

# A Retrospective Study on the Pyogenic Pathogens and Their Antibiotic Susceptibility Patterns along with the ES $\beta$ L Production

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# Abstract

Pyogenic infections are caused by various pathogens leading to pus formation and that can be attributed due to a wound either through accident or during surgery leading to infection spread. There are pathogenic strains that are not uncommon in hospital settings like Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter etc., that are multidrug resistant (MDR) and are a cause of concern. The bacteriological profile in the pyogenic infections tends to be same but there is a vast difference with the antibiotic resistant patterns in different hospital settings. Hence, the aim was to study the antibiotic susceptibility profiles and Extended spectrum  $\beta$ eta Lactamases (ES $\beta$ L) production in these pathogens. A prospective study was carried out in Silchar Medical College and Hospital Assam, India, over a four-month period from February to May 2021. The samples were processed using Blood and MacConkey's agar. Further, these isolated pathogens were identified by standard morphological, cultural and biochemical tests. The antibiotic susceptibility test was conducted by Kirby Bauer disc diffusion method and  $ES\betaL$  production was detected by using combined disk diffusion test. It was observed that the identified pathogens had an incidence rate of 84.2% and further revealed that Gram negative had a higher incidence rate compared to Gram positive with 59.8%. The pathogens isolated from pus samples had a maximum of Klebsiella sps (19.64%) and the lowest was E. coli with 5.36%. Antibiotic susceptibility test (AST) of Gram-negative bacterial isolates showed the highest incidence with aztreonam (40.6%) and the lowest was observed in Piperacillin/Tazobactam with 7.5%. The only Gram positive

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was observed in our study, *Staphylococcus aureus* had the highest resistance in amikacin with 80% and interestingly, all the isolates were sensitive to Linezolid with 100%. There is a high rise and spreading with the multi-drug resistance (MDR) strains along with ES $\beta$ L production and it was observed in our studies that these pathogens had an incidence rate of 18.5%. The highest was 58.1% in *Pseudomonas sps*. None of *Proteus sps* were found to be ES $\beta$ L producers. To combat resistance, the irrational use of antibiotics should be avoided and surveillance of the rising multidrug species regularly helps in implementing better therapeutic options to reduce the morbidity and mortality.

## **Keywords**

Antibiotic Susceptibility Test, Pus, ES $\beta$ L, MDR

## **1. Introduction**

Infectious diseases are an important cause of morbidity and mortality in humans, particularly in developing countries like India. Microbial pathogens can enter the human skin and the soft tissues causing infections during trauma, burn injuries and surgeries that lead to pus formation which consists of white to yellow fluid consisting of dead WBCs, cellular debris and necrotic tissues [1] [2] [3]. It has been reported that the incidence rate of wound sepsis in India is from 10% to 33% [4]. The diversity and the causative agents are basically influenced by the predisposing factors like the anatomical location: type and level of perfusion in tissues and the efficacy of antimicrobial agents in host response [5]. These pathogens in hospital acquired tend to prolong the hospitalisation, increase morbidity and are a huge economic burden to the country [6]. Antibiotic resistance has emerged drastically and rapidly spreading by the pathogens that are a threat to the public health worldwide. Hospital acquired infections in pus have seen a steep rise with these MDR Gram-negative bacterial strains that include A. baumannii, E. coli, K. pneumoniae, P. aeruginosa, and Gram-positive methicillin-resistant S. aureus (MRSA) which is due to the inadequate dose and misperception of antibiotics [7] [8].

Pyogenic infections are significant as they encounter infectious diseases in hospitals worldwide that are associated with high morbidity and to reduce long term complications, the antimicrobial regimes are recommended [9]. The advancement in diagnostic treatment and treatment options in developing countries are further challenging due to the MDR strains that are fast evolving. There has been a rapid emergence of MDR Gram positive and Gram-negative bacterial isolates that are increasing with pyogenic infections in recent years [10]. The crisis of resistance among the pyogenic pathogens can be attributed due to the inappropriate use of antibiotic agents particularly in developing countries [11]. With the rapid emerging MDR strains and limited treatment options and no new discovery of new classes of antibiotics [12], the objective of this study is to characterize the bacterial pathogens from pus samples and to determine their antibiotic susceptibilities to various generations of antibiotics commonly used in chemotherapeutic interventions.

 $\beta$ -lactam antibiotics are the most common drugs that are used for the treatment of Gram-negative bacteria and due to their continuous misuse, have led to the resistance in these pathogens worldwide [13]. Further, these pathogens have induced mutations and continuous production of  $\beta$ -lactamases to counter the effects of these beta lactam drugs. There has been a significant increase in these ES $\beta$ L producers causing various infections worldwide [14] [15]. These ES $\beta$ L producers causing nosocomial infections have resulted in a steep rise in their incidence in recent years with high morbidity-mortality rates due to these  $\beta$ -lactamases resistant MDR strains. Rapid identification of ES $\beta$ L pathogens and their antibiotic resistance patterns will help the clinicians to select appropriate drug regimens like combination therapy and reduce their further spread [16].

## 2. Materials and Methods

**Sample Collection and Characterization**: The pus samples were collected by sterile syringe aspiration and by sterile swabs from inpatients and outpatients of different wards from Silchar Medical College and Hospital Assam, India, over a four-month period from February to May 2021. A total of 133 pus samples were collected over a period of three months from January 2021 to March 2021 with accordance to ethical guidelines. The pus samples were collected from different departments such as Orthopaedic, Surgery, ENT, Paediatric and Dermatology (furuncles, pustules and abrasions). The samples were maintained in Cary-Blair transport media until further processing. Isolation of the pathogens was carried on Blood agar (with 5% sheep blood), Chocolate agar and MacConkey's agar plates (Hi-Media, Mumbai, India), incubated aerobically at 37°C for 24 - 48 h. Further, clinical isolates were identified and characterised by microscopic, cultural characteristics and biochemical tests using standard microbiological methods.

#### Antibiotic Sensitivity Test (AST).

Antibiotics discs containing Amoxyclav (AMC-30  $\mu$ g), Ciprofloxacin (CIP-5  $\mu$ g), Clindamycin (CD-2  $\mu$ g), Cefoxitin (CX-30  $\mu$ g), Linezolid (LZ-10  $\mu$ g), Cotrimoxazole (COT-25  $\mu$ g), Azithromycin (AZM-15  $\mu$ g), Cefuroxime (CXM-30  $\mu$ g), Amikacin (AK-30  $\mu$ g), Cefixime (CFM-5  $\mu$ g), Piperacillin/Tazobactam (TZP-100/10  $\mu$ g), Levofloxacin (LE-5  $\mu$ g), Ceftriaxone (CTR-30  $\mu$ g), Cefepime (CPM-5  $\mu$ g), Aztreonam (AT-30  $\mu$ g), Cef0perazone (CPZ-75  $\mu$ g), Imipenem or Meropenem (IPM or MRP-10  $\mu$ g) were used for the studies. Antibiotic susceptibilities of the bacterial isolates were determined according to the method recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines. Briefly, inoculates were prepared for each bacterial isolate by adjusting the turbidity to 0.5 McFarland standard and spread on Muller-Hinton agar plates. Antibiotic discs (Hi-Media, Mumbai, India) were placed on the agar plates and incubated overnight at 37°C for 24 h. The zones of inhibition were measured and the isolates were classified as sensitive, intermediate, and resistant according to CLSI guidelines [17].

#### Phenotypic $ES\beta L$ detection:

Detection of ES $\beta$ L-producing organisms was performed by Double Disc Synergy Test (DDST) method following the CLSI recommendations. The test suspension was prepared for each pure bacterial isolate according to 0.5 McFarland standard that was swabbed on Mueller–Hinton agar. After 15 minutes the cultured plates were placed with pairs of antibiotic disks containing Cefotaxime (30 µg) with Cefotaxime/Clavulanic acid (30/10 µg) at a distance of 20 mm apart from each other. The plates were incubated for 24 h at 37°C. The results were interpreted by measuring the diameter of zone of inhibition. According to CLSI guidelines, an increase in ≥5 mm in the zone diameter around the clavulanic acid combination disks versus the same disks alone confirmed the organism as ES $\beta$ L producers.

#### **3. Results**

The distribution pattern of the collected pus samples on the basis of gender ratio showed that there were 82 male and 51 female patients. It was observed that the greater percentage of 62% was observed in males than females (38%). Out of the 133 samples processed 112 (89.2%) were found to be culture positive. The highest incidence was observed in the age group of 21 - 40 years with an incidence of 43.6%, followed by, 1 - 20 years with 23.3% and the low incidence was observed in the age group of 60 years and above with 12%.

Gram negative isolates had an incidence of 59.8% (67 isolates) while, Gram positive isolates were 40.18% (45 isolates). Our study revealed that the most predominant Gram negative pathogen was *Klebsiella sps* with an incidence rate of 19.64%, followed by *Pseudomonas sps* (15.17%), *Acinetobacter sps* 10.71%, *Proteus sps* 8.93% and *E. coli* 5.36%. Among the Gram positive bacteria, the most predominant pathogen isolated was *Staphylococcus aureus* with a rate of 40.18% (Figure 1).

Types of microbes isolated from pus sample

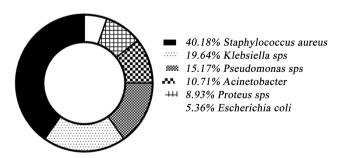


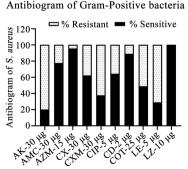
Figure 1. Percent incidence rate of different microbes isolated from pus samples.

The antibiotic susceptibility test results of *S. aureus*, the only Gram positive bacteria isolated, revealed a highest resistance rate to the antibiotic Amikacin with 80.4%, followed by Levofloxacin (71.32%), Cefuroxime (61.7%), Cotrimoxazole (53.3%), Cefoxitin (37.77%) and Ciprofloxacin (36%). While, Amoxyclav (22.2%), Clindamycin (11.11%) showed moderate resistance, and Azithromycin (4.4%) showed a low resistance rate. Interestingly, all the Gram positive isolates were sensitive to Linezolid with 100% (**Figure 2**).

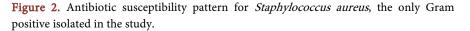
The AST of Gram negative bacilli revealed that a total of 40.6% showed a highest resistance rate to Aztreonam, followed by cefepime (30%), Levofloxacin (25.5%), Amikacin (20.3%), Ceftriaxone (17.2%), Cefaperozone (13.5%). A moderate resistance rate was observed with Imipenem/Meropenem (13.5%) and Cefixime (15.7%). Lowest was observed in Piperacillin/tazobactam with 7.5%.

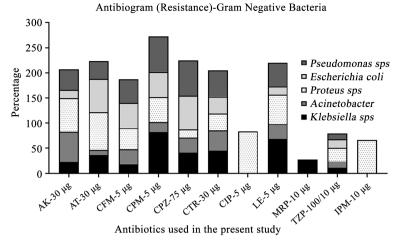
With respect to the different pathogens, AST results were found to be varying. The study showed that in case of *Klebsiella sps*, the highest resistance is observed with cefepime (81.82%) and lowest in Piperacillin/Tazobactam (9.09%) however, *Acinetobacter sps* showed highest with Ciprofloxacin (83.3%) and lowest in ceftriaxone (16.66%). *Proteus sps* were found to be resistant to amikacin antibiotic with a rate of 60% and all isolates were sensitive to and Meropenem. The results in *E. coli* showed resistant to Cefaperazone (66.66%) and sensitive to Meropenem with (100%). *Pseudomonas sps* showed higher resistant rate to some of the  $\beta$ -lactam antibiotics, the highest being to Cefaperazone (70.59%) and the lowest in Piperacillin/tazobactam (11.7%) as observed in **Figure 3** and **Figure 4** for all the Gram negative pathogens. The only Gram positive isolated from pus samples, *S. aureus* has a highest resistant to Amikacin with 79.24% and Linezolid showed no resistant.

The misuse of  $\beta$ -lactam drugs has given rise to MDR pathogens which is alarming situation worldwide. It has been observed that the overall incidence rate of ES $\beta$ L production with respect to the Gram negative pathogens was found to be 18.5%. The highest ES $\beta$ L producers were found to be 58.1% in *Pseudomonas sps*, followed by *Acinetobacter sps* (50%), *Klebsiella sps* (30.13%) and *E. coli* (33.3%). However, none of the *Proteus sps* were found to be ES $\beta$ L producers (**Figure 5**).

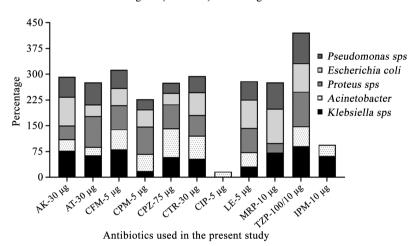


Antibiotics used in the present study



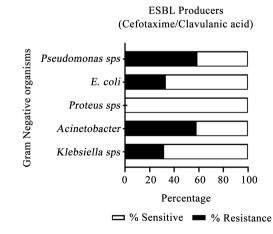


**Figure 3.** Antibiotic susceptibility pattern of Gram negative bacteria with respect to the resistance rate.



Antibiogram (Sensitive)-Gram Negative Bacteria

Figure 4. Antibiotic susceptibility pattern of Gram negative bacteria with respect to the sensitivity.



**Figure 5.** Percent incidence rate of  $\text{ES}\beta L$  producers in different pathogens isolated from pus samples.

### 4. Discussion

Pyrogenic infections that are characterised by local and systemic inflammations can be either due to single organism or multiple pathogens. The study revealed that 84.2% (112 samples) were positive for the isolation of pathogens from pus samples that was higher compared to the reports of [18]. The aetiological agents include Gram positive and Gram negative bacteria like *Staphylococcus aureus, Enterococci, Pseudomonas aeruginosa, Escherichia coli, Klebsiella sps and Proteus sps.* [19]. The study conducted includes both Gram positive and Gram negative bacteria (59.8%) that was almost in accordance with the other findings [20] [21].

The most common Gram negative pathogens isolated include *Klebsiella sps* (40.18%), followed by *Pseudomonas sps* (15.17%), *Acinetobacter sps* 10.71%, *Proteus sps* 8.93% and *E. coli* 5.36%. These are the most commonly found pathogens in hospital settings and are found to be MDR pathogens. However, there is a variation in the aetiological agents in different studies [20]. Among the Gram positive bacteria, the only and most predominant pathogen isolated was *S. aureus* with a rate of 40.18%. While, the studies showed by [20] and [22] had other strains like CONS and *Enterococci* which was absent in the present studies.

Several reporters have shown the implications by different microorganisms that include *Pseudomonas, Staphylococcus, Streptococcus, Klebsiella*, and *E. coli* in wound infections [7]. It has been very well noted that *S. aureus* and specially MRSA are the major cause of soft tissue infections in hospitalised settings [23].

The study revealed that most of the Gram negative isolated in the pus samples had the lowest resistance to Piperacillin/Tazobactam (7.5%) and Meropenem and Imipenem (13.5%) which is similar to the other reports [20]. The only isolated Gram-positive cocci, S. aureus was found to be totally sensitive to Linezolid and Vancomycin with 100% as compared to the results conducted with a lesser resistance rate by [24] and same as our study [24]. With the increasing antibiotic resistance with the pathogens, it becomes mandatory to select and prescribe the antibiotics with appropriate dosage and duration. Our study reported a high MDR in various organisms like E. coli, S. aureus, K. pneumoniae and P. aeruginosa isolated from pus samples. Therefore, it becomes utmost essential to formulate the antibiotic policies and control measures that are suitable and essential [26]. The study also revealed the Gram negative isolates were resistant to cephalosporins but were well susceptible to amikacin and imipenem as was observed in the other study too [25].  $\beta$ -lactamases are responsible for resistance to the  $\beta$ -lactam antibiotics and thus rendering these antimicrobials ineffective. The present study revealed  $\text{ES}\beta$ L producers with an incidence of 18.5%. The highest 58.1% in *Pseudomonas sps* and 100% susceptibility was seen in *Proteus sps*. [27].

## **5.** Conclusion

It has been observed that pyogenic infections are the most prevalent in the

hospital settings and MDR pathogens being *Staphylococcus aureus*, followed by *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, and others. These MDR pathogens exhibit different levels of antibiotic resistance to different classes of antibiotics. There also has been an alarming rise in the ES $\beta$ L producers by these pathogens and their spread throughout the world which is a serious problem. To combat the resistance by these microbes, it becomes necessary to stop the irrational use of antibiotics. There needs to be proper susceptibility data that can be used to implement hospital antibiotic policy. Strict health policies, continuous monitoring and reporting of antibiotic resistance will help in implementing better therapeutic drugs to combat diseases and reduce morbidity and mortality.

## **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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