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Microbiological evaluation of air quality and surface contamination in the surgical room at West Kalimantan Regional General Hospital

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ABSTRACT

Aims: This study aimed to evaluate the microbiological quality of airborne and surface bacterial contamination in surgical rooms.

Methods: This descriptive study was conducted at West Kalimantan Regional General Hospital (WKRGH) from October to December 2023 to evaluate microbiological contamination of air and floor surfaces. Air samples were collected using the settling plate method, while floor surface samples were obtained using the swab method. Surgical rooms with the highest frequency of procedures were selected through purposive sampling.

Results: The airborne bacterial counts ranged from 52.37 colony forming unit (CFU)/m^3 to 995.13 CFU/m^3, while surface bacterial counts ranged from 2.66 CFU/m^2 to 48 CFU/m^2. Post-disinfection, the airborne bacterial counts were, i.e., median 0 CFU/m^3 and surface bacterial counts ranged from median 0 CFU/m^2 to 1.33 CFU/m^2. According to the Italian National Institute for Occupational Safety and Prevention, surgical rooms 1 (median: 209.5) and 2 (median: 995.13) did not meet the criteria for airborne microbial contamination during surgery, as the levels exceeded 180 CFU/m^3. Post-surgical evaluations of both air and floor surface samples showed complete compliance with the established criteria.

Conclusions: Overall, the quality of airborne and surface bacteria in the surgical rooms at WKRGH meets the acceptable standards. However, certain rooms still exceed this limit and require improvement to comply with the recommended standards.

Introduction

The cleanliness standards of hospital environments must be carefully addressed due to the potential transmission of infection-causing bacteria through droplets, airborne particles, and direct contact. The presence of bacteria in hospital air can contribute to diseases, including nosocomial infections, which are infections acquired within healthcare facilities and can impact patients undergoing treatment (1). Nosocomial infections can result from the transmission of pathogenic bacteria originating within the hospital environment, facilitated by air contamination, surfaces such as floors and walls, and medical equipment (2).

Patients face a heightened risk of acquiring nosocomial infections, particularly when surgical room conditions do not meet established standards. Surgical site infections (SSIs) contribute 14-17% of hospital-acquired infections and 38% in surgical patients (3). According to the World Health Organization, up to 77% of deaths among surgical patients are associated with SSIs (4).

The urgency of evaluating microbiological contamination is underlined by a significant increase in surgical procedures in the Central Surgery Unit of West Kalimantan Regional General Hospital (WKRGH) which rose from 1,690 in 2021 to 3,501 in 2022 (5). The increase by more than 50% may indirectly elevate the risk of nosocomial infections. If not adequately monitored, surgical rooms can harbor airborne and surface pathogens that compromise surgical outcomes and patient safety.

Microbiological monitoring is essential to identify pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa*, both of which are common causes of postoperative infections (6,7). Furthermore, consistent evaluation of microbial contamination supports infection control protocols by determining the source of contamination, allowing for targeted interventions (8,9).

Despite the well-established importance of environmental monitoring, no previous study has evaluated the microbiological quality of air and floor surfaces in the surgical rooms of WKRGH. This represents a critical knowledge gap, as hospital infection prevention strategies often rely on general guidelines without supporting regional and local microbiological data.

This study aims to address that gap by evaluating the microbiological contamination of air and floor surfaces in the surgical rooms of WKRGH. This study will provide baseline data for hospital infection prevention policies and inform future improvements in surgical room hygiene practices, in line with national and international health standards.

Methods

Study design, setting and sampling

This study was conducted at WKRGH from October to December 2023 using a descriptive observational research design. The population under investigation comprises the central surgical installation at WKRGH, and the sample selection uses purposive sampling. The selection criteria were determined based on the most frequently performed surgical procedures, specifically ophthalmic and orthopedic surgeries, as documented in the 2022 surgical activity report of WKRGH. These surgeries are conducted in operation room No. 1, No. 2, No. 3 of the hospital. The sample collection area in each room was determined based on the surgical room's area. In this study, seven areas were identified for each room, using the formula number of areas (NL)=√A, where NL denotes the number of areas, and "A" represents the area of the room in square meters (m^2) (10). As a result, seven sampling areas were identified per room, leading to 14 samples per room (7 during surgery and 7 after disinfection); and 84 samples in total from all rooms.

Disinfection and post-disinfection sampling

Airborne bacteria sampling in the surgical room was conducted both during surgery and after disinfection using the settling plate method on blood agar. The sampling areas in a room should encompass areas with minimal air movement, zones of airflow convergence, or turbulence. These locations typically include spots near the entrance, air grilles, high-efficiency particulate arrestance filters, and the corners of the room (Figure 1) (10). Petri dishes were strategically positioned at predefined locations with lids placed adjacent to the dishes to ensure full exposure of the agar surface to the room air. Care was taken to avoid any contact with the agar surface and to prevent any object from passing over the open dish. The petri dishes were left open for 15 minutes and then closed. Following exposure, the area surrounding the dish was cleansed by spraying alcohol, to eliminate any residual media or condensation from the lid that might compromise the room's cleanliness (11).

Bacterial sampling of the surgical room floor surface was conducted using the swab method on blood agar, both during surgery and after disinfection. The positioning of the sample collection area is determined by potential sources and pathways of bacterial exposure, as well as the activities occurring around the area to be swabbed (Figure 1). Care was taken to confirm that the surface at each sampling area was dry. A sterile cotton

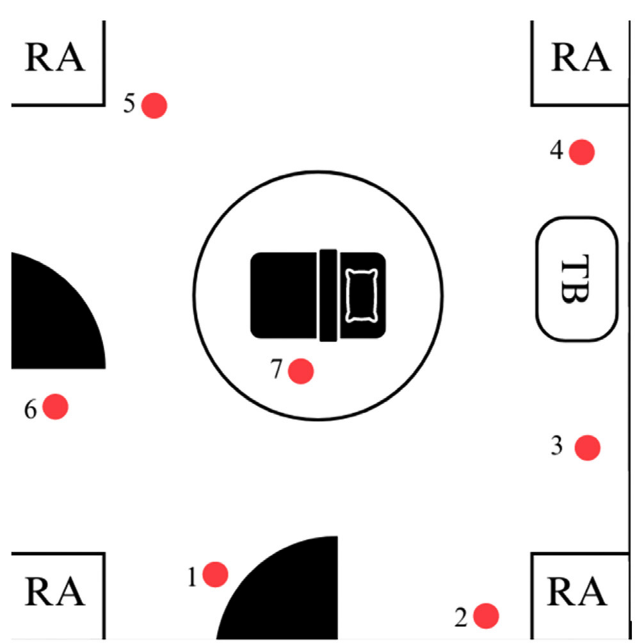

Figure 1. Sampling area RA, TB (●)
RA: Return air, TB: Trash bin

swab was immersed in a 0.9% Natrium Chloride (NaCl) solution and then gently pressed against the side of the tube to remove excess liquid. The sterile cotton swab was then used to swab the sampling area, covering a wide area of 5x5 cm, in horizontal, vertical, and diagonal directions for 30 seconds at a 30° angle (10). Bacterial cultivation at each sampling area is performed in three replications. Incubation is carried out at 37ºC for 24 hours (12). The number of bacterial colonies that grew was subsequently counted.

The disinfection process at WKRGH involved the use of ultraviolet (UV) light, and the floor surfaces were cleaned with 0.5% chlorine solution. Post-disinfection sampling was conducted 30 minutes after the completion of the disinfection procedure.

Microbiological analysis and identification

All collected samples were incubated at 37°C for 24 hours. Following the colony count, additional macroscopic observations were carried out, including examining the shape, size, margin, color, and elevation of the bacterial colonies on the blood agar plate. Subsequently, Gram staining was conducted to examine the cell morphology. Blood agar plates were prepared using Nutrient Agar (Merck, Germany) supplemented with fresh sheep blood. The cultivation of microorganisms from surface samples was conducted using the spread plate method. Swab samples suspended in a tube containing 0.9% NaCl solution were collected and 0.1 mL of the suspension was dispensed onto the agar surface, evenly spread and subsequently incubated.

The maximum concentration of airborne microorganisms in a surgical room during activity is 180 colony forming unit (CFU)/m^3, while after the disinfection process, it is reduced to 10 CFU/m^3 (13). In contrast, the permissible concentration for floor surfaces is only specified for post-disinfection conditions, with a maximum of 5 CFU/m^2 (14).

Ethics considerations

This research has been reviewed and approved by the Health Research Ethics Committee of Dr. Soedarso Regional General Hospital (approval no.: 89/RSUD/KEPK/X/2023, date: October, 2023). As it did not involve human subjects, the research focused on the microbiological evaluation of air quality and surface contamination in the surgical room, with additional institutional authorization provided through Research Permit No. 000.9/20308/RSUD.

Statistical Analysis

IBM SPSS Statistics for Windows, version 30.0 (IBM Corp., Armonk, NY, USA, 2023) was used for statistical analysis. The normality of the data was assessed using the Shapiro-Wilk test. According to the normality test results, normally distributed data are reported as the mean with standard deviation, while non-normally distributed data are reported as the median along with the minimum and maximum values (15).

Results

The results of airborne bacterial count observation

At each designated air sampling area, the findings exclusively indicated the presence of bacterial colonies. The air bacterial counts recorded during surgical procedures are detailed in Table 1. This investigation demonstrated that observable bacterial colonies vary across different sampling areas. For instance, in surgical room No. 1 during the second scheduled operation,

Table 1. Airborne bacterial levels in the surgical room by sampling time and number of personnel

Sample area	CFU/m^3					
	During operation			After disinfection		
	R1	R2	R3	R1	R2	R3
1	471.38	628.5	261.87	0	52.37	157.12
2	209.50	2.514,02	0	0	0	0
3	52.37	419	52.37	0	0	0
4	157.12	523.75	209.5	52.37	0	0
5	733.25	1.099,88	52.37	0	0	0
6	314.25	995.13	52.37	0	0	0
7	0	2.566,40	0	0	0	0
CFU/m^3 [Median (Range)]	209.50 (0-733.25)	995.13 (419-2.566,40)	52.37 (0-209.5)	0 (0-52.37)	0 (0-52.37)	0 (0-157.12)

Room	Samples were taken during the operation	Number of personnel in the room
1	2	4
2	4	6
3	1	5

CFU: Colony forming unit

sampling was conducted at a temperature of 17.60°C with four personnel present during ophthalmic surgery. The highest bacterial count of 733.25 CFU/m^3 was observed at area 5, whereas area 7 showed the lowest bacterial count at 0 CFU/m^3.

In surgical room 2, sampling was conducted during the fourth scheduled operation at a temperature of 17.70°C. Six personnel were present during the orthopedic surgery, with individuals entering and exiting the room during the sampling process. The highest bacterial count, 2,566.40 (CFU/m^3), was recorded at area 7, while the lowest bacterial count, 419 (CFU/m^3), was observed at area 3.

In surgical room 3, sampling was performed during the first scheduled operation at a temperature of 17.70°C. Five personnel were present during the orthopedic procedure. The highest bacterial count, 209.50 CFU/m^3, was detected at area 4, while the lowest bacterial count, 0 CFU/m^3, was found in areas 2 and 7.

The Table 1 presents the bacterial counts in surgical rooms 1, 2, and 3 during surgical procedures. The highest bacterial count, 2,556 CFU/m^3, was recorded in area 7 in room 2. Conversely, the lowest bacterial count, 0 CFU/m^3, was observed in several areas. According to the Italian National Institute for Occupational Safety and Prevention (ISPESL), the acceptable air quality threshold during surgery is 180 CFU/m^3. Surgical rooms 1 and 2 did not meet this hygiene criterion, whereas surgical room 3 complied with the standard (13).

The growth of airborne bacterial colonies in surgical rooms 1, 2, and 3 was significantly reduced after disinfection compared to during surgery. In surgical room 1, the number of bacterial colonies at each air sampling area decreased following the disinfection process. Area 4 exhibited the highest bacterial count at 52.37 CFU/m^3, whereas no bacterial colonies were detected in the other sampling areas.

In surgical room 2, area 1 recorded the highest bacterial count at 52.37 CFU/m^3, with no bacterial colonies detected in the other sampling areas. In surgical room 3, area 1 had the highest bacterial count, measuring 157.12 CFU/m^3.

The highest bacterial count, 157.12 CFU/m^3, was observed in area 1 in room 3. According to ISPESL, the acceptable air quality threshold during surgery, which is 10 CFU/m^3; surgical rooms 1, 2, and 3 met the air hygiene standard.

Observation results of floor surface bacterial counts

The timing and type of operation for floor surface sampling in each room followed the same protocol as air sampling. Table 2 shows the bacterial counts on the floor surfaces of surgical rooms 1, 2, and 3 during surgery. The highest bacterial count, 170.66 CFU/m^2, was recorded at area 2 in room 2. Additionally, several areas showed no bacterial colonies. It is important to note that there are neither local regulatory standards nor international guidelines governing floor surface contamination during surgical procedures.

Following the disinfection process, sampling was conducted sequentially in surgical rooms 1, 2, and 3. The results from these rooms demonstrated a decrease in bacterial counts compared to those collected during surgery. Table 2 displays the bacterial counts in surgical rooms 1, 2, and 3 during surgery. The highest bacterial count, 6.66 CFU/m^2, was observed at area 6 in room 2, with several areas showing no bacterial colonies. Notably, the limits set by Dancer (14) for floor surface contamination following disinfection are set at 5 CFU/m^2, a threshold that was met by all three rooms in the study.

Table 3 presents the cell morphology findings, which included the most frequently identified bacteria: gram-positive cocci, comprising 49.4% of the total, followed by gram-negative cocci

Table 2. Floor bacterial levels in the surgical room

Sample area	CFU/m^3					
	During operation			After disinfection		
	R1	R2	R3	R1	R2	R3
1	6.66	208	4	0	0	1.33
2	2.66	170.66	2.66	1.33	1.33	1.33
3	2.66	4	0	1.33	0	0
4	8	49.33	0	1.33	0	0
5	22.66	46.66	4	0	2.66	1.33
6	25.33	48	6.66	0	66.6	0
7	38.66	24	2.66	0	66.6	0
CFU/m^3 [Median (Range)]	8 (2.66-38.66)	48 (4-170.66)	2.66 (0-6.66)	0 (0-1.33)	0 (0-66.6)	1.33 (0-1.33)
Room	**Samples were taken during the operation**			**Number of personnel in the room**		
1	2			4		
2	4			6		
3	1			5		

CFU: Colony forming unit

Table 3. Morphological characteristics of bacterial cell according to gram staining

Gram type	Cell shape		Total n (%)
	Coccus	*Basil*	
Positive	78 (49,4%)	23 (14,5%)	101 (63,9%)
Negative	36 (22,8%)	21 (13,3%)	57 (36,1%)

at 22.8%. Based on gram staining, gram-positive bacteria constituted the predominant type (64%) of the bacteria found in the surgical rooms of WKRGH.

Discussion

Airborne and surface bacterial contamination

The median concentrations of airborne bacteria in surgical rooms 1, 2, and 3 during surgery were 209.50 CFU/m^3, 995.13 CFU/m^3, and 52.37 CFU/m^3, respectively. According to the ISPESL guidelines, which set a maximum acceptable limit of 180 CFU/m^3 for airborne microorganisms during surgical procedures, rooms 1 and 2 exceeded the permissible threshold, while room 3 remained within acceptable limits. Most countries have established a bacterial threshold limit of 50-150 CFU/m^3 for surgical procedures conducted in conventionally ventilated surgical rooms, with some regulations allowing a maximum of 180 CFU/m^3 (16). According to ISPESL recommendations, the acceptable concentration of airborne microorganisms in surgical environments during general surgery is 180 CFU/m^3 (16). The findings indicate that while room 3 adhered to these microbiological safety standards, rooms 1 and 2 exhibited microbial contamination levels surpassing the acceptable threshold, potentially posing a risk to surgical sterility and patient safety. According to the acceptable limits set by Dancer (14), the contamination level should not exceed 5 CFU/m^2.

These findings align with previous research conducted at the Central Surgical Installation of Dr. Moewardi Hospital, where airborne bacterial levels similarly did not meet the established quality standards for air bacterial concentration (17).

The median airborne bacterial counts in surgical rooms 1, 2, and 3 at WKRGH after disinfection are 0 CFU/m^3, 0 CFU/m^3, and 0 CFU/m^3, respectively. The results for rooms 1, 2, and 3 decreased to within the normal range for bacterial counts of 10 CFU/m^3. The findings from this study in the surgical room were believed to be influenced by the duration of disinfection, which raised concerns about suboptimal disinfection procedures that may not have aligned with recommended practices.

The median floor surface bacterial counts in surgical rooms 1, 2, and 3 during surgery were 8 CFU/m^2, 48 CFU/m^2, and 2.66 CFU/m^2, respectively. These results indicate that bacterial counts vary across different area during surgery, The results of this study suggest that bacterial counts fluctuate across different areas during surgical procedures.

The median floor surface bacterial counts in surgical rooms 1, 2, and 3 after disinfection, were 0 CFU/m^2, 0 CFU/m^2, and 1.33 CFU/m^2, respectively, all of which fall within the acceptable limits for bacterial density.

The findings of this study revealed a noticeable reduction in airborne bacterial counts both during surgical procedures and following the disinfection process. All recorded measurements met the acceptable bacterial limits outlined by the ISPESL, which provides guidelines specifically for infection prevention and control in healthcare facilities. This regulation sets specific thresholds for microbial contamination in medical environments to ensure patient safety and minimize the risk of healthcare-associated infections. The results indicate that, despite the presence of some microbial activity, the surgical rooms met the prescribed standards for bacterial levels, suggesting that both the surgical practices and disinfection protocols employed were effective in maintaining a controlled and sterile environment (14).

Factors affecting microbial load and disinfection efficacy

The findings of this study are likely influenced by multiple factors. First, the level of activity in the surgical area during procedures may contribute to microbial contamination, as bacteria tend to accumulate in high-traffic areas. Second, the duration of the surgical procedure at the time of sampling could also play a significant role, as prolonged operations may increase exposure to airborne and surface contaminants. Third, the effectiveness of the disinfection process during surgical breaks is a crucial factor, as inadequate or incomplete disinfection may leave certain areas untreated, thereby compromising sterility (18).

Research conducted at RGH Brigjend H. Hasan Basry Kandangan suggested that the most effective UV sterilization duration for reducing airborne bacteria was 2 hours, whereas the UV sterilization duration in this study was 1 hour (19).

The study results showed a reduction in airborne bacteria during surgery and after disinfection. This suggests that room disinfection can influence airborne bacterial levels and is anticipated to decrease the risk of surgical wound infections in patients. Several factors can impact the effectiveness of disinfection, including the concentration and potency of the disinfectant. Higher concentrations of disinfectant can enhance effectiveness and shorten the time required to kill microbes. Additionally, physical and chemical factors such as temperature, pH, and humidity also play a role in disinfection efficacy (20). Higher temperatures generally enhanced the effectiveness of most disinfection processes in the past, although excessively high temperatures could lead to degradation of the disinfectant and reduce its activity. Increased pH is known to enhance the antimicrobial activity of certain disinfectants like quaternary ammonium compounds, but it could decrease the activity of others, such as phenol, hypochlorite, and iodine. Additionally, humidity played a crucial role in influencing the activity of disinfectant gases like chlorine dioxide and formaldehyde (21).

According to standard disinfection protocols, specific cleaning procedures must be performed during each surgical break. These include floor cleaning, disinfecting the operating table surface, and properly disposing of medical waste. However, observations in the surgical room revealed that by the end of the procedure, the floor had not been adequately cleaned, and medical waste remained uncollected. Furthermore, subsequent surgeries were scheduled immediately after the previous ones, without sufficient time allocated for thorough sterilization. This lack of proper disinfection and waste management compromises the sterility of the surgical room, increasing the risk of post-surgical infections in subsequent patients.

Several factors may influence the bacterial load on floor surfaces, including the number of surgeries performed, the timing of sample collection, and the number of individuals present in the surgical room prior to sampling. These variables can significantly impact microbial contamination levels. For instance, in room 3, samples were collected during the first surgical procedure of the day, whereas in room 2, sampling occurred during the fourth operation. This difference in sampling time may have contributed to variations in bacterial counts, as surgical rooms subjected to multiple consecutive procedures are more likely to accumulate microbial contamination due to increased exposure to personnel movement, surgical activity, and airborne particulates. Additionally, the number of samples collected at each time point differed, further influencing the observed bacterial distribution. These findings highlight the importance of considering procedural timing and room utilization when assessing microbial contamination in surgical environments (22).

Additionally, the number of bacterial colonies is affected by the activities of surgical room personnel, such as walking, which can deposit microorganisms on the floor. The presence of microorganisms on the floor is also influenced by the cleanliness of the surgical room.

Bacterial identification and clinical relevance

The observation of bacterial colonies in the surgery rooms, based on cell morphology, revealed that gram-positive cocci were the most commonly found at 49.4%, followed by gram-negative cocci at 22.8%, gram-positive bacilli at 14.6%, and gram-negative bacilli at 13.2%. According to gram staining, gram-positive bacteria (63.9%) were predominantly present in the surgical rooms of the hospital, compared to gram-negative bacteria (36.1%). These findings are consistent with the study conducted by Abdilah et al. (23), which also found a higher prevalence of gram-positive bacteria in surgical rooms.

Gram-positive bacteria found in surgical rooms are part of the normal skin flora, which can be released into the air from the skin of patients, doctors, and medical staff during surgery. These bacteria have the potential to cause wound infections, particularly post-surgery infections. These bacteria commonly form spores to protect themselves from adverse environmental conditions. Bacterial spores are highly resistant to disinfectants and high temperatures (24). Because these bacteria are normal flora, almost everyone has them on the skin, nose, or throat.

Based on the Gram staining results, the majority of the gram-positive cocci bacteria are presumed to be *Staphylococcus* species. *Staphylococcus* colonies typically appear round, smooth, raised, and shiny on culture media. Specifically, colonies of *Staphylococcus aureus* usually exhibit a gray-to-yellow coloration, whereas colonies of *Staphylococcus epidermidis* typically appear gray to white (25). In this study, gram-positive *cocci* exhibited colony morphology characterized by round, convex shapes and a white to yellow coloration. Therefore, it can be suspected that the gram-positive cocci bacteria found in this surgical room are *Staphylococcus* aureus and *Staphylococcus* epidermidis.

The findings of Spagnolo et al. (3) indicated that *Staphylococcus* was the most frequently detected bacterium in surgical room examinations. This high prevalence was attributed to *Staphylococcus* being a normal flora on human skin. This finding aligned with research conducted at Jimma University Specialized Hospital, which identified bacteria contaminating the walls and floors, revealing that 33.3% of the bacteria were *Staphylococcus aureus*. Additionally, gram-negative bacteria, specifically *Escherichia coli* and *Klebsiella* spp. each with 11.1% (26).

Microorganism identification was conducted solely based on macroscopic colony morphology and microscopic Gram staining, to provide a general characterization of the bacteria, as this study focused on evaluating the bacterial load in surgical rooms before and after surgery. This study was conducted in the operating rooms of a single hospital; therefore, the results cannot be generalized to all healthcare facilities with different operational characteristics or infection control systems.

Conclusion

The microbiological quality of airborne and surface bacteria in the surgical rooms at WKRGH generally falls within acceptable standards. However, some rooms, specifically rooms 1 and 2, exceeded the recommended threshold of 180 CFU/m^3 during surgical procedures. Inadequate conditions within the surgical room can lead to infections in patients' surgical wounds and facilitate the transmission of diseases to healthcare workers. Consequently, it is essential to enforce standardized procedures within the surgical unit, alongside effective sanitation practices, to ensure a hygienic environment. This is a critical component of infection prevention and control strategies. Infection prevention and control programs play a vital role in minimizing the occurrence of healthcare-associated infections and ensuring patient and healthcare worker safety.

Ethics

Ethics Committee Approval: This research has been reviewed and approved by the Health Research Ethics Committee of Dr. Soedarso Regional General Hospital (approval no.: 89/RSUD/KEPK/X/2023, date: October, 2023).

Informed Consent: As it did not involve human subjects, the research focused on the microbiological evaluation of air quality and surface contamination in the surgical room, with additional institutional authorization provided through Research Permit No. 000.9/20308/RSUD.

Footnotes

Authorship Contributions

Concept: A.R., M.M., M.M., Design: A.R., M.M., M.M., D.F.L., R.A.M., Data Collection or Processing: A.R., M.M., M.M., D.F.L., R.A.M., Analysis or Interpretation: A.R., M.M., M.M., D.F.L., R.A.M., Literature Search: A.R., M.M., M.M., D.F.L., R.A.M., Writing: A.R., M.M., M.M., D.F.L., R.A.M.

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