Research Paper

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Detection of ESBL producing Klebsiella species in Burn Patients and their Antimicrobial Resistance Pattern

Microbiology

KEYWORDS : Burn wound, ESBL, Klebsiella.

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ABSTRACT

Infection is a central cause of mortality in burns. There is a higher rate of nosocomial infections in burn wards. Among them Klebsiella has been found to be the common species to produce extended spectrum β-lactamases (ESBL). The present study was aimed to determine the prevalence of ESBL producing Klebsiella and their susceptibility pattern.

A retrospective study from burn ward infected patients admitted to burn unit of Amravati and Akola district's Government hospitals was carried out to determine the occurrence and resistance pattern of ESBL producing Klebsiella species. Klebsiella species were isolated from 214 burn wound swabs taken from burn patients and identified by conventional biochemical methods and screened for ESBL production by phenotypic confirmatory disc diffusion test and antibiogram was tested. From 214 specimens, 130 Klebsiella species were isolated. Among them 117 isolates were identified as K. pneumoniae and 13 isolates were identified as K. oxytoca. ESBL production was detected in 18.46% of Klebsiella species. All (100%) ESBL positive Klebsiella isolates were found to be sensitive to Chloramphenicol and meropenem, while isolates were resistant to penicillins and cephalosporins classes of antibiotic. Klebsiella species were the common organism causing burn wound infection in the present study showing high resistance pattern against different classes of antibiotics, while drug of choice for therapy is limited and carbapenems was the most effective treatment option for ESBL producing Klebsiella infection.

INTRODUCTION:

Burn is a thermal injury of the skin although electrical and chemical injuries may also result in burn (1). Infection is an important cause of morbidity and mortality in burn patients (2) and 75% of all deaths following thermal injuries are related to infections in patients with burn over than 40% of the total body surface area (3).

The rate of hospitalized infection and cross infection is higher in burns patients due to various factors like nature of burn, injury itself, compromised immune responses of the patient (both humeral and cellular), age of the patient, extent of injury, depth of burn injury in combination with microbial factors such as pattern of bacterial pathogen, enzyme and toxin production and systematic dissemination of the colonizing organism (4, 5).

Burn wounds can be classified as cellulites which involve unburned skin at margin of the burn or invasive wound infection which is characterized by microbial invasion of viable tissue beneath the burn eschar and results in lethal bacteremia. The denatured protein of the burn eschar provides nutrition for the organism. Avascularity places the organism beyond the reach of the host defense mechanism and systematically administered antibiotics (6). There is need to access the pattern of bacterial pathogens responsible for the burn wound infection and diagnosis can be made from wound swabs (7, 8). Pattern of antimicrobial agent usage and bacterial resistance in each community should be known and considered when prescribing antimicrobial agents (9).

Bacteria belonging to the genes *Klebsiella* frequently cause human nosocomial infections because of their ability to spread rapidly in hospitals environment (10). The gastro-intestinal tract and the hands of hospital personnel are the major pathogenic reservoirs for transmission of *Klebsiella*. The incidence of ESBL producing clinical *Klebsiella* strains has been steadily increasing and results in the limitations on the therapeutic options for the management of *Klebsiella* infection (11).

Therefore, the aim of this study was to determine the incidence of the ESBL producing *Klebsiella* from burn wounds and to determine their antimicrobial susceptibility pattern.

Materials and Methods:

The study was carried out in the Department of Microbiology, Adarsha Mahavidyalaya, Dhamangaon Railway, Dist. Amravati. A total 214 burn wound swabs were taken from burned patients, who presented invasive burn wound infection from both sexes admitted to burn unit of Government District's Hospital, Amravati and Government District's Hospital, Akola (MS), India during July 2010 to Feb 2011. The pus was collected on plain sterile swabs and transported to the laboratory. All specimens were inoculated on Mac Conkey agar and Mac Conkey agar modified specially for isolation of *Klebsiella* and incubated overnight (aerobically) at C. Suspected Gram negative organisms were identified by colony characteristics, Gram staining, motility, citrate utilization, indole production, MR-VP, catalase, oxidase tests malonate utilization, lysine decarboxylase test and sugar fermentation reactions.

Antimicrobial sensitivity was performed by the Kirby Bauer disk diffusion method according to the CLSI guidelines. ESBL production by *Klebsiella* isolates was confirmed by demonstrating synergy using ceftazidime disc and combination of ceftazidime plus clavulanic acid (12, 13).

RESULT:

A total of 214 samples were collected from burns unit during the period July 2010 to Feb 2011. Out of 214 samples, 61 samples (28.50 %) were collected from males and 153 (71.50 %) samples were collected from female. Flame burn samples were most common in both male and females in our study followed by scalds and electric burns, while no cases or patients found to be burn due to chemical during study period.

Among 214 specimens, 130 (60.70%) were culture positive. In burn swabs, no significant difference in colonization of *Klebsiella* in male and female patients was found in the present study. Two different types of *Klebsiella* species were identified among *Klebsiella* isolates from burn unit of Amravati and Akola Govt. district hospital. Out of 130 *Klebsiella* isolates, 117 isolates were *K. pneumoniae* and 13 were identified as *K. oxytoca*. Percent distribution of *Klebsiella* species in both districts is shown in Table No.1.

Table No.1: District wise isolation rate and percent distribution of *Klebsiella* species

District	Amravati	Akola
Total number of Specimens	127	87
Positive culture for *Klebsiella*	76	54
Isolation Rate of *Klebsiella*	59.84%	62.06%
No and % Distribution of *K. pneumoniae*	65 (85.52%)	52 (96.29%)
No and % Distribution Of K. oxytoca	11 (14.47%)	02 (3.70%)

A total 24 (18.46%) *Klebsiella* isolates found to be ESBL producing as they were confirmed by using ceftazidime disc and combination of ceftazidime plus clavulanic acid discs. Among burn swabs collected from Amravati city hospital, total 12 isolates of ESBL producing *K. pneumoniae* and 1 isolate of ESBL producing *K. oxytoca* were detected, while 11 isolates of ESBL producing *K. pneumoniae* was recovered from burn swabs collected from Akola city hospital. None of *K. oxytoca* was found to be positive for ESBL production isolated from Akola city.

ESBL producing *Klebsiella* species isolated from burn swabs were tested for antimicrobial resistance against various antibiotics belonging to different to classes of antibiotics showed different resistance pattern as shown in Table No. 2. It is worth to note that 59.64% of ESBL positive *Klebsiella* isolates were resistant to various antibiotics, while near about 20% isolates found to be intermediate and susceptible to various antibiotics.

Table No. 2. Antibiotic resistance pattern of ESBL Producing *Klebsiella* species.

Antibiotic name	% Resistance	Antibiotic name	% Resistance
Piperacilin/ Tazobactam	25	Cefotaxmine	50.00
Cefaperazone/ Sulbactam	8.33	Ceftrioxome	41.67
Penicillin	100.00	Lomefloxacin	33.33
Piperacillin	91.67	Ampicilin	91.67
Methicillin	83.33	Ampicilin/ Sulbactam	58.33
Imipenem	8.33	Tetracycline	66.67
Meropenem	0.00	Ofloxacin	66.67
Timentin	33.33	Chloramphenicol	0.00
Ticarcillin	33.33	Ciprofloxacin	8.33
Ceftazidime	91.67	Nalidixic acid	91.67
Amoxycilin	100.00	Trimethoprime	91.67
Augmentin	100.00	Kanamycin	100.00
Cefepime	25.00	Norfloxacin	75.00
Azithromycin	83.33	Nitrofurantoin	33.33
Levofloxacin	41.67	Gentamycin	75.00
Co-trimoxazole	91.67		

DISCUSSION*:*

The burn wound is considered one of major health problem in the world and infection is one of the frequent and severe complications in patients who sustained burns (14, 15). Control of invasive bacterial burn wound infection, strict isolation technique and infection control policies have significantly minimized the occurrence of burn wound infection (16).

In the present study culture positivity was found to be 60.70% which indicates that wound infections are the common cause of nosocomial infection in burns another studies (17, 18) also reported the same. Our study showed much high prevalence of *Klebsiella* infection among burn patients as compared to other studies noted (1, 19, 20 21, 22, 23) they showed incidence of *Klebsiella* from 0.96% to 31%. It may be due to use of selective media for the isolation of *Klebsiella.*

This study included 142 females, 49 male and 23 children. Among children 12 were male and 11 female children. Overall the most common cases were in 25-45 years age group in both adult males and females. In case of children's (≥14) majority of cases were in 8-12 years age group. The sex wise distribution of specimen and culture positivity among Amravati and Akola district's is shown in Table No.3.

Table No. 3. Sex wise distribution of specimens and culture positivity

District	Male		Female Male		Children			
					Female			
	No. of specimen	Positive	No. of specimen	Positive	No. of specimen	Positive	No. of specimen	Positive
Amravati	29	16	79	53	11	4	8	3
Akola	20	15	63	47	1	--	3	1

In the present study the prevalence of burn wound infection was much higher in females (71.50%) as compared to males (28.50%). It is correlated with (23, 24, 25) from India. They noted female preponderance 56.2%, 55.33% and 53%, respectively. Studies by other researchers (1, 14, 19, 22) reported higher incidence of burn wound infections in males.

Only two types of *Klebsiella* species were isolated in our study. Present investigation noted the high incidence of *K. pneumoniae* (90%) and *K. oxytoca* (10%), which clearly indicates that, *K. pneumoniae* is the most common causative species among genus *Klebsiella.* Mohammed SW (26) reported *Pseudomonas spp.* (36.7%) and *Klebsiella spp.* (36.7%) as the most common pathogenic bacteria from burn wound infections.

The present study showed 18.46% of *Klebsiella* as ESBL producers which was lower than what was reported in another studies (23, 27). Among the *Klebsiella, K. pneumoniae* was the most common ESBL producers in both Amravati and Akola districts. Strains of ESBL producing *Klebsiella* were not only resistant to penicillins but also to all other beta lactam antibiotics including cephalosporins.

The widespread use of topical antimicrobial agents in hospital settings has led to the emergence of multidrug resistant organisms such as *Klebsiella,* causing serious opportunistic infections (28). The change in the pattern of bacterial resistance in the burn unit is important to clinical settings and epidemiological purposes. The widespread use of topical antimicrobial agents in hospital settings has led to the emergence of multidrug resistant organisms such as *Klebsiella,* causing serious opportunistic infections. Currently used antimicrobial agents are not effective in treating burn wound infections and fail to control many bacterial infections due to the development of super-resistant strains.

The result of antimicrobial sensitivity in the present investigation showed that ESBL producing *Klebsiella* was highly resistant to most of the antibiotics tested, while chloramphenicol and meropenem followed by imipenem and cefaperazone/sulbactum found to be the most sensitive drugs. The result of antibiotic resistance pattern of ESBL positive *Klebsiella* against 31 different antibiotics tested in this study are in tandem with various studies (21, 22, 29, 30, 31, 32). Earlier studies by Mohammed SW (26) reported high resistance of *Klebsiella* species to Cefixime (100%) and sensitivity to Norfloxain (67.7%, 56.3%) and Ciprofloxacin (41.7%, 53.8%).

CONCLUSION:

To conclude, *Klebsiella* is highly prevalent in burn wound swab and also significant ESBL producer. Antibiotic policy should be strictly adhered to and followed by clinicians and indiscriminate use of antibiotics should not be practiced. It may help in the prevention and treatment of multidrug resistant pathogens in burn infection.

REFERENCE

1. Ekrami, A., Kalantar, E. (2007): Bacterial infections in burn patients at a burn hospital in Iran. Indian Journal Medical Research, 126: 541-544. | | 2. McManus T., Mason D., McManus F., Prutt A. (1994). A decade of reduced Gram-negative infections andmortality associated with improved isolation of burned patients. Arch. Surg. 129:1306-1309. | | 3. Vindences H., Bjerknes R. (1995). Microbial colonization of large wounds. Burns.21: 575-579. | | 4. Pruitt A., McManus T., Kim H., Goodwin W. (1998). Burn wound infections: current status. World J Surg. 22: 135-145. | | 5. Environmental and Nutritional diseases, chapter- 8, Robbins basic Pathology, 8th ed, 2007. Elsevier. 299. | | 6. Imran M., Faheem, M., Viqar Aslam, Awal Hakeem, Inayat Rehman and Azhar Shah (2009). Wound infections and culture sessitivity pattern in pediatric burn patients. J P M I, Vol. 23 (4): 304-308. | | 7. De macedo J. L., Santos, J. B. (2005). Bacterial and fungal colonization of burn wounds. Mem Inst Oswaldo Cruz, 100: 535-539. | 8. Barian L. M., Vasen Wala S. M., Malik A., Ansari G. H. Choudhary T. E. (1997). A clinicopathological study of infections in burn patients and importance of biopsy. J Ind Med Assoc 95: 573-575. | | 9. Winn Jr. W. C., Allen S. D., Janda W. M., Koneman E. W., Procop G. W. Schreckenberger P. C. (2006). The nonfermentative gram- negative bacilli. Koneman's color atlas and textbook of Diagnostic Microbiology, 6th ed, Philadelphia: Lippincott Williams and Wilkins. 303-391. | | 10. Poduschum, R., Ullman, U. (1998). Klebsiella species as nosocomial pathogens: Epidemiology, Taxonomy, Typing methods, and pathogenicity factors. Cli Microbiol Rev 11:589 – 603. | | 11. John N., Sao, S. and Panda P. (2014). Antibiogram, ESBL production and carbapenemase detection of Klebsiella spp. in hospital acquired infection. Bio Med Res Microbiol Vol.1 (2): 1-10. | | 12. National Committee for Clinical Laboratory standards, 2005. Performance standards for antimicrobial susceptibility testing., 15th international supplement (M100-S15). National Committee for Clinical Laboratory standards, Wayne, Pa. | | 13. Paterson, D., Bonomo, R. (2005). Extended spectrum beta lactamase: A clinical update. Cli Microbiol Rev 18(4): 657-86. Pub Med: 16223952. | | 14. Agnihotri N., Gupta V., Joshi R. (2004). Aerobic bacterial isolates from burn wound infections and their antibiograms a five year study. Burns., 30: 241-243. | | 15. Amin M., Kalantar E. (2004). Bacteriological monitoring of hospital borne septicemia in burn patients in Ahvaz, Iran. Burn Surgical Wound Care., 3: 4-8. | | 16. Zorgani A., Zaidi M., Ranka R., Shahen A. (2002). The pattern and outcome of septicemia in a burns intensive care unit. Ann. Burns Diasters; 15: 179-182. | | 17. Rastegar A., Alaghehbandan R., Akhlaghi L.(2005). Burn wound infection and antimicrobial resistance in Tehran, Iran: An increasing problem. Ann Burn Fire Disaters., 18: 1115-1118. | | 18. Askarian M., Hosseini R. (2004). Incidence and outcome of nosocomial infections in female burn patients in Shiraz, Iran. Am J Infect Control., 32: 25-28. | 19. Oncul O., E Ulkur, A Acar, V Turhan, E Yeniz, Z Karacaer and F Yildiz (2009). Prospective analysis of nosocomial infections in a Burn Care Unit, Turkey. Indian J Med Res 130: 758 -764. | | 20. Alghalibi S., Humaid A., Alshaibani E., Alhamzy E. (2011). Microorganisms associated with burn wound infections in Sanàa, Yemen, Egypt. Acad. J. Biolog. Sci., 3(1): 19-25. | | 21. Magnet M., Arongozeb M., Khan G., Ahmed Z. (2013). Isolation and identification of different bacteria from different types of wound infections and study their antimicrobial sensitivity pattern. Res App Nat Soc Sci; Vol 1 (3). 125-132. | | 22. Rajput, A., Singh, K.P., Kumar, V., Sexena, R., Singh, R. K. (2008): Antibacterial resistance pattern of aerobic bacteria isolates from burn patients in tertiary care hospital. Biomedical Research, 19 (1): 1-4. | | 23. Rajeshwar Rao, L Jaya Lakshmi1, S Pavani, Vijendra Kawle, S Jaya Prakash (2014). Bacteriological profile, antibiogram of burn wound isolates and detection of MRSA and ESBL production at tertiary care hospital, Hyderabad. W J P P Sci. Volume 3, Issue 10, 1691-1698. | | 24. Fouzia B., Damle A., Maher G. (2013). Changing patterns of burn infections. J D M S Volume 5, Issue 4, PP 11-14. | | 25. Chaudhary Uma, Nidhi Goel, Madhu Sharma, Mahavir Singh Griwan, Vikas Kumar (2007). Methicillin resistant Staphylococcus aureus infection / colonization at the burn care unit of a medical school in India. J Infect Dis Antimicrob agents. 24: 29-32. | | 26. Mohammed S. W. (2007). Isolation and identification of aerobic pathogenic bacteria from burn wound infections. Journal of Al-Nahrain University Vol.10 (2) :94-97. | | 27. Bhat S., Deshmukh A., Damle A., Karyakarte R. (1991). Incidence of beta lactamase producer strains ofStaphylococcus aureus and their biological properties. Indian Medical Gazette; 346 -- 348. | 28. Shukla I., Tiwari R., Agrawal M. (2004) Prevalence of extended spectrum β-lactamase producing Klebsiella pneumoniae in a tertiary care hospital. Ind J Med Microbiol 22: 87-91. | | 29. Revati G., Puria J., Jaid K. (1998). Bacteriology o9f burns. Burns; 24: 347-349. | | 30. Nagoba B., Deshmukh S., Wadher B., Pathan A. (1999). Bacteriological analysis of burn sepsis. Ind J Med SCR; 53: 216-219. | | 31. Kehinde O., Ademola A., Okesola O., Oluwatosin M. (2004). Pattern of bacterial pathogens in burn wound infections in Ibadan, Nigeria. Annals of Burns and fire disasters. Vol XVII: 348-355. | | 32. Manjula Mehta, Priya Dutta, Varsha Gupta (2007). Bacterial isolates from burn wound infections and their antibiograms: A eight-year study. Indian J Plast Surg.40(1): 25 -28. |