Occurrence of Multidrug Resistant Pseudomonas Aeruginosa from Dermal Infections in Toba Tek Singh

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Objectives*: Pseudomonas aeruginosa* is one of the most important nosocomial pathogens and ranked 4th among the nosocomial pathogens. It is an opportunistic Gram-negative bacterium. It is a non-glucose fermenter rod. It has an aerobic nature. It has mucoid polysaccharide capsule usually arranged in pairs. It causes nosocomial infections in intensive care units (ICU). Dermal infection is the major nosocomial problem of well-developed countries and one-third operated cases in developing countries having nosocomial infections. Various antibiotics are used as a first line agents to treat these infections. Resistance induced when the enzymes beta lactamases and carbapenemases are produced. Irrational use of antibiotics leads to antibiotic resistant. These resistant bacterial strains cause high mortality and morbidity worldwide. The study was conducted to search the occurrence of MDR *Pseudomonas aeruginosa* and its antimicrobial resistance pattern from Toba Tek Singh. Samples was collected from dermal infections and isolation was done by culturing on cetrimide agar using streak plate method. Identification of *Pseudomonas aeruginosa* was done by Gram staining and further biochemical tests. Molecular identification of the isolates was carried out through PCR by using specific primers against OprL gene of *Pseudomonas aeruginosa.* Antimicrobial susceptibility testing was achieved through Kirby-Bauer disk diffusion technique. Out of total 100 samples 21 (21%) isolates were identified as *Pseudomonas aeruginosa*. Out of positive 21 samples 12 samples were identified as multidrug-resistant *Pseudomonas aeruginosa.* Out of 12 MDR samples, pus wounds has the highest occurrence (50%) but the least occurrence (17%) found in the surgical wounds. It was concluded from the recent study that pus wound has the highest occurrence of MDR *P. aeruginosa*. Appropriate steps to mitigate this danger to public health should be taken.

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INTRODUCTION:

Nosocomial infections are infections that occur in a patient during a hospital stay or in other health care facilities that were not present at admission. It may become clinically evident either before or after discharge. The most common nosocomial infections include dermal infections, urinary tract infection, surgical wound and other soft tissue

infections, respiratory tract infections, Meningitis and gastroenteritis (Bereket et al., 2012).

The pathogens cause nosocomial infections are known as nosocomial pathogens. The most common nosocomial pathogens include *Staphylococcus aureus, Acinetobacter baumanni, Bacillus cereus, Pseudomonas aeruginosa, Enterococcous faecium, E. coli,* and *Klebsiella pneumonia.* But nosocomial pathogens that customarily described are *S. aureus, Pseudomonas aeruginosa* and *Enterococcous faecium* (Weinstein et al., 2005; Bereket et al., 2012).

Pseudomonas aeruginosa is usually obtained from the environment, and is rarely transferred from person to person. Contaminated respiratory treatment devices, irrigation solutions, catheters, infusions, cosmetics, dilute anti-septics, cleaning materials and even soaps are identified as transmission vehicles (Yetkin et al., 2006). It may rapidly colonize to hospitalized patients' respiratory and gastrointestinal tracts, especially those treated with broad spectrum antibiotics, exposed to respiratory therapy equipment, or hospitalized for prolonged periods of time (Bereket et al., 2012).

Pathogenesis is triggered by *Pseudomonas aeruginosa* when the natural defense mechanism is compromised. When body boundaries are broken by direct damage to the tissue such as intravenous or urinary catheter; or where there is neutropenia, as in cancer chemotherapy. The bacterium binds and colonizes to the mucous membrane or skin. Then it invades locally and induces systemic disease. Various virulent factors such as pili, enzymes (elastases, proteases, phospholipase C), and toxins (exotoxin A) mediate these processes (Bereket et al., 2012).

Pseudomonas aeruginosa can produce infection almost in any part of the body, although it typically does not trigger a healthy host to become infected. People most susceptible to infections with *Pseudomonas aeruginosa* are those whose mucous membranes or skin have become so impaired that they no longer serve as a physical barrier to infection (e.g., in patients with burns). Being neutropenic or immunodeficient makes patients predisposed to infection with many different organisms and *Pseudomonas aerginosa* is one of them. The unusual lung condition that develops in patients with cystic fibrosis facilitates a chronic *Pseudomonas aeruginosa* infection in which the organism exhibits a characteristic mucoid phenotype due to the alginate development that surrounds the organism's micro colonies. Hospitalized patients with cardiovascular disease, cancer or diabetes, and mechanical respirator patients in particular, are likely to develop pneumonia or bacteremia due to *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* infections in hospitalized patients lead to increased length of stay (LOS) and cost increases, as well as severe morbidity and mortality. The type of infection caused by *Pseudomonas aeruginosa* varies depending on health conditions of the person whether it is healthy or has underlying disease or has some form of involvement in the healthcare services (Moore et al., 2011).

In *Pseudomonas aeruginosa,* several specific mechanisms may be used to mediate antimicrobial resistance (Livermore, 2002; Tam et al., 2010). Production of β-lactamase, outer membrane or target-site modifications and efflux pumps are resistance mechanism of *Pseudomonas aeruginosa* (Zavascki et al., 2010). β-lactamases are enzymes generated by bacteria that are determined though plasmid-born or by genes of chromosomes , and

save microorganisms across the murderous effects of β-lactam antibiotics by dissolving the β-lactam rings, thus analyzing the drug effect (Yu et al., 2006). The enzymes called extended spectrum β-lactamases (ESBLs) negotiate resistance to ESCs (extended spectrum cephalosporins), such as ceftriaxone, cefotaxime, monobactam aztreonam and the ceftazidime (Jiang et al., 2006). β-lactamases inhibitor (clavulanate) inactivate extended-spectrum β-lactamases (Yu et al., 2006). when different mechanisms combine only in mono isolate then multiple drug resistance is usually produce (Zavascki et al., 2010).

The clinical isolates of *Pseudomonas aeruginosa* develop antimicrobial resistance, may make difficult the cure of infections and can adversely affect treatment costs and clinical outcomes for patients. There are no availability of new antimicrobial drugs in the near future, being active across *Pseudomonas aeruginosa*, making ongoing inspection of the activities of presently available agents is critically important (Flamm et al., 2004).

In most of the developing countries of the world, antibiotic distribution and its use as treatment, cattle breeding and use in agriculture are improperly regulated, or the laws are not properly enforced. Furthermore, population movement contribute to antibiotic resistance around the globe. Due to above mentioned all factors have created to higher level of resistance in bacteria. On the other hand, in the countries with pure defined and implemented legal map concerning antibiotic prescription, distribution and use is under eyes of the authorities. This helped greatly to minimize the antimicrobial resistance. As result, antimicrobial resistance could be minimized by enforcing of long-term and systematic steps at all levels of health-care as well as country level. In order to minimize antibiotic resistance, use of antibiotics to be properly regulated and the rules and regulations should be consistent with practice. The international health community must show a more active part in resolving this biggest global issue.

MATERIALS AND METHODS

Sample Collection:

a total of 100 samples were collected from patients carrying multifarious dermal infections, admitted in the dermatology ward and operation theater of D.H.Q. Hospital Toba Tek Singh, T.H.Q. Hospital Pirmahal, T.H.Q. Hospital Kamalia and T.H.Q. Hospital Gojra. Standard microbiological protocols were followed for the collection of samples. Samples was collected aseptically and was transported immediately to the Post Graduate Laboratory of Microbiology Department of Government College University, Faisalabad (Choudhary et al., 2019).

Isolation of *Pseudomonas aeruginosa*

For the isolation of *Pseudomonas aeruginosa* dermal samples were spread on cetrimide agar with the help of sterile cotton swab by using spread plate method.

Identification of *Pseudomonas aeruginosa*

On the basis of their colony characteristics, pigment production, gram's staining and motility test, *Pseudomonas aeruginosa* was recognized. Further spotting was done via using different biochemical tests. (Hassan et al., 2012).

Motility test for the identification of *Pseudomonas aeruginosa*

A fine colony of *Pseudomonas aeruginosa* was picked from petri plate and inoculated it into nutrient broth for 24 hours at 37°C. Moist preparations are used for motility test of bacteria. Motility test helps in detection of microbes. As *Pseudomonas aeruginosa* is a motile bacterium so that motility test was mandatory for its identification.

Biochemical tests:

Biochemical tests including Catalase and Oxidase was performed according to standard protocol for its confirmation (Koeing et al., 2006).

Molecular identification of *Pseudomonas aeruginosa*

All the identified bacterial isolates were further identified through Polymerase Chain Reaction (PCR) by amplification of bacterial DNA using OprL Gene specific primers (Hassan et al., 2012)

DNA Extraction:

Thermo Scientific GeneJET Genomic DNA Purification kit was used for the extraction of DNA.

Agarose Gel Electrophoresis:

Following the PCR, amplified product was subjected to electrophoresis in I% agarose gel at 100 V for 40 minutes. 0.5µg/ml Ethidium Bromide containing 0.5X TAE buffer was used in the process. Along with the PCR product, a marker indicating the molecule size of DNA was also electrophoresed.

Antimicrobial Susceptibility Testing (AST)

It was performed with the help of Kirby-Bauer disc diffusion method, on Muller Hinton agar (MHA) in accordance with CLSI guidelines 2017.Susceptibility and resistivity of isolates of *Pseudomonas aeruginosa* was evaluated against different classes of antibiotics like aminoglycosides, carbapenems, monobactams, fluoroquinolones and cephalosporins (Yayan et al., 2015).

RESULT AND DISCUSSION

Incidence of *Pseudomonas aeruginosa*

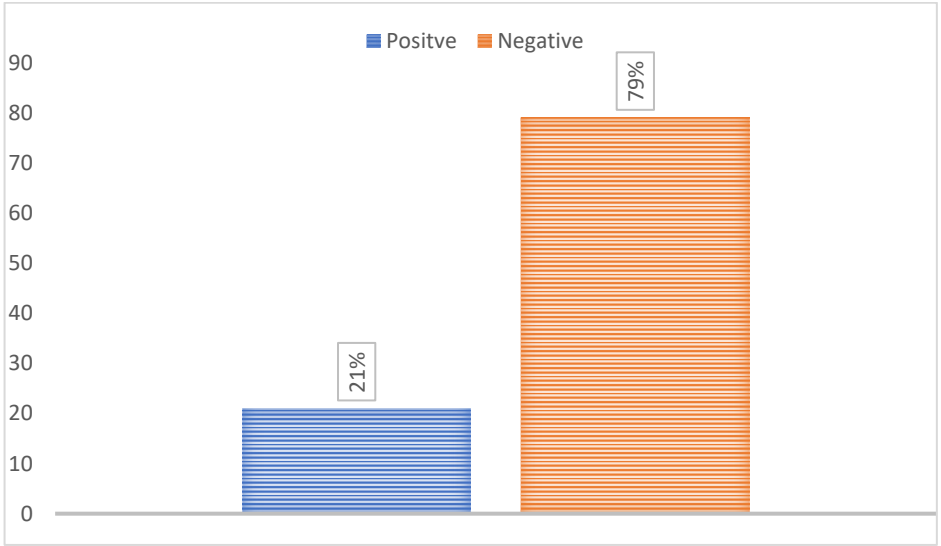

Figure 4.1: Percentage of positive samples and negative samples out of total samples.

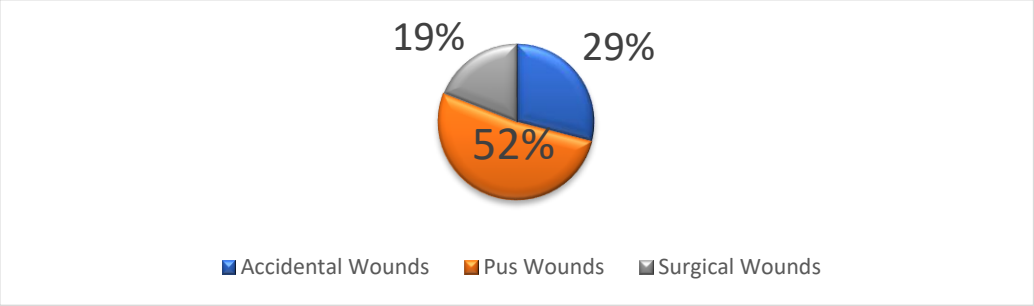

Figure 4.2: Frequency of *Pseudomonas aeruginosa* with respect to type of dermal infection in all hospitals under study.

: Incidence of *Pseudomonas aeruginosa* in DHQ Hospital Toba Tek Singh

A total of 41 samples were collected from District Head Quarter Hospital Toba Tek singh. Only 9 samples considered as *Pseudomonas aeruginosa* comprising accidental wounds (22%), pus wounds (67%) and surgical wound (11%). Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in District Head Quarter Hospital Toba Tek Singh as shown in figure 4.3

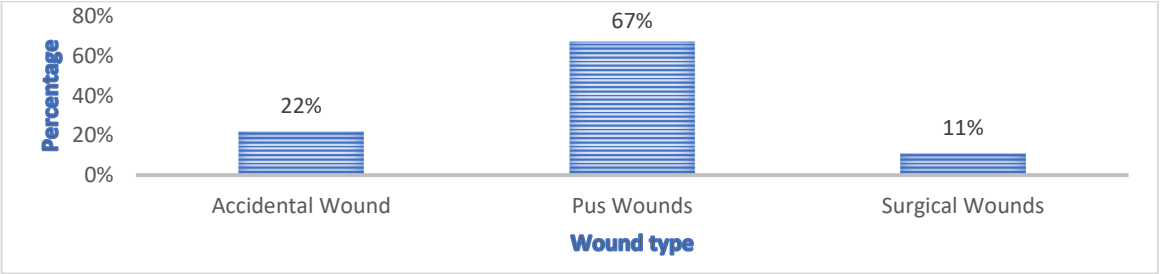

Figure 4.3: Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in District Head Quarter Hospital Toba Tek Singh.

Incidence of *Pseudomonas aeruginosa* in THQ Hospital Pirmahal

A total of 21 samples were collected from Tehsil Head Quarter Hospital Pirmahal. Only 5 samples considered as *Pseudomonas aeruginosa* comprising accidental wounds (20%), pus wounds (40%) and surgical wound (20%). Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in Tehsil Head Quarter Hospital Pirmahal as shown in figure 4.4.

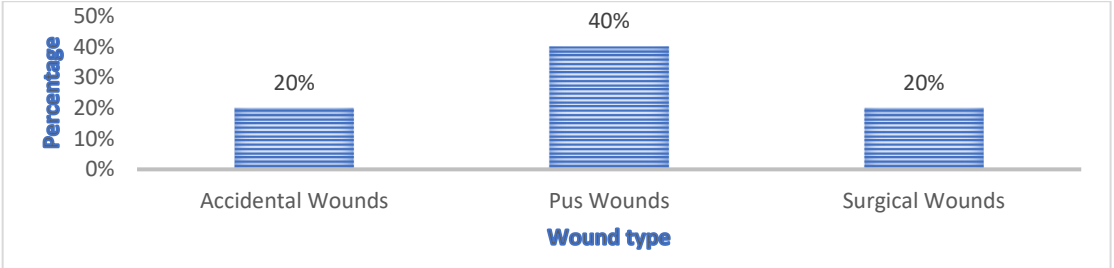

Figure 4.4: Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in Tehsil Head Quarter Hospital Pirmahal.

Incidence of *Pseudomonas aeruginosa* in THQ Hospital Kamalia

A total of 22 samples were collected from Tehsil Head Quarter Hospital Kamalia. Only 4 samples considered as *Pseudomonas aeruginosa* positive comprising accidental wounds (50%), pus wounds (25%) and surgical wound (25%). Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in Tehsil Head Quarter Hospital Kamalia as shown in figure 4.5.

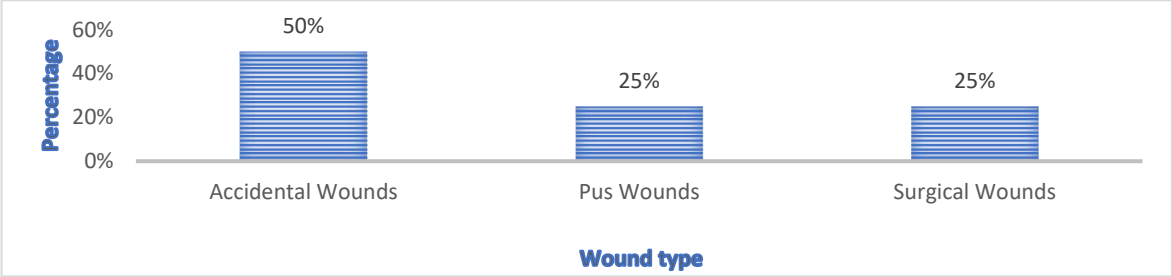

Figure 4.5: Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in Tehsil Head Quarter Hospital Kamalia.

Incidence of *Pseudomonas aeruginosa* in THQ Hospital Gojra

A total of 16 samples were collected from Tehsil Head Quarter Hospital Gojra. Only 3 samples considered as *Pseudomonas aeruginosa* positive comprising accidental wounds (33%), pus wounds (67%) and surgical wound (0%). Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in Tehsil Head Quarter Hospital Gojra as shown in figure 4.6.

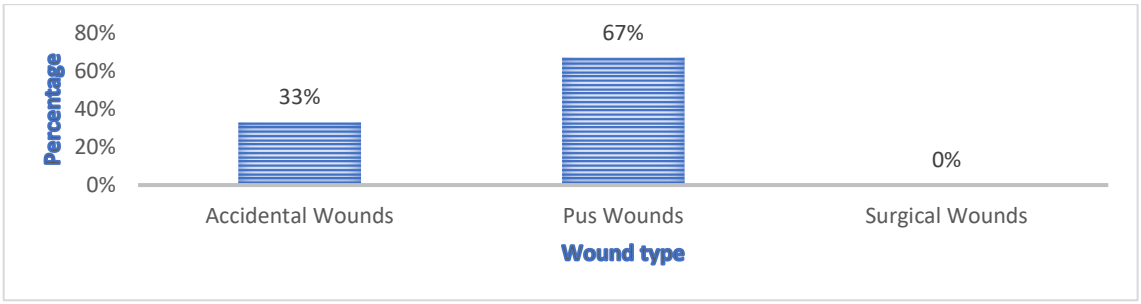

Figure 4.6: Association of *Pseudomonas aeruginosa* isolation with respect to type of dermal infection in Tehsil Head Quarter Hospital Gojra.

Colony Morphology and Macroscopic Characteristics of *Pseudomonas aeruginosa*

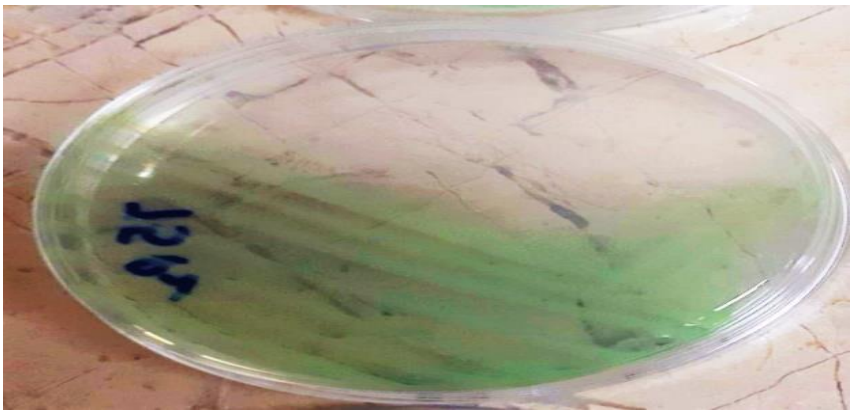

Figure 4.7 Growth of isolated bacteria on Pseudomonas agar

Microscopic Characteristics of *Pseudomonas aeruginosa*
Gram negative small rods with pink stain were seen under a microscope as shown in Figure

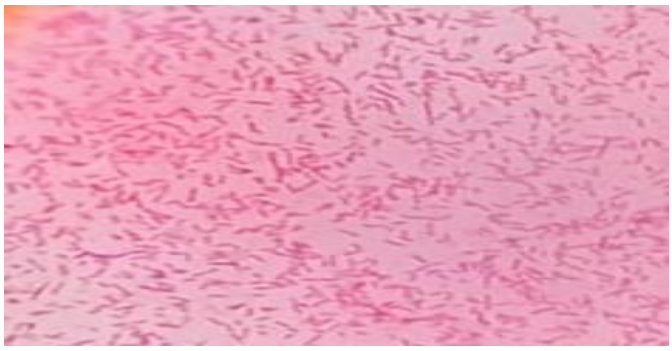

Figure 4.8: Gram negative reaction of isolated bacteria

Biochemical Analysis for the identification of *Pseudomonas aeruginosa*

Figure 4.9: Oxidase test showing positive result.

Catalase Test

Figure 4.10: Catalase Test showing positive result.

Molecular Identification of *Pseudomonas aeruginosa*

Bacterial samples were collected over a 6-month period from the dermal infections of individuals hospitalized in a separate Toba Tek Singh hospital. Our findings showed that *Pseudomonas aeruginosa* isolates have been recovered from 21 (21%) patients with dermal infection based on standard phenotypic and biochemical tests; whereas there were

also 21 (21%) positive samples for *Pseudomonas aeruginosa* using molecular techniques. PCR assays employing each primer pair produced DNA products of the predicted sizes. The OprL amplicon genes were found simultaneously in all 21 isolates of the *Pseudomonas aeruginosa*.

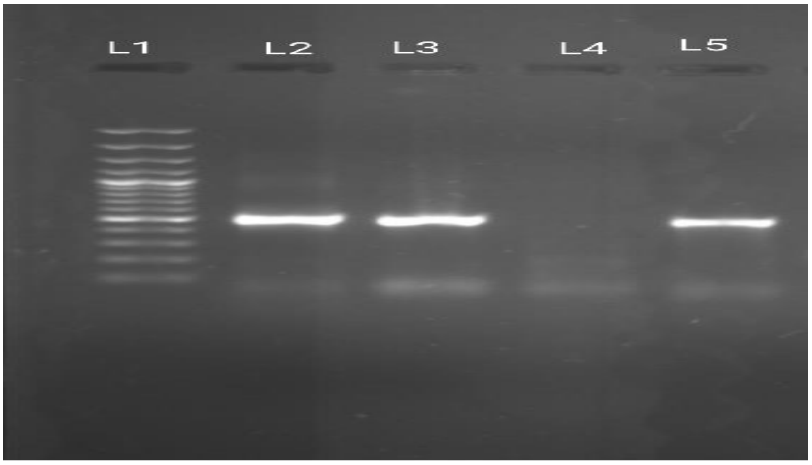

Figure 4.11: PCR based detection of OprL (504). Lane 1: 100bp ladder, Lane 4: Negative Control and Lane 2,3,5 Positive samples for OprL genes confirming *Pseudomonas aeruginosa*.

Antibacterial susceptibility profile

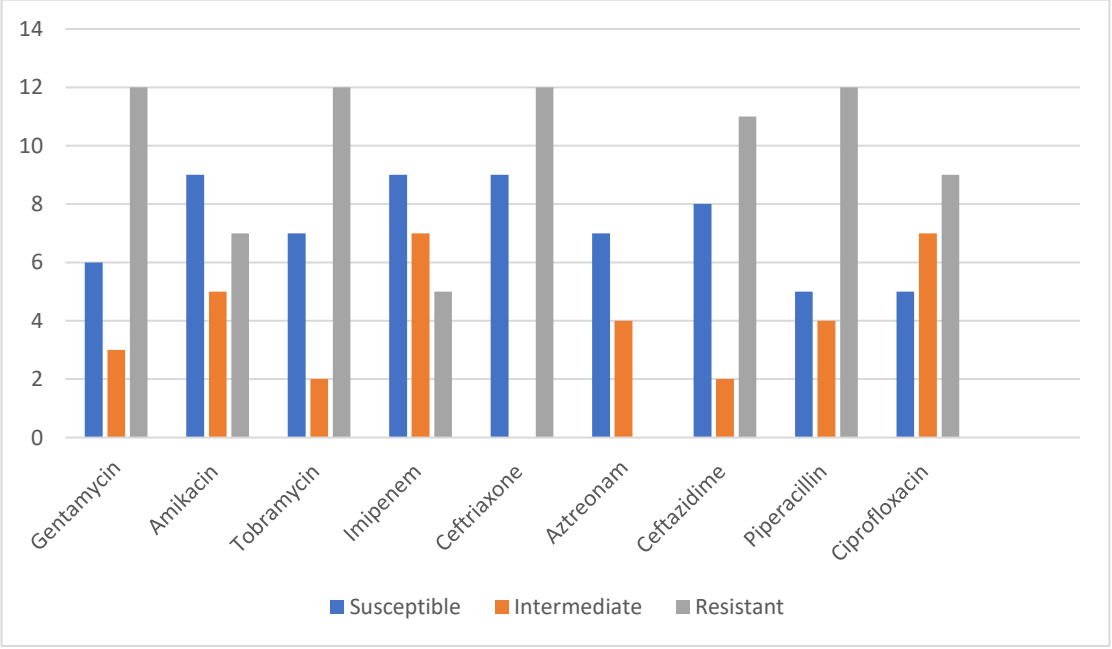

Figure 4.13: Antimicrobial susceptibility profile of the *Pseudomonas aeruginosa*

Table 4.3: Frequency of MDR *Pseudomonas aeruginosa* isolation with respect to type of dermal infections

Hospitals	Accidental wounds (%)	Pus wounds (%)	Surgical wounds (%)	Total (%)
D.H.Q. T.T. SINGH	01 (16.5%)	04 (67%)	01 (16.5%)	06 (100%)
T.H.Q. Pirmahal	0 (0%)	01 (33%)	02 (67%)	03 (100%)
T.H.Q. Kamalia	0 (0%)	01 (50%)	01 (50%)	02 (100%)
T.H.Q. Gojra	01 (100%)	0(0%)	0 (0%)	01 (100%)
Total	02 (17%)	06 (50%)	4 (33%)	12(100%)

Pseudomonas aeruginosa is one of the most important nosocomial pathogens and ranked 4th among the nosocomial pathogens (Abdulhaq et al., 2020). It is an opportunistic Gram-negative bacterium (Karami et al., 2019). It is a non-glucose fermenter rod (Corona et al., 2001). It has an aerobic nature (Driscoll et al., 2007). It has mucoid polysaccharide capsule usually arranged in pairs (Bereket et al., 2012). It is a challenging and growing public health issue specifically in developing countries around the world. In humans, it is still a leading cause of mortality and morbidity (Abdulhaq et al., 2020).

The object of recent study was to estimate the occurrence of multi drug resistant *Pseudomonas aeruginosa* from dermal infections. The result of this study showed that out of 100 dermal samples which was taken from different hospitals of Toba Tek Singh. 21 samples recorded as *Pseudomonas aeruginosa* positive.

In the recent study significant occurrence (21%) of *Pseudomonas aeruginosa* was estimated from dermal infections. Occurrence of *Pseudomonas aeruginosa* in the previously conducted study was slightly high as compared to present study because of large sample size, high contamination, poor cleanliness of hospital environment and inappropriate management of dermal infections. Almost similar findings were found in previously conducted study which was recorded as 32% as positive isolates of *Pseudomonas aeruginosa* (Anupurba et al., 2006). In another previously conducted study same findings were found with the 33.3% occurrence of *Pseudomonas aeruginosa* (Turner

et al., 2014) from dermal infection patients, while hani and his colleagues also estimated 27.78 occurrence of *Pseudomonas aeruginosa* from dermal infections.

Recent findings suggested that Multidrug-resistant (MDR) *Pseudomonas aeruginosa* are frequently found in the multifarious types of dermal infections and the highest percentage recorded in the pus wounds (52%) and least in the surgical wound patients (19%). In the recent study % of MDR *Pseudomonas aeruginosa* is high in pus wound patients because of poor management, ability of *Pseudomonas aeruginosa* to colonize in the tissues, negligence of the patients and unhygienic environment of healthcare center. A study was conducted in the past which estimated the occurrence of MDR *Pseudomonas aeruginosa* in pus wound patients is much greater than other wounds and it dependent on age, sex and duration of the stay in hospital (Ahmed, 2016; Buhl et al., 2015).

Multidrug-resistant *Pseudomonas aeruginosa* are those bacteria who are resistant against at least two specific antibiotics of minimum two classes of antibiotics (Park et al., 2011). It was found resistant against multifarious antibiotics that were used in the recent study like Amikacin, Gentamycin, Ciprofloxacin, Piperacilline, Ceftraxone, Ceftazidime, Imipenem and Tobramycine. It may be due to irregular use of antibiotics, development of mutation in *Pseudomonas aeruginosa,* genetic background of the organism and environmental condition of the specific region. It complies with the studies that were conducted in the past in Russia 75 % isolates were found gentamycin resistant (Montero et al., 2009). Another study in the past from Bangladesh revealed that 49% of *Pseudomonas aeruginosa were* sensitive to Tobramycin and 79 % to Ciprofloxacin (Ansary et al., 1994). This study also complies with the study that was conducted in past 29.60% *Pseudomonas aeruginosa* were resistant to ciprofloxacin, 19.26% *Pseudomonas aeruginosa* were resistant to amikacin and every *Pseudomonas aeruginosa* were susceptible to imipenem (Anil et al., 2013).

In clinical healthcare settings we noticed multifarious malpractices regarding infection management during study. Key associated risk factors in local healthcare centers which perform a key role in the transmission of resistant pathogens include negligence of medical staff, inadequate sterilization techniques and poor sanitization.

Finally, it is concluded in this study significant occurrence of multidrug-resistant *Pseudomonas aeruginosa* in hospitalized dermal infection patients was estimated. Irregular use of antibiotics, unhygienic condition of the environment and malpractices from health associated professionals were recorded as the influencing factors which may increase the occurrence of MDR *Pseudomonas aeruginosa* in hospitalized patients. To hinder the spreading of MDR *Pseudomonas aeruginosa* in healthcare facilities a standard control strategy should be implemented.

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