

## IDENTIFICATION AND DRUG SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES OF CHRONIC WOUND IN PAKISTAN

Kashif Rahim<sup>1\*</sup>, Naila Younas<sup>1</sup>, Abdul Basit<sup>1</sup>, Ilyas Khan<sup>1</sup>, Iqbal Ahmad<sup>1</sup>, Saiqa Sardar<sup>1</sup> and Tanzeela Younas<sup>1</sup>  
1- Department of Microbiology, Kohat University of Science and Technology (KUST), Khyber Pakhtunkhwa, 26000, Pakistan  
2- Khyber Medical College, Peshawar, Khyber Pakhtunkhwa, 26000, Pakistan

**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 contaminated the wounds. The present study was conducted to determine frequency and antibiograms of pathogens associated with wound infections among the patients of Combined Military Hospital, Kohat and Hayat Medical Complex, Peshawar. A total of 107 different kinds of wound samples were collected randomly from patients. Isolation, purification and identification procedure were carried out by standard microbiological methods. Out of total samples, 100 were found culture positive and 07 samples showed no growth furthermore, among all the isolates obtained total 134 isolates, among these 34(25.3%) were of *Staphylococcus spp*, 24 (14.96 %) were of *E.coli*, 19 (14.1 %) isolates of *Pseudomonas aeruginosa* were obtained, 15 (11.1%) were of *Klebsiella spp*, *Streptococcus spp* 29(21.6%) *Serratia merscence*, *Morgenella morgani* were 8(5.9%) and 05(3.7%). Antibiotics susceptibility was checked by disc diffusion method. Out of all the antibiotics, augmentin (81.1%) and ceftriaxone (81.4%) susceptible for chronic wound isolates and were considered the best effective antibiotics against all the wound pathogens. It was concluded that the prevalence of wound infections was higher and 99% of wounds were infected.

**KEYWORDS:** Wound infections, Bacterial flora, Antibiotics.

### INTRODUCTION

As a wound is a cut or injury of the skin layer and the exposure of subcutaneous tissue following loss of skin integrity. So it provides a moist, warm, and nutritive environment that is suitable for microbial colonization and proliferation (Bowler *et al.*, 2001; Shittu *et al.*, 2003). Hence all wounds are contaminated by both pathogens and body commensals ranging from bacteria and fungi to other parasites (Anguzu and Olita, 2007). Thus many different types of bacterial pathogens proliferate in wounds and cause infections most frequent of which is *Staphylococcus aureus* which is also referred to as skin normal flora. Other infectious agents found in infected wounds are beta hemolytic *Streptococci spp* and *Pseudomonas aeruginosa*. The facultative anaerobes include *Enterobacter species*, *Escherichia coli*, *Klebsiella species*, *Proteus species* and fungi associated with wound infections are *Candida species* and *Aspergillus species* (Gonzalez *et al.*, 2009; Mordi and Momoh, 2009). Wound infections are categorized as per the type of wound which includes surgical wound infections, burn wound infections, bite wound infections and trauma infections (Bowler *et al.*, 2001). Wound infections account for 5-34% of the total nosocomial infections (Girri *et al.*, 2008). Wound

infections with special reference to surgical site infections are projected to increase in number at an annual rate of 3.1% and these infections are considered to be second most nosocomial infections (Nwomeh *et al.*, 2010). Moreover, 77% deaths of surgical patients are related to surgical site infections (Mangram *et al.*, 1999). In United States, around 6.5 million patients develop chronic wounds every year. It is claimed that an excess of US \$ 25 billion is spent annually on treatment of chronic wounds and the burden is growing rapidly due to increasing health care costs associated with diabetes and obesity worldwide (Chandan *et al.*, 2009). Wound infections especially surgical site infections were observed to be 7.23% in Abbottabad which is however lower than the percentage found in Kashmir which is 11.3% (Safia *et al.*, 2001). In another study a higher prevalence of 11% is found in public sector of Karachi and 13% in Jamshoro (Safia *et al.*, 2001). Therefore, the purpose of our study was to investigate the frequency of different types of wound infections causing pathogens and associated risk factors in both inpatients and outpatients of hospitals. The study also aimed to check the antibiotic susceptibilities of the isolated wound pathogens.

### MATERIALS AND METHODS

**Corresponding Author:** Kashif Rahim, Department of Microbiology, Kohat University of Science and Technology (KUST), Khyber Pakhtunkhwa, 26000, Pakistan. E-mail: [kashifbangash73@yahoo.com](mailto:kashifbangash73@yahoo.com)

Present study was conducted for time duration of four months (September – December 2012). During this period, 107 different wound samples were collected from Combined Military Hospital, Kohat and Hayat Medical Complex, Peshawar. Samples were processed for culturing and identification in Department of Microbiology, Kohat University of Science and Technology and inoculated into nutrient broth tubes.

2.1. Sampling

Samples were collected from different wounds and wounded organs from 107 patients with the help of sterile cotton swabs and immediately inoculated into nutrient broth tubes. The tubes were incubated for 24 – 48 hours at 37°C to obtain growth.

2.2. Morphological Analysis

2.2.1. Isolation and Purification of Isolates from Samples

Samples from nutrient broth tubes were further cultured upon selective and enriched media such as MacConkey agar, blood agar and mannitol salt agar respectively and incubation was done at 37°C for 24 hours. Further sub-culturing upon same media was done to obtain pure cultures.

2.2.2. Gram Staining

Gram staining technique was performed to differentiate Gram positive and Gram negative

bacteria and studied their morphology. Slides were then observed under 100x of light microscope.

2.2.3. Biochemical Characteristics

Identification of bacterial species was carried out by biochemical profile, which includes Citrate Utilization Test, Catalase Test, Indole Test, DNase Test, Oxidase Test, Triple Sugar Fermentation Test, Motility Test, Coagulase Test (Adejuwon et al., 2011).

2.3. Antibiotic susceptibility test

2.3.1. Disk Diffusion Method

The Kirby-Bauer’s disk diffusion method was used to check the antibiotic susceptibilities of isolated pathogens in aseptic conditions and check the resistance pattern of bacterial isolates toward antibiotics.

RESULTS

3.1. Identification of bacterial isolates on Morphological characteristics

Bacterial isolates were identified on the basis of their colonies morphology, Gram reaction and their hemolysis on blood agar. *Staphylococcus spp* and *Streptococcus spp* species were cultured on Blood agar and Mannitol salt agar. The enteriobacteracea family gram negative microbes were identified and cultured on Macconkey agar (Table 1).

Table 1: Morphological characteristics of genera

Species	MAC	Blood Agar	MSA	Gram Staining
<i>Staphylococcus spp</i>	-	Golden yellow colonies	Yellow colonies	Gram +ive
<i>Streptococcus spp</i>	-	White colonies	Colorless colonies	Gram +ive
<i>E. coli</i>	Pink smooth colonies	-	-	Gram -ive
<i>Pseudomonas aeruginosa</i>	Colorless colonies	-	-	Gram -ive
<i>Morgenella morgani</i>	Colorless colonies	-	-	Gram -ive
<i>Klebsiella spp</i>	Slight pink mucoid colonies	-	-	Gram -ive
<i>Serratia spp</i>	-	-	-	Gram -ive

Table 2: Biochemical characteristics of genera

Bacterial isolates	Motility	Indole	Citrate	DNase	Coagulase	Oxidase	Catalase	TSI			
<i>Streptococcus spp</i>	-		+	-	-	-	-	Slope	Butt	H <sub>2</sub> S	Gas
<i>Staphylococcus spp</i>	-		+	-	+	-	+				
<i>E.coli</i>	+	+	-	-	-	-	-	Y	Y	-	+
<i>Klebsiella spp.</i>	-	-	+	-	-	-	-	Y	Y	-	-
<i>Pseudomonas aeruginosa</i>	+	-	-	-	+	-	-	R	R	-	-
<i>Morgenella morgani</i>	+	-	-	-	-	-	+	Y	B	+	-
<i>Serratia mersence</i>	+	-	+	-	-	-	+				

Key: + = Positive, - = Negative, Y = Yellow (Acid reaction), R = Red-Pink (Alkaline reaction), B = Black (Hydrogen sulfide production), H<sub>2</sub>S = Hydrogen sulfide, Empty=test not Performed

3.2. Biochemical characteristics

Bacteria isolated from chronic wounds were identified with biochemical characteristics using different biochemical tests as given in (Table 2).

3.3. Bacterial Pathogen distribution

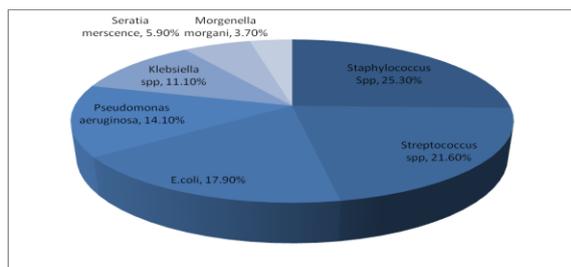
Chronic wound isolates were distributed such as *staphylococcus spp*, *streptococcus spp*, *E.coli* and *psuedomonas aeruginosa* were 25.30%, 21.60%,

17.90% and 14.10% respectively and the rest percentages are also given in (figure 1).

3.4. Antibiotic Patteren

The kirby baur disc diffusion method was used to check the antibiotic patteren on bacterial isolates the augmentin (amoxil+c.acid) and ceftrixone showed highly level of susceptibility 81.1% and 81.4% on the other hand bacterial

isolated showed resistance to erythromycin, norflaxacin and ciproflaxacin 30.5%, 31.3 and 29.1% as shown in (Table 3).



**Figure 1:** Bacterial pathogen distribution

**Table 3:** Antibiotic Resistance and susceptibility pattern

Sample No.	Antibiotics	Resistance (%)	Susceptible (%)
1	Augmentin(30µg)	24(17.9)	110(82.1)
2	Ciproflaxacin(5µg)	29(29.1)	105(70.9)
3	Amikacin(30µg)	20(14.9)	114(85.1)
4	Piperacillin(100µg)	31(23.1)	103(76.9)
5	Cefepime(30µg)	35(26.1)	99(73.9)
6	Ceftriaxone(30µg)	25(18.6)	109(81.4)
7	Norflaxcin(30µg)	42(31.3)	92(68.7)
8	Ceftazidine(30µg)	36(26.8)	98(73.2)
9	Erythromycin(15µg)	41(30.5)	93(69.5)

## DISCUSSIONS

There are many factors that influence long-term healing of chronic wounds include disturbed circulation, hypoxia, ischemia, oedema of tissues and colonization of the wound by bacteria. It is assumed that chronic ulceration of lower extremities was never septic. In a recent study chronic wound isolates were distributed such as *Staphylococcus spp*, *Streptococcus spp*, *E.coli* and *Psuedomonas aeruginosa* were 25.30%, 21.60%, 17.90% and 14.10% respectively. [Marek \*et al.\* \(2002\)](#) also reported in a study that bacterial flora found in leg ulceration of diabetic origin had a different composition. The most common found bacterium were *Staphylococcus aureus*, which was found in 30 (71.43%) ulcerations. Meanwhile, *Pseudomonas aeruginosa* strain was detected in 29 (69.05%) wounds of patients. The next most frequent bacteria were *Enterococcus faecalis* in 28 (66.66%) ulcerations. *Proteus vulgaris* was present in 18 (42.86%) ulceration in patients while *Streptococcus pyogenes* and *Escherichia coli* were found in 15 (35.71%) ulceration of diabetic origin ([Marek \*et al.\* 2002](#)). [Mazur \*et al.\* \(2006\)](#) also evaluate the bacterial flora from 30 venous ulcerations of lower leg. *Staphylococcus aureus* was most frequently isolated bacterium. The Kirby Baur Disc diffusion method was used to check the antibiotic pattern on bacterial isolates the augmentin (amoxil + C.acid) and ceftriaxone showed high level of susceptibility 81.1% and 81.4% on the other hand bacterial isolated

showed resistance to erythromycin, norflaxacin and ciproflaxacin 30.5%, 31.3 and 29.1% respectively. [Peter. \(2009\)](#) also reported sensitivity pattenen of antibiotics that amikacine 73.5%, piperacillin 83.7%, ceftriaxone 79.5%, ciproflaxacin 87.2%, augmentin 76.9% and erythromycin 44.7%. Hence it was concluded from the present study that chronic wounds contain diverse bacterial flora on it and due to these infection wound delay to heal and showing resistance to antibiotics.

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