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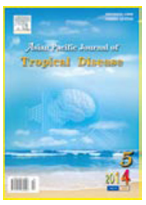

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Isolation of pathogenic microorganisms from burn patients admitted in Dhaka Medical College and Hospital and demonstration of their drug-resistance traits

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PEER REVIEW

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Comments

The study findings are interesting, methods are clearly described, sufficient literatures have been cited, results have been reported and interpreted clearly. The manuscript is succinct to read and easily understandable. The authors detected the bacterial proliferation, pondered their drug-resistance properties, and finally evaluated the disinfectant efficacy.

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ABSTRACT

Objective: To isolate and quantify the microflora from the burn patients admitted in the Division of Plastic Surgery and Burns outdoor patients in Dhaka Medical College Hospital, Bangladesh.

Methods: Thirty wound surface swab samples of first and second degree burn patients were collected and the microbial analysis as well the study of antibacterial susceptibility was conducted. Microbial inhibitory concentration of tobramycin was tested to be applied as effective antimicrobial agent in burn patients. Activity of four disinfectants was also tested against the pathogens.

Results: Among all samples, 28 was found to be populated with the total viable bacteria up to 10^7 CFU/mL. The predominant pathogen was *Pseudomonas* spp., followed by *Staphylococcus aureus* and *Kebsiella* spp. Three of the samples harbored *Enterobacter* spp. while 2 were found to be proliferated with *Escherichia coli*. Most of the pathogens were found to be drug-resistant while several isolates were noted to be multi-drug resistant. Dettol partly showed efficacy among the tested disinfectants to prevent pathogenic proliferation.

Conclusions: 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.

KEYWORDS

Antimicrobial activity, Burn wounds, Microorganisms, Public health

1. Introduction

Burn injuries, either non-invasive or invasive, are frequently exposed to microbial infection together with a general state of immune suppression[1-4]. Nosocomial or hospital-acquired infections, caused by microorganism present as part of the normal flora of the patient, or exogenous infections acquired through exposure to the hospital environment, hospital personnel or medical devices, are mostly known to be associated with burn wound infection[5-10].

Any bacterium could be a likely pathogen in burn wounds; however, coagulase-negative staphylococci, *Staphylococcus aureus (S. aureus)* and *Enterococcus* spp. have been reported to be the most common Gram positive pathogens, and *Pseudomonas aeruginosa (P. aeruginosa)*, *Escherichia coli (E. coli)*, *Klebsiella pneumoniae* and *Acinetobacter* spp. are the most common Gram negative microorganisms[8,11-

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13]. The risk of invasive burn wound infection is influenced by the extent and depth of the burn injury, various host factors, and the quantity and virulence of the microbial flora colonizing the wound[14,15].

Patients suffering from severe burn (>20 percent total body surface area) are at a high risk of developing an invasive burn wound infection with a concomitant burn wound sepsis, leading to multi-organ dysfunction and death[16,17]. Nosocomial infections and emerging multi-drug resistant microorganisms further contribute to burn wound infections, sepsis, and associated death[10,16,18]. Despite the significant advances in antimicrobial treatment, fatality is still the topmost problem in case of burn patients. The worldwide emergence of drug-resistant bacteria has also limited the medication efficacy[8,13,15,19]. Emergence of multi-drug resistant *P. aeruginosa*, multi-drug resistant Gram-negative bacill, *Enterobacter* spp., *Serratia* spp. and *Citrobacter freundii* are well known[20-22]. Besides, most of the nosocomial *S. aureus* infections are known to be caused by methicillin-resistant *S. aureus* strains resulting in fatality[23,24].

Being a developing and densely populated country with lack of health awareness, onset of infectious diseases with the incidence of drug-resistance are very likely in Bangladesh[25-27]. Burn cases are also very frequent, however, with an inadequate management in the burn units in context of proper medication, spatial nursing and complete elimination of nosocomial infections. Several local studies have been conducted in this regard, and the prevalence of several drug-resistant pathogenic microorganisms has been evident. Nevertheless, the frequency of monitoring of wound prevailing microorganisms needs to be heightened for the betterment in burn wound sepsis control[28,29]. The resultant knowledge may be employed with the goal of burn wound management which is in turn to reduce the onset and density of bacterial growth and proliferation within the wounds. Along these lines, current investigation was designed to determine the bacterial diversity and their resistance patterns in burn and infected patients.

2. Methods and materials

2.1. Study population

Patients with open burntube wounds admitted in Dhaka Medical College Hospital burn unit (within October 2012-February 2013) for skin grafting were included in the study. Patients with chronic burn wounds and those admitted with burn wound contracture were excluded from the study. A total of 30 patients (18 female and 12 male patients) of first degree (n=20) and second degree (n=10) burns were included during the study period.

2.2. Ethical approval

Ethical permission was obtained from the Ethical Review Committee of Dhaka Medical College Hospital prior to starting this research project (Supplement I). Patient consents were obtained in patient consent form (Supplement II) before collection of samples and were kept confidential. A questionnaire was filled up before collecting any patient sample (Supplement III).

2.3. Sampling

Wound samples were aseptically collected on Day 7 after admission. Multiple samples from several areas of the burn (especially from the chest, hands and legs of the patients) were collected in order to obtain the most accurate assessment. Surface swabs were collected from burn wounds after the removal of dressings and topical antimicrobial agents and cleansing of the wound surface with 70% alcohol[12]. An area of about 4 cm^2 will be swabbed using two sterile cotton swabs. Swab samples were taken from the wound area where the degree of burn is highest. Samples were homogenized in 4 mL sterile saline.

2.4. Microbiological and biochemical analysis

Samples were immediately cultured on blood agar and MacConkey agar plates. Pathogenic microorganisms were isolated and identified following the standard procedures[30]. MacConkey agar was used for the isolation of Gram negative bacteria while the blood agar was used for isolation and identification of Gram positive bacteria. Nutrient agar was used for the general cultivation and maintenance of bacteria. After inoculation, plates were kept at 37 °C for 24-48 h. A series of several biochemical tests were performed following the standard protocol to identify the bacteria isolated from the wound samples[31].

2.5. Study of antibiogram

The standard agar disc diffusion method known as the Kirby-Bauer method was applied[32-34]. A suspension of the test organisms were prepared by adjusting the turbidity of the broth in phosphate buffer saline by comparing with McFarland 0.5 solutions. A uniform lawn of bacterial growth was prepared on Muller Hinton agar plates. Before inoculation, the swab was passed against the wall of the tube to drain out the excess fluid. Commercially available antimicrobial discs (Oxoid, Hampshire, UK) were applied aseptically (amikacin 30 μg, cefepime 30 μg, gentamicin 10 μg, imipenem 10 μg, erythromycin 15 μg, neomycin 30 μg, streptomycin 10 μg and tobramycin 10 μg) on the surface of the inoculated plates at appropriate spatial arrangement by means of a sterile needle. Susceptibility to the specific antibiotic was interpreted by the presence of clear zone around the disc[35].

2.6. Disinfectant susceptibility test

The susceptibility of the isolates towards antiseptic were tested by using the cup-plate diffusion technique described by Kavanagh[36], on Muller Hinton agar. Four cups were cut using sterile cork borer and filled with 0.1 mL of each antiseptic solution (Savlon, Lizol, Dettol) by using adjustable volume digital pipette and allowed to diffuse at room temperature for 2 h. As the positive control, imipenem disc