ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 09 | September - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANTIBIOTIC RESISTANCE PATTERN OF BACTERIAL PATHOGENS ISOLATED FROM PUS IN A TERTIARY CARE HOSPITAL OF CENTRAL INDIA

Medical Microbiology

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ABSTRACT

Pyogenic infections are commonly caused by various microorganisms and mixed infections that require antibiotic therapy. Emergence of antimicrobial drug resistance among susceptible individuals due to injudicious use of antibiotics has made the treatment of wound infections very difficult. This study was undertaken to determine the antibiotic resistance pattern among aerobic bacteria isolated from pus samples. A total of 312 pus samples were received in the Department of Microbiology, GMCH Nagpur from January to March 2021. The pus isolates were identified and processed by standard bacteriological techniques. The Antimicrobial Susceptibility Testing (AST) was performed by Kirby-Bauer disc diffusion technique and the drugs used were in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines 2020.Out of total 312 pus samples, 182 (58.33%) yielded significant bacterial growth. The most prevalent pathogen isolated were Klebsiella pneumoniae 60 (19.23%) followed by Escherichia coli 42 (13.46%). Further, the study shows that Methicillin Resistant Staphylococcus Aureus (MRSA) and Inducible Clindamycin Resistance (ICR) were 29.41% and 11.76% respectively; moreover 70% isolates were Multi Drug Resistant (MDR). Therefore, this study indicates the prevalence of resistance to different classes of antibiotics in bacterial isolates and highlights the need for effective surveillance, regular reporting and antibiogram-guided antibiotic prescription.

KEYWORDS

Pus, MRSA, ICR, MDR

1. INTRODUCTION

Wound infection is one of the most common and serious complications among the hospital acquired infections [1]. The invasion of pyogenic bacteria during or after trauma, burn injuries and surgical procedures can result in inflammation and pus formation [2]. The defense mechanism of body recruits immune cells into the infection site to fight against the bacteria [3]. Accumulation of these cells produces pus, causing pyogenic infection which delays the wound healing process causing wound dehiscence or breakdown. Both aerobic and anaerobic bacteria have been implicated in wound infections which commonly occur under hospital environment and result in significant morbidity, prolonged hospitalization, and huge economic burden[4]. Rational use of antibiotics can hasten the patient recovery, prevents severe complications and misuse of broad-spectrum antibiotics [5]. However, the above-mentioned literature reported a narrow spectrum of data that would be unreliable to give a concrete conclusion regarding the number of pathogens that are becoming MDR.

2. Research Significance

Inadvertent and inappropriate use of antibiotic leads to emergence of drug resistant pathogen. So, background knowledge of local microbial profile and antibiogram of isolates causing wound infection will encourage the clinicians to treat wound infection empirically. The primary focus has been given to identify the pyogenic bacteria and to determine their antibiotic resistance pattern from pus samples. Therefore, the present study is constituted to fill the void and bridge the gap of knowledge left in the above-mentioned literature.

3. Experimental program

3.1. Test details

A total of 312 non replicate clinical and invasive isolates were obtained from pus samples derived from superficial wounds and deep-seated abscesses using standard bacteriological techniques and identified by conventional methods[6]. This observational study was conducted from January 2021 to March 2021.

3.2. Experimental method

The AST of these clinical isolates were determined by Kirby Bauer disc diffusion method and analyzed according to CLSI 2020 guidelines. The following AST methods were adopted for screening Staphylococcus aureus (MRSA and ICR) and detecting gram negative bacterial isolates [Extended spectrum ß-lactamases (ESBL)] [7].

I) MRSA: Cefoxitin disc (30ug) were used. Zone size ≤ 21mm shows resistance to Cefoxitin indicating MRSA[7].

ii) ICR: D-test :- Erythromycin (15ug/ disc) was placed at a distance of 15mm (edge to edge) from Clindamycin (2ug/disc) on Muller Hinton agar plate, previously inoculated with bacterial suspension equivalent to 0.5 McFarland standards and incubated overnight at 37 C. The flattening of zone (D-shaped) around Clindamycin in the area between the two discs, indicate ICR[7].

iii) ESBL: Combined disk diffusion method was Ceftazidime (30µg) disc alone and in combination with Clavulanic acid disc (30/10µg). The test organisms were inoculated on Muller Hinton Agar plate, discs were placed and incubated overnight at 37°C. The isolates showing zone of inhibition of Ceftazidime plus Clavulanic acid disc ≥ 5mm than those of Ceftazidime disc alone was interpreted as ESBL producers[7].

4. RESULTS AND DISCUSSION

It is observed that 182 pus samples (58.33%) yielded significant bacterial growth out of 312 pus samples (Fig. 1). The similar findings were reported with 60% growth by the Roopa.C *et al* [8]; however, Banker *et al* [9] have shown a much higher growth (78.5%). The reason can be attributed to suppurative infection of the skin, ear, and eye as the common occurrences between the hospitalized patients and the outpatient department. From Fig. 2, it can be noticed that 14.28% (26) of the samples were polymicrobial; whereas, Rahangdale *et al.* [10] reported 5% polymicrobial growth. Open wounds can get easily colonized, proliferated and invaded by numerous bacteria as they provide a warm and moist environment. This might be the reason for polymicrobial growth in few samples in our study.

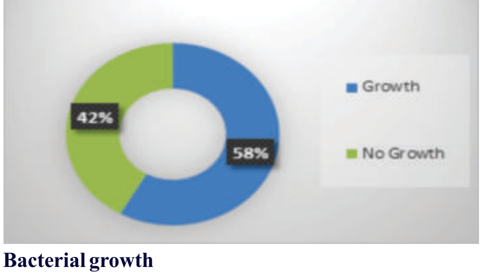

Fig. 1. Bacterial growth

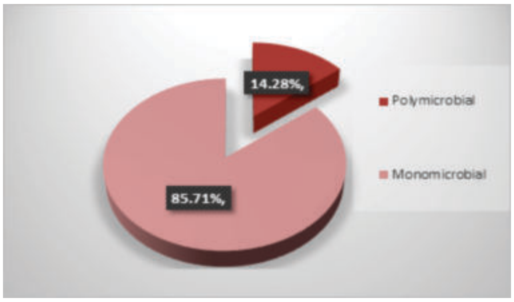

Fig. 2. Distribution of growth (n = 182)

From Fig. 3, the most prevalent gram-negative bacteria isolated were *Klebsiella pneumoniae* 60 (19.23%), followed by *Escherichia coli* 42 (13.46%), *Pseudomonas aeruginosa* 37 (11.85%), *Acinetobacter lwoffii* 22 (7.05%), *Acinetobacter baumannii* 19 (6.08%), and *Proteus mirabilis* 07 (2.24%). Khan *et al*.[11] also reported *Klebsiella pneumoniae* (36.2%) and *Escherichia coli* (16.5%) as the most common gram-negative isolates; moreover, Habsi *et al*.[12] reported the similar percentage of *Pseudomonas aeruginosa* (13.7%) in their study which is compared to the present study. *Acinetobacter* species (13.13%) observed in the present study were much higher compared to Rai *et al*.[13] (7%); They also reported similar percentage of *Proteus mirabilis* (3%) compared to the present study. Further, the gram-positive bacteria isolated in the present study was *Staphylococcus aureus* 17 (5.44%) that correlate with the findings of the Batra *et al.* [14] (10%).

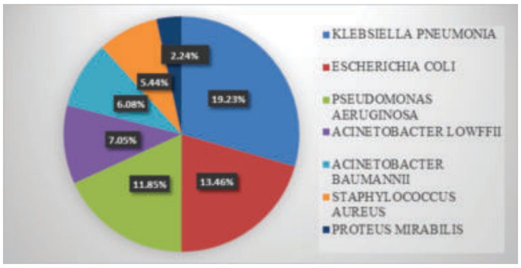

Fig. 3. Different Pathogens from Pus

Among Enterobacteriaceae, maximum resistance was shown against Ampicillin (98%), Cefazolin (95%) and Cefuroxime (94%) (Fig. 4). The similar results were also shown by Biradar *et al* [15]. The least resistance was shown against Gentamicin (50-65%) & Amikacin (50-60%) that is supported by the findings of Singh *et al*. [16] (55%).

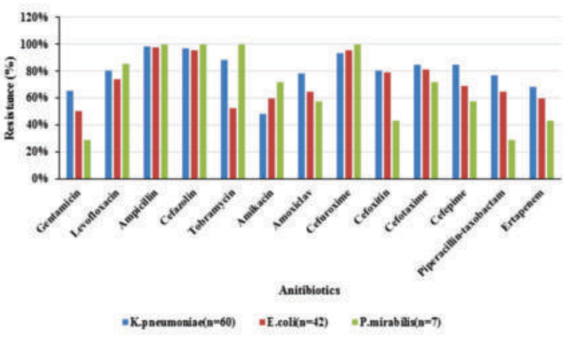

Fig. 4. Antibiotic Resistance Pattern of Enterobacterales

Among non-fermenters, it can be observed from Fig. 5 that 51.35% of *P.aeruginosa* have shown the higher resistance against Gentamicin, Tobramycin, Ceftazidime and Netilmicin. The similar observations were also reported by Hosimin *et al* [17]. The least resistance was shown by *P.aeruginosa* against Astreonam (24%), Ceftazidime (29.72%) in present study; however, Bhatnagar *et al*. [18] reported 39.8% and 46% effectiveness of Astreonam and Ceftazidime, respectively to *P.aeruginosa*. Acinetobacter species have shown maximum resistance against Cefepime (90%), Ceftazidime (90%) and Gentamicin (85%); whereas, the least resistance was shown against Minocycline (25%). The above observations were also noted by Rahangdale *et al*. [10].

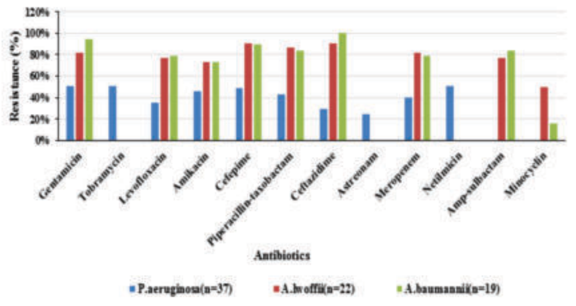

Fig. 5. Antibiotic Resistance Pattern of Non-Fermenters

For *S. aureus*, maximum and least resistance have been observed against Penicillin (100%) and Gentamycin (23%) respectively. We found 29% of *S. aureus* to be resistant against Linezolid & Doxycycline (Fig. 6). These results are in good agreement with the results reported by Biradar *et al* [15] & Bhatnagar *et al*[18]. Naimi *et al.* [19] reported 77% sensitivity of *S. aureus* to Doxycycline and Batra *et al*. [14] observed 100% sensitivity of *S. aureus* to Linezolid.

As shown in Table 1, the ESBL production of Enterobacterales was found to be 9.8% in the present study. Previous studies conducted in India have reported the prevalence of the ESBL producers to be 6.6% to 53%[10]

Table 1: ESBL Production in Enterobacterales

Number of isolates	Number of ESBL producing isolates	Percentage (%)
K.PNEUMONIAE (n=60)	5	8.33
E.COLI (n=42)	5	11.90
TOTAL (n=102)	10	9.8

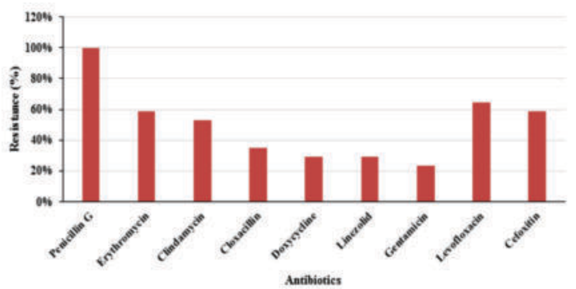

Fig. 6. Antibiotic Resistance Pattern of Staphylococcus aureus

Table 2 shows that 29.41% of *S. aureus* were found to be MRSA in this study, which is in concordance with Biradar *et al*. [15] (26.9%). Various studies conducted in India also reported the MRSA rate between 20 and 45% [10]. Also, in the present study, 11.76% of *S. aureus* were ICR; whereas, 22.58% of ICR were reported in the study of Rahangdale *et al* [10].

Table 2: Screening of Staphylococcus aureus (MRSA and ICR)

Staphylococcus Aureus (N=17)	Number	Percentage (%)
MRSA	5	29.41
ICR	2	11.76
MRSA + ICR	3	17.64

In this study, 70% isolates were MDR, which correlates with Muluye *et al.* [20] that reports 73% isolates were resistant to two or more drugs tested and 20.6% isolates were resistant to more than 5 antibiotics tested.

5. CONCLUSION

Klebsiella pneumoniae was the most common pathogen isolated in the present study. Majority of the isolates displayed resistance to commonly used antibiotics and were MDR, which suggests that the antibiotic sensitivity testing should be carried out of all isolates of wound infections before chemotherapy to avoid emergence of drug resistant strains.At the same time, strict health policies should also be implemented to regulate the purchase and prescription, and to restrict the unsupervised antibiotic use as well as continuous monitoring and reporting antibiotic resistance is recommended.

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