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# Bacteriological profile and antibiogram of the gram negative clinical isolates from a tertiary care centre

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**Abstract:**

**Background:** Indiscriminate use of antibiotics has led to a state where multi drug resistant bacteria have become increasingly prevalent. The knowledge of antibiotic resistance patterns is necessary to stop the continued emergence of resistance. **Objective:** This study thus aims to provide the antibiotic resistance patterns of the gram negative clinical isolates. **Method:** Five hundred and seventy nine gram negative isolates from various clinical samples such as pus, urine, sputum, blood etc., were tested for their antibiotic sensitivity profiles. The clinical data was obtained from the respective units and wards of the patients. **Results:** Majority of the gram negative organisms isolated were found to be sensitive to Imipenem [9.28% resistant], Amikacin [20.84% resistant] and Piperacillin + Tazobactam [24.69% resistant]. Most of the gram negative organisms were resistant to the routinely used drugs, Norfloxacin [96.49%], Nitrofurantoin [94.92%], Carbenicillin [85.98%] and Amoxiclav [78.28%]. Conclusion: The results of the study conducted demonstrate the distribution and susceptibility pattern of the commonly used antimicrobial agents. The importance of strict antibiotic policies, evidence-based empirical therapies and rigorous surveillance programs to prevent the further spread of resistant bacteria cannot be overstated.

**Key words:** Antibiogram; Gram Negative Clinical Isolates; Imipenam; Klebsiella; Norfloxacin

## Introduction

In the past 60 years, antibiotics have been critical in achieving a dramatic rise in life expectancy and significant improvements in public health. However, disease-causing microbes have become increasingly resistant to the antibiotics commonly in use. It has been clearly shown that use of antimicrobials leads to selection of resistant strains both in the individual and in the community, and overuse or inappropriate use only increases this risk. History suggests that microbes will never run out of ways of developing resistance, but we may run out of effective antimicrobials.

The gram-negative enteric bacilli are common causes of a wide variety of infections involving diverse anatomic sites in both healthy and compromised hosts. In general, among adults, the incidence of infection due to these agents increases with age. Thus, as the mean age of the population increases, so will the number of these infections.

Drug resistance is a serious medical problem. We have noticed progressive increases in resistance to commonly used antibiotics with many GNBs being multidrug-resistant. The emergence of antibiotic resistance in the management of infections is a serious public health issue, particularly in the developing world where apart from high level of poverty and ignorance , there is also high prevalence of fake and spurious drugs of questionable quality in circulation [1]. This has led to a significant increase in morbidity.

There also appears to be a significant lack of studies highlighting the susceptibility patterns of locally prevalent organisms. . Knowledge of etiological agents of infections and their sensitivities to available drugs is of immense value to the rational selection and use of antimicrobial agents and to the development of appropriate prescribing policies [1]. Thus, this study aims to bridge the gap in knowledge and provide the clinician with the tools to provide safe and effective empirical therapy.

## Material and Methods

The study was conducted in the department of microbiology of a tertiary care centre for a period of 4 months. The clinical samples received from various departments of the hospital were included in the study. The samples were mainly pus, sputum, urine, blood, ET secretions and other body fluids such as ascitic fluid, pleural fluid, cerebro-spinal fluid that were sent to the lab for culture and sensitivity testing. The clinical data was obtained from the requisition forms and from the respective units and wards of the patient.

Standard operating procedures were used to collect the samples. The samples were inoculated on Nutrient agar, Mac Conkey's and Blood agar plates and incubated aerobically at 37 $^{0}$ C for 24 hours. The isolates were identified by their colonial morphology, gram staining and different biochemical reactions using standard techniques [2]. The antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method [3]. Gram negative bacilli were tested against Amikacin (30 mcg), Amoxiclav (20/10 mcg), Ampicillin-sublactam (10 mcg), Aztreonam (30 mcg), Ceftazidime (30 mcg), Ceftazidime/Clavulanic acid (30/10 mcg), Cefepime (30 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (5 mcg), Cotrimoxazole (25 mcg), Gentamicin (10 mcg), Imipenam (10 mcg), Piperacillin/Tazobactam (100/10 mcg), Carbenicillin (50 mcg), Netlimicin (10mcg) and Azithromycin(15 mcg).

Nitrofurantoin (300mcg) and Norfloxacin (10 mcg) were used for urine isolates. The results were interpreted according to standard CLSI criteria [4]. Control strains were used for checking the quality of discs and reagents.

### Statistical Analysis

The data was analysed and evaluated on the basis of percentage values and the results were presented in the form of tables and figures.

## Results:

From a total of 1942 different clinical samples processed, 777 (40.01%) organisms were isolated. 192 (24.71%) were Gram Positive isolates, 571(73.48%) Gram negative isolates (Table 1) and 14 (1.80%) were Candida species. Of the 73.48% of GNB isolated in the study, 30.82% were isolated from pus samples, 29.24% sputum and 25.56% were urinary isolates as in table 1.

**Table 1: Gram negative isolates from the clinical samples**

| | Sample | Number of Samples n =571 | Percentage |
|---|---|---|---|
| 1 | Pus | 176 | 30.82% |
| 2 | Sputum | 167 | 29.24% |
| 3 | Urine | 146 | 25.56% |
| 4 | Fluids | 41 | 7.18% |
| 5 | ET | 21 | 3.67% |
| 6 | Blood | 20 | 3.50% |

Among the Gram Negatives the most prevalent organisms isolated were *Klebsiella* [37.4%] followed by *E.Coli* [24.5%] and *Pseudomonas species* [13.6%]. Other organisms isolated were *Citrobacter, Enterobacter, Proteus, gram negative non fermenter (GNNF) and Salmonella*. (Table 2)

*Klebsiella* 49[27.8%], *Pseudomonas* 39[22.1%] and *E.Coli* 31[17.6%] were the most prevalent isolates from pus. In sputum, *Klebsiella* 93[55.6%] and *E.Coli* 28[16.7%] were the major isolates. *E.Coli* 71[48.6%] and *Klebsiella* 40[27.3%] were the predominant isolates in Urine. 20% of blood samples showed growth of *Salmonella* 4(0.70%).

**Table 2: Distribution of bacterial isolates in different clinical samples n =571**

| **n = 571** | Klebsiella 214 37.48% | E.Coli 140 24.52% | Pseudomonas 78 16.66% | Citrobacter 45 7.88% | Enterobacter 34 5.95% | Proteus 32 5.60% | GNNF 24 4.20% | Salmonella 4 0.70% |
|---|---|---|---|---|---|---|---|---|
| Pus [176] 30.82% | 49 | 31 | 39 | 16 | 15 | 18 | 8 | 0 |
| Sputum [167] 29.24% | 93 | 28 | 18 | 14 | 5 | 2 | 7 | 0 |
| Urine [146] 25.56% | 40 | 71 | 4 | 10 | 7 | 11 | 3 | 0 |
| Fluids [41] 7.18% | 10 | 7 | 13 | 3 | 3 | 1 | 4 | 0 |
| ET [21] 3.67% | 12 | 1 | 3 | 2 | 1 | 0 | 2 | 0 |
| Blood [20] 3.50% | 10 | 2 | 1 | 0 | 3 | 0 | 0 | 4 |

Also it is seen that among gram negative isolation, *Klebsiella* (93) was the predominant organism isolated from sputum, *E coli* (71) from urine, *Pseudomonas* (39), *Citrobacter*(16) and GNNF typically from pus samples.

**Table 3: Percentage of Resistance among gram negative isolates**

| | Klebsiella | E.Coli | Pseudomonas | Citrobacter | Enterobacter | Proteus | GNNF |
|---|---|---|---|---|---|---|---|
| Norfloxacin | 99.53 | 87.76 | 98.73 | 93.33 | 97.14 | 93.75 | 100.00 |
| Nitrofurantoin | 98.13 | 83.67 | 100.00 | 93.33 | 20.83 | 93.75 | 100.00 |
| Carbenicillin | 87.38 | 84.35 | 83.54 | 88.89 | 77.14 | 75.00 | 91.67 |
| Amoxiclav | 81.78 | 80.95 | 67.09 | 80.00 | 65.71 | 71.88 | 70.83 |
| Ceftazidime | 75.23 | 75.51 | 53.16 | 80.00 | 65.71 | 71.88 | 70.83 |
| Cotrimoxazole | 71.50 | 25.17 | 89.87 | 84.44 | 62.86 | 71.88 | 58.33 |
| Ciprofolxacin | 69.16 | 76.19 | 59.96 | 73.33 | 51.43 | 62.50 | 54.17 |
| Ampicillin Sublactam | 65.42 | 69.39 | 75.95 | 62.22 | 65.71 | 56.25 | 54.17 |
| Netlimicin | 64.02 | 44.22 | 82.28 | 71.11 | 51.43 | 56.25 | 70.83 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Aztreonam | 63.08 | 70.07 | 46.84 | 57.78 | 51.43 | 65.63 | 53.13 |
| Cefepime | 61.68 | 63.95 | 56.96 | 62.22 | 51.43 | 53.13 | 54.17 |
| Gentamycin | 61.21 | 53.06 | 53.16 | 75.56 | 51.43 | 62.50 | 54.17 |
| Chrloramphenicol | 51.87 | 42.18 | 67.09 | 35.56 | 34.29 | 46.88 | 45.83 |
| Piperacillin/Tazobactam | 28.97 | 19.05 | 29.11 | 35.56 | 17.14 | 6.25 | 16.67 |
| Amikacin | 19.16 | 10.88 | 25.32 | 37.78 | 31.43 | 28.13 | 20.83 |
| Imipenam | 10.75 | 5.44 | 15.19 | 6.67 | 8.57 | 6.25 | 8.33 |

The study showed a very high percentage of resistance among the organisms to beta lactam antibiotics, combination of beta lactam/beta lactamase inhibitors and all generations of cephalosporins. 75.5% of E. Coli were resistant to Ceftazidime and 72.7% to the combination of Ceftazidime and Clavulanic acid. 99.5% of *Klebsiella spp*, 98.7% of *Pseudomonas*, 97% *Enterobacter spp* were resistant to Norfloxacin. Resistance to Amikacin and Gentamycin was 19.1% and 61.2% respectively in *Klebsiella spp*, 10.8% and 56% in *E.Coli* and 25.3% and 56.1% in *Pseudomonas spp* respectively. 10.7% of *Klebsiella*, 15.1% of *Pseudomonas spp* were resistant to Imipenam. 28.9% *Klebsiella*, 29.1% Pseudomonas and 19% *E.Coli* were susceptible to the combination of Piperacillin and Tazobactam. The combination of Piperacillin and Tazobactam was 3.4 times more effective than Carbenicillin.

**Table 4: Overall percentage of resistant organisms**

| | Number resistant | Percentage resistant |
|---|---|---|
| Norfloxacin | 551 | 96.49 |
| Nitrofurantoin | 542 | 94.91 |
| Carbenicillin | 491 | 85.98 |
| Amoxiclav | 447 | 78.28 |
| Ceftazidime | 416 | 72.85 |
| Ciprofloxacin | 390 | 68.30 |
| Ampicillin Sublactam | 385 | 67.42 |
| Cotrimoxazole | 358 | 62.69 |
| Aztreonam | 356 | 62.34 |
| Netlimicin | 354 | 31.99 |
| Cefepime | 348 | 60.94 |
| Gentamycin | 337 | 59.01 |
| Piperacillin/Tazobactam | 141 | 24.69 |
| Amikacin | 119 | 20.84 |
| Imipenam | 53 | 9.28 |

In our study we found that most of the organisms were resistant to Norfloxacin [96.49%], Nitrofurantoin [94.91%], Carbenicillin [85.98%] and Amoxiclav [78.28%] and sensitive to Imipenam [9.28% resistant], Amikacin [20.84% resistant] and Piperacillin and Tazobactam [24.69% resistant].Table 4.

## Discussion

The study was undertaken to evaluate the susceptibility patterns of the Gram negative isolates to understand the prevalent resistance patterns and to determine the effectiveness of prescribed drugs for treatment of infections.

Our study showed that 73.4% of the isolates were Gram negative bacteria similar to a study by Javeed *et al* [68.9%] [5]. In the present study the most common organism isolated was *Klebsiella* [37.4%] followed by *E.Coli* [24.5%] and *Pseudomonas* [16.6%]. This is similar to a study conducted by Romanos et al [6]. *E coli* was the most common organism isolated from urine similar to the studies conducted in Coimbatore [7] and Kathmandu [8] . *Klebsiella* 55% was the predominant species isolated from sputum, which is similar to the prevalence noted in Bhopal [9].

Our study showed a high level of resistance to Norfloxacin, Nitrofurantoin, Carbenicillin and Amoxiclav by *Klebsiella* (99.5%, 98.1%, 87.3% and 81.7% respectively), *E.Coli* (87.7%,83.6%,84.3% and 80.9% respectively) and *Pseudomonas* (98.7%,100%,83.5% and 67% respectively).

Norfloxacin which has been shown to be effective in studies [10-12] conducted in developed countries is inactive against 94.7% of Gram negative isolates in our study [*Klebsiella* (99.5%), *Pseudomonas* (98.7%), *Enterobacter* (93.3%) and other non-fermenting bacteria (100%)].

*Klebsiella* species and *E.Coli* showed 75.2% and 75.5% resistance to Ceftazidime, *Pseudomonas species* were found to be 53.1% resistant correlating to the study of Balan *et al* [13]. The addition of Clavulanic acid to Ceftazidime does not appear to change the resistance pattern significantly with 70.5% and 72.7% still being resistant. 60.8% of the bacterial isolates were found to be resistant to the fourth generation cephalosporin – Cefepime [61.6% *Klebsiella*, 63.9% *E.Coli*, 56.9% *Pseudomonas*]. *Pseudomonas species* was also found to be resistant to Cotrimoxazole(89.8%), Netilmicin (82.2%) and Ampicillin/Sublactam (75.9%). Cotrimoxazole was found to be inactive against 71.5% *Klebsiella*, 89.8% *Pseudomonas* and 84.4% *Citrobacter spp*. This pattern has also been observed in Romanos et al [6]. However, Cotrimoxazole has been found to be quite effective against *E.Coli* (25.1% resistant) in the study.

As seen in studies by Balan K *et al* [13], Panta *et al* [14] and other studies in India and abroad, majority of the gram negative organisms isolated were found to be sensitive to Amikacin, Imipenem, Piperacillin and Tazobactam as found in our study. However, the percentage of resistant organisms to Imipenam and Piperacillin/Tazobactam is slightly higher in our study than in the studies [5,13,14] mentioned above.

## Conclusion

This pattern on increasing resistance is alarming. A continued rise of antibiotic resistance in a country that still owes a significant portion of its mortalities to infectious diseases, mainly bacterial, could be catastrophic. A thorough understanding of the susceptibility patterns and a central antibiotic monitoring authority has become necessary.

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