

Retrospective Bacterial Profile in Pus Sample and their Susceptibility Pattern in a Tertiary Care Hospital in Dhaka, Bangladesh.

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ABSTRACT

Pyogenic infections can be caused by various common microorganisms that require antibiotic therapy. The inappropriate use of antibiotics has resulted in the development of antibiotic resistance. This study aimed to identify bacterial isolates with pus infection and to determine their susceptibility pattern. This retrospective study was conducted in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, between January 2023 and December 2023. 1320 pus samples were collected and all samples were cultured in blood agar and MacConkey agar media. The isolated bacteria were identified by colony morphology, gram staining, and biochemical reactions. Antibiotic susceptibility was tested using the Kirby Bauer disc diffusion method as per the National Committee for Clinical Laboratory Standards guidelines. Among culture-positive cases, the majority 232 (43.78%) were in the age group (21-40) years; male 360 (67.93%) were more commonly affected than female 170 (32.07%) patients. Out of 1320 samples, 530 (40.15%) yielded growth of organisms of which 397 (74.91%) were Gram-negative bacteria, and 133 (25.09%) were Gram-positive bacteria. *Klebsiella spp.* (31.70%) was the prevailing isolate followed by *Staphylococcus aureus* (25.09%), *Pseudomonas* spp. (20.70%), *Acinetobacter* spp*.* (10.95%), *Proteus* spp*.* (1.70%) and *Enterobacter* spp. (0.37%). Among gram-negative isolates, most *Klebsiella spp.* were resistant to amoxicillin (97.62%) followed by Cefuroxime (78.58%), cefotaxime (76.19%), cotrimoxazole (73.21%), and ciprofloxacin (71.42%). The highest sensitivity was exhibited for colistin, which demonstrated 10.72 % resistance among *Klebsiella* spp*.*, and the least resistant to meropenem (26.79%), same resistance to amikacin and gentamycin (30.96%) and piperacillin-tazobactam (40.47%); respectively. Among gram-positive isolates, *staphylococcus aureus* was susceptible to linezolid (100%), vancomycin (100%), and cloxacillin (60.15%). They are highly resistant to amoxicillin (93.99%), Erythromycin (77.44%), ciprofloxacin (73.69%), cotrimoxazole (69.93%), and gentamicin (58.65%). These results indicate that the isolation rate from the pus sample was high and the increasing trend of antibiotic resistance in both gram-positive and gram-negative bacteria is alarming, which may lead to treatment failure.

Keywords: Pus samples, Bacterial pathogens, and antimicrobial resistance.

INTRODUCTION

Pus is a collection of thick, opaque, usually yellowish-white, fluid matter that is formed as part of an inflammatory response typically associated with an infection and is composed of exudate chiefly containing

dead white cells such as neutrophils, tissue debris, and pathogenic microorganisms such as *Klebsiella* spp. (Rao et al., 2014). Both aerobic and anaerobic bacteria have been implicated in wound infections which commonly occur in hospital environments and result in significant morbidity, prolonged hospitalization, and economic burden (Dryden, 2010). Pyogenic infections are characterized by local and systemic inflammationusually with pus formation which may be either endogenous or exogenous and polymicrobial ormonomicrobial (Gowsalya, 2017). The organisms acquire multiple routes to enter the body such as breaks inthe skin or mucous membranes, traumatic wounds or bites or surgical complications with foreign bodyimplants are the various modes of entry of microorganisms. Wound infections can spread to tissues andorgans via the hematogenous route (Duggal et al., 2015) and can even lead to fatal sepsis (Rai et al., 2017).The most common organism likely to be encountered from pus are gram-positive organisms such as *Staphylococcus aureus* and gram-negative organisms are *Klebsiella* spp*.*, *Pseudomonas* spp*.*, *Acinetobacte*r spp., *E. coli*, *Enterobacter* spp*.,* and *Proteus* spp*.* respectively (Bass, 2001). The causative organisms of wound infection differ from country to country because of climate changes, hygiene of the people,awareness, etc., and even from hospital to hospital within the same region. The key factor governingmicrobial recurrence is the irregularity in antibiotic treatment that alters the emergence of multidrug-resistant pathogens that cannot be treated by common antibiotics in use (Al-Battat et al., 2022). Therefore,Knowledge of risk factors associated with infections could help to strengthen the efforts towards decliningthe complications and their recurrence. Standardization of protocol for the selection of antibiotics, dosage,and course of treatment is required to reduce morbidity and mortality resulting from pyogenic infection (Alkhafaji et al., 2020). Broad-spectrum antibiotics are typically used empirically in life-threateningcircumstances. In particular, control of wound infections has become very challenging due to widespread bacterial resistance to antibiotics such as infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA), and extended-spectrum Beta-Lactamase (ESBL) producers among gram-negative bacteria(Ebenezer et al., 2019). So, this study is carried out to identify bacterial pathogens associated with woundinfections and determine their resistance to commonly used antibiotics by culture and sensitivity testingamong the patients with wound infection isolates.

MATERIALS AND METHODS

A retrospective study was done in the Department of Microbiology at the Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January 2023 to December 2023. A total of 1320 wound swabs were collected. The skin around the wound was sterilized with 70% ethyl alcohol using a sterile cotton-wool swab to avoid touching the surrounding tissues to prevent swab contamination with endogenous skin flora. The wounds were carefully cleaned using sterile gauze moistened with sterile physiological saline. Each sample was represented using two sterile swabs from the wound ground and edge using the Levine technique. The sample was placed in an Amies transport medium, labeled, and transported to the clinical microbiology laboratory without any delay. The smear was prepared directly from the swab which was collected first and stained with a gram stain. The culture was done from the swab which was collected later in blood and MacConkey agar media. All samples were collected from outpatients and inpatients of BSMMU. Pus samples were received in non-sterile containers, and dry samples and samples from patients on antibiotics were rejected.

Microbiological methods:

Culture of pus: All samples were cultured in blood agar and MacConkey agar media, and incubated overnight at 370C for 24 hours. Organisms were identified by a standard microbiological procedure, including colony characters and gram staining In life-threatening situations are usually empirical in employing broad-spectrum antibiotics (Sader et al., 2002).

Isolation and identification of bacteria: Gram staining works by differentiating bacteria by their cell walls'

chemical and physical properties. However, not all forms of bacteria like *Mycobacterium tuberculosis* can be tested using the gram stain method and biochemical reactions (Sader et al., 2002).

Antimicrobial susceptibility test: All the isolates were tested for antimicrobial susceptibility testing using Muller Hinton agar by modified Kirby-Bauer disc diffusion methods according to the Clinical Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2012; Limbago, 2019). The following antibiotics were used for gram-negative bacteria: amoxicillin, amoxicillin-clavulanic acid, ciprofloxacin, ceftriaxone, gentamicin, cefotaxime, ceftazidime, cotrimoxazole, cefuroxime, amikacin, aztreonam, meropenem, netilmicin, tazobactam + piperacillin, cefepime and colistin. For gram-positive bacteria, the following antibiotics are used: amoxicillin, ciprofloxacin, Cotrimoxazole, cefalexin gentamicin, cloxacillin, erythromycin, cefoxitin, vancomycin, and linezolid. *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 were included as control strains.

Detection of MRSA: Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected by a cefoxitin 30 microgram disc as a surrogate marker for identifying MRSA. *Staphylococcus aureus*, which showed a zone of inhibition $\langle 21 \rangle$ mm with cefoxitin on Mueller Hinton Agar after overnight incubation at 37⁰C, was considered MRSA (Limbago, 2019).

Detection of ESBL: ESBL-producing organism detection was performed by double disc synergy test (DDST) method following the CLSI recommendation. The test suspension was prepared for each pure bacterial isolate according to 0.5 McFarland standard that was swabbed on Muller-Hinton agar. After 15 minutes the cultured plates were placed with pairs of antibiotic disks containing amoxicillin with clavulanic acid at a distance of 20 mm apart from each other. The plates were incubated for 24 hours at 370 C. The results were interpreted by measuring the diameter of the zone of inhibition. According to CLSI guidelines, an increase of >5 mm in the zone diameter around the clavulanic acid combination disks versus the same disks alone confirmed the organism as ESBL procedures.

RESULT

A total of 1320 pus samples were collected, of which 530 (40.15%) showed positivity for microbial growth (**Table -1**). Out of 530 positive-growth, Gram-negative bacteria were 397 (74.91%) and Gram-positive were 133 (25.09%). (**Table -3**)

Culture Frequency Percentage (%) Growth $\frac{1530}{40.15}$

Table 1: Frequency of Culture-positive and culture-negative pus samples (n=1320)

Fig 1: Frequency of Culture-positive and culture-negative pus samples

A greater proportion of male patients (360, 67.93%) than female patients (170, 32.07%) were afflicted among culture-positive cases; the majority, 232 (43.78%), were in the age range of 21 to 40 years (**Table -2**).

Table 2: Age and Gender distribution of culture-positive pathogens (n=530)

Fig 2: Age and Gender distribution of culture-positive pathogens

Regarding 530 isolated organisms, the most common isolate was *Klebsiella spp.* which is about 168 (31.70%) of all the bacterial isolates, followed by *Pseudomonas* spp. 110 (20.76%), *staphylococcus aureus* 133 (25.09%), *Acinetobacter* spp. 58 (10.95%) and *Escherichia coli* (9.43%) respectively. The least isolated organisms were *Proteus* spp. 9 (1.70%) and *Enterobacter* spp. 2 (0.37%); respectively (**Table-3**).

Among the isolated *Staphylococcus aureus*, 12.03% showed resistance to Cefoxitin, 69.93% were resistant to cotrimoxazole and 58.65 % to gentamicin. *S. aureus* showed 39.85% resistance to cloxacillin, followed by 30.07% resistance to clindamycin. However, 93.99% of isolates resisted amoxicillin, and 77.44% and 73.69% were resistant to erythromycin and ciprofloxacin, respectively. At the same time, the isolated *Staphylococcus aureus* were 100% sensitive to vancomycin and linezolid. MRSA was detected in 16 (12.03%) *S. aureus* isolates and was susceptible to vancomycin (100%) and linezolid (100%).

Table 4: Antibiotic resistance pattern of *Staphylococcus aureus* to different antibiotics (n=133)

The antibiotic resistance pattern of six gram-negatives isolated from the pus sample is shown in (**Table -5**). Among isolated gram-negative bacteria, *Klebsiella* spp. were highly resistant to amoxicillin (97.62%), amoxicillin-clavulanic acid (83.33%), cefotaxime (76.19 %) cefuroxime (75.58%), cotrimoxazole (73.21%) and moderately resistant same to ceftriaxone, ceftazidime (68.45%) and cefepime (46.42%) and netilmicin (41.67%); respectively. The highest sensitivity was exhibited for colistin, which had only 10.72% resistance among the isolates. However, *Klebsiella* spp. were least resistant to meropenem (26.79%), tazobactam+piperacillin (40.47%), gentamicin, and amikacin (30.96%); respectively. The most sensitive antibiotic against all other gram-negative bacteria was colistin 89-100%, against *Pseudomona*s spp., *Acinetobacter* spp*., Escherichia coli,* and *Enterobacte*r spp. *Proteus* spp. was 100% resistant to colistin because it is intrinsically resistant to colistin due to the constitutive expression of genes that lead to the modification of the LPS and an increase in its charge (Torres et al., 2021).

Resistance was higher for cephalosporins like ceftriaxone, ceftazidime, cefuroxime, and cefotaxime for all the gram-negative isolates, between (60-100)% except *Proteus* spp*.*, which showed 44.44% resistance to cefepime whereas 50% *Enterobacter* spp. were resistance to cefepime. All isolated *Escherichia coli*, *Proteus* spp., and *Enterobacter* spp*.* exhibited resistance to amoxicillin, amoxicillin-clavulanic acid, and ciprofloxacin 66-100%. In the case of *Pseudomonas* spp. and *Acinetobacter* spp*.;* they showed similar resistance to ciprofloxacin and aztreonam (68.97%) and (86.20%); respectively and also, similar resistance to amikacin (68.97%). But moderate resistant to cefepime (59.09%, 55.17%) and tazobactam-piperacillin (47.27%, 51.73%); respectively. The least resistant to meropenem was 30.91% and similar to amikacin, gentamicin (34.55%) against *Pseudomonas* spp*.*

ESBL positivity was seen 30(7.56%) in *Klebsiella* spp. and *Escherichia coli* of gram-negative isolates and most were susceptible to meropenem, amikacin, and gentamicin 26.79%, 30.96%, 30.96% respectively. Among *Klebsiella* spp., ESBL positivity was 18(4.54%) and *Escherichia coli* 12(3.02%); respectively.

Table 5: Antimicrobial resistance patterns of isolated gram-negative bacteria in pus sample (n=397)

DISCUSSION

Pyogenic infections refer to infections that cause pus formation and are characterized by several local inflammations, usually the multiplication of microorganisms (Murugesan et al., 2017). It may be either monomicrobial or polymicrobial. Gram-negative bacteria such as *Klebsiella* spp.*, Pseudomonas* spp.*, Acinetobacter* spp.*, Escherichia coli, Proteus* spp.*,* and *Enterobacter* spp*.,* and Gram-positive cocci such as *Staphylococcus aureus* are the most causative agents (Thangavel et al., 2017). Knowledge of bacterial pathogens and the choice of appropriate antibiotics are crucial in effectively treating purulent infections. With the increasing numbers of different organisms being recognized in pus samples and the finding of resistance to multiple antimicrobial agents in common isolates. Therefore, correctly identifying organisms and determining antimicrobial susceptibility patterns is crucial for appropriately managing wound infection.

In our study, out of 1320 samples from pus, 40.15% of samples showed positive growth. A study conducted by **Arundhati** and **Subha M** showed similar results of 49.02%, 53 %, and 56.6% growth from pus (Jamatia et al., 2017; Biradar et al., 2016). **Muley** and **Subha** reported a greater isolation rate of 65.6 and 56.6 percent, respectively, in contrast to this study (Mukherjee et al., 2020; Subha & Srinivasagam, 2018). This difference in bacterial isolation rate may be due to differences in the types of specimen collection procedures, specimen quality, antibiotic intake of the patients, or microbial techniques used such as automated culture and sensitivity testing.

In this study, the highest rate was observed in male 360 (67.93%) as compared to female 170 (32.07%). Similar male predominance was reported by Khanam et al., (2018) and Khan et al., (2018) 56.1% and 56.6%; respectively. The reason may be the greater participation of men in outdoor physical work for a living compared to women and the higher risk of trauma and injuries during activities. In our study, the majority (43.78%) of cases were within 21-40 years of age group. This is in agreement with another study where it was reported that people in their second to fourth decades of life are prone to wound infection (Mohammed et al., 2017a). This is the vulnerable age group; people are involved in different types of work and have a higher risk of exposure to a variety of wounds.

In the present study, of total bacterial isolates, 397(74.91%) were gram-negative and 133(25.09%) were gram-positive bacteria. In a similar study conducted by Shivra Batra et al., (2020) and Manmeet Karu et al., (2019), gram-negative bacteria were found to be predominant, which was 70.76% and 76.44% respectively (Kaur Gill & Sharma, 2019; Fatima et al., 2022). The higher rate of gram-negative isolates in our study may be attributed to the inclusion of hospitalized patients only. Data shows that gram-negative bacteria are responsible for more than 30% of hospital-acquired infections (Anton, 2011). The other cause may include regional variations in geographical location and economic status of the study population (Fisman et al., 2014).

Among the isolated bacteria, *Klebsiella* spp. was the most predominant (31.69%) among total gram-negative isolates followed by *Pseudomonas* spp. 20.76%, *Acinetobacter* spp. 10.95%, *Escherichia coli* 9.43%, *Proteus* spp. (1.70%) and *Enterobacter* spp. 0.37%. At the same time, *Staphylococcus aureus* was (25.09%) predominant among gram-positive bacteria. A similar study conducted by Sajjanar et al., (2023), *Klebsiella* spp*.* was the most predominant one (28.8%) among the total isolates of gram-negative bacteria, while *S. aureus* was the predominant (34.45%) among the isolated gram-positive bacteria. Another study conducted by Fatima et al., (2022) reported predominance of organisms was *E. coli* followed by *Klebsiella spp.* and *Pseudomonas spp.* in pus samples. In another study by Jamatia et al., (2017) in Punjab, India *S. aureus* (30.11%) was the predominant pathogen in pus samples (Jamatia et al., 2017), which differs from other studies in India reporting *E. coli* (29.23%) as the predominant bacterial isolate followed by *S. aureus* spp., *Klebsiella* spp*.,* and *Pseudomonas* spp*.* This disparity might be due to the endogenous infection source or

pus contamination from the environment or skin surface.

In our study, *Staphylococcus aureus* showed 100% sensitivity to vancomycin and linezolid followed by cloxacillin (60.15%) and gentamicin (41.35%) whereas amoxicillin, erythromycin, ciprofloxacin, and cotrimoxazole were more resistant (93.99%, 77.45%, 73.69% and 69.93%) respectively. Another two studies showed 100% sensitivity to linezolid and vancomycin followed by cloxacillin (70.11%), gentamicin (53.33%), and cotrimoxazole (53.33%) respectively whereas organisms showed maximum resistance to amoxicillin, ciprofloxacin, erythromycin (Murugesan et al., 2017; Thangavel et al., 2017; Sajjanar et al., 2023). The above two findings are nearly similar to our study findings.

In our study, 12.03% of *S. aureus* isolates were MRSA and emerged as a multidrug-resistant pathogen worldwide. Studies on MRSA have shown their wide variation. Naik and Deshpande (2011) showed 8.0 % MRSA, consistent with our study. Another study done Mohanty et al., (2004) detected 38.56% MRSA, which was higher than our study. Similarly, higher detection was also observed in other studies by Shoaib et al., (2023), Fatima et al., (2022), Kaur Gill & Sharma (2019), 45%, 76%, and 75%; respectively. This finding shows that the prevalence of MRSA is increasing. The most effective drugs for MRSA were linezolid and vancomycin, which were 100% sensitive among those isolates, and the finding was similar to the study done by Sajjanar et al., (2023).

The remarkable susceptibility of *S aureus* to vancomycin, linezolid, and gentamicin might be due to the lesser use of these antibiotics owing to their low availability, cost, and adverse effects (Sultana et al., 2015). Low activities of commonly used antibiotics such as cefradine, erythromycin, and ciprofloxacin might be due to increased consumption of these antibiotics, which leads to selection pressure, giving rise to a multiplication of resistant organisms. Increasing resistance might also result from mutation at drug target sites or the disturbance of drug accumulation in the cytoplasm due to cell wall or membrane rearrangement (Barker, 1999; Pinho et al., 2001). As a result, they have lost their efficacy in treating wound infections.

The antibiotic susceptibility pattern of gram-negative isolates revealed high resistance to selected antimicrobials. Bacterial isolates were mainly resistant to amoxicillin (96-100%) and cotrimoxazole (72- 100%). Similar results were also reported in other studies (Mohammed et al., 2017b; Fisman et al., 2014). Widespread and non-judicious use of antibiotics without sensitivity testing and self-medication, availability of antibiotics, and low cost might promote the development of resistance to these antibiotics. Similarly, resistance to third-generation cephalosporin like ceftriaxone, cefotaxime, and ceftazidime was higher (50- 83% vs 50-80% vs 75-100% respectively). These findings agreed with Sultana et al., (2015). The resistance pattern may be due to the widespread and frequent overuse of third-generation cephalosporins for an extended period in this country. Similar studies by Goswami et al., (2023) and Sultana et al., (2015) supported these findings (Goswami et al., 2023; Sultana et al., 2015). In our study among gram-negative bacteria ciprofloxacin resistance was (50-87%). However, other studies reported higher sensitivity to ciprofloxacin (81.2%), (91.8%) and (75.3%) respectively (Mohammed et al., 2017b; Goswami et al., 2023; Mama et al., 2014). This reduced sensitivity in the present study might result from extensive use of these drugs in clinical practice without susceptibility testing. The most effective antibiotics in our study were colistin, meropenem, amikacin, and gentamicin. Bacterial isolates were reasonably sensitive to these antimicrobial agents, which agrees with other studies (Mohammed et al., 2017b; Jahan et al., 2023; Sultana et al., 2015). This may be attributed to the fact that these antibiotics are less commonly prescribed for empirical treatment and are only used in hospitalized patients, according to susceptibility reports.

The lowest resistant antibiotic against *Pseudomonas spp.* was colistin, meropenem, amikacin, and gentamicin (9.09 %, 30.91 %, 34.55% 47.27%) respectively but highly resistant to ciprofloxacin was 68.18%. This study agrees with Mudassar et al., (2018). The resistance against ceftriaxone, ceftazidime, and cefepime was high in our study. The study was done by Giacometti et al., (2000) had shown variable susceptibility patterns with imipenem/meropenem, piperacillin plus tazobactam, ciprofloxacin, and

ceftazidime (100%, 87.71%, 85.71% and 71.42%) respectively for *P. aeruginosa*. *P. aeruginosa* has a high intrinsic and acquired resistance mechanism to counter most antibiotics.

In the present study, isolates of *Acinetobacter spp.* were highly resistant to commonly used antibiotics Ciprofloxacin, ceftriaxone, ceftazidime, amikacin, and gentamicin (86.20%, 82.76%, 79.31% and 68,975) respectively. Whereas meropenem and tazobactam-piperacillin were sensitive 46.56% and 51.17% respectively. Manyahi, (2012) reported that Acinetobacter *spp.* was highly resistant to ceftazidime, ciprofloxacin, and gentamicin 40% of them being resistant to carbapenems. Rasmussen et al., (1993) also reported all tested antibiotics are resistant to *Acinetobacter spp.* except for carbapenem.

Resistance to Penicillin and cephalosporins by Gram-negative bacteria is most commonly due to the production of beta-lactamase, either chromosomally encoded or more often, plasmid-mediated (Levy & Bonnie, 2004). Other important mechanisms of resistance include alteration in penicillin-binding protein (PBPs), decreased penetration of the antibiotic to the bacterial cells, or combinations of these resistance strategies (Hirukawa et al., 2018). Active efflux pumps in Gram-negative bacteria which excrete drugs including multidrug efflux pumps, can also confer resistance to beta-lactams.

Due to the retrospective nature of this study, we were unable to present detailed clinical data on a patient to identify predictors of all forms of pus sample infection and antimicrobial resistance. This calls for improvements in patient documentation and record keeping.

CONCLUSION

The present study reports the most common organism encountered in pus is *Klebsiella spp.* followed by *S. aureus*, *Pseudomonas spp.*, *Acinetobacter spp.*, *Proteus spp.,* and *Enterobacter spp*. Most of the isolates were found to be resistant to commonly used drugs. Colistin, vancomycin, Linezolid, meropenem, and aminoglycoside could be used as empirical therapy to cover these organisms. Hence continued monitoring of susceptibility patterns needs to be carried out to detect the true burden of antibiotic resistance in organisms and prevent their further emergence by judicious use of drugs.

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DATA AVAILABILITY

The data is contained within the manuscript and supplementary material.

COMPETING INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

SMA drafted the manuscript. SMA provided statistical analysis of the data.

REFERENCE

1. Al-Battat, R. A., Fadheel, B. M., & Al-Bayati, S. (2022). Rifampicin versus Doxycycline in Prevention of recurrent Boils (A Comparative Therapeutic Study). *Research Journal of Pharmacy and*

Technology, 15(7), 3041–3046.

- 2. Alkhafaji, S. L., Kashamar, A. M., & Alkhafaji, I. H. (2020). Chemical composition and antimicrobial activity of different solvent extracts of carthamus tinctorius flowers. *Research Journal of Pharmacy and Technology*, 13(12), 6055–6060.
- 3. Barker, K. F. (1999). Antibiotic resistance: A current perspective. *British Journal of Clinical Pharmacology*, 48(2), 109–124.
- 4. Bass, D. (2001). Doctors must learn to let others treat them and their families. *British Medical Journal* , 323(7303), 47.
- 5. Biradar, A., Farooqui, F., Prakash, R., Khaqri, S. Y., & Itagi, I. (2016). Aerobic bacteriological profile with antibiogram of pus isolates. *Indian Journal of Microbiology Research*, 3(3), 245.
- 6. Clinical and Laboratory Standards Institute. (2012). *Performance standards for antimicrobial disk susceptibility tests: Approved standard – Eleventh edition,* 32(1), 1-57.
- 7. Dryden, M. S. (2010). Complicated skin and soft tissue infection. *Journal of Antimicrobial Chemotherapy*, 65(SUPPL. 3), 35–44.
- 8. Duggal, S., Khatri, P. K., Parihar, R. S., & Arora, R. (2015). Antibiogram of various bacterial isolates from pus samples in a tertiary care centre in Rajasthan. *International Journal of Science and Research (IJSR)*, 4(5), 1580–1584.
- 9. Ebenezer, R., Princess, I., Vadala, R., Kumar, S., Ramakrishnan, N., & Krishnan, G. (2019). Microbiological profile of infections in a tertiary care burns unit. *Indian Journal of Critical Care Medicine*, *23*(9), 405–10.
- 10. Fatima, A., Gohar, H., Dawood, K., Siddiqui, H. Z., Sajjad, M., & Naseem, S. (2022). Bacteriological Profile and Antimicrobial Susceptibility Pattern of Pus Isolates from Tertiary Care Hospital. *Journal of the Liaquat University of Medical and Health Sciences*, 21(3), 190–195.
- 11. Fisman, D., Patrozou, E., Carmeli, Y., Perencevich, E., Tuite, A. R., Mermel, L. A., Quirós, R. E., Vilches, V., Korman, T. M., Miyakis, S., Boutlis, C. S., Reid, A. B., Gales, A. C., Schandert, L., Affini, R., Oliveira, A. M., Marra, A. R., Camargo, L. F. A., Edmond, M. B., … Russo, A. J. (2014). Geographical variability in the likelihood of bloodstream infections due to gram-negative bacteria: Correlation with proximity to the equator and health care expenditure. *PLoS ONE*, 9(12), 1–18.
- 12. Giacometti, A., Cirioni, O., Schimizzi, A. M., Prete, M. S. D. E. L., Barchiesi, F., Errico, M. M. D., Petrelli, E., & Scalise, G. (2000). *Epidemiology and Microbiology of Surgical Wound Infections*. 38(2), 918–22.
- 13. Goswami, A. G., Basu, S., Banerjee, T., & Shukla, V. K. (2023). Biofilm and wound healing: from bench to bedside. *European Journal of Medical Research*, 28(1), 1–18.
- 14. Gowsalya, S. K. . R. P. (2017). Studies on the Effect of Antibiotics on Bacteria Isolated from Diabetic Wound Infection. *International Journal of Science and Research (IJSR)*, 6(5), 2184–2186.
- 15. Hirukawa, S., Sagara, H., Kaneto, S., Kondo, T., Kiga, K., Sanada, T., Kiyono, H., & Mimuro, H. (2018). Characterization of morphological conversion of Helicobacter pylori under anaerobic conditions. *Microbiology and Immunology*, 62(4), 221–228.
- 16. Jahan, T., Yusuf, M. A., Shahid, S. Bin, Sultana, S., Mollika, F. A., & Rahman, M. M. (2023). Comparison of Bacteriological Profiles from wound Swab Isolates among Hospital Acquired Infection and Community Acquired Infection in a Tertiary Care Hospital, Bangladesh. *Bangladesh Journal of Medical Microbiology*, 16(2), 53–59.
- 17. Jamatia, A., Roy, D., Shil, R., & Prabhakar, P. K. (2017). Bacteriological profile and antimicrobial resistance patterns isolates in pus samples at Agartala Government Medical College. *Asian Journal of Pharmaceutical and Clinical Research*, 10(1), 335–337.
- 18. Kaur Gill, M., & Sharma, S. (2019). Bacteriological profile and antibiotic sensitivity patterns of aerobic pus isolates: A study conducted in tertiary care hospital of North India. *IP International Journal of Medical Microbiology and Tropical Diseases*, 5(2), 99–102.
- 19. Khan, R. A., Jawaid, M., & Khaleel, M. (2018). Bacteriological Profile and Antibiogram of Isolates from Pus Samples in a Tertiary Care Centre. *International Journal of Current Microbiology and Applied Sciences*, 7(1), 387–394.

- 20. Khanam, R. A., Islam, M. R., Sharif, A., Parveen, R., Sharmin, I., & Yusuf, M. A. (2018). Bacteriological Profiles of Pus with Antimicrobial Sensitivity Pattern at a Teaching Hospital in Dhaka City. *Bangladesh Journal of Infectious Diseases*, 5(1), 10–14.
- 21. Levy, S. B., & Bonnie, M. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. *Nature Medicine*, 10(12S), S122–S129.
- 22. Limbago, B. (2019). CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019. *Clinical Microbiology Newsletter*, 23(6), 49.
- 23. 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. *Annals of Clinical Microbiology and Antimicrobials*, 13(1), 1–10.
- 24. Manuscript, A. (2011). *NIH Public Access*. 362(19), 1804–1813.
- 25. Manyahi, J. (2012). Risk of Post-Operative surgical site contaminations from word environment in in Khartoum Bahri Teaching Hospital orthopedic words from February to May 2016. *Journal of Dental and Medical Sciences,*18(9), 25-31.
- 26. Mohammed, A., Seid, M. E., Gebrecherkos, T., Tiruneh, M., & Moges, F. (2017a). Bacterial Isolates and Their Antimicrobial Susceptibility Patterns of Wound Infections among Inpatients and Outpatients Attending the University of Gondar Referral Hospital, Northwest Ethiopia. *International Journal of Microbiology*, 2017(1), 1-10.
- 27. Mohammed, A., Seid, M. E., Gebrecherkos, T., Tiruneh, M., & Moges, F. (2017b). Bacterial Isolates and Their Antimicrobial Susceptibility Patterns of Wound Infections among Inpatients and Outpatients Attending the University of Gondar Referral Hospital, Northwest Ethiopia. *International Journal of Microbiology*, 2017(2), 1-10.
- 28. Mohanty, S., Kapil, A., Dhawan, B., & Das, B. K. (2004). Bacteriological and antimicrobial susceptibility profile of soft tissue infections from Northern India. *Indian Journal of Medical Sciences* , 58(1), 10–15.
- 29. Mudassar, S., Khan, S. W., Ali, M., & Mahmood, F. (2018). Aerobic Bacteriological Profile and Antimicrobial Susceptibility Pattern of Pus isolates in a Teaching Hospital, Lahore, Pakistan. *International Journal of Contemporary Medical Research [IJCMR]*, 5(4), 5–7.
- 30. Mukherjee, S., Mishra, S., & Tiwary, S. (2020). Microbial Profile and Antibiogram of Pus Isolate in a Tertiary Care Hospital of Western Odisha. *Journal of Evolution of Medical and Dental Sciences*, 9(16), 1325–1330.
- 31. Murugesan, K., Radha, R. S., & Vijayan, H. (2017). Study on Antibiotic Susceptibility Testing against Pyogenic Organisms from Wound Infections. *Int J Pharm Sci Rev Res*, *45*(1), 262–265.
- 32. Naik, G., & Deshpande, S. R. (2011). A study on surgical site infections caused by staphylococcus aureus with a special search for methicillin-resistant isolates. *Journal of Clinical and Diagnostic Research*, 5(3), 502–508.
- 33. Pinho, M. G., De Lencastre, H., & Tomasz, A. (2001). An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. *Proceedings of the National Academy of Sciences of the United States of America*, 98(19), 10886–10891.
- 34. Raghav Rao, D. V. M. V. S. V., Basu, R., & Biswas, D. R. (2014). Aerobic Bacterial Profile and Antimicrobial Susceptibility Pattern of Pus Isolates in a South Indian Tertiary Care Hospital. *IOSR Journal of Dental and Medical Sciences*, 13(3), 59–62.
- 35. Rai, S., Yadav, U. N., Pant, N. D., Yakha, J. K., Tripathi, P. P., Poudel, A., & Lekhak, B. (2017). Bacteriological Profile and Antimicrobial Susceptibility Patterns of Bacteria Isolated from Pus/Wound Swab Samples from Children Attending a Tertiary Care Hospital in Kathmandu, Nepal. *International Journal of Microbiology*, 2017, 1-16.
- 36. Rasmussen, B. A., Bush, K., & Tally, F. P. (1993). Antimicrobial resistance in bacteroides. *Clinical Infectious Diseases*, 16(4*)*, 390–400.
- 37. Sader, H. S., Jones, R. N., & Silva, J. B. (2002). Skin and soft tissue infections in Latin American

medical centers: Four-year assessment of the pathogen frequency and antimicrobial susceptibility patterns. *Diagnostic Microbiology and Infectious Disease*, 44(3), 281–288.

- 38. Sajjanar, V., Premalatha, D. E., Siddesh, K. C., & Prakash, N. (2023). Study of bacteriological profile and antibiotic susceptibility pattern of pus isolates in tertiary care hospital. *IP International Journal of Medical Microbiology and Tropical Diseases*, 9(4), 253–257.
- 39. Shoaib, M., Aqib, A. I., Muzammil, I., Majeed, N., Bhutta, Z. A., Kulyar, M. F. e. A., Fatima, M., Zaheer, C. N. F., Muneer, A., Murtaza, M., Kashif, M., Shafqat, F., & Pu, W. (2023). MRSA compendium of epidemiology, transmission, pathophysiology, treatment, and prevention within one health framework. *Frontiers in Microbiology*, 13(January), 1-30.
- 40. Subha, M., & Srinivasagam, M. (2018). Microbial Profile and Antimicrobial Susceptibility Pattern of Pus Culture Isolates from a Teaching Tertiary Care Hospital, South India. *International Journal of Current Microbiology and Applied Sciences*, 7(04), 1149–1153.
- 41. Sultana, S., Mawla, N., Kawser, S., Akhtar, N., & Ali, M. K. (2015). Current Microbial Isolates from Wound Swab and Their Susceptibility Pattern in a Private Medical College Hospital in Dhaka city. *Delta Medical College Journal*, 3(1), 25–30.
- 42. Thangavel, S., Maniyan*, G., S., V., & C., V. (2017). A study on aerobic bacteriological profile and antimicrobial susceptibility pattern of isolates from pus samples in a tertiary care hospital. *International Journal of Bioassays*, 6(03), 5317.
- 43. Torres, D. A., Seth-Smith, H. M. B., Joosse, N., Lang, C., Dubuis, O., Nüesch-Inderbinen, M., Hinic, V., & Egli, A. (2021). Colistin resistance in Gram-negative bacteria analysed by five phenotypic assays and inference of the underlying genomic mechanisms. *BMC Microbiology*, 21(1), 1–12.