Prevalence and antibiogram of aerobic bacterial isolates from pus samples in a tertiary care hospital of north Kerala, India

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Abstract

Assessment of pathogens diversity and evolving drug-resistant pattern is quite essential in the systematic management of infections. To regulate the pyogenic infection, 1350 (783 males and 567 females) pus samples collected from individuals attending a tertiary care hospital in Northern Kerala. Pathogens isolated from the collected pus samples were identified based on the colony morphology, microscopic examination, and biochemical characteristics. About 84.44% of samples showed significant bacteria. The causative organisms were Staphylococcus aureus (28%), Escherichia coli (13%), Pseudomonas aeruginosa (12%), Klebsiella pneumonia (10%), coagulase negative Staphylococcus sp. (8%), Proteus mirabilis (6%), Streptococcus sp. (2%), Enterococcus faecalis (2%), Acinetobacter baumanii (1%), Citrobacter koseri (2%), Enterococcus faecium (2%), Morganella morganii (1%), Proteus vulgaris (2%), and other less prominent bacteria (3%). The drug-resistant pattern of pathogens analyses against 29 contemporary antibiotics. Pathogenic Gram-negative bacteria (GNB) were sensitive to amikacin > imipenem > meropenem > tazobactum > gentamycin > chloramphenicol > ciprofloxacin > levofloxacin and resistant to clindamycin, erythromycin, linezolid, oxacillin, penicillin, and vancomycin. Gram-Positive Bacteria (GPB) were susceptible to linezolid > vancomycin > tetracycline > clindamycin > chloramphenicol > gentamycin > ciprofloxacin, and resistant to amikacin, imipenem, meropenem, and tazobactum. Overall, the study concludes that MDR S. aureus was the predominant cause of pyogenic infections, drug resistance pattern of the pathogens in the selected region and raises concerns for the need to analyze signaling mechanism that transforms a susceptible strain into a resistant to develop a suitable treatment strategy.

Keywords: bacterial pathogen; drug resistance; pyogenic infections; Staphylococcus aureus; surgical site infection

Introduction

The normal intact skin protects the body against invasive microorganisms by serving as mechanical barrier and retaining microbicidal activity (Perciva et al., 2012). It prevents underlying tissues from becoming colonized and invaded by the potential pathogens. Any injury that causes physical disruption of the skin integrity leads to exposure of subcutaneous tissue, resulting in an infection of the wound by external pathogens.
Exposed inflamed tissues in the wound provide a moist environment, and release dead cells (nutritious environment) that are conducive for microbial colonization and proliferation (Oladeinde et al., 2013). Pathogens release cytolytic metabolites like leukocidins that exterminates the macrophages and neutrophils resulting in abscess, suppuration, and discharge of pus. The progression of a wound to a systemic and highly infected state involves a multitude of factors of the host conditions including old age, repeated trauma, blood perfusion, immune suppression, balance between normal flora and exogenous flora, level of antimicrobial peptides from normal skin flora, and co-existing morbidity which impair wound healing and increases the risk of infection (Aitcheson et al., 2021; Crane et al., 2018). Apart from the type, site, size, and depth of the wound and the virulence of the microorganisms facilitate further complications in wound infection (Rodrigues et al., 2019). The order of immune response against pyogenic infections in host are; immune suppressed patients under cancer treatment or undergoing transplantation > diabetic patients (foot ulcers (84%) - poor macrophage phagocytosis) > microbial infections (mycobacteria/ fungi/ bacteria) > mechanism of immune response (Dropulic et al., 2016; Mak et al., 2006; Maoura et al., 2019). For analyzing the key bacterial pathogens, samples from drainage or deep incisions are the suitable samples, and for scheduled retrospective treatment (Hakkarainen et al., 2014).

The etiology of wound infection differs from country to country and from hospital to hospital even within the same region. The key factor governing microbial recurrence is the irregularity in antibiotic treatment that accelerates the emergence of multidrug resistant pathogens which cannot be treated by common antibiotics in use (Rzan Al Battat et al., 2022). Therefore, knowledge of risk factors associated with infections could help to strengthen the efforts towards declining the complications and their recurrence. Standardization of protocol for selection of antibiotic, dosage, and course of treatment are required to reduce morbidity and mortality resulting from pyogenic infections (Alkhafaji Sura et al., 2020).

Even though treatments, especially in life-threatening situations are usually empiric in employing broad-spectrum antibiotics. Especially, 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), Extended Spectrum Beta-Lactamase (ESBL) producers, carbapenem resistant Enterobacterales/pseudomonads, and AmpC Beta lactamase producers among Gram negative bacteria (Gupta et al., 2018). Thus, the current work is important to generate findings that would guide the formulation of policies on infection control, empirical antibiotic treatment and control of antibiotic to use.

### Materials and Methods

Ethical approval for the present study was issued as per the standard protocols of the committee for the purpose of control and supervision of experiments (CPCSC) on animals and humans, strictly maintained and followed by the Institutional Ethics Committee of MES Medical College Hospital, Kerala (IEC/MES/10/2022; Dated: 20/07/2022). According to the CPCSE guidelines, pus samples were collected from the MES Medical College Hospital, Kerala during January 2021 and December 2021.

#### Sample collection

All pus samples from patients attending the outpatient department or admitted in wards with infected surgical wounds which were collected following standard procedures and received to the Microbiology lab were used for the study. During the period of study, around 1350 pus samples from wounded sites of both diabetic and non-diabetic patients with complications like venous ulcers, decubitus ulcers, superficial abscesses, and traumatic injuries were collected and included in the study (Figure 1). The patients were belonging to both genders and age group of 01 to 90 years. The criteria used for the sample collection include;
i) The inclusion criteria: The pus sample (pus aspirate and wound swab) from patients attending the outpatient department (OPD) or patients admitted in wards (in patients) with wound infections was collected and stored in the Microbiology clinical laboratory of MES Medical College Hospital, Kerala, India, between January 2021 and December 2021.

ii) Exclusion criteria: Samples collected without following standard guidelines, pus samples collected from patients with more than one complication prescribed by the Association of Indian Medical Society, 2019.

![Figure 1](image.png)

**Figure 1.** Types of samples Collected for the study; a. Pus sample collected from diabetic foot ulcer; b. Pus sample collected from bed sore; c. Pus sample collected from acne

**Analysis of sample**

1) Collected samples were streaked on selective media such as 5% sheep blood agar, Mannitol Salt Agar, and MacConkey agar and incubated at 37 °C for 24h.
2) Direct microscopic examination of Gram’s-stained smears of isolates.
3) Additional test included were Coagulase test, Sorbitol fermentation, Arabinose fermentation test, other sugar fermentation test, species specific identification tests, Optochin and Bacitracin sensitivity test, and specific biochemical tests to identify Enterobacteriaceae members.

After 24 hours of incubation, the plates were examined for the growth of the bacteria. Morphological characterization accomplished based on the appearance of the colony on agar plates like colour differences, β or α hemolysis etc. Bacterial characterization of biochemical reactions includes catalase test, oxidase test, indole production test, methyl red test, Voge-Proskauer test, citrate utilization test, urease test, coagulase test, nitrate reduction test, H$_2$S production test, Bile Esculin test, and the triple sugar iron agar test.

**Antibiotic sensitivity testing**

Antimicrobial susceptibility of the isolates was assessed on Muller Hinton Agar plates using Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards institute (CLSI) guidelines. The list of antibiotics tested include amikacin (Amk-30 µg), ampicillin (Amp-10 µg), bacitracin (Bac-10 µg), cefpime (Cef-30 µg), ceftazidime (Cez-30 µg), cefoxitin (Cex-30 µg), cepotaxim (Cet-30 µg), ceftriaxone (Ceo-30 µg), cefuroxime (Ceu-30 µg), chloramphenicol (Chl-30 µg), ciprofloxacin (Cip-5 µg), clindamycin (Cli-2 µg), colistin (Col-10 µg), erythromycin (Ery-15 µg), gentamycin (Gen-10 µg), gentamycin high (GeH-120 µg), imipenem (Imp-10 µg), levofloxacin (Lev-5 µg), linizolid (Lin-30 µg), meropenam (Mer-10 µg), oxacillin (Oxa-1 µg), penicillin (Pen-10 µg), piperacillin (Pip-10 µg), tazobactum (Taz -10 µg), polymixin B (Pol-300 µg), tetracyclin (Tet-30 µg), clotrimazole (Clo-1.25 µg), teicoplanin (Tei-30 µg), and vancomycin (Van-30 µg).
Results

Sample collection

According to the inclusion criteria, around 1350 samples were collected aseptically from the Microbiology laboratory of MED Medical College Hospital, Perinthalmanna, Kerala, India. From the collected 1350 samples, 1140 samples showed significant bacterial growth, indicating a microbial infection in the patients. It was confirmed that 84.44% of the wounded sites were further aggravated by microbial infections from external sources (Table 1).

Table 1. Gender wise distribution of pathogens

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of samples collected</th>
<th>Culture positive</th>
<th>Percentage of positive cases</th>
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<tbody>
<tr>
<td>Total</td>
<td>1350</td>
<td>1140</td>
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<tr>
<td>Male</td>
<td>783</td>
<td>673</td>
<td>85.95</td>
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<tr>
<td>Female</td>
<td>567</td>
<td>467</td>
<td>82.36</td>
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</table>

The complications associated with wound infections are determined by the patient’s age, gender, and immune strength. Among the 1140 positive samples, the majority were obtained from the 51 to 60 years old age group (270, 24%). Even though predominantly male patients attending in this age group (140, 13%) were affected during this age group, whereas in female patients, the largest number (101, 8.8%) of female samples belong to the age group 61 to 70 years. The age-wise distribution of samples is given in Figure 2. Samples from surgical wounds, open wounds with pus, diabetic foot ulcers, and pus from burn wounds were collected. Among them, the patient’s age is the major factor influencing the microbial infection at the wounded site, as the immune system is in a deprived condition.

Figure 2. Age-wise (in years) wound infection rate in collected samples
Isolation of bacteria from pus samples

The samples streaked on selective agar plates were observed for growth, colour of the colony, and colony morphology after 24 hours of incubation (Figure 3). Gram positive bacteria (GPB) and Gram-negative bacteria (GNB) were isolated from the samples; most of the GPB belonged to the cocci, and the GNB were rod shaped. Gram staining indicated 512 (44.91%) Gram positive bacteria (GPB) and 628 (58.09%) Gram negative bacteria (GNB). As the skin’s normal flora is *Staphylococcus* sp., most of the pus isolates were belonging to the opportunistic GPB, *Staphylococcus* sp. Growth in culture media, cultural characteristics, and the colour of the colony were recorded. Typical colony morphology with colour change of the colonies on selective media indicated the immediate identification of a specific isolate, and the isolate, which did not respond to common identification tests and in ambiguous result, was further subjected to specific confirmatory tests such as growth at 44 °C, niacin sensitivity, arginine utilization, etc., to identify the organism.

![Figure 3](image1.png)

**Figure 3.** Bacterial growth on positive culture plates; a) *S. aureus* growth on blood agar; b) *Klebsiella* sp., growth on MacConkey agar; c) *E. coli* growth on MacConkey agar

Biochemical tests for identification of pathogens

Biochemical tests were performed, and the results are recorded in Table 2. Routine microscopic examination in combination with colony morphology and certain biochemical tests confirmed the commonly occurring pathogens. The predominant bacterial isolates were *Staphylococcus aureus* in 317 samples (28%), *Staphylococcus* sp. Coagulase negative in 95 samples (8%), *Streptococcus* sp., in 20 samples(2%), *Escherichia coli* in 149 samples(13%), *Klebsiella pneumonia* in 118 samples (10%), *Proteus mirabilis* in 70 samples (6%), *Enterococcus faecalis* in 26 samples (2%), *Pseudomonas aeruginosa* in 139 samples (12%), *Acinetobacter baumanii* in 16 samples (1%), *Citrobacter koseri* in 21 samples (2%), *Enterococcus faecium* in 21 samples (2%), *Enterococcus sp.*, in 24 samples (2%), *Morganella morganii* in 17 samples (1%), *Proteus vulgaris* in 21 samples (2%), and 36 samples of bacteria (3%) belonging to other less prominent bacterial isolates. *Staphylococcus aureus* (28%) was the predominant bacterial flora isolated from the pus samples received in the Microbiology Clinical Laboratory, MES Medical College Hospital, in 2021. This was followed by *E. coli* with 13% positivity. Therefore, the *S. aureus* infection was considered primary among the patients (Figure 4).
Table 2. Microscopic, colony morphology and biochemical characteristics used to identify the pathogens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Tests</th>
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</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>Oval shaped</td>
<td>GNB</td>
<td>GNB</td>
<td>GNB</td>
</tr>
</tbody>
</table>
| Enterococcus faecalis | gram-positive | cocci in pairs | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Escherichia coli | GNB | GNB | GNB | GNB |
| Enterobacter aerogenes | Oval shaped | gram-positive | cocci in pairs | Gram |
| Burkholderia sp. | oval shaped | gram-positive | cocci in pairs | Non- | motile |
| A. baumannii | oval shaped | gram-positive | cocci in pairs | Non- | motile |
| Acinetobacter sp. | oval shaped | gram-positive | cocci in pairs | Motile |
| Acinetobacter sp. | oval shaped | gram-positive | cocci in pairs | Motile |
| Burkholderia sp. | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Escherichia coli | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Enterobacter aerogenes | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Acinetobacter baumannii | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Burkholderia cepacia | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Pseudomonas aeruginosa | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Morganella morganii | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Klebsiella oxytoca | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Enterococcus sp. | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |
| Enterobacter sp. | oval shaped | Gram | Negative Bacilli in bipolar 
safety pin appearance |

Key words: “+” = positive test result; “T” = Negative test result; “A” = acid production; “K” = alkaline condition; “G” = Gas production; “NR” = non-reactive, “GNB” = Gram negative bacteria; “GPB” = Gram positive bacteria; MSA = Mannitol salt agar, BA = Blood Agar, MC = Mec-Conkey Agar.
Isolation of bacteria from diabetic foot samples

Of the 1350 wound samples, a total of 597 diabetic foot ulcer samples were streaked on the agar plates and observed for growth. A total of 581 bacterial growth positives were observed. These isolates were examined morphologically and biochemically to identify the pathogen. The pus samples were predominantly males (60.4%) compared to females (39.6%) in cases of diabetic foot ulcers. Aerobic Gram-negative organisms were more frequently (63.5%) isolated compared to Gram-positive organisms (36.5%) in our study even though *Staphylococcus aureus* was the most frequently isolated Gram-positive bacteria from diabetic foot infections (Sannathimmappa *et al.*, 2021) in other studies. With regard to age-wise distribution among culture-positive samples, patients between 51 and 60 years old contributed 35.8% of culture-positive samples (Figure 5).

Antibiotic sensitivity pattern of pathogen

Each isolate was examined for antibiotic sensitivity against common antibiotics. Among the 317 clinical isolates of *S. aureus*, 136 (43%) were methicillin-resistant and among the 149 isolates of *E. coli*, about 36
(24.1%) was belong to the extended spectrum of β-lactamase resistant pathogens. These results were concerning (Figure 4 and Table 3). The isolated Gram-negative bacteria (GNB) were sensitive to antibiotics such as amikacin > imipenem > meropenem > tazobactum > gentamycin > chloramphenicol > ciprofloxacin > levofloxacin and were resistant to clindamycin, erythromycin, linezolid, oxacillin, penicillin, and vancomycin. In Gram Positive Bacteria (GPB), susceptibility to linezolid > vancomycin > tetracycline > clindamycin > chloramphenicol > gentamycin > ciprofloxacin, and resistance to amikacin, imipenem, meropenem, and tazobactum were recorded (Figure 6).

Table 3. Antibiotic resistant pattern of isolates from pus samples of wound infections

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Amikacin</th>
<th>Ampicillin</th>
<th>Bacitracin</th>
<th>Cefazolin</th>
<th>Cefotaxime</th>
<th>Ceftriaxone</th>
<th>Chloramphenicol</th>
<th>Ciprofloxacin</th>
<th>Gentamycin</th>
<th>High</th>
<th>Imipenem</th>
<th>Meropenam</th>
<th>Moxifloxacin</th>
<th>Ositamycin</th>
<th>Polimixin B</th>
<th>Tazobactum</th>
<th>Teicoplanin</th>
<th>Vancomycin</th>
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<td>Streptococcus sp.</td>
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The distribution of aerobic bacteria in pus samples from infected individuals between January 2021 and December 2021 was analyzed. Out of 1350 samples, 1140 (84.44%) samples showed significant microbial infection in the patients. Surgical and non-surgical wound infections are caused by the normal flora, like *Streptococcus* sp., *Propionibacter* sp., and *Staphylococcus* sp., from the near-by area introduced to the infected area through cross-contamination. In 2018, the Centre for Disease control (CDC, WHO) reported that 20% of the women who underwent caesarean sections contracted wound infections, and 11% of the males developed pyogenic infections immediately after surgery from drainage from nearby sites or the use of immune suppressive medications. In the current study, around 80% of the samples showed microbial infections in the wound, either from severely wounded patients attending the clinic or due to poor hygienic practices or poor knowledge of the patient’s medical practices. These are the key governing factors responsible for health care-associated infections (Mohan *et al*., 2021).

The distribution of GPB and GNB was 512 (45%) and 628 (55%), respectively. GPB and GNB cause pyogenic infections in individuals. The bacterial incidence was highly based on the severity of the disease, the immunity of the patient, and also the effect of predisposing factors (Mohan *et al*., 2021). GNB outnumbered GPB in a general hospital in the nearby states of North Kerala, like in Andhra Pradesh, India (Sumanth *et al*., 2020), but in this study, GPB dominated GNB. Among the Gram-positive cocci, *S. aureus* was the most predominant organism (Roopshree *et al*., 2021) in Andra Pradesh, Bangalore, and Karnataka respectively. According to them, the major isolate from pyogenic infection was MRSA (43%). In the present study, among the 317 *S. aureus* positive cases 136 (42.90%) were MRSA. This may be because the three are neighboring states and may have a common protocol in the use of antibiotics. Moreover, maintenance of wound hygiene is important, and correct empirical monitoring is required to control the evolution of multidrug-resistant strains among individuals.

*S. aureus* produces various toxins; among them, the δ-toxin induces the granulation of mast cells, which promotes both innate and adaptive type 2 immune responses; another α-toxin also induces IL-1β production from monocytes, which may consequently promote a T_h17 response; or from CD4⁺ T cells, making the cytokine IL-17. By contrast, when exposed to *S. aureus*-derived cell wall component lipoteichoic acid, T cells neither proliferated nor produced cytokines, indicating that *S. aureus* products activate the immune system and also temporarily paralyses it. In addition to targeting immune cells, *S. aureus* has also been shown to trigger adipocytes to rapidly proliferate and to produce increased levels of the antimicrobial peptide cathelicidin as a
host defence mechanism (Byrd et al., 2018). These are the ways in which the bacteria outnumber others and proliferate at the infected site, thereby predominately causing the pyogenic infections.

It has also been reported that apart from Staphylococcus aureus many GNB are involved in causing pyogenic infections; these include E. coli (Sumanth et al., 2020; Roopa Shree et al., 2021), Pseudomonas aeruginosa, Klebsiella sp., (Divya Shanthi et al., 2015), Citrobacter sp., Proteus sp., etc. Pyogenic infections are common among those with poor hygiene. GNB are susceptible to amikacin and meropenem (Roopashree et al., 2021; Divya Shanthi et al., 2015), and in GPC, the pattern shows susceptibility to linezolid and vancomycin. In this study, the susceptibility of GNB to amikacin and meropenem was 100%, and susceptibility of GPC to linezolid and vancomycin was 94.7%. Among the Gram-negative isolates (n = 81, 54.73%), 60 (74.07%) were multidrug resistant, with the majority being susceptible to imipenem, meropenem and amikacin (Roopashree et al., 2021). Among the 36 ESBL strains, 32 (88.88%) were susceptible to imipenem, meropenem, and amikacin. The frequency of isolation was predominant among males (65%) (Shivra et al., 2020). Aerobic Gram-negative rods were predominantly (56.5%) isolated compared to Gram-positive organisms (43.5%), especially from male volunteers (60.4%) rather than female volunteers (39.6%). Staphylococcus aureus was the most frequently isolated Gram-positive bacteria from pyogenic infections (Bhumbla et al., 2019; Roopashree et al., 2021). Most of the patients were in the 41 - 60 age group (Byrd et al., 2018; Divyashanthi et al., 2015; Shivra Batra et al., 2020). Possible reported reasons for the evolution of 50% of the drug resistant pathogens were inappropriate use of antibiotics by the patients, high risk of drug-resistant pathogens (especially MRSA) by the health care workers among the patients, and/or public illiteracy on the antibiotic schedule (Mulay et al., 2022). In this study, the most prominent age group was 51 to 60, followed by 61 to 70 and 41 to 50 years. With regard to diabetic foot infections, aerobic Gram-negative rods were predominantly isolated compared to Gram-positive organisms. Among 581 culture-positive samples, 369 (63.5%) were GNB. The isolation frequency was predominant in males. In this study, male volunteers isolated 60.4% of the total culture positive diabetic foot infections causing pathogens. The age group of the patients was 65±11 (Mohan et al., 2021). Regarding the age-wise distribution, 51-60 years olds contributed 35.8%, followed by the 61- to 70-year-old age group. Diabetic foot infections are most prominent due to their diabetic neuropathic effects (El Boullant et al., 2022). This is a retrospective study in which we could not investigate the significant determinants such as the source of infection, the duration of hospital stays, and clinical outcome. In addition, the study was based on the characterization of bacterial isolates based on phenotypic, conventional, and automated methods only.

**Conclusions**

Disturbances caused by changes in the normal flora and the contamination of wounds by pathogenic bacteria may lead to pyogenic infections; this may cause pathogenic microflora to multiply and further lead to diseases. The normal flora of every individual is not the same and depends on wound hygiene. Gram positive bacteria were the predominant causative organisms of pyogenic infection. The predominant bacterial isolates were S. aureus (28%), followed by E. coli in 149 (13%), P. aeruginosa (12%), and Klebsiella pneumonia (10%). The antibiotic sensitivity pattern with culture positive samples was evaluated. The prominent antibiotic susceptibilities for GNB are imipenem (86.1%), followed by amikacin (81.5%), meropenem (83.92%), tazobactum (79.45%), chloramphenicol (44.4%), ciprofloxacin (61.30%), gentamycin (71.33%), levofloxacin (60.82%), and resistance to clindamycin, erythromycin, linezolid, oxacillin, penicillin, and vancomycin. Among GPC, the susceptibility pattern is vancomycin (95.11%), followed by linezolid (94.92%), tetracycline (75.58%), clindamycin (56%), chloramphenicol (63.08%), ciprofloxacin (45.11%), gentamycin (57.42%), and resistance to amikacin, imipenem, meropenem, tazobactum, etc. This study shows chloramphenicol, ciprofloxacin, gentamycin, tetracycline, and clotrimazole are susceptible to some strains of both GPC and GNB. Around half of the isolated Staph. aureus (43%) evolved as MDR strains, especially vancomycin, oxacillin, chloramphenicol,
and ciprofloxacin; in GNB, one fourth of the isolated *E. coli* were MDR and commonly resistant to amikacin, imipenim, meropenem, and tazobactum. So, the treatment of pyogenic infections has become very challenging due to widespread bacterial resistance to antibiotics. The study is important to generate findings that would guide the formulation of policies on infection control, empirical antibiotic treatment, and control of antibiotic use.

Routine surveillance for pathogens and their susceptibility to antibiotics is of paramount importance, not only to reinforce strategies for successful pathogenic bacterial pyogenic infection control and management (Natasya et al., 2021). The study helps to provide guidance for using the appropriate antibiotic regimen for effective treatment and to prevent discriminatory usage of antibiotics.

**Authors’ Contributions**

**RK:** Collection of data, collection of samples, processing of sample, carrying out tests, recording of results and manuscript writing; **PSK:** Correction of the data, results analysis and interpretation, editing of manuscript and correspondence works; **SMA:** Standardization and regulation of methodology. All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Ethical approval for the present study was issued as per the standard protocols of the committee for the purpose of control and supervision of experiments (CPCSC) on humans, strictly maintained and followed by the Institutional Ethics Committee of MES Medical College Hospital, Kerala (IEC/MES/10/2022; Dated: 20/07/2022).

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**Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

**References**


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