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ORIGINAL ARTICLE

ESBL producing Gram Negative Aerobic Bacteria Isolated from Burn Wound Infection with Their Antibiogram in Dhaka

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Abstract

Background: Infection is an important cause of mortality in burns. Rapidly emerging nosocomial pathogens and the problem of multi-drug resistance necessitates periodic review of isolation patterns and antibiogram in the burn ward. **Objective:** The purpose of the present study was to see the frequency of bacteria in burn wound with their antibiotic sensitivity pattern. **Method:** This cross sectional study was conducted in the laboratory of Department of Microbiology at Dhaka Medical College, Dhaka and samples were collected from the burn unit of Dhaka Medical College Hospital, Dhaka. Bacterial isolates from 108 wound swabs taken from burn patients were identified by conventional biochemical methods and antimicrobial susceptibility was performed. **Result:** Out of 98 bacteria *E. coli* (20.4%) was most common and 25.0% of these bacteria were ESBL producer. Out of 14 *Klebsiella* species ESBL producer was in 6(42.9%). ESBL producing *Proteus* species (21.4%) and *Pseudomonas* species (14.9%) were also detected. *Klebsiella* (33.91%) was the predominant organism closely followed by *Pseudomonas* (31.84%). **Conclusion:** *E. coli* is the most common ESBL producing bacteria causing the burn wound infection. [J Sci Found, 2012;10(2):63-69]

Key Words: Burn wound, antibiogram, bacterial pathogens

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Introduction

Burn injury is a major problem in many parts of the world (Srinivasan et al., 2009). It has been estimated that 75% of all deaths following burns are related to infection (Saha et al., 2011). Thermal injury destroys the skin barrier that normally prevents invasion by

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microorganisms; thus this makes the burn wound the most frequent origin of sepsis in these patients (Vindenes and Jerknes 1995).

Initially, the burnt area is considered free of microbial contamination; however, gram-positive bacteria in the depth of sweat glands and hair follicles heavily colonize the wounds within 48 hour of the injury (Srinivasan et al., 2009). Though topical antimicrobials decrease microbial overgrowth, it seldom prevents further colonization with other potentially invasive bacteria and fungi. These are derived from the patient's gastrointestinal and upper respiratory tract and the hospital environment (Monafo and Freedman 1987). Following colonization, these organisms start penetrating the viable tissue depending on their invasive capacity, local wound factors and the degree of the patient's immunosuppression (Hansbrough 1987). If sub-eschar tissue is invaded, disseminated infection is likely to occur (Mooney and Gamelli 1989). Great emphasis must therefore be placed on early identification of local signs of invasive burn wound infection. The causative infective microorganisms in any burn facility change with time (Manson et al., 1992). Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time and only to be replaced by newly arriving microorganisms. In addition to that introduction of new topical agents and systemic antibiotics influence the flora of the wound (Srinivasan et al., 2009).

An in-depth knowledge of the pattern of predominant organisms in the burn wound is essential for the treatment of the patient before getting the result of microbiological cultures (Srinivasan et al., 2009). This would be crucial to reduce the overall infection-related morbidity and mortality. The present study was designed to determine the nature of microbial wound infection with their sensitivity pattern. The aim of the present study was to obtain information about the type of isolates, identification and antimicrobial sensitivity of bacterial wound infections in burn patients.

Methodology

This cross sectional study was done in the Department of Microbiology of Dhaka Medical College, Dhaka from a period of July 2006 to December 2006 for a period of 6 months. All the burn wound patients admitted at the burn unit of Dhaka Medical College at any age with both sexes were included in this study. Bacterial aetiology of all burns wound was assessed. The wound swab from the burn wound was collected from clinically deep areas prior to any cleansing. A sterile cotton swab is moistened with sterile normal saline. This swab is rubbed onto the burn wound surface. Swabs are taken from areas which appear deep, areas with discharge, thick eschar. The swabs were transported to the laboratory for bacteriological isolation and identification. MacConkey agar, nutrient agar and blood agar media were used and were incubated at 37^0 C for 24 hours after inoculation. Identification was carried out by biochemical test in Kligler Iron agar (KIA) media. Antibiotic sensitivity test was carried out by disc diffusion method and interpretation was performed according to CLSI M17. Statistical test was performed by SPSS 16 (USA).

Result

A total number of 108 swab from burn wound were collected of which 98(90.7%) were positive for bacterial growth (Table 1). Interestingly, among the 98 culture positive growth of bacteria Gram negative was 95 bacteria of which 21 (22.1%) bacteria were ESBL producer (Table 2).

Table 1: Distribution of Culture Positivity of Burn wound (n=108)

Bacteria	Frequency	Percentage
Culture Positive	98	90.7
Culture Negative	10	9.3
Total	**108**	**100.0**

Out of 98 bacteria *E coli* was 20(20.4%) of which 5(25.0%) were ESBL producer. Out of 14 *Klebsiella* species ESBL producer was in 6(42.9%). ESBL producing *Proteus* species and *Pseudomonas* species were 3 (21.4%) and 7 (14.9%) in out of 14 and 47 bacterial species respectively (Table 3).

Table 2: ESBL Status among Culture Positive Bacteria Isolated from Burn Wound (n=98)

Type of Bacteria	ESBL Production		Total
	Positive	Negative	
Gram Negative	21 (22.1%)	74(77.9%)	95(100.0%)
Gram Positive	0(0.0%)	3(100.0%)	3(100.0%)
Total	**21 (21.4%)**	**76(78.6%)**	**98(100.0%)**

*Chi-square test has been performed corrected Fisher's exact test
*Chi-square value=0.844; p value=0.358

Antimicrobial resistance pattern among the Gram negative bacteria were determined. *E. coli* were highly resistant to cephradine (89.9%) followed by amoxicillin (80.7%), co-trimoxazole (69.7%) and Cefotaxime (64.2%). *Klebsiella* species were highly resistant to cephradine (88.4%) followed by amoxicillin (86.1%), co-trimoxazole (81.4%) and ciprofloxacin (65.1%).

Table 3: ESBLs producer among the different species of Bacteria isolated from Burn Wound (n=98)

Type of Bacteria	ESBL Production		Total
	Positive	Negative	
E. coli	5 (25.0%)	15(75.0%)	20(100.0%)
Klebsiella species	6 (42.9%)	8(57.1%)	14(100.0%)
Proteus species	3 (21.4%)	11(78.6%)	14(100.0%)
Pseudomonas species	7 (14.9%)	40(85.1%)	47(100.0%)
S. aureus	0(0.0%)	3(100.0%)	3(100.0%)
Total	**21 (21.4%)**	**76(78.6%)**	**98(100.0%)**

* *E. Coli= Escherichia coli; S. aureus= Staphylococcus aureus*
*Chi-square test has been performed corrected Fisher's exact test
*Chi-square value=5.022; p value=0.170

Proteus species showed 93.10% resistance to cephradine followed by amoxicillin (86.2%), co-trimoxezole (79.3%) and gentamicin (75.9%). *Pseudomonas* species showed 81.42% resistance to gentamicin followed by cefotaxime (68.6%) and ceftriaxone (65.7%) (Table 4). Among the ESBL producers, all the *E. coli*, *Klebsiella* species, *Proteus* species and

Pseudomonas species were resistant to amoxicillin, cephradine, ceftriaxone, aztreonam, ceftazidime and cefotaxime. All the Gram negative bacteria were sensitive to imipenam.

Table 4: Antibiotic Resistance Profiles among the ESBL Negative Bacteria

Antibiotics	***E. coli***	***Klebsiella***	***Proteus***	***Pseudomonas***
Amoxicillin	80.7%	86.1%	86.2%	-
Cotrimoxazole	69.7%	81.4%	79.3%	-
Gentamycin	50.5%	62.8%	75.9%	81.4%
Ciprofloxacin	39.4%	65.1%	51.7%	57.1%
Cephradine	89.9%	88.4%	93.1%	-
Aztreonam	55.1%	62.8%	62.1%	62.8%
Amikacin	48.6%	58.1%	55.2%	34.3%
Pipercillin	-	-	-	48.6%
Carbenicillin	-	-	-	62.8%
Ceftriaxone	59.6%	65.1%	62.1%	65.7%
Ceftazidime	60.5%	62.8%	65.5%	62.8%
Cefotaxime	64.2%	62.8%	65.5%	68.6%
Imipenem	0.0%	0.0%	0.0%	0%
Nalidixic acid	60.0%	55.0%	66.7%	-
Netilmycin	32.5%	45.0%	51.7%	56.4%

Discussion

A total number of 108 swab from burn wound were collected of which 98(90.7%) were positive for bacterial growth. Interestingly, among the 98 culture positive growth 95 bacteria were Gram negative of which 21 (22.1%) bacteria were ESBL producer (p value=0.358). The denatured protein of the burn eschar provides nutrition for the organisms. Avascularity of the burned tissue places the organisms beyond the reach of host defense mechanisms and systemically administered antibiotics (Church et al., 2006). In addition, cross-infection results between different burn patients due to overcrowding in burn wards (Atiyeh et al., 2007). Also thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humeral immune responses are pivotal factors contributing to infectious complication in patients with severe burn (Rode et al., 2009).

Out of 98 bacteria *E coli* was 20(20.4%) of which 5(25.0%) were ESBL producer. Out of 14 *Klebsiella* species ESBL producer was in 6(42.9%). ESBL producing *Proteus* species and *Pseudomonas* species were 3 (21.4%) and 7 (14.9%) in out of 14 and 47 bacterial species respectively (p=0.170). Burn wound infections are largely hospital acquired and the infecting pathogens differ from one hospital to another (Pruitt et al., 1998). The burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin; thermal injury destroys the skin barrier that normally prevents invasion by microorganisms. This makes the burn wound the most frequent origin of sepsis in these patients (Peck et al., 1998).

Antimicrobial resistance pattern among the Gram negative bacteria were determined. *E. coli* were highly resistant to cephradine (89.9%) followed by amoxicillin (80.7%), co-trimoxazole (69.7%) and Cefotaxime (64.2%). *Klebsiella* species were highly resistant to cephradine (88.4%) followed by amoxicillin (86.1%), co-trimoxazole (81.4%) and ciprofloxacin (65.1%). *Proteus* species showed 93.10% resistance to cephradine followed by amoxicillin (86.2%), co-

trimoxezole (79.3%) and gentamicin (75.9%). *Pseudomonas* species showed 81.42% resistance to gentamicin followed by cefotaxime (68.6%) and ceftriaxone (65.7%). Agnihotri et al (2004) was performed antibiotic sensitivity test of the aerobic bacteria isolated from burn wound patients and has reported that amikacin was found to be the most effective drug against gram negative bacteria, however, resistance to it was significantly increased over 5 years. In addition to that for *S. aureus* and *P. aeruginosa* netilmicin and piperacillin were found to be the most effective drugs and most of the isolates showed high level resistance to antimicrobial agents which is consistent with the present study.

Table 5: Antimicrobial resistance Pattern of ESBL producing Bacteria

Antibiotics	***E. coli***	***Klebsiella***	***Proteus***	***Pseudomonas***
Amoxicillin	100.0%	100.0%	100.0%	100.0%
Cotrimoxazole	88.5	100.0%	85.7	-
Gentamycin	68.4	85.7	85.7	88.9
Ciprofloxacin	39.4	42.8	28.6	22.2
Cephradine	100.0%	100.0%	100.0%	100.0%
Aztreonam	100.0%	100.0%	100.0%	100.0%
Amikacin	86.8	78.5	100	66.7
Netilmycin	75.0	77.7	75.0	61.3
Piperacillin	-	-	-	77.7
Carbenicillin	-	-	-	100
Ceftriaxone	100.0%	100.0%	100.0%	100.0%
Ceftazidime	100.0%	100.0%	100.0%	100.0%
Cefotaxime	100.0%	100.0%	100.0%	100.0%
Imipenam	0.0%	0.0%	0.0%	0.0%

Among the ESBL producers, all the *E. coli*, *Klebsiella* species, *Proteus* species and *Pseudomonas* species were resistant to amoxicillin, cephradine, ceftriaxone, aztreonam, ceftazidime and cefotaxime. All the Gram negative bacteria were sensitive to imipenem. Burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms (Kehinde et al., 2004), gram-positive bacteria that survive the thermal insult, such as *S. aureus* located deep within sweat glands and hair follicles, heavily colonize the burn wound surface within first 48 h (Ozumba and Jiburum 2000). Topical antimicrobials decrease microbial overgrowth but seldom prevent further colonization with other potentially invasive bacteria and fungi. Following colonization, these organisms start penetrating the viable tissue depending on their invasive capacity, local wound factors and the degree of the patient s immunosuppression (Revathi et al., 1998). If sub-eschar tissue is invaded, disseminated infection is likely to occur, and the causative infective microorganisms in any burn facility change with time (Altoparlak et al., 2004). Individual organisms are brought into the burns ward on the wounds of new patients. These organisms then persist in the resident flora of the burn treatment facility for a variable period of time, only to be replaced by newly arriving microorganisms. Introduction of new topical agents and systemic antibiotics influence the flora of the wound (Bhama et al., 2013).

Every treatment facility has unique to microorganisms and these change with time. It is therefore of paramount importance to have an in-depth knowledge of the resident organisms and their antibiotic sensitivity pattern so that infection-related morbidity and mortality are improved. During the period from 2002 to 2005 *Pseudomonas* species was the commonest

pathogen isolated (51.5%) followed by *Acinetobacter* species (14.28%), *Staph. aureus* (11.15%), *Klebsiella* species (9.23%) and *Proteus* species (2.3%) (Song et al., 2004). When compared with the results of the previous five years i.e., 1997 to 2002, *Pseudomonas* species was still the commonest pathogen in the burns unit. However, the isolation of this organism and other gram-negative organisms had decreased in comparison to previous years. Newer drugs were found to be effective.

Conclusion

E. coli is the most common ESBL producing bacteria causing the burn wound infection. Pseudomonas species are the most common bacteria isolated from burn wound patients; however, the frequency of ESBL among these bacteria is low. Routine microbiological surveillance and careful in vitro testing prior to antibiotic use and strict adherence to hospital antibiotic policy may help in the prevention and treatment of multi-drug resistant pathogens in burn infection.

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