Original article:

The prevalance of anaerobes from cutaneous and subcutanueous wound infections

*** Kamble S [1], Pol S[2], Jose T[3], Gore V[4],Kagal A[5] Bharadwaj R[6]**

[1]*Assistant Proffessor,Department of Microbiology,BJ Medical College Pune,Maharashtra,India.

[2]Asso. Proffessor, Department of Microbiology,BJ Medical College Pune,Maharashtra,India.

[3]Biotechnology student, VIT University, Vellore.

[4]Senior Technichian, Department of Microbiology,BJ Medical College Pune,Maharashtra,India.

[5]Prof. Department of Microbiology,BJ Medical College Pune,Maharashtra,India.

[6]Prof. and HOD, Department of Microbiology,BJ Medical College Pune,Maharashtra,India.

***Corresponding author:** Shital Kamble ; **Email**:ingavaleshital@yahoo.in

Abstract:

Introduction: The role of anaerobic bacteria in human infections has been increasingly appreciated in recent years. Along with deep seated abscesses, anaerobes have reported to cause cutaneous and subcutaneous wound infections. Although, appropriate sample collection and choice of transport media still remains the key of successful recovery of anaerobes. So,the aim of the study was to know the prevalence of anaerobes with its antimicrobial susceptibility testing from cutaneous and subcutaneous wound infections and to compare the yield of anaerobes from four different transport media.

Methodology: A total of 50 samples were collected in four different transport media like Thioglycollate broth, Anaerobic transport medium (ATM), Robertsons Cooked Meat medium (RCM), Stuarts transport medium (STM), and were compared for their ability to recover the anaerobes from patients with cutaneous and subcutaneous wound infections over a period of 6 months (Jan 2012 to June 2012). The anaerobes were isolated, identified and antibiotic susceptibility testing was done as per CLSI guidelines.

Result and conclusion: From 50 samples,9 anaerobes were isolated(18%). Out of this, 88% anaerobes were obtained from swabs sent in Thioglycollate medium followed by ATM (77%), RCM (72%) and STM (60%). The predominant anaerobe isolated was clostridium spp followed by peptostreptococcus spp & propionibacterium spp. The isolates showed maximum sensitivity to clindamycin (55.5%) followed by penicillin, cefoxitin, metronidazole (44.4% each) and piperecillin (33.3%).Considering the increasing resistance in anaerobes, routine sensitivity testing of clinical isolates of anaerobes seems to be the need of hour.

Keywords: Anaerobes, Transport media, cutaneous and subcutaneous wound infections

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INTRODUCTION:

Infections caused by anaerobic bacteria are common & may be serious and life threatening but still usually overlooked. The spectrum of anaerobic infection ranges from orodental regions to life threatening Clostridial myonecrosis or gas gangrene. Along with deep seated abscesses anaerobic bacteria can cause cutaneous & subcutaneous infections in the body [(1)]. The recovery of anaerobes in the clinical specimen depends on the method of proper sample collection and transportation to the laboratory, the quality and selection of the isolation media used and method of anaerobic incubation systems. This is important not only for the reliable recovery of anaerobes, but also for the intelligent interpretation of culture results [(1)]. Isolation and identification of anaerobes can suggest the correct course of clinical treatment and thus reduce the morbidity and length of hospital stay [(1)].

Furthermore,increasing resistance to commonly used antibiotices like metronidazole and penicillin, have been a matter of concern. The broad spectrum antibiotics used as empirical therapy in case of anaerobic infections has masked the antibiotic resistance problem in a similar way as that of anaerobic pathogens [(2)]. The standardized testing methods are used to know the resistant pattern of various antibiotics, which have become essential for the treatment of patients [(2)].

So,the present study was planned to know the prevalence of anaerobes with its antimicrobial susceptibility testing from cutaneous and subcutaneous wound infections, along with the load of aerobic organisms. The sample for anaerobes was collected in four different transport media and the yield of anaerobic bacteria was compared.

Aims and Objectives:

1. To compare the yield of anaerobes from four different transport media.
2. To evaluate the prevalance of anaerobes from cutaneous and subcutaneous wound infections.
3. To identify the anaerobe and study the antibiotic susceptibility pattern of the anaerobe.
4. To evaluate the prevalance of aerobic flora and its antibiotic susceptibility pattern from cutaneous and subcutaneous wound infections.

MATERIAL & METHODS :

The prospective study was conducted for a period of 5 months from January 2012 to May 2012 & a total of 50 samples were collected from patients with cutaneous and subcutaneous wound infections from Sassoon General Hospital, Pune.The study design was approved by the Institutional Ethical committee . The patients were selected after they meet strict inclusion and exclusion criteria.They were given information about the nature of the study and if they are willing then the consent was taken.

Inclusion criteria:

1.Patients with cutaneous and subcutaneous wound infections.

2Both sexes male and female.

3.All age groups.

4.Patients who gave consent.

Exclusion criteria:

1.Patients with deep wound infections.

Sample size: 50 pus samples from the patients with cutaneous and subcutaneous wound infections were included in the study.

Sample collection – 50 pus samples from the patients with cutaneous and subcutaneous wound were from : Cellultis (39), Gas gangrene (3), Diabetic foot (2), Bed sore (2), Appendicular infection (2), Perineal region (1) and perforative peritonitis .

The pus sample was collected with a sterile swab stick and immediately inoculated into four different transport media like Robertson's cooked meat medium(RCM)**,** Thioglycollate broth, Anaerobic Transport Medium(ATM) and Stuart transport medium(STM). These transport media were incubated at 37^0C for 4 hrs.

Gram Stain – A direct smear was prepared from each transport media and stained with Gram Stain to note the microbial flora.

Anaerobic culture – Sample from four transport media was subcultured on Brucella blood agar and Wills & Hobbs medium. These plates were incubated under anaerobic condition in an anaerobic jar at 37^0C for 48 hrs. Colonies obtained were confirmed as obligate and facultative anaerobe by doing aerotolerance test. Further identification of anaerobe was done as per Wardsworth Anaerobic Bacteriology manual [(3)]. Antibiotic susceptibility testing was done by using

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Kirby Bauer Disc Diffusion technique according to CLSI guidelines [4].

Aerobic Culture – The sample was inoculated on Blood Agar (BA) and Mac Conkeys Agar, Chocolate Agar (CA) for aerobic isolation.

Identification of isolated colonies was done as per standard microbiological technique[5].Antimicrobial susceptibility testing for aerobic bacteria was done by Kirby Bauer Disc Diffusion method as per CLSI guidelines [4].

RESULTS :

Table No. 1: Isolation rate of anaerobes from different transport media.

Transport media	**Isolation rate(%)**
Thioglycollate broth	88
ATM	77
RCM	72
Stuarts transport medium	60

The isolation rate of anaerobes was high in Thioglycollate broth (88%)

Table No. 2: Distribution of number of samples collected from different sites and rate of anaerobes isolated.

Type of infection	**No. of samples**	**Anaerobes (%)**
Cellulitis	39	5 (12.8)
Gas gangrene	3	3 (100)
Diabetic foot	2	1 (50)
Bed sore	2	-
Appendicular infection	2	-
Perianal abscess	1	-
Perforative peritonitis	1	-
Total	50	9 (18)

Out of 50 samples, 9 samples (18%) showed the presence of anaerobes, cellulitis (12.8%) being the commonest infection.

Table No. 3: Anaerobes isolated from the samples.

Anaerobes isolated	**No. of isolates**	**Percentage**
Peptostreptococus spp	1	11.11
Clostridium welchii	1	11.11
Clostridium novyii	1	11.11
Clostridium septicum	1	11.11
Clostridium spp	3	33.3
Propionibacterium spp	1	11.11
Unidentified GNB	1	11.11
Total	9	

Out of 9 anaerobes isolated , Clostridium spp.(33.3%) was found to predominant one.

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Table No. 4: Antimicrobial susceptibility pattern for Anaerobes.

Anaerobes Isolated	No.	Pipere cillin	Penicillin	Cefoxitin	Clindamycin	Metro--nidazole
Cl.welchii	1	S(1) R(0)	S(1) R(0)	S(1) R(0)	S(1) R(0)	S(1) R(0)
Cl.septicum	1	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(0) R(1)
Cl.novyii	1	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(0) R(1)
Clostridium sp.	3	S(1) R(2)	S(1) R(2)	S(1) R(2)	S(1) R(2)	S(1) R(2)
Peptosptrepto--coccus spp	1	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(1) R(0)	S(0) R(1)
Unidentified GNB	1	S(1) R(0)	S(1) R(0)	S(1) R(0)	S(1) R(0)	S(1) R(0)

The anaerobes showed maximum sensitivity to Clindamycin 55.5%, followed by 44.4% to Penicillin, Cefoxitin, Metronidazole each and 33.3% to Piperecillin.

Table No. 5: Distribution of aerobes isolated from the samples.

Aerobes isolated	**No. of isolates**	**Percentage**
Pseudomonas aeruginosa	19	32.75
Klebsiella pneumoniae	16	27.58
E .Coli	6	10.34
Citrobacter spp	6	10.34
Proteus spp	3	51.72
Non fermenter GNB	1	1.72
Staphylococcus aureus	7	12
Total	58	

The predominant aerobe isolated was *Pseudomonas aeruginosa* (32.75%), followed by *Klebsiella pneumonia* (27.58%).

Table No. 6 : Antimicrobial susceptibility pattern for Aerobes.

Aerobe (GPC)	no	Penicill-in	Ciproflox-acin	Gentamic-in	Cefoxitin	Co-Trimoxazol e	Clindamy-cin	Erythromyc-in
Staphylo coccus aureus	7	S(0) R(7)	S(0) R(7)	S(2) R(5)	S(2) R(5)	S(1) R(6)	S(6) R(1)	S(2) R(5)

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Aerobe (GNB)	no	Amikacin	Ciprofloxacin	Co-Trimoxazole	Gentamicin	Cefoxitin	Imipenem
K. pneumoniae	16	S(1) R(15)	S(2) R(14)	S(4) R(12)	S(4) R(12)	S(0) R(16)	S(11) R(5)
Proteus spp	3	S(1) R(2)	S(0) R(3)	S(0) R(3)	S(1) R(2)	S(0) R(3)	S(3) R(0)
Pseudomonas aeruginosa	19	S(5) R(14)	S(5) R(14)	S(3) R(16)	S(4) R(15)	S(1) R(18)	S(14) R(5)
E. coli	6	S(2) R(4)	S(0) R(6)	S(0) R(6)	S(0) R(6)	S(0) R(6)	S(3) R(3)
Citrobacter spp.	6	S(2) R(4)	S(3) R(3)	S(2) R(4)	S(1) R(5)	S(1) R(5)	S(4) R(2)
Non fermenter GNB	1	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(0) R(1)	S(1) R(0)

The Gran negative organisms showed maximum sensitivity to Imipenem 62% ,followed by cefoxitin 34.4%, Amikacin 18.9%, Ciprofloxacin and Gentamicin 17.2% each.

DISCUSSION:

Infections caused by anaerobic bacteria are common and may be serious and life threatening if not paid attention. Cutaneous & subcutaneous wound infections are caused by polymicrobial aerobic & anaerobic bacteria. Aerobes are traditionally thought of being the usual cause of these cutaneous & subcutaneous wound infections, but recent reports, however have shown that anaerobes are equally involved[6].

The successful isolation of the anaerobes depends on the proper sample collection and transport to the clinical microbiology laboratory. Great emphasis should laid down on the sample transportation, as during sample transport, protection of the anaerobic bacteria from O_2 exposure is the critical step in the recovery of these organisms [7]. So thioglycollate broth, Robertson's cooked Meat Medium, Stuarts Transport Medium and ATM 'were used in the present study. Thioglycollate broth was found to be efficient among four transport media as could recover 88% of the anaerobes. Cheesborough Monica [8] recommends Thioglycollate broth medium to isolate the strict anaerobes if an anaerobic infection is suspected. In the contrast, Miles et al [9] and Pollock et al [10] documented that, RCM gives higher yield of anaerobic organisms than Stuart's medium. Also, Alfa M and Adria Lee et al, 1982 [11] reported ATM as the most suitable medium for the growth and isolation of both aerobes and anaerobes.

In the present study, anaerobes isolated were 18% (9 out of 50). Clostridium spp (33%) was the most predominant, followed by Peptostreptococcus spp (11%), Clostridium welchii (11%), Clostridium novyii (11%), Clostridium septicum (11%) and Propionibacterium spp (11%) respectively.Brook et al [12] reported 23% of anaerobic isolation, which is slightly higher than that of our findings. Anuradha de et al [13] isolated 7.9% of anaerobes from various clinical samples.

Ajitha M et al, Brook et al and Brook I. Finegold showed 23%, 41.33% and 65% of anaerobic isolation respectively [15, 14 , 13]. The varying recovery rate of isolation of anaerobes in different studies may be due to varying criteria of

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patient selection, differences in site sampled and culture methods employed for the isolation of organisms, geographical differences, early antimicrobial treatment and molecular diagnostic techniques used for the identification of anaerobes. [16]

In the present study, out of 50 patients, 39 (78%) of patient had cellulitis. So the higher incidence of anaerobes was seen in cellulitis patients (78%). Most of the anaerobes isolated in the present study were seen in the age group 41-60 (46%). majority of the patients in this group were with cellulitis (43%) and Gas gangrene (100%). The clinical history suggests that, these patients were suffering from certain predisposing factors like DM, immunosuppressive state which may have been responsible for complication of the wound.

As cutaneous & subcutaneous wounds infections are polymicrobial in nature, aerobes were also isolated along with the anaerobes. All the 50 samples showed the presence of aerobes with the predominance of *Pseudomonas aeruginosa* (38%).Among Gram positive organisms, *Staphylococcus aureus* (14%) was commonly found bacteria. Methicillin Resistance was seen in (71.42%) of *Staphyloccocus aureus*. Bradley W. Frazee et al [17] showed 51% of MRSA in skin and soft tissue infection where as Vincent Ki MD [18] reported 40% of MRSA rate from soft tissue infections respectively . Ajitha Mehta et al [15] reported isolation of aerobes to be 81.33% and anaerobes 41.33% respectively.

Most of the recent efforts in antimicrobial susceptibility testing has been directed towards recommendation of standard reference method for aerobes & anaerobes. Development of resistance to antimicrobial agents has long been seen among aerobic & facultative organisms, a similar situation exists among most of anaerobic pathogens. Heightened attention has been given to the resistance pattern among aerobic bacteria for several decades; awareness among the anaerobic bacteria is needed.[2]In the present study, the anaerobes showed maximum sensitivity to clindamycin(55.5%) followed by (44.4%) to Penicillin, Cefoxitin, Metronidazole each and (33.3%) to piperecillin. Resistance among anaerobes was found to be 55.5% to Metronidazole, Penicillin, Pipericillin, Cephoxitin each and 44.4% to Clindamycin.

The present study co-relates with the study done by Francis P Tally et al [19] who reported 51% sensitivity to Metronidazole. In the present study, all the aerobic organisms were found to be 18.9 % sensitive to Amikacin, 17.2 % to Ciprofloxacin and Gentamicin each, 15.5% to Co trimoxazole, 34.4% to cefoxitin and 62% to Imipenem. Similarly resistance among the aerobes was found to be 68.9% to Amikacin, 70.6% to Ciprofloxacin and Gentamicin, 72.4% to Co trimoxazole, 84.4% to Cefoxitin and 25.8% to Imipenem.

Similar results were observed by Dr. Ravishankar Reddy et al [20] where *Psuedomonas aeruginosa* (39.88%) was the commonest organisms followed by *E.coli* (29.2%). Also Imipenem was the effective drug.

Due to the upcoming resistance among anaerobes to various antimicrobial drugs, more attention should be given for the antimicrobial susceptibility pattern for anaerobes along with aerobes in case of various cutaneous & subcutaneous wound infections.

CONCLUSION:

The present study demonstrates the change in susceptibility patterns of anaerobic organisms and increased resistance to Metronidazole and piperecillin. This indicates a need for periodic active surveillance of wound infections in large number of patients to identify & record the

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resistance pattern. The proper measures taken by the clinicians will help the patients for better treatment and also avoid going for the empirical therapy resulting in over usage of drugs & emergence of resistance to various drugs.

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