BACTERIAL ANALYSIS AND ANTIMICROBIAL DRUG RESISTANCE PATTERN OF PATHOGENS ISOLATED FROM WOUND INFECTION

A THESIS

BY

NIGAMANANDA DEB

REGISTRATION NO. 1505254

SEMESTER: JULY-DECEMBER, 2017

MASTER OF SCIENCE (M.S.)
IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DECEMBER, 2017

Dedicated

To

My Beloved

Parents and

Teachers

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Abstract

A hospital based cross-sectional study was conducted to ascertain bacteriological analysis and antimicrobial drug resistance pattern of pathogens isolated from wound infection. The study was done in St. Vincent Hospital during the period of July 2016 to June 2017. A total of 66 infected wound samples were collected with different variables (sex and age group) and both Gram-positive and Gram-negative bacterial pathogens were isolated by using standard microbiological techniques. *Staphylococcus* spp., *Pseudomonas* spp. and *E.coli* were the predominant causes of wound infections. The overall prevalence of bacterial pathogens in wound infection out of 66 samples was 63.6% *Staphylococcus* spp., 21.2% *Pseudomonas* spp. and 15.2% *E.coli* respectively. Two types of mixed bacterial infections were found. One was *Staphylococcus* spp. and *Pseudomonas* spp. mixed infection with 75% prevalence. Another was *Staphylococcus* spp. and *E.coli.* mixed infection with 25% prevalence. For antibiogram study 8 common antibiotics were used for antimicrobial sensitivity test. Gram-positive isolates were more sensitive to Gentamycine , Cefixime and Levofloxacin . On the other hand Gram-negative isolates were more sensitive to Gentamycin and Cefixime. The isolates were highly resistance to Ampicillin , Vancomycin and Erythromycin.

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LIST OF ABBREVIATIONS AND SYMBOLS

-	:	Negative
%	:	Percentage
@	:	At the rate of
+	:	Positive
µg	:	Microgram
µl	:	Microlitre
^{0}C	:	Degree of celcius
BA	:	BloodAgar
BD	:	Bangladesh
EMB	:	Eosin Methylene Blue
ER	:	Erythromycin
et al.	:	Associated
etc	:	Etcetera
Gm	:	Gram
H_2O_2	:	Hydrogen peroxide
H_2S	:	Hydrogen sulphide
HSTU	:	Hajee Mohammad Danesh Science and Technology University
i.e.	:	That is
Ltd	:	Limited
M.S	:	Master of Science
MC	:	MacConkey Agar
MI	:	Milliliter
MIU	:	Motility Indole Urease
MR	:	Methyl Red
NA	:	Nutrient Agar
No.	:	Number
PBS	:	Phosphate Buffer Saline
Prof.	:	Professor
PSS	:	Physiological Saline Solution
SL	:	Serial number
Sp	:	Species

STVH	:	Saint Vincent Hospital
v/v	:	Volume by volume
VP	:	Voges-Proskauer
w/v	:	Weight by volume

CHAPTER I

INTRODUCTION

Wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity **(Giacometti 2000)**. Wound provides a moist, warm, nutritive environment con-ducive to microbial colonization, proliferation, and infection. Many different bacterial species live on human skin, in the nasopharynx, gastrointestinal tract, and other parts of the body with little potential for causing disease, because of the first line of defense within the body. Despite this, any breach in the skin surface whether trauma, accident, surgical operation, or burn provides an open door for bacterial infections **(Aynalem Mohammed *et al.*, 21017).** The exposed subcutaneous tissues provides a favorable substratum for a wide variety of microorganisms to contaminate and colonize, and if the involved tissue is devitalized and the host immune response is compromised, the conditions become optimal for microbial growth. This is because the host immune response plays a critical role in determining wound infection will arise **(Adegoke 2010).** Wounds can be classified as accidental, pathological or post-operative. Whatever the nature of the wound, infection is the attachment of microorganisms to host cells and they proliferate, colonize and become better placed to cause damage to the host tissues **(Adenike A *et al.*, 2012)**. Wounds can also broadly categorized as having either an acute or a chronic etiology. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries. In marked contrast, chronic wounds are most frequently caused by endogenous mechanisms associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue **(Amare B 2011).**

All wounds are contaminated by both pathogens and body commensals. But The progression of a wound to an infected state is likely to involve a multitude of microbial or host factors (**Anusha S *et al.*, 2010**).

Infections caused by resistant microorganisms often fail to respond to the standard treatment resulting in prolonged illness, higher health care expenditures and a greater risk of death. Antimicrobial resistance in addition hampers the control of infectious diseases by reducing the effectiveness of treatment thus patients remain infectious for a

long time increasing the risk of spreading resistant microorganisms to others **(WHO fact sheet 2014).**

Identification of bacterial isolates was determined by standard microbiological techniques. Organisms commonly found in infected wounds include Gram positive cocci such as *S. aureus*, *Streptococcus* spp, Gram negative bacilli mostly *Acinetobacter*, *Enterobacter, E. coli, Proteus spp, P. aeruginosa* and *anaerobic bacteria* such as *Propionibacterium* spp. and *Klebsiella* spp. **(Taiwo SS *et al.*, 2002).** *Staphylococcus aureus* was the predominant microorganism (40%) followed by *Klebsiella* sp. (33%), *Pseudomonas* sp. (18%), *Escherichia coli* (16%), and *Proteus* sp. (7%). The diversity of microorganisms and the high incidence of polymicrobic flora in this study give credence to the value of identifying one or more bacterial pathogens from pus cultures. Continuous dialogue between the microbiology department and wound care practitioners and education of patients on personal hygiene is strongly advised. (**Int. J. of Pharm. & Life Sci. (IJPLS), Vol. 3, Issue 11: November: 2012, 2107-2110) 2107.**

The antimicrobial agents are of great value for devising curative measures against bacterial infections. The use of antimicrobial agents for prevention or treatment of infections in any dose and over any time period, causes a "selective pressure" on microbial populations. According to some estimates as much as 50% of antimicrobials use is inappropriate because the uses do not benefit the patients. These uses do increase selection pressure for the emergence and spread of antimicrobial resistant bacteria. Indiscriminate prescription coupled with improper use of antimicrobials, the development of resistance inducing mutations and horizontal transfer of genes coding for antimicrobial resistance among bacteria has remained a major cause for development of resistance among microorganisms to previously sensitive antimicrobial agents.

The widespread use of antimicrobials, together with the length of time over which they have been available have led to major problems of resistant organisms, contributing to morbidity and mortality. **(Nwachukwu *et al.*, 2009).**

Wound can be infected by a variety of microorganisms ranging from bacteria to fungus and parasites. Both acute and chronic wounds are susceptible to contamination and colonization by a wide variety of aerobic and anaerobic microorganisms. **(Anusha S 2010)** Isolates that have been incriminated in cases of wound infections include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*,

Streptococcus pyogenes, Proteus mirabilis, Pseudomonas aeruginosa, **(Esebelahie 2013)** *Klebsiella* spp, *Escherichia coli*, *Acinetobacter*, and *Enterobacter*. *Candida albicans* and *C. tropicalis* have also been implicated as etiological agents. The majority of wounds are characterized by a polymicrobial aerobic-anaerobic microflora; therefore, the careful use of broad spectrum antimicrobial agents is likely to be the most successful treatment in the management of infected wound. However, various antibiotics are frequently and sometimes inappropriately prescribed or administered in wound treatments, which often leads to the selection of antibiotic-resistant bacteria strains **(Adenike A.O. 2012**). Antimicrobial resistance among pathogens of wound infections is on the increase **(Sani R. A. 2012).** Antimicrobial drug resistance can be acquired as a result of mutation or acquisition of resistance genes via horizontal gene transfer, or can be an innate feature of an organism that is encoded chromosomally **(Livermore DM. 2002).** Antimicrobial drugs overuse, over dosing, drugs prescription with improper susceptibility test, self-medication and long duration of hospitalization was suggested to augment the problem of multi-drug resistant (MDR) in developing nations. **(Girma Godebo 2013).**

By justfying the research in the context of bangladesh and neaighbour country in the world the present study would help to establish guidelines for the management of wound infections and contribute to planning of surveillance, prevention and control of this infection.

The goal of the present study was to find out common bacterial pathogens responsible for wound infection and to determine their antimicrobial susceptibility pattern in our community.

So objectives of the Study

- To determine the prevalence of bacterial wound infection at St.Vincent Hospital, Dinajpur.
- To isolate and identify the bacterial pathogens form wound infection.
- To determine the antimicrobial resistance pattern of commonly used antibiotics against identified isolates.

CHAPTER II

REVIEW OF LITERATURE

Aynalem Mohammed *et al.,* (2017) This studied aimed to assess bacterial isolates and their drug susceptibility patterns from inpatients and outpatients with pus and/or wound discharge. Methods. A cross-sectional study was conducted at the University of Gondar Referral Hospital from March to May, 2014. Wound swab samples were collected from each study participant and inoculated into appropriate media. The bacterial pathogens were identified using standard microbiological methods. Antimicrobial susceptibility tests were performed using disk diffusion technique following Kirby-Bauer method. Results. A total of 137 study subjects were included in the study with bacterial isolation rate of 115 (83.9%). Of all, 81 (59.1%) were males. Seventy-seven (57%) of the isolates were Gram-negative and 59 (43%) were Gram-positive. From the total isolates, *Staphylococcus aureus* was the most predominant isolate 39/115 (34%) followed by *Klebsiella* species (13%), coagulase negative *staphylococci* spp. (12%) and *Pseudomonas aeruginosa*. Gram-positive isolates were resistant to ampicillin (86.4%), amoxicillin (83%), penicillin (81.3%), oxacillin (74.6%), and tetracycline (59.4%), while Gram-negative isolates were resistant to amoxicillin (97.4%), ampicillin (94.8%), tetracycline (72.7%), trimethoprim/sulfamethoxazole (66%), and chloramphenicol (54.5%). Conclusion. High prevalence of bacterial isolates was found, Staphylococcus aureus being the most dominant. High rates of multiple drug resistance pathogens to the commonly used antimicrobial agents were isolated. Therefore, concerned bodies should properly monitor the choice of antibiotics to be used as prophylaxis and empiric treatment in the study area.

Sushmita Roy *et al.,* (2017) studied aimed to determine the prevalence of different bacterial pathogens and their antibiotic susceptibility in various types of wound infections : A cross-sectional study was conducted to collect 105 wound swabs. All isolated bacteria were identified based on colony characteristics, gram stain and standard biochemical tests, and antibiotic susceptibility testing (AST) with the disc diffusion method. Descriptive statistics were used to present the study findings, and all analyses were performed using Stata Version 13. : The rate of isolation of bacteria was 92.3%. *Staphylococcus aureus* was found to be the most frequent isolate (55.7%), followed by

Escherichia coli (23.7%). *Pseudomonas* spp., *Streptococcus pyogenes* Gram-positive bacteria were mostly (60%) found sensitive to vancomycin, azithromycin, gentamicin, imipenem, cefixime, and ceftriaxone in this study. Among the Gram-negative bacteria, (>60%) showed sensitivity Escherichia coli to cefixime, azithromycin, cefuroxime, ceftriaxone, cefotaxime, gentamycin, and ceftazidime. : The diversity of isolated bacteria and their susceptibility patterns. signify a need to implement a proper infection control strategy, which can be achieved by carrying out antibiotic sensitivity tests of the isolates.

Ibrar Khan *et al.*, (2017) were determined the prevalent aerobic and or facultative anaerobic bacterial types and their antibiogram to commonly prescribed antibiotics. Pus, drainage or wound swabs from various body parts of 200 patients were aseptically collected from Khyber Teaching Hospital (KTH) and processed by standard microbiological techniques for identification of bacterial isolates and later antimicrobial susceptibility profile was determined as per Clinical and Laboratory Standard Institute (CLSI) guidelines by using Kirby-Bauer method. Out of 200 clinical wound specimens processed, *Staphylococcus aureus* was the most common bacterial pathogen isolated (n=100, 50%), followed by *Escherichia coli* (n=45, 22.5%), *Pseudomonas aeruginosa* (n=35, 17.5%), *Enterobacter* species (n=14, 7%), *Proteus* species (n=5, 2.5%) and *Morganella* species (n=1, 0.5%). *Staphylococcus aureus* (n=100) showed highest resistance to amoxicillin (82%), followed by norfloxacin (80%), sparfloxacin (78%), ciprofloxacin (71%), levofloxacin (46%) and Gentamicin (34%). Out of 100 *S. aureus* isolates methicillin and vancomycin resistance was found to be in 1.5 and 2% of the isolates, respectively. Among Gram negative isolates (n=100) the vast majority were resistant to augmentin, followed by cephalosporins, quinolones and almost fairly susceptible to carbapenems, cefoperazone + sulbactam and aminoglycosides. There is a need for judicious use of antibiotics in clinical setup. The periodic monitoring of bacterial pathogens and their susceptibility profile is very helpful in understanding the resistance phenotypes in a given area which ultimately help physicians in selecting suitable empirical therapy.

Zorica Stojanović-Radić *et al.*, (2016) were isolated **s**ix hundred and thirteen bacterial strains from wound swabs. The isolates were identified on the basis of growth on differential and selective media. In order to test the sensitivity of isolated strains to different antibiotics, the disc diffusion method, according to EUCAST protocol v 5.0 was

used. The most common species isolated from wound swabs was *Staphylococcus epidermidis* (18.4%), *followed by Staphylococcus aureus, Pseudomonas aeruginosa and Enterococcus* faecalis (16.8%, 12.7% and 10.4%, respectively). The maximum resistance of Gram-positive cocci was observed to penicillin and the lowest to linezolid. Gram-negative bacteria showed the highest resistance to tetracyclines, while the same strains demonstrated the highest sensitivity to polypeptide antibiotics. Comparison of the resistance patterns of Gram-negative and Gram-positive bacterial strains showed significant difference in the tetracycline efficiency.

Pooja Singh *et al.*, (2015) studied on postoperative wound infection and found that E.coli (14.5%) was the commonest pathogen isolated due to more laprotomy surgical procedures followed by equal rate of *Staphyalococcus aureus* (7%) and *Pseudomonas aeruginosa* (7%), *Klebsiella* (3.5%), *Proteus* (2.5%), *Enterococcus* (2.5%) and *Streptococcus* (2%). First generation of Cephalosporins and most of the Aminoglycosides were sensitive against gram positives whereas successive generations of cephalosporins were sensitive against gram negatives including some penicillin drugs like Ampicillin, Amoxicillin, and Pipracillin-Tazobactum which were sensitive for both gram positive and gram negative organisms.

Hrishikesh Sawdekar *et al.*, (2015) was conducted to determine antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to antimicrobial agents. A retrospective study was conducted among patients with wound infection in Suyash super speciality hospital, from January 2012 to December 2013. Wound swab was collected using sterile cotton swabs and processed for bacterial isolation and susceptibility testing to Systemic antimicrobial agents. In this study 78 bacterial isolates were recovered from 258 specimens showing an isolation rate of 31.2%. The predominant bacteria isolated from wounds were gram positive S*taphylococci* 36 (46.2%), followed by gram negative *Streptococci* 18 (23.1%) gram negative *Pseudomonas* 12 (15.4 %) and gram negative *Proteus* 8 (10.4%). The gram positive and gram negative bacteria constituted 68 (87.2%) and 10 (12.8%) of bacterial isolates; respectively. In the present study most of the pathogens isolated from wound isolates showed high rate of resistance to most commonly used newer antibiotics used to treat bacterial infections. Therefore, rational use of antibiotics should be practiced.

Maria Ayub *et al.*, (2015) worked on sepsis, pus and wound infection. The potential microorganisms isolated were gram positive cooci (Beta haemolytic *Streptococci, Erthrococci, Staphylococci*), gram negative aerobic rods (*Enterobacter* species, *Escherchia coli, Klebsiella* species), anaerobes (*Bacteroides, Clostridium*) fungi (Yeasts, Aspergillus). Most of the pathogens are susceptible to vancomycin and ciprofloxacin that is 36.3% and 33.40% respectively while the most resistant drug was ceftriaxone.

Shahin Sultana *et al.*, (2015) was conducted to isolate and identify the bacteria causing wound infection and to determine the antimicrobial susceptibility pattern. A total of 263 wound swab and pus samples were collected during the period of January to December 2012 from Delta Medical College and Hospital, Dhaka, Bangladesh. Swabs from the wound were inoculated on appropriate media and cultured and the isolates were identified by standard procedures as needed. Antimicrobial susceptibility testing was performed by disk diffusion method according to 'The Clinical Laboratory Standard Institute' guidelines. In this study 220 bacterial isolates were recovered from 263 samples showing an isolation rate of 83.65%. The predominant bacteria isolated from infected wounds were Staphylococcus aureus 89 (40.45%) followed by *Escherichia coli* 62 (28.18%), *Pseudomonas aeruginosa* 34 (15.45%), *Enterococci* 18 (8.18%), *Acinetobacter* 5 (2.27%), *Klebsiella* 9 (4.09%) and *Proteus* 3 (3.36%). *Staphylococcus aureus* was sensitive to linezolid (94.38%), fusidic acid (91.01%), vancomycin (87.64%), amikacin (74.15%) and gentamicin (73.03%). Among the Gram negative isolates Escherichia coli was predominant and showed sensitivity to imipenem (93.54%) amikacin (83.87%) colistin (53.22%) and piperacillin and tazobactum (53.22%) and pseudomonas showed sensitivity to amikacin (73.52%), imipenem (70.58%) and colistin (70.58%). Staphylococcus aureus was the most frequently isolated pathogen from wound swab and the antibiotic sensitivity pattern of various isolates help to assist the clinician in appropriate selection of empirical antibiotics against wound infection.

Ezekiel Olugbenga Akinkunmi *et al.*, (2014) observed that Staphylococcus aureus was the most frequent organism isolated accounting for 23 (18.3%) of a total of 126 isolates. Other organisms were *Pseudomonas aeruginosa* and *Bacillus spp* 11.1% each; *Escherichia coli* 10.3%; *Candida spp* 8.7%; Coagulase negative *staphylococci* 8.7%; *Pseudomonas spp* 6.3%; *Serratia odorifera* 4.7%; *Bacteroides* 4.0%; *Enterococcus spp* 3.2%, the remaining isolates were other enterobacteria. He also observed that, resistance

to the β-lactam antibiotics was above 98%, whilst more than 70% of isolates were resistant to erythromycin, fusidic acid and tobramycin.

Mama M *et al.*, (2014) found wound infection was one of the health problems that are caused and aggravated by the invasion of pathogenic organisms. Information on local pathogens and sensitivity to antimicrobial agents, and topical agents like acetic acid is crucial for successful treatment of wounds.To determine antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital. A cross sectional study was conducted among patients with wound infection visiting Jimma University Specialized Hospital, from May to September 2013. Wound swab was collected using sterile cotton swabs and processed for bacterial isolation and susceptibility testing to antimicrobial agents, acetic acid, hydrogen peroxide and dabkin solution following standard bacteriological techniques. Biochemical tests were done to identify the species of the organisms. Sensitivity testing was done using Kirby- Baur disk diffusion method. Minimum inhibitory and bactericidal concentration was done using tube dilution method.In this study 145 bacterial isolates were recovered from 150 specimens showing an isolation rate of 87.3%. The predominant bacteria isolated from the infected wounds were *Staphylococcus aureus* 47 (32.4%) followed by *Escherichia coli* 29 (20%), *Proteus* species 23 (16%), Coagulase negative *Staphylococci* 21 (14.5%), *Klebsiella pneumoniae* 14 (10%) and Pseudomonas aeruginosa 11 (8%). All isolates showed high frequency of resistance to ampicillin, penicillin, cephalothin and tetracycline. The overall multiple drug resistance patterns were found to be 85%. Acetic acid (0.5%), Dabkin solution (1%) and 3% hydrogen peroxide were bactericidal to all isolated bacteria and lethal effect observed when applied for 10 minutes. On in vitro sensitivity testing, ampicillin, penicillin, cephalothin and tetracycline were the least effective. Gentamicin, norfloxacin, ciprofloxacin, vancomycin and amikacin were the most effective antibiotics. Acetic acid (0.5%), dabkin solution (1%) and H_2O_2 (3%) were bactericidal to all isolates.

Reiye Esayas Mengesha *et al.*, (2014) taken 128 wound swab and were culture aerobically, Out of these the predominant bacterial isolates were *Staphylococcus aureus* 44 (35.77%), *Klebsiella* species 29 (22.76%) and Coagulase negative *Staphylococci* (CoNS) 18 (14.63%).

Irfan Iqbal *et al.*, (2014) worked surgical site infections and found from 12 cases of infected surgical wounds were recorded with an infection rate of 24%. *Pseudomonas aeruginosa* (4 isolates, 36.36%) was the most common isolated organism followed by *Staphylococcus aureus* (3 isolates, 27.27%), *Escherichia coli* (2 isolates, 18.18%), *Klebsiella* (2 isolates, 18.18%) and *Enterobacter* (1 isolate, 9.09%). There was no growth in 38 (76%) samples.

Girma Godebo *et al.*, (2013) A Hospital based cross-sectional study was conducted on 322 wound samples at Jimma University Specialized Hospital, Ethiopia. The overall MDR among gram positive and gram negative bacterial isolates were (77%) and (59.3%) respectively. About, 86.2% *S.aureus* and 28.6% of Coagulase negative Staphylococci became MDR. Nearly 30.1% of *S.aureus* was resistant to six classes of antimicrobials. The average MDR rate of *Proteus, Klebsiella,* and *Providencia* species was 74.8%, 69.6% and 75% in that order. Nearly, 30.8% of *Proteus sp,* 32.6% of *Klebsiella sp* and 61% of *Citrobacter sp* were resistance to 4 classes each. Surprisingly, the average MDR rate for *Citrobacter sp* was 100%. About (76.7%) of *S.aureus* was oxacillin/methicillin resistant while (16.4%) were vancomycin resistant. *Proteus species* was the predominant isolates (27.9%) followed by *P. aeruginosa* and *S. aureus* (19.3%) and (19%) respectively.

Mohammad Shahid Raza *et al.*, (2013) A retrospective study was conducted in Gondar on patterns and multiple drug resistance of bacteria pathogens isolates from wounds infection. Bacterial pathogens were isolated from 79 patients showing an isolation rate of 52%. *S. aureus* was the predominant species 65% followed by *Escherichia coli* (10%), *Klebsiella pneumonia* 9%, *Proteus* species 4% and *Streptococci* species 4%. Amoge gram positive bacteria *S. aureus* shows high level of drug resistance against pencilline 59%, tetracycline 57%, ampicillin 55% and co-trimoxazole 35%. *E.coli* was found to be resistant to ampicillin in 87%, tetracycline also in 87% and co-trimoxazole 63%. The overall multidrug resistance pattern were found to be 78.5%.

Anil Chander *et al.*, (2013) worked on Post-Operative Wound Infections and observed that *Staphylococcus aureus* 36 (37.5%) was the predominant gram positive isolate and Escherichia coli 24 (25%) was the major gram negative isolate. All *S. aureus* isolates were sensitive to aminoglycosides and vancomycin. Out of 36 S. aureus , 15 (41.66%) isolates were methicillin resistant S. aureus (MRSA). *Staphylococcus epidermidis*

showed high resistance (50% - 100%) to all antibiotics but were sensitive to vancomycin. All gram negative isolates showed high resistance against cephalexin (75% - 100%) and ceftriaxone (25% - 100%).

Mohammad Shahid Raza *et al.*, (2013) All *S. aureus* isolates were sensitive to aminoglycosides and vancomycin. Out of 36 *S. aureus*, 15 (41.66%) isolates were methicillin resistant *S. aureus* (MRSA). *Staphylococcus epidermidis* showed high resistance (50%-100%) to all antibiotics but were sensitive to vancomycin. All gram negative isolates showed high resistance against cephalexin (75%-100%) and ceftriaxone (25% - 100%). Overall multi-drug resistant isolates were 66.7%.

Ntsama Essomba C. *et al.*, (2013) studied was done in Cameron to determine the bacterial profile of surgical site infection. Out of 110 (9.2%) patients who developed SSI, the isolated bacteria were Enterobacteriaceae (41.2%), *Staphylococcus aureus* (15.3%), *Pseudomonas spp.* (14.1%), *Enterococcus spps.* (12.9%), *coagulase-negative staphylococci* (CoNS, 5.9%), *Streptococcus spp*s (1.8%), and others (8.8%). These bacteria presented a global-sensitivity rate of less than 30% to the commonly prescribed antibiotics.

Daniel *et al.*, (2013) studied microbiological profile of diabetic foot ulcers and its antibiotic susceptibility pattern in a teaching hospital in Gujarat, revealed that *Pseudomonas aeruginosa* (27%) was the most common isolate causing diabetic foot infections followed by *Klebsiella* species (22%), *Escherichia coli* (19%), *Staphylococcus aureus* (17%), *Proteus* species (7%), *Enterococci* (3%), *Acinetobacter* (2%), CoNS (2%) and *Providencia* (1%) (Mehta *et al.,* 2014). The predominance of gram negative bacilli in diabetic pus has also been reported in another study (Sivakumari *et al.,* 2009). However, *Staphylococcal* species was the primary pathogen in most of wound infections of diabetic patients.

Bayram *et al., (*2013) conducted three year review of bacteriological profile and antibiogram on burn wounds isolates in Van,Turkey revealed the most frequent bacterial isolate was *Acinetobacter baumannii* (23.6%), followed by coagulase negative *Staphylococci* (13.6%), *Pseudomonas aeruginosa* (12%), *Staphylococcus aureus* (11.2%), *Escherichia coli* (10%), *Enterococcus* species (8.8%) and *Klebsiella pneumonia* (7.2%).

Ellen Korol *et al.*, (2013) observed that *Staphylococcus aureus* (*S. aureus*) is a commonly-isolated organism for SSI, and methicillin-resistant *S. aureus* SSI incidence is increasing globally.

Verma (2012) studied on aerobic bacterial profile and antimicrobial susceptibility pattern of pus isolates in a South Indian tertiary care hospital revealed *Staphylococcus aureus* (24.29%) was the most common isolates, followed by *Pseudomonas aeruginosa* (21.49%), *Escherichia coli* (14.02%), *Klebsiella pneumonia* (12.15%), *Streptococcus pyogenes* (11.23%), *Staphylococcus epidermidis* (9.35%) and *Proteus* species (7.47%) (Rao *et al.,* 2014). Another study on isolation of different types of bacteria from pus revealed also *Staphylococcus aureus* to be the predominant microorganism (40%) followed by *Klebsiella* species (33%), *Pseudomonas* species (18%), *Escherichia coli* (16%), and *Proteus* species (7%).

Tigist Alebachew *et al.*, (2012) A Cross-sectional, prospective study conducted by Tigist *et. al.*,indicated that out of 114 burn wound pus sample, bacterial infection was observed in 95(83.3%) of which, 66 (69.5%) had *S. aureus* infection. Overall prevalence of *S. aureus* isolation was 57.8%. Most of them were sensitive to vancomycin, clindamycin, kanamycin and erythromycin, but highly resistant to penicillin G. All isolates were found to be multi drug resistant, and one isolate was resistant to all the tested drugs.

Mulugeta K. *et al.*, (2011) studied aimed at assessing bacteriology and antibiogram of pathogens from wound infections at Dessie, North East Ethiopia, Out of 599 wound swab samples analyzed, 422 (70.5%) were culture positive. Seventy eight (18.5%) of the culture had double infections. *Staphylococcus aureus* was the most frequently isolated pathogen which accounted for 208 (41.6%) of isolates followed by *Pseudomonas* spp. 92 (18.4%), *Escherichia coli* 82 (16.4%), *Proteus* spp. 55 (11.0%), *Enterobacter* spp. 21 (4.2%), and *Citrobacter* spp. 21 (4.2%), *Klebsiella* spp. 12 (2.4%) and *Coagulate negative staphylococcus* (1.8%). Amoxicillin had the highest resistance 8 rate 78.9%, followed by tetracycline 76.1% and erythromycin (63.9%). The sensitivity rates of norfloxacin, ciprofloxacin and gentamicin were 95.1%, 91.8% and 85%, respectively. The overall multiple antimicrobial resistances rate was 65.2% and only 13% of the isolates were sensitive to all antimicrobial agents tested. The most frequently isolated bacteria were sensitive to ciprofloxacin, gentamicin, cloxacillin and norfloxacin.

Egbe CA *et al.*, (2011) was carried out in Nigeria to determine Microbiology of Wound Infections and its Associated Risk Factors. The overall prevalence of wound infections was 64.8%. The prevalence of wound infections was not significantly affected by gender but was significantly affected by age . The prevalence of wound infections was minimum among age group of <5 years old (20.0%) and maximum among the age group of 36-40 years old (77.5%). *Staphylococcus aureus* was the most prevalent etiologic agent (21.5%). β-lactams, fluoroquinolones and gentamicin were the most effective antibacterial agents.

Shriyan *et al.*, (2010) studied done on the bacteriology of surgical site infections in Karachi, revealed the most common pathogen isolate was *Staphylococcus aureus* (50.32%), followed by *Pseudomonas aeruginosa* (16.33%), *Escherichia coli* (14.37%), *Klebsiella pneumonia* (11.76%), *Streptococcus pyogenes* (1.30%), and miscellaneous gram negative rods (5.88%) including *Acinetobacter baumannii*, *Proteus mirabilis* and *Citrobacter diversus* (Mahmood 2010). A cross-sectional study designed to determine the distribution of the bacterial pathogens and their antimicrobial susceptibility from suspected cases of post-operative wound infections, also revealed *Staphylococcus aureus* (63%) was the most frequently isolated pathogenic bacteria, followed by *Escherichia coli* (12%), *Pseudomonas* species (9.5%), *Klebsiella* species (5%), *Proteus* species (3.5%) and coagulase negative *Staphylococcus* species (3.5%).

Yishak Abraham & Biruk L. Wamisho (2009) Cross-sectional prospective study was conducted to determine the bacteriology of open fracture wounds at Black Lion Hospital, Addis Ababa Ethiopia. A total of 162 bacterial pathogens were isolated from the 200 open fracture wounds sampled. *S. aureus* was the dominant isolate (14.8%) followed by *Acinetobacter* spp. (11.4%). Of the culture-positive wounds, 51.2% showed mono-microbial growth (single bacterial type) and 48.8% showed polymicrobial growth. The gram-positive and negative bacteria accounted for 34.0 and 66.0%, respectively ($p < 0.05$). All gram-positive bacterial isolates showed low level of resistance (<60%) to all antibiotics tested except for ampicillin and penicillin to which they showed intermediate level of resistance (60-80%). Most gram-positive isolates, 29/55 (52.7%) showed multiple drug resistance (resistance to three or more drugs). All gram negative bacterial isolates showed low level of resistance (<60%) to all antibiotics tested except for ampicillin and amoxicillin (60 - 80%, intermediate level resistance). Fifty-one percent of the gram negative bacterial isolates were identified as multiple drug resistants (MDR).

Darahi *et al.*, (2008) determined DNA finger printing of ten *Escherichia coli* O157:H7 strains based on random amplified polymorphic DNA (RAPD) technique. Ten *Escherichia coli* O157: H7 strains isolated from children with either hemorrhagic colitis (HC) or hemolytic uremic syndrome (HUS). Their DNA was extracted and further amplified by RAPD-PCR using 53 decamer primers. In addition, genetic distance and cluster analysis were estimated. RAPD-PCR analysis proved to be of great value in designing a variety of molecular based epidemiological studies that focuses on the identification and characterization of *Escherichia coli* O157:H7.

JR Anguzu and D Olila *et al.*, (2007) The study conducted in Uganda on drug sensitivity patterns of bacterial isolates from septic post-operative wounds. Pathogenic bacteria were recovered from 58.5% of the specimens. The isolates were*: S.aureus* (45.1%), *Coliforms* (16.9%), *Proteus mirabilis* (11.3%), *P.aeruginosa* (9.9%), *Klebsiella pneumoniae* (7.0%) and *Enterobacter spp* (2.82%). Most of the organisms were sensitive to gentamicin, ciprofloxacin and ceftazidime. There was resistance to ampicillin, amoxycillin and chloramphenicol. *Staphylococcus aureus* was generally sensitive to gentamicin (87.5%), ciprofloxacin (68.7%) and methicillin (75%), but resistant to erythromycin (56.2%) and ampicillin (97%). Most of the gram-negative bacteria isolated were sensitive to Ciprofloxacin, Gentamicin and Ceftazidime but resistance to Ampicillin, Amoxycillin and Chloramphenicol. Methicillin resistant *Staphylococcus aureus* (MRSA) strains formed 25% of this species. *Pseudomonas aeruginosa* was sensitive to gentamicin (87.5%) and ceftazidime (85.7%) but showed resistance to ciprofloxacin (57.2%). Some organisms e.g. *S.aureus, Pseudomonas aeruginosa* and *Proteus mirabilis* exhibited multi-drug resistance to the antibiotics tested.

Anguzu *et al., (*2007)** studied done in a University teaching hospital in Nigeria, revealed *Staphylococcus aureus* (42.3%), *Pseudomonas aeruginosa* (32.9%), *Escherichia coli* (12.8%) and *Proteus mirabilis* (12.8%) are associated with surgical wound infections (Nwachukwu *et al.,* 2009). These findings agree with those reported in Kenya on surgical site infections, that *Staphylococcus aureus* was the most prevalent bacterial isolate (Dinda *et al.,* 2013). These findings also agree with a study done in Uganda that identified *Staphylococcus aureus* as the commonest causative agent of septic post-operative wounds.

Kehinde *et al.*, (2004) studied done in a tertiary hospital, Pakistan on burn wounds, revealed *Staphylococcus aureus* (57.98%) to be the most causative organism in burn wound infections followed by *Pseudomonas aeruginosa* (19.33%), *Klebsiella pneumonia* (8.4%), *Proteus* species (4.2%), *Staphylococcus epidermidis* (3.36%), *Escherichia coli* and *Enterobacter* (2.52%) each, *Citrobacter* and *Serratia* (0.84%) each (Ahmed *et al.*, 2013). Though a study done in Ibadan, Nigeria on burn wound infections revealed *Klebsiella* species to be the most commonly isolated pathogen, constituting 34.4%, closely followed by *Pseudomonas aeruginosa* (29.0%) and *Staphylococcus aureus* (26.8%).

Gibotti *et al.*, (2004) studied the *Escherichia coli* ipa genes by RFLP-PCR assays for enteroinvasive *Escherichia coli* (EIEC) serotypes.

Guan S *et al.*, (2002) identified *Escherichia coli*by the amplification of 16S rRNA gene by PCR.

Adenike A.O. *et al.*, (2000) A study was carried out to determine antimicrobial Susceptibility Patterns of the Bacterial Isolates in Post-Operative Wound Infections in Nepal. Out of 120 pus swabs processed for culture *Staphylococcus aureus* 36 (37.5%) was the predominant gram positive isolate and *Escherichia coli* 24 (25%) was the major gram negative isolate.

A. Giacometti *et al.*, (2000) Another survey was conducted in Italy to assess Epidemiology and Microbiology of Surgical Wound Infections. This study included 676 surgery patients with signs and symptoms indicative of wound infections. Bacterial pathogens were isolated from 614 individuals. Among the common pathogens were *Staphylococcus aureus* (191 patients, 28.2%), *Pseudomonas aeruginosa* (170 patients, 25.2%), *Escherichia coli* (53 patients, 7.8%), *Staphylococcus epidermidis* (48 patients, 7.1%), and *Enterococcus faecalis* (38 patients, 5.6%).

CHAPTER III

MATERIALS AND METHODS

A hospital based cross-sectional study was conducted at St. Vincent Hospital which is located in Dinajpur, Bangladesh.The laboratory works were conducted in the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science & Technology University, Dinajpur, during the period from July 2016 to June 2017. The detailed outline of Materials and Methods are given below.

3. 1 Materials

3.1.1 Study population

All patients with wound infection who visited St. Vincent Hospital at the study period.

3.1.2 Laboratory Preparations

All items of required glassware including test tubes, pipettes, plate, slides, cylinder, flask, conical flaks, glass and vials soaked in a household diswashing detergent solution overnight. Contaminated glasswares were disinfected with 2% sodium hypochloride solution prior to cleaning. The glassware then were cleaned by brushing, washed thoroughly in running tap water, rinsed within distilled water and finally sterilized either by dry heat at 160^0C for 2 hours or by autoclaving for 15 minutes at 121^0C under 15 Ibs pressure per sq inch. Autoclaved items were dried in a hot air oven at 50^0C. Disposable plastic was sterilized by autoclaving. All the glasswares were kept in oven at 50^0C for future use.

3.1.3 Media for culture

3.1.3.1 Solid media

- Nutrient Agar Medium, (HI-MEDIA, India)
- Mannitol Salt Agar Medium, (HI-MEDIA, India)
- Eosin Methylene Blue, (EMB) (HI-MEDIA, India)
- Cetrimide Agar Medium, (HI-MEDIA, India)
- Blood Agar Medium, (HI-MEDIA, India)
- Mac Conkey Agar medium, (HI-MEDIA, India)

- Staphylococcus Agar No.110

3.1.3.2 Liquid media

- Methyl Red-Voges Proskauer (MR-VP) broth, (HI-MEDIA, India)
- 1% Pepton Water, (HI-MEDIA, India)
- Tetrathionate broth, (HI-MEDIA, India)

3.1.4 Chemicals and reagents

3.1.4.1 Reagents

The chemicals and reagents used during the study were-

- Gram's staining reagents (Crystal violet, Gram's iodine, Acetone alcohol, Safranin)
- Potassium- di-hydrogen phosphate (0.2M, KH_2PO_4 $2H_2O$)
- Dehydrated sodium citrate
- Phosphate Buffered Saline (PBS)
- Physiological Saline Solution (PSS)
- Methylene Blue stain
- Di-sodium hydrogen phosphate (0.2M, $Na_2HPO_4 12H_2O$)
- Voges-Proskauer (VP) Solution
- Sugar media (Dextrose, Maltose, Lactose, Sucrose, and Mannitol) and other chemicals and reagents as when required during the experiment.
- Indol Solution
- Methyl Red Solution

3.1.5 Glass and plastic wares

Different types of glass and plastic ware were used in this research works included, Test tube, Graduated test tube, Centrifuge tube, Graduated centrifuge tube, Screw capped test tube, Pipette, Disposable syringe and needle, Tray, Vials, Petridish, Conical flask, Beaker, Measuring cylinder, Eppendorp tube, Glass spreader, Stone bids, Micropipettes and Microplates etc.

3.1.6 Appliances

The following types of appliances were used in the different steps of the experiment. These included Electric balances, Bacteriological incubator, Refrigerator, Bacteriological

loop, pH meter, Autoclave, Hot air oven, Deep freeze, Hot water bath, Microscope, Centrifuge machine and Test tube stands etc.

3.1.7 Antibiotic sensitivity Discs

Commercially available antimicrobial discs (Oxoid Ltd., UK) were used to determine the drug sensitivity pattern of different bacterial isolate. The method allowed for the rapid determination of the efficacy of the drug by measuring the diameter of the zone of inhibition that result from different diffusion of the agent into the medium surrounding the disc. The followings were the antibiotics that were tested against, the selected organism with their disc concentration.

Table 1. Antimicrobial agents with their disc concentration.

Sl. No.	Name of the Antibiotic	Letter Code	Disc Concentration (µg/disc)	Source
1	Ampicillin	AMP	10	Becton Dickinson, USA
2	Ciprofloxacillin	CIP	5	Oxoid Ltd., UK
3	Vancomycin	VA	30	Oxoid Ltd., UK
4	Gentamicin	GEN	10	Oxoid Ltd., UK
5	Cefixime	CFM	5	Oxoid Ltd, UK
6	Streptomycin	S	30	Oxoid Ltd, UK
7	Erythromycin	E	15	Oxoid Ltd., UK
8	Levofloxacin	LF	5	Oxoid Ltd., UK

Legends

µg =Micro gram

3.2 Methods

The following methods were used for the isolation and identification of bacteria.

3.2.1 Experimental design

The experimental work was divided into two steps: The first step was performed for the isolation and identification of the organisms of the collected sample using cultural, staining and biochemical characteristics. The second step was conducted for the determination of antibiotic sensitivity and resistance pattern of isolated organisms of

various samples by using different antibiotic discs available in the market. The layout of the diagrammatic illustration of the present study is shown in figure.

Schematic representation of the experimental design

Experimental layout

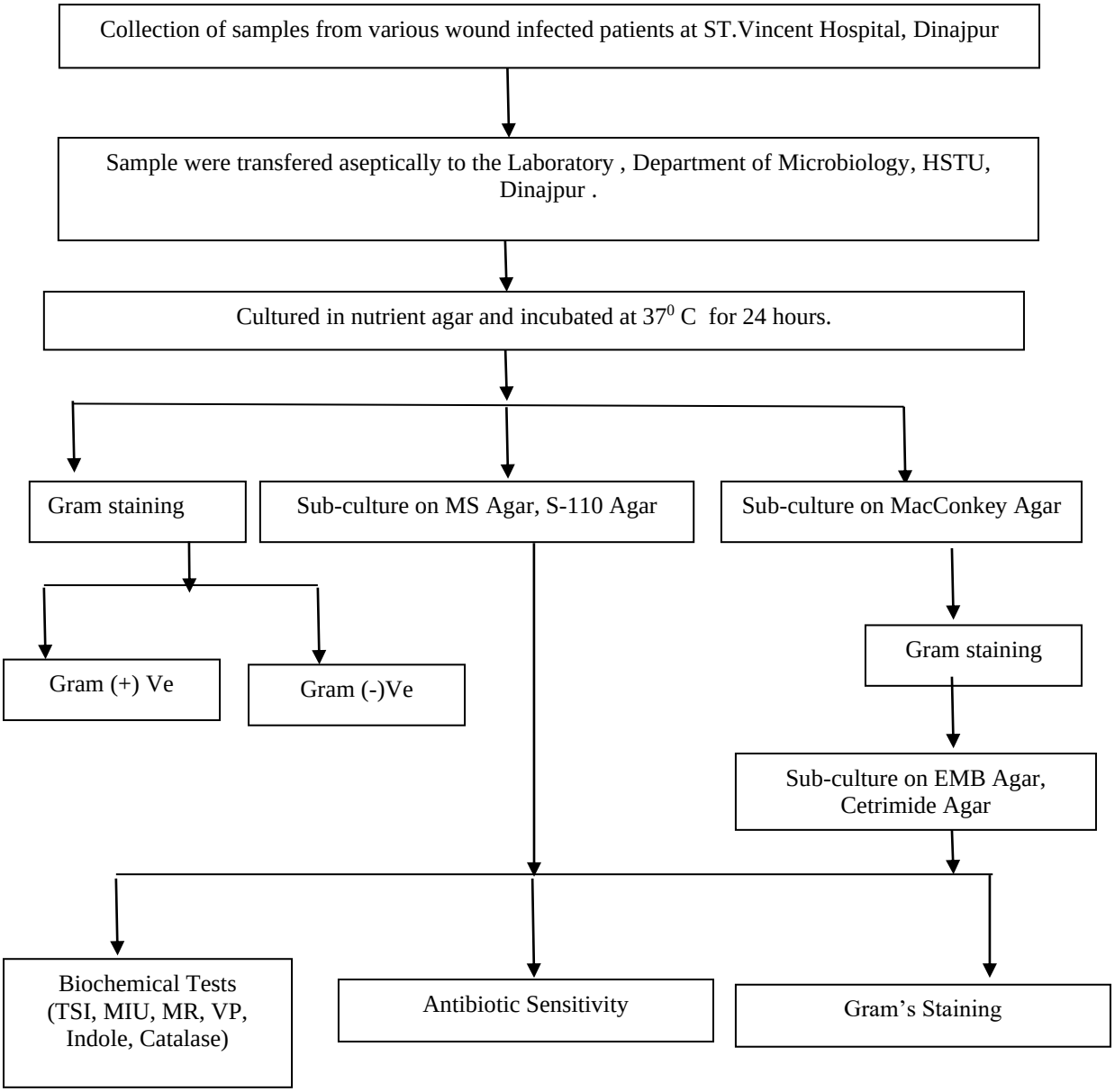

Figure 1. The schematic illustration of the experimental layout

3.2.2 Specimen collection and transportation

Wound beds were prepared before specimen collection by using Levine's technique where the wound surface was cleansed of surface exudates and contaminants with a

moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed with non bacteriostatic sterile normal saline after removing the dressing. This technique is believed to be the best technique for swabbing open wounds and more reflective of tissue bioburden than swabs of exudate or swabs by other techniques. Cleansing the wound prior to obtaining swab specimens was done in an effort to remove immediate surface contaminating organisms (bacteria). The culture was more likely to represent the microbiology in the deep wound compartment. As part of Levine's technique, the end of a sterile cotton-tipped applicator was rotated over 1 cm^2 area for 5 second with sufficient pressure to express fluid and bacteria to surface from within the wound tissue double wound swabs were taken from each wound at a point in time to reduce the chance of occurrence of false-negative cultures. During the study period a total of 66 different types of wound samples was collected.

Figure 2: Specimen collection from Wound Infection

After collection and recording of data , wound specimens were transported to the Microbiological Laboratory, HSTU, Dinajpur within 30 minutes by placing the swabs in to the sterile test tubes having 0.5 ml of sterile normal saline solution.

3.2.3 Preparation of reagents

3.2.3.1 Methyl- Red solution

The indicator MR solution was prepared by dissolving 0.1 gm of Bacto methyl- red in 300 ml of 95% alcohol and diluted to 500 ml with the addition of distilled water.

3.2.3.2 Methyl Red - Voges Proskauer broth

A quantity of 17 gms of MR-VP medium (HI-MEDIA) was dissolved in 1000 ml of distilled water, dispensed in 2 ml amount in each tube and the tubes were autoclaved. After autoclaving, the tubes containing medium were incubated at 37^{o}C for overnight o check their sterility and then in refrigerator for future use.

3.2.3.3 Voges – Proskauer solution

Alpha- naphthol solution was prepared by dissolving 5 gm of Alpha- naphthol in 100 ml of 95% ethyl alcohol.

3.2.3.4 Potassium hydroxide solution

Potassium hydroxide (KOH) solution was prepared by adding 40 grams of Potassium hydroxide crystals in100 ml of cooled water.

3.2.3.5 Phosphate Buffered Saline solution

Eight grams of sodium chloride (NaCl), 2.89 grams of di-sodium hydrogen phosphate Na_2HPO_4, $12H_2O$), 0.2 gram of potassium chloride (KC1) and 0.2 gram of potassium hydrogen phosphate (KH_2PO_4) were suspended in 1000 ml of distilled for the preparation of phosphate buffered saline solution. The solution was heated to dissolve completely. Then the solution was sterilized by autoclaving at 1.2 kg / cm^2 pressure and 121°C for 15 minutes and stored for future use.

3.2.3.6 Preparation of physiological saline solution

For the preparation of this solution procedures suggested by Cowan (1985) were followed. A 0.85% PSS was prepared by dissolving 8.5 gms of chemically pure sodium chloride (NaCl) in 1000 ml of distilled water in a conical flask. The physiological saline solution was then sterilized by autoclaving at l2l^{0}C under 15 lbs, for 15 minutes. Following sterilization, the saline was cooled and then kept at 4^{0}C-8^{0}C in the refrigerator until used.

3.2.4 Preparation of culture media

All the media, broth and reagents used in this experiment were prepared according to instruction of the manufacturer.

3.2.4.1 Nutrient Agar medium

Twenty eight grams of nutrient agar powder (HI-MEDIA) was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, the medium was poured into each sterile Petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 4°C refrigerator for future use.

3.2.4.2 Mannitol Salt Agar media

111 grams manitol salt agar powder (HI-MEDIA) was suspend in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates or dipense as desired. After solidification of the medium in the petridishes, these were incubated at 37°C for overnight to check their sterility and used for cultural characterization or stored at 2-8°C refrigerator for future use.

3.2.4.3 MacConkey Agar media

The MC agar plates were prepared and stored following the procedure of Cowan, (1985). An amount of 51.5 gms of Bacto-MacConkey agar (Hi Media India) suspended in 1000 ml of distilled water were taken in a flask. The suspension was heated up to boiling to dissolve the medium completely and then sterilize by autoclaving at 121^0C under 15 Ibs pressure per square inch for 15 minutes. The media was then poured into sterile petridishes (75 mm diameter) in 20 ml quantities to form thick layer. The sterile of the media was checked by incubating at 37^0C over-night and stored at 4^0C.

3.2.4.4 Blood agar media

The blood agar plates were prepared and stored following the procedure of Cowan, (1935). 40gms of dehydrated BA Base (Hi Media, India) was suspended in 1000 ml of

distilled water and boiled until dissolved completely. It was then sterilized by autoclaving at l2l^{0}C for 15 minutes under 15 lbs pressure per square inch. After autoclaving, the medium was allowed to cool down at 45^{0}C in water both and then 57% defibrinated bovine blood was added. The medium was then poured in the sterile petridishes (75mm diameter) in a volume of 20ml quantities to form thick layer and was kept at room temperature for solidification. After solidification, the Plates were incubated at 37^{0}C in the incubator for 24 hours to check sterility of the media and were kept at 4^{0}C-8^{0}C in the refrigerator until used.

3.2.4.5 Staphylococcus Agar No.110

Suspend 149.5 grams Staphylococcus Agar No.110 in 1000 ml of distilled water. Mix thoroughly. Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Resuspend the precipitate by gentle agitation to avoid bubbles and pour the plates while the medium is hot. Alternatively, cool the medium to 45 - 50°C. This medium may also be used without sterilization; it should be boiled for 5 minutes and used at once . After solidification, the Plates were incubated at 37^{0}C in the incubator for 24 hours to check sterility of the media and were kept at 2^{0}C-8^{0}C in the refrigerator until used.

3.2.4.6 Eosin Methylene Blue agar

Thirty six grams of EMB agar base (HI-MEDIA, India) was added to 1000 ml of water in a flask and boil to dissolve the medium completely. The medium was sterilized by autoclaving at 1.2 kg/cm^2 pressure and 121°C for 15 minutes and I to 50° C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue colour). Then 10 ml of medium was poured into each sterile Petridish sized and allowed to solidify. After solidification of the medium in the petridishs, these were incubated at 37° C for overnight to check their sterility and petridishes without contamination were used for cultural characterization or stored at 4°C in refrigerator for future use.

3.2.4.7 Cetrimide Agar media

The CA agar plates were prepared and stored following the procedure of Crowan, (1985). An amount suspend 46.7 grams in 1000 ml distilled water containing 10 ml glycerol. Heat, to boiling, to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, rehydrated contents of 1 vial of

Nalidixic Selective Supplement (FD130) may be added aseptically to 1000 ml medium. Mix well and pour into sterile Petri plate. The media was then poured into sterile petridishes (75 mm diameter) in 20 ml quantities to form thick layer. The sterile of the media was checked by incubating at 37^0C over-night and stored at 2^0C -8^0C.

3.2.4.8 Mueller Hinton Agar

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. 38 grams of Mueller Hinton agar powder was suspended in 1000 ml of distilled water. It was mixed well. It was heated agitating frequently and boiled for about one minute. It was dispensed and sterilized in autoclave at 116 - 121°C (15 lbs. sp) for 15 minutes. It was cooled to 45° or 50° C (Carter, 1979).

3.2.4.9 MIU medium

18 grams of MIU agar (Difco) was suspended in 950 ml of cold distilled water taken in a conical flask and heated up to boiling to dissolve the medium completely. Ninety five ml was dispensed into flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then was Cooled to about 50-55°C and aseptically add 5ml was added of sterile 40% basal medium. After mixing were dispensed into sterile test tubes. Allow to cool in an upright position. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

3.2.5 Isolation and identification of bacteria

3.2.5.1 Culture of wound samples

Nutient agar, MacConkey agar, Mannitol Salt agar, Blood agar , Staphylococcus Agar No.110 (S-110 agar), Eosin Methylene Blue agar (EMB), Cetrimide Agar media(CA) were used.

3.2.5.2 Culture in ordinary media

Samples were inoculated separately into ordinary media like Nutrient Agar and were incubated at 37^OC for overnight.

3.2.5.3 Isolation of bacteria in pure culture

For isolation of bacteria in pure culture, The colonies on primary cultures were repeatedly sub-cultured on Nutrient Agar by streak plate method (Cheesbrough, 1984) until the pure culture with homogenous colonies were obtained.

Step-1: An inoculum was picked up with a sterile loop and spread on an area of the medium in the petridish.

Step-2: The loop was sterilized by being heated as red hot in a flame.

Step-3: The inoculum was spread over the reminder of the plate by drawing the cooled parallel line.

This method was repeated as many times as necessary to obtain a culture containing only one type of colony and usually at least two more times to ensure purity.

3.2.5.4 Morphological characterization of organisms by Gram's staining method

The grams staining was followed to study the morphological and staining characteristics of bacteria and to provide information about the presumptive bacterial identification as per recommendation of Cowan and Steel (1979).

Technique

A drop of sterile normal saline was taken on the middle of the clear slide. Then a loopful bacterial suspension (young culture) was transferred to the sterile drop of normal saline and a very thin film was prepared on the slide by spreading uniformly. The film was fixed by passing it gently over flame for two or three times.

- The slide was flooded with crystal violet solution for up to one minute. Wash off briefly with tap water (not over 5 seconds). Drained.
- The slide was flooded with Gram’s Iodine solution, and allow to act (as a mordant) for about one minute. Wash off with tap water. Drained.
- Excess water was removed from slide and blotted, so that alcohol used for decolorization was not diluted. Slide was flooded with 95% alcohol for 10 seconds and washed off with tap water. (Smears that are excessively thick may require longer decolorization. This is the most sensitive and variable step of the

procedure, and requires experience to know just how much to decolorize). Drained.

- The slide was flooded with safranin solution and allowed to counter stain for 30 seconds. Washed off with tap water. Drained and blotted with bibulous paper.
- All sides of bacteria were examined under the oil immersion lens.

3.2.5.5 Culture into differential media

3.2.5.5.1 Mac-Conkey agar

Gram negative cultures were sub-culture seperately on Mac-conkey agar media and incubated at 37°C for overnight. After that both lactose fermenter bacteria (rose pink color colony) and lactose non fermenter bacteria (pale color colony) were selected.

3.2.5.5.2 Mannitol Salt Agar media

Gram positive culture were sub-cultured on mannitol Salt Agar. Both mannitol fermenter bacteria (yellow color colony) and mannitol non- fermenter bacteria (pink color colony) were selected.

3.2.5.5.3 Blood Agar

Then colony from Mannitol Salt Agar were subcultured on Blood agar media and incubated at 37^0C for overnight.

3.2.5.6 Culture on selective media

3.2.5.6.1 Staphylococcus Agar No.110

Colonies from Mannitol Salt Agar were taken and sub-culture on S-110 agar media and incubated at 37^OC for overnight. Some S-110 agar plate characteristics by good growth and yellowish colonies.

3.2.5.6.2 Cetrimide Agar media (CA)

Lactose non fermental organism from MacConkey Agar were sub-culture on Citrimide Agar.

3.2.5.6.3 Eosin Methylene Blue (EMB) agar

Samples of positive lactose fermenter were taken and sub-culture on EMB agar media and incubated at 37°C for overnight.

Some EMB agar plate showed slightly circular colonies with dark center metallic sheen. Also in some EMB agar, the growth was indicated by smooth, characteristics mucoid and pink colored colonies which are a consequence of the organism's abundant polysaccharide capsule.

3.2.5.7 Microscopic study for identification of *Staphylococcus* spp., *Pseudomonas* spp. and *E. coli* suspected colonies by Gram's staining

Gram's staining was performed by taking colony from selected media to determine the size, shape, and arrangement of bacteria according to the methods described by Merchant and Packer (1967). Stained slides were examined under light microscope at 100 x magnification.

3.2.5.8 Identification of isolated bacteria by different Biochemical Tests

Isolated organisms with supported growth characteristics of *Staphylococcus* spp., *Pseudomonas* spp. , *E.coli.* were maintained in pure culture and subjected to biochemical tests.

3.2.5.8.1 Procedure of Catalase test

This test was performed by taking 2-3 drops of 3 per cent H_2O_2 on clean grease-free glass slide and single colony was mixed with the help of a wire loop. Immediate formation of gas bubbles was considered as positive test.

3.2.5.8.2 Procedure of Indole test

2 ml of peptone water was inoculated separately with 5 ml of culture of each of the isolated bacteria and incubated for 48 hours. 0.5 ml Kovac's reagent was added, shakes well and examined after 1 minute. A red color ring at the top of the reagent indicated production of the indole by the organisms (Cowan, 1985).

3.2.5.8.3 Procedure of MR test

The test was performed by inoculating separately a colony of the each of the isolated test organisms in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37^0C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of a bright red color. A yellow or orange color was a negative test (Cowan, 1985).

3.2.5.8.4 Procedure of VP test

2 ml of sterile glucose phosphate peptone water were inoculated separately with 5ml of each of the isolated organisms and incubated at 37°C for 48 hours. A very small amount (knife point) of creatine was added and mixed. 3 ml of 40% potassium hydroxide were added and shacked well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases. In negative cases there was no development of pink color (Cowan, 1985).

3.2.5.8.5. Procedure of Motility Indole Urease Test (MIU)

MIU media were prepared in test tubes. Then the isolated organisms were inoculated separately into the media by stabbing method with the help of sterile straight wire. Then the test tubes were incubated 37°C overnight. Single stick that is no turbidity throughout the medium indicate gram negative organism (non motile) and turbidity throughout the medium indicate gram positive case (Cowan, 1985).

3.2.5.8.6 Procedure of Triple Sugar Iron Test (TSI)

Triple sugar iron contains three sugars (Glucose, Sucrose and Lactose). At first TSI agar slant were prepared in a test tube. Then the isolated organisms were inoculated separately into the butt with a sterilized wire and on the slant with a wire loop producing zigzag streaking. The tubes were incubated for 24 hours at 37°C.Yellow color of butt and slant of the test tube indicate fermentation of Glucose, Sucrose and Lactose fermentation and butt shows blacking indicate H_2S production (Cowan, 1985).

3.2.6 Antibiotic sensitivity test

Materials:

- Test tube rack
- Bunsen burner
- Inoculating loop or needle
- Forceps
- Sterile swabs
- Mueller-Hinton or Nutrient agar plates
- Antibiotic discs
- Stock broth cultures of experimental bacteria
- 35°C to 37°C non-CO_2 incubator

Antimicrobial drug susceptibility against eight commonly used antibiotics was performed by disc diffusion or Kirby–Bauer method (Bauer *et al.,* 1966). The procedure of disc diffusion method is presented below:

i. One well isolated colony was selected from the agar plate.

ii. Colony was touched with a sterile loop and streaked onto nutrient agar and incubated overnight at 37^0

iii. 4 or 5 well isolated colonies were transferred into a tube of sterile physiological saline and vortex thoroughly.

iv. A sterile cotton swab was dipped into the bacterial suspension. The excess fluid of swab was removed by pressing firmly against the inside of the tube just above the fluid level.

v. The swab was streaked over the entire surface of Nutrient agar/Mueller-Hinton agar (Himedia, India) medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums.

vi. The antimicrobial discs were placed individually using sterile forceps and then gently press down onto the agar.

vii. The plates were inverted and incubated at 37^0C temperature for overnight .After incubation the diameter of the zone of complete inhibition (including diameter of the discs) was measured in millimeters with a ruler. The measurements were made with a ruler on the undersurface of the plate without opening the lid.

viii. The value was compared with the zone-size table. The zones of growth inhibition were provided by Kirby Bauer (2011) and Clinical and Laboratory Standards Institute (CLSI, 2007).

3.2.7 Recording and interpreting results

The zones of growth inhibition was compared with the zone-size interpretative table standard for *Staphylococcus* spp. ,*Pseudomonas* spp. and *E. coli* (Table 3, 4 and 5) provided by Clinical and Laboratory Standards Institute (CLSI, 2007) and Kirby Bauer (2011). Isolates were classified as sensitive, intermediate and resistant categories based on the standard interpretation tables updated according to the Clinical and Laboratory Standards Institution (CLSI, 2007) and Kirby Bauer (2011). Antimicrobial testing results were recorded as resistant, intermediate and sensitive according to zone diameter interpretive standards provided by CLSI, (2007).

Table 2.: Zone diameter imperative standards for *Staphylococcus* spp.

Antimicrobial agents	**Zone Diameter**		
	Resistant (mm)	**Intermediate (mm)**	**Sensitive (mm)**
Ampicillin (AMP)	≤11	12-14	≥15
Ciprofloxacin (CIP)	≤15	16-20	≥21
Vancomycin (VA)	≤09	10-11	≥12
Gentamycin (GEN)	≤12	13-14	≥15
Cefixime (CFM)	≤15	16-18	≥19
Erythromycin (E) internet	≤13	14-17	≥18
Livofloxacin (LE)	≤15	16-18	≥19

Notes: mm= Millimeter

Table 3. : Zone diameter imperative standards for *Pseudomonas* spp.

Antimicrobial agents	Zone Diameter		
	Resistant (mm)	**Intermediate (mm)**	**Sensitive (mm)**
Ampicillin (AMP)	≤11	12-13	≥14
Ciprofloxacin (CIP)	≤15	16-20	≥21
Vancomycin (VA)	≤09	10-11	≥12
Gentamycin (GEN)	≤12	13-14	≥15
Livofloxacin (LE)	≤13	14-16	≥17
Streptomycin (S)	≤14	15-20	≥21
Erythromycin (E)	≤12	13-14	≥15
Cefixime (CFM)	≤15	16-18	≥19

Notes: mm= Millimeter

Table 4.: Zone diameter imperative standards for *E. coli*

Antimicrobial agents	Zone Diameter		
	Resistant (mm)	**Intermediate (mm)**	**Sensitive (mm)**
Ampicillin (AMP)	≤13	14-16	≥17
Ciprofloxacin (CIP)	≤15	16-20	≥21
Vancomycin (VA)	≤09	10-11	≥12
Gentamycin (GEN)	≤12	13-14	≥15
Livofloxacin (LE)	≤13	14-16	≥17
Streptomycin (S)	≤15	12-14	≥11
Erythromycin (E)	≤13	14-19	≥20
Cefixime (CFM)	≤15	16-18	≥19

Notes: mm= Millimeter

CHAPTER IV

RESULT

The current study was performed as per experimental layout mentiod in page no. 18. Bacterial pathogens were isolated and identified from the 66 clinical wound samples suspected to be infected. The pathogens were confirmed by using morphology (staining), cultural and biochemical techniques and evaluate the sensitivity and resistance pattern of commonly used antibiotics against identified isolates. The results of above mentioned all experiments were presented bellow:

4.1 The age and sex distribution of patients involved in this study

Table 5.: Age distribution of the study population

Age group	N=66	%
10-20 years	12	18.2%
21-30 years	17	25.8 %
31-40 years	15	22.7%
41-50 years	13	19.7 %
>51 years	09	13.6%

Majority of the patients (25.8%) were in the age range group of 21-30 years. Those aged 10-20 year were 18.2 %, 22.7 % were in the age range group of 31-40 years, 19.7 % were in the age range group of 41-50 years, 13.6 % were in the age range group of >51 years.

A total 66 patients with wound infection were included in this study, out of which, 52 (78.8%) were male and 14 (21.2 %) were female, resulting in an overall male to female ratio of 3.7:1 . The age of the patients ranged from 10 year to 68 years. The age group of male and female with wound infections were 21- 30 and 31-40 years, respectively. The infection rate was higher in male (78.8%) than female (21.2%).

4.2 Sex and Age distribution of patents with wound infections at STVH from July 2016 to June 2017

Table-6 Samples Collected From Different Sex Groups

Sex	Number of Samples	Percent (%)
Male	52	78.8
Female	14	21.2
Total	**66**	**100.0**

Table-7 Distribution of positives cases for Wound infecions among different age groups

Age Group	Positive For Wound infections	
	Male	**Female**
10-20	10 (19.2 %)	02 (14.3 %)
21-30	12 (23.1 %)	05 (35.7%)
31-40	14 (27.0 %)	01 (07.1 %)
41-50	10 (19.2%)	03 (21.4%)
>51	06 (11.5 %)	03 (21.4 %)
Total	**52 (100%)**	**14 (100%)**
χ^2 Value	4.231	3.929
Level of Significance	0.376	0.416

4.3 Isolation and identification of bacteria from wound infections

The following bacteria *Staphylococcus* spp., *Pseudomonas* spp., and *Escherichia coli* were isolated and identified from wound infection sample by determining cultural characteristics, staining and different biochemical properties.

4.3.1 Cultural characteristics

The cultural characteristics of *Staphylococcus* spp., *Pseudomonas* spp., and *Escherichia coli* exhibited on the different media are presented in Table. and Fig.

Table 8: Cultural characteristics of the bacterial isolates of wound infections

Sl. No	Name of Bacteria	Name Of Media	Colony Characteristics	Plate No.
01	*Staphylococcus.* spp.	Nutrient Agar	Grey-white to yellowish colonies	Plate-1
		MS agar	Good growth yellowish colonies	Plate-2
		Blood agar	Beta haemolytic colonies	Plate-3
		S-110 agar	Showing yellowish colonies	Plate-4
02	*Pseudomonas* spp.	Nutrient Agar	Smooth, Raised, irregular and semi-translucent colony	Plate-5
		Cetrimide Agar	yellow-green to blue color (Pyocanin color)	Plate-6
03	*E. coli*	Nutrient Agar	White, moist, glistening growth	Plate-7
		MacConkey Agar	Rose Pink colonies	Plate-8
		EMB agar	Metallic sheen (greenish black) colony	Plate-9

Notes: Sl. No= Serial Number, EMB= Eosin Methylene Blue; MS =Mannitol Salt

In this table, it was observed that grey-white to yellowish colonies on Nutrient agar, good growth yellowish colonies on Mannital salt agara, Beta haemolytic colonies on Blood agar, yellowish colonies on Staphylococcus agar no.110 which indicate the organism might be *Staphylococcus* spp. (Plate-1,Plate-2,Plate-3, Plate-4). Smooth ,raised, irregular and semi-translucent colonies on Nutrient agar, pyocanin color on Cetrimide agar which indicatesd the organism might be *Pseudomonas* spp. (Plate-5, Plate-6). White, moist, glistening colonies on Nutrient Agar, rose pink colonies on MacConkey agar, green metallic sheen colonies on EMB agar which indicate the organism might be *E. coli* (Plate-7, Plate-8, Plate-9).

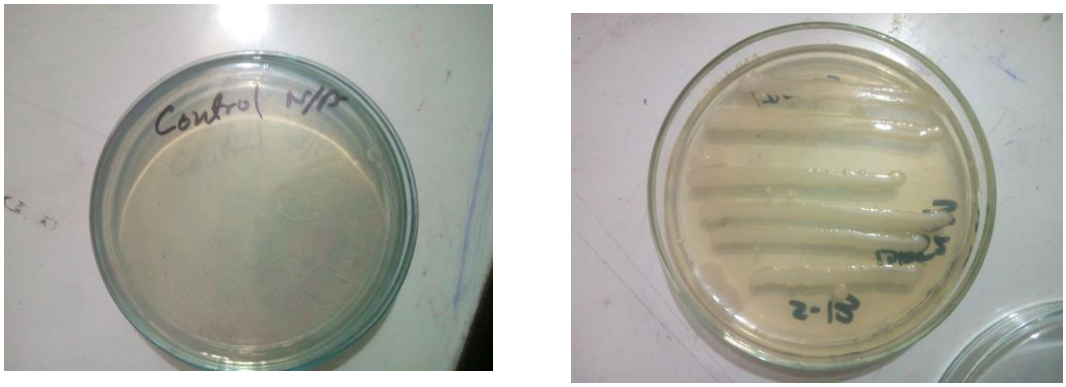

Plate 1: Yellowish colony of *Staphylococcus spp.* on Nutrient agar (right) and uninoculated control (left)

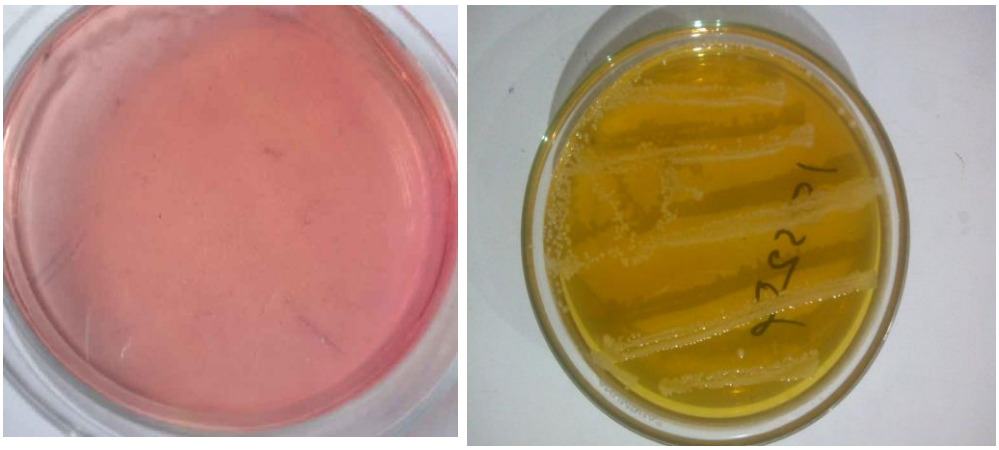

Plate 2: Whitish or yellowish colonies of *Staphylococcus* spp. on MS Agar

Plate 3: *Staphyllococcus* spp. on Blood agar and Beta-haemolysis seen with Control(left)

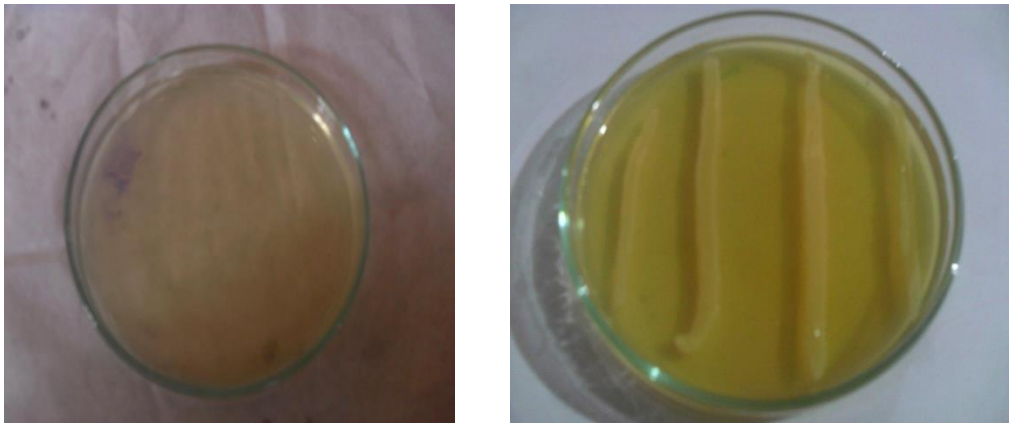

Plate 4: Culture of *Staphylococcus* spp. on *Staphylococcus* agar no. 110. (Right) showing yellowish colonies and uninoculated control (Left).

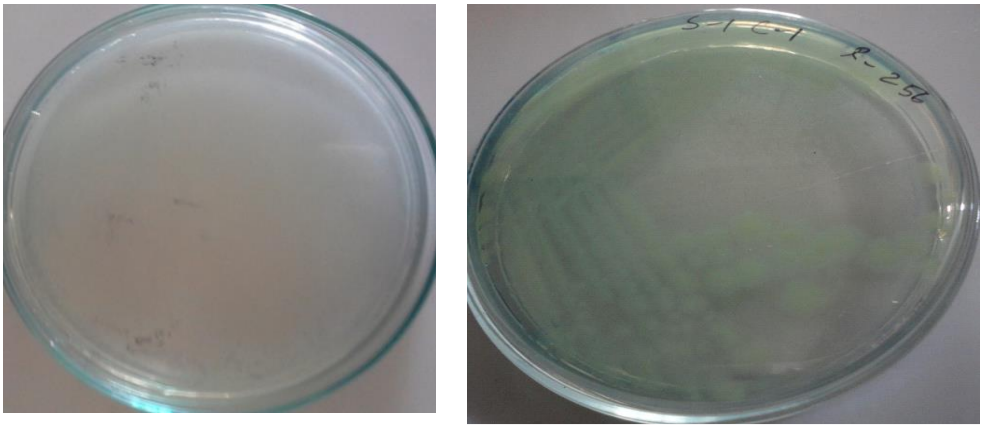

Plate 5 **:** Smooth, raised irregular and semi translucent colonies by *Pseudomonas* spp. on Nutrient agar(right) and uninoculated control (left).

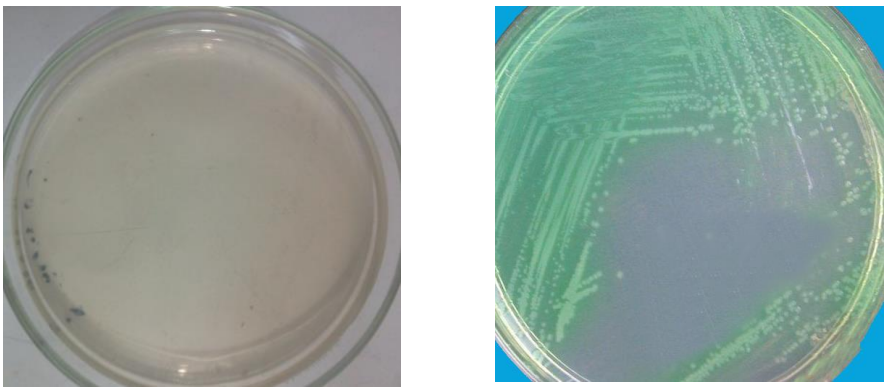

Plate 6 : Smooth, Raised, irregular and semi-translucent colony production *Pseudomonas* spp. (right) and uninoculated control (left).

Plate 7 : *Escherichia* spp. produces white, moist, glistening growth in Nutrient agar and uninoculated control (left).

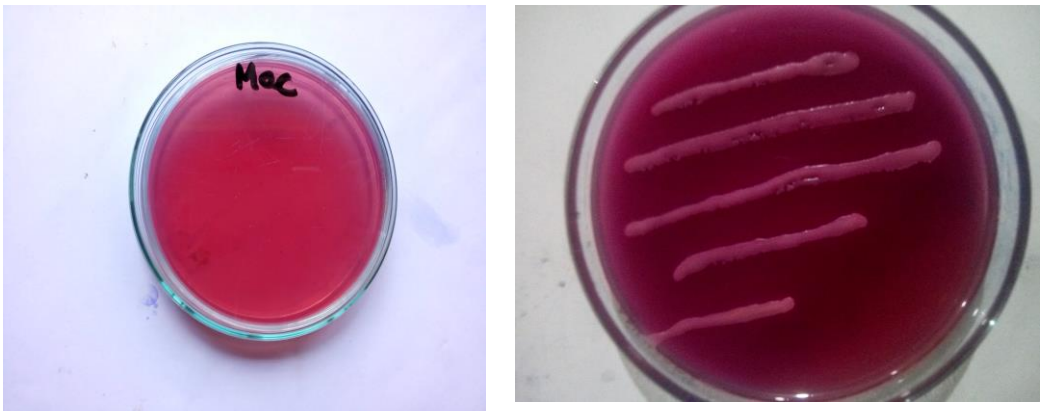

Plate 8 : *Escherichia* spp. produces dark pink color colony in Mac Conkey agar and uninoculated control (left).

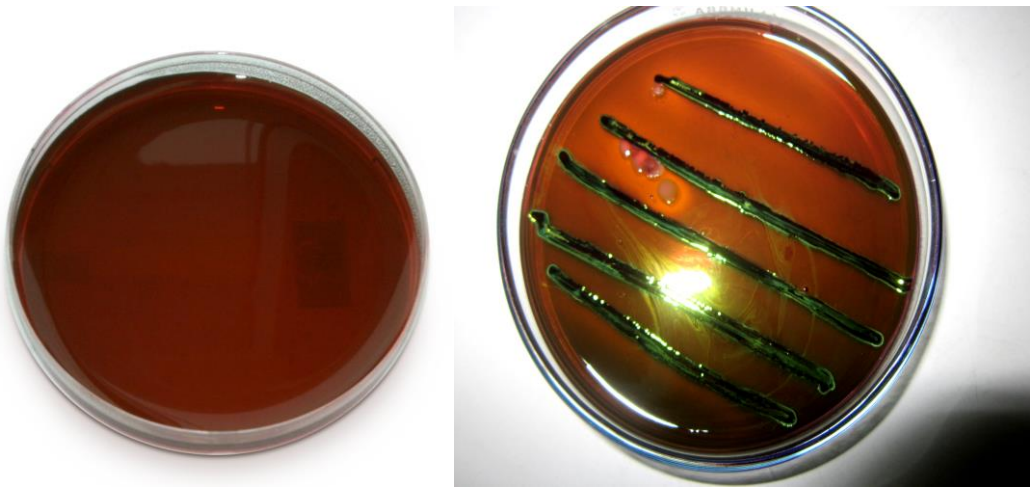

Plate 9 : Metallic sheen colonies of *Escherichia* spp. on EMB agar (right) and uninoculated control (left)

4.3.2 Morphological study of the Isolates

Table 9.: Morphological and staining properties of the bacterial isolates of wound infections by Gram's staining

Characteristics			Bacterial Isolates
Shape	**Arrangement**	**Gram's Staining**	
Cocci in Shape	Arranged in grapes like cluster	Gram positive (+) ve, Violet color	*Staphylococcus* spp.
Rod in shape	Single	Gram negative (-) ve, Pink color	*Pseudomonas* spp.
Short plump rods	Single, paired or in short chain	Gram negative (-) ve, Pink color	*E. coli*

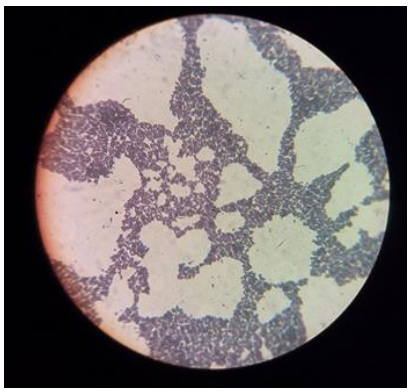

Plate 10 : Gram positive cocci shaped bacteria arranged in grapes like cluster indicate *Staphylococcus* spp. (100 X)

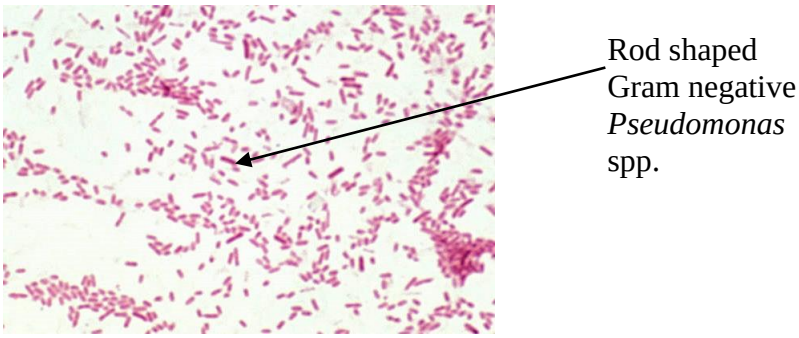

Plate 11: Gram negative single rods of *Pseudomonas* spp.

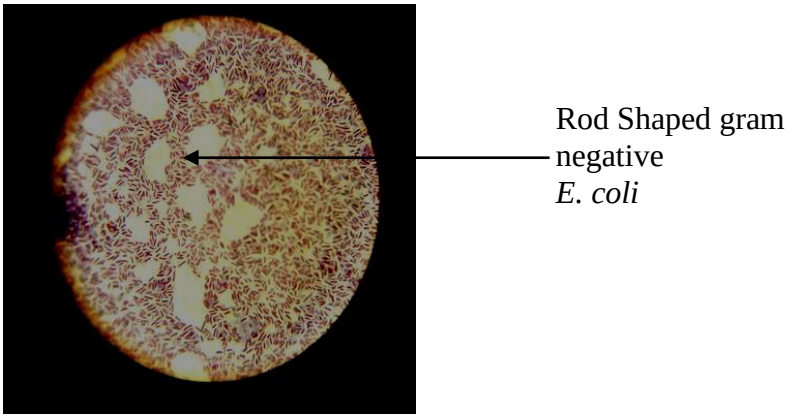

Plate 12: Gram negative single or paired short plump rods of *Escherichia coli* (Oil immersion lens)

4.3.3 Biochemical Characteristics of all of the isolates

4.3.3.1 Identification of *Staphalococcus* spp. by different biochemical tests

The organism might be *Staphylococcus* spp. that were differentiate by observed the growth and morphological characteristics, which was later confirmed by different Biochemical tests (Table-10).

Table: 10. Biochemical characteristics of *Staphalococcus* spp.

Biochemical test	Changes of the reaction	Results	Plate No.
Catalase test	Gas bubble	Positive	Plate-13
Indole test	No color change	Negative	Plate-14
MR test	Red color	Positive	Plate-15
MIU test	No color change	Negative	Plate-16
Triple sugar iron (TSI) test	S-yellow, B-yellow; S-A, B-A, gas (+), H_2S (-)	Positive	Plate-17
VP test	Red color	Positive	Plate-18

(Legends: S=Slant, B=Butt , A= Acid, MR = Methyl-Red test, MIU= Motility Indole Urease**,** VP = Voges-Proskauer test, + = Positive reaction, - = Negative reaction)

In this table it was observed that, all of the Biochemical tests were positive for *Staphylococcus* spp.

4.3.3.2 Identification of *Pseudomonas* spp. by different biochemical tests

The growth and morphology characteristics indicated that the isolated organism might be *Pseudomonas* spp (Table-11), which was later confirm by different biochemical tests.

Table: 11. Biochemical characteristics of *Pseudomonas* spp.

Biochemical test	Changes of the reaction	Results	Plate No.
Catalase test	Gas bubble	Positive	Plate-19
Indole test	No color change	Negative	Plate-20
MR test	No color change	Negative	Plate-21
MIU test	No color change	Negative	Plate-22
Triple sugar iron (TSI) test	S-Al, B-AL	Positive	Plate-23
VP test	No color change	Negative	Plate-24

(Legends: Al= Alkaline, MR = Methyl-Red test, MIU= Motility Indole Urease**,** VP = Voges-Proskauer test).

In this table it was observed that, all of the Biochemical tests were positive for *Pseudomonas* spp.

4.3.3.3 Identification of *E. coli* by different biochemical tests

The growth and morphology characteristics indicated that the isolated organism might be *E. coli* (Table-12), which was later confirm by different biochemical tests.

Table: 12. Biochemical characteristics of *E. coli*

Biochemical test	Changes in reaction	Results	Plate No.
Catalase test	Gas bubble	Positive	Plate -25
Indole test	Pink color ring at the top of the media	Positive	Plate-26
MR test	Bright red color	Positive	Plate-27
MIU test	Diffuse, hazy growth, slightly opaque media	Positive	Plate-28
Triple sugar iron (TSI) test	S-yellow, B-yellow; S-A, B-A, gas (+), H_2S (-)	Positive	Plate-29
VP test	No color change	Negative	Plate-30

(Legends: S=Slant, B=Butt, A = Acid, MR = Methyl-Red test, MIU= Motility Indole Urease**,** VP = Voges-Proskauer test, + = Positive reaction, - = Negative reaction)

In this table it was observed that, all of the Biochemical tests were positive for *E. coli*.

Plate 13 : Catalase test of *Staphylocccus* spp. showing bubble formation indicating positive reaction (right), no bubble formation indicating negative reaction (left)

Plate 14: Indole test showing no change of the medium with the reaction of the *Staphylocccus* spp. (right) and uninoculated control (left)

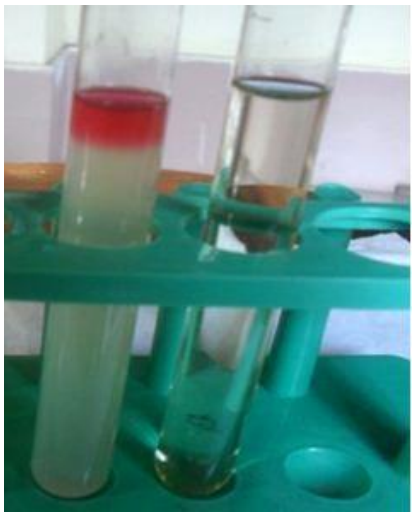

Plate 15 : Methyl-Red test for *Staphylocccus* spp. showing the medium was changed to bright red colour (left) and uninoculated control (right).

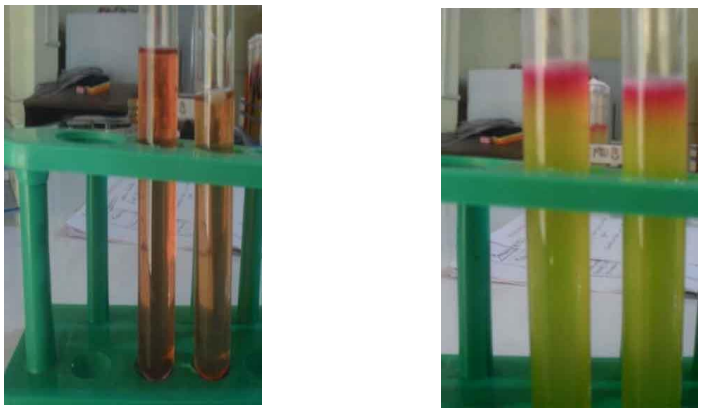

Plate 16 : MotilityIndole Urease test causing no turbidity and Urease production with indole positive by *Staphylococcus* spp. (left) and uninoculated control (right)

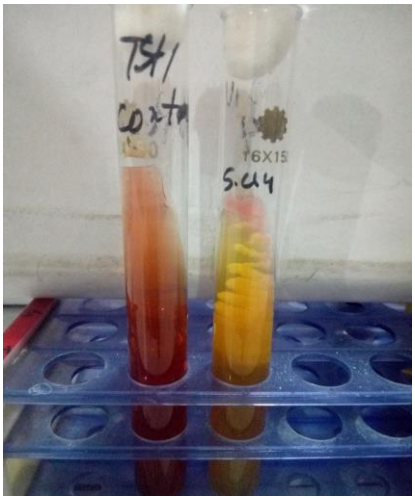

Plate 17 : Culture in Triple Sugar Iron (TSI) agar slant reaction showing yellow slant and yellow butt (right) and production of gas by *Staphylocccus* spp. and uninoculated control (left).

Plate 18: *Staphylocccus* spp. in VP test showing positive result (right) and control (Left)

Plate 19 : *Pseudomonas* spp. in showing bubble formation indicating positive reaction (right), no bubble formation indicating negative reaction (left)

Plate 20 : *Pseudomonas* spp. in indole test showing negative result with control.

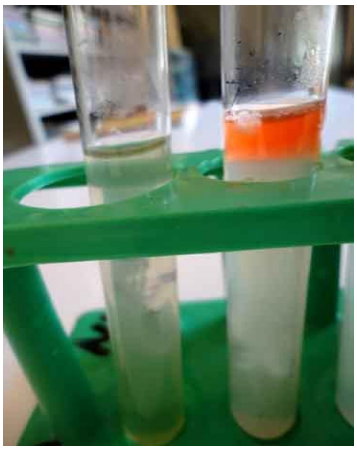

Plate 21 **:** *Pseudomonas* spp. showing MR Negative result (Right) with Control (Left).

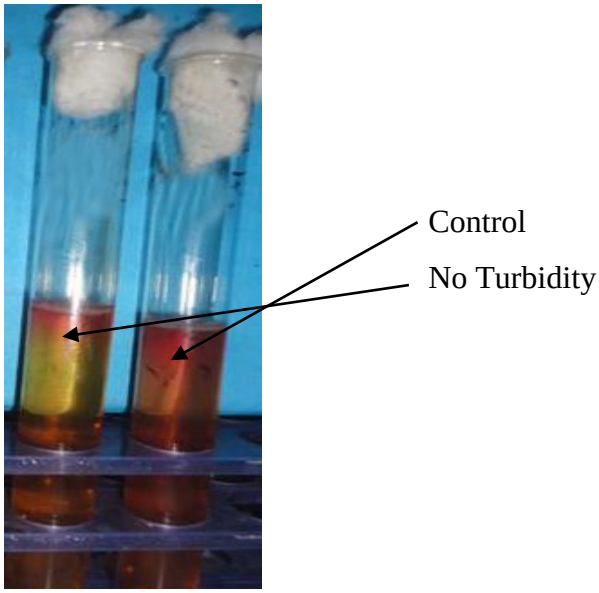

Plate 22 **:** Motility Indole Urease Test. *Pseudomonas* spp. Right one Showing Control & left showing Motility Indole Urease negative test.

Plate 23 : *Pseudomonas* spp. in Triple Sugar Iron (TSI) TSI agar showing slant and butt alkaline right with control(left).

Plate 24 : *Pseudomonas* spp. in VP test showing negative result with control.

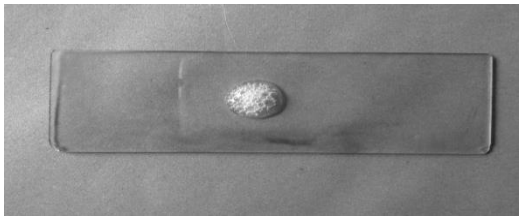

Plate 25 : *E.coli* showing posiive result in catalase test

Plate 26 : *Escherichia coli* showing Positive result in indole test with control uninoculated control (left).

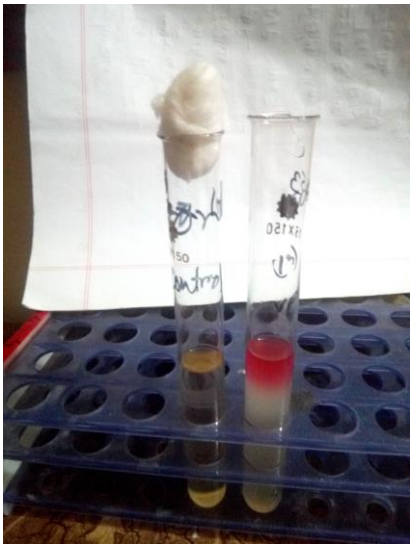

Plate 27 : *E.coli* showing Positive result (right) in MR test with control (left)

Plate 28 : Motility Indole Urease test causing turbidity Urease production with indole positive by *Escherichia coli* (right) and uninoculated control (left).

Plate 29 : Culture in Triple Sugar Iron (TSI) agar slant reaction showing slant and butt (left) and production of gas by *E. coli* with control.

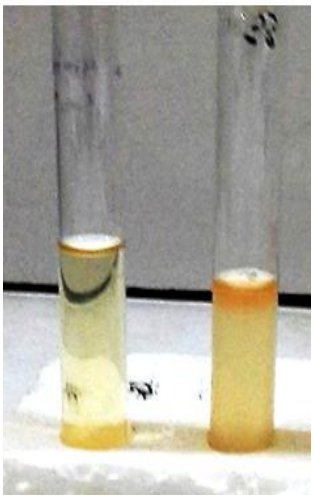

Plate 30 : VP test *E.* coli showing negative result with control (left).

4.4 Prevalence of pathogens isolated from patiens with wound infections

Out of 66 samples, positive wound swab cultures showed *Staphylococcus* spp. 42 (63.6 %) was the most frequently isolated Gram positive bacteria where as *Pseudomonas* spp. 14 (21.2%) and *Escherichia coli* 10 (15.2%) were the most frequently isolated Gram negative bacteria.

Table 13.: Percentage of pathogens isolated from patiens with wound infections at STVH from July 2016 to June 2017.

Bacteria Isolated	Number	Percentage (%)	χ^2 **Value**	**Level of Significance**
Staphylococcus spp.	42	63.6	41.455	0.00
Pseudomonas spp	14	21.2		
E.coli	10	15.2		
Total	66	100		

Table 14.: Percentage of mixed infection in infected wound of patients at STVH from July 2016 to June 2017

Pathogens	Number	Percentage	χ^2 **Value**	**Level of Significance**
Staphylococcus spp. and *Pseudomonas spp.*	3	75.0	2.00	0.157
Staphylococcus spp. and *E.coli*	1	25.0		
Total	4	100		

This table shown mixed infections in our study involved Gram positive and Gram negative bacteria with *Staphylococcus* spp. and *Pseudomonas* spp. show the most common association in 3(75.0%) cases. Infections with *Staphylococcus* spp. and *Escherichia coli* 1(25.0%) respectively were among the mixed infections isolated in this study.

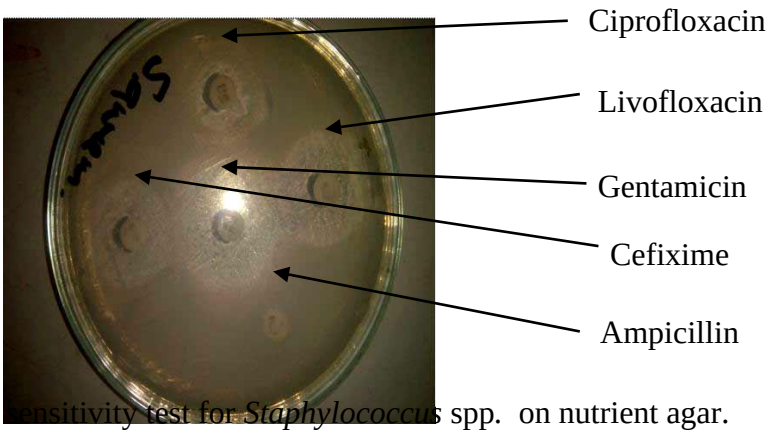

Plate 31: Antibiotic sensitivity test for *Staphylococcus* spp. on nutrient agar. (GM= Gentamicin, CFM= Cefixime, LE= Livofloxacin, CIP= Ciprofloxacin, AMP= Ampicillin

4.5 Antimicrobial susceptibility patterns of different bacterial isolates.

Table 15: Antibiotic resistance pattern (%) of *Staphylococcus* spp.

Antimicrobial agents	Total isolate (42)		Percentage of R & S	
	Resistant	Sensitive	R %	S %
Ampicillin	41	01	97.6	2.4
Ciprofloxacillin	31	11	73.8	26.2
Vancomycin	39	03	92.9	7.1
Gentamicin	05	37	11.9	88.1
Cefixime	08	34	19.0	81.0
Erythromycin	37	05	88.1	11.9
Livofloxacin	09	33	21.4	78.6

Legends: (S= sensitive, R= resistant, R%= Percentage of Resistant, S%= Percentage of Sensitive)

In this table it was observed that, *S.aureus* showed high sensitivity to most of the drugs tested gentamycin (88.1%), Cefixime (81%), Levofloxacin (78.6%).

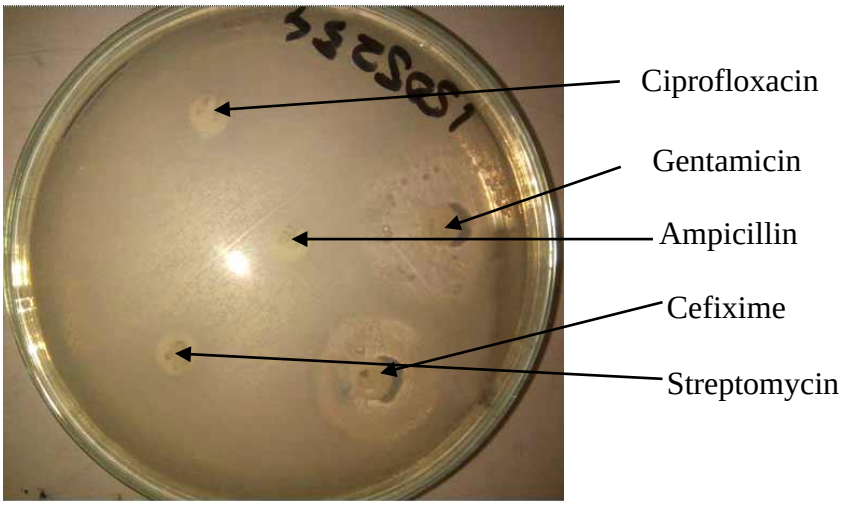

Plate 32 : Antibiotic sensitivity test for *Pseudumonas* spp. on nutrient agar.
(GM= Gentamicin, CFM= Cefixime, CIP= Ciprofloxacin, AMP= Ampicillin, S =Streptomycin.

Table 16: Antibiotic resistance pattern (%) of *Pseudomonas* spp.

Antimicrobial agents	**Total isolate (14)**		**Percentage of Res. & Sen.**	
	Resistant	**Sensitive**	**R %**	**S %**
Ampicillin	13	01	92.9	7.1
Ciprofloxacillin	10	04	71.4	28.6
Erythromycin	11	03	78.6	21.4
Vancomycin	08	06	57.1	42.9
Gentamicin	03	11	21.4	78.6
Levofloxacin	06	08	42.9	57.1
Streptomycin	07	07	50.0	50.0
Cefixime	04	10	28.6	71.4

Legends: (S= sensitive, R= resistant, R%= Percentage of Resistant, S%= Percentage of Sensitive)

In this table it was observed that, *Pseudomonas* spp showed high sensitivity to gentamycin (78.6%) ,cefixime (71.4%). High resistance was showed to ampicillin (92.9%), erythromycin (78.6%).

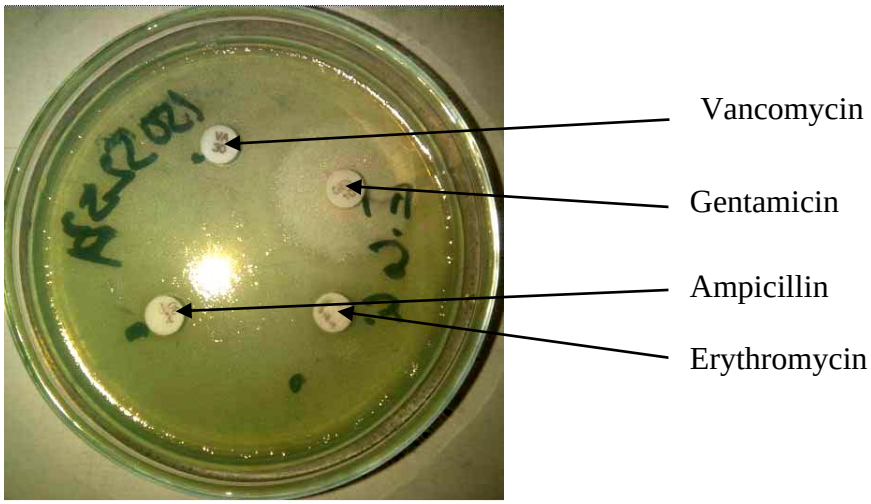

Plate 33 : Antibiotic sensitivity test for *E. Coli* on nutrient agar. (GM = Gentamicin, E= Erythromycin, AMP= Ampicillin, VA =Vancomycin.

Table 17: Antibiotic resistance pattern (%) of *E. coli.*

Antimicrobial agents	**Total isolate (10)**		**Percentage of R & S**	
	Resistant	**Sensitive**	**R %**	**S %**
Ampicillin	09	01	90	10
Ciprofloxacillin	07	03	70	30
Vancomycin	08	02	80	20
Gentamicin	01	09	10	90
Levofloxacin	05	05	50	50
Streptomycin	07	03	70	30
Erythromycin	06	04	60	40
Cefixime	04	06	40	60

Legends: (S= sensitive, R= resistant, R%= Percentage of Resistant, S%= Percentage of Sensitive)

In this table it was observed that, *E.coli* showed high sensitivity to gentamycin (90%) , cefixime (60%). High resistance was showed to ampicillin (90%), vancomycin (80%), ciprofloxacin (70%), erythromycin (60%).

CHAPTER V

DISCUSSION

The research topic was entitle as Bacterial analysis and antimicrobial drug resistance pattern of pathogens isolated from wound infection.The main objectives is covering finding out of common bacterial pathogens responsible for wound infection and to determine their antimicrobial susceptibility pattern in our community. All isolated bacteria were identified on the basis of colony characteristics, gram staining and standard biochemical tests, and antibiotic susceptibility testing (AST) with the disc diffusion method which is similar to the several author **Sushmita Roy *et al.*, 2017 and Mama M *et al.*, 2014.**

In this current study a total 66 wound samples were collected from those patient suffering with wound infection. Out of 66 samples 52(78.8%) were male and 14(21.1%) were female. The infection rate was higher in male than female. This findings supported by several authors (**Hrishikesh *et al.*, 2015, Sushmita Roy *et al.*, 2017** and **Sosina, 2014)** . This findings were disagreed by **Aynalem Mohammed *et al.*,.** The infection rate was relatively high 17(25.8%) in the age group of 21-30 years old followed by 31-40 years of age group 15(22.7%) . This findings were similar to the **Hrishikesh *et al.*, 2015,** (**Sosina, 2014, Dr. Naomi, 2014**).

This study describe the relationships between sex, age and isolated bacterial agents and also described antibiotic resistance of isolated bacteria from woud infection which was similar to the results studied by **Mama *et al.*, 2014**.

Isolation of bacteria was performed by observing the cultural charcteristics. This findings were supported by several authors (**Zorica Stojanović-Radić *et al.*, 2016), (Shahin Sultana *et al.*, 2015)** and (**A. Ananth** and **S. Rajan 21014).**

In gram staining the morphology of the *Staphylococcus* spp. exhibited Gram-positive, cocci shape, grape like cluster(violet color) which was supported by several authors **(Freeman, 1985 and I.A Marchant and Packer 1967)**.

The morphology of the *Pseudomonas* spp. exhibited Gram-negative single, paired or in short chain(pink color) which was supported **Freeman, 1985 and I.A Marchant and Packer 1967)**.

In gram staining, the morphology of the *E.coli* exhibited Gram-negative single rod(pink color) which was supported **Freeman, 1985 and I.A Marchant and Packer 1967)**.

In this present study, biochemical tests were done to confirm the identification of the organisms. This findings was supported by several authors (**Mama M *et al.*, 2014)** and (**A. Ananth** and **S. Rajan 21014).** All of the biochemical tests were positive for *Staphylococcus* spp., *Pseudomonas* spp., *E.coli.* These results were similar to the findinds of other author (**Hrishikesh Sawdekar *et al.*, 2015, Mohammad Shahid Raza *et al.*, 2013).**

In our present study, the observed predomenent organism's isolated were *Staphylococcus* spp.42(63.6%), *Pseudomonas* spp. 14(21.2%) and *E. coli* 10(15.2%), which was supported **Shahin Sultana *et al.*, 2015.**

In this present study the antibiotic sensitivity test performed by according to the procedure describe by Kirby –Bauer and Clinical and Laboratory Standards Institute (CLSI, 2007) disc diffusion methods which agreed with **Aynalem *et al.*, 2017. Shahin Sultana *et al.*, 2015.**

In our present study it was observed that *Staphylococcus* spp. were sensitive to Gentamycin 88.1%, Cefixime 81% and Levofloxacin 78.6% respectively. In this study it was also observed that isolates were resistance to Ampicillin 97.6%, Vancomycin 92.9% and Erythromycin 88.1%. This findings were similar to the findings of **Aynalem *et al.*, 2017.**

In this current study, it was observed that *Pseudomonas* spp. showed high sensitivity to Gentamycin 78.6% and Cefixime 71.4% respectively. In this study it was also observed that isolates were highly resistance to Ampicilin 92.9% and Erythromycin 78.6%. This findings were similar to the several authors **Aynalem *et al.*, 2017, Sushmita *et al.*, 2017.**

In our present study, it was observed that, *E. coli* were sensitive to Gentamycine 90%, Cefixime 60%, Levofloxacin 50% respectively and resistace to Ampicilin 90%, Vancomycin 80% and Streptomycin 70%. This findings were supported by **Sushmita *et al.*, 2017.**

CHAPTER VI

SUMMARY AND CONCLUSION

According to the study *Staphylococcus spp., Psuedomonas* spp. and *Escherichia coli* were the predominant causes of wound infections. *Staphylococcus* spp. was the most frequently isolated Gram positive bacteria where as *Pseudomonas* spp. and *E.coli* were the most frequently isolated Gram negative bacteria. Out of 66 positive samples 42 (63.6%) were Gram-positive while rest 24 (36.4%) were Gram-negative. Mixed infections was found in case of 4 (6.1%) samples. Mixed infections in our study involved Gram positive and Gram negative bacteria. *Staphylococcus* spp. and *Pseudomonas* spp. showed the most common association in 75% cases. Also *Staphylococcus* spp. and *E.coli* showed association in 25% cases.

Gentamicin, Cefixime and Levofloxacin were the most effective drugs against the tested Gram-positive, where as Erythromycin and Ampicillin were the least effective antibiotics against gram positive bacteria. On the other hand Gentamycin and Cefixime were more sensitive against Gram-negative isolates, where as Ampicillin, Vancomycin and Ciprofloxacin were the least effective antibiotics against gram negative bacteria isolates. Gentamicin and Cefixime were the most effective drugs.

REFERENCE

Adegoke, Anthony A, Tom Mvuyo, Okoh Anthony I. and Jacob Steve (2010) Studies on multiple antibiotic resistant bacteria isolated from surgical site infection. *Scientific Research and Essays*;5(24):3876-3881.

Adenike A.O. Ogunshe, Mary T. Niemogha, Gabriel N. Azum & Anthony N. Odikagbue (2012) Microbiological evaluation of antibiotic resistance in bacterial flora from skin wounds. *J Pharm Biomed Sci*.2012;22(6):1-7. 33

Aisha Mohammed, Gbonjubola O Adeshina and Yakubu K Ibrahim and Yakubu K Ibrahim ,(2013) Incidence and Antibiotic Susceptibility Pattern of Bacterial Isolates from Wound Infections in a Tertiary Hospital in Nigeria.

Amare B, Abdurrahman Z, Moges B, Ali J, Muluken L, *et. al*.,(2011) Postoperative Surgical Site Bacterial Infections and Drug Susceptibility Patterns at Gondar University Teaching Hospital Northwest Ethiopia. *J Bacteriol Parasitol*. 2(8):126.

ANDHOGA, A. G. MACHARIA, I. R. MAIKUMA *et al.,* 2002. Aerobic pathogenic bacteria in post-operative wounds at Moi Teaching and Referral Hospital. *East African med journal,* 79(12), 640-644.

ANGUZU, J.R., OLILA D, 2007. Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *African health sciences,* 7(3), 148-54.

Anil Chander, Mohammad Shahid Raza, Abirodh Ranabhat (2013) Antimicrobial Susceptibility Patterns of the Bacterial Isolates in Post-Operative Wound Infections in a Tertiary Care Hospital, Kathmandu, Nepal**.** Open Journal of Medical Microbiology, 3, 159-163.

Anusha S, Vijaya LD, Pallavi K, Manna PK, Mohanta GP, *et. al*.,(2010) An. Epidemiological Study of Surgical Wound Infections in a Surgical unit of Tertiary care Teaching Hospital. *Indian Journal of Pharmacy Practice* ;3(4):8-12.

Aynalem Mohammed, Mengistu Endris Seid, Teklay Gebrecherkos, Moges Tiruneh, and Feleke Moges ,(2017), Bacterial Isolates and Their Antimicrobial Susceptibility Patterns of Wound Infections among Inpatients and Outpatients Attending the University of Gondar Refer ral Hospital, Northwest Ethiopia, 2017.

Bauer A, Kirby W, Sherirs J, Turck M , (1996),Antibiotic susceptibility testing by standard Single disk method. *Am J Clin Pathol* ;45: 493-496.

Biadglegne F., Abera B., Alem A., Anagaw B. (2009) Bacterial isolates from wound infection and their antimicrobial susceptibility pattern in Felege Hiwot referral hospital, Northwest Ethiopia. *Ethiopian Journal of Health Sciences*; 13(3):173-177.

Bindu D, Chitralekhasaikumar, Kiran M *et al.,* 2014. Bacterial profile and antibiotic resistance pattern of aerobic gram positive bacteria isolated from pus sample. *Research journal of pharmaceutical, biological and chemical sciences,* 5(5), 643-646.

Bowler PG, Duerden BI, Amstrong DC, (2001), Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev.* ;14:244-269.

Chandan K. Sen, Gayle M. Gordillo, Sashwati Roy, Robert Kirsner, Lynn Lambert, *et. al.*,(2009), Human Skin Wounds: A Major and Snowballing Threat to Public Health and the Economy. *Wound Repair Regen*. 2009 ;17(6):763–771.

Cheesbrough M: District laboratory practice in tropical countries volume II: microbiology. Cambridge (UK): Cambridge University Press; 2006: 1–479.

Clinical and Laboratory Standards Institute (CLSI): (2010), Performance standards for antimicrobial susceptibility testing. *Twent Informational Suppl* ; 29:1–160.

Cowan, S.T. and Steel, K.T. (1985). Manual for the Identification of Medical Bacteria. 2nd edition. Cambridge University Press, London, 22-122.

Dagnachew Muluye, Yitayih Wondimeneh, Getachew Ferede *et al.,* 2014. Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/ or wound discharge at Gondar university hospital. *BMC research notes,* 7, 1-5.

Darahi KFA., Mahadi LK., Naib KTA., Jubreal J. (2008) Molecular Characterization of *Escherichia coli* O157:H7 strains using random amplified polymorphic DNA (RAPD). *Journal of Dohuk University.* 11: 189-205.

Dinda Victor, Gunturu Revathi, Kariuki SAM *et al.,* 2013. Pattern of pathogens and their sensitivity isolated from surgical site infections at the Agha khan university hospital, Nairobi, Kenya. *Ethiopia J Health sci,* 23(2), 141-149.

Dr. Naomi Kemunto Ratemo, Antimicrobial Susceptibility Pattern of Bacterial isolates from Pus samples at Kenyatta National Hospital, Kenya, 2014.

Eberhardt RT, Raffetto JD. Chronic venous insufficiency. *Circulation*.2005;111:2398–409.

Egbe CA, Omoregie R, Igbarumah IO, Onemu S. Microbiology of wound infections and its associated risk factors among patients of a Tertiary hospital in Benin City, Nigeria. *JRHS.* 2011;11(2):109-113.

Eli N. Perencevich, Kenneth E. Sands, Sara E. Cosgrove, Edward Guadagnoli, Ellen Meara, *et. al.*,Health and Economic Impact of Surgical Site Infections Diagnosed after Hospital Discharge. *Emerging Infectious Diseases*.2003;9(2):196-2003.

Ellen Korol, Karissa Johnston, Nathalie Waser, Frangiscos Sifakis, Hasan S. Jafri, Mathew Lo, and Moe H. Kyaw (2013) A Systematic Review of Risk Factors Associated with Surgical Site Infections among Surgical Patients.; 8(12): e83743.

Esebelahie, N. O. Newton-Esebelahie, F. O. Omoregie R. Aerobic bacteria isolated from infected wound. *African journal of clinical and expremental microbiology*. 2013;14(3):1595-689.

Ezebialu CU, Chukwura EI. Ezebialu IU. Bacterial pathogen associated with wound infection at national orthopaedic hospital, Enugu. *Nigerian J Microbiol.* 2010;24(1):1987-1992

Ezekiel Olugbenga Akinkunmi, Abdul-Rashid Adesunkanmi, and Adebayo Lamikanra (2014) Pattern of pathogens from surgical wound infections in a Nigerian hospital and their antimicrobial susceptibility profiles. Afr Health Sci.; 14(4): 802–809.

Freeman BA. 1985. Burrows Text Book of Microbiology, Twenty Second Edititon. W.B Saunders Company. Philadelphia , London, Toronto, Mexico City, Rio De Jenerio, Sydney, Tokeyo, pp. 464-475.

Gardner SE, Frantz R, Hillis SL, Park H, Scherubel M: Diagnostic validity of semiquantitative swab cultures. Wounds. 2007,;19:31–38.

Giacometti, O. Cirioni, A. M. schimizzi, M. S. Del Prete, F. Barchiesi *et. al.,* (2000), Epidemiology and Microbiology of Surgical Wound Infections. *Journal of clinical microbiology* ;38(2): 918–922.

Gibotti A., Tanaka TL., Oliveria VR., Taddei CR., Martinez MB. (2004) Molecular Characterization of *Escherichia coli* ipa genes by PCR-RFLP analysis. *Brazilian Journal of Microbiology,* 35: 74-80.

Girma Godebo, Gebre Kibru and Himanot Tassew. (2013), Multidrug-resistant bacteria isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. *Annals of Clinical Microbiology and Antimicrobials*.;12(17):1-7.

Gottrup F. (2004), A specialized wound healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. *Am J Surg.* ;187(5):38S–43S. 34

Guan S., Xu R., Chen S., Odumeru J., Gyles C. (2002) Development of a procedure for discriminating among *Escherichia coli* isolates from animal and human sources. *Applied and Environmental Microbiology.* 68: 2690-2698.

Harding K, Renyi R.(2009), The International Wound Infection Institute – a new global platform for the clinical management of infected wounds. *Int Wound J*.; 6(3): 175-78.

Hrishikesh Sawdekar, Radha Sawdekar, Vinod R. Wasnik, (2015), Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to antibiotic agents at super specialty hospital, Amravati city, India.

I Rich, A. & McLachlan, L. (2003), How living with a leg ulcer affects people's daily life: a nurse-led study. *Journal of Wound Care* ; 12(2):51-54.

Ibrar Khan, Naveed Sarwar, Bashir Ahmad, Sadiq Azam, Noor Rehman (2017); Identification and Antimicrobial Susceptibility Profile of Bacterial Pathogens Isolated From Wound Infections in a Teaching Hospital, Peshawar, Pakistan.

Int. J. of Pharm. & Life Sci. (IJPLS), Vol. 3, Issue 11: November: 2012, 2107-2110.

Irfan Iqbal, Aneesa Afzal, Sajad Majid Qazi (2014) Postoperative Wounds and their Antimicrobial Sensitivity Pattern (Hospital-Based Surveillance of Aerobic Bacteria), Indian Journal of Clinical Practice, Vol. 24, No. 9.

Jawad Ahmed, Amjad Ali, Masroor Hussain *et al.,* 2013. Antimicrobial susceptibility pattern of bacteria isolated from burn wounds in a tertiary care hospital in Pakistan. *African journal of microbiology research,* 7(28), 3627-3631.

JR Anguzu and D Olila ,(2007), Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *Afr Health Sci.* ;7(3): 148–154.

Khanal LK, Jha BK: (2010), Prevalence of Methicillin resistant Staphylococcus aureus (MRSA) among skin infection cases at a hospital in Chitwan, Nepal. *Nepal Med Coll J* ; 12:224–28.

Levine NS, Lindberg RB, Mason AD Jr, Pruitt BA Jr: (1976), The quantitative swab culture and smear: a quick, simple method for determining the number of viable aerobic bacteria on open wounds. *J Trauma* ; 16:89–94.

Livermore DM:(2002), Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? *Clin Infect Dis*.;34:634–64.

Mama M, Abdissa A, Sewunet T;(2014) Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University Specialized Hospital, South-West Ethiopia.

Maria Ayu, Huma Rizwan,Safina Siddique and Ushna Maryam (2015) Isolation of Pathogens Causing Sepsis, Pus and Infected Wounds from Critical Care Unit: A Retrospective Study. Ann Clin Lab Res., 3:4.

Mashita K., Shinagawa N., Sato T., Hirata K., Katsuramaki T., Mukaiya M. and Yura J. (2000). Bacteria isolated from surgical infections and their susceptibilities to antimicrobial agents. Special references to bacteria isolated between April 1997 and March 1998, *Japanese Journal of Microbiology*, 53 (80): 533-565

Md. Mehedi hasan magnet1, md. Arongozeb2, golam muktadir khan & zakaria ahmed isolation and identification of different acteria from different types of burn wound infections and study their antimicrobial sensitivity pattern, Vol. 1, Issue 3, Aug 2013, 125-132 © Impact Journals Al-hadithi AB (2007). Isolation and identification of *Pseudomonas aeruginosa* from humans and animals. MSc. Thesis, University of Baghdad.

Merchant IA and Packer RA 1967. Veterinary bacteriology and virology. 07 edition. The Iowa State University Press, Ames, Iowa, U.S.A pp. 211-305.

Mohammad Shahid Raza, Anil Chander, Abirodh Ranabhat, (2013), Antimicrobial Susceptibility Patterns of the Bacterial Isolates in Post-Operative Wound Infections in a Tertiary Care Hospital, Kathmandu, Nepal.

Mohammad Shahid Raza, Anil Chander, Abirodh Ranabhat, (2013), Antimicrobial Susceptibility Patterns of the Bacterial Isolates in Post-Operative Wound Infections in a Tertiary Care Hospital, Kathmandu, Nepal. *Open Journal of Medical Microbiology* ; 3:159-163.

Mordi R. M. and Momoh M.I. (2009), Incidence of *Proteus* species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. *Afr.J. Biotechnol* ; 8(5): 725-730.

Mulu A, Moges F, Tessema B, Kassu A: (2006), Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital. *Ethiop Med J.* , 44:125–131.

Mulu W, Kibru G, Beyne G, Damtie M:(2012), Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. *Ethiop JHealth Sci*. : 22:1–1 36

Mulugeta K. Azene and Bayeh A. Beyene , (2011), Bacteriology and antibiogram of pathogens from wound infections at Dessie Laboratory, North East Ethiopia. *Tanzania Journal of Health Research* ;13(4):1-10.

Nitin Goel Insan, Nikhil Payal, Mahesh Singh, Amod Yadav, B.L Chaudhary,(2013), Post operative wound infection: Bacteriology and antibiotic sensitivity pattern. *Int J Cur Res Rev*.;05 (13):74-79.

Ntsama Essomba C., Avomo J., Essomba R., Esiene A., Leme Banock L., *et. al.*, (2013), Isolation of bacteria in surgical site infections and determination of their sensitivity to commonly prescribed antibiotics .*Sky Journal of Microbiology Research*. ; 1(6):40 – 46.

Nwachukwu, NC, Orji *et al.*, 2009. Antibiotic susceptibility patterns of bacterial isolates from surgical wounds in Abia State university teaching hospital (ABSUTH), Aba-Nigeria. *Research journal of medicine and medical sciences,* 4(2), 575-579.

Pooja Singh.Gangania, Varsha A. Singh, Sunil Sekhar Ghimire (2015) Bacterial Isolation and Their Antibiotic Susceptibility Pattern from Post-Operative Wound Infected Patients. Indian J Microbiol Res 2(4):231-235 231.

Poonam Verma, Int. J. of Pharm. & Life Sci. (IJPLS), Vol. 3, Issue 11: November: 2012, 2107-2110 ,2107

Praveen Kumar Doddmani and Neelima. Bacteriological profile of surgical site infection. *Int J Pharm Bio Sci* .2013 ;4(3):217 – 221.

Ramesh Rao, S.Sumathi, K.Anuradha *et al.,* 2013. Bacteriology of postoperative wound infections. *Int Jpharm biomed res,*4(2), 72- 76.

Reiye Esayas Mengesha,Berhe Gebre-Slassie Kasa,Muthupandian Saravanan, Derbew Fikadu Berhe and Araya Gebreyesus Wasihun (2014) Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. BMC Research Notes 7:575.

Robert H. Demling, and Barbara Waterhouse (2007), The Increasing Problem of Wound Bacterial Burden and Infection in Acute and Chronic Soft-Tissue Wounds Caused by Methicillin-Resistant*Staphylococcus aureus. J Burns Wounds*. 2007;16:1-19.

Rubin, R.H. Surgical wound infection: epidemiology, pathogenesis, diagnosis and management. *BMC Infect. Dis*.2009;6: 171-172.

S. Joseph Christian Daniel, E. Gowthami, S. Sowmiya, 2013. Isolation and identification of bacterial pathogens from wounds of diabetic patients. *International journal of current microbiology and applied sciences*, 2(11), 72-77.

Sani R. A., Garba S. A., Oyewole O. A., Ibrahim A. (2012), Antibiotic Resistance Profile of Gram Positive Bacteria Isolated from Wound Infections in Minna, Bida, Kontagora and Suleja Area of Niger State .*Journal of Health Sciences* ; 2(3): 19-22.

Sepideh Bagheri Nejad, Benedetta Allegranzi, Shamsuzzoha B Syed, Benjamin Ellisc & Didier Pittetd. Health-care-associated infection in Africa (2011) : a systematic review. *Bull World Health Organ*.; 89:757–765.

Shahin Sultana , Nurunnahar Mawla, Shamima Kawser , Nayareen Akhtar, Md. Khoybar Ali; (2015), Current Microbial Isolates from Wound Swab and Their Susceptibility Pattern in a Private Medical College Hospital in Dhaka city. Delta Med Col J. Jan .

Shriyan A, Sheetal R, Nayak N, 2010. Aerobic micro-organisms in post-operative wound infections and their antimicrobial susceptibility patterns. *Journal of clinical and diagnostic research,* 4, 3392-3396.

Sibor JO, Oseni A, Eyaufe A, Osagie R. Turay A. Incidence of aerobic bacteria and Candida albicas in post operative wound infections. *Afr J Microbiol Res*.2008; 2:288-291.

Suchitra Joyce B. and Lakshmidevi N. Surgical site infections: (2009), Assessing risk factors, outcomes and antimicrobial sensitivity patterns. *Afr. J. Microbiol. Res*. ; 3 (4):175-179.

Sushmita Roy, Mejbah Uddin Ahmed,Bhuiyan Mohammad Mahtab Uddin,Zubair Ahmed Ratan, Monali Rajawat, Varshil Mehta, Sojib Bin Zaman; (2017) Evaluation of antibiotic susceptibility in wound infections: A pilot study from Banglade; December .

Taye M. Wound infection in Tikur Anbessa Hospital, surgical department. *EM J*. 2005; 43:167-74.

Theoklis E. Zaoutis. Antibiotic Resistance: Who Will Pay the Bills? *Clinical Infectious Diseases.* 2009; 49:1185–6.

Tigist Alebachew, Gizachew Yismaw, Ayelegn Derabe, Zufan Sisay.(2012), Staphylococcus aures burn Wound infection among patients attending yekatit

2nts hospital burn unit, Addis Ababa, Ethiopia. *Ethiop J Health Sci*. ; 22(3): 209-213.

Taiwo SS, Okesina AB, Onile BA. (2002), In-vitro antimicrobial susceptibility pattern of bacterial isolates from wound infection in University of Ilorin Teaching Hospital. Afr J Clin Experimental Microbiol ; 3(1): 6-10.

WHO- Antimicrobial resistance. *fact sheet N 0 194,* updated April 2014.

Yishak Abraham and Biruk L. Wamisho. (2009), Microbial susceptibility of bacteria isolated from open fracture wounds presenting to the err of black-lion hospital, Addis Ababa University, Ethiopia. *Afr. J. Microbiol. Res*.. ; 3(12):939-951. 35

Zorica Stojanović-Radić, Marina Dimitrijević, Nikola Stanković, Ana Aleksić, Milica Pejčić; (2016), Frequency of isolation and antibiotic resistance patterns of bacterial isolates from wound infections.

APPENDIX-I

Composition of the media used:

Nutrient Agar	**Grams/Liter**
Peptone	5.0
Bacto beef extract	3.0
NaCl	5.0
Agar	15.0
Distilled water	1000 ml
P^H	7.2

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

MacConkey Agar	**Grams/Liter**
Bacto Peptone	17.0
Proteas Peptone	3.0
Lactose	10.0
Bile Salt	1.54
Agar	15.0
Neutral red	0.03
Crystal violet	0.001
Distilled water	1000 ml
P^H	7.1

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Eosine methylene blue(EMB) agar	**Grams/Liter**
Peptone	10.0
Lactose	10.0
K_2HpO_4	2.0
Eosin	0.4
Methylene blue	0.065
Agar	20.0
Distilled water	1000ml
P^H	6.8

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Eosine methylene blue(EMB) agar	**Grams/Liter**
Proteas peptone	10.0
Beef extract	1.0
D-Mannitol	10.0
NaCl	75.0
Phenol red	0.025
Agar	20
Distilled water	1000ml

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Blood agar

Ingredients	**Grams/Liter**
Agar	15.0
Beef extract	10.0
Peptone	10.5
Sodium chloride	5.0
Final pH	7.3±0.2

Normal Saline	**Grams/Liter**
NaCl	0.85
Distilled water	1000 ml

Autoclaved at 121^0C for 15 minutes.

APPENDIX-II

Composition of the media used in biochemical test

MR-VP broth	**Grams/Liter**
Peptone	7.0
Dextrose	5.0
Dipotassium phosphate	5.0
Distilled water	1000ml
P^H	6.9

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Triple Sugar Iron (TSI) Agar	**Grams/Liter**
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Lactose	10.0
Saccharose	10.0
Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0
P^H	7.4

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Urea broth medium	**Grams/Liter**
Urea	20.0
Yeast extract	0.1
KH_2PO_4	9.0
K_2HPO_4	9.5
Phenol red	0.01
Distilled water	1000ml
P^H	6.8

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

Indole tryptopon broth medium	**Gram/Liter**
Urea broth medium	**Grams/Liter**
Tryptone	10.0
Distilled water	1000ml

Sterilized at 121^0C under 151b/in^2 pressure for 15 minutes.

APPENDIX-III

Composition of chemicals and reagents

Crystal violet

Solution-A

Crystal violet (90% dye content)	2.0 g
Ethyl alcohol (95%)	20.0 ml

Solution-B

Ammonium oxalate	0.8
Distilled water	80.0 ml

Note-Mix the solution A and B

Gram's iodine

Iodine	1.0g
Potassium iodide	2.0g
Distilled water	300.0ml
Ethyl alcohol (95%)	
Ethyl alcohol (100%)	95.0 ml
Distilled water	5.0 ml

Safranin

Safranin O	0.25ml
Ethyl alcohol (95%)	10.0ml
Distilled water	100.0ml

Kovac's reagent (for detection of indole)

P-Dimethylaminobenzaldehyde	5.0g
Amyl alcohol	75.0 ml
Hydrochloric acid (concentrated)	25.0 ml

Concentrated P-Dimethylaminobenzaldehyde was dissolved in the amyl alcohol and HCl was added slowly.

Methyl red solution

Methyl red	0.04 g
Ethanol	40.0 g
Distilled water	100 .0 ml

Methyl red dissolved in ethanol and diluted water.

Barrit's reagent

Solution-A

α- naptho	l5.0 g
Ethanol (Absolut)	95.0 g

α- naptho was dissolved in ethanol with constant stirring.

Solution-B

KOH	40.0 g
Creatine	0.3 g
Distilled water	100.0 ml

Hydrogen peroxide

3% aqueous solution of H_2O_2 was prepared from the H_2O_2 absolute solution.