

Detection of Pathogenic Microorganisms from Burn Patients Admitted in Tertiary Medical College Hospital and Their Antimicrobial Patterns

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Abstract

Object: To isolate and identify the microorganisms from the burn patients admitted to the National Institute of Burn and Plastic Surgery Unit in Tertiary Medical College Hospital, Bangladesh. A total number of fifty wound surface swab samples of first and second-degree burn patients were collected and the microbial analysis as well as the study of antibacterial susceptibility was conducted. The study showed the bacterial isolates were found. 45 (90%) of wound swab were positive among 50 and only 5 samples (10%) were negative in bacterial growth, which presented invasive burn wound infection from both sex age groups marked 12 - 60 years. The total viable count TVC-11651 CFU/plate was found and the highest amount in the second-degree burn patients. The results showed that *Pseudomonas aeruginosa* was common in all positive samples 6636 CFU/plate (57%) followed by *Staphylococcus aureus* 4070 CFU/plate (35%), *Klebsiella* spp. 450 CFU/plate (5%), *Proteus* spp. 243 CFU/plate (2%), and *E. coli* 162 CFU/plate (1%). Most of the pathogens were found to be drug-resistant while several isolates were noted to be multi-drug resistant. The growth of multidrug-resistant organisms should be considered as a serious risk factor in a burn unit. Aggressive infection control measures should be applied to limit the emergence and spread of multidrug-resistant pathogens.

Keywords

Pseudomonas aeruginosa, Antimicrobial Activity, Burn Wounds, Microorganisms

1. Introduction

Burn injury is one of the most common and devastating forms of trauma and a major public health concern all around the world. The burn patients have a unique predisposition to different infections which are linked to impaired resistance from disruption of the skin's mechanical integrity and generalized immune suppression. The skin barrier is replaced by a protein-rich, vascular environment that provides a favorable niche for microbial colonization and proliferation by one or more sepsis of microorganisms anywhere and becomes better placed to cause damage to the host sterile tissues. A wound can be infected by a variety ranging from bacteria to fungus and parasites mostly gram-positive and gram-negative microorganisms. Additionally, the migration of the immune cell is hampered, which contributes to the septic process [1]-[6]. It has been estimated that 75% of all deaths following thermal injuries are related to infection. Initially, the burned area is considered free of major microbial contamination. However, gram-positive bacteria in the depths of sweat glands and hair follicles may survive the heat of initial injury and unless topical antimicrobial agents are applied, these bacteria heavily colonize the wounds within the first 48 h post-injury [7] [8] [9]. Although any organisms is a potential pathogen in burned patients, coagulase-negative *Staphylococci* and *S. aureus* were the most common gram-positive pathogens and *Pseudomonas aeruginosa*, *E. coli*, and *K. pneumoniae* and *Proteus vulgaris* were the common gram-negative microorganisms [10] [11]. Thus, the current research study aimed to determine the microorganisms and their susceptibility patterns which were isolated from burn wounds of patients at the National Institute of Burning and Plastic Surgery Unit of Dhaka Medical College Hospital Dhaka. Burns is one of the most common traumas. There are about 2 million fires each year, 1.2 million people with burn injuries, 100,000 hospitalizations, and 5000 patients die from related complications [12]. Nosocomial infections (NI) are common in burn patients due to the typical features of the disease: loss of the first line of defense against microbial invasion; the presence of devitalized, a vascularized tissue that provides a favorable environment for microbial growth; alterations in the specific and nonspecific components of the immune system; gastrointestinal translocation; and extended hospitalization and multiple invasive diagnostic and therapeutic procedures [13] [14]. In recent years, drug resistance to human pathogenic bacteria is being commonly reported from all over the world. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics [15], although pharmaceutical industries have produced a large number of newer antibiotics in the last three decades. The reason behind this is that microorganisms are becoming resistant to both older and newer antibiotics [16]. Besides, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents and transferring the resistance from one bacterium to another. Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses

as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases [17]. However, overuses of antibiotics have become the major factor for the emergence dissemination of multidrug-resistant strains of several groups of microorganisms. Resistant bacteria impact public health in such a way that it increases morbidity and mortality from treatment failures and increases healthcare cost as newer and more expensive antibiotics are needed to treat infections [18]. Resistant bacteria are emerging worldwide as a threat to the favorable outcome of common infections in the community and hospital settings [19]. *Staphylococcus aureus* showed resistance due to the production of penicillinase with the ability to hydrolyzing penicillin, the first generation resistant due to beta-lactamases, and third-generation cephalosporin are resistant due to the production of extended-spectrum beta lactamases (ESBLs). Microorganisms are the enemies to mankind and cause very profound damage to the human body as well as other living organisms. The agents, which can kill the microbes or arrest the multiplication, are called the antimicrobial agent's drugs. There are a lot of antimicrobial drugs of which some are discovered or established [20] [21].

2. Methods and Materials

2.1. Study Population

A total number of 50 wound swab samples were collected from burn patients admitted in the burn and plastic surgery unit in Dhaka Medical College Hospital within (January 2015 to May 2015). Among them, 28 were male patients and 22 were female patients. Age groups were 12 years to 60 years. Degree of burn first degree 24 and second degree 26 were included in this study.

2.2. Ethical Approval

Before starting this research project ethical permission was obtained from the Project Director and an ethical review committee of the national institute of burn and plastic surgery in medical college hospital. Patient consents were obtained from the inpatient consent form. A questionnaire was filled up before collecting any patient sample.

2.3. Samples Preparation

Wound samples were aseptically collected 7 days after admission to the hospital. The sample was collected from the different sites of burn, especially from the chest, hands, and legs.

2.4. Processing of Specimens

The specimen was processed according to the guidelines for the laboratory diagnosis of pathogens. This includes macroscopic, microscopy, Gram's staining, culture, biochemical, and antimicrobial sensitivity testing.

2.5. Study Site/Site

The study was conducted in the Department of Microbiology at Primeasia University, Bangladesh.

2.6. Sample Collection Procedure

At the first preparation of 0.9% sodium chloride solution, each test tube contains 5 ml and then sterilized by autoclave. Surface swabs were collected from burn wounds after removal of the dressing and application of 70% ethanol for cleansing the wound surface. Using sterile cotton swabs an area of 4 cm² where the degree of the burn was highest had been swabbed for each patient. After collection of samples were homogenized in 5 ml sterile normal solution [22].

2.7. Microbiological Study of Burn Wound Samples

Plate culture method: all specimens were inoculated on 5% Blood agar, MacConkey, Mannitol salt agar, and Cetrimide agar plates by spread plate method, under an aseptic condition in a laminar airflow cabinet. Then culture plates were incubated overnight at 37 degree C aerobically. Isolation of microorganisms by total viable count by using colony counter. Blood agar was used for isolation and identification of all kinds of bacteria, MacConkey agar was used for gram-negative bacteria (*E. coli*, *Klebsiella* spp., *Proteus* spp.). Mannitol salt agar for *Staphylococcus* spp. and Cetrimide agar for *Pseudomonas* spp. Then gram staining for gram-positive and gram-negative and microscopic examination for morphology, color, and shape.

2.8. Confirmative Biochemical Study

Identify the bacteria from isolated samples several biochemical tests were done such as catalase, oxidase, IMVIC, TSI test. Then identified bacterial spp was put into nutrient agar slant and subcultures at 37 degree C for 24 hours to perform antibiotic sensitivity test.

2.9. Antibigram Study of Burn Wound Samples

The standard agar disc diffusion method known as the Kirby-Bauer method was applied to study of antibiogram. At first Muller Hinton agar plates were prepared. Before inoculation, the sterile swab stick was passed against the wall of the normal saline solution tube to drain out the excess fluid and moistened. By using sterile technique bacterial cultures were taken by sterile cotton swab stick and a uniform lawn of bacterial growth was prepared on Muller Hinton agar plates. Using sterile forceps, antibiotic discs were placed equally spread apart on the surface of the medium. 5 discs were used on each plate. The plate was incubated overnight at 37 degree C and the results were obtained no more than 24 h from incubation. The antimicrobial pattern was interpreted by the presence or absence of a clear zone around the antibiotic disc and the zone of inhibition was measured in mm by applying an ordinary ruler.

3. Results

Prevalence of microorganisms in burn wound samples (**Figure 1**): Out of 50 samples, 45 were found to be hugely populated with bacterial load from 120 - 500 CFU/plate, among them almost all were found to harbor *Pseudomonas spp.* in the range of 130 - 430 CFU/plate (**Table 1** and **Table 2**). Growth and proliferation of *S. aureus* was observed in 44 samples ranges from 15 - 360 CFU/plate. Among the enteric bacteria, *Klebsiella spp.* was found to prevail among 32 samples in the ranges of 5 - 30 CFU/plate, and the comparative lower frequency was observed in the case of *Proteus spp.* in 23 samples and *E. coli* in 13 samples.

Table 1. Bacterial load (CFU/plate) in burn wound swabs.

Samples	Degree of burning	TVC (CFU)	<i>P. aeruginosa</i> CFU/plate	<i>S. aureus</i> CFU/plate	<i>K. spp</i> CFU/plate	<i>Proteus spp</i> CFU/plate	<i>E. coli</i> CFU
01	1 st	200	75	80	20	15	10
02	1 st	92	90	1	1	0	0
03	2 nd	450	90	360	0	0	0
04	1 st	0	0	0	0	0	0
05	1 st	176	56	100	10	6	4
06	2 nd	415	240	150	20	5	0
07	2 nd	535	310	220	5	0	0
08	2 nd	320	170	80	30	25	15
09	2 nd	226	150	0	26	20	30
10	1 st	150	90	40	10	7	3
11	1 st	67	50	17	0	0	0
12	1 st	80	60	15	5	0	0
13	1 st	120	85	25	10	0	0
14	1 st	230	130	90	5	5	0
15	2 nd	310	230	80	0	0	0
16	2 nd	300	130	170	0	0	0
17	2 nd	420	350	70	0	0	0
18	2 nd	500	430	70	0	0	0
19	2 nd	340	260	60	10	5	0
20	1 st	70	40	30	0	0	0
21	2 nd	430	260	120	20	10	10

*TVC (total viable count). Among 50 samples (1 - 28) were male patients and rest of the samples (29 - 50) were female patients. All the experiments have been done three times and the results were reproducible. One representative data have been shown.

Table 2. Confirmative biochemical identification of the isolates.

Pathogenic organisms	Catalase test	Oxidase test	TSI test				Indole Production	MR test	VP test	Citrate utilization test
			slant	Butt	Gas	H ₂ S				
<i>P. aeruginosa</i>	+	+	R	R	-	-	-	-	-	+
<i>S. aureus</i>	+	-	Y	Y	-	-	-	+	+	-
<i>K. spp.</i>	+	-	Y	Y	+	-	+	+	+	+
<i>Protus spp.</i>	+	-	Y	Y	+	+	+	+	-	+
<i>E. coli</i>	+	-	Y	Y	+	-	+	+	-	-

TSI = Triple Sugar Iron, R = Red (alkaline), Y = Yellow (acidic), MR = Methyl Red, VP = Voges Proskauer.

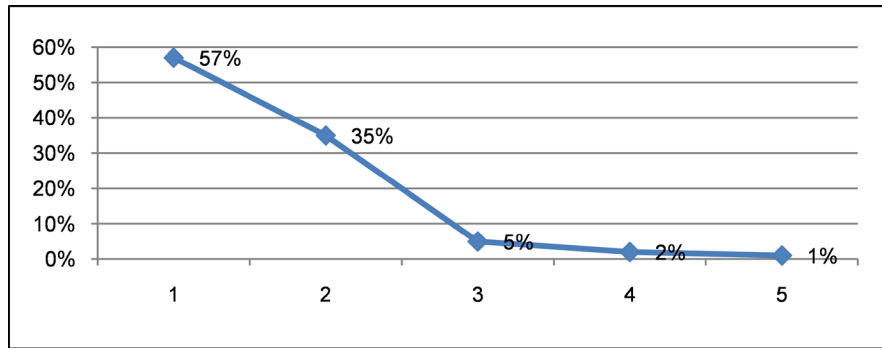
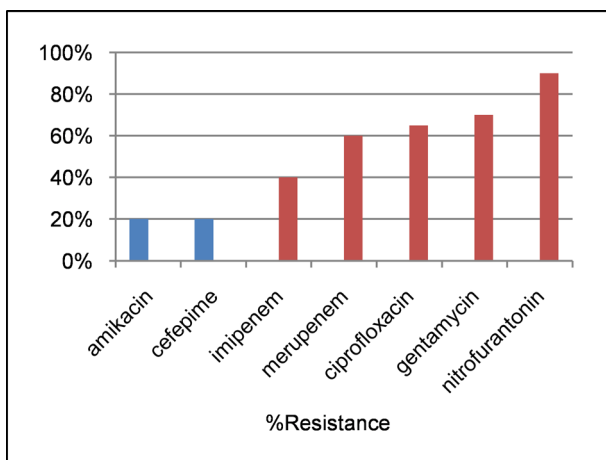
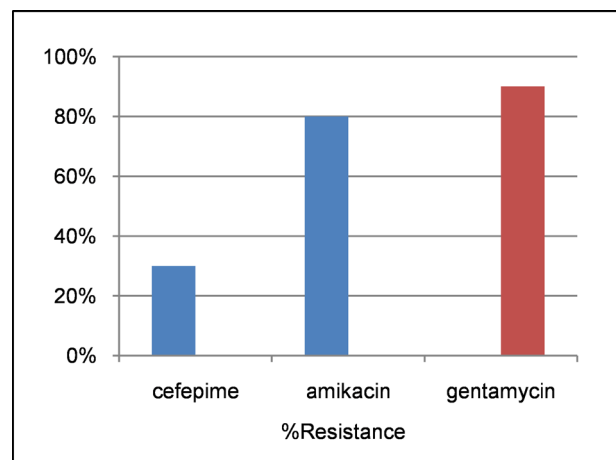


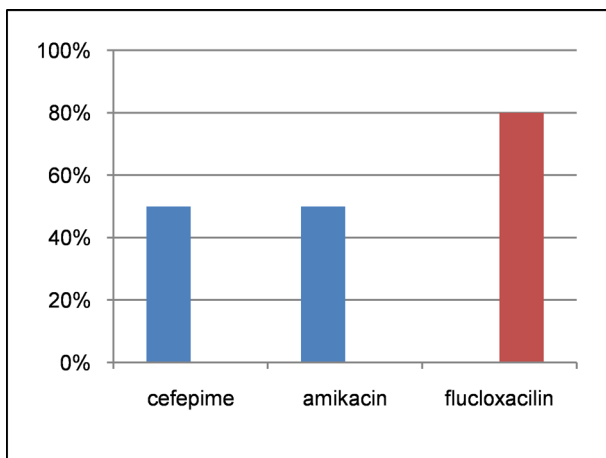
Figure 1. Percentage of isolated organisms from the collected samples: TVC = 11,641. CFU/plate. 1. *Pseudomonas aeruginosa*. 6636 CFU/plate (57%); 2. *S. aureus*. 4070 CFU/plate (35%); 3. *Klebsiella* spp. 450 CFU/plate (5%); 4. *Proteus* spp. 243 CFU/plate (2%); 5. *E. coli* 152 CFU/plate (1%).



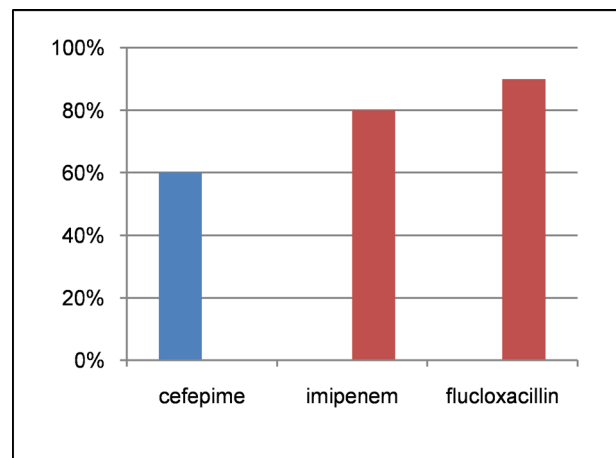
(a)



(b)



(c)



(d)

Figure 2. Antimicrobial % of resistance pattern of *Pseudomonas* spp. (a), *Staphylococcus aureus* (b), *E. coli* (c), and *Klebsiella* spp. (d) against Ciprofloxacin (cip5), Amikacin (Ak30), Cefepime (CP30), Gentamycin (CN10), Nitrofurantoin (NF), Imipenem (IP10), Meropenem (MP), flucloxacillin (FX). The presented data were statistically analyzed by showing standard errors consider as 5%. All experiments were carried out three times and 95% accuracy was found. However, sensitivity of *S. aureus* was scored towards imipenem (IP10) and meropenem (MR) and partially sensitive to flucloxacillin (FX). *E. coli* was found 100% sensitive to ciprofloxacin, and *Klebsiella* spp was found to be sensitive to amikacin (AK30) and gentamycin (CN10) respectively.

Drug-resistance traits of the isolates: There are 8 common drug used, amikacin (n = 45, 20%), cefepime (n = 45, 20%), imipenem (n = 45, 40%), meropenem (n = 45, 60%) ciprofloxacin (n = 45, 65%), gentamycin (n = 45, 70%), nitrofurantoin (n = 45, 90%) were found to be ineffective against *Pseudomonas spp.* (Figure 2(a)), cefepime (n = 44, 30%), amikacin (n = 44, 80%), gentamycin (n = 44, 90%) were found to be ineffective against *S. aureus.* (Figure 2(b)), cefepime (n = 13, 50%), amikacin (n = 13, 50%), and flucloxacillin (n = 13, 80%) were found to be ineffective against *E. coli* isolates (Figure 2(c)). Cefepime (n = 23, 60%), imipenem (n = 23, 80%), flucloxacillin (n = 23, 90%), were found to be ineffective against *Klebsiella spp.* (Figure 2(d)).

4. Discussion

The prototype of bacterial resistance appears to be imperative for epidemiological study. It is worth noting that the multidrug-resistant isolates of *Acinetobacter baumannii* and *P. aeruginosa* are particular concern in burn care units. In our study, almost all the isolates exhibited the multi-drug resistance trait against commonly used antibiotics. However, an important clinical consideration has to be taken on the fact that since *E. coli* and *Klebsiella* pneumonia are well known to be the extended spectrum β -lactamase producers; these isolates found in our study may be further subjected for study [23] [24] and the prevalence of bacteria in 50 burn wound swabs was shown in the bacterial isolates were found in 45 (90%) wound swab samples, and only 5 samples (10%) were negative in bacterial growth. In this study the total viable count CFU/plate was found the highest amount in the second-degree burn patients. The results showed that *Pseudomonas aeruginosa* was common in all positive samples (57%) followed by *Staphylococcus aureus* (35%), *Klebsiella spp.* (5%), *Proteus spp.* (2%), and *E. coli* (1%). All detected pathogens were Catalase positive and Oxidase negative but *Pseudomonas spp.* was Oxidase positive. Most of them were Indole, MR, VP test positive but *Pseudomonas spp.* was Indole, MR and VP negative, *S aureus* Indole negative, *Klebsiella* was MR negative, *Proteus*, and *E. coli* VP negative. In Citrate Utilization Test all were positive except *S. aureus* and *E. coli*. In Triple Sugar Iron Agar Test all organisms were positive except *Pseudomonas aeruginosa* and *S. aureus* among them *Proteus* was unable to produce H₂S. *Pseudomonas* and *S. aureus* were resistant to all antibiotics but *S. aureus* was sensitive to Imipenem and Meropenem and partially sensitive to flucloxacillin. *E. coli* were 100% sensitive to ciprofloxacin, *Klebsiella spp.* were sensitive to Amikacin and. The pattern of bacterial resistance is important for epidemiological and clinical purposes [22] [25]. The results of the antimicrobial pattern give serious cause for concern because the predominant bacterial isolates were highly resistant to the commonly available antimicrobial agents [20] [26]. Our study revealed huge proliferation of bacteria in the burn wound samples studied, and traced a number of multi-drug resistant isolates despite the sex and degree of tissue damage of the patients. The results are in accordance with the contemporary studies and further implicate

the necessity of stringent care in burn unit in hospitals. Routine monitoring of burn infections with antibiogram profile employing the primary experiments described here would be effective in delivering the detailed profile of burn wound prevailing microorganisms and hence would be implicative in context of overall public health management [21] [27] [28] [29] [30].

5. Conclusion

In conclusion, gram negative bacteria were the dominating bacteria all over the study period especially *P. aeruginosa*, most of which were multidrug resistant. Amikacin was the drug of choice for most gram negative bacteria and vancomycin was found to be effective against gram positive bacteria (*S. aureus* and coagulase negative *Staphylococci*). Present investigation seems to be helpful in providing useful guidelines for choosing effective therapy against isolates from burn patients. Huge bacterial onset with an alarming threat of multidrug resistance would potentially raise the necessity of proper care and management of burn wound patients in hospital.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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