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FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF MRSA IN CLINICAL SAMPLES

Original Research

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare- and community-acquired infections worldwide, posing a significant challenge to effective treatment due to its multidrug-resistant nature. It commonly causes skin, wound, and bloodstream infections, and its rapid spread through direct contact or contaminated surfaces complicates infection control. The increasing antibiotic resistance of MRSA highlights the urgent need for ongoing surveillance and the rational use of effective antimicrobials such as vancomycin to limit morbidity and mortality associated with these infections.

Objective: To determine the frequency and antimicrobial susceptibility pattern of MRSA isolated from various clinical specimens collected in a tertiary care hospital.

Methods: A descriptive cross-sectional study was conducted at the Microbiology Laboratory of Arif Memorial Teaching Hospital, Lahore, from August 2024 to January 2025. A total of 149 clinical samples, including pus, blood, urine, and wound swabs, were collected using a convenient sampling technique after obtaining informed consent. Bacterial identification was performed through standard microbiological techniques, including Gram staining, catalase, and tube coagulase tests. Antimicrobial susceptibility testing was carried out using the Kirby–Bauer disc diffusion method following CLSI 2018 guidelines. MRSA detection was based on cefoxitin (30 µg) disc diffusion, with isolates showing a zone of inhibition ≤21 mm considered resistant. Data were analyzed using SPSS version 25.

Results: Out of 149 patients, 75 (50.3%) were males and 74 (49.7%) were females. Positive bacterial growth was observed in 105 samples, yielding a culture positivity rate of 70.5%. *S. aureus* was the most frequent isolate (70.5%), predominantly from pus (43%) and blood (28.2%) specimens. All isolates exhibited complete resistance to penicillin (100%), while significant resistance was noted against levofloxacin (54.4%) and septran (53.7%). Conversely, maximum sensitivity was observed to vancomycin (100%), cefoxitin (57.7%), and linezolid (28.2%). The overall frequency of MRSA was recorded as 2.7%.

Conclusion: The study confirms *S. aureus* as the leading pathogen in clinical infections, exhibiting widespread resistance to commonly used antibiotics but retaining high sensitivity to vancomycin. These findings emphasize the urgent need for rational antibiotic prescribing, regular resistance monitoring, and strict infection control measures to mitigate the rising threat of MRSA in healthcare settings.

Keywords: Antimicrobial susceptibility, hospital-acquired infections, MRSA, pus samples, Staphylococcus aureus, vancomycin, wound infections.

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as one of the most formidable challenges in modern clinical practice, contributing significantly to increased patient morbidity, mortality, and healthcare costs (1). Initially considered a hospital-acquired pathogen, MRSA has now extended its reach to community settings, making it a global public health concern (2). Its ability to cause a wide spectrum of infections—from localized skin and soft-tissue infections to severe systemic diseases such as pneumonia, sepsis, and endocarditis—underscores its clinical importance (3). The persistence of MRSA within healthcare environments and its growing prevalence worldwide highlights the need for continuous surveillance and stringent infection control practices (4,5). Historically, *S. aureus* was highly sensitive to β-lactam antibiotics, which once served as the cornerstone of therapy (6). However, the misuse and overuse of antibiotics have accelerated resistance mechanisms, rendering many conventional antimicrobial agents ineffective (7). Methicillin resistance in *S. aureus* is primarily mediated by the *mecA* gene located on the staphylococcal cassette chromosome mec (SCCmec), which encodes penicillin-binding protein 2a (PBP2a) (8). This altered protein exhibits reduced affinity for β-lactam antibiotics, effectively neutralizing their bactericidal activity (9). As a result, treatment options have become increasingly limited, often relying on last-line agents such as vancomycin, linezolid, or daptomycin—drugs that are expensive, potentially toxic, and not readily available in resource-constrained settings.

In developing countries like Pakistan, antimicrobial resistance surveillance is hindered by dependence on conventional disc diffusion methods and limited access to molecular diagnostic tools. The scarcity of advanced laboratory facilities, high costs of reagents, and shortage of trained personnel further exacerbate the challenge. Consequently, clinicians often lack real-time data on local resistance trends, leading to empirical treatment failures and prolonged hospital stays. The alarming emergence of resistance even to last-resort antibiotics amplifies the urgency to investigate current resistance patterns and guide rational antimicrobial use. Given these challenges, it becomes imperative to assess the prevalence and resistance profile of MRSA within clinical settings. Understanding local epidemiological trends can support evidence-based antibiotic stewardship, optimize therapeutic strategies, and contribute to infection control policies. Therefore, the present study aims to determine the frequency of MRSA among clinical isolates and evaluate its antimicrobial susceptibility pattern to aid in developing effective treatment protocols tailored to the regional resistance landscape.

METHODS

This descriptive cross-sectional study was conducted in the Department of Microbiology at Arif Memorial Teaching Hospital, Lahore, from August 2024 to January 2025, after obtaining ethical approval from the Institutional Review Board (IRB) and informed written consent from all participants prior to sample collection. The study aimed to determine the frequency and antimicrobial resistance profile of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical specimens. A convenient sampling technique was employed due to feasibility constraints within the study period. Both male and female patients aged between 1 and 70 years, presenting to the inpatient and outpatient departments with wound infections or purulent discharges, were included in the study. Patients with known cardiac diseases or other significant comorbidities, such as diabetes mellitus or chronic renal failure, were excluded to minimize potential confounding variables that could influence infection susceptibility or antibiotic response. Clinical specimens, primarily pus and wound swabs, were collected under aseptic conditions and immediately transported to the microbiology laboratory for culture and sensitivity testing. Samples were inoculated on blood agar plates and incubated aerobically at 37°C for 48 hours. Bacterial isolates were identified using standard microbiological procedures, including colony morphology assessment, Gram staining, and confirmatory biochemical tests such as catalase and tube coagulase. The identification process followed recognized microbiological guidelines to ensure diagnostic accuracy and reproducibility.

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller-Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines. Methicillin resistance was phenotypically determined using cefoxitin (30 µg) discs. Isolates exhibiting a zone of inhibition of ≤21 mm were classified as MRSA. A panel of antibiotics was used for susceptibility profiling, including clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), tetracycline (30 µg), minocycline (30 µg), ciprofloxacin (5 µg), penicillin (10 µg), chloramphenicol (30 µg), linezolid (30 µg), fusidic acid (10 µg), and trimethoprim-

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sulfamethoxazole (30 µg) (10-12). The inoculated plates were inverted and incubated at 37°C for 24 hours. After incubation, the diameter of inhibition zones was measured using a Vernier caliper, and interpretations were made in accordance with CLSI breakpoints to ensure standardized and reproducible results. Data obtained were entered and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0. Descriptive statistics, including means, frequencies, and percentages, were used to summarize the data. Results were tabulated to present the prevalence of MRSA and the corresponding antibiotic resistance patterns. All procedures were conducted following ethical principles outlined in the Declaration of Helsinki. Patient confidentiality was maintained throughout the study, and no personal identifiers were recorded.

RESULTS

A total of 149 patients were included in the study, comprising 75 males (50.3%) and 74 females (49.7%), indicating an almost equal gender distribution. The majority of patients belonged to the 16–30-year age group (35.6%), followed by those aged 31–45 years (23.5%) and 46–60 years (22.1%), whereas the least representation was observed among individuals aged below 15 years (10.7%) and those aged up to 70 years (6.7%). Clinical specimens collected for microbiological culture included pus (43%), blood (28.2%), urine (15.4%), and wound swabs (13.4%). Among the total samples processed, *Staphylococcus aureus* was the most frequently isolated pathogen, identified in 105 (70.5%) of the positive cultures. Antimicrobial susceptibility testing revealed that all *S. aureus* isolates (100%) were resistant to penicillin. Despite this, complete sensitivity was observed to vancomycin (100%), making it the most effective antibiotic against the isolates. The isolates exhibited moderate sensitivity to cefoxitin (57.7%), linezolid (28.2%), and clindamycin (26.2%), while comparatively lower sensitivity was recorded for doxycycline (25.5%) and azithromycin (15.4%). Resistance was predominant to levofloxacin (54.4%), septran (53.7%), ciprofloxacin (47.7%), and azithromycin (45.6%). Notably, colistin demonstrated poor sensitivity (0.7%), suggesting limited efficacy in treating *S. aureus* infections. Among the total isolates, the frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) was 2.7%, based on cefoxitin resistance patterns. The antimicrobial resistance pattern of MRSA varied considerably across different antibiotic classes, reflecting diverse mechanisms of resistance. The predominance of *S. aureus* among clinical specimens underscores its significant role as a causative agent of infection within the study population.

Out of the 149 clinical specimens processed, 105 yielded positive bacterial growth, establishing an overall culture positivity rate of 70.5%. Among these, *Staphylococcus aureus* was the predominant pathogen isolated. An analysis of the distribution of culture positivity across demographic variables showed no significant gender-based difference, with 52 of 75 males (69.3%) and 53 of 74 females (71.6%) exhibiting positive cultures ($p > 0.05$, Chi-square test). Similarly, age did not show a statistically significant association with culture positivity ($p > 0.05$). The highest frequency of positive cultures was observed in the 16–30-year age group (35.6%), followed by the 31–45-year group (23.5%), indicating that younger adults were more likely to present with infections during the study period. Regarding specimen type, pus samples demonstrated the highest positivity rate (43%), followed by blood (28.2%), urine (15.4%), and wound swabs (13.4%). Although no statistically significant association ($p > 0.05$) was observed between sample type and culture positivity, the predominance of pus samples highlights the frequent occurrence of skin and soft-tissue infections in the hospital setting. These findings reinforce that infection prevalence was consistent across genders and age groups but was more frequently associated with wound and purulent samples, underscoring the clinical relevance of *S. aureus* as a primary pathogen in localized infections.

Table 1: Age Wise Distribution of Patients

Age group	Total patients (N=149)4.
Up to 15	16(10.7%)
16-30	53(35.6%)
31-45	35(23.5%)
46-60	33(22.1%)
<70	10(6.7%)

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Table 2: Gender Wise Distribution Among Patients

Gender	Frequency	Percentage
Female	74	49.7%
Male	75	50.3%
Total	149	100%

Table 3: Types and Frequency of Samples Submitted for Culture (n=149)

Type of Sample	No. of Samples Submitted	No of Positive Cultures (%)
Pus	64	43%
Blood	42	28.2%
Urine	23	15.4%
Wound	20	13.4%

Table 4: Frequency of Pathogen Isolated (n=149)

Pathogen	No of positive cultures	Frequency
Staphylococcus aureus	105	70.5%

Table 5: Antimicrobial Sensitivity of S. aureus (n=149)

Antimicrobial	Resistant (%)	Sensitive (%)
Penicillin	149 (100%)	0 (0%)
Levofloxacin	81 (54.4%)	8(5.4%)
Septran	80 (53.7%)	5 (3.4%)
Ciprofloxacin	71 (47.7%)	6 (4%)
Azithromycin	68 (45.6%)	23 (15.4%)
Clindamycin	57 (38.3%)	39 (26.2%)
Colistin	56 (37.6%)	1 (0.7%)
Doxycycline	34 (22.8%)	38 (25.5%)
Linezolid	23 (15.4%)	42 (28.2%)
Cefoxitin to detect MRSA	4 (2.7%)	86 (57.7%)
Vancomycin	0 (0%)	149 (100%)

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Table 6: Distribution of Culture Positivity by Gender, Age Group, and Sample Type

Variable	Category	Total Samples (n)	Positive Cultures (n)	Positivity Rate (%)	p-value
Gender	Male	75	52	69.3	>0.05
	Female	74	53	71.6	
Age Group (Years)	Up to 15	16	10	62.5	>0.05
	16–30	53	37	69.8	
	31–45	35	25	71.4	
	46–60	33	23	69.7	
	<70	10	7	70.0	
Sample Type	Pus	64	45	70.3	>0.05
	Blood	42	30	71.4	
	Urine	23	16	69.6	
	Wound Swab	20	14	70.0	

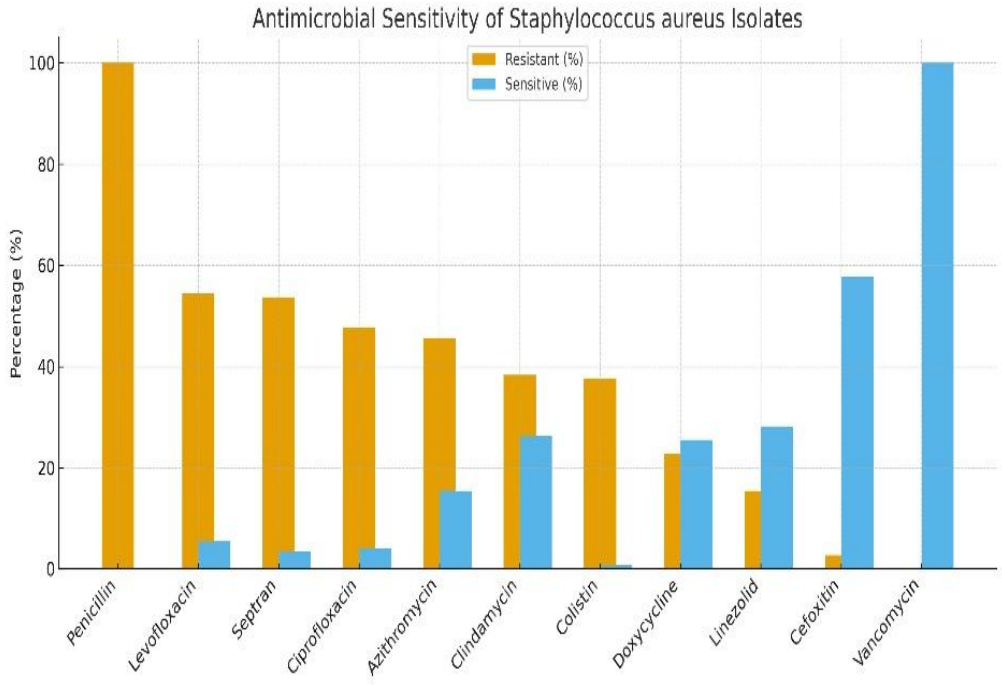

Figure 1 Antimicrobial Sensitivity of Staphylococcus Aureus Isolates

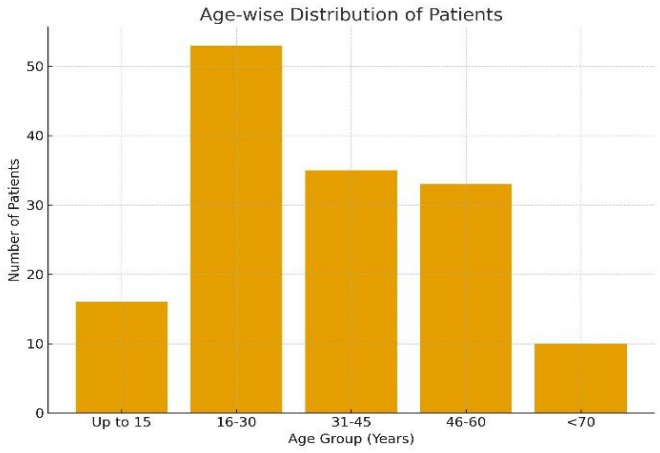

Figure 3 Age-wise Distribution of Patients

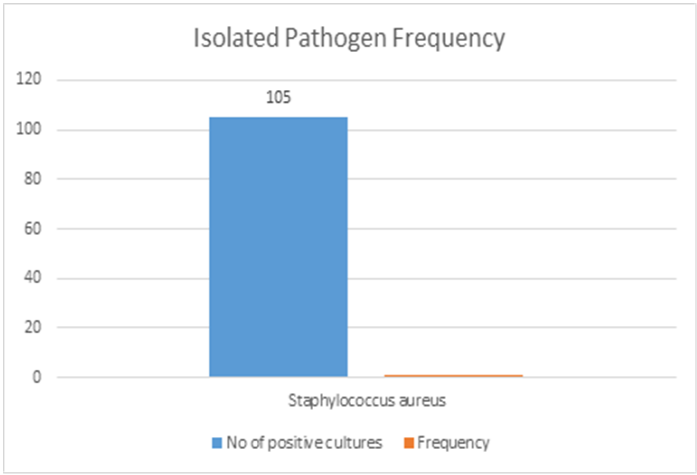

Figure 2 Isolated Pathogens Frequency

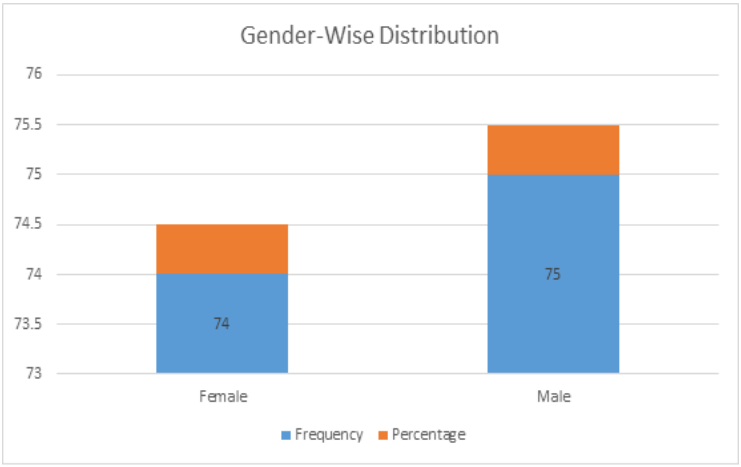

Figure 4 Gender-wise Distribution

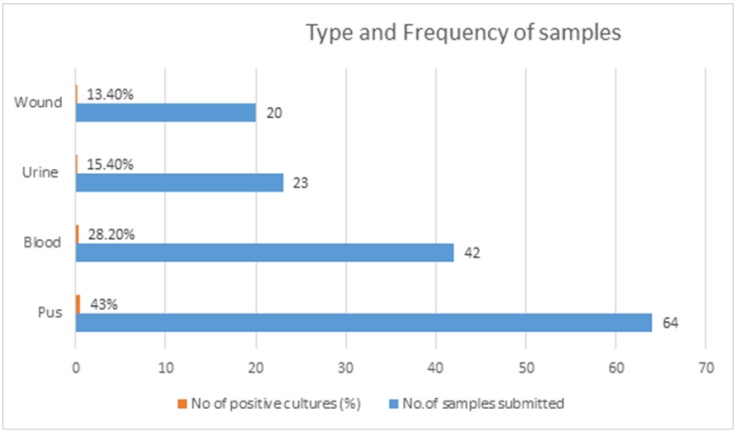

Figure 5 Type and Frequency of Samples

DISCUSSION

The findings of the present study demonstrated that *Staphylococcus aureus* was the predominant pathogen isolated from clinical specimens, accounting for a culture positivity rate of 70.5%, with methicillin-resistant *S. aureus* (MRSA) identified in 2.7% of isolates. This relatively low prevalence of MRSA contrasts with several reports from other regions of Pakistan and abroad, where significantly higher frequencies have been documented. Comparable investigations conducted in Peshawar and Karachi reported MRSA frequencies of 36.1% and 50%, respectively, while another study from Lahore observed a prevalence of 55% among *S. aureus* isolates (11-13). More recent surveillance in Peshawar in 2023 revealed an even higher frequency of 57% (14). The markedly lower frequency in the current study may reflect improved infection prevention practices, rational antimicrobial use, or institutional variations in sample populations and diagnostic methodologies. Globally, MRSA prevalence exhibits considerable regional variation, largely influenced by infection control policies, antibiotic stewardship programs, and surveillance systems. In Europe, MRSA rates range between 1% and 50%, with countries such as Denmark, Iceland, the Netherlands, Sweden, and Norway maintaining rates below 5% due to robust surveillance and stringent antibiotic regulations (15,16). In contrast, southern European nations—including Italy, the United Kingdom, Romania, Spain, Turkey, and Greece—continue to report rates exceeding 25%, reflecting ongoing challenges in containment (17). The United States has documented a steady decline in MRSA-related hospitalizations due to effective public health interventions and the widespread adoption of infection control protocols (18). Conversely, Asian countries continue to report higher MRSA burdens, with prevalence estimates of 28% in Hong Kong and Indonesia, 75% in Korea, and approximately 43% in India (19). These discrepancies underscore the influence of healthcare infrastructure, public health policy, and antibiotic utilization patterns on resistance trends. The results of the present study

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reinforce that *S. aureus* remains a dominant pathogen in clinical infections, with high resistance to commonly used antibiotics such as penicillin, levofloxacin, and septran, yet universal susceptibility to vancomycin. This pattern aligns with global trends, indicating that glycopeptides remain the cornerstone for MRSA treatment. However, reliance on such last-line agents heightens the risk of future resistance emergence (20,21). The detection of resistance to multiple antibiotic classes emphasizes the need for continued local and national surveillance, development of hospital-specific antibiograms, and reinforcement of antimicrobial stewardship programs.

Despite its clinical relevance, the study had certain limitations. The use of a convenient sampling technique may have introduced selection bias, limiting the generalizability of the findings. Furthermore, molecular confirmation of the *mecA* gene through polymerase chain reaction (PCR) was not performed due to resource constraints, which could have strengthened diagnostic accuracy. The absence of inferential statistical analyses to correlate MRSA prevalence with demographic or clinical variables also restricted deeper epidemiological interpretation. Future studies should incorporate molecular typing, multicentric data, and longitudinal follow-up to better delineate temporal resistance trends and transmission dynamics (22). Nevertheless, the strengths of this study lie in its structured approach to local antimicrobial profiling, providing valuable insight into the resistance patterns within a tertiary care setting. The findings serve as an evidence base for clinicians to rationalize empirical therapy and inform hospital infection control policies. To curb the escalating threat of antimicrobial resistance, strict regulatory enforcement against irrational antibiotic use and over-the-counter dispensing must be prioritized. Collaborative efforts between clinicians and microbiologists to design hospital antibiograms and monitor emerging resistance trends are essential. Moreover, ensuring proper hygiene, enforcing infection control measures, and implementing nationwide surveillance frameworks remain pivotal strategies for preventing MRSA transmission and safeguarding the efficacy of existing antimicrobials.

CONCLUSION

The study concluded that methicillin-resistant *Staphylococcus aureus* remains a significant clinical concern within the hospital setting, posing challenges to effective treatment due to its resistance to commonly used antibiotics. The sustained sensitivity of isolates to vancomycin and cefoxitin highlights these agents as reliable therapeutic options in managing MRSA infections. In contrast, the poor efficacy of penicillin emphasizes the necessity for judicious antibiotic selection guided by local susceptibility data. The growing prevalence of resistant strains underscores the urgent need for robust infection control measures, rational antibiotic stewardship, and ongoing surveillance programs to curb the spread of MRSA and preserve the effectiveness of available antimicrobial therapies.

AUTHOR CONTRIBUTION

Author	**Contribution**
Areesha Akram	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Shabana Nawaz	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Maryam Akbar	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Tanzeela Iftikhar	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Uswa Nawaz	Contributed to Data Collection and Analysis

Author	Contribution
	Has given Final Approval of the version to be published
Anas Jahangir*	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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