

Evaluation of bacteriological profile and their antibiogram from pus culture isolates in a tertiary care hospital

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Abstract: *Background:* Pyogenic infections refer to infections that leads to formation of pus. Selection of an effective antibiotic has a great role in reducing the morbidity and mortality among such infections. The awareness of the microbial profile causing pyogenic infections and their antibiogram is crucial for its management. *Objective:* To identify the bacteria, isolated from pus culture of suspected pyogenic infections, received in this hospital and to determine their antimicrobial susceptibility pattern. *Materials and Methods:* In this hospital-based retrospective study, a total of 1040 pus samples were subjected to culture according to standard microbiological procedure. Bacterial isolates were identified using conventional biochemical tests. Antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method and interpreted as per CLSI guideline. *Results:* Out of these 1040 samples, 59.4% were culture positive. Most of the positive samples were from Surgery Department (39.2%). The most common isolate was *Staphylococcus aureus* 39%. Overall, the Gram-positive bacteria demonstrated higher susceptibility to Doxycycline, Linezolid and Vancomycin. Whereas, the Gram-negative bacteria showed good susceptibility to Aztreonam, Carbapenems, and Piperacillin/Tazobactam. *Conclusion:* The study provides an insight into the diverse bacterial etiology of pyogenic infections, and antibiogram revealed varying degree of resistance to some commonly used antibiotics.

Keywords: Pyogenic Infection, Pus Culture, Bacterial Profile, Antimicrobial Susceptibility, Antibiotic-Resistant.

Introduction

Pyogenic infections refer to infections that leads to formation of pus. It is a collection of dead tissues, cellular debris, white blood cells and the microorganisms. It is considered among the major infective syndromes. These may be monomicrobial or polymicrobial. The infection results from invasion and multiplication of pathogenic microorganisms due to breach in the continuity of the anatomical barrier of skin. It may result after any trauma, bite wound, burn, gunshot injury or any surgical procedure [1].

In severe cases, these infections may spread to other sites by hematogenous route and may result in sepsis. It is characterized by local inflammation of skin, soft tissue and bodily parts. Pus is considered among the common clinical samples that may be collected from infections in

skin, soft tissues, surgical site infections, abscess, osteomyelitis, septic arthritis, otitis media [2]. The most common pyogenic bacteria include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococci species*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus species*, *Pseudomonas aeruginosa*, *Citrobacter species*, *Acinetobacter species* [3-4].

Selection of an effective antibiotic has a great role in reducing the morbidity and mortality among such infections. However, one of the major concerns in the management is the evolution of antibiotic-resistant strains due to inadvertent and inappropriate use of antibiotics. This in turn leads to long hospital stay, other medical complication and rise in the healthcare expenses. Although time to

time various studies are conducted across the country to determine the bacterial profile by culture and their antimicrobial susceptibility pattern in pus samples, but the pattern may vary from place to place.

This emphasizes the need for awareness of the regional microbial profile causing pyogenic infections and their antibiogram, which is crucial in determining the empirical therapy and further management of such condition. Therefore, the present study was undertaken to detect and identify the bacteria, isolated from pus culture of suspected pyogenic infection cases received in the department of Microbiology, from various clinical departments of Tezpur Medical College and Hospital and to determine the antimicrobial susceptibility pattern among the isolated bacteria.

Material and Methods

This hospital based retrospective study was conducted in Department of Microbiology, Tezpur Medical College and Hospital, Assam, between January 2023 to December 2023. The study was approved by Institutional ethic committee (IEC Sl.no: 2024/063/TMC&H). A total of 1040 pus samples of suspected pyogenic infection cases received at Microbiology laboratory from various out patient and inpatient department of this tertiary care hospital were included in the study.

Inclusion criteria: All the properly labelled, aseptically collected pus samples, including all age group and irrespective of gender, collected from suspected pyogenic infection cases received at Microbiology laboratory were included in the study.

Exclusion criteria: Sample collected in unsterile container and duplicate samples from the same patient were excluded from the study.

Sample collection: Pus samples were collected with sterile disposable cotton swab stick or aspirated by sterile syringe. The aspirated samples were collected in sterile leak-proof container. The collected samples were transported immediately to the laboratory without delay and processed immediately. All specimens were subjected to direct microscopy by gram stain and culture was done by inoculating the pus sample onto Blood agar media and MacConkey agar

media which were incubated for 24-48 hours at 37°C under aerobic condition. Bacterial isolates were identified based on colony morphology, gram stain, motility test, and conventional biochemical tests following standard laboratory techniques. Antibiotics susceptibility testing was done by Kirby-Bauer disk diffusion method using Mueller Hinton agar CLSI M100 guideline 2023.

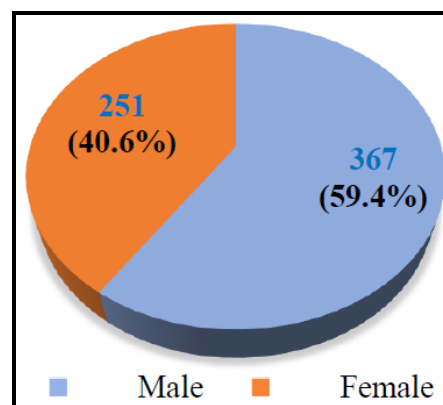
Gram negative isolates were evaluated for susceptibility to Amikacin, Gentamycin, Ciprofloxacin, Ofloxacin, Levofloxacin, Cotrimoxazole, Cefotaxime, Ceftazidime, Imipenem, Meropenem, Piperacillin/Tazobactam, and Aztreonam. Whereas, the Gram- positive isolates were tested against Ampicillin, Erythromycin, Clindamycin, Ciprofloxacin, Ofloxacin, Levofloxacin, Cotrimoxazole, Clindamycin, Doxycycline, Vancomycin, and Linezolid. Cefoxitin disc (30µg) was used to detect the isolates of Methicillin resistance *Staphylococcus aureus* (MRSA). Interpretation of the susceptibility testing was done according to CLSI M100 guideline 2023. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as quality control strain.

Statistical Analysis: Data were entered in Microsoft Excel sheets, followed by analysis and interpretation of the data. Data has been presented by appropriate tables.

Results

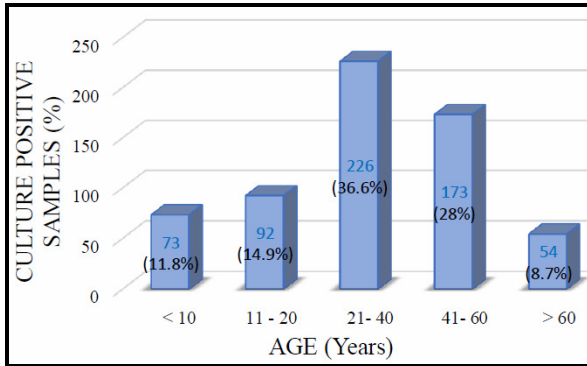
Of the 1040 pus samples received for bacterial culture, 589 (56.6%) were from male and 451 (43.4%) were from female patients.

Fig-1: Gender wise distribution of culture positive samples (%).



Out of these 1040 samples, 618 (59.4%) showed positive culture and remaining 422 (40.6%) showed no bacterial growth. Among the positive samples, 367(59.4%) were from male, whereas 251 (40.6%) were from female patients (Fig-1).

Fig-2: Age wise distribution of culture positive samples (%).



Most of the positive samples were from age group 21-40 years, which accounts for 226 (36.6%) followed by 176 (28%) from 41-60 years of age (Figure 2). The highest number of culture positive cases were from Surgery Department with 242 (39.2%), followed by ENT with 121 (19.6%), Medicine with 114 (18.4%) and Orthopaedics with 76 (12.3%). Department wise distribution of positive cases is given in Table 1.

Departments	Culture positive samples (%)
Surgery	242 (39.2%)
ENT	121 (19.6%)
Medicine	114 (18.4%)
Orthopaedics	76 (12.3%)
Paediatrics	34 (5.5%)
Gynaecology	19 (3.1%)
Dermatology	12 (1.9%)

Of the 618 positive isolates, Gram-positive bacteria accounts for 257(41.6%) and Gram-negative bacteria 361(58.4%). Among these Gram-negative bacteria, non-fermenting Gram-negative bacilli (NFGNB) accounts for 104 (29.4%). The most common isolate was *Staphylococcus aureus* 241 (39%). This was followed by *Klebsiella pneumoniae* 131 (21.2%), *Pseudomonas aeruginosa* 95 (15.4%), *Escherichia coli*, 78 (12.6%). The less frequent

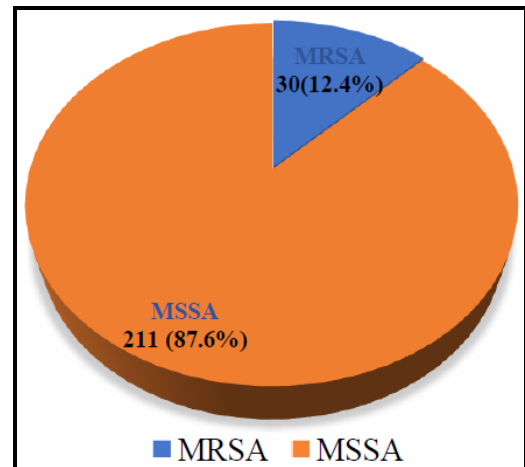
isolates include *Proteus vulgaris* 21(3.4%), *Enterococcus species* 16 (2.6%), *Citrobacter species* 13 (2.1%), *Proteus mirabilis* 12 (1.9%), *Acinetobacter species* 11 (1.8%) (Table 2).

Bacterial isolates	Total (%)
<i>Staphylococcus aureus</i>	241 (39%)
<i>Klebsiella pneumoniae</i>	131 (21.2%)
<i>Pseudomonas aeruginosa</i>	95 (15.4)
<i>Escherichia coli</i>	78 (12.6%)
<i>Proteus vulgaris</i>	21 (3.4%)
<i>Enterococcus species</i>	16 (2.6%)
<i>Citrobacter species</i>	13 (2.1%)
<i>Proteus mirabilis</i>	12 (1.9%)
<i>Acinetobacter baumannii</i>	11 (1.8%)
Total	618 (100%)

Footnotes: Among the 241 isolates of *Staphylococcus aureus*, 30 (12.4%) were Methicillin resistant *Staphylococcus aureus*

Among the isolates of *Staphylococcus aureus*, 30 (12.4%) were Methicillin resistant *Staphylococcus aureus* (Figure 3).

Fig-3: Distribution of MRSA and MSSA among the isolates of *Staphylococcus aureus*



The isolates of *Staphylococcus aureus*, were 95.7% susceptible to Doxycycline, Linezolid (94.8%), Clindamycin (88.7%), and Levofloxacin (85.2%). A higher resistance rate was observed against Ciprofloxacin (65%) and Erythromycin (56.7%).

Enterococcus species were 100 % susceptible to Linezolid and Levofloxacin followed by Vancomycin (76.9%), Ampicillin (75%), and

Doxycycline (50%). A higher resistance was observed against Ciprofloxacin (66.7%) (Table 3).

Table-3: Antibiotic susceptibility pattern among Gram positive bacteria from culture positive cases

Antibiotics	<i>Staphylococcus aureus</i>		<i>Enterococcus species</i>	
	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)
Ampicillin	78.6 %	21.4	75.0 %	25%
Ciprofloxacin	35.0%	65	33.3 %	66.7%
Clindamycin	88.7 %	11.3	-	-
Cotrimoxazole	50.0%	50	-	-
Doxycycline	95.7 %	4.3	50.0%	50%
Erythromycin	43.3 %	56.7	-	-
Levofloxacin	85.2 %	14.8	100 %	0
Linezolid	94.8 %	5.2	100 %	0
Ofloxacin	53.1%	46.9	-	-
Vancomycin	-	-	76.9 %	23.1%

Table-4: Antibiotic susceptibility pattern among Gram negative bacteria from culture positive cases

Antibiotics	<i>Klebsiella pneumoniae</i>		<i>Escherichia coli</i>		<i>Proteus vulgaris</i>		<i>Citrobacter species</i>		<i>Proteus mirabilis</i>	
	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)
Amikacin	63.5%	36.5%	73.7%	26.3%	75.0%	25.0%	71.4%	28.6%	50.0%	50.0%
Aztreonam	90.0%	10.0%	93.0%	7.0%	96.4%	3.6%	80.0%	20.0%	97.0%	3.0%
Cefotaxime	29.3%	70.7%	41.8%	58.2%	71.4%	28.6%	45.5%	54.5%	54.4%	45.6%
Ceftazidime	40.0%	60.0%	60.0%	40.0%	66.7%	33.3%	50.0%	50.0%	100%	0
Ciprofloxacin	48.9%	51.1%	62.5%	37.5%	83.3%	16.7%	50.0%	50.0%	66.7%	33.3%
Cotrimoxazole	33.3%	66.7%	66.7%	33.3%	37.0%	63.0%	48.0%	52.0%	33.0%	67.0%
Gentamycin	69.1%	30.9%	81.3%	18.7%	80.0%	20.0%	75.0%	25.0%	81.9%	18.1%
Imipenem	85.1%	14.9%	92.0%	8.0%	92.9%	7.1%	100%	0	83.3%	16.7%
Levofloxacin	65.7%	34.3%	70.4%	29.6%	80.0%	20.0%	66.7%	33.3%	66.7%	33.3%
Meropenem	83.6%	16.4%	90%	10.0%	100%	0%	71.4%	28.6%	100%	0
Ofloxacin	53.8%	46.2%	54.2%	45.8%	66.7%	33.3%	75.0%	25.0%	57.1%	42.9%
Piperacillin/tazobactam	55.6%	44.4%	75.5%	24.5%	93.8%	6.2%	44.4%	55.6%	83.3%	16.7%

Klebsiella pneumoniae isolates exhibited 90% susceptibility to Aztreonam, Imipenem (85.1%), Meropenem (83.6%), Gentamycin (69.1) and Piperacillin/Tazobactam (55.6%). Around 66.7% resistance was observed against Cotrimoxazole and 70.7% against Cefotaxime. Isolates of *Escherichia coli* were 93 % susceptible to

Aztreonam, followed by Imipenem (92%), Meropenem (90%), Gentamycin (81.3%) and Piperacillin/Tazobactam (75.5%). A higher resistance was observed against Cefotaxime (51.9%). Both the isolates, *Proteus vulgaris* and *Proteus mirabilis*, were 100% susceptible to Meropenem, in addition, *Proteus mirabilis*

is also 100% susceptible to Ceftazidime. This is followed by 96.4% susceptibility to Aztreonam, Piperacillin/ Tazobactam (93.8%), Imipenem (92.9%), Ciprofloxacin (83.3%) among the isolates of *Proteus vulgaris*. Whereas, the isolates of *Proteus mirabilis*, showed susceptibility of 97% against Aztreonam, 83.8% for both Piperacillin/ Tazobactam and Imipenem, and 81.9% against Gentamycin.

Both the isolates, *Proteus vulgaris* and *Proteus mirabilis*, showed resistance rate of 63% and 67% respectively, to Cotrimoxazole. The isolates of *Citrobacter species* showed 100 % susceptibility to Imipenem followed by Aztreonam (80%),

Gentamycin (75%), while 52% resistance to Cotrimoxazole, 54.5% to Cefotaxime, and 55.6% to Piperacillin/Tazobactam (Table 4).

Among the non-fermenting Gram-negative bacilli (NFGNB), *Pseudomonas aeruginosa* showed susceptibility to Piperacillin/ Tazobactam (92.2%), Imipenem (94.3%), Levofloxacin (90%), whereas 53.8% resistant to Ceftazidime. The isolates of *Acinetobacter species* were 80% susceptible to Piperacillin/ Tazobactam, Imipenem (75%), and Meropenem (66.7%), while showed 66.7% resistance to Gentamycin and 72% to Levofloxacin (Table 5).

Table-5: Antibiotic susceptibility pattern among Non -fermenting Gram-negative bacteria from culture positive cases

Antibiotics	<i>Pseudomonas aeruginosa</i>		<i>Acinetobacter species</i>	
	Sensitivity (%)	Resistance (%)	Sensitivity (%)	Resistance (%)
Amikacin	78.6%	21.4%	50.0%	50.0%
Aztreonam	82.6%	17.4%	-	-
Cefepime	68.2%	31.8%	-	-
Ceftazidime	46.2%	53.8%	50.0%	50.0%
Ciprofloxacin	87.5%	12.5%	-	-
Cefotaxime	-	-	45.5%	54.5%
Gentamycin	-	-	33.3%	66.7%
Imipenem	94.3%	5.7%	75.0%	25.0%
Levofloxacin	90.0%	10.0%	28.0%	72.0%
Meropenem	79.4%	20.6%	66.7%	33.3%
Ofloxacin	82.6%	17.4%	-	-
Piperacillin/ Tazobactum	92.2%	7.8%	80.0%	20.0%

Discussion

In the present study, among all the samples, 59.4% showed positive bacterial culture which is comparable to the 58.28% positivity in a study conducted by Rajput K. *et al.* and 60.55% by Kumar M. *et al.* [5-6].

Infection rate was found to be high among males than females which corresponds to the findings of study conducted by Mohanasundari C. *et al.* [7], where higher rate among males was reported. Similarly, various other studies reported that maximum culture positive cases were male [1, 3, 8]. In this study, the higher proportion of pyogenic infections in males may be attributed to their more involvement in outdoor activities,

occupational exposure, more road traffic accidents or may be due to the health care seeking behaviour among them.

Most of the positive samples were from age group 21-40 years, followed by 41-60 years of age, which is comparable to the study conducted by Sejal R. *et al.* [9], with maximum positivity in the 21-40 years, and 41-60 years of age. However, another study by Kursheed F. *et al.* [10], reported higher positivity in 41-60 years and in 61-80 years. The individuals between 20 to 60 years of age, is usually the physically active group, who involves in various outdoor activities, thus making them vulnerable for injury and infections.

The highest number of culture positive cases were from Surgery Department, which could be due to increase number of trauma wounds and surgical site infections. This was followed by ENT, Medicine and Orthopaedics. The humid climate of this region of the country, may provide a favourable condition for upper respiratory tract infections and ear infections, thus contributing to a significant number of culture positivity from ENT department. Likewise, increase in underlying disease conditions like Diabetes mellitus usually leads to nonhealing ulcers or pyogenic infections resulting in increased number of positive culture results from non-surgical sections like Medicine department. Similar finding was reported by Dugga S.*et al.* which showed the same distribution patter from various wards as found in this present study [11].

In this study, Gram-negative bacteria were the predominant isolates (58.4%), whereas Gram-positive bacteria accounts for 41.6%. Among the Gram-negative bacteria, non-fermenting Gram-negative bacilli (NFGNB) accounts for 29.4%. The higher rates of gram-negative bacteria in pus culture were reported in many other studies conducted previously [3, 6, 8]. This may be due to their widespread presence of Gram-negative bacteria in the hospital environment, on surfaces, water system and medical equipments.

The most common isolates reported in this study was *Staphylococcus aureus*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*. Similar distribution of isolates was reported in a cross-sectional prospective study by Sajjanar V. *et al.* [12], with *Staphylococcus aureus* as the predominant isolate followed by other gram-negative bacilli as isolated in our study. This may be attributed to the fact that, *Staphylococcus aureus* is a colonizer of anterior nares and skin, that serve as reservoir of infection in individual with damaged skin, thus resulting in pyogenic infection.

Among the isolates of *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* accounts for 12.4% which is similar to the findings of Kalita JM. *et al.* [2]. Contrary to this, some other studies reported higher rates of MRSA isolates [10, 13]. The variation in the rates of MRSA may be due to the different infection

control practices across different hospitals, that results in different transmission rates.

In this study, isolates of *Staphylococcus aureus* showed good susceptibility to Doxycycline, Linezolid followed by Clindamycin and Levofloxacin indicating that these drugs can be the therapeutic option against pyogenic infection due to *Staphylococcus aureus*, as well as treatment of MRSA. The resistance rate was higher among the commonly used fluoroquinolone (Ciprofloxacin) and Macrolide (Erythromycin). Similar susceptibility pattern was reported in a prospective study by Khan RA. *et al.* [14].

All the isolates of *Enterococcus species* were susceptible to Linezolid and Levofloxacin which makes them highly effective for its treatment. Also, higher level of susceptibility was seen against Vancomycin, Ampicillin, and Doxycycline, indicating that they still remain an effective therapeutic option. But a higher resistance was observed against Ciprofloxacin, that reflects increasing resistance against this fluoroquinolone.

The result is similar with the susceptibility pattern reported by Kursheed F. *et al.* [10], with the exception that resistance is higher for Ampicillin, whereas our study reported a good susceptibility. The higher resistance of *S. aureus* against Ciprofloxacin, as well as Erythromycin and *Enterococcus species* against Ciprofloxacin may be due to the widespread and frequent use of these antibiotics as empirical therapy, resulting in an increased selective pressure on bacterial population.

Isolates of *Klebsiella pneumoniae* and *Escherichia coli* were highly susceptible to Aztreonam, indicating the potential effectiveness of monobactams in the management of such infections. Similarly, a study by Sajjanar V. *et al.* [12], reported a higher susceptibility of *Klebsiella pneumoniae* to Aztreonam, but among the isolates of *Escherichia coli* resistance rate was higher. Another study showed a contrasting result, where susceptibility against Aztreonam is significantly lower than our finding. In our

study, a good susceptibility was found against Carbapenems (Imipenem and Meropenem), Gentamycin and Piperacillin/Tazobactam in both the isolates. However, a reduced susceptibility was observed against Cotrimoxazole and Cefotaxime among the isolates of *Klebsiella pneumoniae* and a reduced susceptibility against Cefotaxime among the isolates of *Escherichia coli* was found. These findings are in concordance with a study by Deboral A. *et al.* [8]. A higher resistance rate of Cefotaxime against *Klebsiella pneumoniae* and *Escherichia coli* were reported by Sajjanar V. *et al.* [12].

All the isolates of *Proteus vulgaris* and *Proteus mirabilis*, were susceptible to Meropenem, in addition, *Proteus mirabilis* is also 100% susceptible to Ceftazidime. The isolates showed better susceptibility to Aztreonam, Piperacillin/Tazobactam and Imipenem, Ciprofloxacin, and Gentamycin. Similar findings were reported by Bhandari BS. *et al.* [15] and Deboral A. *et al.* [8]. Both, *Proteus vulgaris* and *Proteus mirabilis*, showed higher resistance to Cotrimoxazole, which is comparable to the findings of study conducted by Kursheed F. *et al.* [10]

The isolates of *Citrobacter species* showed 100 % susceptibility to Imipenem, followed by Aztreonam and Gentamycin. However higher resistance against Cotrimoxazole, Cefotaxime, and Piperacillin/Tazobactam was found. In contrary, some other studies reported higher susceptibility to Piperacillin/Tazobactam and Cefotaxime [16].

Among the NFGNB, *Pseudomonas aeruginosa* showed higher susceptibility to Piperacillin/Tazobactam Imipenem, Levofloxacin, whereas higher resistant was found against Ceftazidime, which is comparable to the study of Khanapara P. *et al.* [13] except for Levofloxacin which showed lower susceptibility rate compared to our study. The isolates of *Acinetobacter species* showed good susceptibility to Piperacillin/Tazobactam followed by Imipenem, and Meropenem, whereas higher resistance was observed against Gentamycin and Levofloxacin. Similar finding was reported by Sajjanar V. *et al.* [12] except Gentamycin, which showed 100% susceptibility as compared to our study. Contrary to our study, some studies reported higher resistance rate to multiple antibiotics [8,13].

Overall, the Gram-positive bacteria demonstrated higher susceptibility to Doxycycline, Linezolid, Clindamycin, Levofloxacin, and Vancomycin. Whereas, the Gram-negative bacteria showed good susceptibility to Aztreonam, Carbapenems (Imipenem and Meropenem), and Piperacillin/Tazobactam. Resistance was commonly observed against Cotrimoxazole, Cefotaxime, and Ceftazidime. The variations in the susceptibility pattern of the various antimicrobials may be due to the different regional antibiotic prescribing practices, antibiotic usage pattern, empirical therapy used, and infection control practices. These variations in the susceptibility pattern of these antimicrobials highlights the need of regular surveillance of antimicrobial susceptibility to guide the treatment.

However, the present study has certain limitations. Being a hospital-based study, the findings could not be generalised in the community level, as only patients seeking medical care were the part of the study population. Although, minimum inhibitory concentration (MIC) is more precise in determining the antimicrobial susceptibility, but due to resource constrain, it could not be performed. Additionally, our study has focussed only on aerobic bacteria, anaerobic bacteria were not included, which may not reflect the complete bacterial spectrum in pyogenic infections.

Conclusion

The study provides an insight into the diverse bacterial etiology of pyogenic infections, with *Staphylococcus aureus* being the most predominant organism followed by *Klebsiella pneumoniae*. The antibiogram revealed varying degree of resistance to some commonly used antibiotics, while certain antibiotics like Doxycycline, Linezolid, Vancomycin, Aztreonam, Carbapenems and Piperacillin/ Tazobactam still retained a good therapeutic efficacy against their respective bacterial isolates against which they are used.

Adherence to proper hand hygiene, hospital infection control practices, and prevention of irrational use of antibiotics are important measures to reduce the spread of resistant

organism. Periodic surveillance to determine the local bacterial profile and their antibiogram is the key to initiate an effective empirical therapy and to curb antimicrobial resistance, which is a major global public health concern.

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