The Predominance of Nosocomial Pathogens among Patients with Post-Operative Wound Infections and Evaluation of the Antibiotic Susceptibility Patterns in Rural Hospitals in Bangladesh

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Abstract

Wound infection is considered one of the most common nosocomial infections all over the world. So, the aims and objectives of this study are to identify the pathogenic agents of wound infection infecting hospital admitted patients and commonly used antimicrobial susceptibility profiles. A total of 91 samples were collected for the study. The cultural analysis was done by phenotypic examination as well as Gram staining and biochemical examination to identify the isolates. Finally, antibiotic sensitivity was tested against conventionally used antibiotics. All the samples were aseptically collected from different hospitals in the Tangail area and tests were done in the Lab Zone and Hormone Center, Tangail, Bangladesh between January 2019 and June 2019. 84(92.3%) of the total samples yielded a positive culture and only 7(7.7%) samples were negative growth. Gram-Negative bacteria 64(76.2%) showed more prevalence than Gram-Positive bacteria 20(23.8%). The most predominant isolates were *Pseudomonas* species 54(64.3%) in this study though *Staphylococcus* species 20(23.8%), *Proteus* species 7(8.3%), and *Klebsiella* species 3(3.6%) were also found. Like most of the previous reports, *Pseudomonas* species was predominant; this is also corroborated in this study. However, the antimicrobial result of the detected organisms differed compared to studies that were previously done. The isolates were found to be resistant to most common oral antibiotics used, such as Cefuroxime, Colistin, Cephradine, Ciprofloxacin, Ceftazidime, and Ceftriaxone which was alarming. The pathogens showed remarkable sensitivity against Gentamicin and Meropenem. This research needs further work for validating more reliability. This study is designed to provide information on pathogens which are isolated from wound suspected of wound infection hospital admitted patients in Bangladesh and their antibiotic susceptibility profile against locally available antibiotics which are frequently prescribed by the doctors to find a suitable antibiotic for wound infections.

Keywords: Nosocomial Pathogens; Wound Infection; Bacterial Isolates; Antibiogram Profiles.

1. INTRODUCTION

Wound infections are one of the most common nosocomial infections and important causative agents of morbidity which are responsible for 70-80% mortality [1]. It can be caused by different microorganisms like bacteria, fungi, and protozoa. However, it can be possible to exist different microorganisms in polymicrobial communities especially in the margins of wounds as well as in chronic wounds [2].

Although wound infections can be caused by accidental traumas, post-operative wound infections in the hospital are most common. Some endogenous infections occur by the patient's bacterial flora like *Staphylococcus aureus* from skin and anterior areas from coliforms. But most of the infections are exogenous, skin and anterior areas are important sources of *Staphylococci* which spread from hospital staff and visitors that occur by direct and indirect airborne routes. Most hospital strains are resistant to a wide range of antibiotics, including macrolide and vancomycin [3]. The patient may have been affected by an antibiotic-resistant organism from the environment of the hospital. Such microorganisms (e.g., *Pseudomonas aeruginosa*) may colonize a traumatized skin like a wound, burns, and bedsores [4].

Wound infection is difficult to describe and no clear rules or prediction can be given to find it out from contamination and colonization. The contaminating organisms are at first generally present in comparatively small numbers in the body, as originally introduced, and don't need to multiply subsequently. But infection occurs when one or more of the contaminants evades the clearing effect of the host's defense mechanism, replicates in huge numbers, and then attacks as well as harms the host's tissues. For commensal or low-grade pathogen, the multiplication may cause little or no harm to the host, which can be defined as colonization [5]. However harmful infection or harmless colonization occurs depending on the virulence of the microorganisms and the local as well as general resistance of the host. That's why the patient's general and local condition knowledge is important for assessing the significance of bacteriological findings [6]. It has become a great challenge for physicians to select the perfect antibiotic for patients who are infected by wound infection nowadays. So, this study aims to determine the prevalence of nosocomial pathogens among patients with post-operative wound infections and to evaluate the antibiotic susceptibility pattern of the pathogens to the most commonly used antibiotics.

2. METHOD(S)

2.1. Collection of Samples

This study was conducted from January 2019 to June 2019. A total of 91 samples from the patients with complaints of discharge, pain, swelling, foul smell in the wound after surgery from different hospitals in Tangail district, Bangladesh. The wound samples were aseptically collected using a sterile cotton swab; the inner surface of the infected area was swab gently and then transported to the laboratory named Lab Zone and Hormone Center.

2.2. Microbiological Culture

In the laboratory, each sample was inoculated on MacConkey agar, HiChrome agar, Chocolate agar, and Blood agar. The inoculums were streaked out for discrete colonies with a sterile inoculating loop on the plate. Then the culture plates were incubated at 37°C for 24 hours for growth through the formation of colonies. After 24 hours, all the bacteria were isolated and identified using morphologically, microscopically, Gram staining, and biochemical tests. The biochemical tests were carried out included: coagulase, oxidase, catalase, urease, motility, gas production, carbohydrate utilization, citrate utilization, fermentation, and indole test. The species of bacteria were identified according to the standard microbiological methods [7].

2.3. Antibiotic Susceptibility Tests

According to CLSI guidelines [8], disc diffusion test was performed according to the Kirby-Bauer method, using discs such as Amikacin (30µg), Ciprofloxacin (30µg), Ceftriaxone (30µg), Gentamycin (10µg), Ceftazidime (30µg), Cefuroxime (30µg), Meropenem (10µg), Colistin (10µg), Cefepime (30µg), Cefixime (5µg), Cephradine (30µg), Linezolid (30µg), Azithromycin (15µg), Amoxicillin (25µg), and Trimethoprim (5µg). A lawn of test pathogen (1ml of an 18 hours peptone broth culture) was prepared by spreading 100µl inoculums with a sterilized spreader onto the entire surface of the Muller Hinton agar plate. The plates were dried before applying the antibiotic disc. Then, some commercially available antibiotic discs were gently and firmly placed on the agar plates, and then the plates were incubated at 37°C for 24 hours. If the antimicrobial activity was present on the plates, it was indicated by a zone of inhibition. The diameter of the zone of inhibition was measured in millimeters using an electronic scale. An organism was considered as highly susceptible if the diameter of the zone of inhibition was above 19 mm, intermediate if the diameter was 15-18 mm and resistant if less than 13 mm.

2.4. Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 16.0 and Microsoft Excel was used for the analysis of data. The percentage of frequencies were generated for different categorical variables such as rate of isolation, type of bacteria, rate of antibiotic sensitivity, resistance, intermediate of the organisms.

3. RESULTS AND DISCUSSION

91 samples were collected from the patients with complications in the wound after surgery. Among them, 84 samples were infected which means 92.30% were found as positive shown in the following Figure 1. In the present study, the rate of wound infection was high, which is similar to the other studies [9].

Different types of pathogenic bacteria were isolated from wound culture which is showed in Figure 2, *Pseudomonas* species (54 isolates; 64.29%) followed by *Staphylococcus* species (20 isolates; 23.81%), *Proteus* species (7 isolates; 8.33%), and *Klebsiella* species (3 isolates; 3.57%). In the present study, *Pseudomonas* spp. (64.29%) and *Staphylococcus* spp. (23.81%) were the predominant organisms isolated from wound infections followed by other bacteria. A number of studies on wound infection from different parts of the world reported that both organisms were the most frequent isolates in wound infection which corroborates our analysis [10,11]. The prevalence of Gram-negative bacteria in wound infection was much higher than Gram-positive bacteria which were shown in our study. Gram-negative bacteria were the predominant (64 isolates, 76.19%) followed by Gram-positive bacteria (20 isolates, 23.81%).

Figure 1: Frequency of wound infection and non-infections.

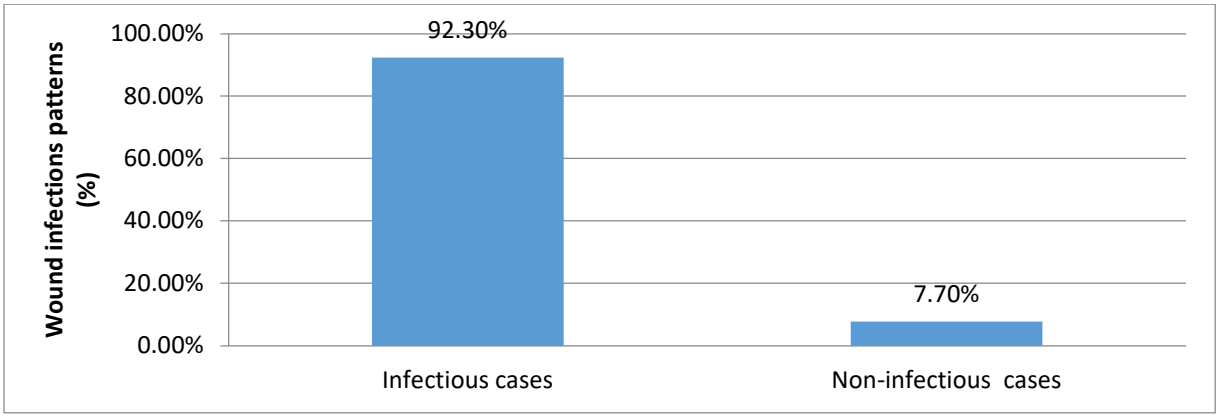

Figure 2: Occurrence of bacterial isolates from wound swab sample.

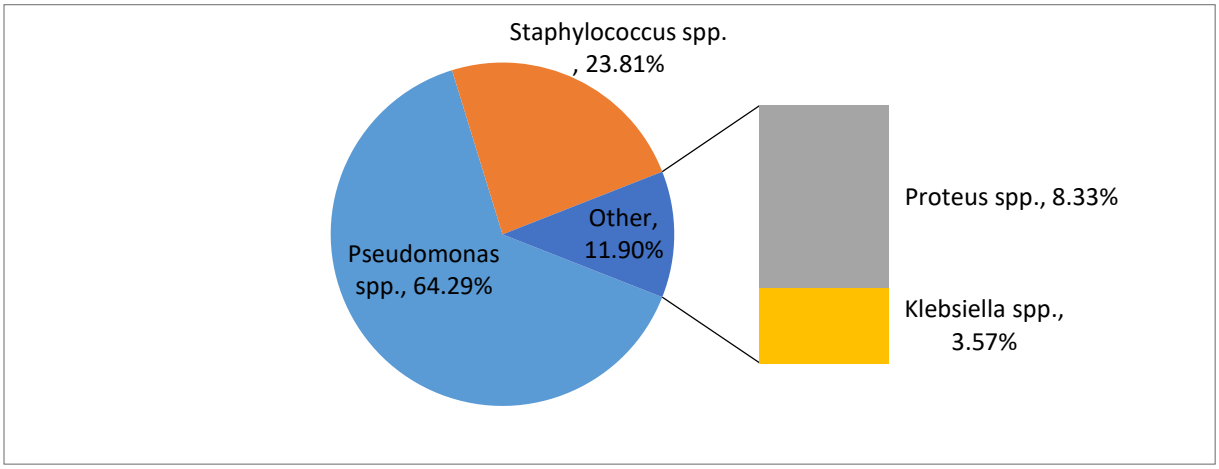

Table 1: *In-vitro* antibiogram profile of gram-negative bacterial isolates.

		Commercially used antibiotics and frequency (%)								
Isolated Bacteria		**AK**	**CIP**	**CRO**	**CN**	**CAZ**	**CFU**	**MEM**	**COL**	**FEP**
Pseudomonas spp. (n=54)	R	1(1.85)	23(42.6)	21(38.9)	3(5.5)	39(72.2)	40(74.1)	0(0.0)	49(90.7)	54(100)
	S	48(88.9)	8(14.8)	15(27.8)	43(79.6)	5(9.2)	4(7.4)	54(100)	1(1.8)	0(0.0)
	I	5(9.25)	23(42.6)	18(33.3)	8(14.8)	10(18.5)	10(18.5)	0(0.0)	4(7.4)	0(0.0)
Klebsiella spp. (n=3)	R	1(33.33)	2(66.67)	1(33.3)	0(0.0)	1(33.3)	2(66.67)	0(0.0)	3(100)	3(100)
	S	1(33.3)	0(0.0)	2(66.6)	1(33.3)	1(33.3)	1(33.3)	3(100)	0(0.0)	0(0.0)
	I	1(33.3)	1(33.3)	0(0.0)	2(66.6)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Proteus spp. (n=7)	R	0(0.0)	2(28.5)	2(28.5)	0(0.0)	5(71.4)	4(57.1)	0(0.0)	7(100)	7(100)
	S	5(71.4)	2(28.5)	3(42.8)	5(71.4)	0(0.0)	0(0.0)	7(100)	0(0.0)	0(0.0)
	I	2(28.5)	3(42.8)	2(28.5)	2(28.5)	2(28.5)	3(42.8)	0(0.0)	0(0.0)	0(0.0)

AK=Amikacin, CIP=Ciprofloxacin, COL=Colistin, CRO=Ceftriaxone, CN=Gentamicin, CAZ=Ceftazidime, CFU=Cefuroxime, MEM=Meropenem, FEP=Cefepime, S=Sensitive, R=Resistant, I=Intermediate.

Staphylococcus spp. isolates exhibited the highest susceptibility against Gentamycin (70%) and Meropenem (100%), moderately sensitive to Ciprofloxacin (40%) and highly resistant to Cefixime (85%), Amoxycillin (55%), Trimethoprim (55%) and Cephradine (45%).

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Antibiotic sensitivity results of the isolated organisms which were shown in Tables 1 and 2 were determined by the standard disk diffusion method. *Pseudomonas* spp. exhibited high resistance to Ceftazidime (72.3%), Cefuroxime (74.1%), Colistin (90.7%), Cephradine (100%). However, the third-generation antibiotics Amikacin (88.9%), Gentamycin (79.6%), Meropenem (100%) proved to be very effective against *Pseudomonas* species but Ciprofloxacin (42.6%) and Ceftriaxone (33.33%) showed moderate sensitivity. Similarly, wound isolates of *Proteus* spp. indicated 100% resistance to Colistin, Cephradine, 71.43% resistance to Ceftazidime, and showed moderate sensitivity to Ciprofloxacin, Cefuroxime but showed highly susceptible to Amikacin (71.43%), Gentamycin (71.4%), Meropenem (100%). The *Klebsiella* spp. isolated from wounds indicated 100% sensitivity to Meropenem, 66.6% sensitivity to Ceftriaxone, and moderate sensitivity to Gentamycin, Amikacin, and Ciprofloxacin. The species showed 100% resistance to Colistin and Cephradine.

Table 2: *In-vitro* antibiogram profile of gram-positive bacterial isolates.

		Commercially used antibiotics and frequency (%)								
Isolated bacteria		**AML**	**TRI**	**CIP**	**CN**	**AZM**	**MEM**	**LIN**	**CEP**	**CFM**
Staphylococcus spp. (n=20)	R	11(55)	11(55)	9(45)	2(10)	7(35)	0(0.0)	6(30)	9(45)	17(85)
	S	1(5)	1(5)	3(15)	14(70)	6(30)	20(100)	8(40)	4(20)	0(0.0)
	I	8(40)	8(40)	8(40)	4(20)	7(35)	0(0.0)	6(30)	7(35)	3(15)

AML=Amoxycillin, TRI=Trimethoprim, CIP=Ciprofloxacin, CN=Gentamycin, AZM=Azithromycin, LIN=Linezolid, CEP=Cephradine, MEM=Meropenem, CFM=Cefixime, S=Sensitive, R=Resistant, I=Intermediate.

Multi-drug resistant pathogens are the major threat to the doctors treating an infected patient. Indiscriminate use of antibiotics is the main reason behind it. That's why, the treatment of any infection is critical and unpredictable due to the scarcity of alternative constructive antibiotics, but Meropenem and Gentamycin showed tremendous susceptibility against all pathogens found in our study, which is a good thing.

4. CONCLUSION

This study is intended to ascertain the existing situation of wound infection and drug resistance among different hospital admitted patients in Tangail district, Bangladesh. From this study, it has been concluded that *Pseudomonas* spp. was the predominant pathogens followed by *Staphylococcus* spp., *Proteus* spp. and *Klebsiella* spp. The prevalence of Gram-negative bacteria in wound infection was higher than Gram-positive bacteria. Gentamicin and Meropenem were found as a reliable therapeutic intervention for investigated pathogens because of their broad-spectrum activity in the current study. The antibiotic selection has to be guided by culture and sensitivity tests and the drug must be decided on the recent antibiogram of a particular geographical area.

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Author Contributions
MZA and AAA conceived and planned the experiments. ABSM carried out the experiments. ABSM and LJ contributed to the sample preparation. MZA, RI, MEU, and RYS contributed to the interpretation of the results. MSUZ, LJ, and MK took the lead in writing the manuscript. MZA, MBA, and RYS supervised and reviewed the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript as a whole.

Conflict of Interest
There is no conflict of interest.

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