Antibiotics sensitivity and resistant pattern in tribal region of Chhattisgarh, India: a retrospective observational study

Rupendra K. Bharti¹, Raj K. Sharma¹*, Sanat K. Sharma¹, Suresh K. Mourya²

¹Department of Pharmacology, Late BRKM Government Medical College, Dimrapal, Jagdalpur, Chhattisgarh, India
²Department of Microbiology, Late BRKM Government Medical College, Dimrapal, Jagdalpur, Chhattisgarh, India

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*Correspondence to:
Dr. Raj K. Sharma,
Email: pinkindiaraj@gmail.com

ABSTRACT

Background: Antimicrobial resistance (AMR) is the biggest health care problem globally, it is responsible for the high rate of mortality worldwide which was commonly observed in developing countries. We tried to find the incidence of antimicrobial resistance in the tribal region of Chhattisgarh, India.

Methods: It was an institutional based retrospective observational study. Out of 3389 samples from inpatient and outpatient department, a total number of 1676 cultured proven micro-organism were isolated from Jan 2017 to Dec 2018. SPSS v23 was used for descriptive analysis.

Results: More than 75% of the samples collected from the inpatient department in which surgical IPD were significantly higher. The commonest isolated were Staphylococcus aureus followed by Escherichia coli, then Klebsiella species. Amoxycillin-clavulanic acid was resistant to the majority of microorganism followed by 3rd generation cephalosporins then Co-trimoxazole.

Conclusions: Antimicrobial resistance was higher even in the tribal region. The incidence of AMR is increasing at an alarming rate. Microorganism targeted antimicrobial therapy with the use of narrow-spectrum antibiotics and avoidance of broad-spectrum antibiotics will possibly overcome the antimicrobial resistance. There is also a need to strengthen laboratory or microbiology department to produce an accurate report to combat antimicrobial resistance.

Keywords: Antimicrobial, Bacter, Cephalosporins, Imipenem, Resistance, Retrospective, Sensitivity

INTRODUCTION

Antimicrobial resistance (AMR) is the biggest health care problem globally, it is responsible for the high rate of mortality worldwide which was commonly observed in developing countries.¹ Many authors demonstrated that misuse or unprofessional indication of antimicrobial agents (AMA) leading to loss of lots of antibiotics efficacy and these are the major risk factor for developing AMR.²-⁴ It can be observed as the failure of drug therapy or prolonged duration of infection. In the modern era, by frequent traveling and trade between countries, there is uncontrollably spreading of resistance strains all over the world, making AMR as a global health care situation. For example, in 2010, Nordmann et al. reported that there was globally spread of carbapenems among the Enterobacteriaceae family of bacteria.⁵

In many diseases, AMR increases with duration of treatment; therefore, it was advocated that the use of a narrow spectrum of antibiotics with, shorter duration of drug therapy may help to reduce the rate of resistance,
reduce cost and improve the patient’s outcomes due to fewer complications. Furthermore, following the similar principle in many diseases such as spontaneous bacterial peritonitis, lung infection in ICU patients, middle ear infection, sinusitis, throat infection, penetrating gut injury and community acquired pneumonia short course regimens were followed.6

To combat AMR, world Health Organization (WHO) initiate a program for the development of a new class of antibiotics to replace the older generation of without losing their effect and a new strategy to protect newer drugs and reduce the intensity of antimicrobial resistance globally in future.3,9 The surveillance of AMR is essential to track changes in microbial populations, estimate the magnitude of the problem and to design and evaluate interventions. In this study, we tried to detect the percentage of antibiotics sensitivity and resistance pattern in the tribal region (Baster) of Chhattisgarh.

METHODS

One thousand six hundred seventy-six culture proven isolates from various resources (blood, urine, stool, CSF, peritoneal fluids, pus, and sputum) were studied retrospectively. The samples were collected from January 2017 to December 2018 from both outpatient and inpatient department of Late. BRKM Government Medical College, Dinnarpal, Jagdalpur, Chhattisgarh. The study was approved by the Institutional Ethics Committee. All specimens were transported to the laboratory and cultured within 3 to 4 hours of collection. A total of 3389 samples were collected, out of which 1676 were culture proven isolate.

The specimens were inoculated on mannitol salt agar (Difco) and streaked with sterilized wire loop so as to obtain discrete colonies. The plates were incubated at 37°C for 24 h under aerobic conditions. After 24 h of incubation, the culture plates were examined according to the appearance, size, colour, and morphology of the colonies. Gram stain reaction, catalase test, and coagulase test were carried out. Isolates that were gram-positive cocci, catalase positive and coagulated human plasma were considered S. aureus in this study.10

An antibiotic sensitivity test was carried out on all isolates using the paper disc diffusion technique. A total of 40 antibiotics shown below were tested. A 0.2 ml of 12-h peptone water culture of the test organism was used to inoculate on a dry sterile nutrient agar plate. This was spread over the entire surface of the nutrient agar using a sterile glass spreader and allowed to dry for about 15 to 30 min. The antibiotic discs were placed on the agar using sterile forceps. Each disc was placed far from each other to avoid their zones of inhibition from each other. The plates with the antibiotic discs were then incubated at 37°C for 24 h to observe the zones of growth inhibition produced by the antibiotics.11 Bacteria in the culture on the left are susceptible to the antibiotic in each disk, as shown by the dark, clear rings where bacteria have no growth.12

**Antibiotic discs and their concentrations per disc (μg) included**

Penicillin (30), Pefloxacin (10), Streptomycin (30), Gentamycin (10), Lincomycin (30), Ciprofloxacin (10), Nalidixic acid (3), Chloramphenicol (25), Cotrimoxazole (25), Tetracycline (30), Ampicillin (25), Methicillin (30), Ampicillin with Cloxacillin (30), Amoxyclillin (10), Cloxacillin (12.5), Netilmicin (10), Nalidixic acid (10), Amikacin (25), Cefotixin (30), Cefazolin (30), Cefazidime (30), Cefuroxime (30), Erythromycin (30), Clindamycin (30), Clindamycin with Azithromycin, (30), Ofoxacin, (30), Meropenem (30), Norfloxacin (30), Tigecycline, (25), Imipenem (30), Meropenem (30), Ertapenem (30), Doripenem (25), Cefoparazone-Sulbactum (25), Amoxyclillin Clavulanic acid (30), Vancomycin (25), Polymyxin-B (30), Teicoplanin (30), Nitrofurantoin (30), Linezolid (30), Colistin (25).

**Statistical analysis**

Data were entered in Statistical Package for Social Sciences (SPSS) version 23 for Chicago Inc. and considered for descriptive analyses. Associations were tested using the Chi-square test.

The age was reported as mean±sd and differences in the mean levels between gender was compared with unpaired t-test. A priori p-value of 0.05 was used throughout the analyses and the results were considered statistically significant at p<0.05.

**RESULTS**

Out of a total of 3389 samples, 1676 samples were culture proven isolates. Among these 1676 isolates 412 patient samples were outpatient (194 male and 218 female) and 1264 (664 male and 600 female) were indoor patients. The mean age of male patients was 32.14±21.67 as compare to 27.0±19.65 year in females patients (p<0.0001).

**Table 1: Various sources of sample between Jan 2017- Dec 2018 (in percentage).**

| Sources of samples   | Male (n=858) | Female (n=818) | 2 tail significant |
|----------------------|-------------|---------------|--------------------|
| Outpatient department (OPD) | 22.61       | 26.65         | 0.0001             |
| Neonatal ICU         | 17.72       | 16.87         |                    |
| Surgery              | 36.36       | 20.05         |                    |
| Medicine             | 10.02       | 13.69         |                    |
| Others (Inpatients)  | 12.58       | 19.55         |                    |

Table legends: >75% of samples collected from In patient department (IPD). Highest number of samples collected from Surgical IPD both in male and female patients followed by neonatal Intensive Care Unit (ICU).
The majority of samples were collected from Inpatient department (IPD). In surgical IPD, 36.36% samples collected from male patients as compared to 20.05% from female patients (Table-1). Secondly, Outpatient Department (OPD) was the second highest source for collecting the patient’s sample for male (22.61%) as well as female (26.65%) followed by neonatal intensive care unit (ICU); p<0.0001. The other department includes for the sample collection was orthopaedics, maternity word, paediatrics, casualty and burn unit (Figure-1).

In this study, we observed that 41.32% of male and 35.43% of female patients, was the commonest isolated organism was Staphylococcus aureus, while in 15.89% male and 18.88% female patients, Escherichia coli was isolated. The third commonest isolated organism was Klebsiella species which was observed 10.27% in male and 8.62% in females’ patients (Table 3).

More than 19% of patients from both the gender isolated organism was acutobacter, Citrobacter, Enterobacter, enterococcus, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, and staphylococcus species (Figure-2).

Table 3: Commonest isolated organism between Jan 2017-Dec 2018 (in percentage).

| Isolated microorganism | Male (n-858) | Female (n-818) | 2 tail significant |
|------------------------|-------------|--------------|-----------------|
| Staphylococcus aureus   | 41.32       | 35.43        |                 |
| Escherichia coli        | 15.89       | 18.88        |                 |
| Klebsiella species      | 10.27       | 8.62         | 0.001           |
| Klebsiella pneumonia    | 5.62        | 7.93         |                 |
| Pseudomonas aeruginosa  | 2.44        | 5.13         |                 |
| Streptococcus pyogenes  | 5.13        | 4.43         |                 |
| Others                  | 19.3        | 19.57        |                 |

Table legends: Staphylococcus aureus was the commonest isolated organism, followed by Escherichia coli then klebsiella species.

Figure legend: the commonest isolated organism was Staphylococcus aureus among both male (41.32%) and female (35.43%), followed by Escherichia coli then Klebsiella species. Other includes Acetobacter, Citrobacter, Enterobacter, Enterococcus, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, and Staphylococcus species.

In this study, out of 40 antimicrobial agents (AMAs) which were tested for antimicrobial sensitivity and resistance, a significant number of antimicrobial agents includes Amikacin, Ampicillin with Sulbactam, Amoxycillin-Clavulanic acid, Aztreonam, Cefazolin, Cefazidime, Cefuroxime, Cefotaxime, Cefoperazone-Sulbactam, Cotrimoxazole, Doripenem, Norfloxacin, Ofloxacin,
Piperacillin, and Teicoplanin were tested more antimicrobial resistant than sensitive both in male and female patients. Only four AMAs includes Chloramphenicol, Imipenem, Nitrofurantoin, and Polymyxin-B were tested more antimicrobial sensitive than resistant (Table 4). Some commonly used antibiotics (not shown in tables) such as Gentamycin, Azithromycin, Tetracycline, and vancomycin was more antimicrobial sensitive than resistant but found to be non-statistically significant.

Table 4: Sensitivity and resistant pattern of various antimicrobial agents between Jan 2017-Dec 2018 (in percentage).

| Characteristics          | Gender       | Sensitive | Resistant | MIC not obtained | 2 tail | significance |
|--------------------------|--------------|-----------|-----------|------------------|--------|--------------|
| Amikacin                 | Female (n=818) | 28.36     | 25.43     | 46.21            | 0.02   |
|                          | Male (n=858)   | 27.04     | 31.24     | 41.72            |        |              |
| Ampicillin Sulbactam     | Female (n=818) | 0         | 8.56      | 91.44            | 0.0001 |
|                          | Male (n=858)   | 0         | 13.99     | 86.01            |        |              |
| Amoxycillin-Clavulanic acid | Female (n=818) | 4.16      | 39.12     | 56.72            | 0.02   |
|                          | Male (n=858)   | 2.33      | 44.29     | 53.38            |        |              |
| Aztreonam                | Female (n=818) | 4.65      | 5.38      | 89.98            | 0.0001 |
|                          | Male (n=858)   | 9.32      | 11.19     | 79.49            |        |              |
| Cefazolin                | Female (n=818) | 0.49      | 2.2       | 97.31            | 0.04   |
|                          | Male (n=858)   | 0.23      | 4.2       | 95.57            |        |              |
| Cefotaxime               | Female (n=818) | 3.18      | 33.25     | 63.57            | 0.005  |
|                          | Male (n=858)   | 5.13      | 38.46     | 56.41            |        |              |
| Cefuroxime               | Female (n=818) | 2.69      | 13.69     | 83.62            | 0.0001 |
|                          | Male (n=858)   | 2.33      | 21.45     | 76.22            |        |              |
| Chloramphenicol          | Female (n=818) | 30.07     | 5.38      | 64.55            | 0.001  |
|                          | Male (n=858)   | 30.3      | 10.26     | 59.44            |        |              |
| Co-trimoxazole           | Female (n=818) | 34.96     | 35.94     | 29.1             | 0.009  |
|                          | Male (n=858)   | 28.44     | 37.3      | 34.27            |        |              |
| Cefoperazone-Sulbactam   | Female (n=818) | 0         | 0         | 100              | 0.05   |
|                          | Male (n=858)   | 0         | 0.47      | 99.53            |        |              |
| Doripenem                | Female (n=818) | 3.18      | 0         | 96.82            | 0.0001 |
|                          | Male (n=858)   | 3.5       | 2.56      | 93.94            |        |              |
| Imipenem                 | Female (n=818) | 27.14     | 2.44      | 70.42            | 0.0001 |
|                          | Male (n=858)   | 35.66     | 4.9       | 59.44            |        |              |
| Norfloxacin              | Female (n=818) | 12.71     | 10.76     | 76.53            | 0.0001 |
|                          | Male (n=858)   | 6.76      | 9.09      | 84.15            |        |              |
| Nitrofurantoin           | Female (n=818) | 16.38     | 10.02     | 73.59            | 0.0001 |
|                          | Male (n=858)   | 10.26     | 7.23      | 82.52            |        |              |
| Ofloxacin                | Female (n=818) | 19.07     | 15.89     | 65.04            | 0.0001 |
|                          | Male (n=858)   | 8.86      | 15.85     | 75.29            |        |              |
| Piperacillin             | Female (n=818) | 3.42      | 23.96     | 72.62            | 0.006  |
|                          | Male (n=858)   | 3.73      | 30.77     | 65.5             |        |              |
| Polymyxin – B            | Female (n=818) | 3.67      | 2.2       | 94.13            | 0.0001 |
|                          | Male (n=858)   | 10.26     | 2.8       | 86.95            |        |              |
| Teicoplanin              | Female (n=818) | 17.60     | 15.16     | 67.24            | 0.02   |
|                          | Male (n=858)   | 13.29     | 13.75     | 72.96            |        |              |

Table legends: Amoxycillin-Clavulanic acid were the most common antimicrobial agent shown more resistant pattern than sensitive toward all microorganism. 3rd Generation cephalosporins and Co-trimoxazole also shown significant high percentage of AMR pattern.

DISCUSSION

In this study, the significant percentage of culture-proven isolates were obtained from pus both in male and female patients followed by urine and sputum in the female patient while blood and urine among male patients. *Staphylococcus aureus* was the commonest isolated organism from Jan 2017 to Dec 2018 in Late BRKM
Government Medical College Jagdalpur and about 13 antimicrobial agents such as Penicillin, Ampicillin, Piperacillin, Aztreonam, Cefoxitin, Cefazolin, Ceftazidime, Cefuroxime, Cefotaxime, Ciprofloxacin, Netilmicin, Ampicillin-Sulbactam, and Amoxycillin-Clavulanic acid was found to be more resistant than sensitive (p<0.004). In 2015, Gandra et al. observed that 26% of isolates were coagulase negative staphylococcus between 2008 to 2014 and he quoted that, Methicillin Resistant Staphylococcus Aureus (MRSA) was 55% during 2014, while we found that during early 2017 to late 2018, there was 25.12% Staphylococcus aureus were resistance to methicillin. We also observed that, In 37.67% Staphylococcus aureus isolates were resistant to vancomycin while 46.83% were sensitive (p<0.001), Menezes et al. also indicated the emergence of Vancomycin Intermediate Resistant Staphylococcus Aureus (VISA) species in southern India.14

More than 40% stool samples were tested positive for Escherichia coli (E. coli) and it was second comments isolated organism both in male and female patients. It was highly resistant to 3rd generation cephalosporins such as Cefotaxime (30.8%) and Ceftazidime (30.5%) followed by Ciprofloxacin (24.35%) and highly sensitive to Imipenem (31%). In 2013, according to Saravanan et al. E. coli was the comment isolated organism and highly resistant against ampicillin/ amoxicillin followed by fluoroquinolones, co-trimoxazole, 3rd generation cephalosporins.15

Klebsiella species and Klebsiella pneumonia together accounting 15.27% among male and 16.55% female patients and the commonest resistant antimicrobial agents were 3rd generation cephalosporin (Ceftazidime, Cefotaxime), piperacillin and amoxicillin-clavulanic acid, while Imipenem and Polynixin-B shown sensitivity towards theses microorganism, similar observation shown by Saravanan et al. Streptococcus pyogenes and Pseudomonas aeruginosa were the four and fifth commonest isolated organism in this study.

We also observed pan drug-resistant microorganisms among many patients. Commonest pan drug-resistant (PDR) organism was E.coli, which was observed in 23 patients followed by Klebsiella species (n-18) and Staphylococcus aureus (n-10) between January 2017 to December 2018. An observed by Bhatt et al. among 1240, 101 (8.1%) isolates were non-susceptible to two or fewer class of antimicrobials and were extensively drug-resistant (XDR) and 0.9% patients were PDR.16

There were 12 patients, whom Staphylococcus aureus was isolated and found to pan drugs sensitive. A significant number of microorganism were sensitive toward gentamycin, clindamycin, and tetracycline but when to compare between male and female, the trend was higher toward gentamycin but was not statistically significant.

CONCLUSION

There is an exponentially rising incidence of antimicrobial resistance in India. Baster is a tribal region of Chhattisgarh state of India, in last two years there was the alarming rate of antimicrobial resistance observed and the incidence of XDR and PDR were also in rising trend with no treatment option available. In view of this, there is a need to develop a very strong and strict policy to address the antimicrobial resistant in India. Microorganism targeted antimicrobial therapy with the use of narrow-spectrum antibiotics and avoidance of broad-spectrum antibiotics will possibly overcome the antimicrobial resistance. There is also a need to strengthen laboratory or microbiology department to produce an accurate report to combat antimicrobial resistance.

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