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Bacteriological Landscape and Antimicrobial Susceptibility Patterns of Pus isolates: A Comprehensive Analysis

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Abstract

Introduction: Pyogenic infections are caused by a variety of microorganisms. Routine surveillance is essential to monitor the distribution and evolving susceptibility patterns of clinically significant pathogens over time. This study aims to highlight the bacteriological profile of pus samples and analyse their antimicrobial susceptibility patterns at a tertiary-care hospital in Eastern India. **Methods:** This was a retrospective study covering an eighteen months period from May 2023 to October 2024. A total of 428 pus samples were included. The samples were inoculated onto Blood, Chocolate and MacConkey agar plates and incubated. Bacteria were identified and tested for antimicrobial susceptibility using an automated system. **Results:** Culture-positivity was 88%, in which Gram-negative (GN) bacteria (58%) outnumbered Gram-positives (GP) (42%). The highest number of samples was obtained from patients with skin and soft tissue infections (40%). Overall, *Staphylococcus aureus* was found to be the most prevalent pathogen (29%). Among GN, *Pseudomonas aeruginosa* was predominant (16.4%). GP isolates were most often susceptible to vancomycin (94%) and linezolid (88.3%). Methicillin-resistant Staphylococcus aureus (MRSA) was 44.4% and vancomycin-resistant Enterococci (VRE) was 20%. Enterobacterales demonstrated a high susceptibility to colistin (80%) and tigecycline (78.5%). ESBL-producing Enterobacterales (ESBL-E) positivity was 48.2% and Carbapenem resistance Enterobacterales (CRE) was 44%. All isolates of *Acinetobacter calcoaceticus-baumannii* complex and 33.3% of *Pseudomonas aeruginosa* isolates were multi-drug resistant (MDR), demonstrating maximum susceptibility to colistin. **Conclusion:** Emergence of resistant strains are the significant concerns of this study. Effective infection control practices and proper antimicrobial stewardship are the utmost needs of the hour.

Keywords: Pus, Bacteriological profile, Antimicrobial susceptibility, Multidrug resistance, MRSA, ESBL

1 Introduction

Pyogenic infections constitute a notable subgroup of infections observed globally. They are primarily caused by the invasion and multiplication of pathogenic microorganisms, which release specific cellular or toxic metabolites and leucocidins that destroy neutrophils, leading to the formation of abscesses and pus[1]. It is a common clinical specimen collected from different types of infections, including skin, soft tissue, surgical site, diabetic wound, and abscesses. Some risk factors for recurrent infections or delayed wound healing include advanced age, malnutrition, obesity, steroid use, poorly controlled diabetes, immunocompromised states, smoking, trauma, procedure sites (intra-abdominal, pelvic, or

extremity), extended preoperative hospitalization, inadequate preoperative skin hygiene, and existing infections at distant sites[2].

The infections can be either monomicrobial or polymicrobial, leading to significant morbidity, disability, extended hospital stays and mortality, with an overall increase in economic burden. The crude mortality rate from infectious diseases in India is approximately 417 per one lakh individuals, with pyogenic infections being one of the major contributors[3].

India confronts one of the world's most significant challenges with drug-resistant pathogens. It is estimated that antimicrobial resistance (AMR) kills at least 1.27 million people every year and it could increase up-to 10 million people per year by 2050[4]. Over the years, poor antimicrobial stewardship and insufficient infection control have led to an upsurge of multidrug-resistant (MDR) strains in both community and hospital settings[5]. Most notably, methicillin-resistant *Staphylococcus aureus* (MRSA) among Gram-positive (GP) and MDR Gram-negative (GN) isolates have increasingly been linked to pyogenic infections in recent years[6]. Elevated rates of extended Spectrum beta-lactamase (ESBL) have been reported in *Escherichia coli* (*E. coli*), and *Klebsiella pneumoniae* (*K. pneumoniae*), along with increased resistance to colistin and carbapenems in *K. pneumoniae*. Additionally, *Acinetobacter calcoaceticus-baumannii* complex (*A. baumannii*) exhibits higher rates of carbapenem resistance compared to *Pseudomonas aeruginosa* (*P. aeruginosa)*[7].

The emergence of high AMR among bacterial pathogens has complicated management and treatment. The prevalence of microorganisms and the pattern of antimicrobial susceptibility vary by geographical region and within hospitals over time. It is essential to have appropriate knowledge of the pathogens and continuously monitor their susceptibility patterns to combat drug resistance[8].

Therefore, this study aimed to (i) highlight the distribution of bacterial etiology from pus samples and (ii) analyse their antimicrobial susceptibility patterns, at a tertiary-care hospital. This could provide the clinicians a valuable insight into appropriate antimicrobial selection and formulate effective treatment strategies.

2 Methodology

Study design: This was a retrospective, cross-sectional study.

Duration of Study: The study was covered for eighteen months from May 2023 to October 2024.

Study Setting: The study was conducted in the Department of Microbiology at a tertiary-care hospital in Eastern India.

Study Samples: All pus samples received in the microbiology laboratory during the study period from various outpatient departments (OPD), inpatient departments (IPD) and intensive care units (ICU) were included. Duplicate samples were excluded from the study.

Study Procedure: The specimens were inoculated onto Blood, Chocolate and MacConkey agar plates and then incubated at 37°C for a period of 18 to 24 hours. They were declared sterile if no growth was observed after 48 hours of incubation. The colony characteristics on culture media were observed, and isolated colonies were subjected to Gram stain and relevant biochemical tests. Bacteria were identified and tested for antimicrobial susceptibility using the MicroScan WalkAway® plus system (Beckman Coulter, California, USA). Antimicrobial susceptibility results were interpreted as per the Clinical and Laboratory Standards Institute (CLSI) 2024, M100 guidelines. MDR for GN infections were interpreted based on Infectious Diseases Society of America (IDSA) 2024 Guidelines.[9] Socio-demographic data, ward of admission and other relevant information were also collected from laboratory register and hospital information system.

Statistical Analysis: Data obtained were entered in Microsoft Excel spreadsheet (Office 2021) and analyzed by SPSS (Statistical Package for Social Sciences) software (version16). Categorical variables were expressed in frequency and percentage and extrapolated using graphs and tables.

Ethical Considerations: The study was conducted in conformity with all ethical guidelines. The protocol was reviewed and approved by the Institutional Ethics Committee. Patient confidentiality was maintained throughout the study by de-identifying all collected data.

3 Result

Demographic characteristics of the study population:

Samples were received from patients of all ages, with the majority in the 40 to 60-year range (32.50%), followed by those over 60 years (28%), ages 20 to 39 years (25.50%), and under 20 years (14%). Male patients (n=249, 66.2%) predominated over female patients (n=127, 33.8%).

Department-wise distribution of the study samples:

About 84.2% of samples were received from the in-patient department (IPD), while 15.8% came from the out-patient department (OPD). The distribution by department indicated that the surgery department (35%) was the primary contributor of pus samples, followed by orthopaedics (26.4%) (Fig. 1).

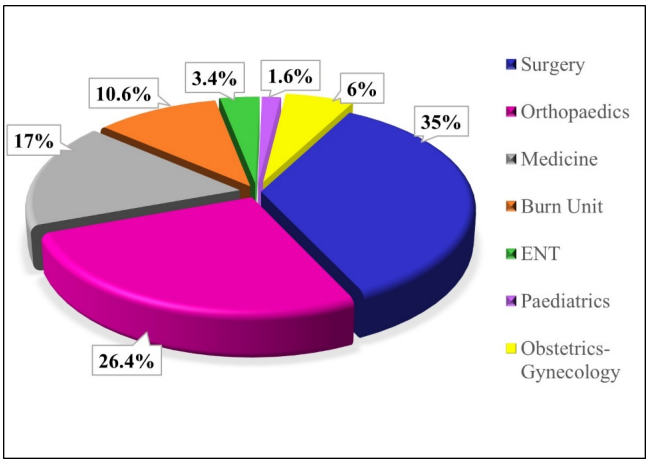


Fig. 1: Department wise distribution of samples

Source of the study samples:

The highest number of samples was obtained from patients with skin and soft tissue infections (SSTI) (40%), followed by tissue or intra-abdominal or internal organ infections (27.4%), surgical site infections (SSI) (16%), and post-burn patients (4.6%), while data was unavailable for 12%.

Prevalence and Bacteriological Profile of Culture Isolates:

Of 428 pus samples collected, 376 (88%) yielded positive cultures, which included 157 (42%) GP and 219 (58%) GN bacteria. Mixed growth was observed in 3.2% and no growth in 9% of the samples. A total of seventeen bacterial species were isolated (Table. 1). Overall, *Staphylococcus aureus (S. aureus)* was the most prevalent pathogen (29%). Among GN, *Pseudomonas aeruginosa (P. aeruginosa)* was the predominant isolate (16.4%), followed by *Klebsiella pneumoniae (K. pneumoniae)* (15%). The occurrence of other isolates in descending sequence are *Escherichia coli (E. coli)* (10.6%), *Acinetobacter calcoaceticus-baumannii* complex (*A. baumannii*) (7.4%), *Proteus spp.* (2.6%), *Enterobacter cloacae* (1.6%), and others (4.3%).

Distribution of isolates from various sources of infections:

The distribution of isolates from different infection sources is compiled (Fig. 2). Among the total *S. aureus* isolates, the highest number was recovered from patients with SSTI (33%). In cases of intra-abdominal or internal organ infections, *E. coli* represented the majority (26.40%). *K. pneumoniae* was the leading isolate among patients with SSI (30%) and *P. aeruginosa* was the most frequently identified organism (38%) in burn unit patients.

Antimicrobial Susceptibility Pattern of Culture Isolates:

GP isolates: They were most often susceptible to vancomycin (94%) and linezolid (88.3%). *Enterococcus spp.* also showed 100% susceptibility to daptomycin (Fig. 3). Methicillin-resistance was detected in 44.4% of *S. aureus* (MRSA) isolates. Vancomycin-resistant Enterococci (VRE) was 20% in our study.

Table 1: Species isolated from pus samples

Microorganism	Species isolated	No. of cases (%)
Gram-positive	*Staphylococcus aureus*	108 (29%)
	Enterococcus spp.	26 (7%)
	Streptococcus pyogenes	5 (1.3%)
	Coagulase-negative staphylococci	18 (4.7%)
Gram-negative	*Pseudomonas aeruginosa*	62 (16.4%)
	Klebsiella pneumoniae	56 (15%)
	Escherichia coli	40 (10.6%)
	Acinetobacter baumannii complex	28 (7.4%)
	Proteus spp.	10 (2.6%)
	Enterobacter cloacae	6 (1.6%)
	Morganella morganii	5 (1.3%)
	Serratia marcescens	4 (1%)
	Citrobacter freundii	2 (0.5%)
	Providencia spp.	2 (0.5%)
	Burkholderia pseudomallei	1 (0.5%)
	Burkholderia cepacia	1 (0.5%)
	Stenotrophomonas maltophilia	2 (0.5%)

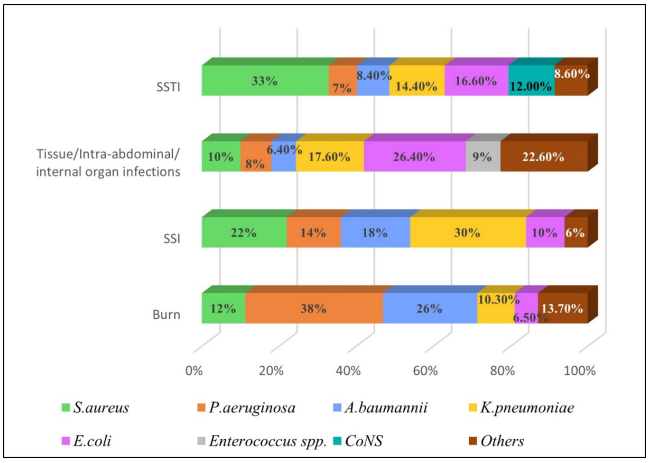
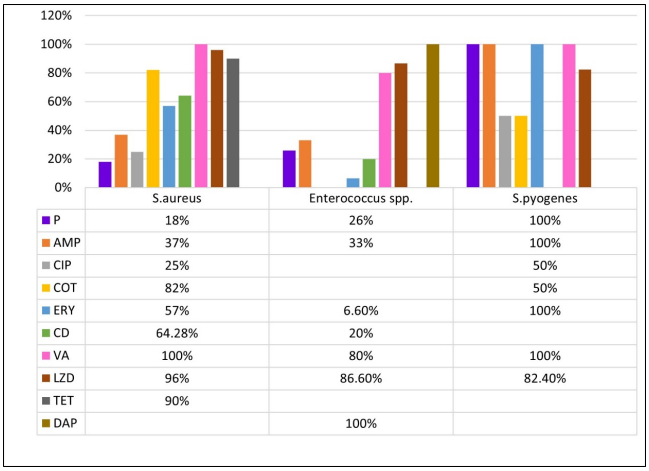


Fig. 2: Distribution of isolates from various sources of infections

*SSTI: Skin and soft tissue infections, SSI: surgical site infections

GN isolates: Members of Enterobacterales demonstrated a high susceptibility to colistin (80%) and tigecycline (78.5%), followed by beta-lactam/beta-lactamase inhibitor (BL-BLI) combination drugs (54.5%) (Fig. 4). ESBL - producing Enterobacterales (ESBL-E) positivity was observed in 48.2% of the isolates. Carbapenem resistance in Enterobacterales (CRE) was 44%, with *K. pneumoniae* exhibiting a higher resistance compared to other isolates. Among non-fermenters (Fig. 5), all isolates of *A. baumannii*

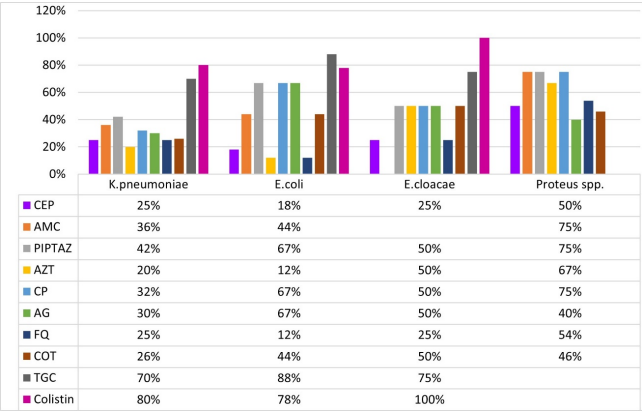
were MDR, showing maximum susceptibility to colistin (86.6%). Carbapenem resistance was detected in 72% of *A. baumannii* isolates (CRAB). *P. aeruginosa* showed highest susceptibility to colistin (94%). MDR *P. aeruginosa* was observed in 33.3% isolates.



	S.aureus	Enterococcus spp.	S.pyogenes
P	18%	26%	100%
AMP	37%	33%	100%
CIP	25%		50%
COT	82%		50%
ERY	57%	6.60%	100%
CD	64.28%	20%	
VA	100%	80%	100%
LZD	96%	86.60%	82.40%
TET	90%		
DAP		100%	

Fig. 3: Antimicrobial Susceptibility Pattern of Gram-positive isolates

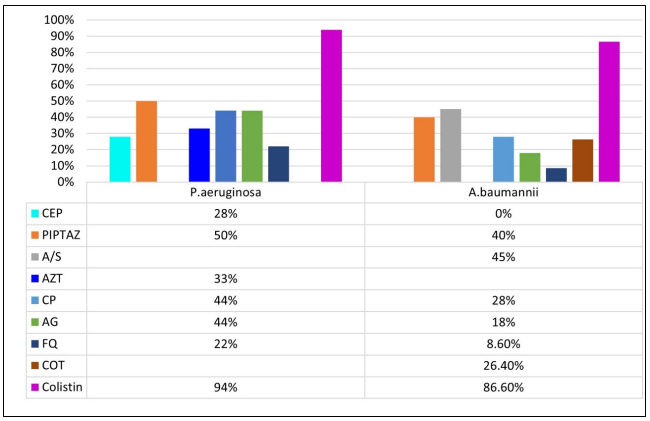
***** P: Penicillin; AMP: Ampicillin; CIP: Ciprofloxacin; COT: Trimethoprim-sulfamethoxazole; E: Erythromycin; CD: Clindamycin; VA: Vancomycin; LZ: Linezolid; TET: Tetracycline; DAP: Daptomycin



	K.pneumoniae	E.coli	E.cloacae	Proteus spp.
CEP	25%	18%	25%	50%
AMC	36%	44%		75%
PIPTAZ	42%	67%	50%	75%
AZT	20%	12%	50%	67%
CP	32%	67%	50%	75%
AG	30%	67%	50%	40%
FQ	25%	12%	25%	54%
COT	26%	44%	50%	46%
TGC	70%	88%	75%	
Colistin	80%	78%	100%	

Fig. 4: Antimicrobial Susceptibility Pattern among members of Enterobacterales

*CEP: 3rd generation (Ceftriaxone, Cefotaxime, Ceftazidime) and 4th generation (Cefepime) cephalosporins; AMC: Amoxicillin/Clavulanate; PIPTAZ: Piperacillin/Tazobactam; AZT: Aztreonam; CP: Carbapenems (Imipenem, Meropenem and Ertapenem); AG: Aminoglycosides (Gentamicin, Amikacin); FQ: Fluoroquinolones (Ciprofloxacin); COT: Trimethoprim/Sulfamethoxazole; TGC: Tigecycline



	P.aeruginosa	A.baumannii
CEP	28%	0%
PIPTAZ	50%	40%
A/S		45%
AZT	33%	
CP	44%	28%
AG	44%	18%
FQ	22%	8.60%
COT		26.40%
Colistin	94%	86.60%

Fig. 5: Antimicrobial Susceptibility Pattern among commonly isolated non-lactose fermenters

*CEP: Cephalosporins (Ceftazidime, Cefepime); PIPTAZ: Piperacillin/Tazobactam; AZT: Aztreonam; CP: Carbapenems (Imipenem, Meropenem); AG: Aminoglycosides (Gentamicin, Amikacin); FQ: Fluoroquinolones (Ciprofloxacin); COT: Trimethoprim/Sulfamethoxazole

4 Discussion

Pyogenic infections are characterized by local and systemic inflammation, usually accompanied by pus formation. Detecting and identifying pathogens is essential for clinicians to initiate appropriate targeted treatment.

In our study, the culture positivity rate was 88%. Several studies across India have been compiled, reporting variations in their culture positivity rates (Table. 2). The highest proportions of samples were obtained from the patients of age group of 40 to 60 years (32.50%), corresponding with studies conducted by Deboral A *et al.* (41.29%) and Kursheed F *et al.* (45%)[7, 10]. Male preponderance was observed in the present study, a finding that has also been reported in other studies[7, 11].

In our study, the majority of the study samples were obtained from the surgery department (35%), followed by orthopaedics (26.6%), which aligns with a study by Biradar A *et al.*[12] Pus from patients with SSTI constituted the largest portion of the samples (40%). A study by Kalita JM *et al.* reported a higher percentage (48.51%)[13].

In this study, both GP (42%) and GN (58%) pathogens were isolated from the study samples, with the latter exhibiting a slight predominance. Similar findings were observed in many other studies[11-14]. In contrast, percentage of GP isolates (61%) outnumbered GN (39%) in a study by Rai S *et al.*[15]. *S. aureus* was the most prevalent isolate in our study (29%), corresponding with findings from several other studies (Table. 2)[11-14]. As a normal component of skin flora, it is often linked to pyogenic infections.

Table 2: Comparison of culture positivity rates among various studies across India

Author	Place of study	Year of publication	Positivity rate	% GP	% GN	Most common isolate
Trojan R *et al.*[6]	Punjab	2016	60.10%	33%	77%	*E. coli* (51.2%)
Biradar A *et al.*[12]	Madhya Pradesh	2016	66%	41.98%	58%	*S. aureus* (41.98%)
Murugesan K *et al.*[1]	Kerela	2017	87.30%	81%	18.90%	*S. aureus* (66.41%)
Subha M *et al.*[14]	Tamil Nadu	2018	59.92%	31.90%	67.12%	S. aureus (26.32%
Sudhaharan S et al. [21]	Hyderabad	2018	93.20%	31.60%	68.30%	S. aureus (29%)
Gill MK *et al.*[22]	North India	2019	66.45%	29.23%	70.76%	*E. coli* (29.23%)
Deboral *et al.*[7]	Puducherry	2020	72.81%	23.50%	76.50%	*Pseudomonas spp.* (24.88%)
Wadekar MD *et al.*[11]	Karnataka	2020	85.50%	33.10%	52.50%	*S. aureus* (22.9%)
Kalita JM *et al.*[13]	Rajasthan	2021	61.54%	45.48%	70.59%	*S. aureus* (30.9%)
Present Study	West Bengal	-	88%	42%	58%	*S. aureus* (29%)

Furthermore, the sources of *S. aureus* in hospitals may arise from its high carriage rates among patients and healthcare workers, as well as from their hands or inanimate objects. It was also the predominant isolate responsible for SSTI in our study population (33%). A report by the Indian Council of Medical Research Antimicrobial Resistance Surveillance Network (ICMR-AMRSN) indicated a higher percentage of *S. aureus* associated with SSTI (73.7%)[16]. The prevalence of CoNS is rising, representing 12% of the SSTI cases in the current study. It was found to be more frequently associated with implant-related infections and diabetic foot ulcers. In our study, *P. aeruginosa* was the most frequently isolated pathogen from post-burn patients (38%), showing similarity with a study by Honnegowda TM *et al.* (35.3%)[17]. Burn injuries compromise the epidermal barrier, leading to the down-regulation of both local and systemic immune responses, which creates a favourable niche for the proliferation of pathogens. The high prevalence of these pathogens in hospital-settings is due to their ability to thrive in moist environments[18]. *S. aureus* and GN bacterial pathogens produce highly potent virulence factors that sustain infections and impede the wound healing process. They are also commonly associated with various nosocomial infections, including SSI[19]. Regular laboratory surveillance and routine cultures are therefore essential for appropriate infection control and antimicrobial therapy.

This study also demonstrated maximum susceptibility to vancomycin and linezolid for GP isolates, while demonstrating the least susceptibility to penicillin, like many other studies[7, 11, 13]. MRSA isolates were detected at 44.4%, comparable to studies by Wadekar MD *et al.* (48.1%) and Kalita JM *et al.* (40.89%)[11, 13]. This data is higher than that reported by Deboral A *et al.* (18.40%) and Rai S *et al.* (19%)[7, 15].

Vancomycin resistance was detected in 20% of the *Enterococcus spp.*, slightly higher than a study by Kalita JM *et al* (16%). An effective infection control program could reduce the rate of MRSA infections[13].

Among the GN isolates, *P.* aeruginosa represented the majority (16.4%), showing consistency with a few studies[7, 11, 20]. However, Kursheed F *et al.* and Sudharna S *et al.* reported *K. pneumoniae* and *E. coli* as the most frequent isolate among GN[10, 21].

Most of the GN pathogens in the current study were MDR, particularly in superbugs like *A. baumannii.* (100%) and *K. pneumoniae.* (70%), which aligns with a study by Kalita JM *et al.* (*Klebsiella spp.*: 74.79% and *Acinetobacter spp.*: 74.32%)[13]. In the current study, Enterobacterales demonstrated a high susceptibility to colistin (80%) and tigecycline (78.5%) and low susceptibility to cephalosporins (22.6%), and fluoroquinolones (31%). This finding is parallel with few other studies[22, 23]. The extensive use of these antimicrobials as the primary line of therapy probably have predisposed for such susceptibility pattern. ESBL-E positivity in our study was observed in 48.2% of the isolates, which is higher as compared to a study with a study by Soniya *et al.* (32.53%)[24]. In this study, MDR *P. aeruginosa* was observed in 33.3% isolates and they were most often susceptible to colistin (94%). This is similar to a study by Farooq L *et al.*, with a similar MDR percentage and 100% of them were sensitive to colistin[25].

As this is a tertiary care hospital, patients might be exposed to several antimicrobials before admission, which might be the cause of rising rates of resistance among these bacteria.

5 Conclusion

This study emphasizes the importance of monitoring antimicrobial susceptibility patterns and understanding the prevalence of pathogenic bacteria in pus samples. The findings underscore the emergence of resistant strains, which are significant concerns of this study. The over-the-counter availability and indiscriminate use of antimicrobials have led

to the development of AMR and MDR superbugs, which are challenging to treat. Therefore, every hospital should create its local antibiogram for empirical therapy and adhere to proper antimicrobial stewardship. By gaining a clearer understanding of antimicrobial susceptibility patterns among pathogens, we can maintain the effectiveness of existing antimicrobials and reduce further AMR emergence.

To further elucidate the dynamics of resistance and guide more precise interventions, future research should focus on molecular typing of these resistant strains. Understanding the genetic relatedness of isolates, identifying specific resistance genes, and tracking the clonal dissemination of pathogens can provide invaluable insights beyond phenotypic susceptibility profiles[26]. Such molecular epidemiological studies are critical for revealing transmission pathways, detecting outbreaks, and informing highly targeted infection control measures and therapeutic strategies in real-time, ultimately strengthening antimicrobial stewardship programs.

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Declaration of patient consent

The authors certify that they have obtained all necessary patient consent forms. In the form the patient has given his/her consent for his/her clinical information to be reported in the journal. The patients understand that due efforts will be made to conceal their identity.

Author Contributions

Concept: Avinash Kumar; Design: Avinash Kumar; Definition of intellectual content: Avinash Kumar; Literature search: Sangeeta Datta; Clinical studies: Sangeeta Datta; Experimental studies: Avinash Kumar; Data acquisition: Avinash Kumar; Data analysis: Sangeeta Datta; Statistical analysis: Avinash Kumar; Manuscript preparation: Avinash Kumar, Sangeeta Datta; Manuscript editing: Biswaroop Chatterjee; Manuscript review: Biswaroop Chatterjee

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