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Research Article

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Retrospective Study of Bacterial Profile in Wound Swab and Their Susceptibility Pattern in A Tertiary Care Hospital in Dhaka, Bangladesh

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Abstract

Increased antibiotic resistance of bacterial isolates from wound infections is a major therapeutic challenge. This study aimed to identify bacterial isolates associated with wound infection and to determine their antimicrobial susceptibility profile. This retrospective study was conducted in the Department of Microbiology, Bangabandhu Sheikh Mujib Medical University, Dhaka, between January 2023 and December 2023. One thousand six hundred thirty wound swabs were collected, and the bacteriological profile was retrieved. The collected wound swab was processed and cultured using standard techniques in a medical microbiology laboratory. The isolated bacteria were identified by colony morphology, Gram staining, and biochemical reactions. Antibiotic susceptibility testing of the detected isolates was performed using the Kirby Bauer disc diffusion techniques as per the National Committee for Clinical Laboratory Standards guidelines. All retrieved profiles were initially recorded into an Excel Sheet and analyzed using SPSS, version 23.

About 1630 wound swab samples were collected, of which 786(48.22%) showed bacterial growth. Out of 786 bacterial growth, the majority (53.94%) of culture-positive cases were in the age group 21-40 years, and 60.56% were male. Of the 786-culture growth, 645 (82.06%) were gram-negative bacteria, and 141(17.93%) were gram-positive. *Pseudomonas* spp. (32.69%) was the prevailing isolate, followed by *Klebsiella* spp (29.26%), *Staphylococcus aureus* (17.93*%), Acinetobacter* spp (11.57%), *Escherichia coli* (5.21%), *Proteus* spp (2.16%), *Enterobacter* spp (1.01*%) and Serratia* spp (0.12%). Among gram-negative isolates, most *Pseudomonas* spp were resistant to ciprofloxacin (83.65%), followed by gentamicin (65.75%) and ceftazidime (61.08%). The highest sensitivity was exhibited for colistin, which demonstrated 10.11% resistance among *Pseudomonas spp*, and the least resistance to meropenem (45.91%), piperacillin+tazobactam (47.85%) and amikacin 47.85%. Among gram-positive isolates, *Staphylococcus aureus* was susceptible to linezolid (100%), vancomycin (100%), cotrimoxazole (59.58%), and gentamicin (58.86%). However, they exhibited resistance to amoxicillin (84.39%), cephradine (73.73%), ciprofloxacin (82.26%), and erythromycin (78.72%). Isolated *Klebsiella* spp were mostly resistant to amoxicillin (94.34%), followed by cefuroxime (80%) and cefotaxime (75.65%). Most *E. coli* was resistant to amoxicillin (95.12%) cefotaxime (75.60%) and ceftriaxone (70.73%) All the *E. coli* isolates were sensitive to colistin. These results indicate that the isolation rate from wound infection was high and the increasing trend of antibiotic resistance in both gram-positive and gram-negative bacteria is alarming, which may lead to treatment failure.

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Introduction

Wound infection is defined as the presence of replicating microorganisms within a wound, leading to host or tissue injury. Agents that cause wound infection can be classified based on the depth of the wound, and they serve as the carriers for organisms that cause infection [1]. The presence of pathogenic bacteria in the wound does not imply infection. Infection occurs when one or more than one contaminant evades the host defenses, replicating in large numbers, attacks, and harms the host tissue. Different microbial organisms can infect wounds [1]. They are likely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* spp*, Acinetobacter* spp*, Escherichia coli, Proteus* spp*,* and *Enterobacter* spp [2,3]. Wound infection is a significant problem in Bangladesh. Complications of wound infection are very common because of poor hospital management and poor aseptic techniques used in the hospitals during surgical procedures and other hospital procedures. It is the most acquired infection in hospitals, which has contributed the majority to prolonged hospitalization and higher costs and is associated with considerable morbidity and mortality rates, especially in the developing world [3,4,5]. Regional and local variations occur among causative microorganisms of wound infection. Thus, clinicians should be aware of common causative agents and their antimicrobial susceptibility profile in their locality [6].

This study was conducted to identify bacterial pathogens associated with wound infections and determine their resistance to commonly used antibiotics among patients with wound infection isolates.

Materials and Methods

A retrospective study was done in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January 2023 to December 2023 for one year. All samples were collected from outpatients and inpatients of BSMMU. A total of 1630 wound swabs were collected. The skin around the surgical wound was sterilized with 70% ethyl alcohol using a sterile cotton-wool swab to avoid touching the surrounding tissues to prevent swab contamination with endogenous skin flora. The wounds were carefully cleaned using sterile gauze moistened with sterile physiological saline. Each sample was collected using two sterile swabs from the wound ground and edge using the Levine technique. The sample was placed in an Amies transport medium, labeled, and transported to the clinical microbiology laboratory without any delay. The smear was prepared directly from the first swab and stained with gram stain. Wound swab samples were received in non-sterile containers; dry samples and samples from patients on antibiotics were rejected. All samples were cultured in blood agar and MacConkey agar media, and incubated overnight at 37^0C for 24-48 hours. Organisms were identified by a standard microbiological procedure, including colony characters and gram staining.

All the isolates were tested for antimicrobial susceptibility by disc diffusion methods according to the Clinical Laboratory Standard Institute (CLSI) guidelines [7,8]. The following antibiotics were used for gram-negative bacteria: amoxicillin, amoxicillin-clavulanic acid, ciprofloxacin, ceftriaxone, gentamicin, cefotaxime, ceftazidime, cotrimoxazole, cefuroxime, amikacin, aztreonam, meropenem, netilmicin, tazobactam piperacillin, cefepime and colistin. For gram-positive bacteria, the following antibiotics are used: amoxicillin, ciprofloxacin, cefradine, cloxacillin, erythromycin, gentamicin, cotrimoxazole, cefoxitin, vancomycin and linezolid. *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 were included as control strains. Cefoxitin 30 microgram was used as a surrogate marker for identifying MRSA. *Staphylococcus aureus*, which showed a zone of inhibition < 21 mm with cefoxitin on Mueller-Hinton Agar after overnight incubation at 37^0C, was considered MRSA [9].

Results

A total of 1630 wound swabs were collected, of which 786 (48.22%) yielded bacterial growth (Table 1). Among them, gram-negative bacteria were 645(82.02%), and gram-positive bacteria were (17.93%) (Figure 1).

Table 1: Frequency of Bacterial isolates in wound swabs (n=1630)

Culture	Frequency	Percentage (%)
Growth	786	48.22
No growth	844	51.77
Total	**1630**	**100**

Out of culture-positive cases, the majority 424(53.94%) were in the age group of 21-40 years (Table 2), and male 476(60.55%) were more commonly affected than female 310(39.44%) patients.

Table 2: Characteristics of the study population with wound swab culture-positive patients (n=786)

Characteristics	Frequency	Percentage
Sex		
Male	476	60.55
Female	310	39.44
Age in years		
<20	38	4.83
21-40	424	53.94
41-60	306	38.93
>60	18	2.29

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Out of 786 isolated organisms, the most common isolate was *Pseudomonas aeruginosa*, which accounted for 257(32.69%) of all the bacterial isolates, followed by *Klebsiella* spp 230 (29.26%), *Staphylococcus aureus* 141 (17.93%), *Acinetobacter* spp 91 (11.57%), *Escherichia coli* 41(5.21%). The least isolated organisms were *Proteus* spp 17(2.16*%), Enterobacter* spp 8 (1.01%), and *Serratia* spp 1(0.12%) respectively (Table 3).

Table 3: Distribution of Bacterial isolates from wound swabs (n=786)

Bacterial isolates (n=786)	Name of isolates	No (%)
Gram-negative bacteria (n=645)	*Pseudomonas aeruginosa*	257(32.69)
	Klebsiella spp.	230(29.26)
	Acinetobacter spp	91(11.57)
	Escherichia coli	41(5.21)
	Proteus spp.	17(2.16)
	Enterobacter spp.	08(1.01)
	Serratia spp	1(0.12)
Gram-positive bacteria (n=141)	*Staphylococcus aureus*	141(17.93)

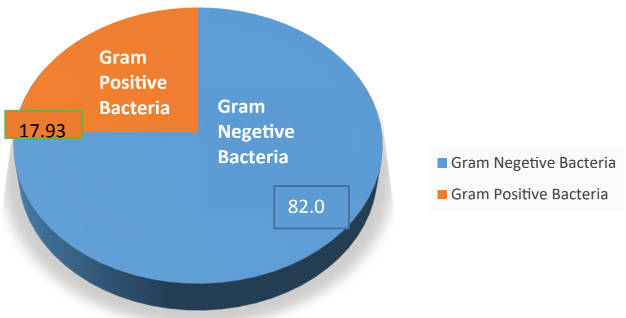

Figure 1: Shows distribution of gram positive and gram-negative bacteria

Among the isolated *Staphylococcus aureus*, 16.31% showed resistance to cefoxitin,73.75% were resistant to cepharadine, and 65.24% to gentamicin. *S. aureus* showed 40.42% resistance to cotrimoxazole, followed by 41.84% resistance to cloxacillin. However, 84.39% of isolates were resistant to amoxicillin, and 82.26% and 78.72% were resistant to ciprofloxacin and erythromycin, respectively. At the same time, the isolated *Staphylococcus aureus* were 100% sensitive to vancomycin and linezolid (Table-4).

Table 4: Antibiotic resistance pattern of gram-positive bacteria to different antibiotics (n=141)

Antibiotic	Resistance	
	Number	Percentage (%)
Amoxicillin	119	84.39
Ciprofloxacin	116	82.26
Erythromycin	111	78.72
Cefradine	104	73.75
Cloxacillin	59	41.84
Gentamicin	58	41.13
Cotrimoxazole	57	40.42
Cefoxitin	23	16.31
Vancomycin	0	0
Linezolid	0	0

The antibiotic resistance pattern of the two hundred fifty-seven *Pseudomonas spp.* isolated from the wound swab is shown (Table 5). Among isolated gram-negative bacteria, Pseudomonas spp. was highly resistant to ciprofloxacin (83.65%), aztreonam (70.03%), gentamicin (65.76**%),** and moderately resistant showed against cefepime (64.20%), and ceftazidime (61.08%) and netilmicin (59.92%). The highest sensitivity was exhibited for colistin, which had only 10.11% resistance among the isolates. However, it is the least resistant to meropenem (45.91%) and piperacillin-tazobactam (47.85%) and amikacin (47.85%) respectively.

Resistance was higher for cephalosporins like ceftriaxone, cefotaxime, and ceftazidime for all the gram-negative isolates, between 61%-83% except for *Proteus* spp, which showed 23.52% resistance to cefepime whereas 50% of *Enterobacter* spp were resistant to cefepime. All isolated *Proteus* and *Enterobacter* exhibited resistance to

Table 5: Antimicrobial resistance pattern of *Pseudomonas spp.* (n=257)

Antibiotic	Resistance	
	Number	Percentage (%)
Amikacin	123	47.85
Aztreonam	180	70.03
Ciprofloxacin	215	83.65
Ceftazidime	157	61.08
Cefepime	165	64.2
Colistin	26	10.11
Gentamicin	169	65.75
Meropenem	118	45.91
Netilmicin	154	59.92
Piperacillin tazobactam	123	47.85

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Table 6: Antimicrobial resistance patterns of isolated gram-negative bacteria (n=786) in wound infections.

Drug tested No (%) of resistance	Microbial species isolated (No %)						
	Pseudomonas spp n=257	*Klebsiella n=230*	*Acinetobacter spp* n=91	*E. coli n=41*	*Proteus spp n=17*	*Enterobacter spp. n=8*	*Serratia spp.* n=1
Amoxicillin	Nt	217 (94.34)	Nt	39 (95.12)	17 (100)	8 (100)	1(100)
Cotrimoxazole	Nt	164 (71.3)	65 (71.42)	25 (60.97)	16 (94.11)	6 (75)	1(100)
Ciprofloxacin	215 (83.65)	165 (71.73)	65 (71.42)	29 (70.73)	15 (88.23)	6 75 (84.4)	0(00)
Gentamycin	169 (65.75)	137(59.56)	67 (73.62)	12 (29.26)	9(52.94)	2 (25)	0(00)
Ceftazidime	157 (61.08)	166(72.17)	62 (68.13)	30(73.17)	11(64.70)	5(62.50)	1(100)
Cefuroxime	Nt	184 (80)	Nt	31(75.6)	15(88.23)	6(75)	1(100)
Amikacin	123(47.85)	127 (55.21)	67 (73.62)	16(39.02)	5 (29.41)	2(25)	0(00)
Aztreonam	180 (70.03)	137(59.56)	67(73.62)	16(39.02)	13 (76.47)	6(75)	Nt
Cefepime	165 (64.2)	Nt	Nt	Nt	4(23.52)	4(50)	Nt
Meropenem	118(45.91)	88(38.26)	41(45.05)	18 (43.9)	8(47.05)	3(37.5)	
Tazobactam Piperacillin	123(47.85)	104 (45.21)	44 (48.35)	20 (48.78)	7 (41.17)	37.5	0(00)
Colistin	26 (10.11)	0 (00)	7 (7.69)	0 (00)	17 (100)	0(00)	0(00)
Ceftriaxone	Nt	166 (72.17)	76 (83.51)	29 (70.73)	11 (64.7)	5 (62.5)	0(00)
Cefotaxime	Nt	174(75.65)	64 (70.32)	31(75.6)	11(64.7)	6 (75)	1(100)

Nt= Not Tested

amoxicillin (100%), but *Klebsiella* and *E.coli* showed almost similar resistance patterns (94.34%) and (95.12%) against amoxicillin respectively. Only one *Serratia* spp was isolated which was sensitive to ciprofloxacin, gentamicin, amikacin, ceftriaxone, and meropenem but resistant to amoxicillin, cotrimoxazole, ceftazidime, cefotaxime, and cefuroxime. The most sensitive antibiotic against all other gram negatives was colistin 100%, against *Klebsiella*, *Acinetobacter*, *E. coli, and Enterobacter.* Proteus was 100% resistant to it because *Proteus* is intrinsically resistant to colistin (Table 6).

Discussion

Wound infection remains a significant concern among healthcare practitioners worldwide, owing to associated morbidity and mortality [10]. It is an important cause of illness that results in prolonged hospital stays and increased treatment costs. It is also likely to play an important role in the development of antimicrobial resistance [11]. Therefore, correctly identifying organisms and determining antimicrobial susceptibility patterns is crucial for appropriately managing wound infection.

In our study, out of 1630 samples from wound infection, 48.22% of samples showed growth. A study by Maharjan, Kartik, Shrestha, and Basnet showed similar results of 50.95%, 47%, and 50% growth from wound infection [1]. In contrast to this study, a higher isolation rate was reported by Mohammed et al. (83.9%) [12]. On the contrary, the rate was lower (61.8%) in the study by Khanam et al. [13]. This difference in bacterial isolation rate may be due to differences in the types of wounds, specimen collection procedures, specimen quality, antibiotic intake of the patient, or microbiological techniques used.

A higher wound infection rate was recorded in males 1120(68.72%) than in females 510(31.28%) of which culture-positive in males were 476(60.56%) and females 310(39.44%) respectively. Similar male predominance was also reported in other studies [14,15]. The reason might be attributed to the fact that male employment is higher in this country. They are involved in occupations such as construction work, farming, transportation, and industry work and were exposed to trauma. In our study, the majority (53.94%) of wound infection cases were within 21-40 years of age group. This agrees with other studies, where it was reported that people in their second to fourth decades of life are more prone to wound infection [12,16]. This is the most vulnerable age group; people are involved in different types of work and have a higher risk of exposure to a variety of wounds.

In our study, out of total bacterial isolates, 82.02% were gram-negative and 17.93% were gram-positive bacteria. In a similar study conducted by Giri et al. (2008), Iregbu et al. (2013), and Eselbelhie et al. (2013), gram-negative bacteria were found to be predominant, which was 56.79%,66%, and 65.45% respectively [17,18,19]. Most hospital-based studies showed that gram-negative bacteria were more prevalent than gram-positive bacteria. Banjara et al. (2002) showed that a higher rate of gram-negative bacteria was found in the

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HAI (Hospital-acquired infection) [20]. A similar study was conducted by Acharya [21] and Yakha et al. [22] where gram-negative bacteria were predominant. The higher number of gram negative isolates in our study may be attributed to the inclusion of hospitalized patients only as it is well known that hospitalization and the procedure undertaken after hospitalization increase the risk of acquiring gram negative infections, The other causes may include the regional variations in geographic locations and socioeconomic status of the study population [22].

Among these isolated bacteria, *P. aeruginosa* was most predominant (32.69%) among total gram-negative isolates followed by *Klebsiella* 29.26%, *Acinetobacter* 11.57%, *E.coli* 5.21%, *Proteus* spp 2.16%, *Enterobacter* spp 1.01% and *Serratia* spp 0.12%. At the same time, *S. aureus* was (17.94%) predominant among total gram-positive isolates. In a similar study conducted by Thanni et al. 2003, *P. aeruginos*a was the most predominant one (29.9%) among the total isolates, while *S. aureus* was predominant (27.5%) among the isolated gram-positive bacteria. In a study conducted by Irebgu et al. (2013) [19], gram-negative bacilli constituted 66% of all pathogens detected, and *P. aeruginosa* was the most frequent (19%). In another study by Pondei K (2013) in Nigeria, *P. aeruginosa* was the predominant pathogen in wound infection [23], which differed from other studies in Nigeria reporting *S. aureus* as the predominant bacteria isolated [24,25]. This disparity might be due to the endogenous infection source or wound contamination from the environment or skin surface.

In our study. *S aureus* showed 100% sensitivity to vancomycin and linezolid, followed by gentamicin (58.86%), whereas amoxicillin and ciprofloxacin were more resistant (84.39% and 82.62%, respectively). Another two studies had shown 100 % sensitivity to Linezolid and vancomycin, followed by gentamicin 78.75% and 73.35%, [26,27], whereas organisms showed maximum resistance to amoxicillin, ciprofloxacin, and erythromycin [28,29]. The above two findings are nearly like our study findings.

In our study, 16.31% of *S. aureus* isolates were MRSA and emerged as a multidrug-resistant pathogen worldwide. Studies on MRSA have shown their wide variation. Naik and Deshpande (2011) showed 8.0% MRSA, consistent with our study [30]. Another study done by Pant et al. (2018) detected 30.70% MRSA, which was higher than our study [31]. Similarly, higher detection was also observed in other studies by Balchandra et al. and Giri et al. 67.6%, and 53.06%, respectively [23,32]. This finding shows that the prevalence of MRSA is increasing. The most effective drugs for MRSA were linezolid and vancomycin, which were 100% sensitive among those isolates, and the finding was similar to the study done by Harsan et al [31].

The remarkable susceptibility of *S aureus* to vancomycin, linezolid, and gentamicin might be due to the lesser use of these antibiotics owing to their low availability, cost, and adverse effects. Low activities of commonly used antibiotics such as cefradine, erythromycin, and ciprofloxacin might be due to increased consumption of these antibiotics, which leads to selection pressure, giving rise to the multiplication of resistant organisms. Increasing resistance might also result from mutation at drug target sites or the disturbance of drug accumulation in the cytoplasm due to cell wall or membrane rearrangement [33]. As a result, they have lost their efficacy in treating wound infections.

The antibiotic susceptibility pattern of isolates revealed high resistance to selected antimicrobials. Bacterial isolates were mainly resistant to amoxicillin (94-100%) and cephradine (73-100%). Similar results were also reported in other studies [12,16,27]. Widespread and non-judicious use of antibiotics without sensitivity testing and self-medication, availability of antibiotics, and low cost might promote the development of resistance to these antibiotics. Similarly, resistance to third-generation cephalosporin like ceftriaxone, cefotaxime, and ceftazidime was higher (60-80% vs 70-100% vs 60-74% respectively). These findings agreed with Sultan et al. [27]. The resistance pattern may be due to the widespread and frequent overuse of third generation cephalosporins for an extended period in this country. Similar studies by Khanam et al. and Sultana et al. supported these findings [13,27]. In our study among gram-negative bacteria, ciprofloxacin resistance was (71-89%). However, other studies reported higher sensitivity to ciprofloxacin (81.2%), (91.8%) and (75.3%) respectively [12,14,15]. This reduced sensitivity in the present study might result from extensive use of these drugs in clinical practice without susceptibility testing. The most effective antibiotics in our study were colistin, meropenem, amikacin, and gentamicin. Bacterial isolates were reasonably sensitive to these antimicrobial agents, which agrees with other studies [13,27]. This may be attributed to the fact that these antibiotics are less commonly prescribed for empirical treatment and are only used in hospitalized patients, according to susceptibility reports.

Among isolated gram-negative bacteria, *Pseudomonas* showed the lowest resistance to colistin, meropenem, piperacillin+tazobactam, and amikacin (10.11%,45.91%, and 47.85% respectively). However, almost all other drugs were resistant. The study done by Albumani et al. [34] showed variable susceptibility patterns with meropenem, piperacillin plus tazobactam, ciprofloxacin, and ceftazidime (100%, 87.71%, 85.71%, and 71.42% respectively) for *P. aeruginosa*. *P. aeruginosa* has a high intrinsic and acquired resistance mechanism to counter most antibiotics.

In our study, *P. aeruginosa* was less resistant to imipenem/meropenem (45.91%) and colistin (10.11%) and maximum

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resistant to ciprofloxacin and ceftazidime. Rajput et al. [35] agreed, in which *Pseudomona*s isolates from wound swabs were less resistant to imipenem/meropenem (26%) and showed maximum resistance to ceftazidime (70%).

Due to the retrospective nature of this study, we were unable to present detailed clinical data on a patient to identify predictors of all forms of wound infection and antimicrobial resistance. This calls for improvements in patient documentation and record keeping.

Conclusion

The present study identified a high frequency of bacterial isolates from wound infections. The predominant isolates were *Pseudomonas, Klebsiella, Acinetobacter*, and *Staphylococcus*. Most of the isolates were found to be resistant to commonly used drugs. Hence, periodic monitoring and surveillance of antimicrobial susceptibility testing are essential for proper wound infection management.

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Author's Contribution

SMA and SA drafted the manuscript. SMA provided statistical analysis of the data. IN, RRK, CKR, AAS, NIS, and SKS validated the results, and revised the manuscript.

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