Research Article

Etiological agents of wounds infection and their antibiogram against various antibiotics in patients of Gilgit-Pakistan

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Abstract

Wound infections are one of the most common infection in which bacterial flora proliferates and delayed the wound healing process. Normally skin normal flora and environment contaminate the wounds. This study was conducted in Gilgit and primary aim of the study was to determine the common causative agents of wound infection and their fluctuating susceptibility pattern against commonly used antibiotics. In this study 185 infected wound samples were taken from patients with high suspicion of wound infection attended at inpatient and outpatient departments of district headquarter (DHQ) hospital Gilgit from December 2014 to September 2015. Swabs from, infected wounds were taken aseptically by using Levine's technique and cultured on blood and MacConkey agars. Bacterial colonies were Gram stained and examined microscopically. Biochemical tests were done to identify pathogens. The antibiotic sensitivity test was performed by disk diffusion method. Both the genders were included in this study and age of patients in the study was ranged from 6 months to 75years. Out of 185 wound swabs, 124 samples yielded significant bacterial growth, the prevalence of wound infection was 67.02 %. *S. aureus* as most prevalent pathogen 59 (39.3%) followed by *Pseudomonas aeruginosa* 31(20.67%). Age group and sex did not affect the prevalence. Most prevalent wound type was trauma 70 (66.67%). All Gram positive *cocci* were susceptible to vancomycin and linezolid (83.33-100%). All Gram negative bacilli showed good response towards piperacillin-tazobactam and imipenem in a range of 90-100%. This study revealed the alarming increase of wound infections by drug resistant bacterial pathogens.

Keywords: Antibiotic resistance; Antibiotic sensitivity; Causative agents; infected wounds

Introduction

For a long time wound, infections have been a common problem in the field of medicine. Even Progresses in control of infections have eliminated this issue because of development of drug resistance. Wounds may be due to

accidents, pathologic agents or post-operative Pondai *et al* [1]. Following a wound, there is exposure of subcutaneous tissue, which leads to the development of a favorable nutritious, moist, warm environment for the multiplication and

colonization of microorganisms. Therefore, any wound is at risk of becoming infected. The other major risk factors for wound infection may include old age, prolonged stay in hospital, immune-compromised patients and infected devices Prabha *et al* [2]. Wound may be infected by a single or multiple organisms. Many of wound infections are caused by the pathogens that are endogenous flora of the patient's skin, mucous membranes or hollow viscera Mangram *et al* [3]. Most of the wound infections are caused by nosocomial pathogens, which may differ country to country, and even from one hospital to another in same region Mulu *et al*. [4]. Most commonly isolated pathogens of wound are *Pseudomona species*, *Staphylococcus aureus*, *Klebsiella species*, *Escherichia coli*, *Streptococcus species*, *Enterobacter species* and *coagulase negative Staphylococci* Akingbade *et al* [5].

In developing countries wound infection are still important causes of morbidity and mortality. The studies done on wound infections, the regional and local variations have been seen in terms of their causative pathogens and their sensitivity. This means that physicians need to identify the prevalent pathogen and its resistance patterns in particular localities Pondai *et al* [1]. Appropriate treatment of wound infection involves treating the right bacteria with right antimicrobial administered in the right manner for right length of the time to prevent the emergence of resistant organisms Micheal *et al* [6].

The United State of America, Center for Disease Control (CDC), the World Health Organization (WHO) and many other researchers have documented the increasing rate of infection by Multidrug Resistance bacteria, new antibiotic resistant pathogens are emerging in clinical environment Davies *et al* [7]. These bacteria pose a greater challenge in the treatment of infections, which often leads to the prolonged illness,

amputation, and even risk of death. Hence, it is necessary to have knowledge about the etiological agents and their antimicrobial susceptibility in particular regions, it will be beneficial for the selection of empirical antimicrobial therapy.

Materials and methods

The specimens were taken from patients with infected wounds of any type including trauma (traffic road accidents, osteomyelitis etc.), post-operative wound infection, and abscess and burn wound infections.

All swabs obtained, by using Levine's technique, were cultured directly on blood and MacConkey agar for isolation of aerobic bacteria Levine *et al* [8]. Cultured plates were examined after overnight incubation at 37°C. Identification of pathogenic bacteria was based on gram stained smear, colony morphology, biochemical test and culture media.

Antibiotics sensitivity was carried out by disk diffusion method Bauer *et al* [9] and Ahmed *et al* [10]. One ml of each identified bacterial isolate was prepared from an overnight culture and adjusted to 0.5 McFarland Standard. A sterilized wooden swab was soaked in inoculums used to streak on Mueller-Hinton agar (MHA) plates and allowed to dry at room temperature. Commercially available sterile disks of antibiotics, Ampicillin (10 µg), Amoxicillin/Clavulanic acid (AMP/Cl- 30 µg), Ciprofloxacin (5 µg), Chloramphenicol (C 30 µg) Gentamycin (GM- 10 µg), Linezolid (Le-30 µg), Vancomycin (V-10µg), Ceftazidime (Ca-30µg) and Erythromycin (E-10 µg) for Gram positive and Amoxicillin/Clavulanic acid (AMP/Cl- 30 µg), Ciprofloxacin (5 µg), Chloramphenicol (C 30 µg) Gentamycin (GM- 10 µg) Ceftazidime (Ca-30µg), Piperacillin- Tazobactam (Pt 100/10µg) and Imipenem (Imp-10µg) for Gram negative were placed aseptically on the pre streaked agar plates with sterilized

forceps and then incubated at 37°C for 18-24 hrs. *E. coli* ATCC 25922, sensitive to all these drugs, was used as a control and the sensitivity of the antibiotics was recorded by measuring the zone of inhibition around the discs for each of the isolated cultures in millimeters (mm). The interpretation of the measurement for sensitive and resistant bacteria was made according to the manufacturer's standard zone size CLSI [11].

Statistical analysis

Differences in antibiotic resistance between wound types and between sexes and age groups were analyzed using Pearson Chi Square test. A difference of $p < 0.05$ was considered significant.

Results

Prevalence of wound infection

During the whole study 185 wound samples were investigated at microbiology department in pathology laboratory (Table 1) at district headquarter hospital Gilgit. There were total 123 male and 62 female subjects. Ages ranged from 6 months to >75 years. The swabs obtained from males were relatively high infected as compare to female and highest cases of wound infections were among the age group of 21-30 years (70.96%). There was greater incidence of wound infection in the age group of 21 to 30

years but there was no significant association between age and the incidence of wound infection ($p = 0.648$). In male the infestation was 83 (67.48%) which was slightly higher than female 41 (66.12%). But statistically there was no significant association between the incidence of wound infection and the sex of the subject ($p = 0.490$) or between the type of wound and the sex of the subject ($p = 0.186$).

A total of 124 samples (Figure 1) had significant bacterial growth revealing wound infection, while 61 samples did not show any significant growth. 101 wounds were infected with a mono organism. However 20 wounds were infected with a couple of bacteria, while three organisms were isolated from 3 specimens. A total of 150 bacteria were isolated.

The prevalence of different pathogenic bacteria isolated from infected wounds (Table 2). *Staphylococcus aureus* (39.3%) was the most predominant isolate followed by *Pseudomonas aeruginosa* (20.67%), *Escherichia Coli* 24 (16.02%). *Coagulase negative Staphylococci* and *Proteus* species 12(8.0%) each *Klebsiella spp.* 10(6.67%) and *Streptococcus spp.* 02 (1.3%). The number of Gram-negative bacteria were higher 77 (51.33%) than Gram Positive 73 (48.67%).

Table 1. Age and gender wise distribution of infected wound patients

Age groups (years)	Culture results		No. of infected swabs	
	No. of Investigated samples (%)	No. of Infected samples (%)	Male (%) N=123	Female (%) N=62
0-10	5 (2.7)	3 (60.0)	02 (2.41)	01 (2.44)
20-Nov	24 (13.0)	15 (62.5)	11 (13.55)	04 (9.76)
21-30	62 (33.5)	44 (70.96)	32 (38.55)	10 (24.39)
31-40	36 (19.5)	24(66.6)	13(15.66)	11 (26.83)
41-50	30 (16.2)	21 (70.0)	13 (15.66)	08 (19.51)
51-60	14 (7.6)	9 (64.28)	6 (7.23)	03 (7.32)
61-70	10 (5.4)	6 (60.0)	4 (4.82)	3 (7.32)
>70	4 (2.2)	2 (50.0)	2 (2.41)	1 (2.44)
Total	185 (100.0)	124 (67.02)	83 (67.48)	41 (66.12)

Figure 1. Number of bacteria isolated showing significant bacterial growth revealing wound infection

Table 2. Prevalence of pathogenic bacteria isolated from infected wounds patients reported at DHQ hospital Gilgit

Isolated Bacterial Pathogens	No. of Isolates (%)
Gram Positive	
<i>Staphylococcus aureus</i>	59 (39.3%)
Coagulase-negative <i>Staphylococcus spp.</i>	12(8.0%)
<i>Streptococcus spp.</i>	02 (1.3%)
Total	73 (48.67%)
Gram negative	
<i>Pseudomonas aeruginosa</i>	31(20.67%)
<i>Escherichia coli</i>	24(16.02%)
<i>Klebsiella spp.</i>	10(6.67%)
<i>Proteus spp.</i>	12(8.0%)
Total	77 (51.33%)
G. Total	150

The frequencies of various wound types and their infestation level was drawn (Table 3). Wound was categorized into four types. Most prevalent type of wound was trauma 105 (56%) and infestation was found 70 (66.67%) followed by surgical wound infection 35(18.9%) with infestation rate of 26(74.28%), burn 25 (13.5%) and infestation was 18 (72.0%) and lowest 20 (10.8%) cases of abscess were reported with infestation rate of 10(50%). High infestation was observed in

surgical wound 26 (74.28) and lowest was observed in abscess 10 (50.0).

Frequency of pathogenic bacteria isolated according to various wound types has been mentioned in (Table 4). The highest Prevalence of pathogenic bacteria 82 (54.67%) was in trauma patients followed by surgical wounds 34 (22.67%), burn 21(14%) and low number of pathogens were isolated from abscess 13 (8.67%). Among the Gram positive bacteria the highly isolated bacteria

was *Staphylococcus aureus* 59 (39.3%) most frequently isolated from trauma patients 37 (62.7% followed by surgical 10 (16.9%), burn 7 (11.86%) and Abscess 5 (8.47%). The *Staphylococcus* spp. Infestation was 12 (8%) and its highest infestation was also found in trauma 7 (58.33%) followed by surgical 3 (25%), burn and abscess 1 (8.33%) each. *Streptococcus* spp infestation was 2 (1.3%) and was isolated only from trauma patients. Among the Gram negative bacteria the *Pseudomonas aeruginosa* was 31 (20.67%) and very highly isolated from trauma patients 14 (45.16%) followed by surgical 11 (35.48%), abscess 4 (12.9%) and in burn 2

(6.45%). The total infestation of *E. coli* in various types of wound was 24 (16.02%) and it was most frequently isolated in trauma 11 (45.8%) followed by surgical 7 (29.1%, burn 4 (16.67%, abscess 2 (8.33%). The overall infestation of *Klebsiella Spp.* was 10 (6.67%) and more frequently isolated from trauma patients 6 (60%) followed by surgical and burn patients 2 (8.33%) each. There was no infestation of *Klebsiella Spp* in abscess. *Proteus* infestation was 12 (8%) and was highest in trauma and burns patients 5 (41.67%) each followed by surgical and abscess 1 (8.33%).

Table 3. Frequency of wound type

Wound type	Frequency (%)	No. of infected samples (%)
Trauma	105 (56)	70 (66.67)
Surgical	35(18.9)	26 (74.28)
Burn	25(13.5)	18 (72.0)
Abscess	20 (10.8)	10 (50.0)
Total	185 (100)	124 (67.02)

Table 4. Frequency of pathogenic bacteria according to wound types

Isolated Bacterial Pathogens	Trauma (%)	Surgical (%)	Burn (%)	Abscess (%)	Total (%)
<i>Staphylococcus aureus</i>	37 (62.7)	10 (16.9)	07 (11.86)	05 (8.47)	59 (39.3)
Coagulase-negative <i>Staphylococcus spp.</i>	07 (58.33)	03 (25)	01 (8.33)	01 (8.33)	12 (8.0)
<i>Streptococcus spp.</i>	02 (100)	00 (0)	00 (0)	00 (0)	02 (1.3)
<i>Pseudomonas aeruginosa</i>	14 (45.16)	11 (35.48)	02 (6.45)	04 (12.9)	31 (20.67)
<i>Escherichia coli</i>	11 (45.8)	07 (29.1)	04 (16.67)	02 (8.33)	24 (16.02)
<i>Klebsiella spp.</i>	06 (60)	02 (20)	02 (20)	00 (0)	10 (6.67)
<i>Proteus spp.</i>	05 (41.67)	01 (8.33)	05 (41.67)	01 (8.33)	12 (8)
Total	82 (54.67)	34 (22.67)	21 (14)	13 (8.67)	150 (100)

As per Antibiotic susceptibility of gram positive cocci (Table 5), *S. aureus* was 59 (100%) sensitive against Linezolid followed

by Vancomycin 58 (98.30%), Ciprofloxacin 30 (50.84%), Ceftazidime 28 (47.45%), Gentamycin 24 (40.67%), Erythromycin 18

(30.50%), Chloramphenicol 13 (22.03%), Amoxicillin/Clavulanic acid 11 (18.64% and Ampicillin 3 (5.08%). *Staphylococcus* sensitivity against Linezolid was 12 (100%) followed by Vancomycin 10 (83.33%), Ciprofloxacin 7 (58.33%), Ceftazidime and Gentamycin 6 (50%) each, Chloramphenicol

3 (25%), Amoxicillin/Clavulanic acid and Erythromycin 2 (16.66%). *Streptococci* spp. sensitive against Linezolid and Vancomycin was 2 (100%) each followed by Chloramphenicol and Ceftazidime 1 (50%) each.

Table 5. Antibiotic susceptibility pattern of gram positive cocci (% sensitivity)

Name of antibiotic discs	<i>Staphylococcus aureus</i> N=59	<i>Staphylococcus spp.</i> N=12	<i>Streptococcus spp.</i> N=02
Gentamycin (GM- 10 µg)	24 (40.67)	06 (50.0)	0
Erythromycin (E-15 µg)	18 (30.50)	02 (16.66)	0
Ciprofloxacin (Cip -5 µg)	30(50.84)	07 (58.33)	0
Chloramphenicol (C-30 µg)	13 (22.03)	03 (25.0)	01 (50.0)
Amoxicillin/Clavulanic acid (AMP/Cl- 30 µg)	11 (18.64)	02 (16.66)	0
Ampicillin (AMP- 10 µg)	03 (5.08)	01 (8.33)	0
Linezolid (Le-30 µg)	59 (100)	12 (100)	02 (100)
Vancomycin (V -10 µg)	58 (98.30)	10 (83.33)	02 (100)
Ceftazidime (Ca-30 µg)	28 (47.45)	06 (50.0)	01 (50.0)

Antibiotic susceptibility of gram negative rods (Table 6), *P. aeruginosa* were sensitive against Imipenem and Piperacillin/Tazobactam 31 (100%) followed by Ciprofloxacin 17 (54.83%), Ceftazidime 16 (51.61%), Amoxicillin/Clavulanic acid 5 (16.12%), Gentamycin and Chloramphenicol 4 (12.90%) each. *E. Coli* sensitivity against Imipenem was 24 (100%), followed by Piperacillin- Tazobactam 23 (95.83%), Ciprofloxacin 8 (33.33%), Chloramphenicol 6 (25%), Ceftazidime 5 (20.83%), Amoxicillin/Clavulanic acid 3 (12.5%) and Gentamycin 2 (8.33%). *Klebsiella* sensitivity was against Imipenem 10 (100%) followed by Piperacillin- Tazobactam 9 (90%), Ciprofloxacin 4 (40%), Chloramphenicol 2 (20%), Gentamycin and Ceftazidime 1 (10%) each.

Discussion

In this study 185 infected wound samples were processed to determine the common

causative agents of wound infection and their fluctuating susceptibility pattern against commonly used antibiotics in Gilgit.

The study revealed that the prevalence of wound infection among males were high than females. These findings are consistent with Nazeer *et al.* [12] but this difference has no significance as Egbe *et al.* [13] and Taiwo *et al.* [14] also reported the same findings in their studies conducted in Nigeria and Africa. Current study reflects wound infection was more prevalent in the age group of 21-30 years 62 (33.5%) followed by 31-41 years age group 36 (19.5%) and 41-50 years age group 30 (16.2%). Which was in contrast with study conducted in Nepal and India, the prevalence was high in age group 41-50 Nazeer *et al.* [12] and Bhatt *et al.* [15]. However, our findings go inconsistent with the results of study conducted by Pondai *et al.* [1] in Nigeria and a study from Ethiopia Mulugeta *et al.* [16]. Present study depicts

that the prevalence of wound infection was 67.02%, this figure is in agreement with study conducted in India (69%) and Nepal (62%) by Nazeer *et al.* [12] and Bhatt *et al.* [15]. However, these findings differ with the study conducted by Pondai *et al.* [1] and Sultana *et al.* [17] where the prevalence was high 86.2% and 83. 65% respectively.

The results of the current study illustrates the high prevalence of Gram negative bacteria in wound infection, whereas same pattern of prevalence was reported by Kehinde *et al.* [18] who documented that the rate of Gram negative bacterial isolates were double than Gram positive isolates in burnt wound infections.

Table 6. Antibiotic susceptibility pattern of Gram-negative rods (% sensitivity)

Name of antibiotic discs	<i>Pseudomonas aeruginosa</i> N=31	<i>E. coli</i> N=24	<i>Proteus Spp.</i> N=12	<i>Klebsiella Spp.</i> N=10
Imipenem(Imp 10µg)	31 (100)	24 (100)	12 (100)	10(100)
Gentamycin (GM- 10 µg)	04 (12.90)	02 (8.33)	02 (16.66)	01 (10.00)
Ciprofloxacin (Cip -5 µg)	17 (54.83)	08 (33.33)	05 (41.66)	04 (40.00)
Amoxycillin/Clavulanic acid (AMP/Cl- 30 µg)	05 (16.12)	03 (12.5)	02 (16.66)	00(0)
Piperacillin- Tazobactam (Pt 100/10µg)	31 (100)	23 (95.83)	31 (100)	09 (90.00)
Chloramphenicol (C-30 µg)	04 (12.90)	06 (25.00)	04 (33.33)	02 (20.00)
Ceftazidime (Ca-30 µg)	16 (51.61)	05 (20.83)	03 (25.00)	01 (10.00)

The most common pathogens isolated in present study was *S. aureus* (39.3%) followed by *Pseudomonas aeruginosa* (20.67%), *E.coli* (16.02%), *Proteusspp.* (8%) and *Klebsiella spp.* (6.67%). The findings of present study are in closer conformity with some earlier studies which were performed in the same study pattern, reported *S. aureus* as the most widespread bacterial isolate of wound infection Mawalla *et al.* [19] and Mama *et al.* [20]. But these fi Jeol *et al.* [21] and Manikandan *et al.* [22] where *Pseudomonas aeruginosa* followed by *S. aureus* were commonest isolates.

The highest prevalence of *S. aureus* may be due to endogenous source of infection and from environmental contamination may also cause infection in addition to infection from surgical instruments. As *S. aureus* is normal flora of skin, with the disruption of this natural barrier it easily enters into wounds.

Antibiotic resistance to commonly prescribed antibiotics was high. This high level of resistance is alarming. The absolute resistance to ampicillin and amoxycillin/clavulanic acid (penicillin class) was not unpredicted as these antibiotic frequently implicated in self-medication in Gilgit- Baltitan. In Current studies Gram positive isolates showed the average resistance of 95.13% and 88.89% to ampicillin and amoxycillin/clavulanic acid (penicillin class) respectively and 86.11% resistance to erythromycin (macrolide class) these findings goes in line to study conducted in Ethiopia where Gram positive isolates showed 82-100% resistance against three classes penicillin , tetracycline and Phenicol class Godebo *et al.* [23]. All the Gram-positive isolates showed 100% sensitivity to linezolid this is in great agreement with the study conducted in Iran by Nazeer *et al.* [12]

and Fatholahzed *et al.* [24] In contrast, study conducted in Bangladesh showed 94.3% sensitivity against linezolid Sultana *et al.* [17] Significant susceptibility of Gram-positive isolates to linezolid and vancomycin, might be due to their low availability, cost and toxic effects Mama *et al.* [20] In present study Gram negative isolates show 100% of average sensitivity to imipenem followed by piperacillin-tazobactam (96.5%) this is similar to the findings of Nazeer et al [12] reported 100% sensitivity to imipenem and 70- 100% sensitivity to piperacillin-tazobactam. Almost similar findings were reported from a study conducted in Bangladesh by Sultana et al [17] who reported 93.54% sensitivity to imipenem. The high frequency of resistance against multiple drugs is a reflection of inappropriate use of antibiotics, lack of laboratory diagnosis and unavailability of guidelines. This inappropriate prophylaxis of antibiotics and unskilled practitioners leads to emergence of resistance in bacteria. As a result, they have lost their efficacy in the treatment of wound infection Barker *et al.* [25].

Conclusion

S. aureus is the primary cause of infection in Gilgit- Baltistan area. The increasing multi-drug resistance may be a reflection of inappropriate use of antibiotics, lack of laboratory diagnosis and unavailability of guidelines. Periodic monitoring of etiological agents and their susceptibility profile of pathogens from infected wounds help physicians to prescribe right drug and discourage emerging resistance.

Authors' contributions

Conceived and designed the experiments: S Yaqub & K Ahmed, Performed the experiments: S Yaqub, R Imran & N Shuja, Analyzed the data: S Yaqub, SU Din & MA Nafees, Contributed materials/ analysis/ tools: R Imran & I Hussain, Wrote the paper: MA Nafees & SU Din.

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