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Prevalence and Antibiotic Susceptibility of MDR Bacteria Isolated from Post-Surgical Wounds

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ABSTRACT

Background: Surgical site infections (SSIs) remain one of the most common healthcare-associated infections worldwide and are increasingly complicated by multidrug-resistant (MDR) bacteria. **Objective:** To determine the prevalence and antibiotic susceptibility patterns of MDR bacteria isolated from post-surgical wound infections in tertiary care hospitals of Peshawar. **Methodology:** An analytical cross-sectional study was conducted from October to December 2022. A total of 174 samples were collected from patients suspected of post-surgical wound infection. Standard microbiological procedures including culture on Blood and MacConkey agar, Gram staining, biochemical tests, API 10E confirmation, and antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method (CLSI 2020 guidelines) were performed. Data were analyzed using MS Excel 2016 and SPSS version 26. **Results:** Out of 174 samples, 139 (79.8%) were culture positive. The mean age of patients was 41.67 ± 12.573 years. Gram-negative bacteria predominated. The most frequent isolates were *Escherichia coli* (32%), *Staphylococcus* species (30%), *Pseudomonas* species (10%), and *Klebsiella pneumoniae* (7%). A high proportion of isolates demonstrated multidrug resistance, including resistance to carbapenems and linezolid in selected strains. The high prevalence of MDR pathogens in post-surgical wound infections highlights the urgent need for routine culture and sensitivity testing, rational antibiotic use, and strict infection control practices.

INTRODUCTION

Surgical site infections (SSIs) are defined as infections that occur in the tissues, organs, or anatomical spaces exposed during a surgical procedure, usually within 30 days after surgery or within one year if an implant is placed [1]. SSIs remain one of the most common healthcare-associated infections worldwide and continue to pose a serious challenge to patient safety and hospital management[2]. Despite improvements in surgical techniques, sterilization procedures, and antibiotic prophylaxis, the burden of post-surgical wound infections remains substantial, particularly in developing countries[3, 4]. SSIs contribute significantly to prolonged hospital stays, increased healthcare costs, delayed wound healing, and higher rates of morbidity and mortality [4, 5]. Globally, millions of surgical procedures are performed each year, and a considerable proportion of patients develop wound infections following surgery. The prevalence of SSIs varies widely, ranging from as low as 2% in developed countries to as high as 41.9% in low- and middle-income regions [6]. Factors such as poor infection control practices, limited resources, overcrowded hospitals, and irrational use of antibiotics contribute to higher infection rates in these settings [7]. In addition to patient-related risk factors such as age, diabetes, obesity, smoking, and immunosuppression, procedural factors including prolonged surgery duration, emergency operations, and contaminated wounds further increase the risk of infection[8]. The microbiological profile of surgical site infections commonly includes both Gram-positive and Gram-negative organisms. Among Gram-positive bacteria, *Staphylococcus aureus* is frequently reported as a leading cause of SSIs. Gram-negative pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter* species are also commonly isolated from infected surgical wounds [9, 10]. The presence of these organisms reflects both endogenous sources, such as the patient's own flora, and exogenous contamination from the hospital environment. A major concern in recent years is the rapid emergence and spread of multidrug-resistant (MDR) bacteria [10]. MDR organisms are defined as bacteria resistant to three or more classes of antimicrobial agents. The overuse and misuse of antibiotics, lack of antimicrobial stewardship programs, and inadequate infection prevention measures have accelerated the development of resistance [11]. Resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, carbapenem-resistant *E. coli* and *Klebsiella pneumoniae*, and multidrug-resistant *Pseudomonas* and *Acinetobacter* species, significantly limit treatment options[12]. In some cases, even last-resort antibiotics such as carbapenems, colistin, and linezolid are becoming less effective [12, 13]. The presence of MDR bacteria in post-surgical wounds complicates management by requiring prolonged hospitalization, repeated surgical interventions, and the use of expensive and potentially toxic antibiotics. This not only increases the economic burden on healthcare systems but also negatively affects patients' quality of life. Therefore, identifying the prevalence of MDR pathogens and understanding their antibiotic susceptibility patterns is crucial for guiding appropriate empirical therapy, improving antimicrobial stewardship, and implementing effective infection control strategies. In this context, the present study was conducted to determine the prevalence and antibiotic susceptibility patterns of multidrug-resistant bacteria isolated from post-surgical wounds in tertiary care hospitals of Peshawar. The findings aim to provide valuable local data that can support evidence-based treatment decisions and contribute to controlling the growing threat of antimicrobial resistance.

MATERIAL AND METHOD

Study Design and Setting

This analytical cross-sectional study was conducted to determine the prevalence and antibiotic susceptibility patterns of multidrug-resistant (MDR) bacteria isolated from post-surgical wound infections. The research was carried out in tertiary care hospitals of Peshawar. Laboratory analysis of collected samples was performed in the microbiology section of Rehman Pathology Laboratory. All procedures were conducted following standard microbiological protocols and CLSI (2020) guidelines. The study focused on identifying common pathogens and evaluating their resistance profiles.

Study Duration and Sample Size

The study was conducted over a period of three months, from October 2022 to December 2022. The sample size was calculated using the formula $n = Z^2p(1-p)/e^2$ at a 95% confidence interval. Based on the calculation, 174 samples were included in the study. Out of these, 139 samples showed positive bacterial growth and were included in the final analysis. The calculated sample size ensured reliability and statistical validity of the results.

Sampling Technique and Eligibility Criteria

A non-probability convenient sampling technique was used to select patients. Individuals of any age and gender who developed signs of surgical site infection after surgery were included in the study. Only admitted patients with clinically suspected post-surgical wound infections were enrolled. Patients without evidence of SSI or those who did not consent were excluded. This ensured that only confirmed cases of post-operative wound infection were analyzed.

Sample Collection and Culture Procedures

Pus samples were collected aseptically using sterile cotton swabs after obtaining informed consent. The samples were transported immediately to the laboratory in sterile tubes. Each specimen was cultured on Blood agar and MacConkey agar plates. The plates were incubated aerobically at 37°C for 24 hours. Colony morphology, hemolysis patterns, pigmentation, and lactose fermentation were observed, and mixed growth samples were sub-cultured to obtain pure isolates.

Identification of Bacterial Isolates

Preliminary identification was performed using Gram staining to differentiate Gram-positive and Gram-negative bacteria. Standard biochemical tests including catalase, coagulase, and oxidase tests were carried out. Gram-negative isolates were further confirmed using the API 10E biochemical identification system. The strips were incubated at 37°C for 24 hours and interpreted according to the API identification chart. Organisms were identified based on biochemical reaction patterns.

Antibiotic Susceptibility Testing and Data Analysis

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar according to CLSI (2020) guidelines. A 0.5 McFarland standard bacterial suspension was prepared, and lawn culture was made on agar plates. Antibiotic discs were placed at appropriate distances and incubated at 37°C for 24 hours. Zones of inhibition were measured and interpreted as sensitive, intermediate, or resistant. Multidrug resistance was defined as resistance to three or more classes of antibiotics.

Data Analysis

Data were entered into Microsoft Excel 2016 and analyzed using SPSS version 26. Descriptive statistics such as frequency, percentage, mean, and standard deviation were calculated. Results were presented in tables and graphs. A 95% confidence interval was applied. Statistical significance was considered at $p\text{-value} \leq 0.05$.

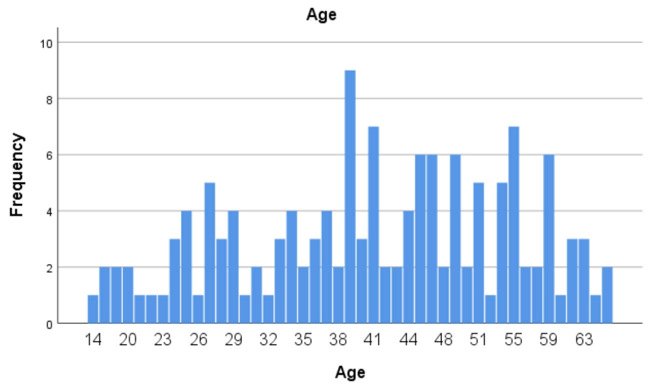
RESULTS

Culture Positivity and Demographic Characteristics

A total of 174 post-surgical wound samples were collected from tertiary healthcare centers in Peshawar during the study period. Out of these, 139 samples (79.8%) showed positive bacterial growth, whereas 35 samples (20.2%) showed no growth on culture. Only the 139 culture-positive cases were included in the final analysis. The mean age of the patients was 41.67 ± 12.573 years. The minimum age recorded was 14 years and the maximum age was 65 years. Most of the patients belonged to the middle-aged group. Statistical analysis showed that the demographic findings were not significant ($p > 0.05$) at a 95% confidence interval. The age distribution indicates that post-surgical wound infections affected a wide age range. The relatively high culture positivity rate reflects a substantial burden of infection in the study population Figure 1. These findings confirm that post-operative wound infections remain a common complication in tertiary healthcare settings.

Figure 1

Age-Wise Distribution of Patients with Culture-Positive Post-Surgical Wound Infections in Tertiary Healthcare Centers of Peshawar.

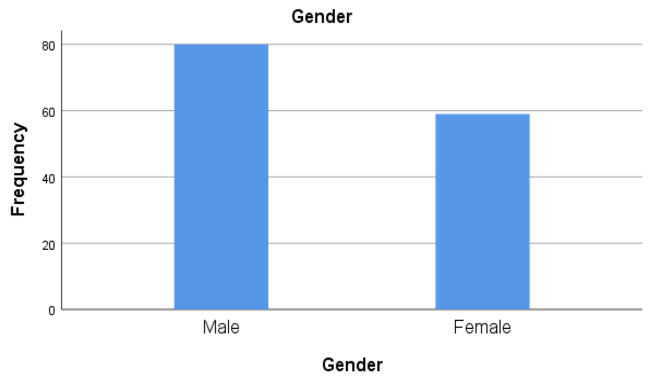


Gender Distribution

Among the 139 culture-positive patients, 80 (57.6%) were males and 59 (42.4%) were females. This demonstrates a slightly higher prevalence of post-surgical wound infections among male patients. The total cumulative percentage reached 100%, confirming complete data recording. Although males were more frequently affected, the difference between genders was not statistically significant ($p > 0.05$). The higher proportion of males may be related to greater exposure to surgical procedures or hospital admissions. Both genders, however, showed considerable susceptibility to infection. The results suggest that post-surgical wound infections are not limited to one gender. The distribution indicates a moderate male predominance in this study Figure 2. These findings are consistent with several regional studies reporting similar trends.

Figure 2

Gender-Wise Distribution of Patients with Post-Surgical Wound Infections, Illustrating a Higher Prevalence Among Male Patients.

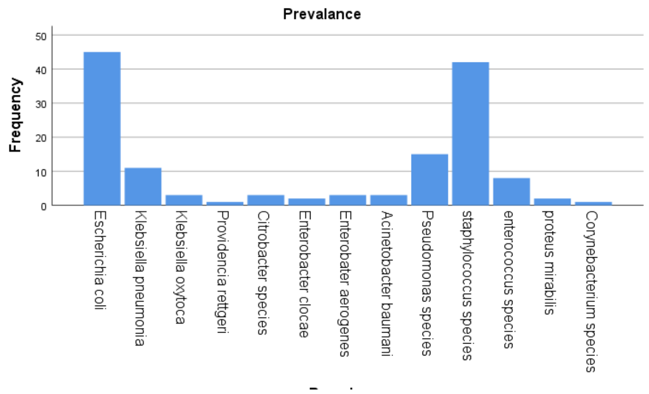


Frequency Distribution of Bacterial Isolates

A total of 139 bacterial isolates were identified from infected wound samples. Gram-negative bacteria were more predominant than Gram-positive organisms. *Escherichia coli* was the most frequently isolated pathogen with 45 isolates (32%). *Staphylococcus* species were the second most common, accounting for 42 isolates (30%). *Pseudomonas* species were identified in 15 cases (10%), while *Klebsiella pneumoniae* was isolated in 11 cases (7%). *Enterococcus* species accounted for 8 isolates (8%). Additionally, *Klebsiella oxytoca*, *Citrobacter* species, *Acinetobacter baumannii*, *Enterobacter aerogenes*, and *Proteus mirabilis* were each isolated in 3 cases (2%). *Enterobacter cloacae* was isolated in 2 cases (1%), while *Providencia rettgeri* and *Corynebacterium* species were each isolated in 1 case (1%) Figure 3. These findings demonstrate a predominance of Enterobacteriaceae and non-fermenting Gram-negative bacilli in post-surgical wound infections.

Figure 3

Frequency-Wise Representation of Bacterial Isolates Identified from Infected Surgical Wounds, Showing Predominance of E. Coli and Staphylococcus Species.

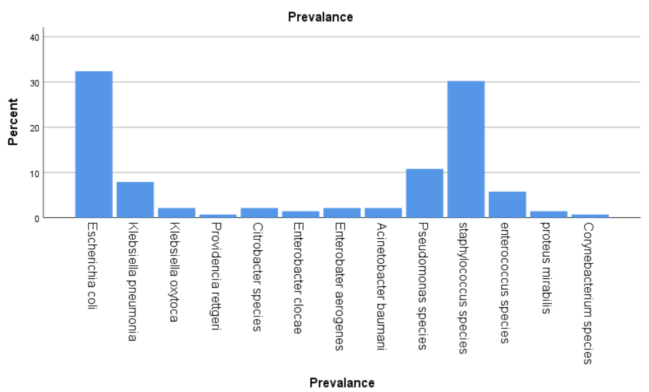


Percentage-Wise Prevalence of Isolates

The percentage distribution revealed that *E. coli* constituted 32% of all isolates, making it the leading pathogen. *Staphylococcus* species followed closely at 30%, and together these two organisms accounted for 62% of total infections. *Pseudomonas* species contributed 10% of isolates. *Enterococcus* species represented 8%, while *Klebsiella pneumoniae* accounted for 7%. Each of the following organisms—*Klebsiella oxytoca*, *Citrobacter* species, *Acinetobacter baumannii*, *Enterobacter aerogenes*, and *Proteus mirabilis*—represented 2% of cases. *Enterobacter cloacae*, *Providencia rettgeri*, and *Corynebacterium* species each accounted for 1% of isolates. Gram-negative organisms collectively contributed approximately 60–65% of infections. Gram-positive organisms accounted for roughly 35–40% Figure 4. These results highlight the dominance of Gram-negative bacteria in the study population.

Figure 4

Percentage-wise Prevalence of Bacterial Isolates, Demonstrating that E. Coli (32%) and Staphylococcus Species (30%) were the Most Common Pathogens.



Microscopic and Biochemical

Gram staining showed that the majority of isolates were Gram-negative rods. Gram-positive cocci were mainly identified as 42 *Staphylococcus* species and 8 *Enterococcus* species. Catalase testing was positive in all 42 *Staphylococcus* isolates. Coagulase testing confirmed coagulase-positive *Staphylococcus aureus* among the staphylococcal isolates. Oxidase testing yielded positive results in all 15 *Pseudomonas* isolates. API 10E biochemical testing confirmed the identity of 45 *E. coli*, 11 *Klebsiella pneumoniae*, and other Enterobacteriaceae members. Colony morphology on Blood agar demonstrated hemolytic patterns in some isolates. MacConkey agar showed lactose fermentation in *E. coli* and *Klebsiella* species Table 1. These laboratory findings confirmed accurate identification of pathogens.

Table 1

Microscopic Characteristics, Biochemical Test Results, and Culture Findings of Bacterial Isolates, Including Gram Reaction, Catalase, Coagulase, Oxidase, and API 10E Confirmation.

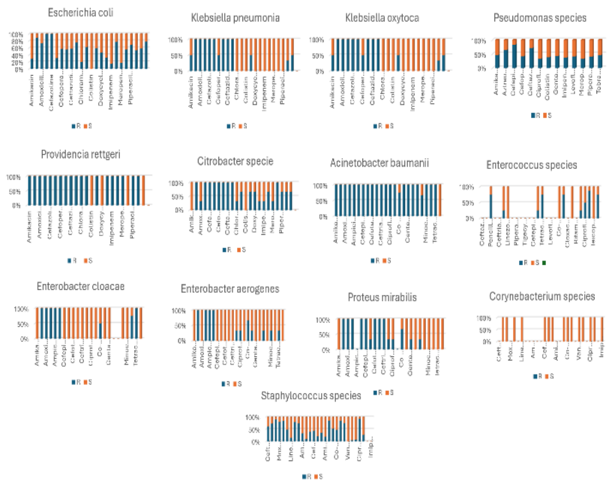
Identification Method	Organism Identified	Number of Isolates (n)	Test Result / Observation
Gram Staining	Gram-negative rods	Majority of isolates	Pink/red rods under microscope
Gram Staining	*Staphylococcus* species	42	Purple Gram-positive cocci in clusters
Gram Staining	*Enterococcus* species	8	Purple Gram-positive cocci in pairs/chains
Catalase Test	*Staphylococcus* species	42	Positive (bubble formation)
Coagulase Test	*Staphylococcus aureus*	Included among 42 Staphylococci	Positive (clumping within seconds)
Oxidase Test	*Pseudomonas* species	15	Positive (purple/blue color change)
API 10E Confirmation	*Escherichia coli*	45	Confirmed by biochemical reaction profile
API 10E Confirmation	*Klebsiella pneumoniae*	11	Confirmed by biochemical reaction profile
Culture on Blood Agar	Various isolates	139	Hemolytic patterns observed in some isolates
Culture on MacConkey Agar	*E. coli, Klebsiella* species	56 (45 + 11)	Lactose fermentation (pink colonies)

Antibiotic Susceptibility Patterns and MDR Prevalence

Antibiotic susceptibility testing revealed a high level of resistance among both Gram-negative and Gram-positive isolates. All *Klebsiella pneumoniae* (11 isolates) were 100% resistant to third-generation cephalosporins and beta-lactam antibiotics and showed 100% susceptibility towards fluoroquinolones, carbapenems, chloramphenicol, gentamicin and minocycline. All the three isolates of klebsiella oxytoca showed 100% resistant towards beta-lactams and cephalosporins while showed 100% towards linezolid and gentamicin. All the *E. coli* isolates showed 100% resistance towards ampicillin and cefazoline. Resistance to carbapenems (imipenem and meropenem) was observed as 75.6% and 86.7% for the E. coli isolates. All 3 isolates of *Acinetobacter baumannii* showed 100 % resistance to almost all antibiotics, including colistin. The 15 *Pseudomonas* isolates demonstrated multidrug resistance patterns with high resistance to cefepime and ceftazidime (83.3% and 66.7%) while meropenem showed the highest sensitivity rate as 66.7%. Among the 42 *Staphylococcus* isolates, high resistance was noted against ceftriaxone (90.2%) and penicillin (73.3%) while highest sensitivity was recorded for linezolid and teicoplanin (100%). The only Providencia rettgeri isolate showed 100% resistant towards all applied antibiotics except tigecylin and colistin. All the 3 isolates of Citrobacter species showed 100% resistance towards beta-lactam and cephalosporins while showing 100% susceptibility towards carbapenems and polymyxins. Both Enterobacter cloacae (2 isolates) and Enterobacter aerogenes (3 isolates) exhibited high-level resistance to a broad spectrum of Beta-lactams, including penicillins and cephalosporins. However, Amikacin, Colistin, and Meropenem remained consistently effective against both species, showing 100% susceptibility. Notably, E. cloacae was more susceptible to fluoroquinolones (Ciprofloxacin) compared to E. aerogenes, which showed emerging resistance in that class. Enterococcus spp. Showed highest resistance (85%) towards gentamycin while showed 100% susceptibility towards vancomycin, teicoplanin and linezolid. The only Corynebacterium specie showed 100% susceptibility to all tested antibiotics. Overall, the majority of the 139 isolates were classified as multidrug-resistant (MDR), being resistant to three or more antibiotic classes Figure 5. Several isolates exhibited resistance to 6–7 different antibiotics, indicating extensive resistance patterns and limited therapeutic options.

Figure 5

Graphical Representation of Antibiotic Susceptibility Patterns of Major Bacterial Isolates, Highlighting High Resistance Rates and MDR Prevalence among Pathogens.



DISCUSSION

The present study demonstrated a high culture positivity rate of 79.8% (139/174) among post-surgical wound samples collected from tertiary healthcare centers in Peshawar. This high prevalence indicates a significant burden of surgical site infections (SSIs) in the study setting. Globally, SSI rates vary widely, with higher rates reported in developing countries due to limited infection control practices, overcrowding, and irrational antibiotic use [14]. Our findings are consistent with previous regional studies reporting elevated SSI rates in tertiary care hospitals [15]. The mean age of affected patients was 41.67 ± 12.573 years, which suggests that middle-aged adults are more susceptible to post-operative infections, likely due to higher surgical exposure. Similar age distributions have been reported by [16], where SSIs were most common among adults between 30 and 50 years. The study showed a slight male predominance, with 57.6% males and 42.4% females affected. Although the difference was not statistically significant ($p > 0.05$), this trend aligns with findings from [16, 17], who also reported higher SSI rates among male patients. This may be associated with occupational risk factors, trauma-related surgeries, or higher hospital admission rates among males. However, SSIs remain a concern for both genders, emphasizing the need for universal infection prevention measures. In terms of microbiological profile, Gram-negative bacteria were predominant, accounting for approximately 60–65% of isolates. *Escherichia coli* was the most common pathogen (32%), followed by *Staphylococcus* species (30%). These findings are consistent with studies conducted by [18, 19], which also identified *E. coli* and *Staphylococcus aureus* as leading causes of SSIs. In contrast, some studies from developed countries report *Staphylococcus aureus* as the primary pathogen [20]. The predominance of *E. coli* in our study may reflect the high number of abdominal or gastrointestinal surgical procedures, where endogenous intestinal flora plays a significant role in wound contamination.

The isolation of *Pseudomonas* species (10%) and *Klebsiella pneumoniae* (7%) further highlights the importance of Gram-negative nosocomial pathogens in postoperative infections. Similar findings were reported by [21], who documented a high prevalence of *Pseudomonas aeruginosa* in SSIs. Additionally, the presence of *Acinetobacter baumannii* (2%) indicates the emergence of opportunistic and hospital-acquired pathogens, which are often associated with multidrug resistance. A reported comparable patterns, emphasizing the rising threat of multidrug-resistant Gram-negative organisms in surgical wards[22]. Antibiotic susceptibility testing in our study revealed alarming resistance patterns. A large proportion of *E. coli* and *Klebsiella pneumoniae* isolates were resistant to third-generation cephalosporins and beta-lactam antibiotics, consistent with the increasing prevalence of extended-spectrum beta-lactamase (ESBL) producers reported in other studies [23, 24]. Resistance to carbapenems observed in selected isolates raises serious concerns, as carbapenems are often considered last-line agents for severe Gram-negative infections. [25] highlighted the global spread of carbapenem-resistant Enterobacteriaceae as a major public health threat. Among Gram-positive isolates, *Staphylococcus* species demonstrated high resistance to penicillin and erythromycin, and some isolates showed resistance to linezolid. These findings suggest the possible presence of methicillin-resistant *Staphylococcus aureus* (MRSA), as reported by [26]. The overall high rate of multidrug resistance (MDR), with many isolates resistant to three or more antibiotic classes and some resistant to 6–7 drugs, is comparable to findings from tertiary care centers in developing countries [27, 28]. The predominance of Gram-negative pathogens and the high level of multidrug resistance observed in this study underscore the urgent need for strict antimicrobial stewardship, routine culture and sensitivity testing, and strengthened infection control measures. Without immediate intervention, treatment options for post-surgical wound infections may become increasingly limited, leading to higher morbidity, prolonged hospitalization, and increased healthcare costs.

CONCLUSION

This study demonstrated a high prevalence (79.8%) of post-surgical wound infections, with Gram-negative bacteria predominating over Gram-positive organisms. *Escherichia coli* (32%) and *Staphylococcus* species (30%) were the most commonly isolated pathogens. A significant proportion of isolates exhibited multidrug resistance, including resistance to third-generation cephalosporins and carbapenems. The presence of MDR organisms highlights limited therapeutic options and increased risk of treatment failure. These findings emphasize the urgent need for routine culture and sensitivity testing, strict infection control practices, and effective antimicrobial stewardship programs.

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