Research Paper MEDICAL SCIENCE

Aerobic Bacterial Isolates in Burns Patients and Their Antibiogram

***Kanagapriya M**	Assistant Professor, Department of Microbiology, Tirunelveli Medical College, Tirunelveli. * Corresponding author
Pandiyaraja S	Department of Microbiology, Tirunelveli Medical College, Tirunelveli
Sucilathangam G	Assistant Professor, Department of Microbiology, Tirunelveli Medical College, Tirunelveli
Revathy C	Professor, Department of Microbiology, Tirunelveli Medical College, Tirunelveli

ABSTRACT

Background and objectives:: Bacterial infections are a serious problem among burns patients. The skin also provides primary protection against infection by acting as a physical barrier. Infection is a major cause of morbidity and mortality in hospitalized burns patients. To find out the aerobic bacterial isolates from the samples of each burn wound case and to observe their antibiotic sensitivity pattern and to formulate a guideline for empirical treatment on the basis of antimicrobial sensitivity pattern of aerobic bacteria causing burn wound infection in our hospital. Materials and Methods: This study was conducted in the Burns ward of Tirunelveli Medical College Hospital from March to June 2014. This study group comprised of 50 burns ward patients. The surface swab specimens were cultured and their antibiotic sensitivity testing was performed by Kirby – Bauer by Kirby – Bauer disc diffusion method on Mueller - Hinton agar plates. Results: The predominant bacteria isolated from the infected wounds werePseudomonas aeruginosa (28%) followed by Klebsiella species (20%), Staphylococcus aureus (8%), Coagulase negative staphylococcus (8%) , Escherichia.coli (4%), Proteus vulgaris (2%) and Acinetobacter Spp. (2%). Pseudomonas aeruginosa. Klebsiella species , Escherichia coli were ESBL producers sensitive to Ciprofloxacin ,Doxycycline,Amikicin, Cotrimoxazole , Piperacillin and Tazobactam and Imipenam. All Staphylococcus aureus isolates were MRSA and also resistant to Erythromycin and were sensitive to Clindamycin and Vancomycin. All the CONS species were MRCONS, they were also resistant to Ciprofloxacin , Erythromycin, Clindamycin and sensitive to Vancomycin and, Doxycycline. Conclusion: . Pseudomonas aeruginosa has emerged as the commonest organism causing infection and is resistantto most of the antibiotics. To keep a check on burn wound infections it is important forevery hospital to have a data on prevalent organisms and their antibiotic susceptibilitypattern to formulatean effective antibiotic policy.

KEYWORDS Burns, antibiotic susceptibility,aerobic bacterial isolates Pseudomonas aeruginosa

Introduction

The skin, one of the largest organs in the body, performs numerous vital functions. The skin also provides primary protection against infection by acting as a physical barrier. When this barrier is damaged, as in burns, pathogens have a direct route to invade the body, possibly resulting in infection.[1] Loss of skin barrier function provides microorganisms with access to viable tissue over a broad area, and the protein-rich, avascular eschar provides them with an excellent culture medium. [2] In addition to the nature and extent of the thermal injury influencing infections, the type and quantity of microorganisms that colonize the burn wound influencethe risk of burns wound infection.

The pathogens that infect the wound are primarily Gram-positive bacteria such asStaphylococcus aureus and Gram-negative bacteria such as Pseudomonas aeruginosa,and Klebsiella species and Acinetobacter species,.These pathogens are notable for their increasing resistance to a broad array of different antimicrobial agents. Methicillin Resistant Staphylococcus Aureus (MRSA) and Extended Spectrum Beta Lactamase (ESBL) producing strains are at the rise.[3,4.]

Infection is a major cause of morbidity and mortality in hospitalized burns patients.[5]It has been estimated that about 75% of the mortality associated with burn injuries is related to sepsis especially in developing countries.[6.] The pattern of infection differs from hospital to hospital, the varied bacterial flora of infected wound may change considerably during the healing period.[7]

This study was done to determine the aerobic bacterial burn wound isolates in our setting and describe their resistance patterns, which would enable the determination of empirical antibiotic strategies for the early treatment of imminent septic events.

Materials and methods:

Study Group

This study was conducted in the Burns ward of Tirunelveli Medical College Hospital from April toSeptember2014. This study group comprised of 50 burns ward patients with more than 10% burns patients. With proper ethical committee clearance and consent the patient's age, sex, address and mode of injury, time taken to reach the hospital, involved body surface area(the percentage of burns was assessed clinically by using Rule of Nine), referral place and length of hospital stay was noted in a predesigned Proforma.

Inclusion Criteria

In this study, patients who are

1. Of all age group
2. Both gender
3. Patients with chronic diseases like tuberculosis, diabetes mellitus were included the study.

Exclusion Criteria
1. Patients with burns less than 10% were excluded.

Sample Collection
A total of 50 surface swabs were takenfrom clinically deep area of the burns wound site prior to any cleansing. Swabs werecollected by using sterile cotton tipped swabs. Specimens were immediately transferred to sterile test tube. In case of collecting samples from dry surface, swabs were moistened with sterile normal saline. After collection, tubes were plugged properly, labelled and carried to the microbiology laboratory.

Isolation and Identification of Pathogen
The specimens collected were sent to the central microbiology laboratory immediately and cultured under aerobic conditions and the sensitivity checked with conventional methods. Samples werecultured on Nutrient agar, MacConkey agar, and Blood agar at 37°c for 24 hrs. The isolates were identified by culture, staining,motility,oxidase test,catalase test and other relevant biochemical tests

Antibiotic sensitivity testing
Antibiotic sensitivity testing was performed by Kirby – Bauer disc diffusion method on Mueller - Hinton agar plates , inoculated with 0.5 Mac Farlands standard bacterial suspension. ThefollowingantibioticsAmpicillin(10mcg),Amikacin(30mcg) Gentamicin(10mcg),Ciprofloxacin(5mcg),Erythromycin(15mcg),Doxycyclin(30mcg),Clindamycin(2mcg),Cotimoxazole(1.25/23.75mcg),Cefoxitin(30mcg),Ceftazidime(30mcg) Cefotaxime(30mcg)Vancomycin (30µgm),Cephelexin(30mcg). Second line drugs used were Imipenem(10mcg),Cefoperazone(75mcg)-Sulbactam(30mcg),Piperacillin -Tazobactum 100/10mcg.) Antibiotic discs were procured from HiMedia India, Private Limited. ,Methicillin resistance was detected by using cefoxitin as a surrogate marker. The resistance pattern including ESBL, MRSA, and Clindamycin resistance were detected accordingto the CLSI guidelines.[8]

ESBL Detection
The screening for extended spectrum beta lactamase (ESBL) was done using, ceftazidime (≤ 22 mm), and cefotaxime (≤ 27 mm).The phenotypic confirmation was done by testing the strain against ceftazidime and ceftazidime with clavulanic acid discs. A >5 mm diameter of the zone of inhibition for ceftazidime with clavulanic acid in comparison to ceftazidime was considered indicative of ESBL production.

MRSA Detection
MRSA Detection was done by Cefoxitin(30µgm) disc diffusion method(zone size ≤ 21 mm for Staphylococcus and ≤ 22 mm for CONS) is resistant.

Clindamycin Resistance detection by D - TEST
Erythromycin (15µg) disc was placed at a distance of 15mm (edge to edge)from clindamycin (2µg) disc on the same plate and were incubated at 37^0C overnight.(6) isolates showing resistance to erythromycin (zone size ≤ 13mm) and sensitive to clindamycin (zone size ≥ 21mm) with a D shaped zone of inhibition around clindamycina with flattening towards erythromycin disc.-D-test positive, same inhibitory diameter as above with no D shaped zone-D-test negative.

Results
In the present study out of the 50 patients, 27 were male (54%) and 23 were female (46%)Males were a few more than the female which was not so significant(p value > 0.05). Most common age group affected was between 20 years to 40 years.(Table-1)Therefore there is no significance or association between age and gender with burns in this study.

Out of the 50 burns ward patientspus culture was positive in36 patients (72 %) while in 14 patients (28%) , pus were sterile . Among the 50 patients with culture positivity,8patients(16%) had gram positive bacterial infection and the rest of them(28 patients) had gram negative bacterial infection(56%).(Table-2)The predominant bacteria isolated from the infected wounds wasPseudomonas aeruginosa (28%) followed by Klebsiella species (20%), Staphylococcus aureus (8%), Coagulase negative staphylococcus (8%) , Escherichia. coli (4%), Proteus vulgaris (2%) andAcinetobacter Spp. (2%). (Table -3)

Pseudomonas aeruginosa isolateswere ESBL producers highly sensitive to Imipenem and Amikicin followed by Ciprofloxacin, Doxycycline and resistance toAmpicillinand Tetracycline, Similarly Klebsiella species were also ESBL producers sensitive to Ciprofloxacin , Doxycycline,Amikicin, Cotrimoxazole , Piperacillin and Tazobactam. Escherichiacoli were also ESBL producers sensitive to Amikacin only.Acinetobacter species were resistant to Ceftazidime , Cephalexin, Ceftriaxone and sensitive to Ciprofloxacin, Cotrimoxazole and Doxycycline.Proteus species was sensitive only to Cefoperozone and Sulbactam.Regarding the antibiotic susceptibility pattern of the gram positive isolates, all theStaphylococcus aureus isolateswere MRSA and also resistant to Erythromycin and were sensitive to Clindamycin and Vancomycin.All the CONS(Coagulase Negative Staphylococcus) species were MRCONS (Methicillin Resistant COagulase Negative Staphylococcus),they were also resistant to Ciprofloxacin , Erythromycin, Clindamycin and sensitive to Vancomycin and Doxycycline.(Chart- 1)

Discussion
In the current study among the 50 patients, 27 were male (54%) and 23 were female (46%) . In a study done in Yemen in 2011,Fifty eight (58%) of patients were males and forty two(42%) were females. This was similar to the present study. Ghaffar et al, in 2002 reported that burn wound infection in males was 189(62.4%) while burn wound infection in females 114 (37.6%).[9] In a similar study by Macedo and Santos in 2005 males were more affected.[10]In an Iranian study done by Shakibaie et al in 2008 found that 77 (64.2%) out of 120burn infection patients were males while 43(35.8%) were females.[11] Male to female ratio was 1.38:1 in a study by SK Saha et al.[12] Few other studies also had a male preponderance.[13,14]In contrast to Rajupt et al.,(2008) showed that burn infection infemales (60%) was more than male (40%) in India.[15]

In this study, it was found that the highest distribution of burn wound infection was within the age group between 20 years to 40 years . This may be due to the increased psychological stress among this age group, moreover this age is the prime age for earning and settlement.This can be the reason for both suicidal and occupational burns.Our study was in concordance with the findings reported by Kwong and Chung who found that the age group 19-40 years were more susceptible to burn wound infection than other age groups.[16] On the other hand, Ghaffar et al and Shakibaie et al found that the age group 10-19 years was more susceptible to burn woundinfection.[9,11] The findings reported byAl- Akayleh et al showed that the age group <10 years had the highest distribution of burn wound infection in burn patients.[17]

In present study,pus culture was positive in 36 patients (72 %) while in 14 patients no pathogens were isolated.Gram negative bacterial infections were more than gram positive bacterial infection in the present study .Among the 36 patients with culture positivity,8 patients(22%) had gram positive bacterial infection and the rest of them had gram negative bacterial infection (77%). Staphylococcal isolates were 16% of the total isolates. There was no Streptococcal infection in this study. This may be due to the fastidious nature of the organism.Similarly the complete absence of B-haemolytic Streptococcus was also reported by some workers1[18,19,20,]. But some other workers found the organism in post burn infection[21,22,]

The predominant bacteria isolated from the infected wounds werePseudomonas aeruginosa (28%) followed by Klebsiella species (20%), Staphylococcus aureus (8%), Coagulase negative staphylococcus (8%) , Escherichia.coli (4%), Proteus vulgaris (2%) and Acinetobacter Spp. (2%).A study done by SK Saha et al was similar to the present study.[12]A study

done by Alghalibi, S. M. S. et al also had a predominance of Pseudomonas infection like this study.[23]These findings were also consistent with those of other centers of different countries[19,20,22]. The frequency of Gram negative organisms was high and the most common isolate was Pseudomonas aeruginosa in a study conducted by Muhammad Naveed Shahzad et al.[24]

In this study, Pseudomonas aeruginosa. Klebsiella species Escherichia coli were ESBL producers sensitive to Ciprofloxacin ,Doxycycline, Amikicin, Cotrimoxazole , Piperacillin and Tazobactam and Imipenem. AllStaphylococcus aureus isolates were MRSA and also resistant to Erythromycin and were sensitive to Clindamycin and Vancomycin.All the CONS species were MRCONS, they were also resistant to Ciprofloxacin , Erythromycin, Clindamycin and sensitive to Vancomycin and, Doxycycline.

Common burn wound pathogens such as Pseudomonas aeruginosa and Staphylococcus aureus produce a number of virulence factors that are important in the pathogenesis of invasive infection. The breached skin barrier is the hallmark of thermal injury.Although burn wound surfaces are sterile, immediately following thermal injury, these wounds eventually become colonized with microorganisms. Significant thermal injuries induce a state of immunosuppression that predisposes burn patients to infectious complications like blood stream infections and pneumonia.Over the last several decades, gram-negative organisms have emerged as the most common etiologic agents of invasive infection by virtue of their large repertoire of virulence factors and antimicrobial resistance traits.

Pseudomonas aeruginosa produces a number of cell-associated and extracellular virulence factors that mediate a number of processes, including adhesion, nutrient acquisition, immune system evasion, leukocyte killing, tissue destruction, and bloodstream invasion.Pseudomonas aeruginosa also carries many intrinsic and acquired antimicrobial resistance traits that make infected burn wounds difficult to treat.[25,26.] Gram-positive bacteria that survive the thermal insult, such as Staphylococci located deep within sweat glands and hair follicles, heavily colonize the wound surface. Staphylococcus aureus also has a diverse array of virulence factors that facilitate adherence to host tissues, immune system evasion and destruction of host cells and tissues, including coagulase, protein A, leukocidins, hemolysins, and super antigens.

Emerging antimicrobial resistance trends in burn wound bacterial pathogens represent a serious therapeutic challenge for clinicians caring for burn patients. Antibiotic-resistant organismssuch as MRSA and multidrug resistant gram-negative rods, including Pseudomonasaeruginosa,Klebsiellaspecies,Proteusspecies, Acinetobacter spp. have been associated with infections of the burn wound in this study. The increased incidence of multidrug resistant isolates is probably due to empirical use of broad-spectrum antibiotics and non-adherence to hospital antibiotic policy. The early detection of isolates is important to prevent treatment failure. Therefore, careful microbiological surveillance and in vitro testing before the start of antibiotic therapy and restrictive antibiotic policy may be of great help in prevention and treatment of MDR isolates in burn units.

Burn units should routinely determine and track the specific pattern of burn wound microbial colonization, time-related changes in the predominant microbial flora of the burn wound in individual patients, the antimicrobial susceptibility profiles of microorganisms implicated in burn wound infections in a given time. Antibiotic utilization should be rotated or changed based on monitoring of antibiotic resistance trends (e.g., antibiogram) within the burn unit. The infection control program for burn centers requires strict compliance with a number of environmental control measures that include strictly enforced hand washing and the universal use of personal protective equipment (i.e., gowns, gloves, and masks). Infection control programs need to document and report burn wound infections.

Conclusion

Bacterial infections are serious problem among burns patients. Pseudomonasaeruginosa has emerged as the commonest organism causing infection and is resistantto most of the antibiotics. To keep a check on burn wound infections it is important forevery hospital to have a data on prevalent organisms and their antibiotic susceptibilitypattern. This study should be done frequently to check the changing pattern of theorganisms and their susceptibility pattern. Based on this, the hospital should formulatean effective antibiotic policy.

Acknowledgement

The author likes to thank The Dean, Department of Microbiology and Surgery, Tirunelveli Medical College for the facilities provided forconducting the study.

TABLE: 1
DISTRIBUTION OF AGE AND GENDER

S.NO	AGE	NO. OF MALE	NO. OF FEMALE	TOTAL
1	0-20	4	6	10
2	21-40	12	14	26
3	ABOVE 40	11	3	14
TOTAL		27	23	50

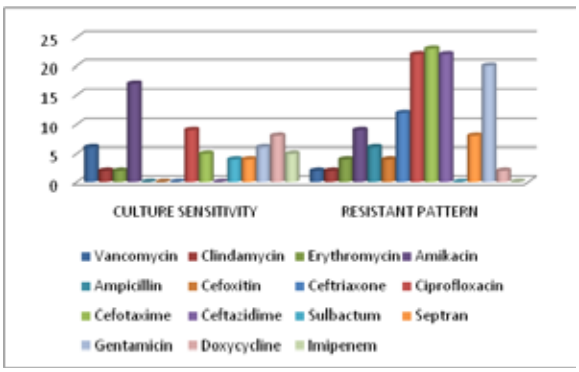
TABLE: 2
DISTRIBUTION OF BACTERIAL ISOLATES GRAM POSITIVES &GRAM NEGATIVES

S.NO	ISOLATES	PERCENTAGE
1	GRAM POSITIVES	16%
2	GRAM NEGATIVES	56%
3	NO PATHOGENS	28%

TABLE:3
ORGANISMS ISOLATED & THEIR PERCENTAGE

S.NO	ORGANISMS	TOTAL NO. PATHOGENS ISOLATED	PERCENTAGE
1	***Staphylococcus aureus***	**4**	**8%**
2	***CONS(MR Cons)***	**4**	**8%**
3	***Klebsiella pneumonia***	**7**	**14%**
4	***Pseudomonas aerunginosa***	**14**	**28%**
5	***Escherichia coli***	**2**	**4%**
6	***Acinetobacter species***	**1**	**2%**
7	***Klebsiella oxytoca***	**3**	**6%**
8	***Proteus vulgaris***	**1**	**2%**
9	***No pathogens***	**14**	**28%**

Chart-1
ANTIBIOTICS WITH CULTURE SENSITIVITY AND RESISTANT PATTERN



REFERENCES

1. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev. Apr 2006; 19(2):403-34. | 2. Order SE, Mason AD Jr,, Switzer WE, Moncreif JA. Arterial vascular occlusion in devitalization of burn wounds. Ann Surg.(1965);161:502. | 3. Keen EF 3rd, Robinson BJ, Hospenthal DR, Aldous WK, Wolf SE, Chung KK, et al. Prevalence of multidrug-resistant organisms recovered at a military burn center. Burns. September 2010; 36:819-25. | 4. Albrecht MC, Griffith ME, Murray CK, Chung KK, Horvath EE, Ward JA. Impact of Acinetobacter infection on the mortality of burn patients. J Am Coll Surg. October 2006; 203:546-50. | 5. Mc Manus AT., Mason AD.. jr, McManus WF., Pruitt BA,Jr: A decade of reduced Gram -negative infections and mortality improved isolation of burned patients. Arch.Surg 1994 ; 129: 1306-1309. | 6. Donati L, Scammazo F, Gervasoni M, Magliano A, Stankow B, Fraschini. Infection and antibiotic therapy in 4000 burned patients in Milani Italy between 1976 and 1988. Burns injury, 1993; 4: 345-8. J Burn Care Rehabil 1985; 129-34. | 7. Kumar.V., Bhatnagar.SK,Singh. AK,Kumar.S. Mishra RK. Burn Wound Infection: A study of 50 cases with special reference to antibiotic resistance. Indian Journal of Bio Research 2001; 46: 66-69. | 8.Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial disk susceptibility tests: Approved StandardM2-A7.11 th ed. Clinical and Laboratory Standards Institute Wayne, Pa; 2005.] | 9. Ghaffar, U. B.; Husain, M. and Rizvi, S. Thermal burn: An epidemiological prospective study. Indian J. Academy of ForensicMedicine,.2002; 30(1):10-14. | 10. Macedo, J. L. S. and Santos, B. (2005):Bacterial and fungal colonization of burn wounds. Mem Inst OswaldoCruz, Rio de Janeiro, 100(5): 535-539. | 11.Shakibaie, MR, Shahchraghi, F,Hashemi A,Adeli S.Detection of TEM, SHV and PER type extended-spectrum B-Lactamasegenes among clinical strains of isolated from burn patients at Shafa-Hospital, Kerman, Iran. Iranian Journal of Basic Medical Sciences,11(2): 105-107 | 12. SahaSK, Muazzam N,Begum SA,Chowdhury A,Islam MS,Parveen R.Study on Time-related Changes in Aerobic Bacterial Pattern of Burn Wound Infection Faridpur Med. Coll. J. 2011;6(1):41-45. | 13.Bagdonas, B,Tamelis A, Rimdeika R,Kiudelis M.Analysis of burn patients and the isolated pathogens. Lithuanian Surgery.2004;2(3):190-193. | 14 Vostrugina, K,Gudavičiene D, Vitkauskiene A.Bacteremias in patients with severe burn trauma. Medicina (Kaunas)2006;42(7):576-578. | 15. Rajput, A,Singh, KP,Kumar V,Sexena, R, Singh RK.Antibacterial resistance pattern of aerobic bacteria isolates from burnpatients in tertiary care hospital. Biomedical Research 2008; 19 (1): 1-4. | 16.Kwong YL. and Chung, CH.The use of tegaderm transparent dressing in the outpatient management of partial thickness burns. Journal of the Hong KongMedical Association1985;37(4): 187-188. | 17.Al-Akayleh AT. Invasive burnwound infection. Annals of Burns andFire Disasters, 1999;XII – (4): 1-3. | 18.Vindenes H, Bjerknes R. Microbial colonization of large wounds. Burns 1995;21:575-9. | 19Husain MT,Karim QN, Tajuri S. Analysis of infection in a burn ward. Burns 1989;15(5):299-302. | 20.Singh NP, Goyal R, Manchanda V, Das S, Kaur I, Talwar V.Changing trends in bacteriology of burns in the burn unit, Delhi, India. Burns 2003;29:129-32. | | 21. Neelam T, Rekha E, Chari PS, Meera S. A prospective study ofhospital - acquired infections in burn patients at a tertiary carereferral center in North India. Burns 2004;30:660-69. | 22. Salah N, Amr M, Ashraf M. Colonization of burn wounds in Ainshams University Burn unit. Burns 2003;29:229-33. | 23. Alghalibi SMS,Humaid, AA,Alshaibani, EAS,Alhamzy EHL,Microorganisms Associated With Burn Wound Infection in Sana'a, Yemen Egypt. Acad. J biolog Sci.2011;3(1): 19-25. | 24.Muhammad Naveed Shahzad ,Naheed Ahmed, Iftikhar Hussain Khan,Arif Baig Mirza Faisal waheed. Bacterial Profile of Burn Wound Infections in Burn PatientsAnn. Pak. Inst. Med. Sci. 2012; 8(1): 54-57. | 25. Torres, A., and S. Ewig. Diagnosing ventilator-associated pneumonia. N. Engl. J. Med.2004;350:433-435. | 26. Van Delden C, Iglewski BH.Cell-to-cell signalling and Pseudomonas aeruginosa infections. Emerg. Infect. Dis.1998;4:551-560. |