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IDENTIFICATION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA IN POST-SURGICAL PATIENTS AND THEIR ANTIBIOTIC SENSITIVITY PATTERN

Original Research

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ABSTRACT

Background: Surgical site infections (SSIs) continue to be a major postoperative complication, particularly in resource-limited healthcare settings. These infections significantly prolong hospitalization, increase morbidity, and escalate healthcare costs. Both Gram-positive and Gram-negative bacteria are implicated, and the rise of antimicrobial resistance has further complicated management. Accurate identification of pathogens and their resistance patterns is essential to guide empirical therapy and strengthen infection control practices.

Objective: To identify Gram-positive and Gram-negative bacteria isolated from post-surgical patients and to evaluate their antimicrobial susceptibility patterns.

Methods: A descriptive cross-sectional study was conducted in the Department of Microbiology, Arif Memorial Teaching Hospital, Lahore, from August 2024 to January 2025. A total of 104 clinical specimens, including pus, wound swabs, and blood samples, were collected using convenient sampling. Standard microbiological procedures such as Gram staining, colony morphology, catalase, coagulase, and biochemical assays were employed for bacterial identification. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method in accordance with CLSI 2018 guidelines. Data were analyzed using SPSS version 25, with frequencies and percentages calculated for isolates and resistance profiles.

Results: Out of 104 collected specimens, 63 (60.6%) were culture-positive, while 41 (39.4%) showed no growth. *Staphylococcus aureus* was the predominant pathogen (23.1%), followed by *Escherichia coli* (19.2%), *Enterobacter* species (7.7%), *Staphylococcus* species (3.8%), *Pseudomonas* (1.9%), *Proteus vulgaris* (1.9%), and *Klebsiella* species (1.9%). Gram-positive isolates showed complete resistance to penicillin and ampicillin (100%) but full sensitivity to vancomycin (100%) and high sensitivity to linezolid (81.8%). Gram-negative isolates exhibited marked resistance to ceftazidime (96.0%), ciprofloxacin (82.4%), and levofloxacin (82.5%), whereas carbapenems such as imipenem (80.0%) and meropenem (74.3%) retained considerable efficacy.

Conclusion: The findings highlight the coexistence of Gram-positive and Gram-negative organisms as major contributors to SSIs, with alarmingly high resistance to first-line antibiotics. Vancomycin, linezolid, and carbapenems remain effective therapeutic options. The results emphasize the urgent need for continuous surveillance, rational antimicrobial use, and stronger infection control strategies to reduce SSI-related morbidity.

Keywords: Antibiotic resistance, *Escherichia coli*, Gram-negative bacteria, Gram-positive bacteria, Surgical site infections, *Staphylococcus aureus*, Vancomycin.

INTRODUCTION

Post-surgical infections, often termed surgical site infections (SSIs), remain one of the most common and challenging complications following operative procedures. They not only contribute to prolonged hospital stays and increased healthcare costs but also significantly impact patient recovery and overall morbidity (1–3). These infections are caused by a diverse spectrum of microorganisms, with Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative organisms including *Escherichia coli* and *Klebsiella pneumoniae* being the predominant culprits (4,5). In recent years, the emergence of antimicrobial resistance has intensified the clinical burden of SSIs. Gram-positive pathogens have increasingly acquired resistance to beta-lactam antibiotics through altered penicillin-binding proteins, while Gram-negative species employ mechanisms such as beta-lactamase production, efflux pumps, and porin modifications, leading to extensive drug resistance (6–9). This growing resistance undermines the effectiveness of standard therapies, complicating treatment and elevating the risk of poor outcomes.

The epidemiology of SSIs varies globally depending on surgical procedures, patient demographics, and infection control measures. In developed countries, strict surveillance and robust antimicrobial stewardship programs have contributed to declining SSI incidence. In contrast, South Asian countries continue to report high burdens of infection, with resistance rates to commonly used antibiotics frequently exceeding 50% (10–13). These regional disparities underscore the importance of contextualizing infection control strategies within local healthcare systems. In Pakistan, the situation is further aggravated by the inappropriate use of antibiotics, limited access to advanced microbiological facilities, and gaps in infection prevention policies (14–16). These factors collectively contribute to the rising tide of antimicrobial resistance, leaving clinicians with fewer effective options for managing SSIs. As such, reliable local data on the bacterial profiles and their susceptibility patterns are urgently required to inform clinical decision-making. This study was therefore undertaken to identify the spectrum of Gram-positive and Gram-negative bacterial pathogens responsible for SSIs in post-surgical patients and to evaluate their antimicrobial susceptibility profiles. The findings are intended to provide evidence-based guidance for empirical therapy and to support the development of antibiotic stewardship policies tailored to the local healthcare context.

METHODS

This descriptive cross-sectional study was carried out in the Department of Microbiology, Arif Memorial Teaching Hospital, Lahore, between August 2024 and January 2025, following approval from the institutional ethical review committee. Written informed consent was obtained from all patients or their attendants prior to specimen collection. Clinical specimens, including pus, wound swabs, and blood samples, were obtained from post-surgical patients presenting with signs and symptoms suggestive of surgical site infection. A non-probability convenient sampling technique was applied to recruit eligible participants. All patients of any age who developed surgical site infections following operative procedures were included in the study. Exclusion criteria comprised patients undergoing contaminated surgeries and those who were immunocompromised, receiving steroid therapy, or undergoing chemotherapy, as these conditions could confound the microbiological and clinical outcomes (3,4). This approach ensured that only representative cases of post-surgical infections were analyzed. Collected samples were transported promptly to the laboratory and cultured aerobically on blood agar and MacConkey agar. Plates were incubated at 37°C for 24 to 48 hours, after which bacterial colonies were identified using standard microbiological methods. Gram staining, catalase, and coagulase tests were employed for the identification of Gram-positive organisms, whereas Gram-negative isolates were characterized using biochemical tests including oxidase and API strip systems. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method in line with Clinical and Laboratory Standards Institute (CLSI) guidelines 2018, which remains a validated benchmark for resistance profiling. The panel of antibiotics tested comprised penicillin, ampicillin, cefoxitin, vancomycin, linezolid, ciprofloxacin, levofloxacin, gentamicin, ceftriaxone, imipenem, meropenem, and colistin, covering a wide range of therapeutic classes relevant to Gram-positive and Gram-negative pathogens. Data were entered and analyzed using SPSS version 25. Descriptive statistics were employed, with means calculated for continuous variables and frequencies with percentages for categorical variables, particularly the distribution of bacterial isolates and their respective susceptibility patterns. This statistical approach allowed clear representation of microbiological trends and resistance rates among the studied population.

RESULTS

The study included 104 post-surgical patients, with an equal gender distribution of 52 males (50.0%) and 52 females (50.0%). The age of participants ranged widely, with the highest proportion of patients observed in the 45–60 year group (29.8%), followed by 31–45 years (27.9%) and above 61 years (22.1%). Younger patients between 16–30 years accounted for 17.3%, while only 2.9% of patients were under the age of 15. This distribution demonstrated that middle-aged and older adults were most frequently affected by surgical site infections. A total of 104 clinical samples were processed, with pus specimens being the most frequently submitted (72.1%), followed by wound swabs (7.7%), blood (5.8%), and ascitic fluid (1.9%). Less frequent sources included soft tissue, pleural fluid, pancreatic abscess, and bone fragments, each accounting for approximately 1.0%. Culture positivity was noted in 63 samples (60.6%), whereas 41 samples (39.4%) showed no bacterial growth. Among the 63 culture-positive cases, both Gram-positive and Gram-negative pathogens were isolated. *Staphylococcus aureus* emerged as the predominant pathogen, detected in 24 isolates (23.1%), followed closely by *Escherichia coli* in 20 cases (19.2%). Other notable organisms included *Enterobacter* species (7.7%), miscellaneous *Staphylococcus* species (3.8%), *Pseudomonas* species (1.9%), *Proteus vulgaris* (1.9%), *Klebsiella* species (1.9%), and *Proteus mirabilis* (1.0%). Antimicrobial susceptibility testing revealed significant resistance trends. Gram-positive isolates demonstrated universal resistance to penicillin (100%) and ampicillin (100%), whereas vancomycin and linezolid retained full or near-full activity, with sensitivity rates of 100% and 81.8% respectively. Cefoxitin also showed considerable effectiveness, with 79.2% sensitivity. Conversely, fusidic acid and doxycycline showed variable performance, with sensitivity rates of 38.5% and 50.0% respectively.

Gram-negative isolates exhibited high resistance to cephalosporins and fluoroquinolones, with resistance rates of 96.0% to ceftazidime, 82.5% to levofloxacin, and 82.4% to ciprofloxacin. Piperacillin also demonstrated poor efficacy, with only 25% sensitivity. In contrast, carbapenems remained the most effective agents, with imipenem showing 80.0% sensitivity and meropenem 74.3%. Colistin also demonstrated reliable activity across resistant Gram-negative organisms. These results highlighted that while carbapenems and colistin remain the most reliable options against Gram-negative pathogens, vancomycin and linezolid continue to be the most effective for Gram-positive isolates. The study revealed important gaps in stratification of antimicrobial resistance across specific bacterial species and sample types. While aggregate resistance profiles were presented, species-specific susceptibility patterns were not clearly delineated. For example, *Staphylococcus aureus* isolates displayed complete resistance to penicillin and ampicillin, yet retained sensitivity to vancomycin and linezolid, whereas *E. coli* and *Klebsiella* species demonstrated high levels of resistance to cephalosporins and fluoroquinolones but preserved sensitivity to carbapenems and colistin. Similarly, the majority of positive isolates originated from pus samples, followed by wound and blood specimens.

Table 1: Age wise Distribution of Patients

Age	Frequency	Percentage
Up to 15	3	2.9%
16-30	18	17.3%
31-45	29	27.9%
45-60	31	29.8%
>61	**23**	**22.1%**

Table 2: Gender Wise Distribution Among Patients

Gender	Frequency	Percentage
Male	52	50.0
Female	52	50.0
Total	**104**	**100%**

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Table 3: Types and Frequency of Samples submitted for Culture (n=104)

Types of samples	No. Of samples submitted	Frequency
Pus	75	72.1%
Wound	8	7.7%
Blood	6	5.8%
Fluid	1	1.0%
Left hand abscess	1	1.0%
Breast drainage	1	1.0%
Right gluteal region	1	1.0%
Left hand discharge	1	1.0%
Soft tissues	1	1.0%
Swab	1	1.0%
Ascitic fluid	2	1.9%
Gluteal abscess	1	1.0%
Pleural fluid	1	1.0%
Pancreatic abscess	1	1.0%
Purulent discharge	1	1.0%
Purulent slough	1	1.0%
Bone fragment femur	1	1.0%
Total	**104**	**100%**

Table 4: Frequency of Isolated Pathogens (n= 104)

Isolated Pathogen	Frequency	Percentage
Staphylococcus aureus	24	23.1%
E. coli	20	19.2%
Enterobacter species	8	7.7%
Staph species	4	3.8%
Pseudomonas species	2	1.9%
Proteus vulgaris	2	1.9%
Klebsiella species	2	1.9%
Proteus mirabilis	1	1.0%
No growth	41	39.4%
Total	**104**	**100%**

Table 5: Summarized Culture Result

Culture	Frequency	Percentage
Positive	63	60.58%
Negative	41	39.42%
Total	**104**	**100%**

Table 6: Antibiotic Susceptibility Pattern of Drugs on Bacterial Pathogens

Sr. No	Antibiotics	Resistant (%)	Sensitive (%)
1	Imipenem	20.00%	80.00%
2	Meropenem	25.70%	74.30%
3	Penicillin	100%	0.00%
4	Ciprofloxacin	82.40%	17.60%
5	Ampicillin	100%	0.00%
6	Fusidic acid	61.50%	38.50%
7	Doxycycline	50.00%	50.00%
8	Vancomycin	0.00%	100%
9	Ceftazidime	96.00%	4.00%
10	Piperacillin	75.00%	25.00%
11	Azithromycin	68.00%	32.00%
12	Levofloxacin	82.50%	17.50%
13	Linezolid	18.20%	81.80%
14	Cefoxitin	20.80%	79.20%
15	**Tetracycline**	**78.90%**	**21.10%**

Table 7: Species-specific Resistance Trends of Major Isolates

Pathogen	Key Resistance Patterns (%)	Key Sensitivity Patterns (%)	Sample Types Most Commonly Involved
Staphylococcus aureus	Penicillin (100%), Ampicillin (100%), Ciprofloxacin (>80%)	Vancomycin (100%), Linezolid (81.8%), Cefoxitin (79.2%)	Pus, wound swabs
Escherichia coli	Ceftazidime (96%), Ciprofloxacin (82.4%), Levofloxacin (82.5%)	Imipenem (80%), Meropenem (74.3%), Colistin (active)	Pus, blood, ascitic fluid
Klebsiella pneumoniae	High resistance to cephalosporins and fluoroquinolones (>80%)	Carbapenems (70–80%), Colistin (active)	Pus, abscess
Enterobacter spp.	Moderate resistance to beta-lactams and quinolones	Variable carbapenem susceptibility	Pus

Pathogen	Key Resistance Patterns (%)	Key Sensitivity Patterns (%)	Sample Types Most Commonly Involved
Other Gram-negative spp. (Proteus, Pseudomonas)	Multidrug resistance to cephalosporins and fluoroquinolones	Retained activity of carbapenems, colistin	Soft tissue, wound drainage

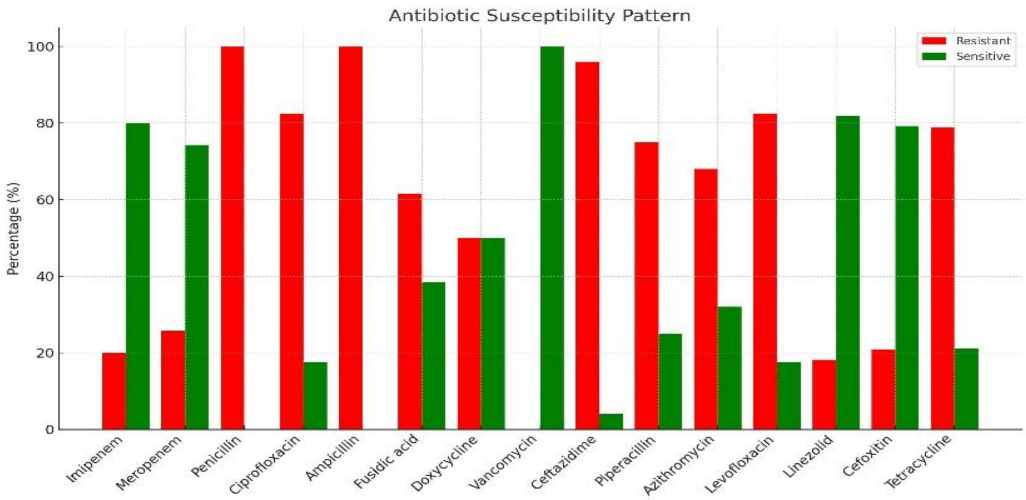

Figure 1 Antibiotic Susceptibility Pattern

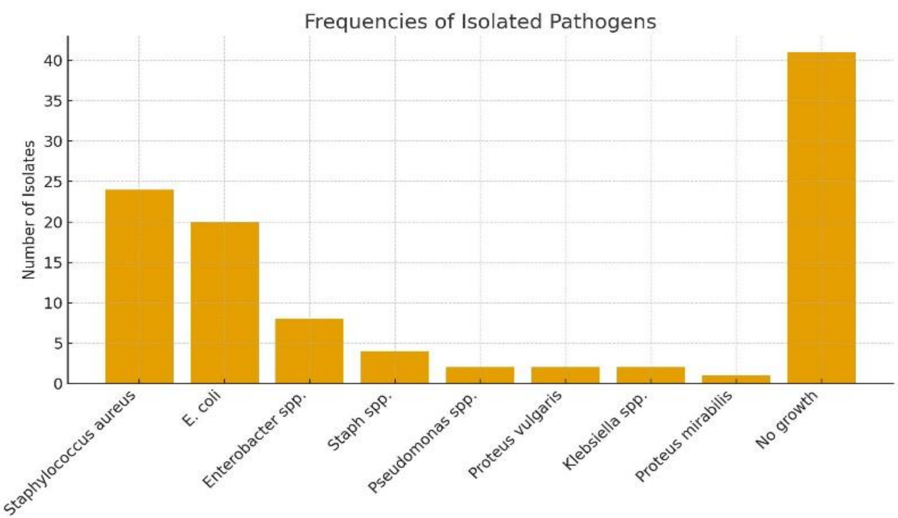

Figure 2 Frequencies of Isolated Pathogens

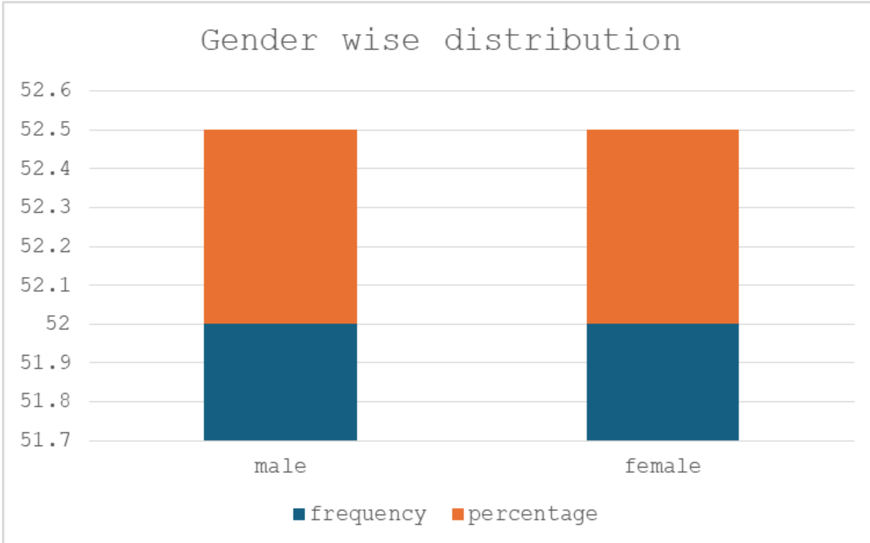

Figure 3 Gender wise Distribution

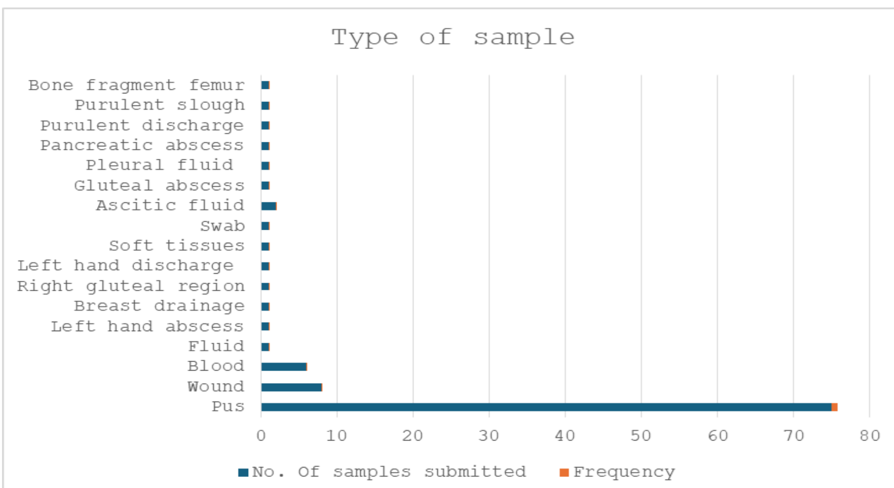

Figure 4 Type of Sampe

DISCUSSION

The present study demonstrated that surgical site infections were caused by a wide spectrum of both Gram-positive and Gram-negative organisms, with *Staphylococcus aureus* emerging as the predominant Gram-positive isolate and *Escherichia coli* and *Klebsiella pneumoniae* being the leading Gram-negative pathogens. This dual microbial burden reflects the complexity of post-surgical infections and is consistent with earlier national and regional studies where similar pathogens were frequently isolated (12–15). The persistence of methicillin-resistant *Staphylococcus aureus* (MRSA) alongside resistance to fluoroquinolones underscored the alarming rise in antimicrobial resistance, particularly in South Asian healthcare settings where antibiotic misuse remains widespread (16,17). The predominance of *E. coli* and *Klebsiella* among Gram-negative isolates corresponded with international reports where Enterobacteriaceae remain the chief contributors to surgical site infections (18–20). The high resistance observed against cephalosporins and fluoroquinolones reflects patterns of overuse and misuse of these drugs, a phenomenon well-documented in low- and middle-income countries. The retained sensitivity of carbapenems and colistin suggests that these agents continue to play a pivotal role in the management of resistant infections. However, their use must be approached cautiously, given their toxicity profiles and the global

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concern over the emergence of carbapenem-resistant Enterobacteriaceae. When compared with global data, the prevalence of multidrug resistance observed in this study exceeded rates reported from many Western countries but paralleled those from South Asia, indicating a region-specific challenge that requires targeted interventions (21,22). The MRSA burden in particular demonstrated striking geographical variation, being reported at less than 5% in Scandinavian nations with stringent infection control, while reaching over 25% in parts of Southern and Eastern Europe, and exceeding 40% across many Asian countries (20–22). Such contrasts reinforce the importance of robust surveillance systems and infection prevention policies in reducing the spread of resistant organisms.

The demographic distribution of infections, with the highest burden among middle-aged and older patients, emphasized the need for careful postoperative monitoring in these age groups. The equal gender distribution indicated that sex was not a major determinant of risk. The predominance of pus samples highlighted the clinical importance of superficial and deep wound infections, though bloodstream infections also represented a significant proportion of cases and warrant closer evaluation due to their potentially severe outcomes. A strength of this study was the comprehensive microbiological profiling of isolates using standard laboratory methods aligned with CLSI guidelines, ensuring reliable antimicrobial susceptibility data. The findings provide valuable local evidence that can guide empirical therapy in similar tertiary care settings. However, the study also carried limitations. The use of convenient sampling may have introduced selection bias, potentially affecting the representativeness of results. Resistance profiles were not stratified by individual species, which limited the precision of clinical interpretation. Furthermore, the absence of data on patient outcomes, including length of hospital stay, morbidity, and mortality, restricted the ability to directly link microbial resistance with clinical impact. These omissions highlight the need for integrated clinical and microbiological studies in the future. Overall, the findings underscored the urgent necessity of antimicrobial stewardship programs and improved infection prevention strategies within tertiary hospitals in Pakistan. Future studies should focus on species-specific resistance trends, stratification by specimen type, and incorporation of patient-centered outcomes to better quantify the burden of surgical site infections. Such measures will be critical in formulating evidence-based strategies for the control of multidrug-resistant organisms and in improving surgical outcomes in resource-limited healthcare systems.

CONCLUSION

This study concluded that both Gram-positive and Gram-negative bacteria play a major role in post-surgical infections, with rising antimicrobial resistance posing a serious challenge to effective management. The persistence of resistance to commonly prescribed first-line antibiotics highlights the narrowing spectrum of therapeutic options, leaving only a few agents with consistent efficacy. Vancomycin remained the most reliable treatment choice for Gram-positive organisms, while carbapenems and colistin demonstrated retained effectiveness against Gram-negative pathogens. These findings emphasize the critical need for ongoing surveillance, prudent antibiotic prescribing, and strengthened infection control measures to curb the spread of resistant pathogens and improve postoperative patient outcomes.

AUTHOR CONTRIBUTION

Author	Contribution
Uswa Nawaz	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Neelam Fatima	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
Shabana Nawaz	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published

Author	Contribution
Hafiz Asif Muhammad Ali	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Areesha Akram	Contributed to Data Collection and Analysis 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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