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RESEARCH ARTICLE

Microbiological Profile in Necrotizing Soft Tissue Infections: A Comparative Study of Blood, Wound Discharge, and Tissue Cultures

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Abstract: ***Background***: Necrotising soft tissue infections (NSTIs) are rapidly progressive, life-threatening infections that involve the skin, subcutaneous tissue, and fascia. Early identification of causative organisms is essential for timely targeted antimicrobial therapy. This study analysed the microbiological profile of NSTIs using three diagnostic approaches—blood culture, wound discharge culture, and tissue culture—to determine the most reliable method for pathogen isolation. ***Methods***: An observational analytical study was conducted at the Department of General Surgery, Subharti Medical College, Meerut, over 18 months. Sixty patients clinically diagnosed with NSTIs were included. Samples from blood, wound discharge, and debrided tissue were collected and cultured using standard aerobic, anaerobic, and fungal techniques. Microorganisms were identified to species level, and antibiotic sensitivity was determined using standard microbiological methods. ***Results***: Of 60 patients, 70% were male, and the mean age was 48.3 years. Diabetes mellitus was the most common comorbidity (55%). Positive culture rates were: tissue culture (91.7%), wound discharge culture (83.3%), and blood culture (8.3%). Escherichia coli (28.3%), Klebsiella pneumoniae (21.6%), Staphylococcus aureus (16.6%), and Pseudomonas aeruginosa (10%) were the most common isolates. Antibiotic sensitivity revealed high susceptibility to colistin, carbapenems, and aminoglycosides, while resistance to cephalosporins and fluoroquinolones was common. Mortality was 6.7%. ***Conclusion***: Tissue culture provided the highest diagnostic yield and should be preferred for microbiological confirmation in NSTIs. Empirical antibiotic therapy should target Gram-negative and anaerobic organisms, guided by local resistance patterns. Early diagnosis, aggressive debridement, and culture-guided antibiotics are crucial for improving survival.

Keywords: Necrotizing soft tissue infections, tissue culture, blood culture, wound discharge culture, microbiological profile, antibiotic sensitivity, NSTI.

INTRODUCTION

Necrotising soft tissue infections (NSTIs) are fulminant, rapidly spreading infections involving the skin, subcutaneous tissue, fascia, and sometimes muscle. They are characterised by widespread tissue necrosis, systemic toxicity, and high mortality. Early recognition and surgical intervention remain critical to reducing morbidity and mortality. This study compares microbial yield and pathogen profiles across blood, wound discharge, and tissue cultures to determine the most reliable sample for pathogen detection in NSTIs.

MATERIALS AND METHODS:

An observational analytical study was conducted at the Department of Surgery, Subharti Medical College, for a period of 18 months on a total of 60 subjects after taking Ethical permission from the Institutional Ethical Committee of the college and hospital. Patients with NSTI were recruited on clinical diagnosis as shown in figure 1. 6 samples were sent for patient, which had Blood, Tissue and Wound discharge culture to see the microbiological profile, which included aerobic, anaerobic and fungal growths.The data, which was collected, included demographic, clinical and microbiological profiles with antibiotic sensitivity and comorbidities. Collected data was analysed using SYSTAT 13.2 software.

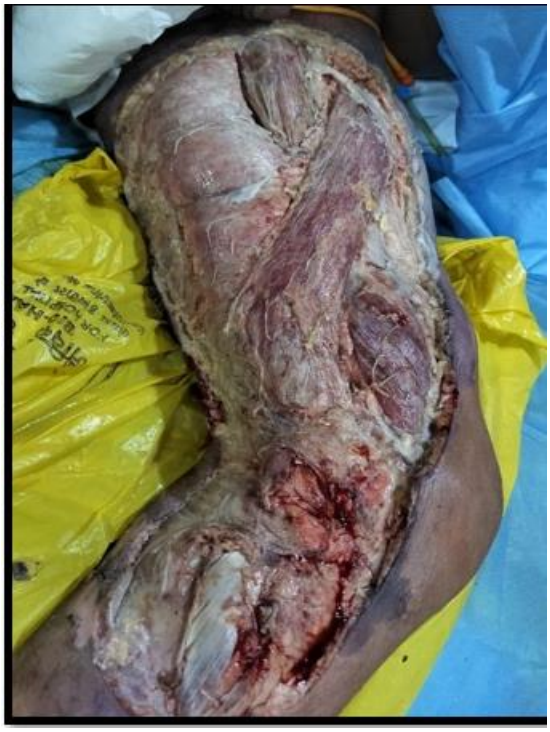


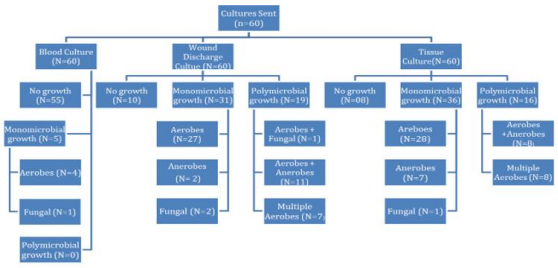
Figure 1

RESULTS:

Table 1: DEMOGRAPHIC AND CLINICAL PROFILE

PROFILE		NUMBER OF PATIENTS
SEX	Male	42(70%)
	Female	18 (30%)
AGE	<30yrs	10 (16.7%)
	30-60yrs	38(63.3%)
	>60yrs	12(20%)
Diabetes Mellitus	yes	33(55%)
	no	27(45%)
Duration of stay	< 1 week	15(25%)
	1-2 weeks	24(40%)
	>2weeks	21(35%)

CULTURE RESULTS:



BLOOD CULTURE:

Blood culture was positive only in 4 patients. The microbial growths were found to be different in all the 4 patients. The complete profile of these patients is given in table 2.

Table 2. Distribution of blood culture pathogens with all cultures with antibiotic sensitivity, TLC, diabetes, other

Pathogen in blood culture	Pathogen in tissue culture (aerobic + anaerobic)	Pathogen in wound discharge (aerobic + anaerobic)	Antibiotic sensitivity	TLC	DM	Other comorbidity	Outcome
Klebsiella pneumoniae	-	-	Colistin	>10,000 cells/mm³	Non-Diabetic	None	Discharged
Klebsiella pneumoniae	-	-	Colistin Chloramphenicol Aminoglycoside	>20,000 cells/mm³	Diabetic	AKI	Discharged
Candida aureus	*Enterobacter aerogenes*	*Enterobacter aerogenes*	Micafungin Caspofungin Chloramphenicol Colistin	>10,000 cells/mm³	Diabetic	DCMP, Metabolic Syndrome	Expired
Granulicatella adiacens	*Granulicatella adiacens*	*Granulicatella adiacens Peptostreptococcus anaerobeus*	Penicillin Erythromycin Linezolid	>30,000 cells/mm³	Diabetic	None	Expired
Proteus mirabilis	*Proteus mirabilis*	*Proteus mirabilis*	Aminoglycoside Colistin Penicillin Carbapenems Cephalosporins	>20,000 cells/mm³	Diabetic	None	Discharged

WOUND DISCHARGE CULTURE:

Table 3: Distribution of Pathogens Identified from Wound Discharge Culture with Antibiotic Sensitivity, TLC, Comorbidities and Percentages (n = 60)

Type	Pathogen	No. of Isolates (n)	% of Total (60)	Antibiotic Sensitivity	TLC (cells/mm³)	Associated Comorbidities
Aerobic Organisms						
	No growth	14	23.3 %	–	–	–
	Escherichia coli	12	20.0 %	Colistin, Aminoglycosides, Chloramphenicol, Carbapenems, Tetracycline, Cephalosporin, Penicillin	<10k–3, 10–20k–8, 20–30k–2, >30k–1	T2DM (9), None (3)
	Staphylococcus aureus	6	10.0 %	Aminoglycosides, Tetracycline, Clindamycin, Chloramphenicol, Erythromycin, Linezolid, Vancomycin	<10k–1, 10–20k–5, >20k–4	T2DM (4), None (2)
	Proteus mirabilis	4	6.7 %	Carbapenems, Penicillin, Chloramphenicol, Aminoglycosides, Colistin, Cephalosporins	<10k–0, 10–20k–4, 20–30k–2	T2DM (3), None (1)
	Acinetobacter baumannii	4	6.7 %	Colistin, Chloramphenicol, Carbapenems, Tetracycline, Aminoglycosides, Clindamycin, Linezolid	<10k–0, 10–20k–3, 20–30k–2, >30k–1	T2DM (3), None (1)
	Klebsiella pneumoniae	3	5.0 %	Colistin, Carbapenems, Aminoglycosides, Chloramphenicol	<10k–2, 10–20k–2, 20–30k–2	T2DM (2), None (1)
	Pseudomonas aeruginosa	2	3.3 %	Colistin (3), Resistant to all (1)	<10k–1, 10–20k–3, 20–30k–1	T2DM (2)

	Granulicatella adiacens	1	1.7 %	Penicillin, Erythromycin, Linezolid	20–30k–1	T2DM (1)
Anaerobic Organisms						
	Anaerococcus prevotii	7	11.7 %	–	<10k–2, 10–20k–4, 20–30k–1	T2DM (5), None (2)
	Bacteroides fragilis	3	5.0 %	–	<10k–2, 10–20k–1	T2DM (3)
	Clostridium perfringens	1	1.7 %	–	>10k–1	None
	Peptostreptococcus anaerobius	2	3.3 %	–	<10k–1, 10–20k–1	T2DM (2)
Total	60	100 %				

TISSUE CULTURE:

Table 4: Distribution of Pathogens Identified from Tissue Culture with Antibiotic Sensitivity, TLC, Comorbidities and Percentages (n = 60)

Type	Pathogen	No. of Isolates (n)	% of Total (60)	Antibiotic Sensitivity	TLC (cells/mm³)	Associated Comorbidities
Aerobic Organisms						
	No growth	8	13.3 %	–	–	–
	Escherichia coli	18	30.0 %	Colistin, Aminoglycosides, Chloramphenicol, Carbapenems	<10k–4, 10–20k–9, 20–30k–3	T2DM (11), None (7)
	Staphylococcus aureus	4	6.6 %	Aminoglycosides, Chloramphenicol, Clindamycin, Linezolid	<10k–4	T2DM (1), None (3)
	Proteus mirabilis	7	11.6 %	Carbapenems, Colistin, Penicillin, Chloramphenicol	10–20k–4, 20–30k–3	T2DM (5), None (2)
	Acinetobacter baumannii	4	6.6 %	Colistin, Aminoglycosides, Carbapenems	10–20k–2, 20–30k–2	T2DM (4)
	Klebsiella pneumoniae	7	11.6 %	Colistin, Carbapenems, Aminoglycosides, Chloramphenicol, Cotrimoxazole	<10k–2, 10–20k–3, >30k–2	T2DM (4), None (3)
	Pseudomonas aeruginosa	2	3.3 %	Colistin (50%), Resistant to all (50%)	10–20k–2	T2DM (1), None (1)
	Enterobacter aerogenes	1	1.6 %	Chloramphenicol, Colistin, Micafungin	>10k–1	T2DM (1)
	Granulicatella adiacens	1	1.6 %	Penicillin, Erythromycin, Linezolid	>30k–1	T2DM (1)
Anaerobic Organisms						
	Anaerococcus prevotii	6	10.0 %	–	>10k–6	T2DM (2), None (4)

	Peptostreptococcus anaerobius	5	8.3 %	–	>10k–5	T2DM (4), None (1)
	Bacteroides fragilis	3	5.0 %	–	>10k–3	T2DM (1), None (2)
	Staphylococcus saccharolyticus	1	1.6 %	–	–	None

DISCUSSION:

Necrotising soft tissue infections (NSTIs) are life-threatening conditions requiring prompt diagnosis and targeted antimicrobial therapy. This study evaluated the microbiological profiles of NSTIs using blood cultures, wound discharge cultures, (aerobic, anaerobic and fungal) and tissue cultures (aerobic and anaerobic) from the site of infection. The study revealed each type of culture's pathogen detection rate and its microbial distribution. The

findings align with previous research while also highlighting key variations in diagnostic yield across different sample types.

The study population exhibited a male predominance (70%), consistent with multiple prior studies. Goh et al. (2014)1 reported a similar male-to-female ratio (2:1) in their retrospective analysis of NSTIs, attributing the disparity to higher rates of trauma, diabetes, and peripheral vascular disease in males. The mean age of our cohort (48.35 years) mirrors findings by Stevens et al. (2014)2, who noted that middle-aged and elderly individuals are at increased risk due to comorbidities such as diabetes and immunosuppression. Notably,68.3% of patients presented within 1 week of symptom onset, reinforcing the rapid progression of NSTIs described by Hakkarainen et al. (2014)3, who found that delays in surgical intervention beyond 24 hours significantly increase mortality. 55% had diabetes as a comorbidity, which made them immunocompromised and more susceptible to Necrotising soft tissue infections, especially with those organisms that are resistant to the first-line antibiotics. Tsai Y-H et al (2015)4 reported diabetes mellitus as most common comorbid condition in 9% of their patients, with a combination of diabetes mellitus and others in 18.9% and Faraklas I et al (2013)5 in a study of 1392 patients had 49% patients with diabetes. Our study showed that 93.93% of diabetics had TLC more than 10,000 cells/mm3, out of which 39.3% had TLC more than 20,000 cells/mm3. In our study, out of 60, 4 cases had expired (Mortality 6.67%). From the 4 expired cases, 3 patients had diabetes mellitus as a comorbidity, which was similar to the findings of both Faraklas I et al (2013)5 and Tiu A et al (2005)6. Average length of stay in the hospital was 12.97+/-7.73 days. Around 75% of the patients stayed for more than 7 days. Out of these 75%(45), 48.33% cases were diabetics. This infers that diabetes with NSTIs need more care and more longer stay in the hospital.

Tissue cultures had the highest pathogen recovery rate (86.67%), followed by wound discharge cultures (83.33%), while blood culture had the least recovery rate (8.33%). These findings align with previous research by Wong et al. (2003)7, who reported that blood cultures are often negative in NSTIs due to the localised nature of infection, whereas tissue culture provides a more accurate microbiological assessment. In our study, polymicrobial growths were seen in 26.67% (16 out of 60) in tissue culture and 31.67% (19 out of 60) in wound discharge culture. The presence of monomicrobial growths in NSTIs is more common than polymicrobial growth in our study. And this finding is in contrast with Chen C et al (2011)15,Giuliano et al. (1977)17 and Nischal et al (2015)16, where they found polymicrobial growth to be 54.8% and 60%, respectively. The presence of polymicrobial growth in a higher number of wound discharge samples than tissue samples is seen in our study, which may be due to higher chances of contamination of wound discharge culture during collection and transport of the sample; therefore, deeper tissue biopsies will be more reliable for studying the microbiological profile in NSTIs. Earlier studies emphasised Streptococcus pyogenes as a dominant pathogen (Stevens et al., 1989)18; our study found Escherichia coli and Staphylococcus aureus to be more prevalent, possibly due to evolving antimicrobial resistance patterns or regional variations in microbial epidemiology.

BLOOD CULTURE: Our study found that 91.68% of blood cultures were sterile, with only 8.3% yielding pathogens. This aligns with Wong et al. (2003)7, who reported that blood cultures are positive in only 5–15% of NSTI cases. The low sensitivity of blood cultures in NSTIs has been well-documented, as bacteremia occurs late in the disease course and is often transient (Stevens & Bryant, 2017)8. The finding of Candida aureus(1.66%) isolate from the blood culture of one patient with a history of diabetes is in contrast with older studies, where fungal isolates were rare (<1%) (Stevens & Bryant, 2017)8. The emergence of C. aureus in our cohort may reflect the growing prevalence of nosocomial fungal infections in critically ill and immunocompromised patients. (Spivak & Hanson, 2018)9 When growth in other cultures of blood culture positive cases was assessed, out of 5 cases, 3 cases showed growth in other cultures as well (wound discharge culture/ tissue culture). This shows dissemination of the local infection into systemic circulation, which is usually a late presentation, or infection by a deadly pathogen, or immunocompromised status. 2 out of these 3 patients expired in our study.

Increased TLC more than 10,000 cells/mm3 and the presence of diabetes as a comorbidity in most of the blood culture-positive patients support our above statement.

WOUND DISCHARGE CULTURE: Wound discharge cultures were sent to see aerobic, anaerobic and fungal growths (polymicrobial), and they came positive (pathogen recovery rate) in 83.33% (50 out of 60) patients. Monomicrobial growths were seen in 51.67% (31 out of 60) and 31.66% (19 out of 60) patients showed polymicrobial growths. This reinforces the polymicrobial aetiology of NSTIs seen by Brook & Frazier, 199510. However, the maximum (74%) of anaerobic wound discharge cultures were sterile. The high rate of anaerobic culture negativity (74%) could be due to: Fastidious growth requirements of anaerobes (Ladhani et al. 1999)11 and inadequate sample transport (prior studies suggest that anaerobic cultures require immediate processing to avoid false negatives) (Jousimies-Somer et al., 2002)12. The most common pathogen identified in our study in wound discharge culture samples was Escherichia coli (28%)(gram-negative) (14/50) followed by Anaerococcus prevotii (14%) (7 out of 50). Staphylococcus aureus (gram positive), Klebsiella pneumoniae(gram negative), Proteus mirabilis(gram negative) and Acinetobacter baumannii(gram negative) each were identified in 12% of the wound discharge culture samples (6 out of 50). In the study by Varsha et al (2008), Staphylococcus aureus was the most common, followed by Pseudomonas aeruginosa in Zarrin et al (2015. Comparing other studies with our study, Escherichia coli or Staphylococcus aureus is either the first or second most expected growth. Anaerobic and fungal growths are rare and usually show the presence of Anaerococcus prevotii (14%) or Bacteroides fragilis (6%), or Candida species (4%). Escherichia coli was seen in 14 wound discharge cultures, either as monomicrobial or polymicrobial growth and showed sensitivity mostly to Colistin (12 out of 14), followed by Aminoglycosides (10 out of 14), Chloramphenicol (6 out of 14) and Carbapenems (4 out of 14). Staphylococcus aureus was the most prevalent gram-positive isolate (6 out of 50) and was mostly sensitive to Tetracycline and Aminoglycosides (4), followed by Clindamycin (3). This data infers that most suspected growth in NSTI wound discharge culture in gram-positive is Staphylococcus aureus, gram-negative is Escherichia coli and Anaerococcus prevotii in anaerobes. Treatment typically should involve a combination of antibiotics such as Meropenem-Sulbactam or Imipenem for gram-negative coverage, supplemented with Aminoglycosides. For gram-positive coverage, Clindamycin or Tetracycline should be considered. Anaerobic coverage should be provided by Metronidazole or Tinidazole. Fungal growths are rarely seen in NSTI, so antifungals should be restricted only to highly suspected cases. TLC is a part of the LRINEC score, which helps in differentiating between necrotising and non-necrotising forms of infections. This score is not routinely used in our hospital, so in our study, we have included TLC to make the inference. The presence of TLC more than 10,000 cells/mm3 was seen in 41 out of 50 wound discharge culture-positive patients, out of which 12 patients had TLC more than 20,000 cells/mm3. Escherichia coli showed a raised TLC of more than 10,000 cells/mm³ in 11 out of 14 cases and more than 20,000 cells/mm³ in 3 cases. Staphylococcus aureus showed more than 10,000 cells/mm3 TLC in 5 cases out of 6 and more than 20,000 cells/mm3 TLC in 4 out of 5 cases. Anaerococcus prevotii showed raised TLC of more than 10,000 cells/mm³ in 5 out of 7 cases and more than 20,000 cells/mm³ in 1 out of 5 cases. TLC is seen to be increased in most of the NSTI cases. Its use, along with other parameters for calculating the LRINEC score, in addition to a carefully recorded patient's history, can help in increasing the diagnostic chances in patients with unclear clinical presentation. In Patients with Escherichia coli infection (14), 9 were diabetics, whereas with Staphylococcus aureus, only 1 patient out of 6 was diabetic. 8 out of 13 anaerobic wound discharge culture patients had a history of diabetes. Diabetes is the most common comorbidity seen in patients with NSTI. There was no mortality in wound discharge culture-negative cases (10). Out of 50 cases, 4 patients expired (8%), of whom 3 were diabetics. This implies that diabetes as a comorbidity in a patient with NSTI had made the patient more immunocompromised and more susceptible to a resistant pathogen, whereas Escherichia coli was seen as the most common growth, but none of them expired. This also implies that, Escherichia coli being the most common pathogen, its prognosis is good.

TISSUE CULTURE: Tissue culture samples from 60 patients were sent to see both aerobic and anaerobic growth. No growths were seen in 8 samples, polymicrobial growths were seen in 16 samples, and the remaining 36 samples showed monomicrobial growths. In tissue culture samples, the most common gram-negative pathogen was Escherichia coli (18 out of 50). In gram-positive, most common was Staphylococcus aureus, isolated in 4 cases. Anaerobic growth was seen in 15 tissue cultures, out of which 6 were Anaerococcus prevotii and 5 were Peptostreptococcus.

COMPARISON OF WOUND DISCHARGE, BLOOD AND TISSUE CULTURES: The high rate of negative blood cultures (91.68%) aligns with data from Sarani et al. (2009)14, who reported that blood cultures are insensitive in NSTIs. The higher yield from tissue cultures than wound discharge culture supports the findings of Wall et al. (2000)13, who demonstrated that deep tissue biopsies improve microbial detection and guide appropriate antibiotic therapy. In 26 patients, tissue and wound discharge culture showed similar growths with similar antibiotic sensitivity. While 4 patients had no growth in tissue and wound discharge culture. This infers that in 50% of the cases, wound discharge culture and tissue culture gave the same yield with respect to the isolated pathogens and their antibiotic

sensitivity. But the pathogen recovery rate of tissue culture is higher than that of wound discharge culture, as discussed earlier. The higher yield from tissue cultures supports the findings of Wall et al. (2000)13, who demonstrated that deep tissue biopsies improve microbial detection and guide appropriate antibiotic therapy.

CONCLUSION:

The study highlights the limitations of blood cultures in diagnosing NSTIs and reinforces the importance of wound and tissue cultures for accurate pathogen identification. Tissue biopsies should be sent for isolation of the pathogen, as it gives the maximum yield. Diabetics are more prone to getting infected with NSTIs. The most common pathogen isolated in aerobes was Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive), and Anaerococcus prevotii in anaerobes. Escherichia coli was the most common growth with a good prognosis, as only 1 patient expired(4%) (21/60). Given the polymicrobial nature of these infections, empirical antibiotic therapy should cover both aerobic and anaerobic organisms until culture results are available (Stevens et al., 2014). Due to the development of antibiotic resistance in commonly seen pathogens (Escherichia coli, Staphylococcus aureus), treatment typically should involve a combination of antibiotics such as Meropenem-Sulbactam or Imipenem for gram-negative coverage, supplemented with aminoglycosides. For gram-positive coverage, Clindamycin or Tetracycline should be considered. Metronidazole or Tinidazole should provide anaerobic coverage. Fungal growths are rarely seen in NSTI, so antifungals should be restricted only to highly suspected cases. Anaerobic organisms show fastidious growth. Sample collection and transportation should be conducted in a controlled, timely manner. The detection of multidrug-resistant organisms such as Proteus mirabilis and Pseudomonas aeruginosa (1.66% and 1.66%, respectively) underscores the need for antimicrobial stewardship to prevent treatment failure. Maximum of the growths from local infection samples (tissue and wound discharge) showed sensitivity to Colistin and Chloramphenicol. Use of these antibiotics in topical formulations can be a new area of research and further studies.

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