

How to Cite:

Wajid, M., Naaz, S., & Hashmiya, S. S. (2022). Evaluation of bacteriological profile of pyogenic infections and their susceptibility profiles at a tertiary care hospital. *International Journal of Health Sciences*, 6(S3), 5237–5246. <https://doi.org/10.53730/ijhs.v6nS3.7079>

Evaluation of bacteriological profile of pyogenic infections and their susceptibility profiles at a tertiary care hospital

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Abstract---Pyogenic infections are an important cause of sepsis and their prompt recognition and therapy are required to prevent inadvertent complications. A retrospective analysis of 302 culture-positive pus and wound samples received in the department of Microbiology between January 2020 to December 2020 was conducted. Data about the pathogen isolated and its antimicrobial susceptibility was subjected to analysis using WHONET software. Identification and susceptibility testing was done using the standard microbiological procedures. Among the samples male to female ratio [M: F-2.02:1] was skewed in favor of males with the majority of patients being between the age group of 40 – 60 yrs. Predominantly Gram-negative bacilli were isolated in 65.2% [197/302]. Among the Gram-negative bacilli, *Escherichia coli* was the most common pathogen isolated [35.5%, 70/197]. Gram-positive organisms were isolated in 34.7% [105/302] of cases and *Staphylococcus aureus* was the predominant organism isolated 56.1%, [59/105]. This study accentuates the need for probing into the pattern of increasing trends of Multidrug-resistant [MDR] bacteria and frames an effective hospital-based policy for providing fruitful treatment in such cases.

Keywords---multidrug resistant, *E. coli*, *S. aureus*, wound infections.

Introduction

Pyogenic infections of wounds are among the leading causes of morbidity and mortality worldwide which may further proceed to sepsis if proper treatment is not initiated. Suppurative wound infections have been reported to vary between 3% and 11% in developed countries and are estimated to be as high as 40% in developing countries [1]. Factors such as wound type, depth, location, and quality, level of tissue perfusion, and the antimicrobial efficacy of the host immune response are decisive in the fate of such infections [2]. In hospital practice, 30–50% of antibiotics are prescribed for surgical prophylaxis and 30–90% of these prophylaxes are inappropriate, further leading to increased drug resistance, as well as prolongs hospital stay, increases trauma, poses risk for disarticulation and amputation, increases need for medical care and increases treatment costs [1,3]. Inappropriate use of antibiotics increases selection pressure favoring the emergence of pathogenic drug-resistant bacteria which chooses empirical antimicrobial agents more complicated [4].

A variety of commonly found drug-resistant bacteria in such infections are Extended-spectrum beta-lactamase [ESBL] producing organisms, MRSA [Meticillin-resistant *Staphylococcus aureus*], and Carbapenamases producing organisms. ESBL producing Gram-negative bacilli [GNBs] have spread all over the world. The prevalence of ESBL producing GNB varies across the world from 50 to 80%. *Staphylococcus aureus* [*S. aureus*] has been reported to be the most common isolated bacteria from such wounds. *Pseudomonas aeruginosa* is commonly isolated in infected wounds following surgeries and burns whereas *Enterococcus* species and *Enterobacteriaceae* are commonly isolated from wounds in immune-compromised patients and abdominal surgeries. Thus, it is imperative to start empirical therapy based on local rolling antibiogram followed by target-specific therapy post the culture and sensitivity reports. This study aims at determining the bacterial isolates in such pyogenic infections followed by their susceptibility pattern to aid in formulating an empirical therapy accordingly and implementation of hospital infection control strategies to prevent the spread of Multidrug-resistant [MDR] bugs.

Materials and Methods

Study design

This retrospective Laboratory record-based study was carried out after approval by the institutional ethics committee for a period of one year [from January 2020 to December 2020]. All Pus and wound swab samples received from In Patients [Department of Surgery, Orthopedics, Obstetrics and Gynecology and various other departments] both adult and pediatric age group, for Culture and sensitivity at the Laboratory, Department of Microbiology, ESIC Medical College and Hospital, Sanathnagar, Hyderabad, during the study period were included. Duplicate samples yielding the same organism and those yielding mixed flora were rejected.

Sample collection & Isolation

The pus samples were aspirated and transported in sterile leakproof containers and Wound swabs were collected from patients with surgical site infections, trauma, infected diabetic wounds and other wounds. To avoid contaminating the swab with skin flora, pus, or necrotic tissue, the wound was thoroughly cleansed with 60–120 ml sterile normal saline before taking the sample. The samples were streaked on 5% Sheep Blood Agar [SBA- Himedia] and MacConkey Agar [MCA] plates and incubated aerobically for 18–24 h at 37 °C. They were then observed for bacterial growth. Plates with no growth and with growth were re-incubated for another 18–48 h for isolation of bacteria that require extended incubation [slow growers].

Identification of Bacterial isolates

Standard microbiological techniques were used for the identification of pathogenic bacteria isolated in pure cultures. Characteristic morphological appearances of colonies on media, Gram stains, and standard biochemical tests.

Antibiotic susceptibility testing

Performed using the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute [CLSI] guidelines [5,6]. Following antimicrobial disks were used ; ampicillin (10 µg), amoxicillin – clavulanate (20/10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), ceftazidime (30 µg), piperacillin-tazobactam (100/10 µg) , aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), gentamycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), trimethoprim-sulphamethoxazole/co-trimoxazole (25 µg), Cefatizidime avibactam E strip and tigecycline (30 µg) from HiMedia Laboratories, India for GNB. The antibiotics tested for Gram-positive bacteria were as follows: Penicillin (10U), Cefoxitin (30µg), High Level Gentamicin (HLG) (120µg) Levofloxacin Trimethoprim/Sulfamethoxazole (1.25/23.75 µg) Clindamycin (2µg), Erythromycin (15 µg), Linezolid (30 µg), Vancomycin (30 µg discs), Teicoplanin (30 µg), Tetracycline (30 µg). Vancomycin E strip was used for *Staphylococcus* & *Enterococcus* isolates.

Detection of MRSA Strains of *Staphylococcus aureus*

MRSA isolates were detected by using ceftazidime disc [30 µg] as per standard detection guidelines mentioned as per CLSI [19]

Detection of ESBL and Carbapenemase Resistant *Enterobacteriaceae*

- Double disk synergy: Synergy between. Ceftazidime (30 µg), Cefotaxime disks (30 µg) and Ceftazidime/Clavulanic acid (30/10µg) discs or amoxicillin-clavulanic acid (20/10 µg/disk) was looked for.
- Combined disk test: When the difference in the zone of inhibition was noted to be >5 mm when comparing the various cephalosporin disks and their disks containing clavulanate it indicated production of ESBL.

Detection of Vancomycin-Resistant Enterococci

The antibiotic sensitivity testing for the *Enterococcus* species [either *Enterococcus faecium* or *Enterococcus faecalis*] was categorized as resistant to Vancomycin when zone size was ≤ 12 mm. Further such isolates were subjected to Minimum Inhibitory Concentration [MIC] for Vancomycin by commercially available Vancomycin E Strip [HiMedia Laboratories, India].

Multidrug resistant bacteria

As per standardized international terminology created by European Centre for Disease Control (ECDC) and Centre for Disease Control & Prevention (CDC), Atlanta, the multidrug-resistant (MDR) bacteria have been defined [7].

Quality Control

For quality control, *S. aureus* [ATCC 25923] and *Escherichia coli* [ATCC 25922] *Pseudomonas aeruginosa* [ATCC 27853], and *Enterococcus faecalis* [ATCC 29212] were used.

Data Analysis

Data were entered directly into WHONET software and analyzed using the same. WHONET is a Windows-based database software package for the management of Microbiology laboratory data and the analysis of antimicrobial susceptibility test results [8]. Categorical variables have been mentioned in numerical and percentages.

Results

A total of 697 pus and wound swab samples were received at the laboratory during the study period. 302/697[43.3%] samples showed significant growth of a single organism. The majority of the patients were in the age group of 40-60 years [52.3%] and a male predominance was observed [67.5%]. (Figure1) High culture positivity was observed among the males in comparison to females with the male: female ratio being 2.02:1 Pus aspirates 268 [78.1%] followed by wound swabs 34 [11.25%] yielded the highest positivity rates. The most common isolate observed was *Escherichia coli* 70/302[23.1%] followed by *Staphylococcus aureus* 59/302[19.5%], *Klebsiella pneumoniae* (*K.pneumoniae*), 40/302[13.2%], *Pseudomonas aeruginosa* 34/302[11.2%], followed by *Coagulase negative staphylococcus* [CoNS], *Proteus mirabilis*, *Enterococcus spp*, *Acinetobacter baumannii*, etc.

The majority of the infections were caused by Gram-negative organisms, 197/302[65.2%] compared to Gram-positive organisms, 105/302[34.7%]. Among the Gram-negative organisms, the most common isolate was *Escherichia coli* 70/197 [35.5%] followed by *Klebsiella pneumoniae* (*K.pneumoniae*), 40/197 [20.3%]. Table 1 shows the distributions of gram-negative organism in various exudates and antibiotic profile described in table 2. Among the 197 Gram-negative isolates ESBL production was observed in 58 isolates [29.5%]. Of the

aforementioned isolates *E. coli* 23/70 [32.8%] and *K. pneumoniae* 24/40 [60%] were ESBL producers. Carbapenemase production was observed in 30.1% [59/197] of the isolates. 10/34 [29.4%] isolates of *Pseudomonas aeruginosa* were Carbapenemase producers and 16/34 [47.1%] were MDR strains.

Among the Gram-positive isolates, *Staphylococcus aureus* was the most common isolate 56.1% [59/105] followed by CoNS, 24/105[24.7%] and *Enterococcus species* 20/105[19%]. Table 3 shows the distribution of gram-positive bacteria in various samples. Antimicrobial susceptibility profile of gram-positive organisms isolated is depicted in Table 4. Methicillin Resistance was observed in only 49.1% [29/59] of *Staphylococcus aureus* isolates whereas 50% [13/26] of CoNS were resistant to Methicillin. All the *Staphylococcus aureus*, CoNS and *Enterococcus species* were sensitive to Vancomycin.

Discussion

Pyogenic infections are one of the most common and serious complications among hospital-acquired infections. Wound infection can increase the length of hospital stay and accounts for the mortality rate up to 70–80% [9]. In the present study, 43.3% of samples showed significant growth of single organisms which was similar to the study by Salu rai et al, [49%] [10] and Shreshta et al, [50%][11]. In the study conducted by SM Regmi et al [12], wound infections were predominant in the age group of 18- 40 years whereas in the present study majority of the patients were in the age group of 40-60 years. Duwadi et al reported infection was most commonly seen in the age group 51 to 60, which is in concordance with this study [13]. In the present study, a male predominance [67.5%] was observed. The study by Masaadeh and Jaran reported predominance of pyogenic infections in males [52.2%] male which is similar to this study [14]. The male: female ratio was skewed towards male predominance [2.02:1], these findings are similar to a study conducted by Sudhaharan S et al [15] where the males to female ratio were found to [M: F-2.5:1].

A higher prevalence of Gram-negative isolates [64.9%] was observed compared to Gram-positive organisms [34.7%] in the present study. Similar findings were observed in the study conducted by M.D Wadekar et al [16] and Swathi Duggal et al [17]. Enterobacteriaceae were the most predominant organisms to be isolated. The most of the GNB were isolated from surgical site infections in cases of intra-abdominal surgeries [fasciotomies, meshplasty for inguinal hernia repair, intestinal obstructions], infected colostomy sites, and cellulitis of the lower limb. Out of the gram-negative bacilli, *E. coli* was the predominant isolate [23 %] followed by *K.pneumoniae* [13.2%]. Among *E.coli* 23 [32.8%] and among *K.pneumoniae* 24 [60%] were ESBL producers. In another South Indian study, 12 [40%] isolates of *Escherichia coli* and 4 [23.5%] isolates of *K.pneumoniae* were found to be ESBL producers. [18].

23 [57.5%] and 31 [44.3%] of the isolates of *K.pneumoniae* & *E.coli* respectively were found to be MDR strains. Resistance in these aforementioned bacteria was predominantly seen towards 3rd generation cephalosporins, β lactamase inhibitors, and fluoroquinolones. These rates of resistance were comparable to studies done by Fatima S et al, Trojan et al and Rijal B et al [18-20]. The reason

for this trend of increasing multidrug resistance may be due to selective pressure resulting from the rampant use of these antibiotics during the preoperative period that was continued post-surgery. A combination of meropenem and amikacin was given with or without colistin in most of the MDR cases. Few complicated intraabdominal infections were treated with ceftazidime avibactam.

10/34 [29.4%] isolates of *Pseudomonas aeruginosa* especially from cases of cellulitis and diabetic foot were Carbapenemase producers 16/34 [47.1%] were MDR strains which is in concordance with a study done by Sudhaharan S et al documenting MDR strains to be 47.2% [15]. Piperacillin-tazobactam with or without clindamycin was given in such cases. The 2nd most common isolate after *E. coli* in the present study was *Staphylococcus aureus* [19.5%]. This is similar to a study by Wadekar et al where *S. aureus* was isolated in 22.9% of samples [16]. Among gram-positive pathogens, *Staphylococcus aureus* [56.1%] was commonly isolated followed by CoNS[25%] and Enterococci[19%] which correlates with the study done by Kumari PH et al[21]. Among Gram-positive isolates, the Methicillin-Resistant *Staphylococcus* was observed in 49.1 % of the isolates which was also slightly higher than the studies conducted by Sanjana et al (39.6%) [22] and Kshetry et al (37.6%) [23]. All the isolates were sensitive to vancomycin. Most of the MRSA cases were responsive to intravenous vancomycin 1g 12th hourly or Tab Linezolid 600mg 12th orally.

Conclusion

This study highlights the need to understand the flora of organisms isolated from wound infections which may vary geographically and their susceptibility pattern as it aids in starting the empirical therapy of patients based on antibiotic susceptibility patterns at the institute. Strict implementation of antimicrobial stewardship with a routinely updated antibiogram along with proper infection control measures may help in decreasing the burden of infections with various resistant organisms in the era of challenges faced due to the globally increasing antimicrobial resistance.

Funding - Did not receive any funding

Conflict of interest – none

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Table 1
Distribution of Gram negative organisms in various samples

Gram negative bacilli	Pus	Wound swabs	Total Number=197	Percentage
<i>Escherichia coli</i>	62	8	70	35.5%
<i>Klebsiella pneumonia</i>	35	5	40	20.3%
<i>Pseudomonas aeruginosa</i>	26	8	34	17.3%
<i>Proteus mirabilis</i>	22	3	25	12.7%
<i>Acinetobacter baumannii</i>	16	2	18	9.1%
<i>Citrobacter</i>	5	0	5	2.5%
<i>Enterobacter</i>	4	0	4	2.0%
<i>Serratia marcescens</i>	1	0	1	0.5%

Table 2
antibiotic susceptibility of gram-negative bacilli [Enterobacteriaceae & Non – fermenters]

No. of isolates	AM P%	AMC %	CX M%	CA Z%	CR O %	FE P%	CZ A%	CIP %	IPM %	ME M%	TZ P %	GEN %	AMK %
<i>Escherichia coli</i> [70]	0.1	55.7	34.2	54.2	55.7	55.7	97.1	17.1	74.2	74.2	62.8	78.5	84.2

Cotrimoxazole- CLI- Clindamycin, ERY – Erythromycin, LNZ- Linezolid, VAN- Vancomycin, TEC- Teicoplanin, TCY- Tetracycline.

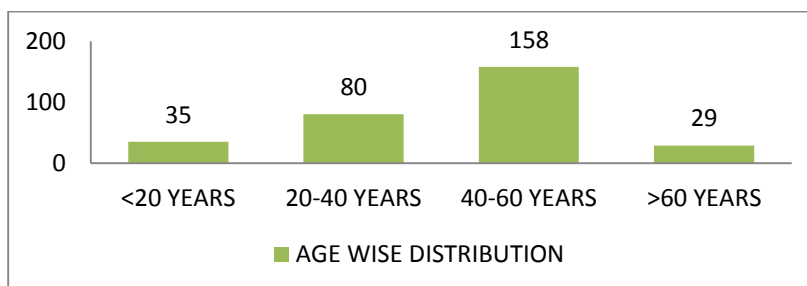


Figure 1. Age wise distribution amongst culture positive samples.