTC).

Before the experiments, each strain was pre-cultured in either solid or liquid medium and incubated at 25°C or 35°C depending on its growth characteristics and the purpose of the experiment. Bacterial growth was monitored by measuring the optical density at 600 nm (OD₆₀₀) using an Epoch 2 Microplate Spectrophotometer (Agilent Technologies, USA).

The strains were grouped according to the experimental purpose. *E. coli* (ATCC 11775) and *E. faecalis* (ATCC 19433) were used to compare antimicrobial susceptibility between MH agar and MA using the disk diffusion method. *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29212) were then used for MIC measurement and quantitative evaluation under different NaCl concentrations. In addition, *S. aureus*, *P. aeruginosa*, *S. enterica*, *B. clausii*, and *B. cereus*, together with *E. coli* (ATCC 11775) and *E. faecalis* (ATCC 19433), were included to examine whether NaCl-dependent responses were consistent across a broader range of strains. This strain set allowed comparison of salinity-related changes in antimicrobial susceptibility across both Gram-positive and Gram-negative bacteria.

Culture media preparation

To evaluate antimicrobial susceptibility under different medium conditions, MHA (Kisan Bio Co., Ltd., Korea) and Mueller-Hinton Broth (MHB, Kisan Bio Co., Ltd., Korea) were used as the basic media. For broth dilution-based MIC testing, cation-adjusted Mueller-Hinton broth (CAMHB) was used according to the CLSI guidelines. In the present study, MHB-based liquid media were therefore prepared with consideration of this CLSI framework for comparison of MIC responses under different NaCl conditions. MA (Kisan Bio Co., Ltd., Korea) was included as a comparison medium to examine differences between standard and marine culture conditions.

To quantitatively evaluate the effects of NaCl concentration, NaCl was added to the test media to create final concentrations of 0.0%, 0.5%, 1.0%, 1.5%, and 2.0% (w/v). Each medium in the NaCl gradient was prepared by changing only the NaCl concentration while keeping the basic medium composition unchanged. This design allowed direct comparison of changes in inhibition zone diameter and MIC values according to increasing NaCl concentration. The NaCl range of 0–2% used in this study was selected to partially reflect marine-related culture conditions while maintaining comparability with existing standard antimicrobial susceptibility testing methods.

Disk diffusion assays on solid media and MIC assays in liquid media were performed using MHA (or MA) and MHB, respectively, according to the purpose of each test. All media were sterilized before use, and media prepared under the same conditions were used throughout the experiments to minimize batch-to-batch variation.

Disk diffusion assay

To evaluate the effects of medium condition and NaCl concentration on antimicrobial susceptibility results, the disk diffusion method was performed based on the CLSI M100 protocol [12]. Bacterial suspensions were adjusted to a 0.5 McFarland standard and evenly spread onto the surface of MHA or MA. Antibiotic disks were then placed on the agar surface, and the plates were incubated at 35°C for 18 h. After incubation, the diameter of the inhibition zone formed around each disk was measured.

The inhibition zone diameter was used as the main indicator of susceptibility, and the diameter of each clear zone (mm) was measured using the AntibioGram (v1.0) program [13]. The main purpose of this experiment was to compare how inhibition zone size changed according to medium type or NaCl concentration. Repeated experiments were performed under the same conditions for each strain to confirm reproducibility.

The antibiotic disks used in the disk diffusion assay were selected based on non-human antimicrobial resistance information for livestock and fishery products provided by the One Health inter-ministerial antimicrobial resistance monitoring program (www.kdca.go.kr). The six antibiotics used were chloramphenicol (CHL, 30 µg), trimethoprim-sulfamethoxazole (TRI/SUL, 25 µg), amoxicillin (AMX, 10 µg), streptomycin (STR, 25 µg), tetracycline (TET, 30 µg), and erythromycin (ERY, 15 µg). These antibiotics were selected because they are relevant to susceptibility testing of marine- or aquaculture-associated microorganisms and represent different antibiotic classes with different modes of action.

Determination of MIC

To quantitatively evaluate changes in antimicrobial susceptibility across the NaCl gradient, MIC testing was performed using the broth dilution method. The antibiotics selected for MIC testing were chloramphenicol, gentamicin, kanamycin, penicillin, tetracycline, and vancomycin, and each antibiotic was used in a stepwise dilution series. Standardized bacterial suspensions were inoculated into liquid media containing each antibiotic, and cultures were incubated at 25°C or 35°C while OD₆₀₀ values were measured. The MIC was defined as the lowest antibiotic concentration at which visible microbial growth was inhibited.

MIC measurements were performed under stepwise NaCl supplementation, and results at 25°C and 35°C were compared when required. *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29212) were used as the main reference strains, and the measured MIC values were evaluated against the CLSI [12,14] or EUCAST (European Committee on Antimicrobial Susceptibility Testing) [15] quality control ranges with repeated measurements for reproducibility.

Statistical analysis

All experiments were basically performed in triplicate, and additional independent repeat experiments were carried out when needed to confirm data consistency and reproducibility. Data for basal growth, inhibition zone diameter, and MIC-related measurements were summarized as means with variation based on repeated measurements, and trends and statistical significance according to changes in NaCl concentration were evaluated.

Statistical analyses were performed using the R program (v4.2.0). Homogeneity of variance between two conditions was evaluated using ‘var.test’, and differences in means between two groups were tested using ‘t.test’. In addition, ‘cor.test’ was used to evaluate the direction and relationship of changes in inhibition zone diameter or MIC values with increasing NaCl concentration.

Data Availability: All data are available in the main text or in the Supplementary Information.

Author Contributions: D.H. designed the study, performed the experiments, interpreted the data, and wrote the manuscript. The author has read and agreed to the published version of the manuscript.

Notes: The author declares no conflict of interest

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Additional Information:

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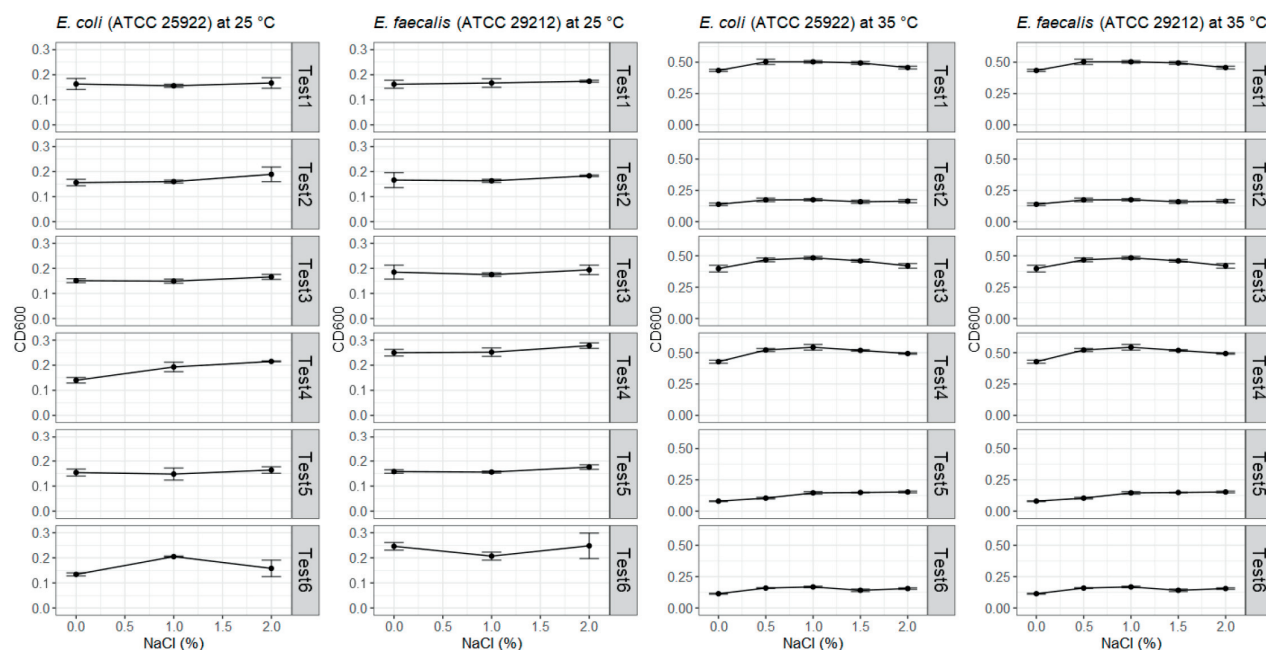


Figure S1. Comparison of the basal growth rates OD600 of *E. coli* (ATCC 25922) and *E. faecalis* (ATCC 29212) at different temperatures (25°C vs. 35°C) and NaCl concentrations (0% to 2.0%). Data represents six independent replicates (Test 1–6).

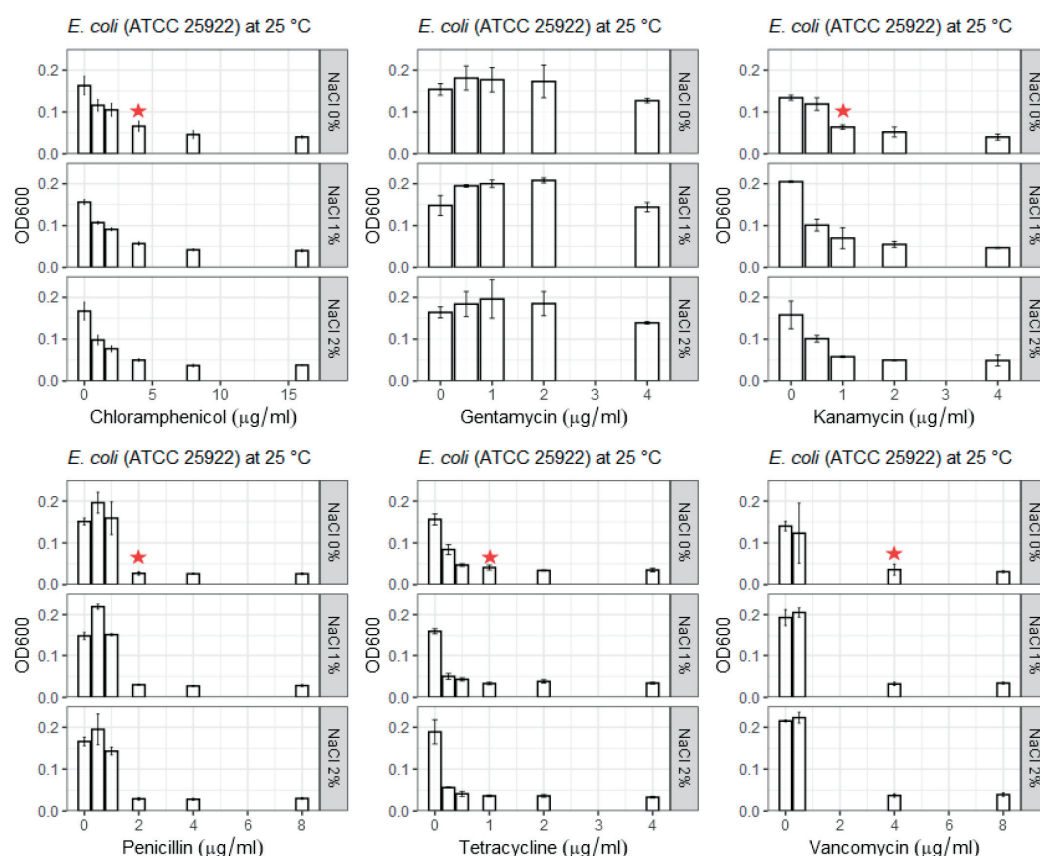


Figure S2. Antimicrobial susceptibility profiles of *E. coli* (ATCC 25922) at 25°C as a function of NaCl concentration (0%, 1%, and 2%). Red arrows indicate the MIC values determined experimentally in this study. For comparison, the reference MIC ranges for the quality control strain ATCC 25922, as established by the CLSI guideline, are provided (Chloramphenicol, 2–8 µg/ml; Gentamycin, 0.25–1 µg/ml; Kanamycin, 1–4 µg/ml; and Tetracycline, 0.5–2 µg/ml). Reference MICs for Penicillin and Vancomycin are not determined by CLSI for this strain.

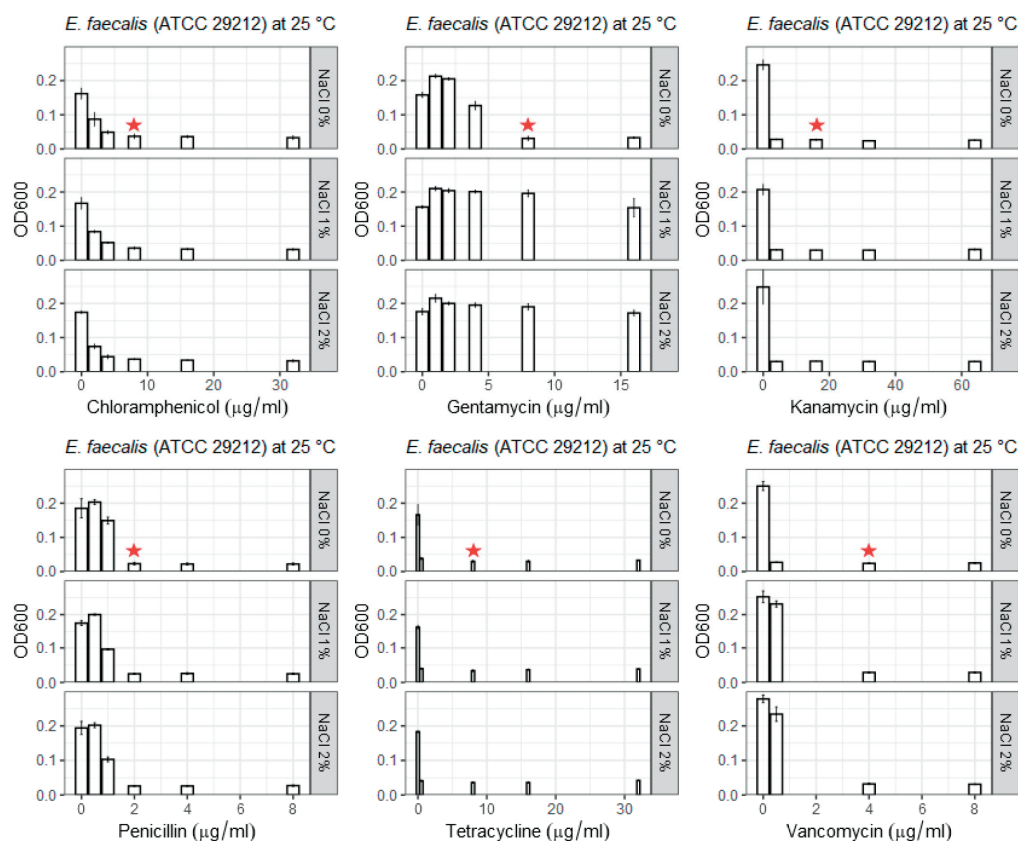


Figure S3. Antimicrobial susceptibility profiles of *E. faecalis* (ATCC 29212) at 25°C as a function of NaCl concentration (0%, 1%, and 2%). Red arrows indicate the MIC values determined experimentally in this study. For comparison, the reference MIC ranges for the quality control strain ATCC 29212, as established by the CLSI guideline, are provided (Chloramphenicol, 4–16 µg/ml; Gentamycin, 4–16 µg/ml; Kanamycin, 32–128 µg/ml; Penicillin, 1–4 µg/ml; Tetracycline, 8–32 µg/ml; and Vancomycin, 1–4 µg/ml).

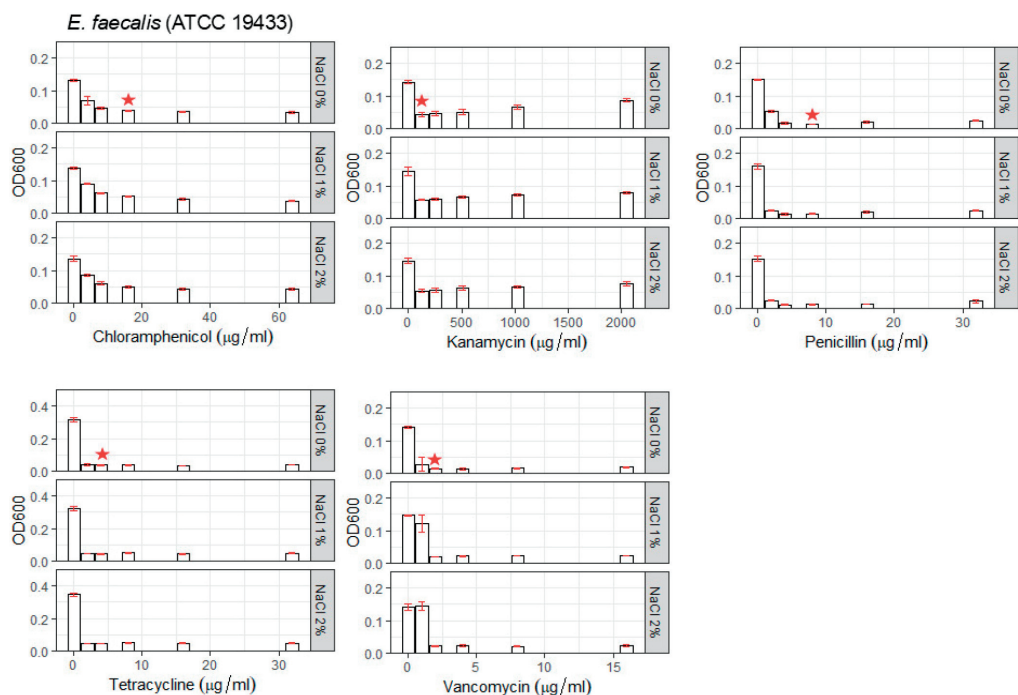


Figure S4. Susceptibility of *E. faecalis* (ATCC 19433) to various antibiotics (CHL, KAN, PEN, TET, and VAN) under different NaCl concentrations (0%, 1%, and 2%).

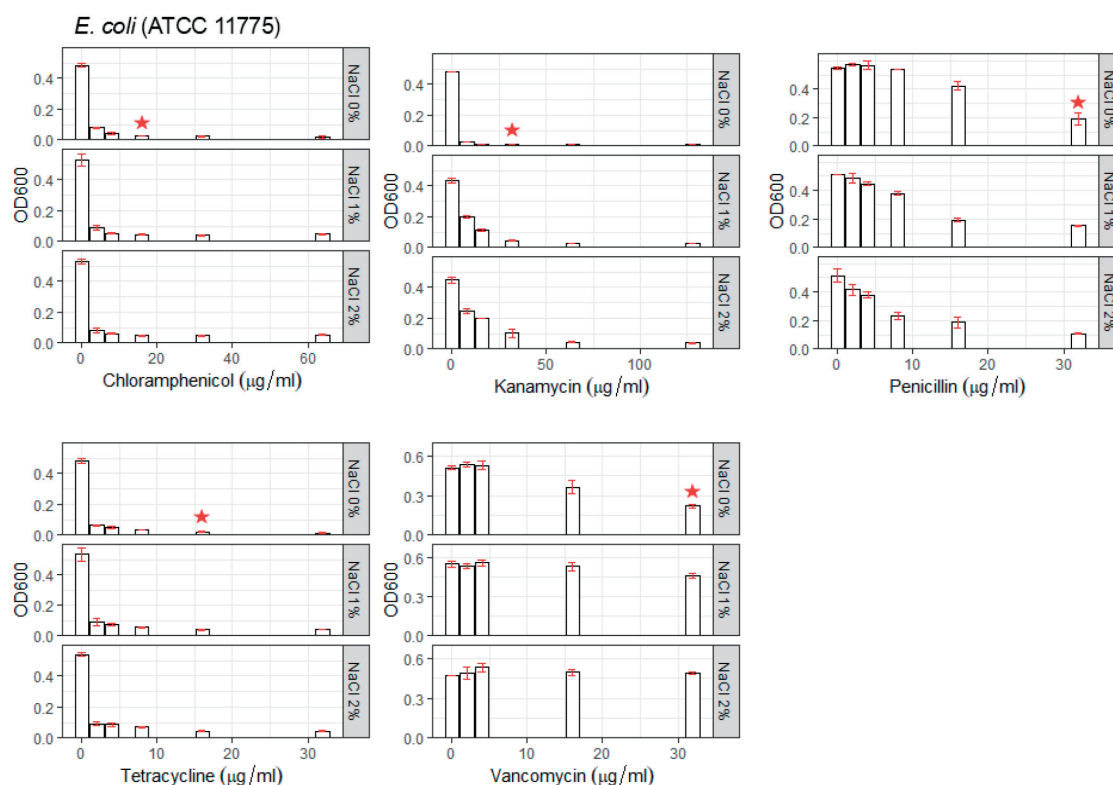


Figure S5. Susceptibility of *E. coli* (ATCC 11775) to various antibiotics (CHL, KAN, PEN, TET, and VAN) under different NaCl concentrations (0%, 1%, and 2%).

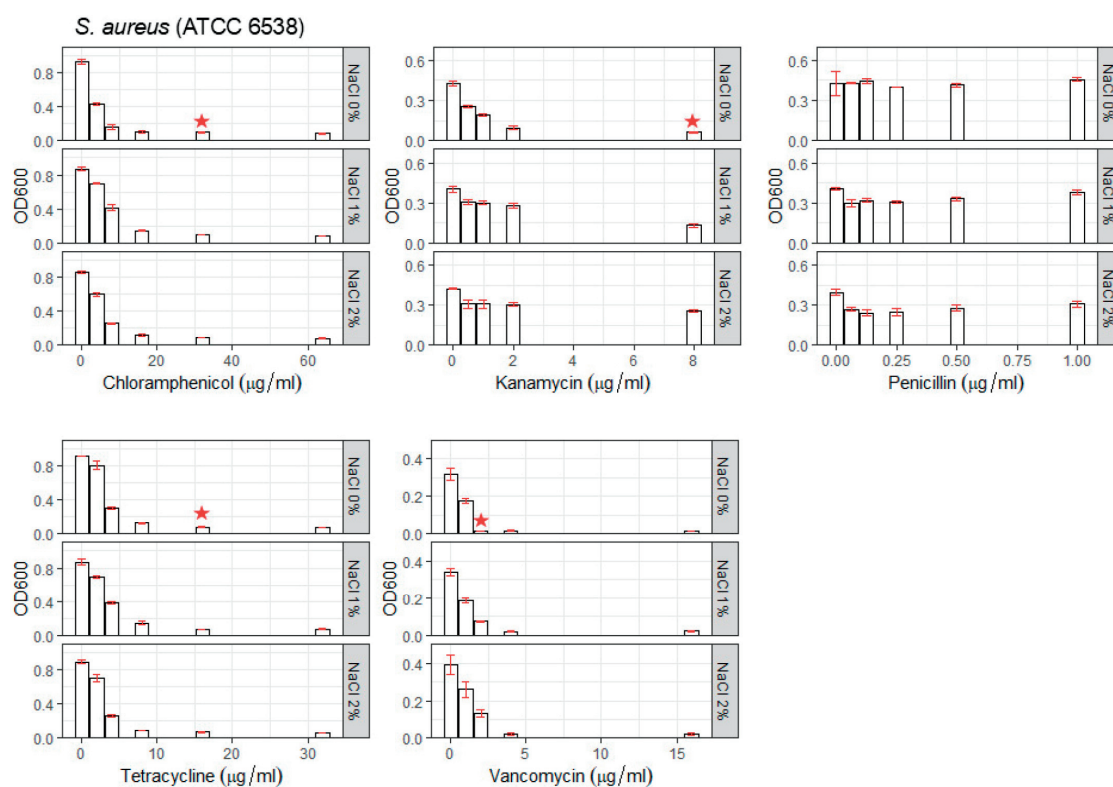


Figure S6. Susceptibility of *S. aureus* (ATCC 6538) to various antibiotics (CHL, KAN, PEN, TET, and VAN) under different NaCl concentrations (0%, 1%, and 2%).

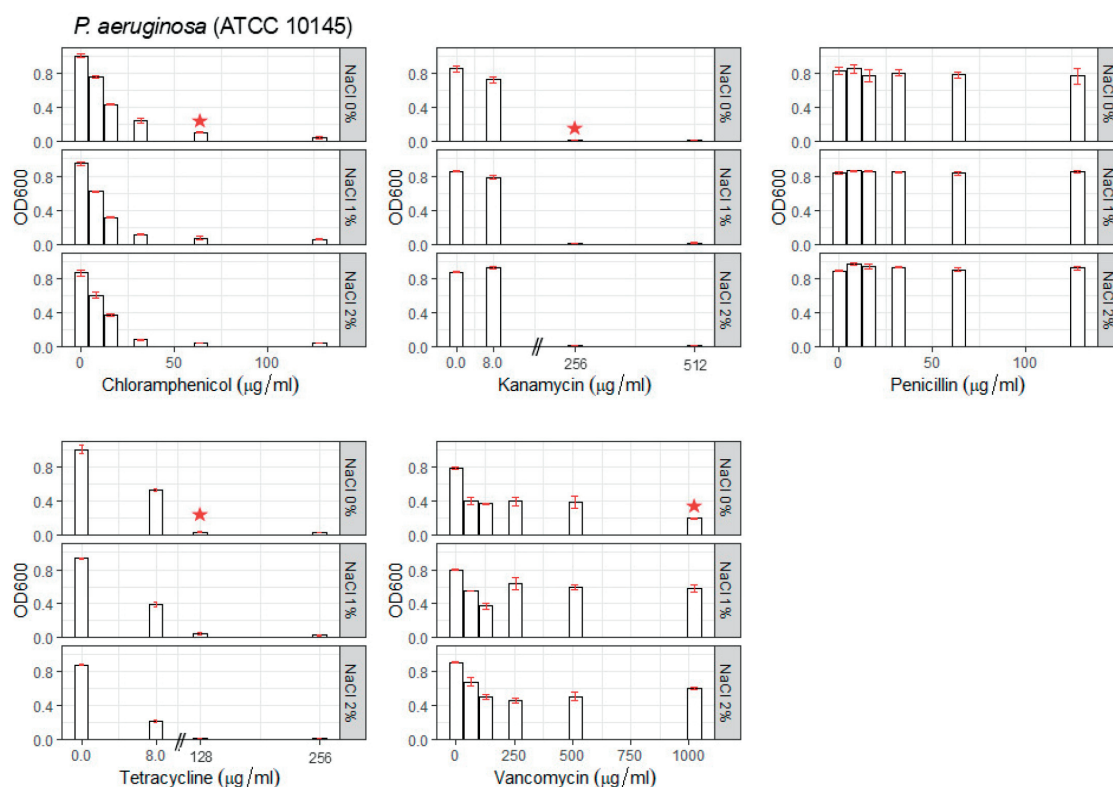


Figure S7. Susceptibility of *P. aeruginosa* (ATCC 10145) to various antibiotics (CHL, KAN, PEN, TET, and VAN) under different NaCl concentrations (0%, 1%, and 2%).

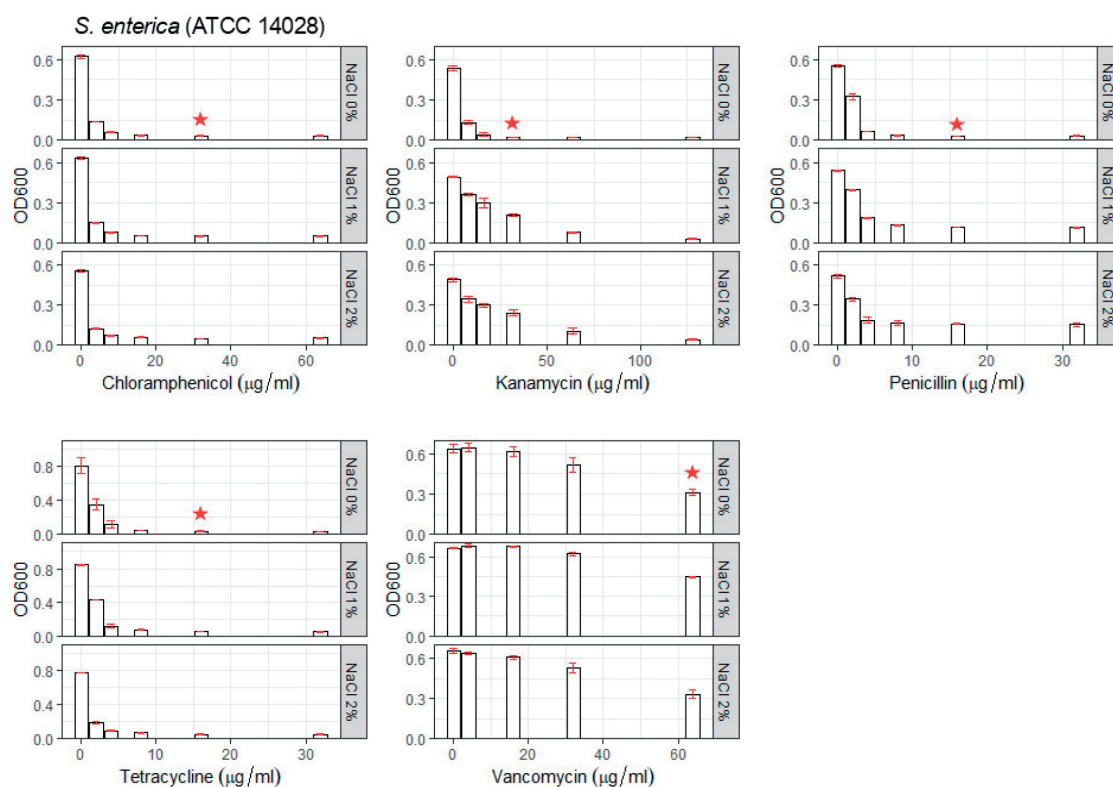


Figure S8. Susceptibility of *S. enterica* (ATCC 14028) to various antibiotics (CHL, KAN, PEN, TET, and VAN) under different NaCl concentrations (0%, 1%, and 2%).

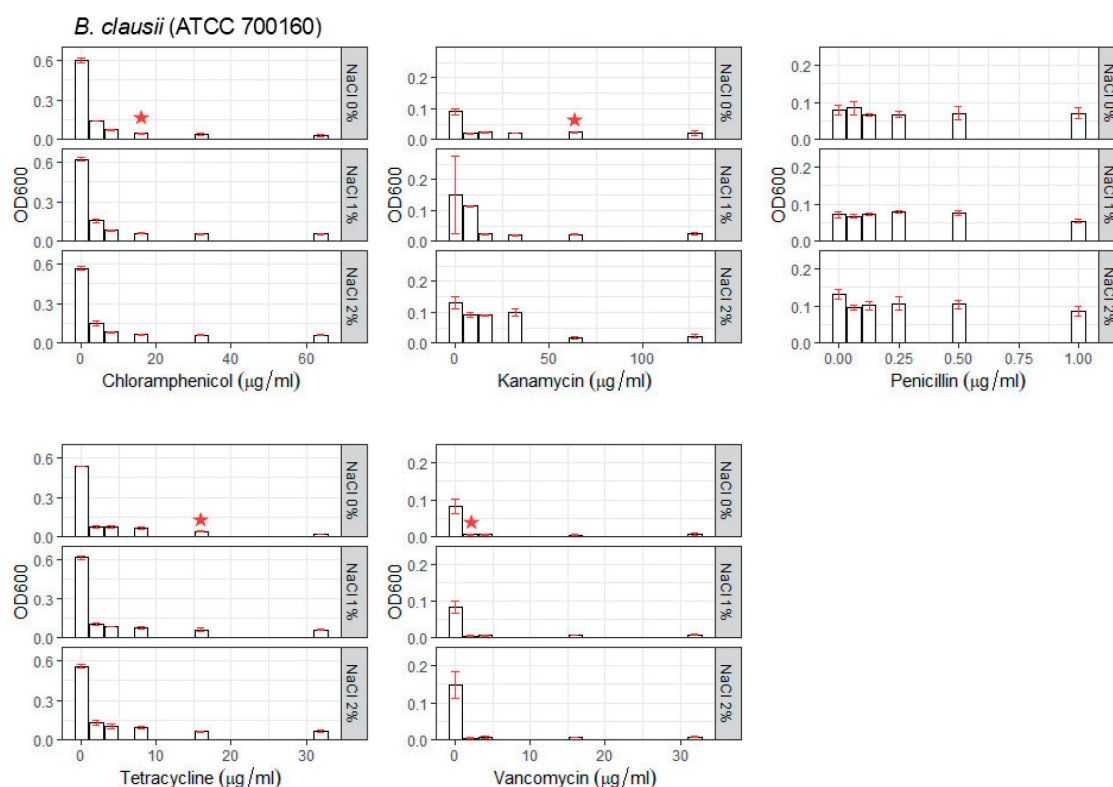


Figure S9. Susceptibility of *B. clausii* (ATCC 700160) to various antibiotics (CHL, KAN, PEN, TET, and VAN) under different NaCl concentrations (0%, 1%, and 2%).

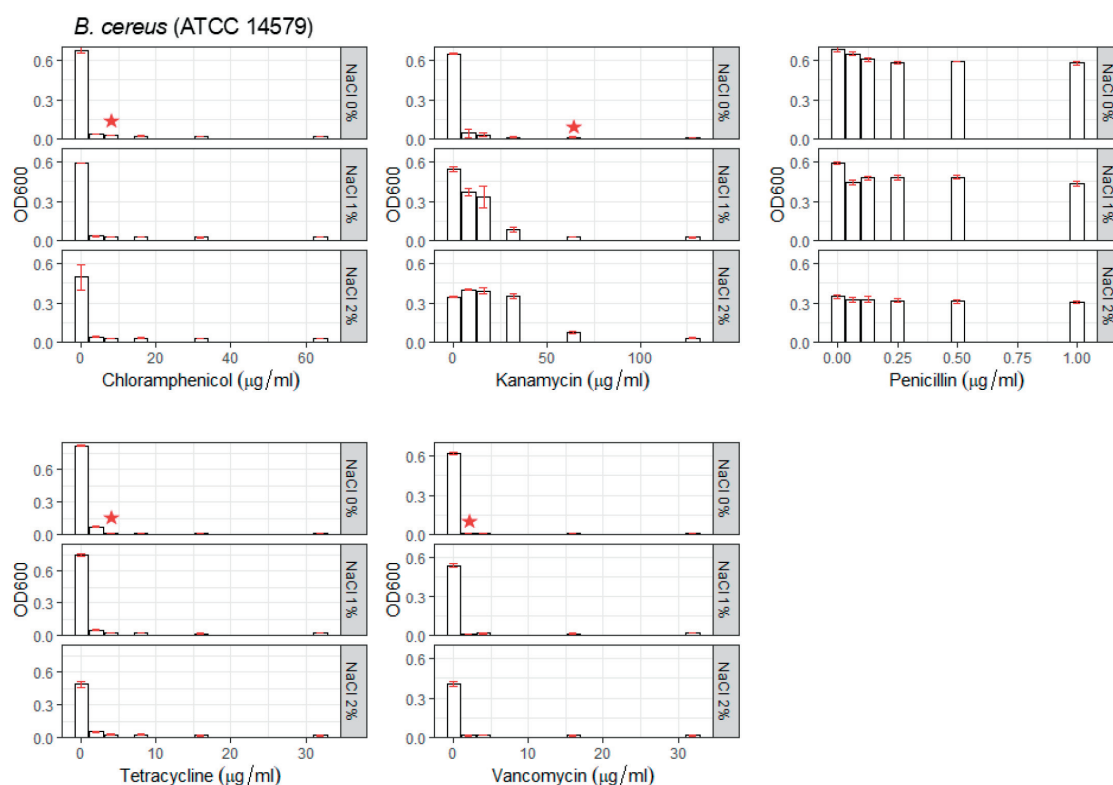


Figure S10. Susceptibility of *B. cereus* (ATCC 14579) to various antibiotics (CHL, KAN, PEN, TET, and VAN) under different NaCl concentrations (0%, 1%, and 2%).