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ASSESSING ANTIBIOTIC SELECTION, TREATMENT SEQUENCING, AND BASELINE SUSCEPTIBILITY ASSOCIATIONS WITH PHAGE ANTIBIOTIC BIOFILM ERADICATION SYNERGY

Kannan Vishwanath* & Lloyd Zulu**
* Garwood Innovation Fellow, Garwood Center for Corporate Innovation,
Haas School of Business, Berkeley
** World Christian University, Lusaka, Zambia

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Abstract:

This analysis examines how antibiotic choice, administration schedule and baseline susceptibility distinguish biofilm synergy patterns relevant to antimicrobial combination screening. Published experimental data comprise 80 treatment conditions across ten clinical Staphylococcus aureus isolates collected between 2015 and 2019. Descriptive stratification and 40 matched schedule comparisons retain isolate identity and source synergy classifications. Synergy occurred in 60% of prior phage conditions versus 30% of simultaneous conditions. Twelve matched pairs showed synergy only under prior phage administration, with none showing the reverse pattern. Fosfomycin accounted for half of these additional classifications, while daptomycin remained synergistic across both schedules. Baseline susceptibility showed antibiotic specific patterns rather than a common response gradient. Omitting each isolate in turn preserved a positive schedule difference of 25.0 to 33.3 percentage points. Longer exposure accompanied prior phage administration, preventing attribution to sequencing alone. The contribution distinguishes persistent synergy, schedule associated gains and persistent absence of demonstrated synergy, refining the descriptive interpretation of antimicrobial complementarity. These findings support combination specific screening and matched exposure protocols for internationally comparable laboratory evaluation.

Key Words: Antimicrobial Combinations; Bacteriophages; Biofilm Eradication; Descriptive Analysis; Treatment Sequencing

1. Introduction:

Selecting a phage and antibiotic combination for a biofilm infection requires more than demonstrating that both agents can kill bacteria. Their joint activity depends on whether antibiotic exposure preserves, enhances or restricts the bacterial processes needed for productive phage infection. A combination that performs well under one administration schedule may therefore perform differently under another. This dependence matters for laboratory screening because a general expectation of synergy can obscure differences among antibiotics, clinical isolates and exposure conditions. Gu Liu et al., 2020, demonstrated that phage and antibiotic interactions vary with antibacterial mechanisms and treatment stoichiometry, establishing the need to evaluate specific combinations rather than assume a common response. Biofilm organisation adds a further constraint to combination selection. Antibiotic susceptibility measured in planktonic cultures does not directly describe the concentration needed to eradicate adherent bacterial populations. Phages may alter this relationship through bacterial lysis and changes in the extracellular matrix. Tkhilaishvili et al., 2018, demonstrated matrix degradation and activity against persister cells using the staphylococcal phage Sb-1. These mechanisms provide a biological rationale for complementary treatment, but their presence does not establish that every antibiotic will benefit equally. The relevant empirical question concerns which combinations exhibit synergy under specified conditions and how consistently those classifications extend across clinical isolates.

Administration timing can reshape these interactions. Prior phage exposure may allow infection and replication before antibiotics restrict host activity, while simultaneous administration exposes both agents to the bacterial population together. Kumaran et al., 2018, demonstrated improved activity when phage preceded selected antibiotics in Staphylococcus aureus biofilms. However, concentration also affects the additional benefit attributable to combination treatment. Dickey and Perrot, 2019, found that adding phage improved activity for several antibiotics at lower concentrations, whereas additional benefits were less frequent at higher concentrations. Together, these findings make antibiotic identity, schedule and baseline susceptibility relevant analytical dimensions, without establishing a universal ordering of treatments. Measurement differences also constrain comparisons across experiments. Chaudhry et al., 2017, assessed synergy through bacterial killing beyond that expected under independent action. The clinical isolate experiments reported by Wang et al., 2020, instead classified synergy using the ratio of combination to antibiotic alone minimum biofilm eradication concentrations. These outcomes address related but distinct questions. The former evaluates departure from independent killing, while the latter identifies a specified reduction in antibiotic concentration required for eradication. Retaining the source definition therefore supports transparent interpretation, whereas pooling these classifications as equivalent outcomes would obscure differences in what synergy represents.

The closest empirical foundation is the investigation by Wang et al., 2020, which already established variation across antibiotics, schedules and rifampin resistant clinical isolates. The present analysis contributes a structured descriptive reanalysis of those published observations rather than a new experimental demonstration of synergy. Its focus is the distinction between combinations that remain synergistic across schedules, combinations classified as synergistic only after prior phage administration, and combinations without demonstrated synergy under either schedule. It also examines whether baseline inhibitory concentrations distinguish these response patterns within individual antibiotics. This separation gives aggregate synergy proportions a clearer interpretation while preserving the experimental conditions underlying each observation. This study aims to characterise how antibiotic selection, administration schedule and baseline antibiotic susceptibility correspond to observed biofilm eradication synergy across clinical Staphylococcus aureus isolates. The analysis uses 80 published treatment conditions from ten isolates collected between 2015 and 2019, organised into 40 matched isolate and antibiotic pairs. Descriptive proportions, matched

classification summaries and antibiotic specific susceptibility profiles address three questions: how synergy varies among antibiotics within methicillin groups, how classifications differ across the recorded schedules, and what susceptibility patterns accompany those outcomes. The findings distinguish persistent from schedule associated synergy and show that baseline susceptibility does not provide a common response rule across antibiotics. Their practical relevance lies in informing combination screening and interpretation. Transferability remains conditional on the phage, isolates, assay and exposure schedules because the comparison does not separate administration order from total treatment duration.

2. Theory, Conceptual Framework and Research Question Development:

This analysis draws on ecological complementarity to explain how phages and antibiotics jointly affect bacteria within biofilms. Phages require susceptible bacterial hosts for infection and replication, while antibiotics disrupt cellular functions that can also sustain phage reproduction. Their interaction therefore creates competing pathways. Antibiotic activity may complement phage killing, but it may also reduce the bacterial activity required for phage amplification. Abedon, 2019, explains this dependence as a central constraint on combination therapy. Complementarity provides a theoretical expectation of improved activity under compatible conditions, rather than a universal prediction of synergy. The empirical framework connects antibiotic selection, treatment sequencing and baseline antibiotic susceptibility with observed biofilm eradication synergy. The dataset contains 80 treatment conditions across ten clinical Staphylococcus aureus isolates. Synergy follows the source classification based on a combination to antibiotic alone minimum biofilm eradication concentration ratio of ≤0.25. Wang et al., 2020, provide the experimental measurements and classification rule. The analysis retains this binary outcome because censored concentrations limit exact ratio comparisons. The observations represent repeated conditions within isolates, and the framework concerns laboratory treatment responses.

Antibiotic selection determines which bacterial functions are disrupted during phage exposure. Agents that interfere with cell wall synthesis, membrane activity or protein synthesis can create different conditions for bacterial survival and phage replication. Drug identity therefore represents a mechanistically relevant treatment characteristic rather than an interchangeable component of combination therapy. Complementary action is plausible when an antibiotic weakens bacterial survival without preventing productive phage infection. Interference is plausible when antibiotic exposure suppresses processes needed for viral replication before phages establish infection. These competing mechanisms imply variation among combinations, but they do not justify a single directional ranking across all antibiotics and isolates.

Chaudhry et al., 2017, demonstrated that some phage and antibiotic combinations reduced viable biofilm populations beyond the effects expected from independent killing. Their findings establish that compatibility varies across combinations. Because those experiments involved Pseudomonas aeruginosa, they support the general interaction mechanism without establishing an antibiotic ranking for Staphylococcus aureus. This analysis therefore compares observed synergy proportions among antibiotics within the methicillin groups in which they were tested.

RQ1: How do observed biofilm eradication synergy proportions differ among antibiotics within each methicillin susceptibility group?

Treatment sequencing concerns when bacterial populations encounter each antimicrobial agent. Administering phage before antibiotics may permit infection and local replication before antibiotic exposure restricts bacterial activity. Simultaneous administration introduces both agents together and may either preserve complementary killing or produce interference from the start. The theoretical advantage of prior phage exposure depends on the compatibility of the selected antibiotic with phage infection. A sequence advantage should therefore remain conditional rather than universal. In the available protocol, prior phage administration also involves a longer total treatment period. The measurable comparison consequently concerns the complete administration schedules, including their durations. Kumaran et al., 2018, found that phage exposure before antibiotics improved killing in Staphylococcus aureus biofilms under selected experimental conditions. This evidence motivates comparison of the two recorded schedules. It does not establish that sequencing alone explains differences when exposure duration also changes. The analysis compares matched isolate and antibiotic conditions and describes the direction of schedule differences without assigning them solely to treatment order.

RQ2: Within matched isolate and antibiotic conditions, how does observed biofilm eradication synergy differ between simultaneous administration and prior phage administration under the recorded treatment schedules?

Baseline antibiotic susceptibility represents the concentration required to inhibit planktonic bacterial growth before combination treatment. It captures an isolate and antibiotic characteristic rather than a property of the administration schedule. Higher minimum inhibitory concentrations indicate lower susceptibility to the same antibiotic, but concentrations cannot provide a common susceptibility ranking across different drugs. Baseline susceptibility may influence how strongly an antibiotic alters the host population available to phages. However, planktonic growth inhibition and biofilm eradication measure different biological responses. Biofilm structure and bacterial physiological state can weaken the correspondence between these endpoints. A directional relationship between minimum inhibitory concentration and synergy therefore requires evidence rather than assumption. Dickey and Perrot, 2019, found that adding phage improved the activity of several antibiotics at lower concentrations, while additional benefits were less frequent at higher concentrations. Their findings concern experimental antibiotic exposure relative to inhibitory concentrations. They do not establish that isolates with higher baseline inhibitory concentrations necessarily show less synergy. This analysis therefore describes baseline susceptibility alongside synergy within individual antibiotics, retaining isolate identity and administration schedule.

RQ3: Within each antibiotic, what descriptive pattern links baseline minimum inhibitory concentration with observed synergy under each administration schedule?

The conceptual framework treats antibiotic identity, administration schedule and baseline minimum inhibitory concentration as separate observed variables. Biofilm eradication synergy is the observed binary outcome. Methicillin susceptibility defines comparable antibiotic groups, while isolate identity preserves the repeated observation structure. The proposed relationships express descriptive associations. The framework supports antibiotic specific comparisons, matched schedule comparisons and susceptibility profiles without treating the 80 conditions as independent biological samples.

3. Data:

This analysis establishes the provenance of the experimental records, documents sample construction and defines the observed variables. It combines antibiotic specific summaries with matched schedule comparisons while preserving isolate identity, concentration censoring and the published synergy classifications.

3.1 Data Sources, Population, and Sample Construction:

The empirical source is the published experimental dataset reported by Wang et al., 2020, in Frontiers in Microbiology. Table 1 supplies baseline antibiotic susceptibility and eradication concentrations, while Table 3 supplies clinical isolate combination outcomes. The version of record is identified by DOI 10.3389/fmicb.2020.602057 and is accessible through the publisher under a Creative Commons Attribution licence. The supplied workbook consolidates these tables rather than introducing an independent experimental sample. The accessible frame comprises ten rifampin resistant clinical Staphylococcus aureus isolates from an institutional prosthetic joint infection biobank in Berlin, Germany. Isolates were collected between 2015 and 2019, and the experimental findings were published in 2020. The eligibility window of 2010 to 2023 therefore describes the permitted evidence period, rather than annual observation coverage. Individual collection dates and experimental calendar dates are unavailable.

The target population concerns clinical isolates relevant to biofilm associated infections. The accessible sample contains four methicillin resistant and six methicillin susceptible isolates. Each contributes four antibiotic conditions under two administration schedules, producing eight records per isolate. The observational unit is an isolate, antibiotic and schedule combination. The data form a repeated experimental cross section with 40 matched schedule pairs. Selection retained every clinical isolate condition in the main combination table. Laboratory reference strains and supplementary rifampin experiments fall outside this extraction frame. These boundaries describe the implemented extraction; a dated preregistered selection protocol is unavailable.

Stage	Isolates	Treatment Conditions	Reason or Rule
Accessible clinical frame in Table 3	10	80	Four antibiotics and two schedules per isolate
Selected extraction	10	80	All clinical conditions retained
Linked baseline measurements	10	80	Matching by isolate and antibiotic
Duplicate or unmatched records removed	0	0	All analytical keys were unique and matched
Final descriptive sample	10	80	Complete outcome and predictor fields
Matched schedule comparison	10	40 pairs	One simultaneous and one prior phage condition per pair

Coverage is balanced across isolates and schedules, but antibiotic coverage differs by methicillin group. Daptomycin and vancomycin occur in four isolates, cefazolin and flucloxacillin in six, and fosfomycin and gentamicin in all ten. The sample supports comparisons within these observed combinations rather than a complete factorial comparison of all antibiotics across all isolates.

3.2 Data Integration, Cleaning, and Quality Assurance:

Baseline measurements were linked to combination outcomes using isolate identity and antibiotic name. Each baseline record corresponds to two schedule records, establishing an intended relationship of one baseline observation to two treatment observations. The resulting dataset contains 80 unique isolate, antibiotic and schedule keys. Repeated isolate identifiers represent experimental dependence rather than duplicate records. Quality checks examined key uniqueness, schedule completeness, binary outcome coding, positive concentrations, measurement units and agreement with published table entries. Every isolate and antibiotic pair contains both schedules. The analytical fields contain no missing values, and no observations were deleted, imputed or interpolated.

Concentrations remain in micrograms per millilitre. Greater than signs were preserved because they identify assay limits rather than exact measurements. Baseline eradication concentrations exceed the reported limit in 52 records, while combination eradication concentrations are censored in 41 records. These counts refer to treatment rows, including repeated baseline measurements across schedules. The workbook contains seven ratio flags. Arithmetic inspection distinguishes one difference consistent with reported rounding from six larger decimal discrepancies. The primary analysis retains the published binary synergy labels rather than treating the reported ratios as exact continuous outcomes. Two auxiliary spreadsheet columns also contain formula errors and were excluded from the descriptive calculations. Their exclusion removes no treatment records because source concentrations, schedule identifiers, baseline susceptibility and synergy labels remain available.

Observed minimum inhibitory concentrations range from 0.25 to 128 micrograms per millilitre. The upper value remains in the sample because it agrees with the source table. No winsorisation, trimming or numerical replacement was applied. The analysis uses published endpoint measurements rather than reconstructed biological trajectories. It preserves both concentration units and treatment durations. This distinction matters because Dickey and Perrot, 2019, showed that combination responses depend on antibiotic exposure relative to inhibitory concentrations, making concentration context essential when comparing experimental outcomes.

3.3 Variable Measurement, Construction, and Validation:

Biofilm eradication synergy is the observed outcome. The source defines synergy through a combination to antibiotic alone eradication concentration ratio of ≤ 0.25. The analytical indicator equals one for published synergistic classifications and zero for classifications without demonstrated synergy. Zero does not imply antagonism. Antibiotic selection is categorical. Treatment schedule distinguishes simultaneous exposure from phage exposure followed by antibiotic exposure. Baseline susceptibility is measured by the minimum inhibitory concentration for planktonic bacteria. It remains antibiotic specific because equal numerical concentrations do not represent equivalent susceptibility across different drugs. The dictionary below identifies the variables used in the analysis. Source identifiers refer to Tables 1 and 3 of the experimental publication.

Variable or Indicator	Analytical Role	Definition and Construction	Source and Identifier	Unit and Frequency	Transformation and Direction	Missing Value Rule	Coverage and Limitation
Biofilm eradication synergy	Outcome	Published synergy classification	Table 3	Binary experimental endpoint	Synergy = 1; otherwise = 0	None missing	80 conditions; assay specific classification
Antibiotic selection	Explanatory grouping	Recorded antibiotic identity	Tables 1 and 3	Six categories per condition	No numerical ordering	None missing	Antibiotic availability differs by methicillin group
Treatment schedule	Paired comparison	Simultaneous exposure or phage followed by antibiotic	Table 3 and protocol	Two schedules per pair	Prior phage administration = 1	None missing	Schedule and total duration vary together
Baseline inhibitory concentration	Susceptibility measure	Lowest antibiotic concentration inhibiting planktonic growth	Table 1	Micrograms per millilitre per isolate and antibiotic	Higher values indicate lower susceptibility to the same antibiotic	None missing	40 distinct measurements, repeated across schedules
Methicillin group	Stratification variable	Resistant or susceptible isolate classification	Tables 1 and 3	Category per isolate	No transformation	None missing	Four resistant and six susceptible isolates
Isolate identity	Dependence identifier	Published clinical strain label	Tables 1 and 3	Identifier per isolate	No transformation	None missing	Ten biological units
Treatment duration	Protocol descriptor	Total antimicrobial exposure time	Experimental protocol	Hours per condition	24 or 48 hours	None missing	Completely aligned with schedule
Eradication concentrations	Measurement audit	Baseline and combination concentrations with censoring retained	Tables 1 and 3	Micrograms per millilitre per condition	Higher thresholds indicate greater concentration requirements	Censoring retained	Not analysed as exact continuous outcomes

The phage identity, initial phage concentration and biofilm formation period are constant across the extracted conditions. They define the experimental setting rather than estimated explanatory effects. All measures are observed laboratory variables; no latent construct or composite index was constructed. Baseline susceptibility summaries use each isolate and antibiotic measurement once, avoiding duplication across schedules.

Antibiotic	Valid Measurements	Mean	Standard Deviation	Median	Minimum	Maximum
Cefazolin	6	0.375	0.137	0.375	0.25	0.50
Daptomycin	4	0.750	0.289	0.750	0.50	1.00
Flucloxacillin	6	0.417	0.129	0.500	0.25	0.50
Fosfomycin	10	19.600	38.387	6.000	4.00	128.00
Gentamicin	10	1.250	2.372	0.500	0.50	8.00
Vancomycin	4	1.250	0.500	1.000	1.00	2.00

All concentration statistics are expressed in micrograms per millilitre. Standard deviations describe variation across isolate and antibiotic measurements within each drug. Across 80 conditions, the synergy indicator has a mean of 0.450, a sample standard deviation of 0.501, a median of zero and a range from zero to one. These statistics describe treatment records rather than independent isolate prevalence. The outcome definition remains distinct from other synergy frameworks. Chaudhry et al., 2017, evaluated killing relative to independent action, whereas the present dataset records an eradication concentration threshold classification. Their findings provide biological context but are not pooled with these observations as equivalent measurements.

3.4 Analytical Framework, Identification, and Reproducibility:

The implemented analysis is descriptive. It reports synergy frequencies by antibiotic, methicillin group and schedule, together with baseline susceptibility distributions. The principal schedule comparison preserves the 40 matched isolate and antibiotic pairs. Equation 1 defines the synergy proportion within an observed group.

$$p_g = \frac{s_g}{n_g}$$

Here, p_g is the observed synergy proportion in group g, s_g is the number of conditions classified as synergistic, and n_g is the number of recorded conditions in that group. Equation 2 expresses the schedule contrast in percentage points.

$$D = 100\left[p_{prior} - p_{simultaneous}\right]$$

Here, D is the descriptive percentage point difference, p_{prior} is the synergy proportion under prior phage administration, and $p_{simultaneous}$ is the proportion under simultaneous administration.

The recorded proportions are 24 of 40 under prior phage administration and 12 of 40 under simultaneous administration, yielding a difference of 30 percentage points. Pairwise inspection identifies 12 pairs classified as synergistic under both schedules, 16 under neither schedule and 12 only under prior phage administration. These counts describe the observed schedules without treating the pairs as independent isolates. The comparison does not isolate a causal sequencing effect because simultaneous administration lasts 24 hours, whereas prior phage administration involves two successive 24-hour exposures. Kumaran et al., 2018, provide experimental support for examining administration order, but the current contrast combines order and total duration. Antibiotic summaries remain stratified by methicillin group to respect the observed allocation of drugs. Baseline susceptibility is described within antibiotics rather than pooled into a common numerical ranking. No regression coefficients, interaction effects, significance tests or causal treatment estimates were produced.

The interpretation also remains conditional on phage compatibility. Abedon, 2019, explains that antibiotics can interfere with bacterial functions needed for productive phage infection. Consequently, descriptive differences among combinations do not establish a universal benefit of combining phages with antibiotics. Reproducibility rests on the published table identifiers, retained

isolate labels, explicit schedule coding and preserved source classifications. The extraction was checked against the same publication rather than an independent dataset. The contribution is therefore a structured secondary analysis that separates antibiotic coverage, matched schedule responses and susceptibility distributions while maintaining a clear boundary between observed laboratory patterns and causal interpretation.

4. Methods:

4.1 Research Design and Analytical Scope:

This analysis uses a descriptive secondary design to examine variation in biofilm eradication synergy across antibiotics, administration schedules and baseline susceptibility profiles. The analytical unit is an isolate, antibiotic and schedule combination. The sample contains 80 conditions from ten clinical isolates, organised into 40 matched isolate and antibiotic pairs. Comparisons retain this structure rather than treating every condition as an independent biological sample. The analytical targets are observed synergy proportions within antibiotic and methicillin groups, differences between matched administration schedules, and susceptibility patterns within individual antibiotics. The source experiments reported by Wang et al., 2020, provide the treatment classifications and measurement definitions. The analysis reorganises these published observations without introducing additional experimental measurements. Schedule comparisons hold isolate identity and antibiotic selection constant within each pair. However, simultaneous treatment involves 24 hours of exposure, whereas prior phage administration involves successive 24 hour exposures. Consequently, the comparison concerns complete administration schedules. It does not identify an isolated causal effect of treatment order. Antibiotic comparisons also remain within the methicillin groups in which the drugs were tested because treatment coverage differs across groups.

4.2 Measurement Alignment and Analytical Sample:

The sample construction table and variable dictionary document eligibility, source identifiers and coding. All clinical isolate conditions in the extracted combination table enter the descriptive analysis. Laboratory reference strains and supplementary rifampin experiments fall outside that extraction frame. No additional observations are lost through analytical processing. The outcome retains the published synergy classification, coded one when synergy is demonstrated and zero otherwise. The source classification uses a combination to antibiotic alone minimum biofilm eradication concentration ratio of ≤ 0.25. A zero classification indicates that the assay did not demonstrate synergy under its stated rule; it does not establish antagonism. Antibiotic identity and administration schedule remain categorical. Baseline minimum inhibitory concentration retains its original unit of micrograms per millilitre. Susceptibility comparisons are conducted within antibiotics because equal concentrations across different drugs do not provide a common biological scale. Each baseline measurement is counted once per isolate and antibiotic when calculating its distribution, avoiding duplication across the two schedules. Methicillin group defines the antibiotic comparison strata, while isolate identity identifies repeated observations. Phage identity, starting phage concentration and biofilm formation duration define the shared experimental setting. No composite index, latent construct, moderator or mediation measure is constructed.

4.3 Descriptive Estimation and Matched Comparisons:

The analysis first checks the cleaned records for unique isolate, antibiotic and schedule keys. It then tabulates condition counts and synergy frequencies by antibiotic, methicillin group and administration schedule. Equation 1 defines each observed synergy proportion, and Equation 2 expresses the difference between schedules in percentage points. Every reported proportion retains its observed denominator. Matched comparisons classify each isolate and antibiotic pair into one of four outcome combinations: synergy under both schedules, synergy only under simultaneous administration, synergy only under prior phage administration, or no demonstrated synergy under either schedule. These classifications distinguish changes within pairs from differences in sample composition. Equation 3 defines the matched classification counts.

$$n_{ab} = \sum_{j=1}^{40} \mathbf{1}_{Y_{j0}=a,\, Y_{j1}=b}, \qquad a, b \in \{0,1\}$$

Here, n_{ab} is the number of pairs with simultaneous classification aand prior phage classification b. The index j identifies an isolate and antibiotic pair. $Y_{j0}$and Y_{j1}denote its binary classifications under simultaneous and prior phage administration, respectively. The indicator equals one when both stated conditions hold and zero otherwise. Baseline susceptibility summaries report valid counts, arithmetic means, sample standard deviations, medians and observed limits. Additional cross tabulations describe synergy at each recorded inhibitory concentration within each antibiotic and schedule. These summaries assess descriptive patterns without imposing a linear relationship. All conditions receive equal weight in condition summaries. Isolate level summaries count the number of synergistic antibiotic conditions under each schedule. Calculations use Python with openpyxl for workbook reading, collections for grouping and statistics for distribution summaries. The procedures are deterministic and require no random seed. No regression, significance test or model-based confidence interval is estimated.

4.4 Quality Checks, Sensitivity and Reproducibility:

Quality assurance checks key uniqueness, completeness of schedule pairs, binary outcome coding, concentration units and consistency with source table entries. Repeated isolate identifiers are retained as legitimate experimental observations. The analytical outcome and predictor fields contain no missing values, so deletion, imputation and interpolation are unnecessary. Greater than concentration values remain censored rather than being replaced with exact thresholds. Valid extreme inhibitory concentrations remain unchanged. Published synergy labels provide the primary outcome because reported ratios include rounding differences and decimal discrepancies. Auxiliary spreadsheet fields containing formula errors are excluded from calculations; their exclusion does not remove treatment records.

An exploratory sensitivity analysis omits each isolate in turn and recalculates the aggregate schedule difference using the remaining complete pairs. This procedure evaluates whether the direction and magnitude of the descriptive contrast depend on any single isolate. It does not estimate sampling uncertainty or establish external validity. The analysis also decomposes the additional synergistic classifications by antibiotic, expressing each contribution as a share of the total observed gain rather than as a mediated effect. Replication requires the published source tables, the supplied workbook, its variable dictionary and the grouping rules

specified here. Source identifiers, isolate labels and original classifications preserve traceability. The methodological contribution is transparent application of established descriptive procedures to matched experimental observations. The design supports comparison within the accessible sample while preserving the distinction between laboratory response patterns, biological mechanisms and clinical effectiveness.

5. Findings:

This analysis examines how observed biofilm eradication synergy varies with antibiotic selection, administration schedule and baseline antibiotic susceptibility. The findings distinguish treatment condition summaries from matched comparisons within isolates. They resolve the research questions through descriptive evidence without interpreting laboratory associations as causal treatment effects.

5.1 Sample Composition and Outcome Distribution:

The analytical sample contains 80 treatment conditions from ten clinical Staphylococcus aureus isolates collected between 2015 and 2019. Four isolates are methicillin resistant and six are methicillin susceptible. Each isolate contributes four antibiotic conditions under two administration schedules, yielding 40 matched pairs. All outcome, antibiotic, schedule and baseline susceptibility fields are complete, and all 80 analytical keys are unique. Synergy is recorded in 36 conditions, representing 45.0% of the sample. The remaining 44 conditions do not meet the published synergy classification. These outcomes retain the assay definition reported by Wang et al., 2020, rather than interpreting absence of demonstrated synergy as antagonism. The observations are a secondary analysis of published experimental results, not independent replication.

Methicillin resistant isolates account for 32 conditions, including 15 synergistic classifications, or 46.9%. Methicillin susceptible isolates account for 48 conditions, including 21 synergistic classifications, or 43.8%. This small aggregate difference conceals substantial variation across antibiotics and schedules. It cannot establish a resistance group effect because the antibiotic sets differ between groups.

5.2 Antibiotic Selection and Observed Synergy:

The antibiotic comparisons answer RQ1 by identifying variation within the methicillin groups in which each drug was tested. Daptomycin shows synergy in every recorded condition among resistant isolates, whereas vancomycin shows none. Fosfomycin and gentamicin occupy intermediate positions, with outcomes depending on the administration schedule. Observed synergy by antibiotic, methicillin group and schedule

Methicillin Group	Antibiotic	Simultaneous Synergy	Prior Phage Synergy	Difference in Percentage Points
Resistant	Daptomycin	4 of 4, 100.0%	4 of 4, 100.0%	0.0
Resistant	Fosfomycin	0 of 4, 0.0%	2 of 4, 50.0%	50.0
Resistant	Gentamicin	2 of 4, 50.0%	3 of 4, 75.0%	25.0
Resistant	Vancomycin	0 of 4, 0.0%	0 of 4, 0.0%	0.0
Susceptible	Cefazolin	0 of 6, 0.0%	4 of 6, 66.7%	66.7
Susceptible	Flucloxacillin	3 of 6, 50.0%	4 of 6, 66.7%	16.7
Susceptible	Fosfomycin	0 of 6, 0.0%	4 of 6, 66.7%	66.7
Susceptible	Gentamicin	3 of 6, 50.0%	3 of 6, 50.0%	0.0

Among susceptible isolates, prior phage administration produces the same synergy proportion for cefazolin, flucloxacillin and fosfomycin, but their simultaneous outcomes differ. Flucloxacillin retains synergy in half of the isolates under simultaneous administration, while cefazolin and fosfomycin show none. Thus, identical proportions under one schedule do not imply equivalent response profiles across schedules. The observed antibiotic variation is consistent with combination specific activity rather than a uniform benefit from adding phage. Chaudhry et al., 2017, similarly identified variation across antibiotic and phage combinations, although their experiments involved Pseudomonas aeruginosa and a different synergy framework. The present findings establish descriptive differences within the available Staphylococcus aureus conditions without transferring rankings across bacterial species.

5.3 Matched Administration Schedule Comparisons:

The matched comparison answers RQ2. Synergy occurs in 12 of 40 simultaneous conditions and 24 of 40 prior phage conditions. The corresponding proportions are 30.0% and 60.0%, yielding an absolute difference of 30 percentage points. Matched synergy classifications across administration schedules

Simultaneous Classification	Prior Phage Classification	Matched Pairs	Share of Pairs
Synergy	Synergy	12	30.0%
No demonstrated synergy	Synergy	12	30.0%
Synergy	No demonstrated synergy	0	0.0%
No demonstrated synergy	No demonstrated synergy	16	40.0%
Total	Both schedules observed	40	100.0%

The schedule difference arises entirely from 12 pairs classified as synergistic only under prior phage administration. No pair shows the reverse pattern. Nevertheless, 16 pairs remain without demonstrated synergy under either schedule. Prior phage administration therefore expands the observed set of synergistic combinations without producing a universal response. The difference is larger among susceptible isolates. Their synergy proportion increases from 25.0% to 62.5%, compared with an increase from 37.5% to 56.3% among resistant isolates. These are descriptive group contrasts because antibiotic coverage differs.

Seven isolates show more synergistic antibiotic conditions under prior phage administration, while three show unchanged counts. An exploratory sensitivity check omitted each isolate in turn. The aggregate schedule difference remained positive, ranging from 25.0 to 33.3 percentage points. Its direction therefore does not depend on retaining any single isolate. These patterns

align with the schedule dependent activity documented by Kumaran et al., 2018. However, the available protocol combines prior phage administration with a longer total exposure period. The comparison identifies a difference between complete treatment schedules and cannot separate sequencing from duration.

5.4 Baseline Susceptibility and Response Heterogeneity:

The susceptibility profiles answer RQ3 through comparisons within individual antibiotics. Fosfomycin provides the clearest descriptive separation. Under prior phage administration, synergy occurs in four of five isolates with a baseline inhibitory concentration of 4 micrograms per millilitre and both isolates with a concentration of 8 micrograms per millilitre. Neither of the two isolates at 16 micrograms per millilitre nor the isolate at 128 micrograms per millilitre shows synergy. Under simultaneous administration, none of these fosfomycin conditions is synergistic. Gentamicin shows a related but incomplete pattern. Among nine isolates with a baseline inhibitory concentration of 0.5 micrograms per millilitre, five show synergy under simultaneous administration and six under prior phage administration. The single isolate with a concentration of 8 micrograms per millilitre shows no synergy under either schedule. Lower baseline concentrations therefore coincide with more observed synergy, but do not identify every responsive isolate.

The other antibiotics do not show a consistent susceptibility gradient. Daptomycin shows synergy at both recorded concentrations. Vancomycin shows none at either concentration. Cefazolin produces identical prior phage synergy proportions at its two concentrations, with two of three isolates responding in each category. Flucloxacillin shows simultaneous synergy in half of the isolates at both concentrations, while prior phage responses remain heterogeneous. These results indicate that baseline planktonic susceptibility alone does not distinguish all synergistic combinations. Dickey and Perrot, 2019, demonstrated that combination activity also varies with antibiotic exposure relative to inhibitory concentrations. Their findings support retaining antibiotic and schedule context when interpreting susceptibility measurements rather than pooling concentrations across drugs. Across the three research questions, antibiotic identity distinguishes observed response profiles, prior phage schedules contain more synergistic conditions, and baseline susceptibility shows antibiotic specific patterns. The evidence does not establish a common susceptibility rule across all combinations. This conditional pattern is compatible with the competing phage and antibiotic interactions examined by Abedon, 2019, but the present data do not directly measure those mechanisms. The empirical conclusion remains bounded to ten isolates, the recorded schedules and the published binary synergy endpoint.

6. Discussion:

The central finding is that greater aggregate synergy conceals distinct antibiotic and isolate response patterns. The antibiotic comparison table separates combinations that remain synergistic across schedules from combinations whose classification changes with administration timing. Equation 1 expresses these differences as observed proportions, while Equation 2 identifies a 30-percentage point schedule contrast. Neither represents an adjusted causal effect. The contribution of this secondary analysis lies in separating persistent synergy, schedule associated gains and persistent absence of demonstrated synergy within matched observations. It does not establish a previously undiscovered biological interaction. Gu Liu et al., 2020, showed that interaction profiles depend on antibiotic action and treatment stoichiometry. The present analysis sharpens that interpretation by demonstrating why an aggregate synergy proportion cannot substitute for an antibiotic and isolate response profile. The paired classification table shows that every discordant pair favours prior phage administration. This asymmetry is compatible with biological preparation of the biofilm before antibiotic exposure, but it does not identify the process responsible. Tkhilaishvili et al., 2018, directly demonstrated matrix degradation and activity against persister cells using the same phage. Those experimental findings provide a plausible explanation for the observed schedule pattern, rather than evidence that either mechanism mediated the current results. The distinction changes the interpretation of combination performance: administration schedules identify conditions under which synergy becomes observable, while direct measurements of matrix disruption, phage replication and bacterial physiology are needed to establish transmission pathways. Here, longer total exposure also accompanies prior phage administration, so the observed advantage belongs to the complete schedule.

A descriptive decomposition of the antibiotic comparison table identifies where the additional synergistic classifications arise. Fosfomycin contributes six of the twelve schedule associated gains, cefazolin contributes four, and flucloxacillin and gentamicin contribute one each. Thus, half of the additional classifications occur with fosfomycin, while daptomycin and vancomycin contribute none for different reasons: daptomycin already shows synergy under simultaneous administration, whereas vancomycin remains without demonstrated synergy. These shares describe contributions to the observed count difference, not biological mediation effects. Ryan et al., 2012, demonstrated improved biofilm control through a specific phage and antibiotic combination, supporting evaluation at the combination level. The present decomposition further distinguishes an absence of room for improvement from an absence of observed response, two patterns that an aggregate schedule contrast would otherwise combine. Baseline susceptibility reveals another constraint on generalisation. The fosfomycin and gentamicin profiles suggest that higher inhibitory concentrations can coincide with fewer synergistic responses, yet cefazolin, daptomycin and vancomycin provide no comparable concentration gradient. Planktonic susceptibility therefore supplies context without functioning as a universal selection rule for biofilm combinations. This interpretation accords with Akturk et al., 2019, who showed that antibiotic concentration and application timing influence combined activity in single and mixed species biofilms. The practical implication is to assess the intended combination and schedule directly rather than infer biofilm synergy from baseline susceptibility alone. The positive schedule difference also survives omission of each isolate in turn, but this sensitivity result establishes stability within the ten isolate sample rather than transportability to broader clinical populations.

The international relevance concerns experimental comparability and treatment screening rather than differences between national economies. Akturk et al., 2023, found that simultaneous treatment delivered through repeated doses improved activity in a mixed species wound model. That result contrasts with the absence of simultaneous synergy for several combinations here and shows why prior phage administration cannot be treated as universally preferable. Differences in dosing, microbial composition and biofilm substrate can change which schedule performs best. Laboratories should therefore compare candidate schedules under matched total exposure periods and retain isolate specific outcomes alongside aggregate summaries. The theoretical refinement is a conditional account of complementarity: antibiotic identity, bacterial susceptibility and administration conditions jointly define

the observed response. The evidence supports this focused screening perspective while leaving clinical effectiveness and the relative contribution of individual biological mechanisms to direct experimental evaluation.

7. Conclusion and Implications:

Effective biofilm treatment requires choosing compatible combinations rather than assuming that combining antimicrobial agents guarantees improvement. This analysis shows that treatment responses depend on the pairing and exposure conditions, while conventional susceptibility alone provides an incomplete guide to combined activity. Its contribution is a descriptive distinction between persistent synergy, schedule associated gains and persistent absence of demonstrated synergy. This distinction refines complementarity theory by showing that an aggregate advantage can conceal different response patterns. The evidence supports conditional interpretation rather than a universal treatment hierarchy or an identified causal pathway. For laboratory decision makers, the practical priority is to screen candidate combinations across relevant clinical isolates and administration schedules before advancing them for evaluation. Screening resources should target combinations with heterogeneous responses, where aggregate summaries provide the least guidance. Research governance can support this approach through consistent reporting of exposure duration, assay thresholds and isolate specific outcomes. These practices would improve comparability across laboratories and help distinguish reproducible activity from protocol dependent findings. International relevance therefore rests on a transferable evaluation approach rather than an assumed treatment recommendation. Better targeted screening could strengthen the evidence used to develop antimicrobial strategies, although patient benefit requires clinical evaluation beyond the present laboratory observations.

The ten isolate sample provides a focused basis for expanding evaluation across bacterial populations, phages and biofilm environments. Experiments with equal total exposure periods would distinguish administration order from duration. Direct measurements of phage replication, matrix disruption and surviving bacterial populations would test the mechanisms suggested by the observed patterns. Access to replicate measurements and uncensored concentration endpoints would support uncertainty estimation and alternative response definitions. Coordinated laboratory comparisons could assess reproducibility under shared protocols, while clinically representative infection models would clarify biological transferability. Prospective clinical investigation would then determine whether laboratory screening patterns inform safe and effective treatment selection.

References:

1. Abedon, S. T. 2019. Phage antibiotic combination treatments: Antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy? Antibiotics, volume 8, issue 4, Article 182. https://doi.org/10.3390/antibiotics8040182
2. Akturk, E., Melo, L. D. R., Oliveira, H., Crabbé, A., Coenye, T., & Azeredo, J. 2023. Combining phages and antibiotic to enhance antibiofilm efficacy against an in vitro dual species wound biofilm. Biofilm, volume 6, Article 100147. https://doi.org/10.1016/j.bioflm.2023.100147
3. Akturk, E., Oliveira, H., Santos, S. B., Costa, S., Kuyumcu, S., Melo, L. D. R., & Azeredo, J. 2019. Synergistic action of phage and antibiotics: Parameters to enhance the killing efficacy against mono and dual species biofilms. Antibiotics, volume 8, issue 3, Article 103. https://doi.org/10.3390/antibiotics8030103
4. Chaudhry, W. N., Concepción-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., & Levin, B. R. 2017. Synergy and order effects of antibiotics and phages in killing Pseudomonas aeruginosa biofilms. PLOS ONE, volume 12, issue 1, Article e0168615. https://doi.org/10.1371/journal.pone.0168615
5. Dickey, J., & Perrot, V. 2019. Adjunct phage treatment enhances the effectiveness of low antibiotic concentration against Staphylococcus aureus biofilms in vitro. PLOS ONE, volume 14, issue 1, Article e0209390. https://doi.org/10.1371/journal.pone.0209390
6. Gu Liu, C., Green, S. I., Min, L., Clark, J. R., Salazar, K. C., Terwilliger, A. L., Kaplan, H. B., Trautner, B. W., Ramig, R. F., &Maresso, A. W. 2020. Phage antibiotic synergy is driven by a unique combination of antibacterial mechanism of action and stoichiometry. mBio, volume 11, issue 4, Article e01462-20. https://doi.org/10.1128/mBio.01462-20
7. Kumaran, D., Taha, M., Yi, Q., Ramirez-Arcos, S., Diallo, J. S., Carli, A., &Abdelbary, H. 2018. Does treatment order matter? Investigating the ability of bacteriophage to augment antibiotic activity against Staphylococcus aureus biofilms. Frontiers in Microbiology, volume 9, Article 127. https://doi.org/10.3389/fmicb.2018.00127
8. Ryan, E. M., Alkawareek, M. Y., Donnelly, R. F., & Gilmore, B. F. 2012. Synergistic phage antibiotic combinations for the control of Escherichia coli biofilms in vitro. FEMS Immunology & Medical Microbiology, volume 65, issue 2, pages 395 to 398. https://doi.org/10.1111/j.1574-695X.2012.00977.x
9. Tkhilaishvili, T., Lombardi, L., Klatt, A. B., Trampuz, A., & Di Luca, M. 2018. Bacteriophage Sb-1 enhances antibiotic activity against biofilm, degrades exopolysaccharide matrix and targets persisters of Staphylococcus aureus. International Journal of Antimicrobial Agents, volume 52, issue 6, pages 842 to 853. https://doi.org/10.1016/j.ijantimicag.2018.09.006
10. Wang, L., Tkhilaishvili, T., Trampuz, A., & Gonzalez Moreno, M. 2020. Evaluation of staphylococcal bacteriophage Sb-1 as an adjunctive agent to antibiotics against rifampin-resistant Staphylococcus aureus biofilms. Frontiers in Microbiology, volume 11, Article 602057. https://doi.org/10.3389/fmicb.2020.602057