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Research Article

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Aerobic and Anaerobic Bacterial Isolates from Deep-Seated Infections: A Microbiological Study at a Tertiary Care Center in Western Rajasthan

Aditya Kundu[1], Sarika P Kombade[2*], Vijaya Lakshmi Nag[2], Amit Goel[3], RamKaran Chaudhary[4], Naveen Dutt[5], Kirti Chaudhary[6] and Vaibhav Kumar Varshney[7]

[1]*Department of Microbiology, All India Institute of Medical Sciences, Kalyani*
[2]*Department of Microbiology, All India Institute of Medical Sciences, Jodhpur*
[3]*Department of ENT, All India Institute of Medical Sciences, Jodhpur*
[4]*Department of General Surgery, All India Institute of Medical Sciences, Jodhpur*
[5]*Department of Pulmonary Medicine, All India Institute of Medical Sciences, Jodhpur*
[6]*Department of Dentistry, All India Institute of Medical Sciences, Jodhpur*
[7]*Department of Surgical gastroenterology, All India Institute of Medical Sciences, Jodhpur*

[*]**Corresponding author**
Sarika P Kombade, Additional Professor, Department of Microbiology, AII India Institute of Medical Sciences, Jodhpur, Rajasthan.

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ABSTRACT

Deep seated pyogenic infections (DSPI) are one of the most common causes of hospitalization resulting in high mortality & morbidity. DSPI are majorly polymicrobial in nature caused by anaerobic and aerobic bacteria. Very few studies have reported anaerobic isolates in DSPI. Thus, this study was formulated to determine both aerobic & anaerobic bacteria from deep seated infections in patients presenting to the institution.

Material And Methods: This was a prospective observational study conducted in a tertiary care hospital of Jodhpur from Jan 2020 – Dec 2021. The sample from deep seated infections like aspirated pus, tissue, biopsy material, etc., were collected and processed in both anaerobic and aerobic conditions and the isolates obtained were identified by both conventional and automated methods. Their antibiotic susceptibility was done according to CLSI 2020.

Result: Out of the 107 samples collected culture positive was seen in 74.8% (n=80) samples, out of which 85% (n=68) were monomicrobial and 15% (n=12) were polymicrobial. Among these 80 culture positive samples, 92 isolates were isolated; in which aerobic isolates were 92.3% (n=85) and anaerobic were 7.7% (n=7). Among aerobes, most common organism was Klebsiella pneumoniae 35% (n=30). Among the anaerobes, Bacteroides fragilis 71% (n=5) was the predominant isolate. Among the aerobes, ESBL and MBL was detected in 15.7% and 43.7% of total GNBs respectively.

Conclusion: This cross-sectional study gives an idea about prevalence of the common aetiology causing deep seated pyogenic infection and also the antibiotic resistance pattern of the isolates in the western part of Rajasthan.

Keywords: Deep Seated Pyogenic Infections, Anaerobic Infection, Aerobic Infection, Bacteroides Fragilis, Klebsiella Pneumonia

Introduction and Review of literature

Deep-seated pyogenic infections (DSPI) are difficult to diagnose and mostly polymicrobial in nature, hence, which leads to significant morbidity and mortality [1]. These infections can occur in various body parts, including fascia, muscles, and internal organs, and are often localized as abscesses or inflammatory necrosis [1]. In DSPI, bacterial invasion can result in tissue necrosis and systemic illness due to the release of toxic metabolites. While pyogenic infections on the skin are usually non-fatal, infections in vital organs like the liver or brain can be life-threatening if untreated. It could be caused by several aerobic and anaerobic bacterial isolates (monomicrobial or

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polymicrobial) [2]. These organisms being endogenous normal human flora when spilled into normally sterile spaces, can cause anaerobic infections due to insufficient vascularity or obstruction [3]. Anaerobic bacteria, which are part of the normal human flora, can cause a wide range of infections, from abscesses and diabetic foot infections to life-threatening conditions like gas gangrene.

DSPI are most commonly caused by Gram-positive bacteria, particularly Staphylococcus spp., though Gram-negative bacteria are increasingly implicated [2]. Additionally, anaerobic bacteria such as Bacteroides, Clostridium, and Prevotella spp. often contribute to polymicrobial infections, particularly following trauma or surgery that disrupts mucosal barriers [3,4]. Overuse of antibiotics has led to growing antibiotic resistance, complicating treatment and increasing healthcare costs. Identifying the pathogens and understanding their antibiotic resistance patterns are crucial for effective treatment.

This study aims to identify anaerobic and aerobic bacterial isolates from deep-seated pyogenic infections in patients at a tertiary care hospital in Western Rajasthan and analyze their antimicrobial susceptibility using both conventional and automated methods. This is the first observation prospective study to identify anaerobic bacterial isolates among DSPI in western Rajasthan. The primary objectives of the study were to evaluate the antibiotic susceptibility patterns of both aerobes and anaerobes in this region.

Material and Methods

A prospective observational study was conducted at the Department of Microbiology, All India Institute of Medical Sciences, Jodhpur, from January 2020 to December 2021. Ethical approval was obtained from ethical committee with ref no AIIMS/IEC/2019-20/970. The study included patients of all age groups with deep-seated pyogenic infections, such as brain abscesses, diabetic foot infections, necrotizing fasciitis, and intraabdominal DSPI from patients admitted in OPD, IPD, and OT settings. Inclusion criteria comprised patients willing to provide consent to be part of study, with or without a history of antibiotic treatment. Exclusion criteria included patients who refused to give consent or submitted inadequate/inappropriate samples.

Two samples from each patient suspected of DSPI were collected in sterile universal container. Samples like aspirated pus, sterile body fluids, drain fluids, tissue, biopsy material, etc., were collected and first sample was processed for aerobic culture and second for anaerobic culture respectively. For aerobic culture, gram`s staining was done for all sample and part of sample was inoculated on Blood Agar, MacConkey Agar and Chocolate Agar and plates were incubated in the incubator at 37˚C for 48 hours. However, for anaerobic culture, first Kopeloff modification of Gram staining and Schaeffer and Fulton spore staining was done. Afterward, sample was transferred in RCM or in Thioglycolate broth were incubated for 48 hours at 37˚C incubation and subculture was done on neomycin blood agar, brain heart infusion agar and Bacteroides bile esculin agar and for gram positive bacilli, subculture was also done on egg yolk agar media (Willis &Hobbs Medium) and plates were kept in the anaerobic jar with gas pack along with resazurin indicator disc and jar was incubated for further 48 hours [5].

After 48 hours of incubation, colony morphology was observed and aerotolerance test was done to identify obligate and facultative anaerobes. Identification of anaerobic bacteria was done by conventional biochemical tests like Catalase, Indole, Urease, Glucose, Lactose, Mannose fermentation, Gelatin liquefaction, Litmus milk test etc. Vancomycin (VA)(5µg), Kanamycin (Ka)(1000µg), Colistin (Cl)(10µg) & SPS disks potency test was used to differentiate between gram positive and gram-negative anaerobes. Peptostreptococcus anaerobius will be sensitive to Vancomycin & SPS while Clostridium species only seen to Vancomycin and resistant to Colistin and kanamycin. Furthermore, Bacteroides fragilis group will be sensitive to colistin but resistant to Va & Ka [5].

Antimicrobial susceptibility testing (AST)of aerobic isolate was done by both conventional and automated method. Conventional AST was done by Kirby-Bauer disc diffusion after adjusting inoculum to 0.5 MacFarland turbidity according to Clinical and Laboratory Standards Institute (CLSI) 2020 guideline using antibiotic disc like Ceftriaxone 30µg, Piperacillin-Tazobactam 100/10µg, Cotrimoxazole 1.25/23.75 µg, Amikacin 30µg, Gentamicin 10µg, Ciprofloxacin 5µg, Meropenem 10 µg, Imipenem 10µg, Ertapenem 10 µg, Aztreonam 30 µg,, Minocycline 30 µg along with additional antibiotics for GPC like Erythromycin 15µg, Clindamycin 2µg, Vancomycin 30 µg, Linezolid 30µg & Teicoplanin 30µg [6].

AST of anaerobic isolates was done on Brucella Blood Agar by MIC detection with E-strip. For anaerobes antibiotic E strips of antibiotics like Piperacillin/Tazobactam, Amoxicillin-Clavulonic acid, Cefoperazone, Meropenem, Imipenem & Metronidazole were tested. Automated identification and AST of aerobic isolate and only identification of anaerobic isolates was done by VITEK 2 Compact using GN, GP and ANC Card respectively. Anaerobic isolates were also confirmed by MALDI TOF MS [6].

Result

Total 107 patients with DSPI were included in this study. Male patients 69% (n=74) were dominated over female patient 31% (n=33). Maximum 32% (n=34) patients were from adult age group ranging from 21-40 years followed by elderly age group 30% and 20% from age group 41-60 years and 61-80 years respectively. In this study, most common DPSI were abscesses 59% (n=63) followed by gangrene on limbs 9% (n=10), necrotizing fasciitis in 5 %(n=5), Grade 3 Bed sore in 6% (n=7) and 22% (n=21) cases of diabetic foot, deep ulcers, gingivitis respectively shown in Figure 1. As shown in Fig 2, most common abscess was the intraabdominal 32% (n=20) followed by on the limb 27% (n=17) and brain in 10% (n=16), maxillo-facial site in 14% (n=9) & abscesses were on back, gluteal region in17% (n=11) cases.

Most common type of sample collected was pus 66% (n=71), tissue biopsy 32% (n=34) and aspirated fluid in 2% cases respectively.

Out of the 107 DSPI samples, 25.2% (n=27) samples were sterile and bacterial growth were present in 74.8% (n=80) samples. Out of the 80 samples polymicrobial infections were found in 15% (n=12) cases and monomicrobial infections were seen in 85%

(n=68) cases. Out of 80 culture positive samples 92 isolates were isolated, among which aerobic bacteria were isolated in 92.3% (n=85) while anaerobic bacteria in 7.7% (n=7) samples respectively. Among the aerobic isolates most common isolates were gram negative bacteria (GNB) 75.2% (n=64) followed by gram positive cocci (GPC) 24.8% (n=21).

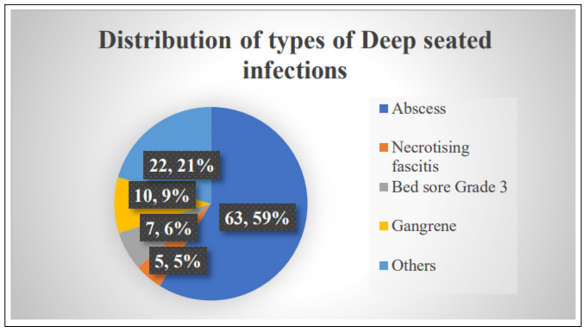

Figure 1: Distribution of type of deep seated infections

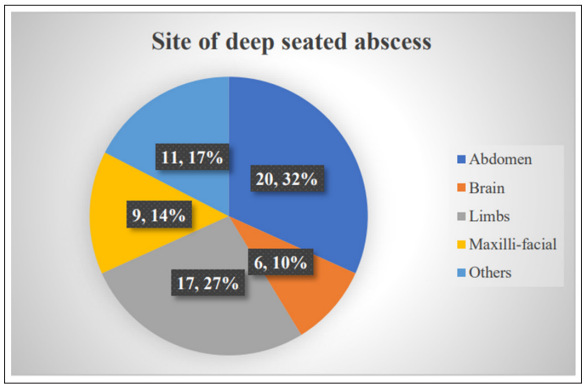

Figure 2: Site of deep seated abscess

Among GNB, Klebsiella pneumoniae 35% (n=30) was most common followed by Escherichia coli which in 26% (n=22) cases and others were Enterobacter aerogenes 2% (n=2), Citrobacter freundii 1% (n=1), Klebsiella oxytoca 2% (n=2), Proteus vulgaris 1% (n=1), Acinetobacter baumannii 5% (n=4), Pseudomonas aeruginosa 2% (n=2). Among the GPCs Staphylococcus aureus was most common in 18% (n=15) followed by Enterococcus faecalis in 6% (n=5) and Streptococcus pyogenes in 1% (n=1) cases respectively. Aerobic profile of the isolated organisms is shown in Figure 3.

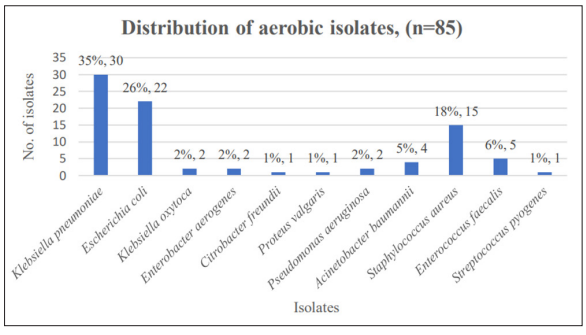

Figure 3: Distribution of aerobic isolates.

Among the 7 anaerobes Bacteroides fragilis 67% (n=5) was the most common isolate followed by *Peptostreptococcus anaerobius* and *Clostridium perfringes* in 14.5% (n=1) cases respectively as shown in Figure 4. Growth of anerobic organisms shown in Figure 5.

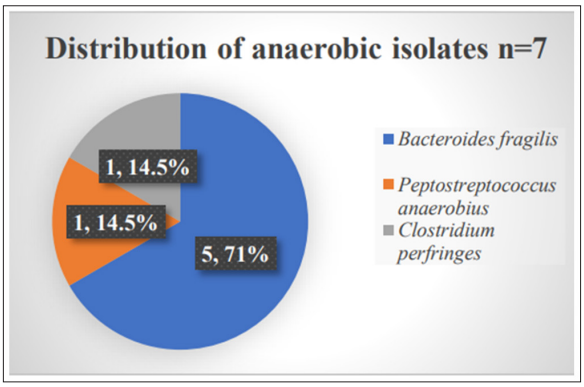

Figure 4: Distribution of anerobic isolates.

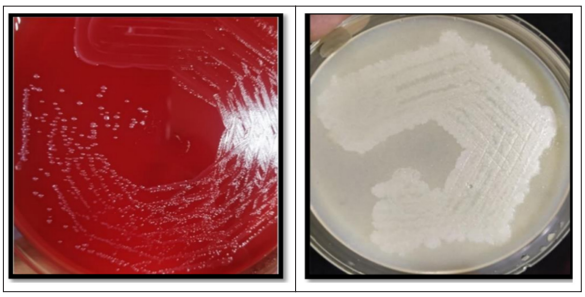

Figure 5: Brucella Blood agar showing growth of *Peptostreptococcus* and Egg yolk agar showing growth of Clostridium perfringes

Among GNB high resistance was observed to 3rd and 4th generation cephalosporins like ceftriaxone 62.5% & cefepime 59.3%. Piperacillin-tazobactam in 46.8% cases and aminoglycosides like amikacin, gentamicin was resistant in 46.8% and 51.5% respectively. Fluoroquinolones like ciprofloxacin was resistant in 56.3% cases of GNBs.

Carbapenems were resistant in 46.8% cases of GNBs. Minocycline was sensitive in 32.8% cases while no resistance was detected in colistin except the intrinsic resistance one. Multidrug resistance was seen in Klebsiella pneumoniae and Escherichia coli. Extended spectrum beta lactamase (ESBL), AmpC beta-lactamase (AmpC) production and metallo-beta-lactamase (MBL) resistance among GNB was 15.7%, 4.6% and 43.7% respectively. High ESBL and AmpC production was detected in Escherichia coli (22.72% & 9%) respectively while high MBL production was seen in 60% Klebsiella pneumoniae as shown in Table 1.

Table 1: Distribution of β-lactamases in the Enterobacterales.

Organisms	ESBL	AmpC	MBL
Klebsiella pneumoniae (n=30)	16.6% (n=5)	3% (n=1)	60% (n=18)
Escherichia coli (n=22)	22.72% (n=5)	9% (n=2)	45.4% (n=10)
Enterobacter aerogenes	0	0	0
Citrobacter freundii	0	0	0

Klebsiella oxytoca	0	0	0
Proteus vulgaris	0	0	0
Total GNB (n=64)	15.7% (n=10)	4.6% (n=3)	43.7% (n=28)

Among GPC, Erythromycin and clindamycin resistance was seen in 33.3% and 26.6%, Ciprofloxacin and gentamicin resistance in 13.3% cases respectively. No resistance was detected to vancomycin, linezolid and teicoplanin in staphylococcus and *Enterococcus feacalis* and *Streptococcus pneumoniae*. Among Staphylococcus aureus, Methicillin resistant Staphylococcus aureus (MRSA) was seen 60% cases & Inducible clindamycin resistance was detected in 26.6% cases.

No resistance to anaerobes were seen to Ceftriaxone 30µg, Piperacillin- Tazobactam 100/10µg, Cotrimoxazole 1.25/23.75 µg, Meropenem 10 µg & Metronidazole.

Discussion

Deep-seated pyogenic infections characterized by the abscess in deeper sites with accumulation of pus in tissues, pose significant challenges in clinical management. The microbial pathogens, as well as, their antibiotic sensitivity pattern, may change from time to time and place-to-place and so local profile of resistant pattern of these organisms is crucial for effective treatment. With the growing incidence of MDR, the management of deep-seated infections has become increasingly complex and a global health concern.

In present study male predominance of high with a male-to-female ratio of 2.2:1. This gender disparity is comparable with other studies, such as those by Rijal BP (66.3% male subjects) and JM Kalita (M: F ratio of 1.6:1) [1,2]. The male predominance can be attributed to greater exposure to outdoor environments and work-related risks, which increase the likelihood of developing DSPI infections like deep abscesses and wounds.

Regarding age distribution, the majority of the participants were from the 21-40 years age group (32%), followed by those in the 41-60 years age group (30%). This finding aligns with similar studies in India and Nepal, which report a higher incidence of infections in the 20-40 age range [1, 3]. Rate of DSPI in this study was 59% was consistent with other study done by Saini (43%) [4].

Among DSPI, intra-abdominal abscesses were the most common site involved in 32% of cases. This finding was consistent with previous research by Basireddy and Brook, who also identified the intra-abdominal region as the primary site for abscess formation due to its rich blood supply and abundant commensal flora [7,8]. The peritoneal cavity's high level of microbial flora makes it a common site for deep-seated infections, which can lead to abscess formation when the mucosal barriers are breached.

In this study,74.8% microbial growth was seen among all DSPI sample processed for culture, which is consistent with the findings of other studies such as Basireddy (70%) and Rana (72.9%) [7,3]. A total of 92 bacterial isolates were identified from the 80 positive cultures, with an average of 1.16 isolates per sample. This is comparable to other studies, such as those by Basireddy and Pramodhini S, who observed rate of bacterial isolates per sample as 1.2 and 1.45, respectively [7, 9].

Out of the 80 positive samples,15% showed polymicrobial infections, and 85% were monomicrobial infections. Polymicrobial infections, included both aerobic and anaerobic bacteria, mostly isolated from samples from oral abscesses, diabetic foot ulcers, and deep venous ulcers.

The most frequently isolated aerobic organisms were GNB in 75.2% cases, while GPC accounted for 24.8% cases. Itzhak brook also found GNB over GPC among DSPI while Brook and Finegold, Saini, Basireddy, S. Pramodhini found GPC as most common aerobic bacteria among DSPI [5-9].

Among GNB, Klebsiella pneumoniae (35%) was the most common followed by Escherichia coli (26%). This is consistent with studies by Verma P and Gill MK, which also identified Klebsiella pneumoniae as the most common Gram-negative pathogen [10, 11]. Comparison is shown in Table 2.

Table 2: Comparison of aerobic isolates in various studies.

	Itzhak Brook et al [8]	Pramodhini S et al [9]	Saini et al [4]	Manmeet kaur Gill et al. [11]	Basireddy et. al [7]	Poonam Verma et al [10]	JM Kalita et al [2]	Present study
Klebsiella pneumoniae	2%	9.7%	9%	12.3%	12%	33%	14.4%	35%
Escherichia coli	15%	17.9%	13%	29.2%	24%	16%	24.7%	26%
Enterobacter aerogenes	-	-	-	4.9%	-	-	1.7%	2%
Klebsiella oxytoca	-	-	-	-	-	-	-	2%
Citrobacter freundii	-	-	-	1.3%	4%	-	1.4%	1%
Proteus vulgaris	9%	4.4%	4%	3.1%	4%	7%	1.5%	1%
Pseudomonas aeruginosa	3%	-	11%	11.1%	9%	18%	16.6%	2%
Acinetobacter baumannii	-	-	-	8%	-	-	8.3%	5%
Staphylococcus aureus	26%	38%	30%	20%	29%	40%	30%	18%
Enterococcus faecalis	7%	-	-	1%	6%	-	4.6%	6%
Streptococcus pyogenes	-	16%	6%	0.2%	6%	-	0.5%	1%

Among the GPC, S. *aureus* was the most common (18%), followed by *E. faecalis* (6%). These findings are in line with other studies, such as those by Manmeet Kaur Gill and Pramodhini, where S. *aureus* accounted for 20% to 38% among GPC isolates [11,9].

In this study only 7.7% anaerobic organisms were isolated which is very low as compared to Basireddy (25.4%), Brook (63%) [11]. The predominant anaerobic isolate in this study was Bacteroides fragilis (71%), followed by *Peptostreptococcus anaerobius* and *Clostridium perfringens* (14.5% each). These findings are consistent with studies by Pramodhini et al. and Saini et al., where Bacteroides fragilis was also the predominant anaerobic isolate among DSPI [9,4]. Compared in Table 3.

Table 3: Comparison of anaerobic isolates in different studies

	Itzhak Brook et al. [8]	Pramodhini S et al. [9]	Saini et al. [4]	Basireddy et. al [7]	Kedar Mohan et al. [16].	Present study
Bacteroides fragilis	16%	59%	60%	23%	-	71%
Peptostreptococcus anaerobius	35%	41%	-	41%	38%	14.5%
Clostridium perfringes	10%	-	-	9%	50%	14.5%

One of the most concerning aspects of this study is the high rate of antimicrobial resistance (AMR) observed, particularly among the Gram-negative pathogens. A significant proportion of isolates, including *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*, showed resistance to commonly used antibiotics, such as third-generation cephalosporins (e.g., ceftriaxone), fluoroquinolones (e.g., ciprofloxacin), and carbapenems (e.g., meropenem). Resistance rates for *Klebsiella pneumoniae* to ceftriaxone, cefepime, and piperacillin-tazobactam were found to be 73.3%, 70%, and 56.6%, respectively [1,2].

Carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* were also prevalent, with 63.3% and 45.4% resistance to meropenem, respectively. Furthermore, extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* and *Escherichia coli* were found in 16.6% and 22.72% of cases, respectively. Metallo-beta-lactamase (MBL) production was also observed in 60% of *Klebsiella pneumoniae* and 45.4% of *Escherichia coli* isolates, Basireddy also found similar findings in his studies [7,12,13].

In present study MRSA was detected in 60% of cases which is compared to other studies like Pramodhini and Basireddy [7,9]. Additionally, this study found inducible clindamycin resistance in 26.6% among *Staphylococcus aureus* which was comparable to study conducted by Walia K, highlighting the challenges in treating infections caused by this pathogen [12].

All the anaerobe isolated were 100% sensitive to the antimicrobials used against them like beta- lactam inhibitors, imidazole, macrolides, carbapenems etc which corresponds to the finding by Pramodhini and Saini [9,4]. In the study conducted by Ritu Garg most of the anaerobic isolates were 100 % sentitive to the antimicrobials, however metronidazole was resistant in 16% cases [13].

The environment of an abscess is detrimental to many antimicrobials. The abscess capsule, the low pH level and the presence of binding proteins or inactivating enzymes such as β-lactamase may impair the activity of many antimicrobial agents. Management of mixed aerobic and anaerobic infections requires surgical correction and drainage of pus and the administration of antimicrobial agents effective against both aerobic and anaerobic bacteria [14-17]. Without adequate therapy DSPI will persist and increase the morbidity.

Conclusions

The findings of this study underscore the growing threat posed by MDR aerobic pathogens in deep-seated pyogenic infections, particularly those caused by Klebsiella pneumoniae, Escherichia coli, and Acinetobacter baumannii.

The high rates of resistance to critical antibiotics like third-generation cephalosporins, carbapenems, and fluoroquinolones highlight the need for more judicious use of antimicrobials & appropriate infection control measures. Drainage of abscesses and the use of combination therapy targeting both aerobic and anaerobic pathogens are essential for effective management.

The findings also emphasize the importance of strengthening antimicrobial stewardship programs, particularly in countries with high rates of antibiotic misuse and overuse, like India. DSPI are caused by a variety of etiological agents and are often difficult to treat. Identifying the etiological agents and knowing its susceptibility pattern will help in the better management of the patients.

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