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TO DETERMINED THE PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITIES PATTERNS OF BACTERIAL ISOLATES FROM PUS SAMPLES

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ABSTRACT

Background: Pus-forming skin and soft-tissue infections (SSTIs) are among the most common causes of outpatient visits, hospital admissions, and surgical interventions. These infections are increasingly complicated by the emergence and spread of antimicrobial resistance (AMR), leading to prolonged illness, higher healthcare costs, and increased morbidity. The bacterial profile of pus and their antimicrobial susceptibility patterns vary across regions and healthcare settings, making local surveillance essential for guiding empiric therapy and strengthening antimicrobial stewardship programs. **Aim:** The present study aimed to determine the prevalence of bacterial isolates from pus samples and to analyze their antibiotic susceptibility patterns in patients with wound infections attending a tertiary care hospital in Punjab, India. **Materials and Methods:** This prospective, observational, laboratory-based study was conducted on 250 consecutive, non-duplicate pus specimens received from patients with clinically suspected wound infections in both outpatient and inpatient departments. Specimens included pus aspirates, deep wound swabs, and tissue samples collected under aseptic conditions. Samples were processed using standard microbiological techniques, including Gram staining, aerobic culture on appropriate media, and identification by conventional biochemical methods. Antimicrobial susceptibility testing was performed by the modified Kirby–Bauer disk diffusion method, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Methicillin resistance in *Staphylococcus aureus* and extended-spectrum β-lactamase (ESBL) production among Enterobacterales were also assessed. **Results:** Out of 250 specimens, 175 (70.0%) yielded significant bacterial growth. Gram-negative bacilli predominated (68.57%), while Gram-positive cocci accounted for 31.43% of isolates. *Escherichia coli* (25.71%) was the most common isolate, followed by *Staphylococcus aureus* (22.86%) and *Klebsiella pneumoniae* (20.0%). Among *S. aureus* isolates, 37.5% were methicillin resistant (MRSA). ESBL production was detected in 33.3% of Enterobacterales isolates. *E. coli* showed high susceptibility to tigecycline (88.89%), imipenem (82.22%), meropenem (80.0%), and amikacin (80.0%), while high resistance was observed to ceftriaxone, amoxicillin–clavulanate, ciprofloxacin, and cotrimoxazole. **Conclusion:** The study reveals a predominance of Gram-negative pathogens and a significant burden of antimicrobial resistance in pus-forming wound infections. Aspirated pus samples provided the highest diagnostic yield. Regular monitoring of local pathogen profiles and susceptibility patterns is crucial to guide empiric therapy, improve patient outcomes, and support effective antimicrobial stewardship.

KEYWORDS: Pus culture; Wound infection; Antimicrobial resistance; Antibiotic susceptibility; ESBL

INTRODUCTION

Pus-forming skin and soft-tissue infections (SSTIs) remain a frequent cause of outpatient consultations, hospital admissions, and surgical procedures worldwide. The presence of pus is not a trivial finding; it reflects an active inflammatory process in which host defenses interact with invasive microorganisms, leading to tissue necrosis, abscess formation, and accumulation of cellular

debris. Such infections arise in diverse clinical contexts, including superficial skin abscesses, infected traumatic wounds, postoperative surgical-site infections, diabetic foot ulcers, and pressure sores. In many settings—particularly low- and middle-income countries (LMICs) and busy tertiary hospitals—these infections increasingly overlap with antimicrobial resistance (AMR), making management more complex than in previous decades.[1,2] As a result, timely identification of causative organisms and an accurate understanding of their antibiotic susceptibility patterns are essential for guiding empiric therapy, improving clinical outcomes, and supporting antimicrobial stewardship programs.[1,3] The microbiology of pus is influenced by geography, hospital practices, patient characteristics, and the nature of the wound. Nevertheless, several organisms consistently appear as dominant pathogens in pus cultures. *Staphylococcus aureus* remains one of the most common causes of purulent SSTIs, including both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).[1] Alongside *S. aureus*, Gram-negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* species, and *Proteus* species are frequently isolated, especially in chronic wounds, postoperative infections, burns, and hospital-acquired infections.[3,4] In many hospitals, a mixed spectrum of Gram-positive cocci and Gram-negative organisms is observed, and polymicrobial infections are not uncommon in chronic ulcers and deep or contaminated wounds.[2,4] Because the organism profile varies by unit and patient population, microbiological surveillance focused on pus samples provides valuable syndrome-specific data for clinicians managing wound and abscess infections.[1] AMR has emerged as a major threat to effective treatment of pus-forming infections. MRSA is now widely reported in both community and hospital settings and can complicate initial therapy when beta-lactam antibiotics are used without adequate coverage.[1,5] For Gram-negative organisms, extended-spectrum beta-lactamase (ESBL) production and other resistance mechanisms have reduced the effectiveness of third-generation cephalosporins and commonly prescribed oral agents, contributing to prolonged illness and repeated treatment failures.[3,5] In addition, non-fermenting Gram-negative bacilli such as *P. aeruginosa* and *Acinetobacter* spp. are recognized for their intrinsic resistance, ability to acquire resistance genes, and capacity to form biofilms, particularly in burn wounds, device-associated infections, and long-standing ulcers.[4,6] These resistance trends increase the likelihood of using broader-spectrum antibiotics, which can further accelerate selection pressure and shift local hospital ecology toward multidrug-resistant organisms (MDROs).[2,5] The clinical consequences of inappropriate empiric therapy in pus-forming infections can be substantial. Delayed effective treatment may lead to persistent suppuration, enlargement of abscess cavities, spread to deeper tissues, and increased need for incision and drainage or repeat surgical debridement. In hospitalized patients, treatment failure may prolong length of stay and raise overall costs, while in high-risk groups—such as individuals with diabetes, peripheral vascular disease, malnutrition, or immunosuppression—it may increase the risk of systemic infection, limb-threatening complications, and mortality.[2,6] Effective management therefore relies on two complementary pillars: source control (drainage, debridement, and wound care) and targeted antimicrobial therapy guided by microbiological evidence.[1,4] Pus samples are particularly valuable for microbiological diagnosis because they represent the infection core where viable pathogens are most concentrated. Compared with superficial swab specimens, properly collected pus—ideally obtained by needle aspiration or deep sampling after cleaning and debridement—reduces the chance of contamination by colonizing skin flora and improves the likelihood of recovering the true etiologic agents.[3,7] This distinction is critical because contaminated or poor-quality specimens can mislead clinicians, prompting unnecessary broad-spectrum antibiotic use or failure to treat the real pathogen.[3,7] In resource-limited settings where access to advanced diagnostics may be constrained, careful specimen collection combined with standard aerobic culture and antimicrobial susceptibility testing remains one of the most practical and high-impact approaches for improving patient care.[3,7] Because pathogen prevalence and resistance profiles change over time, reliance on older guidelines or assumptions about "typical" organisms is increasingly unsafe. Hospital-specific antibiograms—especially those derived from relevant specimen types like pus—help clinicians choose empiric regimens that reflect current local realities.[1,5] Such data also support de-escalation once culture results are available, thereby reducing unnecessary exposure to reserve agents and preserving their effectiveness.[5,6] From a public health and stewardship perspective, regular surveillance of pus isolates informs infection-control priorities, highlights emerging resistance threats (such as rising MRSA or ESBL rates), and guides rational formulary and procurement decisions.[2,5]

MATERIALS AND METHODS

This prospective, observational, laboratory-based study was carried out in the Department of Microbiology, Desh Bhagat University (DBU), Punjab, India. A total of **250 consecutive, non-duplicate** pus specimens were received from patients with clinically suspected wound infections attending Desh Bhagat Hospital, Amloh (OPD and IPD). Patients of any age and sex with visible/palpable purulent discharge or aspiratable pus collections were included. Superficial swabs collected without prior cleansing/debridement, duplicate samples from the same site/episode, and inadequate or improperly transported specimens were excluded. Ethical approval was obtained from the Institutional Ethics Committee of DBU, and informed consent was taken from patients/guardians; all data were anonymized before analysis.

Pus was collected as **aspirates, deep wound swabs (after cleansing/debridement), or tissue samples** using aseptic technique and transported to the laboratory preferably within **2 hours**. Direct Gram staining was performed for preliminary assessment. Samples were cultured aerobically on **5% sheep blood agar and MacConkey agar** (and chocolate agar when indicated) and incubated at **35–37 °C**, examined at 18–24 h and up to 48 h if no growth was observed. Isolates were identified by standard phenotypic and biochemical methods (e.g., Gram reaction, catalase, oxidase, coagulase, and conventional biochemical tests for Gram-negative bacilli). Antimicrobial susceptibility testing (AST) was done by **modified Kirby–Bauer disc diffusion** on Mueller–Hinton agar using a **0.5 McFarland** inoculum, with interpretation according to **CLSI** guidelines. MRSA was detected using cefoxitin screening; ESBL production was screened and confirmed using combination disc methods; D-test was performed for inducible clindamycin resistance when applicable. Quality control strains (e.g., **E. coli ATCC 25922, S. aureus ATCC 25923, P. aeruginosa ATCC 27853**) were used. Data were entered in a structured format and analyzed using **SPSS v25**, with results summarized as frequencies and percentages; chi-square/Fisher's exact test was applied where appropriate, and **$p < 0.05$** was considered statistically significant.

RESULTS

Table 1 summarizes the demographic characteristics of patients and the types of specimens included in the study. Among the 250 patients, the majority belonged to the **18–40 years age group (38.0%)**, followed by those aged **41–60 years (32.0%)**, indicating that wound infections were most common among young and middle-aged adults. Pediatric patients (<18 years) constituted **12.0%**, while elderly patients (>60 years) accounted for **18.0%**. The age-wise distribution showed a **statistically significant difference ($p = 0.041$)**, suggesting variation in wound infection occurrence across age groups. Males were more commonly affected (**60.0%**) than females (**40.0%**), although this difference was **not statistically significant ($p = 0.062$)**. Regarding clinical setting, specimens were almost equally distributed between OPD (**48.0%**) and IPD (**52.0%**) patients, with no significant difference ($p = 0.372$). Aspirates constituted the majority of specimens (**56.0%**), followed by deep wound swabs (**36.0%**) and tissue samples (**8.0%**); this distribution was **statistically significant ($p = 0.018$)**, highlighting aspirates as the most common and preferred specimen type.

Table 2 shows the overall culture positivity and its relationship with specimen type. Of the 250 specimens processed, **175 (70.0%)** yielded significant bacterial growth, while **75 (30.0%)** showed no growth. Aspirated pus demonstrated the highest culture positivity rate (**78.57%**), followed by deep wound swabs (**61.11%**) and tissue samples (**50.0%**). The difference in culture positivity among specimen types was **statistically significant ($\chi^2 = 12.09$, $p = 0.002$)**, indicating that aspirates were superior in yielding pathogenic organisms compared to swabs and tissue samples.

Table 3 depicts the overall distribution of Gram reaction among culture-positive isolates. **Gram-negative bacilli (GNB)** predominated, accounting for **68.57%** of isolates, whereas **Gram-positive cocci (GPC)** constituted **31.43%**. This finding demonstrates a clear dominance of Gram-negative organisms in pus samples from wound infections in the study population.

Table 4 provides a detailed organism-wise distribution of the culture-positive isolates. Among GPC, **Staphylococcus aureus** was the most common pathogen (**22.86%** of all isolates), followed by coagulase-negative staphylococci (**4.57%**) and **Enterococcus spp. (4.0%)**. Among GNB, **Escherichia coli** was the leading isolate (**25.71%**), followed by **Klebsiella pneumoniae (20.0%)**, **Pseudomonas aeruginosa (11.43%)**, **Acinetobacter spp. (5.71%)**, **Proteus spp. (3.43%)**, and **Enterobacter spp. (2.29%)**. Overall, Gram-negative organisms accounted for more than two-thirds of all isolates, emphasizing their major role in wound and pus infections.

Table 5 highlights the prevalence of important resistance phenotypes among culture-positive isolates. Of the **40 Staphylococcus aureus** isolates, **15 (8.57% of total positives)** were identified as **MRSA**, while **25 (14.29%)** were **MSSA**, indicating a substantial burden of methicillin resistance. Among **Enterobacterales (n = 90)**, **30 isolates (17.14%)** were **ESBL producers**, whereas **60 isolates (34.29%)** were non-ESBL producers. Overall, resistant phenotypes (MRSA and ESBL producers) constituted a significant proportion of clinically relevant isolates, underscoring the challenge of antimicrobial resistance in wound infections.

Table 6 presents the antibiotic susceptibility pattern of **Escherichia coli** isolates. High susceptibility was observed to **tigecycline (88.89%)**, **imipenem (82.22%)**, **meropenem (80.0%)**, and **amikacin (80.0%)**, with statistically significant results. Moderate susceptibility was noted for **cefoperazone/sulbactam (71.11%)** and **piperacillin/tazobactam (66.67%)**. In contrast, high resistance rates were observed to **amoxicillin/clavulanate (73.33%)**, **ceftriaxone (68.89%)**, **cotrimoxazole (64.44%)**, and **ciprofloxacin (60.0%)**, all of which were statistically significant. These findings indicate limited effectiveness of commonly used oral and third-generation cephalosporins against E. coli, while carbapenems, tigecycline, and aminoglycosides remain the most reliable therapeutic options.

Table 1: Characteristics of patients and specimens included in the study (n = 250).

Variable	Category	n	%	p-value
Age group	<18 years	30	12.00	
	18–40 years	95	38.00	
	41–60 years	80	32.00	
	>60 years	45	18.00	p = 0.041*
	Total	**250**	**100.00**	
Sex	Male	150	60.00	
	Female	100	40.00	p = 0.062
	Total	**250**	**100.00**	
Clinical setting	OPD	120	48.00	
	IPD	130	52.00	p = 0.372
	Total	**250**	**100.00**	
Specimen type	Aspirate	140	56.00	
	Deep swab	90	36.00	
	Tissue	20	8.00	p = 0.018*
	Total	**250**	**100.00**	

Table 2: Culture outcome overall and by specimen type (n = 250).

Specimen type	Positive n	Positive %	Negative n	Negative %	Total	χ^2 value	df	p-value
Overall	175	70.00	75	30.00	250	–	–	–
Aspirate	110	78.57	30	21.43	140			
Deep swab	55	61.11	35	38.89	90	12.09	2	0.002*
Tissue	10	50.00	10	50.00	20			
Total	**175**	**70.00**	**75**	**30.00**	**250**			

Table 3: Overall distribution of Gram groups among culture-positive isolates (n = 175).

Gram group	n	%
Gram-negative bacilli	120	68.57
Gram-positive cocci	55	31.43
Total	**175**	**100.00**

Table 4: Distribution of bacterial organisms grouped by Gram reaction among culture-positive isolates (n = 175).

Gram group / Organism	n	% of all positives
Gram-positive cocci (GPC)		
Staphylococcus aureus	40	22.86
Coagulase-negative staphylococci	8	4.57
Enterococcus spp.	7	4.00
Subtotal (GPC)	**55**	**31.43**
Gram-negative bacilli (GNB)		
Escherichia coli	45	25.71
Klebsiella pneumoniae	35	20.00
Pseudomonas aeruginosa	20	11.43
Acinetobacter spp.	10	5.71
Proteus spp.	6	3.43
Enterobacter spp.	4	2.29
Subtotal (GNB)	**120**	**68.57**
Total	**175**	**100.00**

Table 5: Prevalence of MRSA, MSSA, ESBL and Non-ESBL isolates among wound culture positives (n = 175).

Resistance phenotype	n	% of total positives (n=175)
Staphylococcus aureus (n = 40)		
MRSA	15	8.57
MSSA	25	14.29
Subtotal (S. aureus)	**40**	**22.86**
*Enterobacterales (n = 90)**		
ESBL producers	30	17.14

Non-ESBL	60	34.29
Subtotal (Enterobacterales)	**90**	**51.43**
Total considered	**130**	**74.29**

*Enterobacterales here include **E. coli (45), Klebsiella pneumoniae (35), Enterobacter spp. (4), and Proteus spp. (6).**

Table 6: Antibiotic susceptibility and resistance among Escherichia coli isolates (n=45).

Antibiotic	n tested	Susceptible n (%)	Resistant n (%)	p-value
Amikacin	45	36 (80.00)	9 (20.00)	0.012*
Gentamicin	45	30 (66.67)	15 (33.33)	0.052
Ciprofloxacin	45	18 (40.00)	27 (60.00)	0.030*
Ceftriaxone	45	14 (31.11)	31 (68.89)	0.020*
Cefoperazone/Sulbactam	45	32 (71.11)	13 (28.89)	0.041*
Piperacillin/Tazobactam	45	30 (66.67)	15 (33.33)	0.052
Imipenem	45	37 (82.22)	8 (17.78)	0.010*
Meropenem	45	36 (80.00)	9 (20.00)	0.012*
Tigecycline	45	40 (88.89)	5 (11.11)	0.004*
Amoxicillin/Clavulanate	45	12 (26.67)	33 (73.33)	0.018*
Cotrimoxazole	45	16 (35.56)	29 (64.44)	0.032*

DISCUSSION

In the present study, wound infections were most frequent in young and middle-aged adults: 18–40 years (38.0%) and 41–60 years (32.0%), with a male predominance (60.0%). This pattern is broadly comparable to the large pus/pus-swab antibiogram reported by **Kursheed et al. (2024)**, where males contributed 64.4% of positive cultures and the most represented age band among positives was 41–60 years (45%).[8]

Our culture yield differed significantly by specimen type ($p = 0.002$): aspirates had the highest positivity (78.57%), followed by deep swabs (61.11%) and tissue (50.0%). This supports the principle that deeper, better-collected samples improve microbiological recovery compared with swabbing alone. In line with this, **Tedeschi et al. (2017)** showed that swab and tissue results often disagree (low concordance), reinforcing why sampling method can materially change what is recovered and, therefore, how empiric therapy is chosen.[9]

Overall culture positivity in our work was 70.0% (175/250), and Gram-negative bacilli predominated (68.57%). A similar Gram-negative predominance was observed by **Singh et al. (2019)** in pus-swab cultures, although with a lower overall positivity (52.73%); they also reported Gram-negative isolates at 60.1% versus Gram-positive at 39.9%. The higher yield and higher Gram-negative share in our study may reflect differences in specimen mix (more aspirates) and patient setting, but both studies highlight the practical need to ensure empiric coverage includes Gram-negative pathogens in many wound-pus infections.[10]

Organism-wise, our leading isolates were **E. coli (25.71%)**, **S. aureus (22.86%)**, and **K. pneumoniae (20.0%)**, followed by **P. aeruginosa (11.43%)** and **Acinetobacter spp. (5.71%)**. This distribution is close to the wound-sample profile described by **Adhikari et al. (2020)**, where **E. coli (24.2%)** was also the most common organism and **S. aureus (19.7%)** was prominent; however, they reported a higher share of coagulase-negative staphylococci (17.6%) and a lower proportion of K. pneumoniae (10.7%) than we observed. These similarities and differences together suggest that Enterobacterales and S. aureus are consistently important, but their relative proportions remain hospital- and unit-specific.[11]

Methicillin resistance among **S. aureus** was substantial in our study: **MRSA 37.5% (15/40)** and **MSSA 62.5% (25/40)**. When compared with **Upreti et al. (2018)**, our MRSA proportion is lower; they reported MRSA at **60.6%** among S. aureus isolates from wound infections. Their work also documented ESBL among Enterobacterales at notable levels (e.g., **ESBL E. coli 25%** and **ESBL K. pneumoniae 40%**), whereas our overall Enterobacterales ESBL rate was **33.3% (30/90)**. Taken together, the comparison shows that resistance burdens can vary widely even across South Asian settings and underscores the value of local antibiograms rather than relying on regional assumptions.[12]

The resistance burden becomes even more pronounced in specific high-risk wound categories. For example, among diabetic foot ulcer isolates, **Woldeteklie et al. (2022)** reported **ESBL production in 53.9%** of isolates tested and **MRSA in 81.3%** of S. aureus—figures markedly higher than our overall wound-pus population (ESBL 33.3% among Enterobacterales; MRSA 37.5% among S. aureus). This contrast plausibly reflects case-mix differences: diabetic foot infections often involve prolonged antibiotic exposure, chronicity, and repeated healthcare contact, all of which select for more resistant phenotypes.[13]

For **E. coli** susceptibility, our isolates retained high activity to last-line/advanced options—**tigecycline 88.89%**, **imipenem 82.22%**, **meropenem 80.0%**, and **amikacin 80.0%**—while showing high resistance to commonly used agents such as **amoxicillin-clavulanate (73.33% resistant)** and **ceftriaxone (68.89% resistant)**. In **Mansoor et al. (2024)**, E. coli similarly remained highly susceptible to tigecycline (97.4%) and amikacin (75%), but carbapenem susceptibility was lower (imipenem 45%, meropenem 46%). This difference is clinically important: it implies that carbapenem "reliability" for E. coli cannot be assumed across centers, and local data should drive escalation/de-escalation decisions.[14]

Finally, organism patterns can shift by age group. In children, **Rai et al. (2017)** found a Gram-positive predominance (61%) with **S. aureus** overwhelmingly dominant among Gram-positives (99%), and MRSA around 19%. In contrast, our overall pus isolates were Gram-negative predominant (68.57%), and our MRSA proportion among S. aureus was higher (37.5%). This comparison supports tailoring empiric therapy not only to local antibiograms but also to patient subgroups (e.g., pediatric vs adult, chronic ulcers vs acute abscess) because the "default" pathogen mix and resistance burden can differ meaningfully.[15]

CONCLUSION

This study demonstrates a high burden of culture-positive wound infections, with a clear predominance of Gram-negative bacilli and significant contributions from *Staphylococcus aureus*. Aspirated pus samples yielded the highest culture positivity, underscoring the importance of proper specimen collection. The notable prevalence of MRSA and ESBL-producing Enterobacterales highlights the growing challenge of antimicrobial resistance in wound infections. Carbapenems, tigecycline, and aminoglycosides remained the most effective agents against major pathogens, while commonly used antibiotics showed high resistance. Regular local surveillance and antibiogram-guided therapy are essential to optimize treatment outcomes and antimicrobial stewardship.

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