

Original research article

Bacteriological profile and their antibiogram of skin and soft tissue infection in a tertiary care health institution in Dahod, Gujarat

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Abstract

Background: Soft tissue infections are one of the most common presenting problems in hospital settings. Microorganisms are commonly found in the hospital environment and are responsible for significant morbidity and mortality. Antibiotic resistance and its rapid spread in bacterial isolates are one of the biggest threats to global health. We have conducted a study to determine the bacterial etiology and its antibiotic susceptibility pattern of skin and soft tissue infection in a tertiary care hospital.

Aim: To find the prevalence, bacterial profile and antimicrobial resistance pattern of skin and soft tissue infection.

Results: A total of 1005 pus samples from patient were collected and cultured. Of them 28.05% positivity rate for the pathogenic bacterial isolate was observed. The most common pathogen was *Klebsiella* spp (23.04%) followed by *Pseudomonas aeruginosa* (19.14%), *Staphylococcus aureus* (15.95%) and *Escherichia coli* (15.95%). Gram positive bacteria showed high resistance to Penicillin-G, Ampicillin, Gentamycin and Cotrimoxazole while Gram negative bacteria were more resistant to Ampicillin, Cefotaxime, Ceftazidime and Cefepime.

Conclusion: Timely identification of the pathogen and its antibiogram from clinical specimen of soft tissue infection is an important function of clinical microbiology laboratory; it is beneficial for the patient in selection of appropriate antimicrobial therapy.

Key words: Skin and Soft tissue infection, Bacterial infection, Antibiogram

Introduction

Soft tissue infections are one of the most common presenting problems in both indoor and outdoor patients of hospitals. Microorganisms play an important role to spread this infection during and after any accidental trauma, burn injuries and during and after surgical procedures. The outcome of this infection leads to pus formation which is a white to yellow fluid made of dead white blood cells, cellular debris, and necrotised tissues^{1, 2}. Wound

infection is caused by both aerobic and anaerobic bacteria; these are commonly found in the hospital environment and are responsible for significant morbidity and prolonged hospitalization. This may lead to huge economic burden³. The prevalence of soft tissue and skin infection in developed countries varies between 3 to 11% , while in developing countries it is estimated to be as high as 40% ^{4,5}. Degree of wound contamination affects the severity of clinical infection and it is estimated that 50% of wounds contaminated by bacteria become clinically infected ⁶. Antibiotic resistance and its rapid spread in bacterial isolates are one of the biggest threats to global health. Multidrug resistant gram positive and gram negative bacterial prevalence associated with pus samples are on high especially in hospital settings because of the inadequate drug dose and misprescription of the antibiotics ^{7, 8}. Inappropriate use of antimicrobials agent creates selection pressure on the microbes which favours the growth of pathogenic drug resistant bacteria leading to complicated empirical antimicrobial therapy ^{9,10}. Infection in soft tissue constitutes several risk like delay in healing of wound, trauma, disarticulation and amputation, prolongs hospital stay, need for increased medical attention and increases treatment costs ¹¹. Therefore infection of soft tissue is a matter of concern and study of the causative agents and their antibiotic pattern becomes very useful to guide hospital infection control and antibiotic usage policy. We have conducted a study to determine the bacterial etiology and its antibiotic susceptibility pattern of soft tissue infection.

Materials and Methods:

Hospital based prospective study was conducted at the department of Microbiology, Zydus Medical College and Hospital, Dahod, Gujarat. Pus samples from the patient of soft tissue infection were collected during the period between January 2019 to December 2020. Patients with post-operative wound infection, burn wound infection, diabetic wound, infected wounds due to trauma, and patients with other infected wounds admitted at Zydus Hospital, were included in this study. Pus samples which were found growth for the skin contaminants were not included in this study.

Sample collection and culture

Wound swab and aspirated pus samples were collected from the patient. Nurses posted in the respective wards were allowed to collect these samples. The wound was thoroughly washed with sterile normal saline prior to the sample collection to avoid any contamination with skin flora or necrotic tissue. Sterile gauze was used to remove excess saline from the wound surface. A sterile swab was placed in middle of the wound and pus was collected by swabbing around the wound. When there were two or more wounds in the same location, separate swabs were used for each wound ¹². Pus swabs were streaked on Blood Agar (BA) and Mac-Conkey Agar (MCA) plates and incubated aerobically for 18–24 h at 37 °C. They were then observed for bacterial growth. Plates with no growth and with growth were re-incubated for another 18–48 h for isolation of bacteria that require extended incubation ^{12, 13}.

Identification of bacteria

Standard culture and identification techniques were used to identify pathogenic bacteria isolated in pure cultures. The characterisation and identification were done by its morphological appearances of colonies on culture media, Gram stains and standard biochemical tests including catalase, coagulase, oxidase, Indole, Triple sugar iron agar, citrate utilisation test, urease production test, etc.

Antimicrobial Susceptibility testing

Antibiotic susceptibility tests were conducted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines¹⁴. Kirby–Bauer disc diffusion method was used to perform antimicrobial susceptibility tests. A suspension of normal saline with the isolated growth was prepared; the density of suspension was fixed to the optical density of McFarland 0.5 Barium sulphate solution. A sterile swab was dipped into the suspension, squeezed free from excess fluid against the side of tube and spread over the Mueller–Hinton agar plate. The appropriate antibiotic disc were placed onto the media and incubated at 37 °C for 16–18 hrs. Zones of inhibition were read and resistance rates to respective antibiotics were determined as per the CLSI guidelines.

Results:

A total of 1005 pus samples from patient were collected and processed for culture and sensitivity. Of them, 282 (28.05%) were found positive for the pathogenic bacterial growth. The most common isolate was *Klebsiella*spp (23.04%) followed with *Pseudomonas aeruginosa* (19.14%), *Staphylococcus aureus* (15.95%), *Escherichia coli* (15.95%), Coagulase negative *Staphylococcus*spp (13.82%), *Enterobacter*spp (5.31%), *Acinetobacter*spp (3.19%), *Streptococcus*spp (2.12%) and *Enterococcus*spp (1.06%), shown in table 1 and figure 1.

Table 1: Distribution of bacterial pathogens isolated from pus samples

S.No	Bacterial Pathogen	No of isolates (%)
	<i>Klebsiella</i> spp	66(23.40)
	<i>Pseudomonas aeruginosa</i>	54(19.14)
	<i>Staphylococcus aureus</i>	45(15.95)
	<i>Escherichia coli</i>	45(15.95)
	Coagulase negative <i>Staphylococcus</i> spp (CONS)	39(13.82)
	<i>Enterobacter</i> spp	15(5.31)
	<i>Acinetobacter</i> spp	9(3.19)
	<i>Streptococcus</i> spp	6(2.12)
	<i>Enterococcus</i> spp	3(1.06)
Total		282

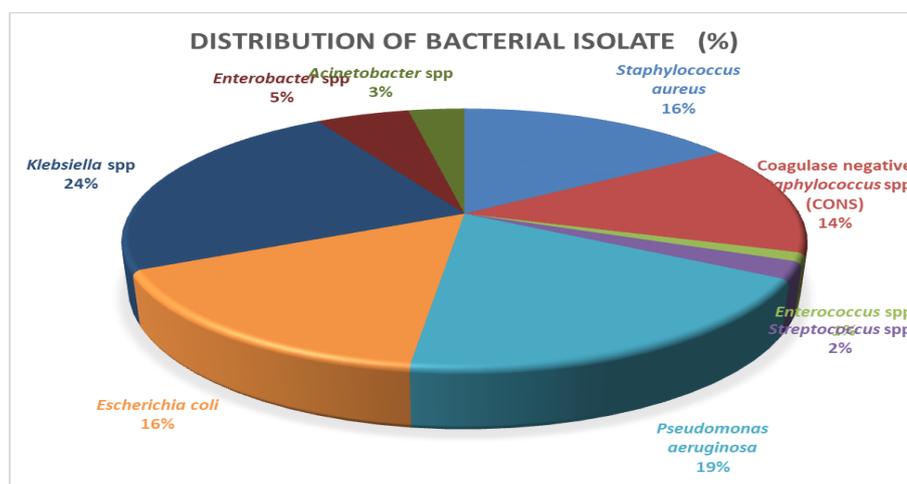


Figure 1:

Antibiogram of Gram Positive isolates

Staphylococcus aureus showed high resistance to Penicillin G (26.66%) while all the isolates of *Staphylococcus aureus* were susceptible to Teicoplanin. 13.33% of *Staphylococcus aureus* strain were found as MRSA (Methicillin Resistant *Staphylococcus aureus*). Coagulase negative *Staphylococci* also showed high resistance to Penicillin G (38.46%) however all the strains of CONS were found susceptible to Linezolid, Teicoplanin and Clindamycin. *Enterococcus* spp and *Streptococcus* spp were all susceptible to routinely employed antibiotics except for vancomycin (100%) which was resistant for all the isolates, shown in table 2 and figure 2.

Table 2: Antibiotic resistance pattern of Gram Positive isolates

Antibiotics (Disc potency)	<i>S.aureus</i> n=45 (%)	CONS n=39 (%)	<i>Enterococcus</i> spp n=3 (%)	<i>Streptococcus</i> spp n=6 (%)
Penicillin–G (10 unit)	12(26.66)	15(38.46)	0(0.00)	0(0.00)
Ampicillin (10 µg)	6(13.33)	12(30.76)	0(0.00)	0(0.00)
Linezolid (30 µg)	3(6.66)	0(0.00)	0(0.00)	0(0.00)
Erythromycin (15µg)	6(13.33)	12(30.76)	0(0.00)	0(0.00)
Clindamycin(2µg)	3(6.66)	0(0.00)	0(0.00)	0(0.00)
Gentamycin (10 µg)	8(17.77)	6(15.38)	Not applied	0(0.00)
Cefoxitin (30 µg)	6(13.33)	3(7.69)	Not applied	Not applied
Teicoplanin(30 µg)	0(0.00)	0(0.00)	Not applied	Not applied
Cotrimoxazole (1.25/23.75 µg)	6(13.33)	12(30.76)	0(0.00)	0(0.00)
Vancomycin (30 µg)	Not applied	Not applied	3(100)	6(100)

n- No of isolates

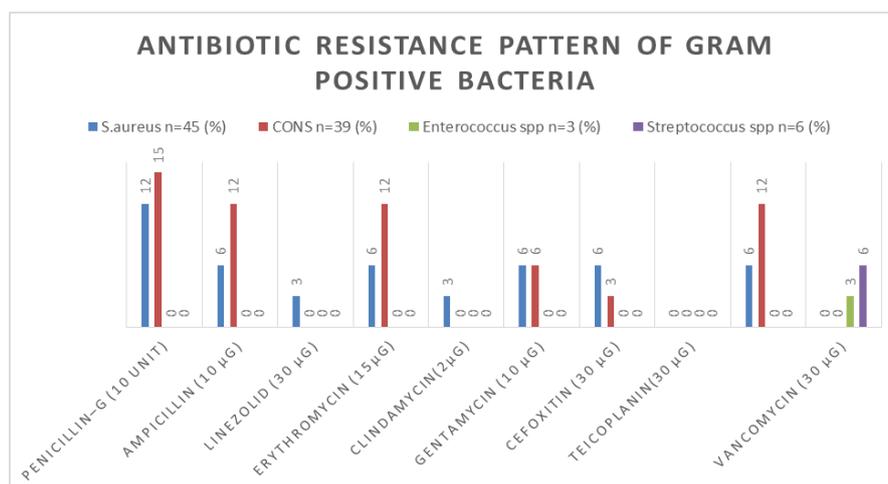


Figure 2:

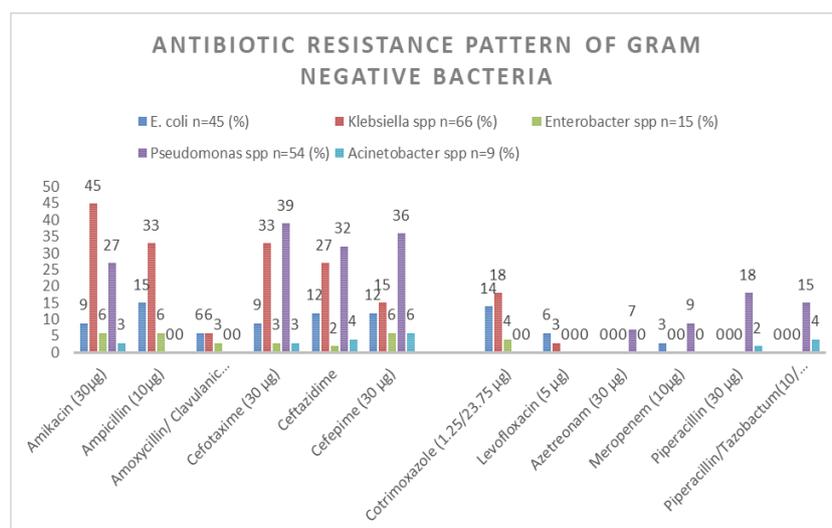
Antibiogram of Gram Negative isolates

Escherichia coli showed high resistance to Ampicillin (33.33%). *Klebsiella* spp showed highest resistance to Amikacin (68.18). *Enterobacter* spp were more resistant for Amikacin and Cefepime (40% each). *Pseudomonas* spp and *Acinetobacter* spp, were found to be more resistant to Cefepime (66.66% each) shown in table 3 and figure 3.

Table 3: Antibiotic resistance pattern of Gram negative isolates

Antibiotics (Disc potency)	<i>E. coli</i> n=45 (%)	<i>Klebsiella</i> spp n=66 (%)	<i>Enterobacter</i> spp n=15 (%)	<i>Pseudomonas</i> spp n=54 (%)	<i>Acinetobacter</i> spp n=9 (%)
Amikacin (30µg)	9(20.00)	45(68.18)	6(40.00)	27(50.00)	3(33.33)
Ampicillin (10µg)	15(33.33)	33(50.00)	6(40.00)	Not applied	Not applied
Amoxycillin/ Clavulanic acid(20/10µg)	6(13.33)	6(9.09)	3(20.00)	Not applied	Not applied
Cefotaxime (30 µg)	9(20.00)	33(50.00)	3(20.00)	39(72.22)	3(33.33)
Ceftazidime (30 µg)	12(26.66)	27(40.90)	2(13.33)	32(59.25)	4(44.44)
Cefepime (30 µg)	12(26.66)	15(22.72)	6(40.00)	36(66.66)	6(66.66)
Cotrimoxazole (1.25/23.75 µg)	14(31.11)	18(27.27)	4(26.66)	Not applied	Not applied
Levofloxacin (5 µg)	6(13.33)	3(4.54)	0(0.00)	Not applied	Not applied
Aztreonam (30 µg)	Not applied	Not applied	Not applied	7(12.96)	Not applied
Meropenem (10µg)	3(6.66)	0(0.00)	0(0.00)	9(16.66)	0(0.00)
Piperacillin (30 µg)	Not applied	Not applied	Not applied	18(33.33)	2(22.22)
Piperacillin/ Tazobactam (10/10 µg)	Not applied	Not applied	Not applied	15(27.77)	4(44.44)

n- No of isolates

**Figure 3:**

Discussion:

Infection of soft tissue is a challenging problem globally; it is responsible for high mortality and morbidity. Antibiotic resistance to the wound pathogens are on the higher side which produces tremendous financial burden to the health infrastructure of a hospital^{15, 16}. Timely identification of the pathogen and its antibiogram from clinical specimen of soft tissue infection is an important function of clinical microbiology laboratory; it is beneficial for the patient in selection of appropriate antimicrobial therapy.

This study showed positivity rate of soft tissue infection to 28.05% which can be correlated with the infection incidence rates of 6.09% to 38.7% in various Indian studies^{17, 18, 19, 20, 21}. Different studies from various developed countries exhibited a lower incidence of skin and soft tissue infection ranging from 2.8% to 19.4%^{22, 23, 24, 25}. A higher infection rate in developing countries indicates that there is need of better formulation and implementation of hospital infection control practices.

In our study, incidence of Gram negative bacterial wound infection was more (67.02%) than the Gram positive bacterial infection (32.97%) which is in concordance with the study of Anvika et.al in 1999 and Agarwal et.al 2001^{26,27}. Staphylococcus aureus (50.56%) and Klebsiellaspp (34.92%) were the most common pathogen isolated among gram positive and gram negative bacteria respectively. Similar findings were observed in a study from Rajasthan, India in which highest percent of Staphylococcus aureus isolates were found in infected clean wounds (37.50%), while highest percent of both Klebsiellapneumoniae both and Pseudomonas spp. isolates were found in infected contaminated wounds (47.62% and 33.33%, respectively)²⁸.

The antibiogram of gram positive bacteria reveals, high resistance to Penicillin-G, Ampicillin, Gentamycin and Cotrimoxazole for both S.aureus and Coagulase Negative Staphylococcus spp. Narula H et al had found high resistant to ampicillin (ranging from 88.9% to 100%), and Cotrimoxazole (ranging from 60% to 100%) among gram-positive isolates (28). Gram negative isolates showed high resistance to ampicillin and other Cephalosporin like Cefotaxime, Ceftazidime and Cefepime which is in concordance with the previous studies^{29,30,31,32}. The higher rate of resistance to beta-lactum group of antibiotics could be due their non-judicious and overuse of these drug triggering production of extended-spectrum beta-lactamases (ESBLs) by microorganisms.

Conclusion:

Based on the findings of the present study following recommendation can be made to decrease the prevalence of skin and soft tissue infection in the hospital set up:-

Strict adherence to the standard safety precaution by all the healthcare personnel to prevent hospital acquired infections.

Proper sample collection, transportation, culture and antimicrobial susceptibility testing of all the suspected patient of skin and soft tissue infections

Encourage clinicians to follow the culture sensitivity report while prescribing empirical antimicrobial therapy.

Active surveillance of antibiotic susceptibility testing data.

Formulation and implementation of antibiotic policy as per the guidelines.

References:

1. Cogen A L, Nizet V, and Gallo R L. Skin microbiota: a source of disease or defence? *British Journal of Dermatology*. 2008; 158(3): 442–455.
2. Scalise A, Bianchi A, Tartaglione C et al., Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors, *Seminars in Vascular Surgery*.2015; 28(3-4):151–159.

3. Bowler P G, Duerden B I, and Armstrong D G, Wound microbiology and associated approaches to wound management, *Clinical Microbiology Reviews*. 2001; 14(2): 244–269.
4. Mayon-White RT, Ducel G, Kereselidze T, Tikomirov E. An international survey of the prevalence of hospital-acquired infection. *J Hosp Infect*. 1988; 11:43–8.
5. Ducel G, Fabry J, Nicolle L. Prevention of hospital-acquired infections. 2nd ed. Geneva: World Health Organization; 2002.
6. Balows A, Truper H, Dvorkin M, Harder W, Schleifer K. The prokaryotes. A handbook on the biology of bacteria: proteobacteria: gamma subclass. 2nd ed. New York: Springer; 1991.
7. Rice L B. Antimicrobial resistance in gram-positive bacteria, *The American Journal of Medicine*. 2006; 119(6): S11–S19.
8. Iredell J, Brown J, and Tagg K. Antibiotic resistance in *Enterobacteriaceae*: mechanisms and clinical implications, *British Medical Journal*. 2016; 352: ID h6420.
9. Al-Momany NH, Al-Bakri AG, Makahleh ZM, Wazaify MMB. Adherence to international antimicrobial prophylaxis guidelines in cardiac surgery: a Jordanian study demonstrates need for quality improvement. *J Manag Care Pharm*. 2009; 15:262–71.
10. Manyahi J. Bacteriological spectrum of post operative wound infections and their antibiogram in a Tertiary Hospital, Dar Es Salaam, Tanzania. Master thesis in Medicine (Microbiology and Immunology). Muhimbili University of Health and Allied Sciences; 2012.
11. Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev*. 1993; 6:428–42.
12. Mohammed A, Adeshina G, Ibrahim YK. Incidence and antibiotic susceptibility pattern of bacterial isolates from wound infections in a tertiary hospital in Nigeria. *Trop J Pharm Res*. 2013; 12(4):617–21.
13. Taiwo S, Okesina A, Onile B. In vitro antimicrobial susceptibility pattern of bacteria isolated from infected wounds at the University of Illorine teaching Hospital. *Afr J ExpExp Microbiol*. 2002; 3:13–6.
14. CLSI. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplements. CLSI document M100-S25. 2015.
15. Bowler P G, Duerden B I, & Armstrong, D G. Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*. 2001; 14:244-269.
16. Mulu A, Moges F, Tessema B & Kassu A. Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital, Northwest Ethiopia. *Ethiopian Medical Journal*. 2006; 44: 125-131.
17. Agarwal P K, Agarwal M, Bal A, Gahlaut YVS. Incidence of postoperative wound infection at Aligarh. *Indian J Surg*. 1984;46:326–33
18. Ganguly P S, Khan Y, Malik A. Nosocomial infection and hospital procedures. *Indian J Community Med*. 2000:990–1014.
19. Sharan H, Misra A P, Mishra R. Determinants of surgical site infection in rural Kanpur. *India J Evol Med Dent Sci*. 2012:1921–8.
20. Rao B N, Rao A R, Prasad KV, Murty DVSS R. A Prospective Study of Postoperative Wound Infections in a Teaching Hospital of Rural Setup. *J Clin Diagn Res*. 2012; 6:1266–71.
21. Saxena A, Singh M P, Brahmchari S, Banerjee M. Surgical site Infection among postoperative patients of tertiary care centre in central India-A prospective study. *Asian journal of Biomedical and Pharmaceutical Sciences*. 2013; 3:41–4.
22. Cruse P J, Foord R. The epidemiology of wound infection. A 10-year prospective study of 62,939 wounds. *Surg Clin North Am*. 1980; 60:27–40.

23. Olson M, O'Connor M, Schwartz M L. Surgical wound infections. A 5-year prospective study of 20,193 wounds at the Minneapolis VA Medical Center. *Ann Surg.* 1984; 199:253–9.
24. Wanger M B, da Silva N B, Vinciprova A R, Becker A B, Burtet L M, Hall A J. Hospital acquired infection among surgical patients in a Brazilian hospital. *J Hosp Infect.* 1997; 35:277–85.
25. Creamer E, Cunny R J, Humphrey H, Smyth E G. Sixteen years' surveillance of surgical sites in an Irish acute-care hospital. *Infect Control HospEpidemiol.* 2002; 23:36–40.
26. Anvikar RA, et.al A one year prospective study of 3280 surgical wounds. *Indian Journal of Medical Microbiology* 1999; 17 (3): 129-32.
27. Aggarwal A, Khanna S, Arora U, Devi P. correlation of beta-lactamase production/ methicillin resistance and phage pattern of *Staphylococcus aureus*. *Indian J Med Sci.* 2001; 55(5):253-5.
28. Narula H, Chikara G, Gupta P. A prospective study on bacteriological profile and antibiogram of postoperative wound infections in a tertiary care hospital in Western Rajasthan. *J Family Med Prim Care.* 2020; 9(4):1927-1934.
29. Lilani S P, Jangale N, Chowdhary A, Daver G B. Surgical infection in clean and clean-contaminated cases. *Indian J Med Microbiol.* 2005; 23:249–52.
30. Tripathy B S, Roy N. Post-operative wound sepsis. *Ind J Surg.* 1984; 47:285–8.
31. Rao A S, Harsha M. Postoperative wound infections. *J Indian MA.* 1975; 64:90–3.
32. Prabhakar H, Arora S. A bacteriological study of wound infections. *J Indian Med Assoc.* 1979; 73:145–8.