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Research Article

BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERNS OF BACTERIAL ISOLATES FROM PUS/WOUND SWAB

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ABSTRACT

The existence of high drug-resistant bacteria wound infection is a serious problem, especially in surgical practice. The inappropriate use of antibiotics has resulted in the development of antibiotic resistance. The bacteriological profile may remain the same, but the antibiotic susceptibility pattern varies. The study's main objective is to identify the bacteriological profile of pus/wound swab samples and their antibiotic susceptibility pattern. This retrospective study was carried out from August 2019 to January 2020. A total of 300 clinical specimens (273 pus samples + 27 wound swab samples) were collected during the study period, in which 103 samples were collected from male patients and 197 samples were collected from female patients. The results showed that 61% were gram-positive and 37% were gram-negative. Samples collected were cultured using standard microbiological techniques and the colonies grown were identified with the help of biochemical tests. The antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion technique. In this study, the frequency of Staphylococcus aureus bacteria was higher. Klebsiella pneumoniae was the predominant Gram-negative bacteria. The penicillin group of antibiotics showed highest resistance in most of the organisms. It is essential to establish an accurate schedule for the use of antibiotics and assess the resistance pattern intermittently in each region based on the antibiotic resistance pattern. The formulation of infection, control measures and appropriate use of antibiotics must be considered compulsory to alleviate wound infection rates and to prevent the further spread of resistance.

INTRODUCTION

Skin is the largest organ in the human body which plays a crucial role in the regulation of water and electrolyte balance and thermoregulation. Skin acts as a protective barrier against invading microorganisms.[1] However, when the epithelial integrity of the skin is disrupted, a wound is formed. Because the underlying subcutaneous tissue provides an environment needed for microbial colonization and proliferation.[2] And due to the proliferation of microorganisms that enter the wound site causes localised inflammation, which leads to pus formation. Pus consists of white blood cells, damaged cells and necrotic tissue.[3] Wound infections can also turn into a life threatening one to an individual unless it is identified timely before the infection enters the bloodstream and spreads to other parts of the body creating a condition called as SEPSIS which could eventually lead to death. Even after identifying the infection, acquired multidrug resistance character of microbes challenges the healthcare providers in their treatment plan to patients. According to WHO, Antimicrobial resistance is listed as the one among top ten worldwide risks to humans. Wound infections are one of the main causes of high mortality and morbidity rate worldwide.[4] Several other factors that delay wound healing process includes age, sex, diabetes, blood pressure, stress, malnutrition, obesity, endocrine or metabolic disorders, microbial load and host defense mechanisms.[5]

Apart from trauma, other causes for wound infections include surgical site infections (SSI). SSI has become a major concern in hospitals, as it increases the length of hospital stay, cause anxiety and discomfort for patients, add to cost of healthcare services.[6] Infection at or near surgical incisions within 30 days of an operative procedure, dubbed surgical site infection, contributes substantially to surgical morbidity and mortality each year.

Surgical site infection (SSI) accounts for 15% of all nosocomial infections.[7] In the majority of SSI cases, the pathogen source is the native flora of the patient's skin, mucous membranes, or hollow viscera. Surgical instrument or theatre atmosphere will contaminate the site during operation leads to

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exogenous causes of SSI.[8] Multiple interventions have been proposed and employed over in an attempt to prevent SSI. These include wound cleaning protocol, maintenance of intraoperative normothermia, preoperative antimicrobial prophylaxis administration, preoperative glycemic control, plastic adhesive skin barriers, high flow oxygen supplementation, sterility of instruments, bowel preparation, length of the incision, and the delayed primary incision closure [9,10].

Wound infection can be due to variety of microorganisms ranging from bacteria, fungi, parasites and virus. Wound infections can either be caused by only one pathogen (mono-microbial) or else by more than one pathogen (poly-microbial). The most commonly found bacterial pathogens are Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter spp. and bacteria belonging to the family Enterobacteriaceae. The control of wound infection has become more challenging due to Anti Microbial Resistance (AMR).[11] Well-known antibiotic resistance microorganisms discovered in hospitals, so called "ESKAPE" cluster composed by Enterococcus faecium, *S. aureus, K. pneumoniae*, *A. baumannii, P. aeruginosa*, and *Enterobacter species*.[12] The most dangerous pathogens include methicillin-resistant S. aureus, vancomycin-resistant *S.aureus*, carbapenem-resistant *Acinetobacter species*, quinolones and carbapenems-resistant P. aeruginosa.[13,14] Staphylococcus aureus is currently the most common cause of SSIs causing as many as 37% of cases of SSIs in community hospitals, with MRSA of particular concern.[15] Since the emergence of methicillin-resistant Staphylococcus aureus (MRSA) in 1960, there have been reports of increasing rate of infection by MRSA and this superbug has established itself as the common cause of nosocomial as well as community acquired infections.[16]The type of organism depend on the site which is either skin incision or opening of the gastrointestinal tract.

Aminoglycosides, Cephalosporins, Tetracyclins, Penicillins, Sulfonamides, Fluoroquinolones, Macrolides, Carbapenems, Glycopeptides and Lincosamides are the major group of antibiotics widely used to treat bacterial infection. Frequent use of antibiotics leads to antimicrobial resistance (AMR). AMR threatens the prevention and treatment of infection.[17] So, appropriate drugs selected by antibiotic sensitivity testing have great importance.[18]

According to a study (2019), about 42.6 % of countries develop AMR. In that 13 gram negative and 5 Gram positive bacteria were dominant and tested sensitive against 37 different varieties of antibiotics. Further, 34% of Haemophilus influenza isolates were resistant to amoxicillin. Escherichia coli resistant to amoxicillin, trimethoprim and gentamicin was 88.1%, 80.7% and 29.8% respectively. Ciprofloxacin resistance in Salmonella Typhi was rare. Carbapenem resistance was common in Acinetobacter baumannii and Pseudomonas aeruginosa but uncommon in Enterobacteriaceae.[19]

The current study is aimed to characterize bacteria associated with wound infection. To provide additional evidence on the antibiotic sensitivity patterns of bacterial isolates against commonly used antibiotics.

MATERIALS AND METHOD

Study Design

A cross-sectional study was conducted using samples collected from various collection centres of "Regenix Super Speciality Laboratories Pvt. Ltd. (NABL accredited)" all over Chennai (Tamil Nadu, India) from August 2019 to January 2020. Then followed for advancement of clinical signs and side effects on careful site and circulation system disease until the hour of release and post release. A wide range of Diabetic, Paediatric, Gynaecology, orthopaedic patients were part of this study. These are mainly hospital attached laboratories with both in-patients and out-patients. A detailed questioner and consent were collected and filed for future reference. The samples considered for this study were pus samples and wound swabs. Exclusion criteria for the study were neonates.

HI - Media sterile cotton swabs were used and the samples were collected. The samples were processed by experienced technician and the swabs were right away dropped into a sterile cylinder and was transported to Regenix Super Specialty Research facility in a separate cooler box at 2° C - 8° C. These samples were cultured for growth and the samples with positive growth were further tested for antibiotic sensitivity.

Isolation, Identification and Antimicrobial identification Testing

Across 300 clinical specimens (273 pus samples; 27 wound swab samples), 103 samples are collected from male patients and 197 samples are collected from female patients. The samples were subjected to bacteriological culture following standard microbiological techniques.[20] Swabs were streaked as quadrant streak and incubated in inverted position in incubator for overnight at 37°C.The colonies grown were identified with the help of colony morphology and Gram's staining. [21]. Depending on the results of Gram staining further biochemical testing was carried out for confirmation of species. Further the colony was isolated and streaked by lawn streak technique.

The antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique following clinical and laboratory standards institute guidelines [22]. The antibiotic discs were placed and incubated overnight. After 24 hours the antibiotic resistance patterns were seen and measured and reported as sensitive, moderate sensitive and resistant and colony count (CFU/g of tissue) was also reported.

Antibiotics

HI - Media drug plates

Ampicillin (AP, 10µg), Amoxycillin (AMX), Penicillin G (P), Chloramphenical (C), Gentamycin (GEN), Tetracycline (TE), Netillin (NET), Levoflax (LE), cloxacillin (COX), Clindamycin (CD), Azteram (AT), Imipenem (IPM), Teicoplanin (TEI), Meropenum (MRP) Ceftriaxone (CTB), Doxycycline (DO), Norfloxacin (NX), Ciprofloxacin (CIP), Erythromycin (E) and Nitrofurantoin (F). These antimicrobial medication plates were chosen in light of Clinical and Laboratory Standards Institute (CLSI) guidelines. [23]

Quality Control

The standard reference strains, *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922) and *P. aeruginosa* (ATCC 27853) were utilized to guarantee testing execution of the power of drug discs as well as the quality of culture media

Data Analysis

The quantitative data was checked for completeness, coded and fed into SPSS version 21 and P - value <0.05 was considered statistically significant for association between variables.

Ethical Consideration

Samples are approved by Hy - care Ethical Committee - reference number 033/HYC/IEC/2019.

RESULT

Bacteriological Profile

Overall, 300 pus/wound swab samples were processed. In the present study Gram-positive Cocci (GPC) were the dominant isolates 61% compared to Gram-negative Bacilli (GNB) 37% (Fig. 2). Gram-positive isolates include Staphylococcus aureus, Coagulase-negative Staphylococci (CoNS) and Streptococcus pyrogenes. Among them highest rate of infections were caused by Staphylococcus aureus(46.7%). Followed by Klebsiella pneumoniae, which is a Gram- negative Bacilli. Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis were the other prominently found Gram-negative bacteria **(Fig. 3)**.

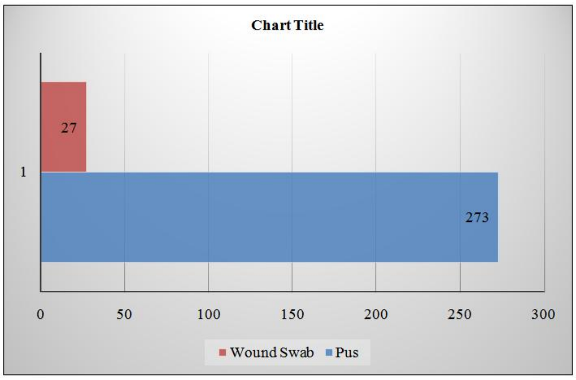

Figure 1: Pus samples (273) and Wound swab samples (27)

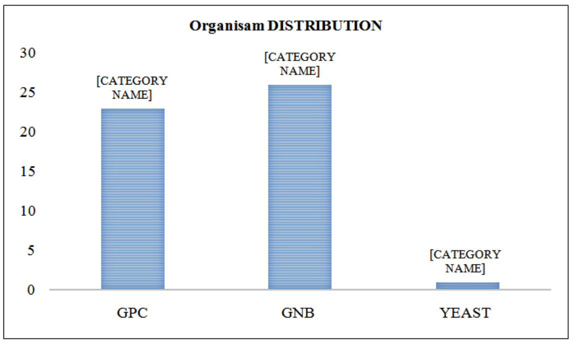

Figure 2: Distribution of Gram-negative Bacillus (37%), Gram-positive cocci (61%), and Yeast (2%) respectively

Antibiotic Susceptibility Patterns

Penicillin group shows more resistant when compared to other antibiotics including Tetracyclines, Cephalosporins, Marcolides and Aminoglycons. Penicillin shows highest resistance in almost all the bacteria increasingly in Staphylococcus aureus followed by Pseudomonas aerginosa, Klebsiella pneumoniae, E. coli, and Proteus mirabilis **(Fig. 4).** Penicillin groups such as Amoxicillin shows topmost resistant comparing to all the other antibiotics **(Fig. 5).** Ampicillin and Cephalosporins shows topmost resistant comparing to all the other antibiotics.

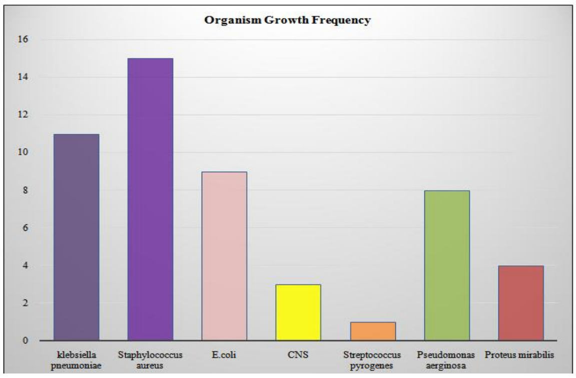

Figure 3: Staphylococcus aureus showed maximum number of growth frequency, followed by Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis

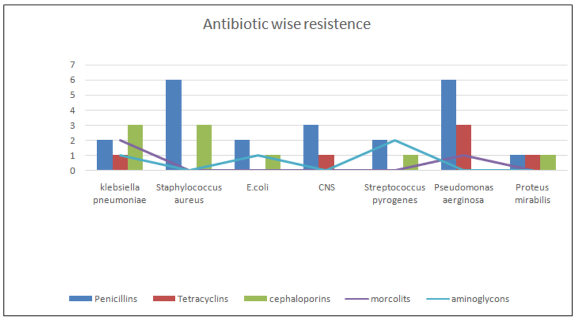

Figure 4 Major classifications of Antibiotics showing Resistance

Penicillins shows high resistance in *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*, *CoNS*, *Klebsiella pneumoniae*, *E. coli*, *Streptococcus pyrogenes* and *Proteus mirabilis*.

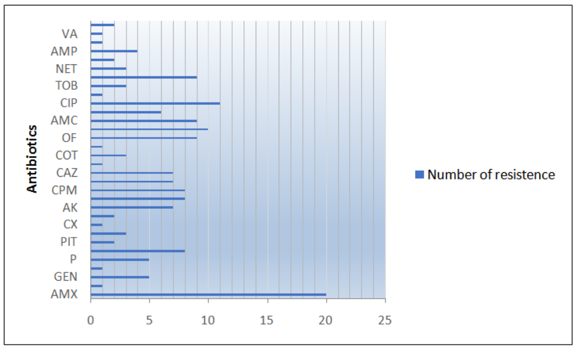

Figure 5: Antibiotic Resistance: Amoxicillin shows uppermost resistance among all the other antibiotics followed by Ampicillin and Cephalosporins.

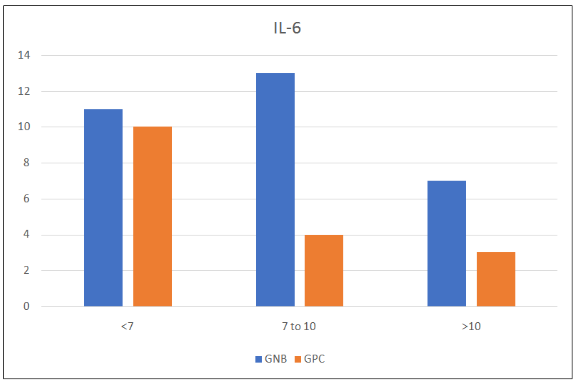

Figure 6 GNB shows an increased IL6 rate compared to GPC.

DISCUSSION

Successful management of patients with different kinds of infectious diseases mainly depends on the timely identification of pathogens and on the selection of effective antimicrobials. This study was conducted to provide additional information about the bacterial profile of pus/wound swabs and to evaluate the antimicrobial resistance pattern of bacterial isolates. In this study, Pus samples show more positive results than wound swabs. Our finding was in accordance with the findings by Dagnachew Muluye*et al*.The culture positivity of pus or wound discharge samples were 2.38 times positive for bacterial isolates when compared to wound swab samples.[24,25] The predominance of Staphylococcus aureus infection was noted in this study. These Gram-positive cocci are found in the majority of cases of wound infections. Also reported in many other similar studies as the predominant microorganism (40–60% of the total microorganisms) isolated from different types of wounds. [26,27,28].

Abebaw Bitew Kifilie*et al*. states that the predominant bacterial isolates were S. aureus (41.6%), E. coli (19.8%), K. pneumoniae (13.9%), coagulase-negative Staphylococcus (12.9%), and Enterobacter spp. (4%). Similarly, P. G. BOWLER *et al*. at Arizona shows Staphylococcus aureus has the highest number infection growth frequency. This is purely based on the climatic condition of the study population, number antibiotics exposed, wound location, patients co - morbidities and poor sanitization.[29] On contrary, Sulochana Khatiwada *et al*. shows Out of 152 pus and swab samples processed for culture, (64.5%) showed culture positivity. In total isolates (65.7%) were Gram negative bacteria and (34.3%) Gram positive bacteria.

Likewise, Gosh*et al*. and Zubair *et al*. have shown E. coli and Pseudomonas species to be the most common isolates.[30] Further, we here relatively found Klebsiella pneumonia as the second most common source of infection. Whereas E. coli was the third most commonly isolated bacteria from SSI (Surgical Site Infection). In Lorsone study in nosocomial infection after cardiac surgery in infants and children found Klebsiella spp. (22%) to be the predominant organism followed by Enterobacter spp., S. aureus and P. aeruginosa.[31]

The bacterial isolates were then examined for their susceptibility pattern towards the most commonly used group of antibiotics in therapy. Despite increasing concerns about antibiotic-resistant bacteria, appropriate use of systemic antibiotics is still recommended.[32] The majority of isolates were resistant to amoxicillin, ampicillin and tetracycline but susceptible to ceftriaxone, amikacin and chloramphenicol. Lined up with the above study, Penicillin shows highest resistance in all the bacteria increasingly in Staphylococcus aureus followed by Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli, and Proteus mirabilis. Tetracyclines and Cephaloporins are the other two antibiotics shows highest resistance in the bacterial organisms. Rao *et al*. revealed that Staphylococcus aureus was resistant to penicillin (84.62%) which was close to our findings.[33] Same has been reported by Tiwari and Kau that penicillin, amoxicillin was highly resistance against Staphylococcus aureus.[34]

High levels of IL-6 in the blood can indicate inflammation, infection, cardiovascular diseases or autoimmune disorders. This Interleukin-6 will increase once we get a bacterial infection this is high in GNB bacteria when compared with GPC. Christian Leli *et al* 2015says the the GNB and fungi have more correlation with IL6.

CONCLUSION

Study about bacteriological profile of a sample helps us to identify the specific bacterial species causing the infection, allowing doctors to prescribe the most appropriate antibiotic treatment. The antibiotic sensitivity testing of bacterial isolates determines which antibiotics are most effective against the specific bacteria causing the infection. This information can guide clinicians in choosing the most appropriate antibiotic treatment for their patients, while also helping to prevent the development and spread of antibiotic resistance.

In this study, the prevalence of antibiotic resistance was high in most common pathogenic organisms. The results of this study demonstrated that antibiotics with a high resistance pattern must be less used for the treatment of bacterial contamination. Moreover, to prevent the spread of resistance among various strains and to improve the effectiveness of antibiotics, it is suggested to establish a precise schedule for antibiotic use in each region based on their antibiotic resistance pattern.

Reference

1) Enoch, S. and Price, P. 2004. Cellular, molecular and biochemical differences in the pathophysiology of healing between acute wounds, chronic wounds and woundsin theaged.
2) M Pushpa Bacteriological Profile of Wound Infection and Antibiotic Susceptibility Pattern of the IsolatesJMicrobiol Exp20174512610.15406/jmen.2017.04.00126
3) D Bindu Chitralekhasaikumar M Kiran R Praveena V Illamani Bacterial profile and antibiotic resistance pattern of aerobic gram-positive bacteria isolated from pus sampleRes J Pharma Biol Chem Sci2014556436\
4) Hailu, D., Derbie, A., Mekonnen, D., Zenebe, Y., Adem, Y., Worku, S., &Biadglegne, F. (2016). Drugresistance patterns of bacterial isolates from infected wounds at Bahir Dar regional Health Research Laboratory center, Northwest Ethiopia. Ethiopian Journal of Health Development, 30 (3), 112 - 117.
5) SN Gomatheswari T Jeyamurugan Bacteriological Profile and the Antibiotic Susceptibility Pattern of Microorganisms Isolated from Pus/Wound Swab Isolates in Patients Attending a Tertiary Care Hospital in South IndiaInt J Curr Microbiol Appl Sci201761014051310.20546/ijcmas.2017.610.166
6) R Naz SM Hussain Q Ul Ain Bacteriological profile of surgical site infections and their antibiotic susceptibility pattern Int J Sci20195222249
7) Watanabe A, Kohnoe S, Shimabukuro R, *et al*. Risk factors associated with surgical site infection in upper and lower gastrointestinal surgery. SurgToday.2008; 38: 404–412. [Pubmed] [Google Scholar]
8) Mangram AJ, *et al*. Guideline for prevention of surgical site infection, 1999. Infect Control Hosp Epidemiol.1999; 20 (4): 247–80.
9) Horan TC, Culver DH, Gaynes RP, *et al*. Nosocomial infections in surgical patients in the United States. January 1986-June 1992. National Nosocomial Infections Surveillance (NNIS) System. Infect Control Hosp Epidemiol.1993; 14: 73993.

10) Perencevich EN, Sands KE, Cosgrove SE, *et al*. Health and economic impact of surgical site infections diagnosed after hospital discharge. Emerg Infect D is 2003; 9: 1969–196.
11) Balchandra MH, Naik SD, Verma PK. Aerobic Bacterial profile of wound infections and its sensitivity pattern at Tertiary care hospital. International Journal of Current Microbiology and Applied Sciences. 2018; 7(6): 1668-79. DOI: https://doi.org/10.20546/ijcmas.2018.706.198
12) Rice L.B. Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE. J. Infect. Dis. 2008; 197: 1079–1081. doi: 10.1086/533452. [PubMed] [CrossRef] [Google Scholar]
13) Boucher H.W., Talbot G.H., Bradley J.S., Edwards J.E., Gilbert D., Rice L.B., Scheld M., Spellberg B., Bartlett J. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. Clin. Infect. Dis. 2009; 48: 1–12. doi: 10.1086/595011. [PubMed] [CrossRef] [Google Scholar]
14) Li B., Webster T.J. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. J. Orthop. Res. 2018; 36: 22–32. doi: 10.1002/jor.23656. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
15) Anderson DJ, Sexton DJ, Kanafani ZA, Auten G, Kaye KS. Severe surgical site infection in community hospitals: Epidemiology, key procedures, and the changing prevalence of methicillin-resistant Staphylococcus aureus. Infect Control Hosp Epidemiol. 2007; 28: 1047–53. [PubMed] [Google Scholar]
16) Khanal L. K., Jha B. K. Prevalence of methicillin resistant Staphylococcus aureus (MRSA) among skin infection cases at a hospital in Chitwan, Nepal. Nepal Medical College Journal. 2010; 12(4):224–228. [PubMed] [Google Scholar]
17) Mulu, W., Kibru, G., Beyene, G., &Damtie, M. (2012). Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at FelegeHiwot Referral Hospital, Bahirdar, Ethiopia. Ethiopian journal of health sciences, 22 (1), 7 - 18.
18) Pant M, Shrestha D, Thapa S. Antibiogram of bacterial species causing skin wound infections. Novel Research in Microbiology Journal. 2018; 2(3):53-7. DOI: https://doi.org/10.21608/ NRMJ.2018.8153.
19) Antimicrobial Resistance Collaborators (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet (London, England), 399 (10325), 629–655. Https: //doi. org/10.1016/S0140 - 6736 (21) 02724 – 0
20) M. Cheesbrough, District Laboratory Practice in Tropical Countries, Part II, Cambridge University Press, New York, NY, USA, 2nd edition, 2006.
21) J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams, Bergey's Manual of Determinative Bacteriology, Williamsons and Wilkins, Baltimore, Md, USA, 1994.
22) Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing: Twenty Second Informational Supplement, CLSI Document M100-S22, CLSI, Wayne, Pa, USA, 2012.
23) CLSI. Performance standards for antimicrobial susceptibility testing. 2017.
24) Muluye, D., Wondimeneh, Y., Ferede, G., Nega, T., Adane, K., Biadgo, B., Tesfa, H., &Moges, F. (2014). Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/or wound discharge at Gondar university hospital. BMC research notes, 7, 619. https: //doi. org/10.1186/1756 - 0500 – 7 - 619.
25) Langelotz, C., Mueller - Rau, C., Terziyski, S., Rau, B., Krannich, A., Gastmeier, P., &Geffers, C. (2014). Gender - Specific Differences in Surgical Site Infections: An Analysis of 438, 050 Surgical Procedures from the German National Nosocomial Infections Surveillance System. Viszeralmedizin, 30 (2), 114–117. https : //doi. org/10.1159/000362100.
26) Bowler PG, DaviesBJ. The microbiology of infected and noninfected leg ulcers. Int J Dermatol 1999; 38: 573– 8.
27) Gjødsbøl K, Christensen JJ, Karlsmark T, Jørgensen B, Klein BM, Krogfelt KA. Multiple bacterial species reside in chronic wounds: a longitudinal study. Int Wound J 2006; 3: 225– 31
28) Körber A, Schmid EN, Buer J, Klode J, Schadendorf D, Dissemond J. Bacterial colonization of chronic leg ulcers: current results compared with data 5 years ago in a specialized dermatology department. J EurAcad Dermatol Venereol 2010; 24: 1017– 25.
29) Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. Clinical microbiology reviews, 14 (2), 244–269. https: //doi. org/10.1128/CMR.14.2.244 - 269.2001
30) Ghosh A, Karmakar PS, Pal J, Chakraborty N, Debnath NB, Mukherjee JD, *et al*. Bacterial incidence and antibiotic sensitivity pattern in moderate and severe infections in hospitalized.
31) Larson E. “Differences in Skin flora Between Inpatient and Chronically ill Outpatients”. Heart Lung 29.4 (2000): 298-305. patients. J Indian Med Assoc. 2009;107(1):21–2
32) European Wound Management Association (EWMA). Position document: management of wound infection. London: MEP Ltd, 2006.
33) Rao DR, Basu R, Biswas DB. Aerobic bacterial profiles and antimicrobial susceptibility pattern of pus isolates in a south Indian tertiary care hospital. IOSR J Dent Med Sci. 2014;13(3):59–62
34) Tiwari P, Kaur S. Profile and sensitivity pattern of bacteria isolated from various cultures in a Tertiary Care Hospital in Delhi. Indian J Public Health 2010; 54(4):213-5.
35) Christian Leli, Marta Ferranti, Amedeo Moretti, Zainab Salim Al Dhahab, Elio Cenci, Antonella Mencacci, "Procalcitonin Levels in Gram-Positive, Gram-Negative, and Fungal Bloodstream Infections", Disease Markers, vol. 2015, Article ID 701480, 8pages, 2015. https://doi.org/10.1155/2015/701480