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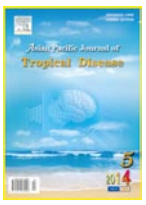

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Isolation of pathogenic microorganisms from burn patients admitted in Dhaka Medical College and Hospital and demonstration of their drug-resistance traits

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PEER REVIEW

Peer reviewer

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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

(30 μg) was used. Plates were incubated in upright position at 37 °C for 18 h. Growth inhibitory zones were then measured.

3. Results

3.1. Prevalence of microorganisms in burn wound samples

Out of 30 samples, 28 were found to be hugely populated with bacteria ranging from 10^5–10^7 CFU/mL, among which almost all were found to harbor *Pseudomonas* spp. in the range of 10^4–10^7 CFU/mL (Table 1 and 2). Growth and proliferation of *S. aureus* was observed in 25 samples (10^3–10^6 CFU/mL). Among the enteric bacteria, *Klebsiella* spp. was found to prevail among 16 samples in the range of 10^3–10^7 CFU/mL and a comparative lower frequency was observed in case of *E. coli* (in 2 samples).

Table 1

Bacterial load (CFU/mL) in burn wound samples.

Samples*	Degree of burning	TVC (CFU/mL)	*Pseudomonas*spp. (CFU/mL)	*S. aureus* (CFU/mL)	*E. coli* (CFU/mL)	*Klebsiella* spp. (CFU/mL)
01	First	1.38×10^5	5.60×10^4	1.4×10^4	0	0
02	First	6.80×10^6	1.70×10^7	2.4×10^6	0	1.40×10^7
03	First	7.20×10^6	1.70×10^7	0	0	1.02×10^5
04	First	2.30×10^5	4.60×10^4	4.0×10^3	0	7.00×10^3
05	Second	7.20×10^6	2.10×10^7	4.0×10^5	0	4.60×10^4
06	Second	1.00×10^3	1.20×10^4	9.0×10^3	0	0
07	Second	7.80×10^6	1.05×10^7	1.2×10^6	0	3.20×10^6
08	First	1.48×10^7	5.20×10^6	2.3×10^6	0	6.60×10^6
09	First	5.60×10^6	7.20×10^6	2.0×10^5	0	2.80×10^6
10	First	4.80×10^6	7.10×10^6	1.2×10^6	0	0
11	First	1.40×10^7	1.23×10^7	0	0	0
12	Second	2.42×10^5	1.80×10^5	4.0×10^3	0	1.56×10^5
13	First	1.07×10^5	1.26×10^5	1.2×10^4	0	4.80×10^4
14	First	5.60×10^7	7.20×10^4	0	0	1.30×10^3
15	First	8.20×10^6	5.60×10^6	1.1×10^6	0	0
16	Second	1.10×10^5	6.80×10^4	1.2×10^4	0	4.80×10^4
17	Second	1.34×10^5	1.05×10^5	3.0×10^3	3.2×10^4	3.20×10^4
18	Second	5.70×10^6	4.20×10^6	4.0×10^5	0	0
19	First	4.80×10^6	3.20×10^6	1.2×10^6	0	9.00×10^5
20	First	4.80×10^6	5.20×10^6	1.0×10^5	0	1.20×10^4
21	First	4.30×10^6	3.80×10^6	8.0×10^5	0	0
22	First	3.60×10^6	7.60×10^6	2.0×10^5	0	0
23	Second	5.60×10^6	5.60×10^6	1.3×10^6	0	4.00×10^5
24	Second	5.20×10^6	6.20×10^6	3.0×10^5	0	0
25	First	4.50×10^6	4.80×10^6	8.0×10^5	0	0
26	First	3.00×10^6	1.02×10^7	2.2×10^6	0	0
27	Second	7.80×10^6	4.70×10^6	1.5×10^6	0	5.20×10^6
28	First	1.07×10^5	7.20×10^4	8.0×10^3	1.2×10^4	0
29	First	0	0	0	0	0
30	First	0	0	0	0	0

TVC: total viable count.

*First 18 samples (1–18) were of female patients and the other 12 samples (19–30) were of male patients. All the experiments have been done three times and the results were reproducible. One representative data have been shown.

3.2. Drug-resistance traits of the isolates

Out of 8 common drugs used, amikacin (*n*=2, 50%), cefepime (*n*=2, 50%) and neomycin (*n*=2, 50%) were found to be ineffective against *E. coli* isolates (Figure 1a), amikacin (*n*=28, 25%), cefepime (*n*=28, 25%), gentamicin (*n*=28, 39.29%), imipenem (*n*=28, 50%), erythromycin (*n*=28, 96.42%) and neomycin (*n*=28, 89.28%) were found to be ineffective against *Pseudomonas* spp. (Figure 1c), cefepime (*n*=16, 62.50%), erythromycin (*n*=16, 81%) and imipenem (*n*=16, 81.25%) were found to be ineffective against *Klebsiella* app. (Figure 1b), and gentamicin (*n*=25, 36%), erythromycin (*n*=25, 92%) and imipenem (*n*=25, 96%) were found to be ineffective against *S. aureus* (Figure 1d). Notably, all the bacterial isolates were found to be sensitive against tobramycin 10 μg.

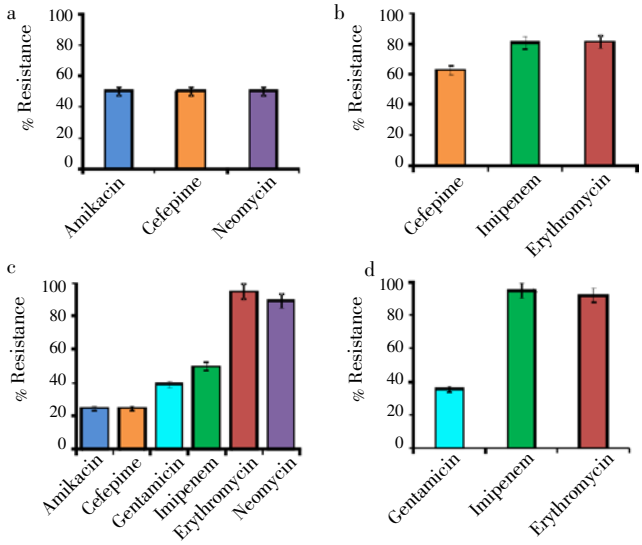

Figure 1. Antimicrobial resistance pattern of *E. coli* (a), *Klebsiella* spp. (b), *Pseudomonas* spp. (c) and *Staphylococcus aureus* (d). against amikacin 30 μg, cefepime 30 μg, gentamicin 10 μg, imipenem 10 μg, erythromycin 15 μg, neomycin 30 μg. The presented data were statistically analyzed by showing standard errors considered as 5%. All experiments were carried out three times and 95% accuracy was found.

However, sensitivity of *E. coli* was scored towards gentamicin (10 μg) and imipenem (10 μg). *Klebsiella* spp. was found to be sensitive to amikacin (30 μg), gentamicin (10 μg) and streptomycin (10 μg), respectively. *Pseudomonas* was was found to be sensitive against streptomycin (10 μg) and staphylococcal isolates were found to be sensitive to amikacin (30 μg), cefepime (30 μg) and streptomycin (10 μg).

Table 2

Confirmative biochemical identification of the isolates.

Identified pathogenic microorganisms	TSI				Motility	Indole production	MR	VP	Citrate utilization	Catalase	Oxidase
	Slant	Butt	Gas	H_2S							
Pseudomonas spp.	R	R	−	−	−	−	−	−	+	+	−
S. aureus	Y	Y	−	−	−	−	+	+	−	+	−
Klebsiella spp.	Y	Y	+	−	+	−	−	+	+	+	−
E. coli	Y	Y	+	−	+	+	+	−	−	+	−

TSI: triple sugar iron test, Y: yellow (acidic), R: red (alkaline), MR: methyl red, VP: Voges-Proskauer.

3.3. Susceptibility of the isolates towards common disinfectants

Among the disinfectants studied, Dettol was found to be most active against the isolates, especially against *Klebsiella* spp. and *E. coli*. However, *S. aureus* was found to be most susceptible to Savlon and Lizol. *Pseudomonas* spp. was moderately susceptible to all of the disinfectants employed (Table 3).

Table 3
Effects of disinfectants on the different isolates.

Microorganisms	Zone of inhibition (mm)				
	Phenyl	Savlon	Lizol	Dettol	Imipenem 30 μg (control)
S. aureus	10	22	20	11	32
E. coli	10	10	15	22	28
Pseudomonas spp.	13	11	11	14	26
Klebsiella spp.	16	10	10	29	29

4. Discussion

Due to the lack of appropriate regulatory encounter on health risk issues in Bangladesh, which is developing country with dense population, commencement of acute complications including the burn cases are very much frequent[25-27]. The regular demonstration of burn associated microorganisms thus stands as a major public health concern[29]. Several studies showed that in context to the presence of *P. aeruginosa*, *S. aureus*, and *E. coli* (as have also been identified in the current study), *Acinetobacter baumannii* was indeed the most prevalent bacterium in the burn wounds[8,11,37]. Isolation of this bacterium would suffice the scenario of burn pathogen cases in Bangladesh as well. In our study, more than 90% samples were found to be massively propagated with *Pseudomonas* spp. with a maximal bio-burden of 10^7 CFU/mL. Around 80% samples revealed the presence of *S. aureus* and more than 50% samples were found to harbor *Klebsiella* spp. Such a high frequency of pathogenic dissemination within the burn cases examined clearly demonstrates the further possibility of the onward onset of opportunistic complications.

Moreover, the prototype of bacterial resistance appears to be imperative for epidemiological study. It is worth to note that the multidrug-resistant isolates of *Acinetobacter baumanni* and *P. aeruginosa* are particular concern in burn care units[8,20,21,37]. 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 of extended spectrum β-lactamase activity[21,26]. Studies on methicillin resistant *S. aureus* together with extensive environmental monitoring would also be interesting as well[6,24,38].

Together with the dangers associated with the burn injury, the hospital-acquired infections may take place due to insufficient disinfection of hospital surfaces, instruments and rooms[5,6]. Generation of multi-drug resistant microorganisms may limit the ability of the usual disinfectants to inhibit or kill the infectious agents. Being led by such idea, we also tested some of the common disinfectants for the microbial growth inhibition. As stated earlier, *Pseudomonas* spp. was found to be insignificantly susceptible to all of the disinfectants which is indeed in cohort with the *Pseudomonas* burden as found in our study.

Modern disease diagnosis and medication by means of antimicrobials have heightened the management of numbers of complications. However, the burn associated morbidity and mortality couldn't be combated properly till date, unfortunately merged with the problem of evolving the drug-resistant pathogens with a concomitant risk of medication inefficiency[8,13,15,19-24]. Legislative actions in these regards especially in the underdeveloped or developing countries like Bangladesh therefore urge immediate measures. In accordance to our study rationale, the findings of our study sufficiently may address the objective of burn wound management in order to trim down the bacterial prevalence within the burn wounds and hence lessen the possibility of the opportunistic infections.

Our study revealed huge proliferation of bacteria in the burn wound samples studied, and traced a number of multi-drug resistant isolates despite of 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.

Conflict of interest statement

Authors have declared no conflict of interest.

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Comments

Background

Burn wound infections are largely known to be assisted by further infections acquired from the hospital environments, and with a possible onset of further opportunistic infections. Besides, the drug-resistant pathogenic dissemination may further complicate the medication strategies. Burn cases in Bangladesh are reported to be very frequently and stand as a major cause of fatality. Careful monitoring of burn wound prevailing microorganisms with adequate hospital hygiene is therefore essential for a sound management of burn cases.

Research frontiers

The study was designed to isolate and identify the burn wound prevailing microorganisms with a further quantification of the specific bio-burden. Also, in order to depict on the hospital associated complications within the burn patients, a microbiological test of the disinfectants was conducted.

Related reports

The study appears as a reflection of one of the current fatal cases as has been done earlier by Noor et al in 2012 and 2013 on the onset of opportunistic infections and on tuberculosis. Regarding burn cases, the resulting data achieved from the current study is significant like the studies especially conducted by Chim *et al*. in 2007 and with a more recent study conducted by Yasemin *et al*. in 2013. While the the identification of *Pseudomonas* spp. and staphylococcal species from the current study is nearly identical to those investigations, the study of the bacterium *Acinetobacter baumannii* is lacking. However, authors have clarified the related reports and the overall microbiological studies are sound and in cohort with the previous studies. The most important part of the current study was the identification of the drug-resistant microorganisms within the burn wounds which is in cohort with some other studies conducted by Guggenheim *et al*. in 2009 and Giske *et al*. in 2008 . Overall, the research presented in the current study is quite relevant in the associated fields and the citations are adequate enough to explain the current data.

Innovations & breakthroughs

Compared to other relevant studies, the major significance of the study lies over the identification of multi-drug resistant pathogenic bacteria within the burn cases. Another important point is to ponder on the relative inefficiency of the disinfectants within the hospital environment which in turn focuses on the hospital associated opportunistic infections among the burn patients.

Applications

The findings of the current study derived the concept not only on the huge bio-burden within the burn wounds but also on the drug-resistance properties of the pathogens. The second aspect is about the effective antimicrobials for medication as well as the converse impact of the disinfectants. Such knowledge is indeed applicable for the well regulation of burn case management especially in the other developing countries. The overall hospital hygiene may also be improved, and the burn unit care management can be upgraded in Bangladesh using the current findings.

Peer review

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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