Research Article

Characterization of isolated bacteria from burn patients and its susceptibility against different antibiotics in district Swat

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Abstract

Burn is an injury to the skin or other body tissues caused by various agents like thermal injury, scalds, fire, electrical shock and chemical injury. The aims of current study were isolation, identification and sensitivity of isolated bacteria against antibiotics from burn infected patients of District Swat. A total of 50 swab samples were collected from 50 burn infected patients. On the basis of colony morphology and Biochemical tests, including Oxidase, Catalase, Coagulase, Indole, and Motility eight different bacterial isolates were identified, including *Bacillus*, *Enterobacter*, *Epidermis*, *E.Coli*, *Klebseilla*, *Pseudomonas Aeruginosa*, *Proteus* and *Staphylococcus aureus*. The frequency range were *Bacillus* (0.3%), *Enterobacter* 6.97%), *Epidermis* (6.97%), *Proteus* (9.30%), *E.coli* (11.62%), *Klebsiella* (13.95%), *Staphylococcus aureus* (20.93%) and *Pseudomonas aeruginosa* (30.23%). The maximum infections, i.e. 38% were reported in children's age group 1-5 years. The infections reported in females were 65% while males 35%. A total of 12 antibiotics were tested in which levofloxacin and ciprofloxacin were found more effective. Moderate against Amikacin, Chloramphenicol and Gentamycin. Whereas Imipenem, Amoxicillin, Ceftriaxone were found less effective. Attention and concentration is need to avoid the infection in burn patients at distract swat. A further detail and comprehensive study is needed to avoid or reduce these infections.

Keywords: Antibiotic sensitivity; Bacteria; Burn Patients; District Swat

Introduction

Burn is an injury to the skin or other body tissues caused by various agents like thermal injury, scalds, fire, electrical shock and

chemical injury [1]. Skin is the thin layer of tissue form the natural outer covering of body of a person or animal. It contains certain specific component of the innate immune

system, which help to protect the host from pathogens. The skin has an immunological and neurosensory function. It may have other function like Vitamin D metabolism [2]. According to literature the burn injuries are classified into three different categories. These categories are based on the depth of injuries. The first degree burn is surficial burn that affects the upper surface of skin. It appears as red spots of the skin and sensitive to touch for three to five days [3]. Second degree are subcutinic and has effect on the under layer of the skin and causes blisters. In such injury the function of sweat glands is affected and may feel pain for six to eight weeks. Systemic burn injury is classified as third degree. In this class the injury extends to all layers of the skin, e.g. epidermis, dermis and hypodermis. A sever burn have many effect on the body and can penetrate deep skin layers, which causing muscle or tissue damage that effect every system of the body. It has effect on human immune system. The function of immune system is to protect cells from invasion by microorganisms. The body has three important immune defense systems, all of which are disrupted with burn injury [4]. The response of immune system to burn injury is immediate, prolonged and or severe. The effect of burn in individuals is immune suppression, with increased susceptibility to fatal systemic burn wound or sepsis. The burn wound has a greater incidence of infections because in burn wound the skin barriers is destructed extensively and also alteration of the cellular and humoral immune responses. Burns can also cause emotional problems such as depression. Moreover age, drug use, gender, seasonal, smoking, socio-economic status and sun exposure are the high risk factors for burns [5].

Globally, Burn is serious public health problem and approximately 265,000 deaths occur each year from fire alone. However, death rate from other form of burn, *i.e.* scalds,

electrical burn, etc. are not reported. The mortality rates due to burn are high in developing countries than developed countries. The possible reasons may be poverty, overcrowding, illiteracy, undoubtedly limited treatment resources and inaccessibility to sophisticated skills makes the management of burns more challengeable. In developed countries with high gross national per capita spends more on health sector which reflected a better health status [6].

Initially the burn site is free from all types of microbial contamination. Infection usually starts after 48 hours [7]. Major microbes, e.g. fungi and Bactria colonized in wounds. These microbes usually present as normal flora, or occur from external environments and from hospital environment [8]. The use of topical antimicrobials may retard microbial growth or prevent further colonization. When colonized, these microbes start penetrating the viable tissues. This penetration depends on their invasive capacity, local wounds and degree of immune response of the patients [9]. Among the fungal pathogens *Aspergillus niger*, Penicillin and *Candida albicans* are the most common cause of infection [10]. In different centers large numbers of deaths (63-75%) from burns occurs due to sepsis. This causes the indiscriminate use of broad spectrum antibiotics causing multidrug resistant virulent flora [11].

Different microorganisms associated with burn infection are bacteria and fungi [12]. The percentage of fungal infection with burn wounds is about 6-15% among the fungal pathogens. *Aspergillus niger*, *Candida albinos*, and Zygomycetes are the most common causative agent of infection [3]. The fungal colonization may also depend on body parts of patients, like candida is rapidly colonized on skin and mucus membrane of nose, throat, and gastrointestinal tract [13].

Bacteria both gram positive and gram negative are the most common cause of burn

wound infections. The gram negative bacteria colonized in wound in first few days. Common gram positive organisms are methicillin-resistant *S. aureus* (MRSA), *Enterococcus*, group a beta-hemolytic *Streptococcus* and Coagulase Negative *Staphylococcus* [9]. *Staphylococcus aureus* is a group of bacterial strains gram positive and as non-motile small round shaped cocci. Additionally, it can cause various skin and soft tissue infections [14]. Normally it is catalase positive. *Enterococcus* are gram positive Cocci [15]. They are facultative anaerobic organisms, non-motile, non-spore forming and catalase negative *Streptococcus pyogenes* are non-motile, catalase negative non-spore forming round bacteria, causing many skin infections to life threatening systemic diseases [16]. *Propionibacteria* are gram positive anaerobic rod shaped bacteria. They are non-motile, not filamentous, catalase positive and are secondary agents of infected wounds and abscesses in humans and other animals.

Gram negative bacteria are the most common and serious infectious source in burn patients. Among gram negative bacteria the important microbes are *Pseudomonas aeruginosa*, a rod shaped motile bacterium [17]. It produces water soluble pigments which diffuse through the medium, e.g. *Pyocyanin*, *Pyoverdine* and *Pyorubin* [18]. *Klebsiella pneumoniae* is a non-motile, encapsulated, facultative anaerobic, lactose fermenting and rod shaped bacteria. It is catalase positive and oxidase negative. *E. coli* is rod shaped, facultative anaerobic, motile normal flora. It is Catalase positive and Oxidase negative [19]. *Proteus* motile, rod shaped bacteria. It is catalase and Urease positive and Oxidase negative [13].

Materials and methods

Study Area

The current study was carried out in laboratory of Centre for Biotechnology and Microbiology at University of Swat with the

collaboration of Research and Clinical laboratory, Kanju. The study time was from January to August 2017.

Sample collection

The 50 sterile swabs samples were collected from burn sites aseptically at Saidu teaching hospital, Matta hospital, Khwazakhela Hospital and Kanju Burn Care Centre. Samples were collected from different body parts (hands, leg, chest, and abdomen) of burn patients and properly labeled with patients Name, Age, and Gender. The samples were taken to laboratory of Centre for Biotechnology and Microbiology at University of Swat for further processing.

Media preparation

Different media's were used for the growth of isolated agents such as Nutrient agar, Blood agar, and MacConkey's agar. The media was autoclaved for 15 minutes at 121°C. Then antifungal was added to inhibit fungal growth and poured in sterilized petri dishes under aseptic environment in laminar flow hood (LHF).

Swab culture

Swab samples were streaked on Nutrient agar media. After streaking plates were incubated for 48 hours on 37°C.

Sub culture and pure culture

Different Morphological colonies were appeared on Nutrient agar plates. To obtain pure culture subcultures of separated colonies were again sub cultured for 48 hours at 37°C.

Identification of bacteria

All cultures were examined for the growth and colony morphology. Gram staining and biochemical tests, e.g. oxidase, catalase, coagulase, indole, and motility were used for identification

Antibiotic sensitivity test

Antibiotic disk preparation

For inoculums preparation a sterile distilled water ampoules were used. The inoculum of pathogenic bacteria were adjusted to 0.5 McFarland standards. In bacterial suspension a sterile cotton swab were dipped. In order to

get a uniform growth of isolates, the isolated samples were streaked on Mueller Hinton agar with cotton swab smoothly. After 15 minutes of inoculation the antibiotic discs of known concentration were placed on the media surface with the help of disc dispenser. The list of antibiotics used in present research work is given in table 1. The plates were then incubated for 24 hours at 37°C in inverted position. The zones of inhibition were measured after 24 hours.

Antibiotic susceptibility test Disc diffusion Method

Antibiotic susceptibility test Disc diffusion technique [Kirby-Bauer method] was used to test antimicrobial sensitivity. Sterile swab was soaked in the test organism suspension and swab was streaked over the surface of Muller Hinton agar. The Petri-dish was allowed for 3-5 minutes to dry, by using sterile forceps the antimicrobial discs were placed evenly distributed on inoculated plate. The plates were incubated aerobically at 37°C for 24 hours. The zones of inhibition around each disc were measured in millimeters and the results were reported as sensitive, intermediate and or resistant.

Antimicrobial sensitivity Assay

The antibiotic sensitivity tests were performed for isolated bacteria, e.g. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella*, *Epidermidis*, *Enterococcus* and *Proteus*. Large numbers of antibiotics were used.

Results

During current research work the patients were distributed on the basis of various categories given below.

Gender wise distribution of burn patients

The Gender wise distribution for all 50 Burn patients were made and found that 35% were male while the remaining 65 % were found female (Figure 1).

Age group wise distribution of Burn patient's

During age wise distribution of patients it were found that patients having age group 1-5 years were (34%) followed by 10-15 years (18%), 15-25 years (24%) and 25-35 years (6%) (Table 2).

Burning source wise distribution of bacterial infection

The isolated bacteria were distributed on the basis of different source of burns. The result indicates that six bacterial isolates, i.e. *Pseudomonas*, *Staphylococcus Aureus*, *E.coli*, *Klebsiella*, *Proteus*, *Epidermis* were found in high number. The bacteria including *Pseudomonas*, *Staphylococcus Aureus*, *E. coli*, *Klebsiella*, *Proteus*, *Epidermis* were recorded in burns of direct heat. *Pseudomonas*, *Klebsiella*, *Staphylococcus Aureus*, *Epidermis*, *E.coli*. Isolated from burn caused by accident. The *Pseudomonas*, *E.coli*, *Proteus*, *Enterobacter Epidermis* were recorded in burn caused by petroleum.

Susceptibility of isolates against various antibiotics

During study the *pseudomonas aeruginosa* was associated with maximum number of patients, i.e. 26, followed by *S. aureus*, *Klebsiella*, *Proteus*, and *E.coli* 18, 12, 10 and 8 respectively while *Enterococcus* and *epidermis* both were associated with 6 patients (Table 3). The susceptibility of *Pseudomonas aeruginosa* against various antibiotics were determined and found 60% sensitive to levofloxacin (LEV) followed by TZP and AK 45% and 35% respectively, 10% was recorded against MP while no activity were shown against IPM and TGC each (Figure 2).

Figure 3 revealed that *klebsiella* were found more sensitive against CIP, 55% and LEV 65%. Against AK, TZP, CPO recorded moderate while against E no activities were recorded. The bacteria *proteus* susceptibility were determined against various antibiotics and found susceptible against TZP (60%),

LEV (70%) and CIP (65%). While against AK, CPO, SCF moderate, i.e. 45%, 35% and 40% respectively. Similarly CAZ, AMC showed no effect against proteus (Figure 4). In case of *E.coli* showed high resistance against Amoxicillin (AMC) 100% (Figure 5). The *Staphylococcus aureus* showed high resistance against Amoxicillin (AMC) 97.1% as compare to the other antibiotics (Figure 6). The bacteria *Enterococcus* showed 100% resistance to Erythromycin while less resistant to Ciprofloxacin (CIP) and Levofloxacin (LEV) recorded as 10% (Figure 7).

Discussion

Burn infection is a serious public health problem of Swat, Khyber Pakhtunkhwa. Burn injuries have body disinfection, lifelong difficulty even cause death [20]. The burn infection explores the tissue for microorganisms of normal flora and contaminated environment. The virulence factor for infection including adhesion, nutrients and immune system of the patient. To prevent and treat burn infection numbers of antibiotics can be used to administrate directly as topical cream taken by orally or by injections [21].

The results of current study showed 80% infection in burn patients. These results are in agreement with the study conducted by Mooney et al; 2004, who investigated the microbial association with burn patients [20]. Manjula et al; in 2004 isolated a number of different bacterial isolates from post burn infections, including *S. Aureus*, *Epidermis* and *klebsiella* [22]. These results are concise with our study, in which we isolated eight different bacteria including *pseudomonas aeruginosa*, *staphylococcus aureus*, *klebsiella*, *E.coli*, *epidermis*, *Enterobacter*, *proteus* and *bacillus*.

Adeyemi et al; in 2006 recorded in his study that most common burn infections were due to association of *pseudomonas auregenousa*, *klebsiella*, *staphylococcus aureus*, *E.coli* and

epidermis [23]. The current study is in agreement with previous researcher. It is also in line with research work conducted by Barret et al; in 2002 [7].

In present study the higher frequency were recorded for *pseudomonas auregenousa* 30.23% followed by *staphylococcus aureus* 20.93%, *klebsiella* 13.95%, *E.coli* 11.62% *Proteus* 9.30%, *Enterobacter* 6.97% and *epidermis* 6.97% (Figure 8). These results are in line with the results of [23]. The reasons for this high frequency of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsella* may be that they are present abundantly in air, can survive in waste range of environmental conditions and showing resistance against a number of antibiotics.

The present research work is also in conformations with the reports of [24]. The lowest frequencies of our results were noted in *Enterobacter* and *E.coli* that was 6.97% each which is in agreement with [25]. It is well documented that children's of age group 1-15 years are in high risk of getting burn injuries [5].

The results of our study revealed that highest percentage of burn injuries in age group 1-5 years were recorded followed by age group 5-10 years 18%. These results are in line with the research work conducted by Cheesbrough and his colleagues [26]. The reasons for high percentage are unaware about consequences of fire and carelessness of parents. The report of QUECH supports our results Komolafeet al; 2003 stated that likewise burn infection higher percentage were reported in age group 1-15 years of children's [27]. Our results are agreed with the previous studies of Zetola and friends and Church et al; 2006 [3, 14]. The reasons for this high infection percentage may be that child's are more active in daily life exposed to outdoor contaminated environment and unprotected wounds parts. Similarly 65% infections were reported in females which are higher than male 35%.

According to this study hands are more prone to burn injuries, i.e. highest percent infection 16% were noted in hands, followed by legs 13% chest 9%, abdomen 8%, face 04%, arm fit 2% and back 1% the reports of the QECH also shows the same percentage [28].

Antibiotics resistance is the ability of bacteria to resist against antibiotics to which they were once susceptible. Bacteria associated with burn patients have high level of

resistance to many antibiotics as burn patients generally take broad spectrum of antibiotics. About ten antibiotics with different generation and mode of action were tested against important bacterial isolates *Pseudomonas*, *Staphylococcus aureus* and *Klebsella* are more resistive to antibiotics. The current study is in agreement with previous researchers [14].

Table 1. List of antibiotics with chemical name and Generation

Serial no	Commercial name Antibiotics	Chemical name	Generation	Concentration
1	CM	Gentacycol	2 ND	10 mcg
2	AML	Amoxicillin	2 ND	30 µg
3	AMC	Amoxillin	2 ND	30 µg
4	ZOX	Ceftizoximum	3 RD	30 mcg
5	MEM	Meropenemtrihydrate	3 RD	10 mcg
6	AK	Amikacin	3 rd	30 mcg
7	CE	Cefixime	3 RD	30 µg
8	E	Abomacetin	3 RD	30 mcg
9	SCF	Cefoperazine	3 RD	75 µg
10	KAZ	Fortaz	3 RD	30mcg
11	CPO	Cefepime hydrochloride	4 th	30mcg
12	TZP	Piperacillin anhydrous	4 TH	110µg
13	LEV	Levofloxacin	3 rd	5mcg

Figure 1. Gender wise distribution of burn infections

Table 2. Age group wise distribution of Burn patient's

S. No	Age	No of persons	Percentage
I	1-5	17	34
II	5-10	10	18
II	10-15	07	14
IV	15-20	08	16
V	20-25	06	12
VI	25-30	02	4
VII	30-35	01	2

Table 3. Source and types of bacteria in burn wounds

S. No.	Source of burn	Bacteria name
1	Direct heat	Pseudomonas, Staphylococcus Aureus , E.coli, Klebsiella, Proteus , Epidermis
2	Fire	Pseudomonas , Staphylococcus aureus
3	Hot water	Proteus, Pseudomonas , Klebsiella
4	Hot Milk	Staphylococcus aureus , Epidermis , E.Coli , Klebsella
5	Accident	Pseudomonas, Klebsella , Staphylococcus Aureus, Epidermis, E.Coli
6	Cooking oil	Proteus , E.Coli
7	Hot tea	Staphylococcus aureus , E.coli
8	Electricity	Pseudomonas , E.coli , Klebsella
9	Petrol	Pseudomonas, E.coli, Proteus , Enterobacter Epidermis

Figure 2. Susceptibility of *Pseudomonas aeruginosa* against antibiotics

Figure 3. Antibiotic sensitivity tests against *Klebsiella*

Figure 4. Antibiotics sensitivity test against *Proteus*

Figure 5. Antibiotic sensitivity tests against *E.coli*

Figure 6. Antibiotic sensitivity against *S.aureus*

Figure 7. Antibiotic sensitivity against *Enterobacter*

Figure 8. Numbers of Bacteria Isolated From Burn Infection

Conclusion

Burn is a skin or other body tissues injury caused by various agents including thermal injury, scalds, fire, electrical shock and chemical injury. Various bacteria, e.g. *Bacillus*, *Enterobacter*, *Epidermis*, *E. coli*, *Klebsella*, *Pseudomonas Aeruginosa*, *Proteus* and *Staphylococcus aureus* were

isolated lavishly from these injuries. During current research work 12 antibiotics were tested against these bacteria's in which levofloxacin and ciprofloxacin were found more effective than the left over antibiotics.

Authors' contributions

Conceived and designed the experiments: MN Uddin, Performed the experiments: B

Jabeen, Q Khan & S Naeem, Analyzed the data: W Khan & S Mehmood, Contributed materials/ analysis/ tools: M Rizwan, Wrote the paper: Muhammad

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