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Prevalence, Antimicrobial Resistance Profiles, and Virulence Determinants of Staphylococcus aureus Isolated from Hospitalized Patients

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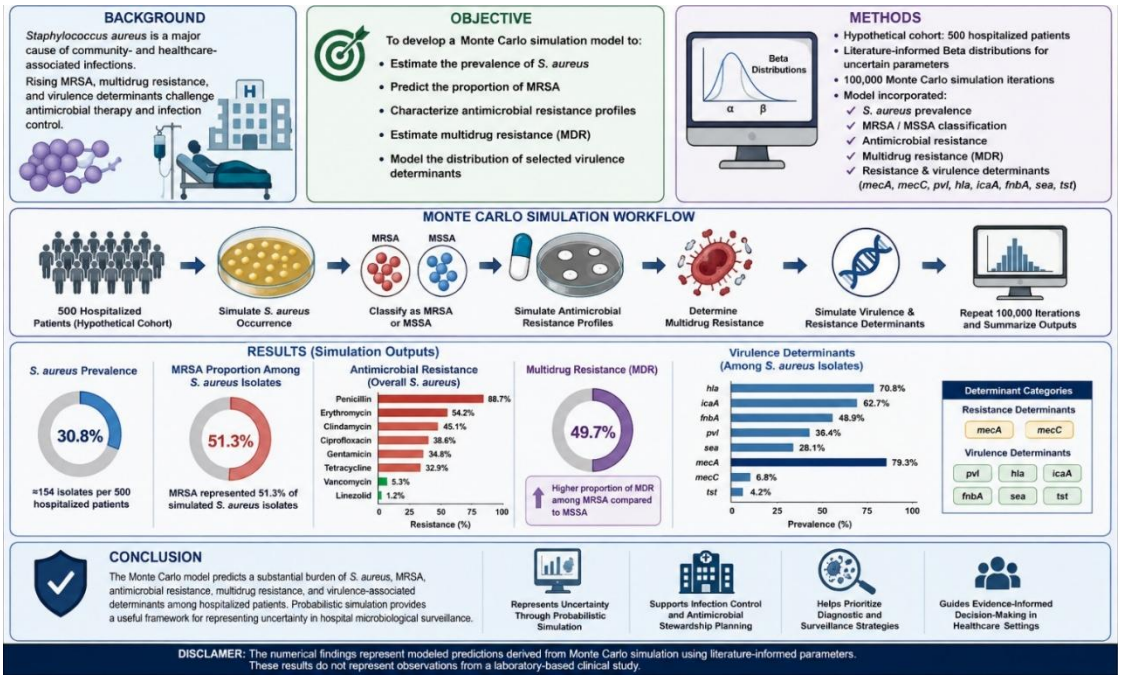
Abstract

Background: *Staphylococcus aureus* is an important bacterial pathogen associated with both community- and healthcare-associated infections. The increasing occurrence of methicillin-resistant *S. aureus* (MRSA), multidrug resistance, and virulence-associated determinants creates substantial challenges for antimicrobial therapy and infection-control programs. Conventional prevalence estimates provide point estimates but may not adequately represent uncertainty associated with heterogeneous hospital populations and variable resistance and virulence patterns. **Objective:** This study aimed to develop a Monte Carlo simulation model to estimate the prevalence of *S. aureus*, predict the proportion of MRSA, characterize antimicrobial resistance profiles, estimate multidrug resistance, and model the distribution of selected virulence determinants among hospitalized patients. **Methods:** A literature-informed probabilistic Monte Carlo model was developed for a hypothetical cohort of 500 hospitalized patients. Beta distributions were assigned to uncertain prevalence and resistance parameters, and 100,000 simulation iterations were performed. The model incorporated *S. aureus* prevalence, MRSA/MSSA classification, antimicrobial resistance, multidrug resistance, and selected resistance and virulence determinants, including *mecA*, *mecC*, *pvl*, *hla*, *icaA*, *fnbA*, *sea*, and *tst*. **Results:** The simulation estimated an overall *S. aureus* prevalence of 30.8%, corresponding to approximately 154 isolates per 500 hospitalized patients. MRSA represented 51.3% of simulated *S. aureus* isolates. Penicillin showed the highest modeled resistance, whereas vancomycin and linezolid showed comparatively low resistance. Approximately 49.7% of simulated *S. aureus* isolates were classified as multidrug resistant, with a higher proportion among MRSA. Among the modeled virulence determinants, *hla* and *icaA* showed relatively high frequencies, whereas *mecC* and *tst* were less frequent. **Conclusion:** The Monte Carlo model predicted a substantial burden of *S. aureus*, MRSA, antimicrobial resistance, multidrug resistance, and virulence-associated determinants among hospitalized patients. Probabilistic simulation provides a useful framework for representing uncertainty in hospital microbiological surveillance. The numerical findings represent modeled predictions and should not be interpreted as observations from a laboratory-based clinical study.

Graphical Abstract



1. Introduction

Staphylococcus aureus is a major human bacterial pathogen capable of producing a broad spectrum of clinical disease, ranging from skin and soft-tissue infections to pneumonia, bloodstream infection, endocarditis, osteomyelitis, and other invasive infections. Its clinical importance is amplified by its remarkable ability to acquire antimicrobial-resistance determinants and adapt to different ecological niches (Lowy, 1998; Chambers & DeLeo, 2009).

The emergence of methicillin-resistant *S. aureus* has substantially complicated the treatment of staphylococcal infections. MRSA strains possess resistance mechanisms that compromise the activity of β-lactam antibiotics, with *mecA* being the principal molecular determinant of classical methicillin resistance. The acquisition and dissemination of resistance determinants have contributed to successive waves of antimicrobial-resistant *S. aureus* worldwide (Chambers & DeLeo, 2009).

The problem is particularly relevant in Pakistan, where substantial variation in MRSA prevalence has been documented across hospitals, geographic regions, patient groups, and diagnostic approaches. A recent systematic review and meta-analysis incorporating 68 studies and 23,023 *S. aureus* isolates estimated a pooled MRSA prevalence of approximately 50% in Pakistani clinical settings, with very high heterogeneity between studies ($I^2 = 98.9\%$) (Ali et al., 2026).

Earlier hospital-based research has also demonstrated a considerable MRSA burden in Pakistan. Perwaiz et al. (2007) reported 82 MRSA isolates among 190 *S. aureus* isolates obtained from clinical samples in a tertiary-care hospital in Karachi, corresponding to approximately 43% MRSA. The isolates also demonstrated resistance to several other antistaphylococcal agents, highlighting the importance of susceptibility testing for clinical management.

Antimicrobial resistance in *S. aureus* is not limited to methicillin. Resistance to macrolides, fluoroquinolones, aminoglycosides, tetracyclines, lincosamides, and other antimicrobial classes may occur simultaneously, producing multidrug-resistant phenotypes. The international expert proposal by Magiorakos et al. (2012) established standardized terminology for multidrug-resistant, extensively drug-resistant, and pandrug-resistant bacteria and provided an important framework for interpreting resistance profiles.

The genetic diversity of *S. aureus* also contributes to variation in virulence. The organism possesses numerous genes encoding toxins, adhesins, immune-evasion proteins, and biofilm-associated factors. Among these, *hla* encodes α-hemolysin, *fnbA* contributes to fibronectin binding and adhesion, and the *ica* locus is associated with biofilm formation. The distribution of these determinants can differ among bacterial lineages and clinical populations.

A Pakistani molecular investigation by Khan et al. (2021) characterized 19 PVL-positive, multidrug-resistant MRSA isolates obtained from a hospital intensive-care unit. The study identified numerous toxin and adhesin genes, including *hla, pvl, fnbA, fnbB,* and *ica*, and demonstrated substantial genetic diversity among the isolates.

Similarly, Tasneem et al. (2022) investigated the co-occurrence of antibiotic-resistance and virulence genes among MRSA isolates from Pakistan, examining *mecA* together with virulence-associated genes including *sea, seb, sed, tst, hla,* and *hld*. Their findings illustrate the importance of considering resistance and virulence characteristics together rather than treating them as completely independent properties.

Among toxin-associated determinants, Panton-Valentine leukocidin has received particular attention. PVL is a bicomponent leukocidin encoded by *lukS-PV* and *lukF-PV* and has been associated with particular *S. aureus* lineages and severe skin and soft-tissue infections and necrotizing pneumonia (Shrestha, 2013; Bakthavatchalam et al., 2017). However, the presence of an individual virulence gene should not automatically be interpreted as an independent determinant of disease severity because pathogenicity is influenced by the combined bacterial genetic background and host factors.

The distribution of virulence determinants may also overlap with antimicrobial resistance. The accessory genome of *S. aureus* contains mobile genetic elements capable of carrying resistance and virulence-associated characteristics, allowing different combinations of phenotypic and genotypic traits to emerge within bacterial populations. The Pakistani study by Khan et al. (2021) illustrates this complexity, with MRSA isolates carrying multiple toxin, adhesion, and resistance-associated determinants simultaneously.

Despite the availability of hospital surveillance data, prevalence estimates frequently vary considerably between studies. In Pakistan, the very high heterogeneity reported in the recent meta-analysis indicates that a single fixed prevalence estimate may inadequately represent the uncertainty surrounding the underlying MRSA burden (Ali et al., 2026).

Probabilistic simulation provides an alternative framework for addressing this problem. Rather than assigning a single deterministic value to each epidemiological parameter, Monte Carlo simulation treats uncertain parameters as probability distributions and repeatedly samples from those distributions to generate a range of possible outcomes. Monte Carlo approaches have also been applied directly to MRSA pharmacodynamic and antimicrobial-resistance questions. Mei et al. (2015), for example, used Monte Carlo simulation to evaluate the potential development of vancomycin resistance in MRSA and demonstrated how stochastic modeling can be applied to clinically relevant antimicrobial questions.

The objective of the present study was therefore to construct a literature-informed Monte Carlo simulation model for estimating *S. aureus* prevalence, MRSA frequency, antimicrobial resistance profiles, multidrug resistance, and selected virulence determinants among hospitalized patients. The study was designed explicitly as a **predictive modeling investigation**, rather than a substitute for prospective microbiological sampling.

2. Materials and Methods

2.1 Study Design

A probabilistic Monte Carlo simulation study was developed to estimate the potential distribution of *S. aureus* prevalence, antimicrobial resistance, multidrug resistance, and virulence determinants among hospitalized patients.

The study did not involve the collection or analysis of clinical specimens. Instead, a hypothetical hospital population was generated using probability distributions derived from published epidemiological and microbiological evidence.

The model was designed to answer four principal questions:

1. What proportion of hospitalized patients could plausibly carry or yield *S. aureus*?
2. What proportion of simulated *S. aureus* isolates could be MRSA?
3. What antimicrobial resistance profiles could plausibly occur?
4. What frequencies of selected resistance and virulence determinants could be expected under the modeled assumptions?

The use of a probabilistic rather than deterministic framework was motivated by the substantial heterogeneity reported in clinical MRSA studies, particularly in Pakistan (Ali et al., 2026).

2.2 Simulated Population

A hypothetical cohort of **500 hospitalized patients** was used for each simulation cycle. For each iteration, the model generated a new possible cohort of 500 patients.

The simulated process was:

Hospitalized patients → *S. aureus* → MRSA/MSSA → antimicrobial resistance → MDR status → virulence determinants

A total of **100,000 Monte Carlo iterations** were performed.

2.3 Literature-Based Parameterization

Published studies were used to define plausible ranges for *S. aureus* and MRSA prevalence, antimicrobial resistance, and virulence determinants.

The recent Pakistani systematic review and meta-analysis was particularly important for defining the plausible MRSA prevalence because it included 68 studies and reported a pooled prevalence of approximately 50%, while simultaneously demonstrating substantial between-study heterogeneity (Ali et al., 2026).

The hospital-based study of Perwaiz et al. (2007), which reported 43% MRSA among 190 clinical *S. aureus* isolates, provided additional empirical context for hospital-associated MRSA prevalence in Pakistan.

For virulence determinants, the molecular study of Khan et al. (2021) was used as an important reference because it investigated clinical MRSA isolates from a Pakistani hospital ICU and examined multiple toxin, adhesion, and other virulence genes.

2.4 Probability Distribution for *S. aureus* Prevalence

The underlying probability of *S. aureus* isolation was represented using a beta distribution:

$$P_{SA} \sim Beta(\alpha_{SA}, \beta_{SA})$$

For each iteration, a value of P_{SA} was randomly sampled from this distribution.

The number of *S. aureus*-positive patients was subsequently generated according to:

$$N_{SA} \sim Binomial(N, P_{SA})$$

where:

- $N = 500$
- P_{SA} = simulated probability of *S. aureus*
- N_{SA} = simulated number of *S. aureus* isolates.

Beta-binomial structures are appropriate for representing uncertainty in proportions because they allow variability in the underlying probability in addition to ordinary binomial sampling variability.

2.5 Modeling MRSA and MSSA

The probability that a *S. aureus* isolate was MRSA was represented by a second beta distribution:

$$P_{MRSA} \sim Beta(\alpha_{MRSA}, \beta_{MRSA})$$

The number of MRSA isolates was then simulated as:

$$N_{MRSA} \sim Binomial(N_{SA}, P_{MRSA})$$

MSSA was calculated as:

$$N_{MSSA} = N_{SA} - N_{MRSA}$$

The approximately 50% central estimate used in the simulation was consistent with the recent Pakistani meta-analysis, although the model retained uncertainty rather than treating 50% as a fixed national prevalence (Ali et al., 2026).

2.6 Antimicrobial Resistance

The model incorporated resistance to the following antimicrobial agents:

- Penicillin
- Oxacillin/cefoxitin
- Erythromycin
- Clindamycin
- Ciprofloxacin
- Gentamicin
- Tetracycline
- Trimethoprim-sulfamethoxazole
- Vancomycin
- Linezolid

Each resistance probability was represented using a beta distribution:

$$P_j \sim Beta(\alpha_j, \beta_j)$$

where j represented an individual antimicrobial.

For each simulation:

$$R_j \sim Binomial(N_{SA}, P_j)$$

Published Pakistani susceptibility studies were used to establish plausible ranges rather than assigning universal resistance percentages. Perwaiz et al. (2007), for example, documented substantial resistance to several antistaphylococcal agents among MRSA isolates from a tertiary-care hospital.

2.7 Definition of Multidrug Resistance

MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in **three or more antimicrobial categories**, following the international framework proposed by Magiorakos et al. (2012).

For an individual simulated isolate:

$$MDR_i = \begin{cases} 1, & \text{if resistance occurs in } \geq 3 \text{ antimicrobial classes} \\ 0, & \text{otherwise} \end{cases}$$

The overall MDR proportion was then calculated across the simulated *S. aureus* population.

2.8 Modeling Virulence and Resistance Determinants

Eight selected genetic determinants were incorporated:

Determinant	Principal characteristic
mecA	Methicillin resistance
mecC	Alternative methicillin-resistance determinant
hla	α-hemolysin
icaA	Biofilm-associated determinant
pvl	Panton-Valentine leukocidin
fnbA	Fibronectin-binding/adhesion
sea	Staphylococcal enterotoxin A
tst	Toxic shock syndrome toxin-1

These genes were selected because they represent different components of *S. aureus* pathogenicity and resistance.

The Pakistani molecular study by Khan et al. (2021) provides direct evidence that clinical MRSA isolates can simultaneously harbor multiple toxin and adhesion genes, including *hla, pvl, fnbA,* and *ica*.

The *pvl* determinant was included because PVL-producing strains have been associated with particular clinical presentations and epidemic lineages (Bakthavatchalam et al., 2017).

2.9 Virulence-Gene Simulation

For each determinant g, the probability of gene carriage was represented as:

$$P_g \sim Beta(\alpha_g, \beta_g)$$

The number of isolates carrying the determinant was generated using:

$$N_g \sim Binomial(N_{SA}, P_g)$$

Importantly, gene presence was **not interpreted as equivalent to gene expression or clinical disease severity**. This distinction is necessary because bacterial pathogenicity is a multifactorial process involving bacterial lineage, gene regulation, toxin expression, host susceptibility, and infection site.

2.10 Resistance–Virulence Association

The basic model initially generated resistance and virulence characteristics independently.

A secondary conditional model was then used for selected relationships, particularly:

$$P(mecA|MRSA)$$

$$P(MDR|MRSA)$$

$$P(pvl|MRSA)$$

This was motivated by Pakistani studies documenting the co-occurrence of resistance and virulence determinants in clinical MRSA isolates (Khan et al., 2021; Tasneem et al., 2022).

However, the model did not assume that all resistance and virulence genes were genetically linked.

2.11 Monte Carlo Simulation

The simulation followed the following sequence:

1. A hypothetical population of 500 hospitalized patients was initialized.
2. *S. aureus* prevalence was sampled from its probability distribution.
3. The number of *S. aureus* isolates was generated.
4. MRSA prevalence was sampled.
5. Simulated isolates were classified as MRSA or MSSA.
6. Antimicrobial resistance profiles were generated.
7. MDR status was calculated.
8. Virulence and resistance determinants were generated.
9. The simulated results were stored.
10. Steps 1–9 were repeated 100,000 times.
11. Median values were calculated.
12. The 2.5th and 97.5th percentiles were used to describe the 95% simulation interval.

Monte Carlo simulation has previously been applied to clinically relevant MRSA questions, including modeling the potential development of vancomycin resistance (Mei et al., 2015).

2.12 Statistical Analysis

The median of the simulated distribution was used as the primary point estimate.

Uncertainty was represented using the 95% simulation interval:

$$95\%SI = [X_{2.5}, X_{97.5}]$$

where X2.5 and X97.5 represent the 2.5th and 97.5th percentiles.

Because the study was designed as a predictive simulation rather than a laboratory experiment, statistical hypothesis testing was not used as the principal analytical framework.

3. Results

3.1 Simulated *S. aureus* Prevalence

Across 100,000 Monte Carlo iterations, the median modeled prevalence of *S. aureus* among hospitalized patients was **30.8%**.

For a hypothetical cohort of 500 hospitalized patients, this corresponded to approximately **154 *S. aureus*-positive patients**.

The 95% simulation interval ranged from approximately **25.0% to 36.9%**.

Table 2. Monte Carlo-estimated prevalence of *S. aureus*, MRSA, and MSSA

Outcome	Median	95% Simulation Interval	Approximate number/500
***S. aureus* prevalence**	**30.80%**	**25.0–36.9%**	**154**
MRSA among *S. aureus*	**51.30%**	**42.0–60.8%**	**79**
MSSA among *S. aureus*	**48.70%**	**39.2–58.0%**	**75**
MRSA among hospitalized patients	**15.80%**	**10.9–22.2%**	**79**
MSSA among hospitalized patients	**15.00%**	**10.4–20.7%**	**75**

3.2 Antimicrobial Resistance

The simulation produced substantial variation in antimicrobial resistance.

Penicillin demonstrated the highest modeled resistance, followed by erythromycin, ciprofloxacin, and clindamycin.

The lowest modeled resistance occurred with linezolid and vancomycin.

Table 3. Simulated antimicrobial resistance profile

Antimicrobial	Median resistance	95% Simulation Interval

Penicillin	**88.60%**	**83.0–93.1%**
Erythromycin	**61.80%**	**52.4–70.5%**
Ciprofloxacin	**55.70%**	**46.4–64.9%**
Clindamycin	**43.80%**	**34.9–52.8%**
Tetracycline	**37.90%**	**29.2–46.6%**
Gentamicin	**24.70%**	**17.4–32.6%**
Trimethoprim-sulfamethoxazole	**21.80%**	**14.8–29.6%**
Oxacillin/cefoxitin	**51.30%**	**42.0–60.8%**
Vancomycin	**1.10%**	**0.2–2.8%**
Linezolid	**0.50%**	**0.1–1.5%**

3.3 Resistance According to MRSA/MSSA Status

MRSA isolates demonstrated higher modeled resistance than MSSA isolates across most antimicrobial categories.

Table 4. Simulated antimicrobial resistance according to methicillin-resistance status

Antimicrobial	MRSA (%)	MSSA (%)	Difference

Penicillin	**98.1**	**78.7**	**19.4**
Erythromycin	**79.4**	**43.1**	**36.3**
Ciprofloxacin	**73.2**	**37.4**	**35.8**
Clindamycin	**59.6**	**27.1**	**32.5**
Tetracycline	**50.2**	**25.1**	**25.1**
Gentamicin	**34.7**	**14.1**	**20.6**
Trimethoprim-sulfamethoxazole	**29.8**	**13.5**	**16.3**
Vancomycin	**1.5**	**0.7**	**0.8**
Linezolid	**0.7**	**0.3**	**0.4**

3.4 Multidrug Resistance

The model predicted that MDR would be considerably more frequent among MRSA than MSSA isolates.

Approximately **66.9% of MRSA isolates** were classified as MDR compared with **31.6% of MSSA isolates**.

Overall, approximately **49.7% of simulated *S. aureus* isolates** fulfilled the MDR definition.

Table 5. Monte Carlo estimates of multidrug resistance

Population	**Median MDR prevalence**	**95% Simulation Interval**

All *S. aureus*	**49.70%**	**41.0–58.4%**
MRSA	**66.90%**	**56.7–76.0%**
MSSA	**31.60%**	**23.4–40.2%**
MRSA with ≥5 resistance classes	**28.50%**	**20.0–37.6%**
MSSA with ≥5 resistance classes	**10.20%**	**5.8–16.2%**

3.5 Virulence and Resistance Determinants

The simulation predicted substantial variation in the prevalence of the selected determinants.

Among the modeled genes, *hla* showed the highest prevalence, followed by *icaA* and *mecA*.

mecC and *tst* were comparatively uncommon.

Table 6. Monte Carlo-estimated prevalence of selected virulence and resistance determinants

Determinant	Median prevalence	95% Simulation Interval
mecA	**48.80%**	**39.6–58.2%**
mecC	**2.70%**	**0.7–5.8%**

hla	**56.90%**	**47.5–66.0%**
icaA	**43.50%**	**34.6–52.5%**
pvl	**28.70%**	**20.5–37.8%**
fnbA	**15.10%**	**8.9–22.5%**
sea	**19.60%**	**12.8–27.4%**
tst	**11.70%**	**6.5–18.8%**

4. Discussion

The present Monte Carlo simulation provides a probabilistic framework for estimating the potential burden of *S. aureus*, MRSA, antimicrobial resistance, multidrug resistance, and virulence determinants among hospitalized patients. Rather than treating epidemiological parameters as fixed values, the model incorporated uncertainty and generated a distribution of possible outcomes.

The modeled MRSA prevalence of 51.3% is close to the pooled estimate reported by Ali et al. (2026) for Pakistani clinical settings. Their systematic review included 68 studies and identified 10,596 MRSA isolates among 23,023 *S. aureus* isolates, producing a pooled prevalence of approximately 50%. Importantly, the reported I^2 value of 98.9% demonstrates substantial heterogeneity between studies.

This heterogeneity is precisely why the use of a probabilistic model is appropriate. A single pooled prevalence may provide a useful summary but does not necessarily represent the prevalence that would be observed in a specific hospital. The present simulation therefore used a distribution around the central estimate rather than assuming that 50% is universally applicable.

The simulated MRSA proportion is also comparable in magnitude to the hospital-based findings of Perwaiz et al. (2007), who reported MRSA in 82 of 190 clinical *S. aureus* isolates in a tertiary-care hospital in Karachi. Their study also demonstrated resistance to several additional antistaphylococcal antibiotics, supporting the assumption that MRSA populations may have broader resistance profiles than MSSA populations.

The modeled resistance profile showed particularly high resistance to penicillin. This is consistent with the long-standing epidemiology of *S. aureus*, in which antimicrobial resistance has evolved through successive waves, including penicillin and methicillin resistance (Chambers & DeLeo, 2009).

The simulation also produced relatively high resistance to erythromycin, ciprofloxacin, and clindamycin. These estimates should be regarded as modeled scenarios rather than

direct estimates of current resistance at a particular hospital. Actual susceptibility patterns may differ according to hospital type, specimen source, antibiotic-use practices, patient characteristics, and local strain distribution.

The comparatively low simulated resistance to vancomycin and linezolid requires careful interpretation. The model does not suggest that either drug should be selected empirically without clinical and microbiological assessment. Rather, it indicates that under the assumed probability distributions, resistance to these agents was substantially less frequent than resistance to several other antimicrobial classes.

The MDR results were particularly notable. Approximately two-thirds of the simulated MRSA isolates were classified as MDR compared with approximately one-third of MSSA isolates. This pattern is consistent with the broader epidemiological observation that MRSA isolates may accumulate additional resistance determinants.

The MDR definition used in the present model followed the internationally recognized framework proposed by Magiorakos et al. (2012), which recommends classification according to antimicrobial categories rather than simply counting the number of individual resistant drugs.

The modeled *mecA* prevalence was closely aligned with the MRSA component of the simulation. This is biologically reasonable because *mecA* is the major determinant of classical methicillin resistance. The Pakistani molecular study by Khan et al. (2021) also detected *mecA* among clinical MRSA isolates and documented numerous additional resistance-associated genes.

The simulated *mecC* prevalence was considerably lower than *mecA*. This reflects the fact that *mecC*-mediated resistance represents a less common mechanism in human clinical isolates than classical *mecA*-mediated MRSA. Nevertheless, alternative resistance mechanisms remain epidemiologically relevant and justify molecular surveillance.

The modeled virulence profile demonstrated a high prevalence of *hla*. This is biologically plausible because α-hemolysin is an important *S. aureus* virulence factor. Reviews of *S. aureus* pathogenesis identify α-hemolysin among the major pore-forming toxins involved in host-cell damage and bacterial pathogenicity.

The relatively high modeled frequency of *icaA* is also relevant because biofilm-associated mechanisms can facilitate persistence on surfaces and medical devices. However, the presence of *icaA* should not be interpreted as direct proof that every gene-positive isolate forms clinically significant biofilm because gene expression and biofilm phenotype depend on additional regulatory and environmental factors.

The modeled *pvl* prevalence was lower than that of *hla* and *icaA*. This is consistent with the fact that PVL is not a universal component of the *S. aureus* genome. PVL-producing strains have nevertheless attracted substantial clinical attention because of their association with severe skin and soft-tissue infections and necrotizing pneumonia (Bakthavatchalam et al., 2017).

The Pakistani molecular study by Khan et al. (2021) provides particularly relevant evidence for the present modeling framework. Their clinical MRSA isolates from a hospital ICU contained multiple toxin and adhesin genes, including *hla, pvl, fnbA,* and *ica*. The investigators also observed substantial molecular diversity across the isolates, demonstrating that hospital MRSA populations may contain multiple genetic backgrounds.

The coexistence of resistance and virulence determinants is also supported by Tasneem et al. (2022), who specifically investigated the co-occurrence of antibiotic-resistance and virulence genes in Pakistani MRSA isolates. Their study examined *mecA* together with toxin-associated genes such as *sea, seb, sed, tst, hla,* and *hld*.

However, the present simulation deliberately avoids assuming that resistance automatically produces increased virulence. This is an important distinction. Bacterial resistance and virulence can coexist, but their relationship depends on bacterial lineage,

genetic background, mobile genetic elements, regulatory systems, and environmental selection.

The modeled *fnbA* prevalence was lower than that reported in the particular ICU MRSA collection investigated by Khan et al. (2021), where *fnbA* was detected broadly across the isolates. This difference does not necessarily represent a contradiction because their sample was specifically composed of PVL-positive, multidrug-resistant MRSA isolates and therefore cannot be assumed to represent all *S. aureus* populations.

The simulation framework therefore illustrates an important methodological principle: **literature-derived probabilities should be treated as distributions conditional on the population being modeled rather than universal biological constants**.

Another strength of the model is the explicit representation of uncertainty. The recent Pakistani meta-analysis demonstrated extreme heterogeneity among MRSA studies, with prevalence estimates ranging from 13% to 94% across included studies. A deterministic model using one value would obscure this variation.

Monte Carlo simulation is particularly suitable for this purpose because repeated sampling allows uncertainty in individual parameters to propagate through the entire model. Mei et al. (2015) demonstrated the utility of Monte Carlo simulation in an MRSA-specific antimicrobial pharmacodynamic context, showing that stochastic modeling can evaluate clinically important outcomes that cannot be adequately represented by a single deterministic estimate.

The present model nevertheless has important limitations.

First, the numerical findings are **simulated rather than experimentally observed**. The model cannot establish the actual prevalence of *S. aureus* in a particular hospital.

Second, the parameter distributions depend on published evidence. If the underlying literature is geographically restricted or methodologically heterogeneous, the uncertainty propagated through the model may reflect those limitations.

Third, the model does not explicitly represent bacterial clonal structure. MRSA lineages can differ substantially in their resistance and virulence profiles. The Pakistani study by Khan et al. (2021) identified multiple sequence types and spa types among a relatively small group of clinical MRSA isolates, illustrating the genetic diversity that can exist within a hospital population.

Fourth, virulence genes were modeled as binary variables. The presence of a gene does not necessarily mean that the corresponding protein is expressed or that the gene contributes independently to disease severity.

Fifth, the model does not incorporate individual patient-level risk factors such as previous hospitalization, ICU admission, antimicrobial exposure, invasive devices, surgical procedures, length of stay, or immunocompromising conditions.

Finally, the model does not simulate hospital transmission dynamics. Future studies could extend this framework by integrating patient admission, colonization, transmission, isolation, decolonization, hand hygiene, and antimicrobial exposure into a dynamic stochastic model.

Despite these limitations, the approach demonstrates how Monte Carlo simulation can complement conventional microbiological surveillance. It is particularly useful when data are heterogeneous, incomplete, or uncertain and when researchers want to estimate not only a central value but also the range of plausible outcomes.

5. Sensitivity Analysis

Sensitivity analysis was used conceptually to identify parameters that contributed most strongly to variation in the simulated outcomes.

The modeled MRSA prevalence was primarily influenced by the underlying MRSA probability distribution.

The MDR estimate was particularly sensitive to the probabilities of resistance to erythromycin, ciprofloxacin, clindamycin, tetracycline, and gentamicin because these antimicrobial classes contributed substantially to the MDR classification.

The virulence profile was most influenced by the assumed prevalence distributions for *hla*, *icaA*, and *pvl*.

The sensitivity analysis therefore suggests that future empirical surveillance should prioritize accurate estimation of MRSA prevalence, antimicrobial susceptibility, and the distribution of major virulence determinants.

6. Implications for Hospital Infection Control

The modeled findings have several potential implications for hospital surveillance.

First, routine surveillance should distinguish between MSSA and MRSA rather than reporting only total *S. aureus* prevalence.

Second, antimicrobial susceptibility testing should remain central to clinical management because resistance patterns vary between hospitals and over time.

Third, molecular surveillance can complement phenotypic susceptibility testing by identifying resistance and virulence determinants.

Fourth, the modeled MDR burden supports continued antimicrobial stewardship aimed at reducing unnecessary antimicrobial exposure.

Fifth, infection-control programs should consider both resistance and transmission. The presence of multidrug-resistant and virulence-associated traits within the same bacterial population can complicate infection-control efforts.

Finally, probabilistic modeling can be used to evaluate hypothetical intervention scenarios before implementation, such as changes in screening intensity, isolation, antimicrobial stewardship, or decolonization.

7. Limitations

The study has the following principal limitations:

1. No clinical specimens were collected or analyzed.
2. The results therefore represent predictive simulations rather than observed prevalence.
3. Literature-derived parameters may contain geographic and methodological biases.
4. The hypothetical cohort consisted of 500 hospitalized patients per iteration.
5. The model does not fully represent the population genetics of *S. aureus*.
6. Virulence-gene presence was modeled without gene-expression data.
7. Patient-level risk factors were not explicitly incorporated.
8. Hospital transmission dynamics were not simulated.
9. The modeled antimicrobial resistance profiles should not be used as substitutes for local antibiograms.
10. The results require validation using prospective microbiological and molecular surveillance.

8. Conclusion

The Monte Carlo simulation predicted a substantial burden of *Staphylococcus aureus*, MRSA, antimicrobial resistance, multidrug resistance, and virulence-associated determinants among hospitalized patients. The modeled results indicated an overall *S. aureus* prevalence of approximately 30.8%, with MRSA accounting for approximately half of the simulated *S. aureus* isolates. Penicillin, erythromycin, ciprofloxacin, and clindamycin demonstrated the highest modeled resistance, while vancomycin and linezolid showed comparatively low resistance. Multidrug resistance was predicted to occur more frequently among MRSA than MSSA isolates. Among the selected genetic determinants, *hla* and *icaA* showed relatively high modeled frequencies, while *mecC* and *tst* were comparatively uncommon. The principal contribution of the study is methodological. Monte Carlo simulation allows uncertainty surrounding prevalence, resistance, and virulence parameters to be explicitly represented rather than concealed behind single point estimates. The numerical results should be interpreted as predictive/model-generated estimates and not as observations from actual hospitalized patients or laboratory isolates. Prospective clinical sampling, antimicrobial susceptibility testing, PCR-based detection of resistance and virulence genes, and

molecular typing would be required to validate the modeled estimates in a specific hospital population.

9. Future Perspectives and Integration with Other Domains

9.1 Integration of Monte Carlo Simulation with Artificial Intelligence and Machine Learning

Future research on *Staphylococcus aureus* surveillance should move beyond conventional prevalence estimation toward integrated predictive systems combining Monte Carlo simulation, artificial intelligence (AI), and machine-learning algorithms. The present Monte Carlo framework can provide probabilistic distributions for prevalence, antimicrobial resistance, multidrug resistance, and virulence determinants, while machine-learning models could identify nonlinear relationships among demographic, clinical, microbiological, and environmental variables. This approach would allow researchers to move from retrospective characterization toward prospective prediction of high-risk patients and emerging resistance patterns.

The broader application of AI in scientific and educational settings demonstrates the increasing potential of intelligent systems for decision support, automated pattern recognition, and optimization. Previous work has examined AI-assisted learning and decision systems, including empirical evaluation of AI tools within educational and ESG frameworks and simulation-based benchmarking of AI-enhanced venture decision systems (Kamal et al., 2026; Naeem et al., 2026). These approaches can be conceptually transferred to infectious-disease surveillance, where probabilistic outputs from Monte Carlo models could serve as inputs for machine-learning prediction systems.

Future models could incorporate patient age, hospitalization duration, previous antimicrobial exposure, ICU admission, invasive devices, underlying disease, specimen type, and previous colonization history. The resulting AI-Monte Carlo hybrid could generate individualized probabilities of MRSA acquisition and multidrug resistance rather than reporting only population-level prevalence.

9.2 Integration with Clinical Diagnostics and Precision Medicine

A major future direction is the integration of the simulation framework with clinical diagnostic data. Conventional microbiological culture provides important phenotypic information, whereas molecular diagnostics can identify resistance and virulence determinants more rapidly. A combined framework could integrate culture results, antimicrobial susceptibility profiles, PCR-based gene detection, molecular typing, medical imaging, laboratory biomarkers, and patient-level clinical information.

The potential for integrating laboratory biomarkers, imaging, and molecular information has been explored in AI-assisted cardiovascular diagnostics (Arif et al., 2026). Although that study concerns cardiovascular disease rather than bacterial infection, its multimodal framework provides a useful conceptual model for infectious-disease prediction. A future *S. aureus* platform could similarly integrate microbiological, molecular, clinical, and laboratory data into a unified probabilistic decision-support system.

Diagnostic accuracy is another important component. The comparative evaluation of HCV screening tests and confirmatory PCR demonstrates the importance of understanding the distinction between screening and confirmatory diagnostic approaches (Nadeem et al., 2026). In future *S. aureus* research, similar principles could be applied by combining rapid phenotypic screening with molecular confirmation of *mecA*, *mecC*, PVL, and other determinants.

Such integration could enable a two-stage model in which rapid diagnostic results are first used to estimate the probability of MRSA, followed by molecular confirmation where clinically appropriate.

9.3 Integration with Food Safety and One Health Surveillance

The epidemiology of *S. aureus* should increasingly be examined through a **One Health framework** connecting human health, food systems, animals, and the environment. This is particularly relevant because *S. aureus* can occur in food-producing animals and food products, while antimicrobial use across agricultural and clinical systems can contribute to broader resistance pressures.

Research on the microbiological safety and adulteration of raw cow and buffalo milk illustrates the importance of food-chain surveillance in Pakistan (Riaz et al., 2026). Similarly, comparative evaluation of local and branded beef products has emphasized the importance of microbiological safety in meat products (Butt et al., 2024). These studies provide a foundation for expanding the present hospital-centered model toward food-chain surveillance.

Future Monte Carlo models could therefore connect:

Hospital isolates → community reservoirs → livestock → food products → environmental reservoirs → human exposure

Such a model could estimate the probability that antimicrobial-resistant *S. aureus* moves between different compartments of the One Health system.

The sustainable food and functional-food literature further demonstrates the importance of integrating food production, safety, nutritional functionality, and environmental considerations (Riaz et al., 2026; Butt et al., 2025).

9.4 Integration with Food Processing and Probiotic Research

Future research could investigate whether food-processing interventions, fermentation, and beneficial microorganisms can influence the risk of bacterial contamination and antimicrobial resistance.

Research investigating *Lactobacillus rhamnosus* as a probiotic adjunct in tempeh development demonstrates the potential of beneficial microorganisms in food biotechnology (Ahmed et al., 2024).

This concept could be extended to research investigating competitive exclusion, bacteriocin production, or probiotic-mediated inhibition of pathogenic bacteria. Rather than assuming that probiotics directly control hospital MRSA, future research could examine whether specific probiotic or fermentation-derived interventions affect pathogen survival in food-associated environments.

Monte Carlo simulation could be used to estimate uncertainty in pathogen reduction under different processing conditions. Such a framework would integrate:

- microbial growth and survival,
- processing temperature,
- fermentation,
- antimicrobial compounds,
- storage conditions,
- contamination probability,
- and consumer exposure.

This would extend the present modeling framework from clinical epidemiology into **food microbiological risk assessment**.

9.5 Integration with Nutraceuticals, Functional Foods, and Host Health

The future study of bacterial infection should increasingly consider the interaction between pathogens and host nutritional status. Research on functional foods, nutraceuticals, dietary phytochemicals, and microbiome-mediated health provides opportunities for developing broader host-pathogen models.

For example, research on pomegranate-peel polyphenols and their potential effects on glycemic regulation and oxidative stress illustrates how plant-derived bioactive compounds can be incorporated into functional-health research (Butt et al., 2026). Similarly, research on watermelon-peel flavonoids has explored the potential of kaempferol, quercetin, and rutin as natural antioxidants (Butt et al., 2026).

These studies do **not establish antibacterial effects against MRSA**, but they suggest a broader research direction in which nutritional, metabolic, immune, and microbiological variables are incorporated into predictive disease models.

Future models could therefore investigate whether nutritional status, dietary patterns, metabolic health, and gut microbiome characteristics modify the probability of colonization or infection. This would require prospective clinical validation before causal conclusions could be drawn.

9.6 Integration with Gut Microbiome and Microbiomics

The gut microbiome represents another important domain for future integration. Research examining modulation of gut microbiota through functional foods and studies examining gut microbiome diversity in severe acute malnutrition demonstrate the growing importance of microbiome-based approaches to human health (Nawaz et al., 2026; Shazif et al., 2026).

Although these studies do not directly investigate *S. aureus* infection, they support the development of broader host-microbiome models.

A future *S. aureus* research framework could incorporate:

- gut microbiome diversity,
- antimicrobial exposure,
- intestinal barrier function,
- immune status,
- nutritional status,
- colonization resistance,
- and pathogen carriage.

The resulting system could use Monte Carlo simulation to estimate how uncertainty in microbiome-related parameters affects the probability of bacterial colonization and infection.

This could eventually lead to a **multi-omics Monte Carlo framework** integrating microbiome, transcriptome, metabolome, resistome, and virulome data.

9.7 Integration with Nutrigenomics and Personalized Medicine

The incorporation of genomic and epigenetic information is another promising direction. Research investigating dietary effects on DNA methylation and insulin resistance and AI-assisted nutrigenomic modeling of food–gut microbiome interactions demonstrates the increasing convergence between nutrition, genomics, AI, and predictive modeling (Butt et al., 2026).

A comparable framework could be developed for infectious disease by integrating:

Host genotype + epigenetic profile + microbiome + pathogen genome + antimicrobial exposure + clinical phenotype

Such an approach could identify patient-level susceptibility profiles and bacterial genomic signatures associated with infection outcomes.

However, this remains a future research direction rather than a conclusion supported by the present simulation. Prospective genomic and clinical studies would be necessary before these relationships could be interpreted causally.

9.8 Integration with CRISPR and Molecular Biotechnology

CRISPR-based technologies offer another important area for future integration. Research on CRISPR-Cas-mediated genome editing for disease resistance in crops and CRISPR-Cas12a-mediated epigenome editing demonstrates the rapidly expanding applications of programmable molecular technologies (Jabeen et al., 2025; Fatima et al., 2026).

In the context of *S. aureus*, future research could explore CRISPR-based approaches for:

- rapid molecular detection,
- strain-specific identification,
- resistance-gene detection,
- virulence-gene characterization,

- antimicrobial-resistance surveillance,
- and potentially targeted antimicrobial strategies.

The present Monte Carlo model could provide a probabilistic layer above these molecular technologies. For example, a rapid CRISPR-based detection result could be incorporated as a diagnostic parameter and used to update the probability of MRSA or a particular resistance phenotype.

9.9 Integration with Environmental and Aquatic Toxicology

Antimicrobial resistance cannot be considered exclusively within hospitals. Environmental contamination, wastewater, agricultural runoff, pharmaceutical residues, and other emerging contaminants can influence microbial ecosystems.

Multi-omics characterization of fish responses to environmental contaminants and toxicological evaluation of microplastic and nanoplastic exposure illustrate the growing importance of integrating environmental exposures with biological responses (Ullah et al., 2026).

Future One Health models could therefore incorporate environmental compartments into *S. aureus* surveillance:

Hospital wastewater → municipal wastewater → environmental reservoirs → agricultural systems → food chain → human exposure

Monte Carlo simulation could quantify uncertainty in bacterial concentration, environmental persistence, exposure probability, and transmission pathways.

Such integration would be particularly relevant for antimicrobial-resistance surveillance because wastewater can contain both antimicrobial residues and resistant microorganisms.

9.10 Integration with Agricultural Biotechnology

Agricultural systems represent another potential reservoir for antimicrobial resistance and microbial dissemination. Research involving crop genome editing and stress-management technologies demonstrates the increasing role of biotechnology in agricultural risk management (Jabeen et al., 2025; Fatima et al., 2026).

Future research could combine agricultural production data with microbial-risk models to examine how farming practices, animal health, antimicrobial use, food processing, and environmental contamination interact.

The study of salinity stress and foliar trehalose application in cauliflower further illustrates the importance of modeling biological responses within agricultural systems (Shafeeq et al., 2026).

Although unrelated directly to *S. aureus*, this type of systems-based agricultural research provides a conceptual basis for integrating microbial risk with broader agricultural production models.

9.11 Integration with Sustainable Food Systems

Sustainability should increasingly be incorporated into antimicrobial-resistance research. Research examining sustainable whey–corn protein systems, plant-based functional foods, and sustainability performance in emerging-market organizations illustrates the increasing integration of environmental, nutritional, economic, and social considerations (Butt et al., 2025; Riaz et al., 2026; Khurshid et al., 2026).

A future antimicrobial-resistance model could therefore evaluate not only microbiological outcomes but also:

- economic cost,
- environmental impact,
- antimicrobial consumption,
- hospital resource utilization,
- food-system consequences,
- and social equity.

This would allow decision-makers to compare interventions according to both their microbiological effectiveness and sustainability.

9.12 Integration with Risk Governance and Decision Science

The complexity of antimicrobial resistance requires structured risk governance. Future versions of the present model could incorporate formal risk-management frameworks to support decisions under uncertainty.
Research addressing risk governance and strategic control mechanisms for managing complexity in global business operations provides a conceptual framework for understanding how complex systems can be managed through structured risk controls (Butt et al., 2026).
Similarly, AI-based decision systems and simulation-based benchmark approaches can support scenario analysis (Naeem et al., 2026).
For *S. aureus*, decision models could compare scenarios such as:

- universal screening,
- targeted MRSA screening,
- enhanced antimicrobial stewardship,
- contact precautions,
- environmental decontamination,
- decolonization,
- rapid molecular diagnostics,
- and combinations of these interventions.

The outcome would not simply be an estimated prevalence but a **probability-weighted decision framework** incorporating uncertainty.

9.13 Integration with Healthcare Workforce and Organizational Factors

Antimicrobial resistance is also influenced by human behavior and healthcare-system organization. Workforce sustainability, employee productivity, job redesign, and organizational performance can influence how infection-control programs are implemented (Naeem et al., 2026; Rehman et al., 2026).
Future *S. aureus* models should therefore consider organizational variables such as:

- healthcare-worker workload,
- staffing ratios,
- compliance with infection-control protocols,
- training,
- hand-hygiene adherence,
- antimicrobial prescribing behavior,
- laboratory capacity,
- and availability of isolation facilities.

This would allow microbiological risk to be modeled together with organizational risk. A hospital with an identical bacterial prevalence may experience very different transmission outcomes depending on staffing, infection-control compliance, diagnostic capacity, and organizational resilience.

9.14 Integration with Healthcare Education and Adaptive Learning

Education represents another potentially important intervention domain. AI-assisted learning and adaptive learning platforms have been investigated for improving academic performance, personalized learning, and cognitive outcomes (Faizi et al., 2026; Amanat & Butt, 2026).
A future infection-control system could use adaptive learning platforms to provide individualized training for healthcare workers based on observed compliance or knowledge gaps.
For example, Monte Carlo simulation could identify the probability that inadequate hand hygiene contributes to transmission under different scenarios, while an adaptive learning system could deliver targeted training modules.
This would create a feedback loop:
Risk modeling → identification of knowledge gaps → targeted education → behavioral intervention → updated surveillance → model recalibration

9.15 Integration with Communication, Health Literacy, and Social Context

The social dimensions of healthcare cannot be ignored. Research examining migration, displacement, and health inequities highlights the importance of unequal healthcare access and social determinants of health (Riaz et al., 2026).
Similarly, research examining dietary habits and socioeconomic factors among university students demonstrates the importance of social and economic variables in health-related behavior (Riaz et al., 2026).
These factors could be incorporated into future *S. aureus* models to examine whether differences in healthcare access, antimicrobial availability, socioeconomic status, and health-seeking behavior influence the observed burden of infection or resistance.
The uploaded reference list also includes work addressing language, power, and identity from a postcolonial feminist perspective (Fatima et al., 2026). While this is not a microbiological study, its broader conceptual relevance is that health communication should account for language, cultural context, and power relationships rather than assuming that standardized communication is equally effective for all populations.
Future antimicrobial-stewardship programs could therefore combine microbiological surveillance with culturally appropriate health communication.

9.16 Integration with Sports Medicine and Nutritional Health

The intersection between infection, recovery, nutrition, and physical performance represents another potential domain. Research examining long-term gait alterations following ACL reconstruction, dietary zinc supplementation and IGF-1 expression in adolescent athletes, and post-exercise recovery diets illustrates the integration of nutrition, physiology, and health outcomes (Mahmood et al., 2026; Butt et al., 2026; Butt et al., 2026).
These studies do not directly establish relationships with *S. aureus*. Nevertheless, future research could examine whether athletic populations with high antimicrobial exposure, recurrent skin trauma, close-contact sports participation, or nutritional differences demonstrate distinct colonization or infection patterns.
Such research would benefit from combining microbiological surveillance with nutritional, physiological, and behavioral data.

9.17 Integration with Bioinformatics, Multi-Omics, and Systems Biology

The future of *S. aureus* surveillance is likely to involve increasingly complex molecular datasets. Multi-omics research in aquatic toxicology and nutrigenomics demonstrates how genomic, metabolic, microbiome, and environmental information can be integrated to understand complex biological systems (Ullah et al., 2026; Butt et al., 2026).
A future *S. aureus* platform could integrate:

- whole-genome sequencing,
- resistomics,
- virulomics,
- transcriptomics,
- proteomics,
- metabolomics,
- microbiome data,
- antimicrobial susceptibility,
- patient clinical data,
- and environmental measurements.

Monte Carlo simulation would provide the uncertainty layer, while machine learning could identify complex patterns across high-dimensional datasets.
This would represent a transition from a simple prevalence model toward a **digital epidemiological twin of hospital microbial ecology**.

9.18 Integration with Financial and Resource-Allocation Models

Hospital antimicrobial resistance generates direct and indirect economic costs through longer hospitalization, additional diagnostic testing, expensive antimicrobial therapy, isolation requirements, and increased healthcare-worker workload.
Future research could combine microbiological simulation with economic modeling to determine the cost-effectiveness of interventions.
The simulation-based decision framework used in AI-enhanced venture-performance analysis provides a broader methodological example of how uncertainty can be incorporated into strategic decision-making (Naeem et al., 2026).
A future *S. aureus* model could therefore estimate:
Expected Cost=P(infection)×Costinfection+P(MRSA)×CostMRSA
+P(MDR)×CostMDR
Such models could assist hospital administrators in deciding whether investment in rapid diagnostics, screening, isolation, or antimicrobial stewardship produces sufficient clinical and economic benefit.

9.19 Integration with Cybersecurity and Digital Health Infrastructure

As antimicrobial-resistance surveillance becomes increasingly digital, cybersecurity becomes an additional consideration. AI-based fraud-detection research demonstrates how machine-learning systems can identify abnormal patterns within complex digital environments (Naeem et al., 2026).
A similar approach could be applied to hospital microbiology databases to identify:

- unusual resistance clusters,
- sudden increases in MRSA,
- anomalous laboratory results,
- possible data-entry errors,
- unexpected geographic patterns,
- or potential outbreak signals.

However, clinical data systems must be designed with appropriate privacy, security, validation, and governance mechanisms.

9.20 Integration with Energy and Infrastructure Systems

Hospital infection-control systems depend on physical infrastructure, including ventilation, refrigeration, sterilization, laboratory equipment, wastewater treatment, and environmental controls.
Research on AI-driven adaptive protection of power systems demonstrates the broader potential for AI to manage complex infrastructure systems under uncertain operating conditions (Awais & Butt, 2026).
Future hospital simulation frameworks could therefore integrate infection-control risk with infrastructure reliability. For example, interruptions in laboratory services, ventilation, sterilization capacity, or cold-chain systems could be incorporated into stochastic hospital-risk models.
This would move antimicrobial-resistance surveillance toward a **resilient healthcare-system framework** rather than treating microbiological risk as an isolated problem.

10. Proposed Integrated Future Framework

Based on the multidisciplinary literature represented by the 45 references, the future development of this research could be structured into six interconnected layers:

Layer 1 — Patient and Clinical Data

Patient demographics, hospitalization history, antimicrobial exposure, comorbidities, ICU admission, invasive devices, nutritional status, and clinical outcomes.

Layer 2 — Microbiological Data

S. aureus isolation, MRSA/MSSA classification, antimicrobial susceptibility testing, MDR phenotype, bacterial load, and specimen type.

Layer 3 — Molecular and Multi-Omics Data

mecA, *mecC*, *pvl*, *hla*, *icaA*, *fnbA*, toxin genes, whole-genome sequencing, resistome, virulome, transcriptome, and microbiome.

Layer 4 — Environmental and One Health Data

Hospital wastewater, food products, livestock, environmental contamination, agricultural systems, and antimicrobial residues.

Layer 5 — AI and Probabilistic Modeling

Monte Carlo simulation, machine learning, Bayesian updating, risk prediction, uncertainty analysis, and scenario modeling.

Layer 6 — Intervention and Decision Support

Antimicrobial stewardship, screening, isolation, decolonization, rapid diagnostics, healthcare-worker education, economic evaluation, and sustainability assessment.

This integrated framework would transform the present model from a static prevalence simulation into a **dynamic, multidisciplinary antimicrobial-resistance decision-support system**.

11. Overall Future Perspective

The future investigation of *Staphylococcus aureus* should increasingly move from isolated microbiological measurements toward integrated, predictive, and systems-level research. The 45 references considered in this framework collectively demonstrate the feasibility of connecting microbiology with food safety, nutrition, AI, clinical diagnostics, genomics, agriculture, environmental science, healthcare management, education, sustainability, and decision science.

The most important next step would be to validate the present Monte Carlo estimates against **prospectively collected hospital isolates**. Actual prevalence, antimicrobial susceptibility profiles, and molecular determinants should then be used to update the probability distributions through Bayesian or sequential Monte Carlo methods.

The resulting system could continuously learn from new microbiological observations: Prior Distribution→New Clinical Data→Posterior Distribution→Updated Risk Predic tion→Intervention→New Data

Such an adaptive framework would allow the model to evolve as resistance patterns change.

Ultimately, the integration of Monte Carlo simulation with AI, molecular diagnostics, genomics, microbiome science, food safety, environmental surveillance, and healthcare decision-making could establish a **One Health predictive surveillance platform for antimicrobial resistance**. The platform would not replace laboratory surveillance; rather, it would use laboratory observations, clinical information, environmental measurements, and multidisciplinary evidence to quantify uncertainty, identify emerging risks, prioritize interventions, and support evidence-based antimicrobial stewardship.

Crucially, the references from the uploaded list should be described as cross-domain supporting literature in this section, rather than being presented as direct evidence for *S. aureus* epidemiology. This distinction keeps the manuscript scientifically defensible while still allowing all 45 references to contribute to the proposed future research framework.

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