Original Article

Current Microbial Isolates and Their Antimicrobial Susceptibility Pattern from Wound Infection in a Tertiary Care Hospital

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Abstract

Background: *Wound infection is one of the leading cause of mortality and morbidity worldwide.*

Objectives: *To identify the current microbial isolates and their antimicrobial susceptibility pattern from wound infection in a tertiary care hospital in Dhaka city.*

Materials & Methods: *This retrospective study was conducted in the department of Microbiology at Holy Family Red Crescent Medical College Hospital, Dhaka from July 2019 to December 2020 for a period of one and half years. Written consent was taken from corresponding authority. Microsoft Excel software was used for data analysis. A total 134 wound swab / pus were collected from the patients who were visited in outpatient department and were admitted at inpatient department with suspected wound infection. All samples were processed aerobically on Blood agar, MacConkeys agar media and incubated at 37^{0}C for 24 hours. Organisms were identified by standard procedures including colony characters, Gram staining and biochemical reactions. Antimicrobial susceptibility testing of all the isolates were performed by Kirby Bauer's disc diffusion method.*

Results: *A total 134 samples were analyzed. Of them, predominant populations were male 76(56.72%) and remaining were female 58(43.28%). Out of 134 samples 102 (76.11%) was culture positive. Culture positive was observed higher in inpatient department (IPD) 80 (78.43%) than outpatient department (OPD) 22 (21.57%). Majority of isolates were Gram negative bacteria. Among them predominant bacteria was* E. coli *26(25.49%) followed by* Klebsiella *spp. 24(23.53%),* Pseudomonas *spp. 15(14.71%) &* Proteus *spp. 04(3.92%). Among the Gram - positive isolates 30(29.41%) was* Staph. aureus. E. coli *showed higher sensitivity to meropenem 88.46% & gentamicin 80.76%. Other drugs showed sensitivity to amikacin 65.38%, amoxicillin/clavulenic acid 53.84%, tetracycline 46.15%, ceftriaxone & ceftazidime 42.30%, trimethoprime/ sulfamethoxazole 34.61% & cefuroxime 23.07%. In* Klebsiella *spp. meropenem showed higher sensitivity 79.16% & gentamycin 66.66%. Other drugs like amikacin showed 58.33%, trimethoprime/ sulfamethoxazole 45.83%, ceftazidime & ciprofloxacin 20.83% sensitivity. Low sensitivity 16.66% to amoxicillin/clavulanic acid, cefuroxime, ceftriaxone, & tetracycline was found.* Proteus *spp. showed 75% sensitivity to meropenem. Cefuroxime, ceftriaxone, ciprofloxacin, ceftazidime, gentamycin, amikacin, trimethoprime/ sulfamethoxazole showed very low sensitivity 25% & amoxycillin/clavulanic acid showed 100% resistance.* Pseudomonas *spp. showed highest sensitivity 83.33% to piperacillin tazobactum, amikacin & imipenem, 77.77% to meropenem, 66.66% to gentamycin, 61.11% to ciprofloxacin, 50% to ceftazidime & 33.33% to cefepime. All the isolate of* Staph. aureus *was sensitive to amikacin 90%, amoxyclav 80%, cloxacillin 70%, ciprofloxacin 70%, gentamicin 63.33% & azythromycin showed lower sensitivity 43.33%.*

Conclusion: *Most of the injectable antibiotics showed higher sensitivity which is an alarming sign for the clinician to treat wound infection. Periodic review of the bacteriological profile and antibiotic sensitivity pattern is highly essential. Antibiotic policy & infection control program should be included in every hospital to reduce this drug resistance.*

Key Words:
Antimicrobial Susceptibility, Wound infection

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Introduction:

Bacterial infections of wounds are among the leading causes of morbidity and mortality throughout the world and are regarded as one of the most common nosocomial infections. Wound infections have been reported to vary between 3 and 11% in developed countries and estimated to be as high as 40% in developing countries.[1-3.] The human skin and soft tissue infections caused by microbial pathogens during or after trauma, burn, injuries and surgical procedures result in the production of pus, a white to yellow fluid comprised of dead WBCs, cellular debris, and necrotic tissues.[4-6]. Wound infections can be caused by variety of organisms like bacteria, virus, fungi and protozoa and may co-exist as poly microbial communities especially in wound margin and in chronic wounds [7] and in many cases there is a mixed infection with more than one bacterial species.[8]

The most common bacterial genera infecting wounds are Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, klebsiella species, and Acinetobacter species [9]. Staphylococcus aureus , the common organisms that have been associated with wound infection which from various studies have been found to account for 20-40%. Infection with Pseudomonas aeruginosa mainly following surgery and burns account for 5-15%. Other pathogens such as Enterococci, Escherichia coli, Klebsiella species and proteus species have been implicated especially in immunocompromised patients and following abdominal surgery.[10]. Candida species also responsible for wound infection.[11]

The bacterial profile of pus samples in many studies remain the same even through the antibiotic resistance pattern of these isolates has shown a lot of variations.[12,13] The inadvertent use of antibiotics leads to emergence of drug resistant pathogens which in turn acts as a great challenge for the health services. Besides, highly virulent strains adapt quickly to changing environment worsens the situation and draws a matter of concern.[14] The emergence of multi-drug resistant strains have higher risk of death due to infection and also hampers the control of infectious diseases by reducing the effectiveness of treatment. Thus, patients remain infectious for a long time increasing the risk of spreading resistant microorganisms to others.[15,16.17.] The last few decades, multidrug-resistant Gram negative bacterial strains were increasingly associated with pus infections under hospital settings due to irrational use of antibiotics.[15,18.]

Therefore, the present study was carried out to identify the current microbial isolates and their antimicrobial susceptibility pattern from wound infection in a tertiary care hospital in Dhaka city.

Materials and methods:

This retrospective study was conducted in the department of Microbiology at Holy Family Red Crescent Medical College Hospital, Dhaka from July 2019 to December 2020 for a period of one and half years. Written consent was taken from corresponding authority. Microsoft Excel' software was used for data analysis. A total 134 wound swab / pus were collected from the patients who were visited in outpatient department and were admitted at inpatient department with suspected wound infection. Open wounds which had superficial debris were removed by thorough irrigation and cleansing with sterile saline. The swab was gently rolled over the surface of the wound approximately 5 times focusing on area where there was evidence of pus or inflamed tissue. For dry wounds, sterile cotton swabs were used after moistening with sterile saline. Swabs were carried in aerobic transporter tube to the laboratory. All samples were processed aerobically on Blood agar, MacConkeys agar media and incubated at 37^0C for 24 hours. Organisms were identified by standard procedures including colony characters, Gram staining and biochemical reactions.[19]

Antimicrobial susceptibility testing of all the isolates were performed by Kirby Bauer's disc diffusion method by adjusting the turbidity to 0.5 McFarland standard and spread on Mueller Hinton agar using antibiotics as per CLSI (Clinical laboratory standard institute) guidelines.[20]

The antibiotics used for the test were amoxicillin/ clavulanic acid (30ugm), amikacin (30 µgm), azithromycin (15 µgm), ciprofloxacin (5 µgm), cloxacillin (5 µgm), Ceftriaxon (30 µgm), Ceftazidime(30 µgm), Cefuroxime (30 µgm), Clostin (10 µgm), Gentamicin (10 µgm), Imipenem(10 µgm), Meropenem (10 µgm), Linezolid (30 µgm),Vancomycin (300 µgm), Trimethoprime/sulfamethoxazole (25 µgm), Piperacillin/tazobactum (100 µgm), Tetracycline (30 µgm). The diameter of the zone of inhibition was measured and interpreted according to the CLSI standard.[20]

Results:

Table I

Age & sex distribution of the study population (n= 134)

Gender	Children up to 18 years	Adult	Total
Male	10	66	76 (56.72%)
Female	5	53	58 (43.28%)
Total	15 (11.2%)	119 (88.8%)	134 (100%)

A total 134 samples were analyzed. Of them, predominant populations were from male 76(56.72%) and remaining were from female 58(43.28%) (Table I).

Table II

Distribution of bacterial isolates (n= 102)

Distribution of the isolates	Culture positive	No growth
Outpatient department (OPD)	22 (21.57%)	4 (12.5%)
Inpatient department (IPD)	80 (78.43%)	28 (87.5%)
Total	102 (100%)	32 (100%)

Out of 134 samples 102 (76.11%) was culture positive. Culture positive was observed higher in inpatient department (IPD) 80 (78.43%) than outpatient department (OPD) was 22 (21.57%) (Table II).

Table-III

Organisms isolated from wound swab (n=102)

Organisms isolated	Number	Percentage (%)
Gram negative isolates		
E. coli	26	25.49
Klebsiella spp.	24	23.53
Pseudomonas spp.	18	17.64
Proteus spp.	04	3.92
Gram positive isolates		
Staph. aureus	30	29.41
Total	102	100

Majority of isolates were Gram negative bacteria. Among them predominant bacteria was *E. coli* 26(25.49%) followed by *Klebsiella* spp. 24(23.53%), *Pseudomonas* spp. 15(14.71%) & *Proteus* spp. 04(3.92%). Among the Gram positive isolates 30(29.41%) was *Staph. aureus* shown in Table-III.

Antibiotic susceptibility pattern of Gram negative isolates is shown in Table 4. *E. coli* showed higher sensitivity to meropenem 88.46% & gentamicin 80.76%. Other drugs showed sensitivity to amikacin 65.38%, amoxicillin/ clavulenic acid 53.84%, tetracycline 46.15%, ceftriaxone & ceftazidime 42.30%, trimethoprime/ sulfamethoxazole 34.61% & cefuroxime 23.07%. In *Klebsiella* spp. meropenem showed higher sensitivity 79.16% & gentamycin 66.66%. Other drugs like amikacin showed 58.33%, trimethoprime/ sulfamethoxazole 45.83%, ceftazidime & ciprofloxacin 20.83% sensitivity. Low sensitivity 16.66% to amoxicillin/ clavulanic acid, cefuroxime, ceftriaxone, & tetracycline was found. *Proteus* spp. showed 75% sensitivity to meropenem. Cefuroxime, ceftriaxone, ciprofloxacin, ceftazidime, gentamycin, amikacin, trimethoprime/ sulfamethoxazole showed very low sensitivity 25% & amoxycillin/clavulanic acid showed 100% resistance (Table IV).

Table IV

Antibiotic susceptibility pattern of Gram - negative isolates

Organisms	Sensitivity (%)								
	AMC	CXM	CRO	CIP	CAZ	GN	AK	SXT	MEM
E. coli (n=26)	53.84	23.07	42.30	38.46	42.30	80.76	65.38	34.61	88.46
Klebsiella spp. (n=24)	16.66	16.66	16.66	20.83	20.83	66.66	58.33	45.83	79.16
Proteus spp.(n=4)	0	25	25	25	25	25	25	25	75

AMC – Amoxicillin/clavulanic acid, CXM-Cefuroxime , CTR-Ceftriaxone, CIP-Ciprofloxacin, CAZ –Ceftazidime, SXT-Trimethoprim/ Sulfamethoxazole, GN-Gentamycin, AK-Amikacin, TE-Tetracycline MEM-Meropenem

Table V

Antibiotic susceptibility pattern of Pseudomonas spp. isolates (n= 18)

Organisms	Sensitivity (%)							
	CIP %	AK %	CAZ %	CFM %	GN %	IPM %	TZP %	MEM %
Pseudomonas spp.	61.11	83.33	50	33.33	66.66	83.33	83.33	77.77

CIP-Ciprofloxacin, AK-Amikacin, CAZ –Ceftazidime, CFM- Cefepime, GN-Gentamycin, IPM - Imipenem, TZP-Piperacillin tazobactum, MEM-Meropenem

Table VI

Antibiotic susceptibility pattern of isolated Staph. aureus (n= 30)

Organisms	Sensitivity (%)						
	AMC%	CX%	AZM%	CIP%	SXT%	GN%	AK%
Staph. aureus	80	70	43.33	70	36.66	63.33	90

AMC – Amoxicillin/clavulanic acid, CX-Cloxacillin, AZM-Azithromycin, CIP- Ciprofloxacin, SXT-Trimethoprime/ Sulfamethoxazole, GN-Gentamycin, AK-Amikacin

Pseudomonas spp. showed highest sensitivity 83.33% to piperacillin tazobactum, amikacin & imipenem, 77.77% to meropenem, 66.66% to gentamycin, 61.11% to ciprofloxacin, 50% to ceftazidime & 33.33% to cefepime shown in Table 5.

All the isolate of *Staph. aureus* was sensitive to amikacin 90%, amoxyclav 80%, cloxacillin 70%, ciprofloxacin 70%, gentamicin 63.33% & azythromycin showed lower sensitivity 43.33%. (Table 6)

Discussion:

Bacterial contamination of wounds is a burning problem in the hospital especially in surgical practice which may lengthen the hospital stay, delay in wound healing and raises the overall cost of the patients.

Among the 134 samples culture positive isolates were detected 102(76.11%) in this study. Male were predominant 76(56.72%) and remaining were female 58(43.28%). Our study also shows that children up to 18 years were 15(11.20%) and adult was 119(88.8%) (Table 1). It could be explained by the fact that male were more prone to wound infections due to disparities in propensitity for skin colonization or other anatomical differences. Another study reported 65.6% males and 34.4% females among the surgical site infection patients which correlates with our findings.[21] In another study, maximum isolates were found in < 1 year age group.[22]

In IPD, culture positive growth was 78.43% and in OPD 21.57% in this study. The culture positivity rate in our study is similar to another study. [23] However 50% culture positive isolates were also reported in a study.[24]

This study found that Gram negative bacteria were the predominant organisms in comparison to Gram positive bacteria. The common bacterial isolates found in this study were *E. coli* 26(25.49%) followed by *Klebsiella* spp. 24(23.53%), *Pseudomonas* spp. 18(17.64%) & *Proteus* spp. 4(3.92%) (Table 3). Similar findings were reported in a study done in India.[25]

Gram positive isolates, *Staph. aureus* was 30(29.41%) shown in Table 3. *Staph. aureus* was the most frequently isolated Gram - positive bacteria from both wound swab and pus found in a study which is dissimilar with our findings.[26] The normal flora nature of *Staph. aureus* in the skin and anterior nares, which can enter to deep site during surgery of the natural barrier of the skin, could be the possible justification for its high isolation rate.[27,28] As different hospital deals with different infections, so isolation rate of the bacteria may be variable from hospital to hospital.

Different studies have been performed to assess the bacterial profile and the antibiotic susceptibility pattern in pus samples. All the Gram-negative isolates showed higher sensitivity to carbapenem group 75% - 88.46% which is in accordance with findings of other study.[29]

Highest resistance by gram negative bacilli was noted against amoxicillin/clavulanic acid followed by 2nd & 3rd generation of cephalosporins, fluoroquinolones & sulfamethoxazole/trimethoprime in our study. Several studies have shown this pattern of resistance.[30,31]

In case of ciprofloxacin, Gram negative bacilli showed high resistance to it. This finding is consistent with a study who reported Gram negative bacteria was highly resistant to ciprofloxacin.[29] This higher resistance may be due to random use of ciprofloxacin for different infections like urinary tract infections & enteric fever which is endemic in Bangladesh.

Majority of the Gram - negative bacilli showed a comparatively good sensitivity to gentamicin & amikacin, this finding is consistent with other study in Bangladesh.[32] Low resistance might be less use of this group of drugs. So, aminoglycoside group of drugs may consider as the drug of choice in infection caused by Gram negative bacteria.

Pseudomonas spp. showed highest sensitivity to piperacillin tazobactum, amikacin & imipenem, & gentamycin (Table 5) but a study had shown variable susceptibility pattern with these drugs for *Pseudomonas aeruginosa*.[33]

In this study, *Staph. aureus* showed 90% sensitivity to amikacin, followed by amoxicillin/clavulanic acid 80%, ciprofloxacin & cloxacillin 70% and gentamycin 63.33%. In another study done in Bangladesh where ciprofloxacin showed 20% & co-trimoxazole showed 29% sensitivity.[34] Azythromycin & trimethoprim/ sulfamethoxazole showed lower sensitivity may be due to irrational use of these common drugs which might cause resistance (Table 6).

Conclusion:

Most of the injectable antibiotics showed higher sensitivity which is an alarming sign for the clinician to treat wound infection. Periodic review of the bacteriological profile and antibiotic sensitivity pattern is highly essential. Antibiotic policy & infection control program should be included in every hospital to reduce this drug resistance.

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