STUDY ON CHANGE IN THE TREND OF ANTIBIOTIC SUSCEPTIBILITY PATTERN OF KLEBSIELLA PNEUMONIAE ISOLATES FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Klebsiella pneumoniae colonizes skin, nasopharynx, gastrointestinal tract, and oropharynx of hospitalized individuals. Klebsiella causes Pneumonia, Meningitis (neonates), Urinary tract infections, Intra-abdominal infections, Skin and Soft tissue infections in both community and health-care settings. It became a challenge to the ID (Infectious Disease) physicians to treat Klebsiella infections due to increasing resistance to various antibiotics which led to significant morbidity and mortality. This study was done to know the change in the trend of antibiotic susceptibility pattern of K. pneumoniae for a period of 2 years and to identify Extended Spectrum β-lactamase and AmpC β-lactamase producing organisms.

Materials and Methods: This is a prospective study done in the Department of Microbiology, Siddhartha Medical College, Vijayawada for a period of 2 years (August 2019 – July 2021). Blood, pus, and urine specimens received from both Out-patients and In-patients of Government General Hospital, Vijayawada during the study period were subjected to culture according to CLSI guidelines. Antibiotic susceptibility testing was done by modified Kirby-Bauer disc diffusion method on Muller-Hinton agar. ESBL and AmpC β-lactamase producing organisms were identified using Cefotaxime (30 µg), Ceftazidime + Clavulanic acid (30 µg/10 µg) and Cefoxitin (30 µg) discs respectively.

Results: Out of the 3021 and 3558 samples received during period 1 (August 2019 – July 2020) and period 2 (August 2020 to July 2021), 254 and 320 K. pneumoniae were isolated respectively. 167 (65%) isolates to Piperacillin-Tazobactam (PIT), 13 (5.1%) isolates to Amikacin (AK) and 6 (2.36%) isolates to Meropenem (MR) were resistant during period 1. 240 (75%) isolates to PIT, 40 (12.5%) isolates to AK and 28 (8.75%) isolates to MR were resistant during period 2. More than 50% resistance was observed to Cefoxitin, Co-trimoxazole, and Ciprofloxacin during both periods 1 and 2. 2.57 ESBL, 8 AmpC β-lactamase producing organisms were identified during period 1 and 108 ESBL, 25 AmpC β-lactamase producing organisms during period 2.

Conclusion: Antibiotic resistance has increased during period 2 when compared with period 1. It is important to monitor and optimize antibiotic use through antibiotic stewardship programmes.
collaboration of Microbiologists and clinicians is also necessary for the effective management of infectious diseases.

Introduction:
Klebsiella pneumoniae belongs to the family Enterobacteriaceae. It is a Gram-negative encapsulated bacterium that grows in the mucosal surfaces of humans and soil, vegetation and water. In humans, K. pneumoniae colonizes the oropharynx and gastrointestinal tract, from where it can easily enter the circulation and other tissues, causes infections such as bacteremia, septicemia, surgical site infections, urinary tract infections, hospital-acquired pneumonia and ventilator-associated pneumonia. K. pneumoniae can form biofilms. It became a challenge to the ID (Infectious Disease) physicians to treat Klebsiella infections due to increasing resistance to various antibiotics which led to significant morbidity and mortality. One major drug resistance mechanism of K. pneumoniae is through the production of \( \beta \)-lactamases like Extended-Spectrum \( \beta \)-Lactamases (ESBLs) and/or AmpC \( \beta \)-lactamases or both. K. pneumoniae is often resistant to several classes of non-\( \beta \)-lactam antibiotics. Intensive and prolonged use of antibiotics is another reason for the emergence and spread of highly resistant nosocomial infections.

Methods:
Blood, pus and urine specimens were received from both Out-patients and In-patients from different wards of Government General Hospital, Vijayawada for a period of 2 years from August 2019 - July 2021 which was divided into 2 periods. Period 1 is from August 2019 to July 2020. Period 2 is from August 2020 to July 2021. The samples were inoculated on fresh Nutrient agar, 5% sheep Blood agar and MacConkey’s agar. The plates were incubated aerobically at 37°C for 24 hrs. The organisms were identified phenotypically by cultural characteristics, Gram’s stain, Motility and standard biochemical tests as per CLSI guidelines. Antibiotic susceptibility testing of the isolated bacteria was done by Modified Kirby-Bauer Disc Diffusion method on Muller Hinton’s agar with proper standardization using ATCC control strains.

Phenotypic screening of ESBL producing Klebsiella isolates was done using cefotaxime (30µg) disks and confirmation was done using ceftazidime (30µg) disk and clavulanate (10µg) on Mueller-Hinton agar. A difference of more than or equal to 5 mm between the zone diameters of Ceftazidime disks and Ceftazidime/clavulanate disks was taken to be phenotypic confirmation of ESBL production.

AmpC \( \beta \)-lactamase producing isolates were detected using 30µg cefoxitin disks on inoculated Muller Hinton’s agar. Isolates producing zone diameters less than 18 mm were confirmed as AmpC \( \beta \)-lactamase producers.

Results:
Out of 3021 samples received during period 1, 1442 were pus samples, 594 were blood samples, 985 were urine samples. The no. of Klebsiella pneumoniae isolates from the pus, blood and urine samples were 139, 67 and 48 respectively during period 1, which accounts for a total of 254.

Out of 3558 samples received during period 2, 1778 were pus samples, 612 were blood samples, 1168 were urine samples. The no. of Klebsiella pneumoniae isolates from the pus, blood and urine samples were 175, 82 and 63 respectively during period 2, which accounts for a total of 320.

Out of the 254 Klebsiella pneumoniae isolates during period 1, 1167(65%) isolates were resistant to Piperacillin-Tazobactam (PIT), 13(5.1%) isolates were resistant to Amikacin (AK) and 6(2.36%) isolates were resistant to Meropenem (MR) respectively. The no. of isolates resistant to Amoxicillin + Clavulanic acid, Cefoperazone + Sulbactam, Ceftaxime, Cefotaxin, Ceftriaxon, Ciprofloxacin, Co-trimoxazole, Tetracycline were 195(76.7%), 165(65%), 87(34.25%), 186(73.2%), 205(80.7%), 145(57%), 221(87%) and 200(78.7%) respectively.

Out of the 320 Klebsiella pneumoniae isolates during period 2, 240(75%) isolates were resistant to PIT, 40(12.5%) isolates were resistant to AK and 28(8.75%) isolates were resistant to MR respectively. The no. of isolates resistant to Amoxicillin + Clavulanic acid, Cefoperazone + Sulbactam, Cefotaxime, Cefoxitin, Ceftriaxon, Ciprofloxacin, Co-trimoxazole, Tetracycline were 274(85.6%), 242(75.6%), 233(72.8%), 249(77.8%), 285(89.06%), 199(62.1%), 288(90%) and 293(91.5%) respectively.
Resistance to Nitrofurantoin was tested only for urine samples. 33(68.75%) urine samples out of 48 during period 1 and 45(71.4%) urine samples out of 63 during period 2 were resistant to Nitrofurantoin respectively.

57 ESBL (22.4%), 8 (3.14%) AmpC β-lactamase producing organisms were identified during period 1. 1108(33.75%) ESBL, 25 (7.8%) AmpC β-lactamase producing organisms were identified during period 2.

**Discussion:**
The present study shows the antibiotic resistance pattern of Klebsiella pneumoniae over a period of 2 years.

The above study shows 34.25% resistance to Cefotaxime during period 1, which increased to 72.8% during period 2 which correlates with 20% resistance to cefotaxime in the group B of Hyun M et al. study, 73.2% in Guo et al. study and 79.2% in Effah et al. study respectively. Cefoxitin resistance increased from 73.2% to 77.8% in the present study which is in line with the Zorgani et al. study with 71% resistance. Amikacin resistance increased from 5.1% during period 1 to 12.5% during period 2 which were almost similar to 4.9% resistance in group B and 7.1% in group A of Hyun M et al. study respectively.

Piperacillin+ Tazobactam resistance increased from 65% to 74% in the present study which is in line with the Amikacin resistance increased from 5.1% during period 1 to 12.5% during period 2 which were almost similar to 4.9% resistance in group B and 7.1% in group A of Hyun M et al. study respectively. Piperacillin+ Tazobactam resistance increased from 65% to 74% in the present study showing similarity with 67% and 73.13% resistance during periods 2 and 3 of the Sharma et al. study. A.F. Saleem et al. study showed decreased resistance to Piperacillin+ Tazobactam (41.34%). Meropenem was the least resistant drug with 2.36% resistance during period 1 and 8.75% during period 2 among all the drugs tested in the above study which correlates with 5.8% resistance in Guo et al. study.

In the present study, Cefaperazone+ Sulbactam resistance increased from 65% (period 1) to 75.6% (period 2) which is in line with the Sharma et al. study showing 60.19% resistance. The present study shows an increase in resistance from 80.7% to 89.06% for ceftriaxone which correlates with 78.5% and 81% resistance during periods 2 and 3 of the Sharma et al. study respectively. Ciprofloxacin resistance increased from 57% to 62.1% in the present study which has similarity with 59.8% resistance in Effah et al. study and 76.8% in Zorgani et al. study. Sharma et al. study showed less resistance to fluoroquinolones (31.12%). There is an increase in the resistance from 87% to 90% for Cotrimoxazole in the present study which is in line with 90.78% resistance during period 1 and 95% during period 2 of the Sharma et al. study. Zorgani et al. study showed 76.5% resistance to Co-trimoxazole. In the present study, resistance observed to Tetracycline was 78.7% during period 1 and 91.5% during period 2 which correlates with 89% resistance in Mitra et al. study. Amoxicillin+ Clavulanic acid resistance increased from 76.7% to 85.6% in the present study which is in line with 73.79% resistance during period 1, 84% during period 2 of the Sharma et al. study and 76.1% in Zorgani et al. study.

Nitrofurantoin resistance increased from 68.75% (period 1) to 71.4% (period 2) which correlates with Zorgani et al. study showing 72.3% resistance.

In this study 22.4% (57 samples) ESBL producing organisms were identified during period 1 which correlates with group B (19.6%) of Miri Hyun et al. study. 8 (3.14%) AmpC β-lactamase producing organisms were identified during period 1 which correlates with Song W et al. study which shows the isolation of 2.9% AmpC β-lactamase producing organisms.

During period 2, ESBL producing organisms identified in the present study were 108, that corresponds to 33.75% which is similar to 33.2% ESBL producing organisms in Antonella Agodi et al. study during 2013. 25 (7.8%) AmpC β-lactamase producing organisms were identified during period 2 that correlates with Abdulaziz et al. study which shows the isolation of 7.9% AmpC β-lactamase producing organisms.

**Table 1:** No. of samples received during the study period.

| Year of study       | No. of Pus samples received | No. of Blood samples received | No. of Urine samples received | Total no. of samples received in a year |
|---------------------|-----------------------------|-------------------------------|-------------------------------|----------------------------------------|
| AUGUST 2019 – JULY 2020 (Period 1) | 1442                         | 594                           | 985                           | 3021                                   |
| AUGUST 2020 – JULY 2021 (Period 2) | 1778                         | 612                           | 1168                          | 3558                                   |
Table 2: Antibiotic susceptibility pattern of K. pneumoniae isolates during the study period.

| ANTIBIOTICS | PERIOD 1 (n=254) | PERIOD 2 (n=320) |
|-------------|------------------|------------------|
|             | SENSITIVE | RESISTANT | SENSITIVE | RESISTANT |
| Amikacin    | 241       | 13        | 280       | 40        |
| Amoxicillin+clavulanic acid | 59        | 195       | 46        | 274       |
| Cefaperazone + Sulbactam | 89       | 165       | 78        | 242       |
| Cefotaxime  | 167       | 87        | 87        | 233       |
| Cefoxitin   | 68        | 186       | 71        | 249       |
| Ceftriaxone | 49        | 205       | 35        | 285       |
| Ciprofloxacin | 109     | 145       | 121       | 199       |
| Cotrimoxazole | 33      | 221       | 32        | 288       |
| Meropenem   | 248       | 6         | 292       | 28        |
| Piperacillin + Tazobactam | 87      | 167       | 80        | 240       |
| Tetracycline| 54        | 200       | 27        | 293       |

Figure 1: No. of Klebsiella isolates from different clinical samples.

Figure 2: Details of ESBL and AmpC β-lactamase producing isolates during the study.
Conclusion:-
There is an increase in the antibiotic resistance during period 2 when compared with period 1. Intensive and prolonged use of antibiotics is likely the main underlying factor in the transmission of antibiotic-resistant nosocomial infections. So it is very important to monitor and optimize antibiotic use through antibiotic stewardship programmes. The collaboration of Microbiologists and clinicians is also necessary for the effective management of infectious diseases.

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