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Pathogenic Bacterial Isolates from Wound Swab and Pus with their Antibiotic Susceptibility Pattern at a Tertiary Care Hospital in Dhaka, Bangladesh: A Retrospective Study

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Abstract

Background: The issue of antimicrobial resistance is one of the worldwide health concerns since more and more organisms are becoming resistant to common antibiotics. **Objective:** The objective of the study was to determine the pattern of common organisms isolated from pus and wound swabs in relation to their antibiogram. **Methodology:** This retrospective analysis was conducted in the Department of Microbiology at Green Life Medical College & Hospital Ltd., Dhaka, Bangladesh which was carried out between July 2023 and June 2024 for a period of one year. Pus samples and wound swabs were subjected to culture and sensitivity testing. Information about the patients, isolated organisms, culture reports, and sensitivity analyses was gathered from the record book of the Department of Microbiology. **Results:** Gram-positive bacteria comprised 13.7% and gram-negative bacteria accounted for 86.3% of the organisms that grew in 71.7% of the total 1245 samples. The most often isolated bacterium from wound swabs and pus samples was *Pseudomonas* species (34.9%). *Staphylococcus aureus* (14.3%), *Klebsiella* species (12.5%) and *Escherichia coli* (10.5%) were the next most frequently isolated organisms. Among gram-negative bacteria, 14.8% were ESBL-producing organisms, and *Klebsiella* species were the most commonly isolated ESBL producers. The majority of the microorganisms had significant antibiotic resistance. The majority of gram-negative bacteria were resistant to amoxicillin, fluoroquinolones, co-trimoxazole, and cephalosporins. The most effective antibiotics against gram-negative bacteria were colistin, tigecycline, carbapenems, and piperacillin/tazobactam. Most gram-positive bacteria were resistant to co-trimoxazole and fluoroquinolones, while 100.0% of *Staphylococcus aureus* was susceptible to linezolid and vancomycin, and 35.2% of it was methicillin-resistant (MRSA). **Conclusion:** The most common isolated bacteria are *Pseudomonas* species followed by *Staphylococcus aureus*, *Klebsiella* species and *Escherichia coli*, which are resistant to the majority of the commonly used antibiotics. [*Bangladesh Journal of Infectious Diseases, June 2025;12(1):34-41*]

Keywords: Antibiotic susceptibility; pus; resistance; wound swab

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Introduction

Localized and systemic inflammation, typically accompanied by pus development, is indicative of pyogenic infections. The skin serves as a barrier of protection to prevent pathogen invasion. Consequently, altering the skin's activities through chemical, physical, mechanical, or thermal events or surgical procedures that disturb the normal anatomical structure might lead to the entry of surface organisms that begin to multiply locally. The body deploys immune cells as part of its defence strategy to combat the microorganisms. The accumulation of these cells eventually results in pus, a thick, white substance[1]. Skin is more prone to pathogen colonization because it is exposed to wounds, scrapes, and interaction with the outside world[2-4].

The term "wound infection" refers to the occurrence of bacteria that reproduce within a wound and cause damage to the host or tissue. Depending on the depth of the wound, agents that cause wound infection can be categorized. These agents act as carriers of the organisms that cause infection. When one or more contaminants overcome the host's defenses, they multiply rapidly, assault the host tissue, and cause damage; then it is called an infection. Wounds can become infected by a variety of microorganisms. 70.0% to 80.0% of surgical patients die from wound infections, which account for one-third of nosocomial infections[5-7]. Regardless of the kind of lesion, wound infections are linked to patient morbidity and mortality, particularly in developing nations[5,6,8]. Failure to treat a patient results in increased healthcare expenses since it necessitates longer hospital stays for diagnostic testing, extensive antibiotic medication, and perhaps invasive surgery[8].

Many bacteria can cause skin infections because the deeper skin tissues provide an ideal habitat for their colonization and growth[4,9,10]. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Acinetobacter baumannii* are the most frequent bacterial species that cause wound infections. Gram-positive bacteria, particularly *S. aureus*, seem to be the most common colonizers, especially during the first week of infections[11,12]. Gram-negative bacteria, like *P. aeruginosa* and *A. baumannii*, begin to colonize the wound around the start of the second week. If these bacteria get into the lymphatic system or blood arteries, they can cause sepsis.

Global public health is seriously threatened by the rise of antibiotic resistance and the fast spread of these germs among harmful ones. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and other multidrug-resistant bacterial species have been linked to infections in hospital settings throughout the past few decades[13]. The prevalence of extended-spectrum beta-lactamases (ESBLs) in gram-negative bacteria has grown recently. ESBLs are enzymes that mediate resistance to extended-spectrum antibiotics, such as third-generation cephalosporins[14].

Before learning the results of the microbiological culture, a thorough understanding of the pattern of dominating organisms in pus and wounds is crucial for the patient's care. This would be essential to lower the total morbidity and death from infections. To ascertain the current microbiological spectrum of wound infection, together with their pattern of antibiotic sensitivity, the current study was designed.

Methodology

Study Settings and Population: This retrospective investigation was carried out in the Department of Microbiology at Green Life Medical College & Hospital Ltd., Dhaka, Bangladesh. The record was gathered over a year, from July 2023 to June 2024. This study included all wound swabs and pus samples, regardless of age, sex, or antibiotic intake, delivered to the microbiology laboratory for culture and sensitivity testing from the Tertiary Care Hospital's inpatient and outpatient departments. Information was gathered from the laboratory records on the patient's identity, referring departments, specimen type, and sensitivity reports.

Microbiological Laboratory Procedure: Samples were inoculated in blood agar and MacConkey's agar media and incubated aerobically at 37ºC for 24 to 48 hours. The inoculated plates were examined for bacterial growth, and organisms were identified by colony morphology, hemolytic criteria, pigment production, Gram staining, and different biochemical tests per standard protocols. Kirby-Bauer's Disk Diffusion method was used to test antimicrobial susceptibility and interpreted by the Clinical Laboratory Standard Institution (CLSI) guidelines[15]. Sensitivity was done using commercially available antibiotic discs (Oxoid, UK); amoxicillin (30 mcg), amoxyclav (20/10 mcg), amikacin (30 mcg), gentamicin (10 mcg), vancomycin (30 mcg), cefoxitin (30 mcg), ciprofloxacin (5 mcg), ceftriaxone (30 mcg), cefipime (30 mcg), cephradine (30 mcg), cotrimoxazole (25 mcg), piperacillin/tazobactam (100/10 mcg), meropenem (10mcg), aztreonam (30 mcg), imipenem(10mcg), linezolid (30mcg), colistin (10mcg), cloxacillin (5mcg), ceftazidime (30mcg), cefixime (5mcg), cefotaxime (5mcg), cefuroxime (30mcg), tigecycline (15mcg). The zone of inhibition was measured according to the CLSI guideline[16]. ESBL production in gram-negative bacteria was detected by a double disc synergy test following CLSI guidelines using amoxiclav and 3rd-generation cephalosporin discs[16]. Cefoxitin 30 micrograms was used as a surrogate marker for identifying MRSA. Staphylococcus aureus, which showed a zone of inhibition < 21 mm with cefoxitin on Mueller-Hinton Agar after overnight incubation at 37 °C, was considered MRSA[17].

Statistical Analysis: Version 21.0 of the SPSS program was used to enter and analyze the data. Whereas the quantitative data were presented as mean with standard deviation, the qualitative data were provided as frequency and percentage.

Ethical Clearance: The local ethics review committee (ERC) granted this project ethical clearance.

Results

A total number of 1245 pus and swabs from wounds were cultured. Of those, 767 were swabs from wounds; 599 (78.09%) of those samples showed bacterial growth, and 478 (61.5%) of the pus samples showed positive results in a culture. Pus and wound swab isolation rates of organisms differ statistically significantly (p<.001, Chi-Square test, df=1; 95% CI). Bacteria grew in 893 (71.72%) of the samples (Table 1).

Table 1: **Growth of Bacteria in Different Samples**

Samples	Growth	No Growth	P value
Wound Swab	599(78.1%)	168(21.9%)	<0.0001
Pus	294(61.5%)	184(38.5%)	<0.0001
Total	893(71.7%)	352(28.3%)	

Differences between the growth rate of wound swabs and pus are statistically significant (p<0.0001).

Among the culture-positive cases, 541 male patients (60.58%) were more commonly affected than the 352 female patients (39.41%), with the majority of 482 (53.97%) falling into the age range of 21 to 40 years (Table 2).

Table 2: **Characteristics of the Study Population with Wound Swab and Pus Culture-Positive Patients (n=893)**

Variables	Frequency	Percent
Gender		
• Male	541	60.58
• Female	352	39.41
Age Groups		
• Less than 20 Years	43	4.81
• 21 to 40 Years	482	53.97
• 41 to 60 Years	347	38.85
• More than 60 Years	21	2.35

Of the 893 culture-positive samples tested, 771(86.34%) produced gram-negative bacterial growth, and 122(13.67%) produced gram-positive bacterial growth. 543(90.65%) gram-negative and 56(9.34%) gram-positive bacteria were found in the wound swab; 228(77.55%) gram-negative and 66(22.45%) gram-positive bacteria were found in the pus sample (Figure I).

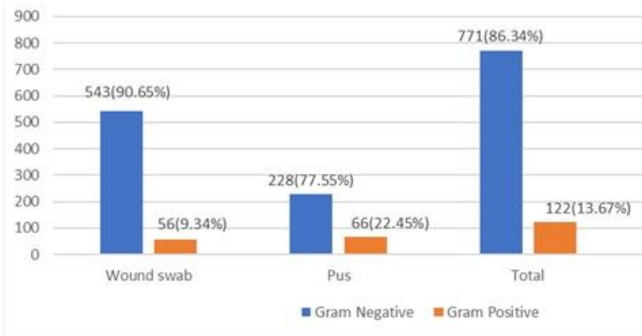

Figure I: **Showing Isolated Bacteria in Different Samples according to Gram reaction**

The most frequently isolated bacterium (34.94%) from pus samples and wound swabs was *Pseudomonas* spp. 20.49% of the samples had *Escherichia coli* isolated from them, and 12.54% had *Klebsiella* spp. growing, and 11.42% had *Proteus* spp. isolated. The most common gram-positive bacteria found in both pus and wound swabs were *Staphylococcus aureus* (Table 3).

Table 3: **Pattern of Isolated Bacteria from Wound Swabs and Pus**

Bacteria	Wound Swab	Pus
Pseudomonas spp	205(34.22%)	107(36.39%)
Escherichia coli	109(18.19%)	74(25.17%)
Staph aureus	59(9.85%)	69(23.47%)

Bacteria	Wound Swab	Pus
Klebsiella spp.	84(14.02%)	28(9.52%)
Proteus spp.	78(13.02%)	24(8.16%)
Acinetobacter spp.	88(14.69%)	2 (0.68%)
Citrobacter spp.	2(0.33%)	4 (1.36%)
CoNS	1(0.17%)	3 (1.02%)

Note: The total number of organisms was greater than the number of samples because co-infections, based on the colonization of different/more species, have been detected.

The presence of only one species was the most frequent condition, instead of co-infection by different species found in 44 samples, corresponding to 3.53% of culture-positive samples. Among the isolated gram-negative bacteria, 14.8% (114/771) were ESBL-producing organisms. *Klebsiella* species were the most commonly isolated ESBL producers, 38% of which produced ESBLs.

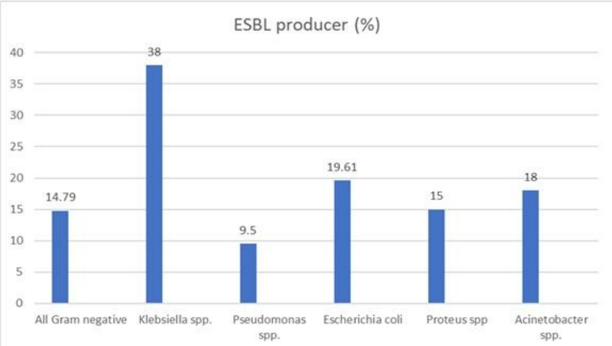

Figure II: Distribution of ESBL-producing Gram-negative organisms from wound swabs and pus

Among *Pseudomonas* species, 9.5% isolates were ESBL producers; 19.6% of *Escherichia coli*, 15.0% isolates of *Proteus* spp., and 18.0% of isolated *Acinetobacter* species were ESBL-producing organisms (Figure II).

About 100.0% of *Staphylococcus aureus* and CoNS were sensitive to vancomycin and linezolid (Table 4).

Table 4: Antibiotic sensitivity pattern of gram-positive bacteria isolated from wound swab and pus

Antibiotic Name	*Staphylococcus aureus*	CoNS
Amikacin	41.2%	62.5%
Amoxicillin	19.1%	21.2%
Ceftriaxone	34.4%	62.5%
Cephradine	32.6%	38.3%
Cloxacillin	59.7%	62.7%
Gentamicin	29.5%	38.4%
Cotrimoxazole	39.9%	69.9%
Cefoxitin	64.8%	62.7%
Vancomycin	100.0%	100.0%
Linezolid	100.0%	100.0%

CoNS- Coagulase-negative *Staphylococcus aureus*

Carbapenems were effective antibiotics against *E. coli*, *Klebsiella* spp., and *Proteus*; colistin and tigecycline were the most effective antibiotics in vitro against all gram-negative bacteria except *Proteus* spp. and *Pseudomonas* spp. respectively. Piperacillin/tazobactam was also an effective antibiotic in vitro against gram-negative bacteria (Table 5).

Table 5: Antibiotic Sensitivity Pattern of Gram-Negative Bacteria from Wound Swabs and Pus

Antibiotic name	*Pseudo* spp.	*E. coli*	*Klebsiella* spp.	*Proteus* spp.	*Acineto* spp.	*Citro* spp.
Amoxicillin	0.0%	5%	6.7%	40.0%	0.0%	7.2%
Amoxyclav	14.0%	36.8%	8.8%	5.3%	0.0%	14.3%
Amikacin	27.2%	43.3%	48.2%	33.7%	15%	48.8%
Aztreonam	14.9%	48.7%	41.3%	17.1%	14.4%	52.4%
Cefixime	7.7%	23.1%	11.5%	23.1%	7.9%	33.5%
Cefipime	37.2%	27.5%	13.4%	28.2%	19.3%	37.2%
Cefotaxime	51.0%	50.0%	0.0%	0.0%	0.0%	0.0%
Cefuroxime	11.3%	13.2%	7.5%	30.2%	0.0%	17.7%
Ceftriaxone	10.2%	21.5%	6.7%	24.6%	3.1%	9.3%
Ciprofloxacin	15.3%	12.2%	6.8%	20.3%	1.7%	16.3%
Cotrimoxazole	13.8%	1.1%	14.0%	13.8%	8.0%	29.1%
Ceftazidime	9.8%	37.3%	3.9%	9.8%	3.9%	6.9%
Gentamicin	11.8%	21.5%	4.3%	28.1%	0.0%	34.2%
Imipenem	60.7%	77.7%	69.3%	67.1%	21.2%	77.2%
Meropenem	60.4%	78.7%	67.5%	69.7%	24.6%	79.4%

Antibiotic name	*Pseudo* spp.	*E. coli*	*Klebsiella* spp.	*Proteus* spp.	*Acineto* spp.	*Citro* spp.
Colistin	93.2%	98.2%	94.3%	0.0%	98.4%	100%
Tazobactam/piperacillin	67.5%	79.4%	87.4%	87.3%	79.2%	98.3%
Tigecycline	0.0%	100%	100%	100%	100%	100%

Pseudo spp.-*Pseudomonas* spp., *E. coli-Escherichia coli, Acineto* spp.-*Acinetobacter* spp., *Citro* spp.-*Citrobacter* spp.

Discussion

Even with the implementation of fundamental wound care concepts, many patients experience infections that call for accurate organism identification to provide the right course of treatment. A common characteristic is a shifting pattern of isolated organisms and their variability in susceptibility to antibiotics across different hospitals. Adequate use of antibiotics, stringent infection control measures, and ongoing surveillance can all help prevent the emergence and spread of antibiotic resistance[18].

Approximately 75.0% of the samples in this investigation had bacterial growth on culture, which was comparable to the growth rate seen in a number of prior studies[19,20]. In line with previous research, gram-negative bacilli accounted for the majority of the organisms recovered from wound swabs (90.65%) and pus (77.55%)[21,22]. Several investigations have found nearly comparable amounts of gram-positive and gram-negative bacteria from pus and wound swabs, which contradicts the current findings[22,23]. The type of hospital setting and surgical technique used will determine the pattern of bacteria that cause wound infection[24].

Males had a higher rate of wound infection than females, with males having a culture-positive rate of 60.58% and females having a culture-positive rate of 39.41%, respectively. Other investigations also revealed a similar male predominance[25,26]. One possible explanation for this could be that there are more men working in this nation. They worked in industries, farms, construction, and transportation, among other things, and they experienced trauma. The majority of wound infection cases (53.97%) in our study involved people in the age range of 21 to 40. This is consistent with the study, which found that individuals in their second to fourth decades of life have a higher risk of developing wound infections[27]. This age group is the most susceptible since more people in it are employed in a variety of jobs and are more likely to sustain various types of injuries.

Pseudomonas species were the most frequently isolated gram-negative bacteria in this investigation, followed by *E. coli*, *Klebsiella* species, and *Proteus* species. Accordingly, an Indian investigation indicated that *Pseudomonas* spp. were the most prevalent gram-negative bacteria[28]. In Bangladesh, *Pseudomonas* spp. was shown to be the main agent of wound infection in 28% of cases in 2011 and 26.5% of cases in 2016, which is lower than the findings of this study[29,30]. One of the most common causes of wound infection is *Pseudomonas* spp., a widespread and adaptable human opportunistic pathogen that can originate from the environment or the body's natural gut flora[31,32]. *Pseudomonas* is an effective agent for infection in wounds because it produces extracellular and cell-associated virulence factors that mediate a number of activities, including adhesion, leukocyte death, tissue degradation, immune system evasion, and bloodstream invasion[33,34]. *E. Coli* and *Klebsiella* spp. were identified as the most common gram-negative bacterial pathogens[21,22,35], which is in contradiction to the current findings. Because various hospitals treat different types of infections, there may be variations in the prevalence of infection-causing bacteria between hospitals, which could account for the variance in the isolation rate.

Similar to previous research conducted in Bangladesh and India[21,22,36], *Staphylococcus aureus* was the most commonly isolated gram-positive bacterium from both wound swabs and pus in our investigation. Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for about 36% of the identified strains of *Staphylococcus aureus*. According to several studies conducted in Bangladesh, the isolation rate in this study corresponds to the MRSA infection rate, which varies from 32.0% to 63.0%[37]. MRSA is a multidrug-resistant bacterium that is immune to most cephalosporins, carbapenems, β-lactam/β-lactamase inhibitor combination, and methicillin and other penicillin[17].

In the current investigation, 3.53% of the culture-positive samples had co-infections. According to data from earlier research, *Staphylococcus aureus* and *Pseudomonas* spp. were the most commonly associated bacteria with polymicrobial illnesses[11,12]. The freeing of organisms is more challenging when polymicrobial illnesses are present. Chronic wounds' polymicrobial makeup is probably going to create an environment that is favorable for bacteria to transfer genes horizontally[38].

This study's data on antibiotic susceptibility revealed that the effectiveness of some conventional antibiotics in treating wound infections is quite restricted. In our investigation, we found that gram-negative bacteria exhibited the greatest resistance to amoxicillin, with fluoroquinolones, co-trimoxazole, and third-generation cephalosporins following suit. Numerous investigations have demonstrated this resistance pattern[39,40]. Despite being a useful antibiotic, gram-negative bacteria have demonstrated significant resistance to ciprofloxacin. This result is in line with research showing gram-negative bacteria to have a high level of ciprofloxacin resistance[41,42,43]. The increased prevalence of ciprofloxacin resistance may be attributed to the drug's widespread use in Bangladesh for a variety of illnesses, including endemic enteric fever. Good sensitivity to amikacin was demonstrated by most of the gram-negative bacilli, and this trend is similar to previous studies conducted in Bangladesh[41,42,43]. The minimal usage of this antibiotic at this facility may be the cause of the poor resistance. The outcome suggests that in this situation, amikacin might be taken into consideration as a substitute medication for illnesses brought on by gram-negative bacilli. Carbapenems were effective antibiotics exhibiting 60-80% sensitivity against gram-negative bacilli except for *Acinetobacter* spp. in this investigation, which conforms with the findings of other studies[41,42]. Even though there were few instances of resistance, carbapenem resistance is a serious problem when treating infections. Due to its intrinsic resistance to colistin, *Proteus* spp. exhibited 100% resistance to the drug. This study demonstrated a higher percentage of sensitivity for colistin and piperacillin/tazobactam. These two injectable medications are used for patients who are resistant to the majority of conventional antibiotics and are often reserved for emergencies. Except for *Pseudomonas* species, all gram-negative isolates were 100% sensitive to tigecycline.

According to a 2017 study[44], 14.79% of the gram-negative bacteria in this investigation were found to produce ESBL. The current findings are not consistent with the 23% and 24% rates of ESBL-producing bacteria reported in Bangladesh in 2008 and 2012, respectively[42,45]. The decreased percentage of ESBL producers in the current study may be attributed in part to the decreased usage of cephalosporins and the relatively increased use of carbapenems and colistin in the treatment of gram-negative bacterial infections in this hospital context. The fact that all indoor and outdoor samples were included in this analysis, whereas prior research focused solely on infected surgical wounds or ICU patients, may also have contributed to the low occurrence of ESBL. In this investigation, the most frequently isolated ESBL-producing microbes were *Escherichia coli* and *Klebsiella* species. Due to the scarcity of available treatments, ESBL-mediated resistance detection is crucial[46].

Most gram-positive bacteria were susceptible to linezolid and vancomycin. Every *S. aureus* was shown to be susceptible to vancomycin and linezolid and most of the *S. aureus* showed reduced sensitivity to ciprofloxacin and co-trimoxazole. This pattern of sensitivity was found to be comparable to that of *S. aureus* in a 2019 study conducted in Bangladesh[47]. With very few exceptions, the study's overall data on antibiotic sensitivity reveals that bacteria were generally found to be more resistant to oral antibiotics. Antibiotics, particularly oral antibiotics, are frequently used indiscriminately in Bangladesh; in certain locations, they are available over the counter, and anyone can buy them even without a prescription. Moreover, drug marketers often offer other oral antibiotics instead of the prescribed ones. Additionally, some patients are unaware of the importance of continuing to take antibiotics for the recommended length of time and at the prescribed dose. In Bangladesh, antibiotic resistance is highly prevalent due to these factors.

Conclusion

One issue that is often overlooked but leads to chronic illness is wound infections. The information presented in this study may assist medical professionals in developing guidelines for selecting the best course of treatment to treat wound infections and prevent the emergence of multidrug-resistant strains. These guidelines can be based on microbiological analysis. The discovery of the antibiotics that work best against a particular species of microorganism may lead physicians to prescribe certain antimicrobials over others, which may reduce the amount of less effective medications used to treat wound infections. A public health aim that should be pursued and supported by the WHO is limiting the spread of antibiotic resistance, which calls for the management of multi-resistant bacteria and the provision of updated treatment plans.

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Conflict of Interest
We do not have any potential conflicts of interest.

Financial Disclosure
None

Authors' contributions
Conception and design: Shaila Akhtar, Rafia Afreen Jalil; Acquisition, analysis, and interpretation of data: Shaila Akhtar, Nooriya Haque; Manuscript drafting and revising it critically: Shaila Akhtar, Mahnaz Tabassum Raisa; Approval of the final version of the manuscript: Shaila Akhtar, Rafia Afreen Jalil; All authors revised the manuscript for important intellectual content, approved the final version, and agreed to be accountable for all aspects of the work.

Data Availability
Data are available from the corresponding author upon reasonable request and subject to approval by the relevant institutional authorities.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a retrospective study, written informed consent was not obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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