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Bacteriological profile and antibiogram patterns of pyogenic bacteria isolated from pus specimens at a tertiary care hospital, Islamabad

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Abstract

Pyogenic wound infections are a significant source of morbidity, due to the development of different resistant strains. Assessing antibiotic sensitivity patterns for pyogenic bacterial isolates from pus samples is essential for the identification of suitable antibiotic treatments for patients. Antibiotic resistance among various bacteria develops and spreads due to the careless use of antibiotics for treating diseases. The present six-month study was conducted to establish the bacteriological profile and antibiogram patterns through the analysis of bacterial isolates obtained from various pus-infected patients in the Department of Microbiology at a tertiary care hospital, Islamabad. Total of 373 pus samples were collected and processed by standard microbiological techniques for the identification of bacterial isolates by culturing them on selective and differential media. According to Clinical and Laboratory Standard Institute (CLSI) guidelines, the antibacterial sensitivity profiling was performed by using the Kirby-Bauer method. The most prevalent bacterial isolate identified was *Staphylococcus aureus*, accounting for 46% of cases, followed by *Pseudomonas aeruginosa* (23%), *E. coli* (7%), *Klebsiella pneumoniae* (6%), *Proteus mirabilis* (3.5%), *Enterococcus* (2%), *Providencia stuarti* (1.8%), *Acinetobacter baumannii* (0.39%), and *Stenotrophomonas maltophilia* (0.38%). The results indicated that Gram-positive bacteria were highly sensitive to antibiotics like linezolid, vancomycin, rifampicin, teicoplanin, and minocycline. In contrast, Gram-negative bacteria showed greater susceptibility to ciprofloxacin, tigecycline, amikacin, and levofloxacin. The study provides the foundation for evidence-based therapy to reduce the unnecessary use of antibiotics, thereby ensuring successful treatment for pyogenic infections and helps in preventing the emergence of drug-resistant strains.

Keywords: pyogenic infection, antimicrobial resistance, morbidity, antibiotic susceptibility pattern, Kirby Bauer method

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1. INTRODUCTION

Pus-forming infections are referred to as pyogenic infections and are recognized by a number of localized and systemic inflammations, production of pus and bacterial growth[1], it could be either exogenous or endogenous. The surface bacteria can enter through a skin crack, and, as a result, start growing locally with immune cells being introduced into the area as part of the body's defense mechanism to fight off bacteria, and these cells eventually accumulate to form pus, which is a thick, white liquid[2]. This may involve a mixture of both aerobic and anaerobic bacteria, either alone or in mixed combinations, and requires the use of antibiotics. The risks for wound infections are mainly determined by local wound conditions, bacterial burden and host immune response. *Staphylococcus aureus, Pseudomonas aeruginosa, P. stuartii, E. coli, K. pneumoniae, P. mirabilis, Enterobacteriaceae, A. baumannii,* and *S. maltophilia* are the most commonly isolated pyogenic strains[3]. Wound infections have not entirely been eradicated because of high antibiotic-resistant strains, despite advancements in infection control[4]. Drug-resistant bacteria evolved because of the misuse of antibiotics, posing a significant burden to the healthcare system[5]. Moreover, extremely virulent strains and their ability to quickly respond to changes in environments make the problem worse and raise serious concerns[6].

Regardless of improvements in diagnostic techniques, multidrug-resistant (MDR) organisms have emerged, making it difficult to treat infections in developing nations[7]. In recent years, pyogenic infections associated with MRSA and MDR Gram-negative bacterial isolates are becoming more common and spreading rapidly. The problem of resistivity patterns in bacterial diseases is due to the misuse of antibiotics, particularly in underdeveloped countries. The main causes of the increasing prevalence of antibiotic-resistant microbes include unnecessary, excessive, and incorrect antibiotic drug prescriptions[8]. Learning about the causes of wound infections is useful for selecting the most effective antimicrobial treatment[9]. Hence, an efficient treatment strategy was chosen to deal with pyogenic wound infections, and the main purpose of the current study was to identify the impact of isolated bacterial strains and their antimicrobial susceptibility patterns.

2. MATERIALS AND METHODS

2.1. Study area and period

Cross-sectional research was conducted for a time period of six months (August 2022- January 2023) in the Department of Microbiology at the pathology laboratory in a tertiary care hospital (n=373), in accordance with ethical guidelines and standard procedures. Pus samples were taken from the patients who were admitted to various wards, operation theaters, burn centers, OPD, and ICUs of the hospital.

2.2. Inclusion and exclusion criteria

The inclusion criteria for this study specified that only wound pus samples would be collected, while the exclusion criteria required that patients with parasitic or fungal infections or those undergoing antibiotic treatment would not be included in the sample collection process[10].

2.3. Sample collection and culture of the specimen

Different pus samples were obtained from the skin (abrasions, furuncles and pustules), ear discharge, nasal wounds, legs, catheters, and internal organs (kidney, bladder, and lungs). Pus sample was collected using a swab; a well-soaked swab contains at least 1mL of pus, if possible, a few mL of pus stored in a sterile tube or bottle, even a few drops still in a syringe is better than a swab. Pyogenic bacteria are living entities that grow, reproduce, and die quickly. These three processes are supposed to be prevented or slowed via transport media. If any of the three occur before the specimen can be grown in the laboratory, it could lead to false positive or false negative results. Before samples were prepared for culture and Gram staining, specimens were stored in Cary-Blair transit media. The samples were aseptically inoculated on MacConkey and blood agar plates and then incubated aerobically for 24 to 48 h at 35°C to 37°C[11].

2.4. Identification and characterization of bacterial pathogens

Initial identification of bacterial isolates was done by colony characteristics (following are the key features that are observed in colony on media, size, shape, color, texture, elevation, margin and opacity), including hemolysis on blood agar, lactose and non-lactose fermenters on MacConkey agar, difference in physical appearance in differential media (Fig.1) and biochemical tests such as catalase, coagulase and oxidase. Identification of Gram positive cocci was performed using Gram staining, coagulase, catalase, and DNase activity whereas for Gram-negative bacilli (GNR), oxidase, citrate, urease, and triple sugar iron (TSI) tests were performed using standard biological methods. API (Analytical profile index) strips provide reliable identifications using extensive databases and it is a standardized, fast, secure, simple, and accessible test device. These API strips contain up to 20 micro biochemical assays that provide additional confirmation of tests performed to validate the identification results[10].

2.5. Antibiotic susceptibility testing

Bacterial inoculum was created by making a suspension in distilled water, comparing the turbidity to the 0.5 McFarland standard and spreading them on plates of Muller-Hinton agar. Plates with antibiotic discs were then incubated for 24 h at 37°C. The Clinical and Laboratory Standards Institute (CLSI) recommended the disc diffusion method for antimicrobial susceptibility testing on Muller-Hinton agar plates. The following drugs were tested against these bacterial isolates. For Gram-positive cocci: Erythromycin (15µg), Penicillin (10 units), Teicoplanin (30µg), Clindamycin (2µg), Linezolid (30µg), Cotrimoxazole (25µg), Chloramphenicol (30µg), Gentamicin (10µg), Vancomycin (30µg), Tetracycline (30µg) and Ciprofloxacin (5µg). For Gram-negative bacilli: Meropenem (10µg), Tetracycline (30µg), Linezolid (30µg), Amikacin (30µg), Cephotaxime (30µg), Ciprofloxacin (5µg), Cefoxitin (30µg), Cefepime (30µg), Cotrimoxazole (25µg), Ceftriaxone (30µg), Gentamicin (10µg), Ceftazidime (30µg), Cefazoline (30µg), Cefuroxime (30µg), Aztreonam (30µg), Chloramphenicol (30µg), Piperacillin (100µg), Amoxyclavulanic acid (30µg). For Non-fermenters: Ampicillin (10µg), Amoxyclavulanic acid (30µg), Amikacin (30µg), Ciprofloxacin (5µg), Tobramycin (10µg), Cefoxitin (30µg), Cefepime (30µg), Ceftriaxone (30µg), Cephotaxime (30µg), Ceftazidime (30µg), Piperacillin+Tazobactam (100/10µg), Cefazoline (30µg), Cefuroxime (30µg), Aztreonam (30µg), Piperacillin (10µg), Meropenem (10µg), Levofloxacin (5µg), Gentamicin (10µg), Ticarcillin (75µg), Polymyxin B (30 units)[12].

2.6. Quality control and quality assurance

Data quality management was performed using different techniques which included standardization, training, and supervision during data collection. For laboratory analysis, the Tertiary Care Hospital's Laboratory strictly follows its standard operating practices for quality assurance, where each sample is defined with standardized information, such as the date, location of collection, and the type of sample, with the aid of predetermined data entry templates ensuring the consistent and accurate recording of this information; to prevent confusion and duplication, serial numbers are assigned to all samples. The identification process was carried out by skilled lab technicians; in order to authenticate assay performance, the investigations involved the use of positive and negative control samples strains of *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922). Experiments are replicated to examine variability and to ensure accuracy; for precision, the laboratory's equipment and instruments are calibrated on a daily or weekly basis; furthermore, the reduction of human error in sample handling and media preparation is achieved through the application of automated pipetting technology; and, lastly, a Laboratory Information Management System (LIMS) is used to manage data storage, sample monitoring, and retrieval processes. The reagents for Gram staining and biochemical testing were examined, and the sterility test was confirmed by incubating 5% of the prepared media at 37 °C for 24-48 h. Adherence to well-established best practices and stringent quality control methods also improves the credibility and reliability of clinical research[13].

2.7. Ethical approval

Ethical approval was taken from the ethical committee of the International Islamic University Islamabad via letter no. 987646747.

2.8. Statistical analysis

Data analysis was done through Excel and GraphPad Prism software. Chi-square or Fisher's test and paired t-test are specific statistical tests used to analyze the data and to identify the prevalence of bacterial isolates and sensitivity trends. p-values > 0.05 were considered significant. The distribution and prevalence of the bacterial isolates based on sex (female and male), age range, and samples (Pus from both surgical and non-surgical sites of the human body) were displayed using graphs or pie charts. The sensitivity trends of the eight microbial isolates against the widely used antibiotics were described using a frequency table displaying percentages and absolute numbers.

3. RESULTS AND DISCUSSIONS

Pus samples are used to test antibiotic susceptibility because pyogenic wound infections are more likely to endure polymicrobial growth and have a higher number of different bacterial strains reported than urine, stool, body fluids, blood, spinal fluid, ear discharge, and mucus cultures, whereas polymicrobial growth is less common and only a few common bacterial isolates are associated with it. The use of antibiograms is crucial for identifying and tracking changes in antimicrobial sensitivity and resistance patterns. Bacteria from pus samples can be examined and their variations in antibiotic susceptibility over time can be monitored. Out of a total of 373 samples collected from various body sites (Table 1), 254 were growth-positive culture (68%). The results were observed after an overnight-incubated period of 1-2 days, whereas 117 samples (32%) showed no growth (Fig. 1). With ages ranging from 1 month to 83 years, 153 (41%) were females and 220 (58.98%) were males (Table 2). The eight bacterial species were isolated based on Gram staining reaction, morphological characteristics, culture properties, and biochemical testing (Fig. 2). 146 (57%) samples were Gram-positive whereas, 110 (43%) were Gram-negative. Monomicrobial growth was observed in 98% (249/254) samples whereas, 1.6% (7/254) samples had polymicrobial growth. Among Gram-positive isolates, 117 (46%) were *Staphylococcus aureus,* 23(9.1%) were CoNS, and *Enterococcus* 6(2%). Similarly, the most prevalent bacteria identified in Gram-negative isolates were *Pseudomonas aeruginosa* 59 (23%), followed by *Escherichia* coli 18(7%), *Klebsiella pneumoniae* 16(6%), *Proteus mirabilis* 9(3.5%), *Acinetobacter baumannii* 4(2%), *Providencia stuartii* 3(1.1%), and *Stenotrophomonas maltophilia* 1(0.39%) (Fig.3). The most prevalent isolate in *Enterobacteriaceae* group was *E. coli* 18(7%) followed by *Klebsiella pnuemoniae* 16(6.2%).

Our study correlates with the study done by Jain[14]. In comparison to Gram-negative bacterial strains, Gram-positive bacteria were the most common (57%) in pus samples, which is consistent with earlier reported research. Our study correlates with the study of Afshan and Shahid who reported a predominance of *S. aureus, Pseudomonas aeruginosa, Klebsiella species, E. coli,* and *Proteus spp* isolated from pus[15]. In a study, *P. aeruginosa, P. mirabilis*, and *E. coli* were the next most common bacteria found in wounds, followed by *S. aureus* [16]. In 2009 Dryden reported that the major causes of soft tissue infections in hospital-admitted patients include *S. aureus* and MRSA[17]. Other studies have linked wound infections to *Pseudomonas, Staphylococcus, Streptococcus, Klebsiella, and E. coli* [18,19]. This also correlates with the results of the previous study [20]. It has also been reported that MRSA is associated with wound infectionss[21]. Antibiotic susceptibility profile of isolated Gram-positive and Gram-negative strains demonstrate differences in response to various antibiotics. According to the result of antibiograms in this study, *S.aureus* was resistant to penicillin, cefoxitin, levofloxacin, erythromycin, and azithromycin, whereas Linezolid and vancomycin were also entirely efficient against Gram-positive isolates (Fig. 4 & Fig. 5). The results of statistical analysis indicated that LZD, TEC, VA, MH, and RD are highly effective against Gram-positive isolates ($p < .0001$).

The antimicrobial susceptibility of Gram-negative isolates in present study is shown in Fig. 6. Highly significant sensitivity ($p < .0001$) was indicated by LEV, CIP, TGC and AK against Gram-negative isolates. Notably, even though both organisms displayed cephalosporin resistance, the studied antibiotics demonstrated a higher level of effectiveness against *P. aeruginosa* compared to *K. pneumoniae*. In contrast to our study *P. aeruginosa* isolates have been identified in earlier research from Canada, Croatia, and Latin America to be resistant to carbapenems, ciprofloxacin, and aminoglycosides, but not to piperacillin[22,23], this might be due to demographic difference. *Escherichia coli* exhibited minimal resistance against cefatrizine and minocycline but maximal resistance against ceftriaxone, ampicillin, amoxicillin-clavulanic acid, and cephalosporins. Similar result was reported by Trojan[11]. High drug resistance due to negligence of patients, incomplete treatment schedules, self-prescription, overuse of antibiotics, a lack of information on regional antibiograms, and less knowledge of isolates with multiple drug resistance are all contributing factors. It leads to the side effects of MDR that are mild to severe. Therefore, knowing the antibiotic susceptibility profile of different bacterial isolates from pus specimens will help in the best dosage formulation and regimen in the effective treatment of pyogenic wound infections. It also helps to suppress the emergence of drug resistance.

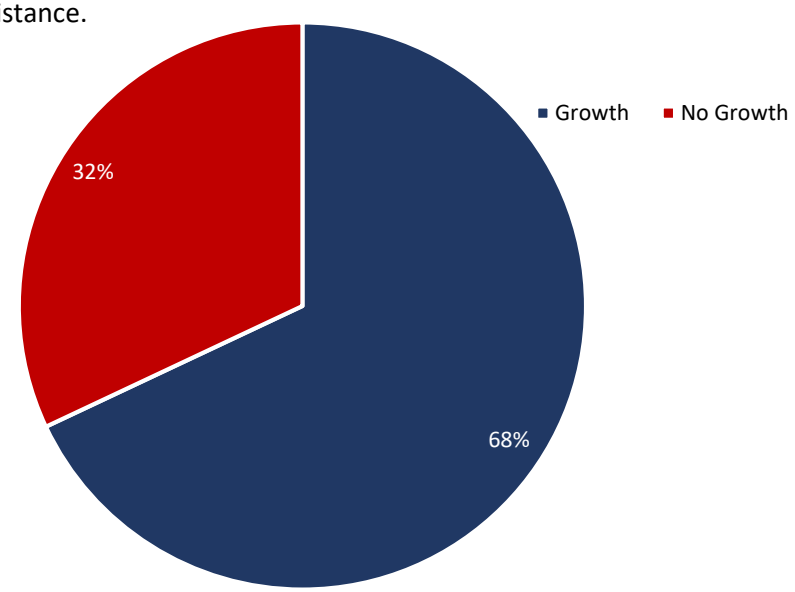

Fig. 1: Culture positivity in pus samples for pyogenic bacteria (n=373).

Table 1. Collection of pus samples from different body sites.

Body sites	Number of samples received	Percentage (%)
UTI	5	1.34
Surgery	205	55.0
Orthopedic	53	14.2
Tooth abscess	15	4.02
OBG	65	17.42
ENT	26	6.97
Pleural	4	1.07
Total	**373**	**100**

Table 2. Age wise and gender wise distribution of patients with pyogenic infections.

Age	Male	Female	Total
>1	7	6	13
1-15	39	14	53
16-30	35	19	54
31-45	50	57	104
41-60	63	36	99
51-75	22	15	37
75<	4	6	10
Total	**220 (59%)**	**153 (41%)**	**373 (100%)**

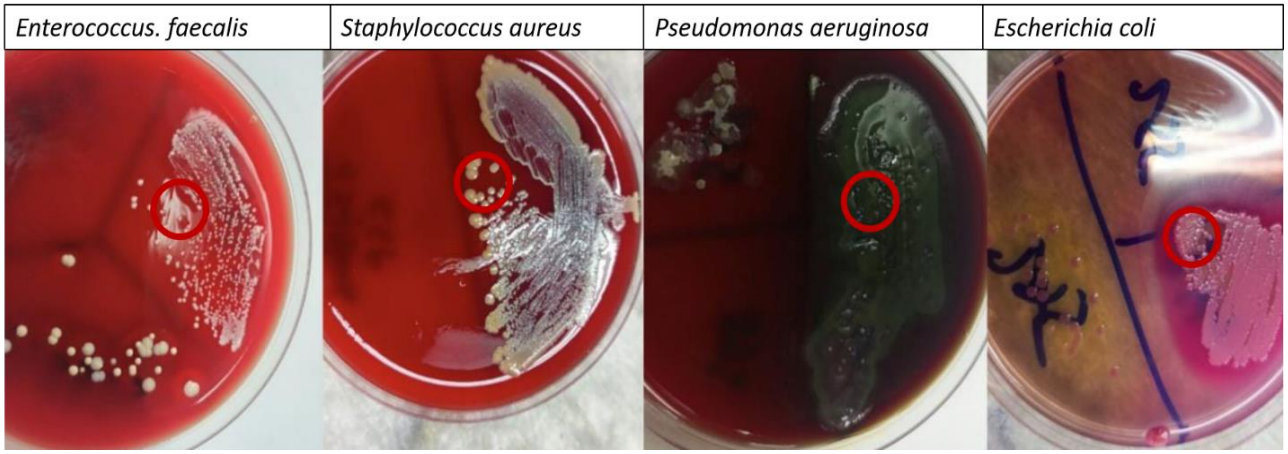

Fig. 2: Pus samples of patients were taken from surgical wounds, tooth abscesses, and OBG cultured on Blood agar & MacConkey agar**.**

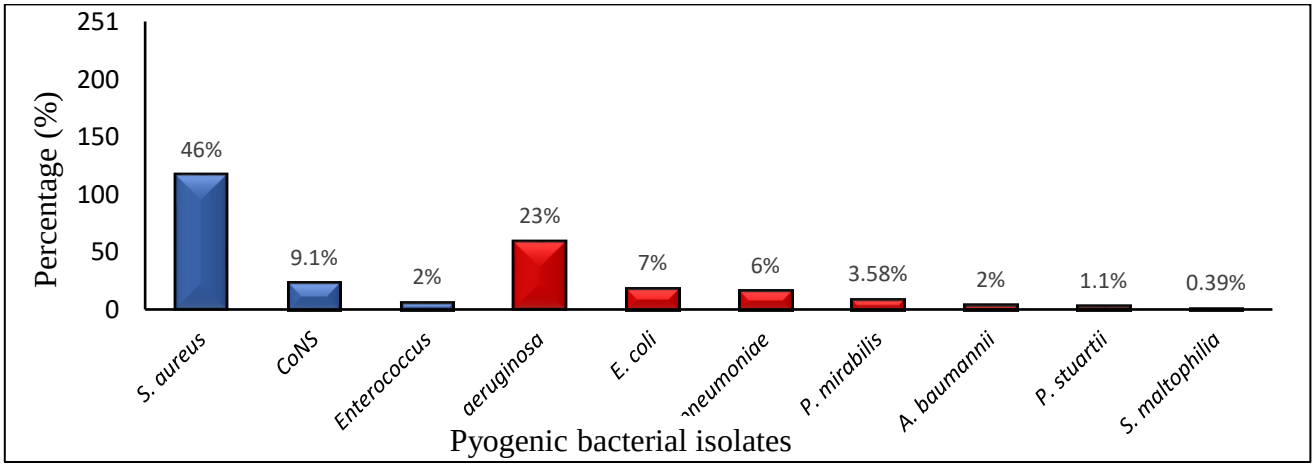

Fig. 3: Spectrum of pyogenic bacterial isolates from pus specimen.

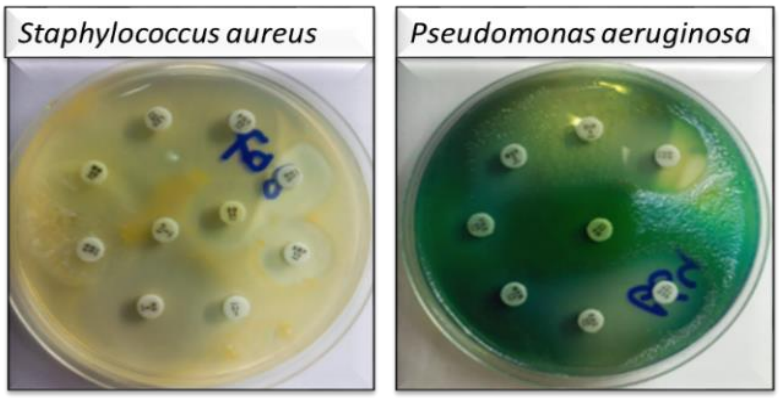

Fig. 4: Antibacterial sensitivity profile of pyogenic bacterial isolates.

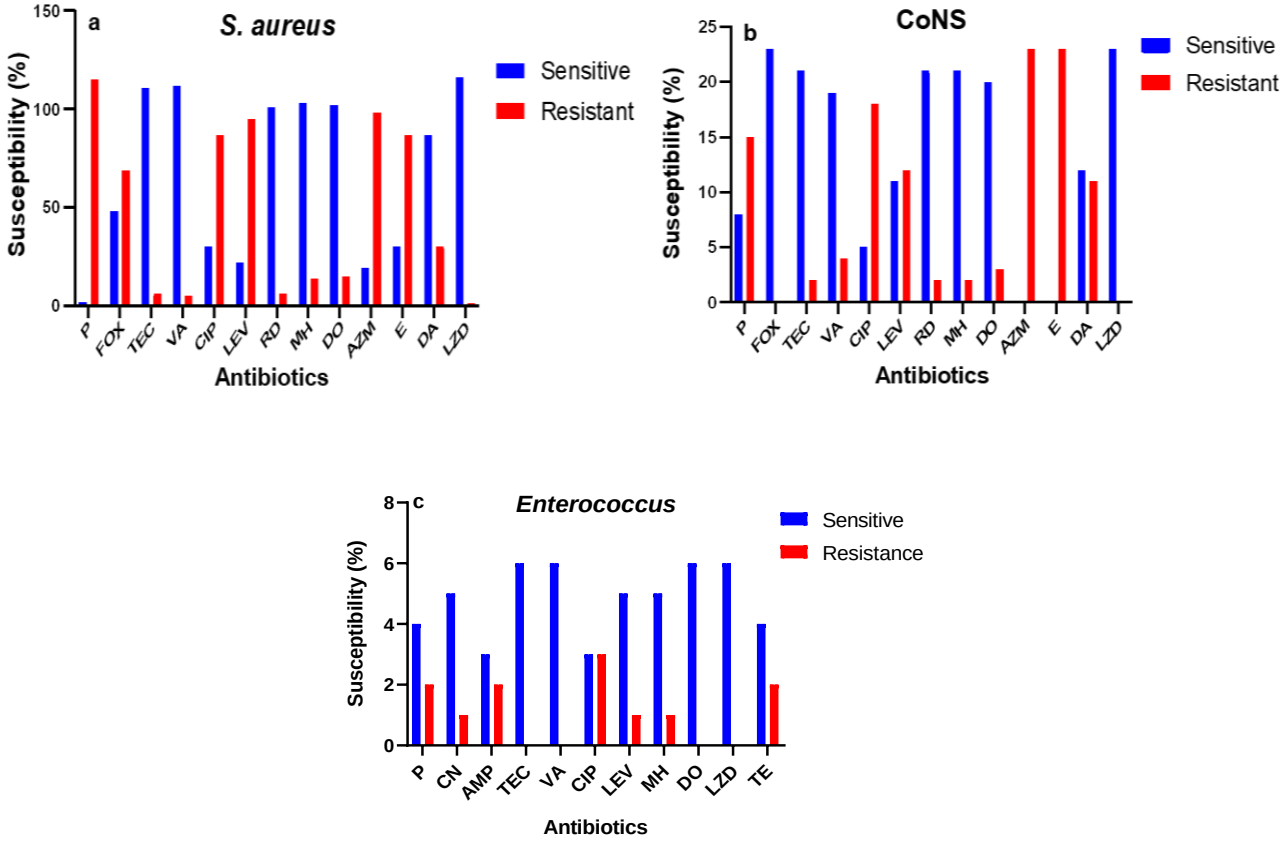

Fig. 5: Antibiogram of Gram-positive bacterial isolates from pus samples**.**

Key; P. Penicillin, **CN.** Gentamycin, **AMP.** Ampicillin, **TEC.** Teicoplanin, **VA.** Vancomycin, **CIP.** Ciprofloxacin, **LEV.** Levoflaxacin, **MH.** Minocycline, **DO.** Doxycyclin, **LZD.** Linzolod, **TE.** Tetracyclin **FOX.** Cefoxitin, **RD.** Rifampicin, **AZM.** Azithromycin, **DA.** Clindamycin, **E.** Erythromycin.

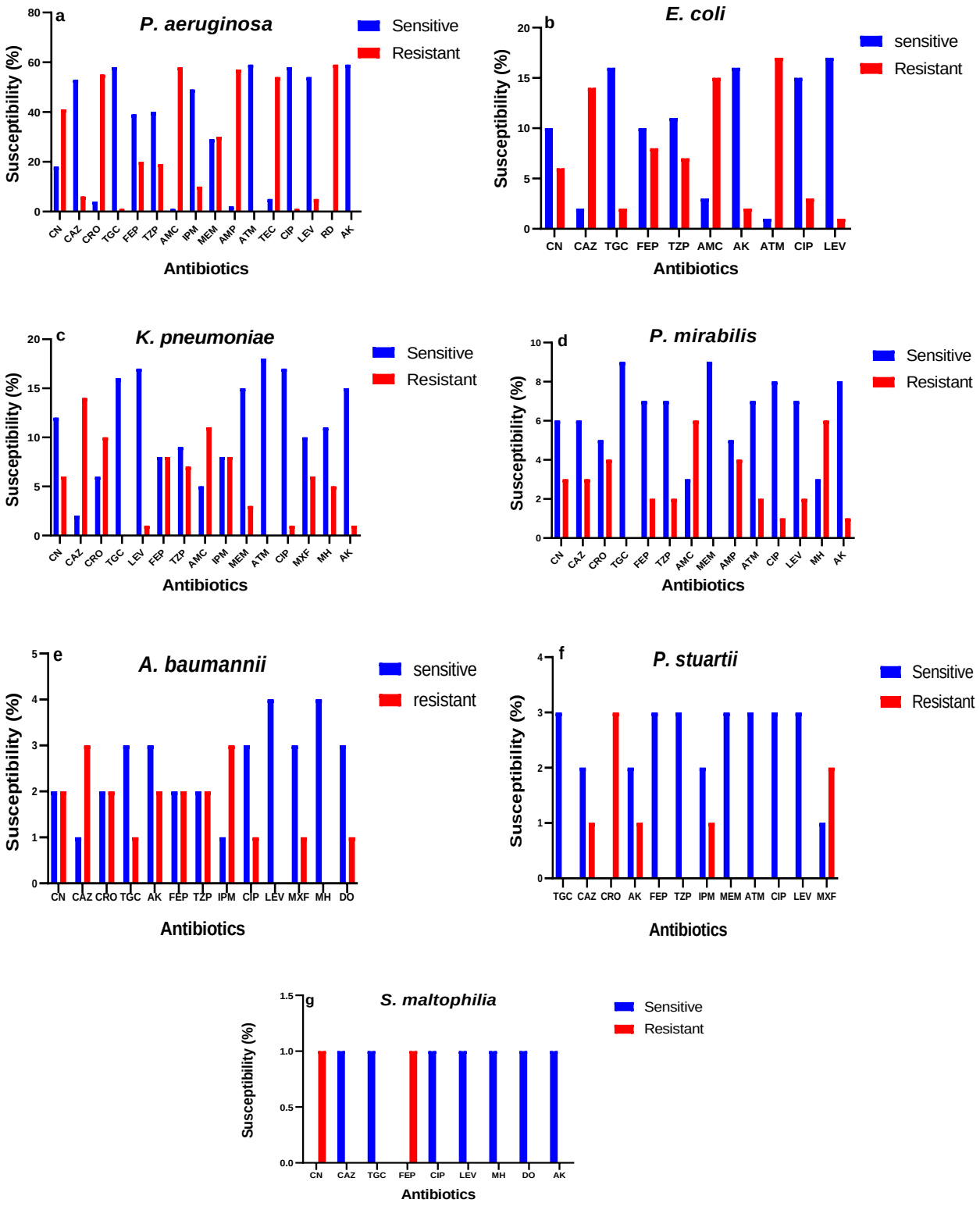

Fig. 6: Antibiogram of Gram-negative bacterial isolates from pus samples. **Key; MEM.** Meropenem, **MH.** Tetracycline, **AK.** Amikacin, **CIP.** Ciprofloxacin, **FEP.** Cefepime, **CRO.** Ceftriaxone, **CN.** Gentamicin, **CAZ.** Ceftazidime, **ATM.** Aztreonam, **AMC.** Piperacillin+Amoxyclavulanic acid **TGC.** Tigecycline, **LEV.** Levofloxacin, **DO.** Doxycyclin, **TZP.** Piperacillin+Tazobactam, **IPM.** Imipenem, **AMP.** Ampicillin, **TEC.** Teicoplanin, **MXF.** Moxifloxacin.

4. CONCLUSIONS

Pyogenic infections are the significant contributing factor to morbidity in post-surgical wounds, among Gram-positive isolates, *S. aureus* was found to be the most pus-causative agent 117(46%), the most common pyogenic bacterial isolates from pyogenic infections and shows multiple drug resistance against azithromycin, levofloxacin, ciprofloxacin, penicillin and erythromycin. Whereas in the case of Gram-negative isolates, *Pseudomonas aeruginosa* was the most prevalent organism 59 (23%) out of 373 pus samples, it shows MDR against gentamycin, rifampicin and amoxicillin+clavulanic acid. The emergence of antibiotic resistance among these pyogenic bacterial isolates has an adverse effect on the treatment of such MDR. However, effective use of antibiotics plays an important role in reducing the emergence of MDR strains. This study will highlight the antibiotic susceptibility patterns and guide clinicians for appropriate antibiotics based on individual isolates. Consequently, it can help prevent antibiotics that are sensitive from being misclassified as resistant. Hence, resisting antibiotic resistance will lead to the maintenance of antimicrobial efficacy.

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CONFLICT OF INTEREST

Regarding this article, none of the authors declares any conflicts of interest.

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