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## Original Research Article

## Bacteriological profile and antibiogram analysis from a tertiary care centre in eastern India: Time to Act / ReAct

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## ABSTRACT

**Background:** Development of antimicrobial resistance imposes a serious threat to the timely and efficient healing of mankind who fall prey to the infectious agents. It is increasingly being reported day by day throughout the world. Irrational and overuse of antibiotics coupled with unavailability of regional or local antibiogram aggravates the situation further.

**Aim:** To understand and present the local antibiogram data for this region and bridge the existing knowledge gap about the same for this particular region.

**Materials and Methods:** Two year data was retrospectively analyzed from a 750 bedded tertiary care centre.

**Results:** A total of 1143 non repeat isolates were studied, their antibiogram profiles were analyzed. Percentage of Gram negative bacteria obtained were 59.6%, while Gram Positive bacteria were obtained in 40.4% of the isolates. *Escherichia coli* (22.8%) was the most common organism isolated, followed by *Staphylococcus aureus* (14.2%), Other *Staphylococcal* sp(12.8%), *Enterococcus* sp(12.6%), *Klebsiellapneumoniae* (9.4%), *Pseudomonas aeruginosa* and *Acinetobacter baumannii calcoaceticus* complex(7.4%), Miscellaneous Bacteria(13.4%). An alarming number of isolates were found to be Multidrug resistant isolates(MDRs). With the panel of antibiotics tested for Gram negative bacterial isolates, only Fosfomycin(1.5%), Nitrofurantoin(16%) & Chloramphenicol (19%) were the only antibiotics to exhibit <20% resistance. Antibiotics for Gram positive bacteria for which low resistance was observed were Vancomycin (0%), Linezolid (0.1%), Teicoplanin (<1%), Aminoglycosides (18%), Nitrofurantoin(15%), Chloramphenicol(6%) & Tetracyclines(11%). Extended Spectrum Beta lactamase(ESBL) prevalence was estimated to be 63.6% in *E.coli*, 82.4% in *Klebsiellapneumoniae*. Methicillin resistant *Staphylococcus aureus* (MRSA) was seen in 54% of the isolates, while Inducible clindamycin resistance was observed in 35.9% of the isolates.

**Conclusion:** It is high time that we shift our focus from internationally and nationally available data on antibiogram to locally prevalent antibiotic resistance pattern. It will ensure faster patient recovery at a lesser cost and also prevent undue development of drug resistance.

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### 1. Introduction

Antimicrobial resistance happens to be one of the top ten global public health menace threatening humanity and a

major impediment in achieving the sustainable development goals. The price that the community has to pay has been aptly described by William Forster Lloyd(1794-1852) in his famous “Two lectures on the checks to Population”, which was further explained by Garrett Hardin’s article

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“The tragedy of the Commons”.<sup>1</sup> Antibiotic resistance has increased to an alarmingly high levels in the past two decades and the main cause for the same can be safely attributed to irrational usage, overuse and over the counter availability of these drugs.<sup>2,3</sup> In spite of various agencies working towards the cause of rationalizing antibiotic usage and promoting safe prescription practices, a lot of Knowledge, attitude & practice gap remains amongst the healthcare professionals especially in underdeveloped or developing nations. Whilst the need to provide faster treatment (Hit Hard. Hit Fast) has become rule of the day in most of the health care setups partly attributed by the increasing and sometimes exorbitant cost of availing healthcare facilities. Lack of dynamic data of antibiogram at Global, Nationwide and regional levels often force clinicians to choose regimens based on their wisdom and prevalent local practices. Providing them with these recent updates for their region will definitely help the clinicians and act as a guide to rationally choose from their armamentarium. Stringent guidelines from the Government, better regulations and penal provisions to stop quacks, practitioners other than those of Allopathic medicine to prescribe antibiotics will essentially go a long way in ensuring reversal of the current trends of rapidly increasing antibiotic resistance. This study was designed with the aim to understand and present the local antibiogram data for this region and to bridge the existing knowledge gap about the same for this particular region. Various initiatives have been taken at international and national levels to bring a wholesome data while maintaining the uniformity of reporting techniques. Few of the notable ones are Global antibiotic resistance partnership (GARP), Global outbreak alert & response network (GOARN), Global antimicrobial resistance surveillance system (GLASS), Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR), the European Antimicrobial Resistance Surveillance Network (EARS-Net), the Latin American Network for Antimicrobial Resistance Surveillance (Rede Latino Americano de Vigilancia de la Resistencia a los Antimicrobianos (ReLAVRA), and the Western Pacific Regional Antimicrobial Consumption Surveillance System (WPRACSS), Infection control Africa network (ICAN), Action on antibiotic resistance (ReAct) & Indian Council of Medical Research initiated Antimicrobial resistance surveillance & research network (AMRSN).

## 2. Materials and Methods

### 2.1. Study design

The study was carried out at a 750 bedded tertiary care Centre and teaching hospital located in Eastern part of Uttar Pradesh, India. The study was carried during the period of June 2019 to May 2021. Samples received for Bacterial Culture and Sensitivity were retrospectively analyzed using

WHO NET version 5.6 and subsequently data for 1143 isolates were tabulated on Microsoft Excel software.

### 2.2. Inclusion criteria

Non repeat samples for bacterial Culture and sensitivity from all age groups of patients visiting this hospital either in Outpatient department or as Inpatient were included in the study.

### 2.3. Exclusion criteria

Repeat samples from the same patient and Surveillance samples were excluded from the study.

### 2.4. Study protocol

Various clinical samples including Blood, Urine, Pus, Tracheal aspirates, Stool, Sputum, Body Fluids etc were subjected to culture plating using standard microbiological techniques and incubated at 37°C and was analyzed for growth after 24 – 48 h. Culture positive samples were subjected to Antibiotic susceptibility testing by disk diffusion method using M-100 CLSI guidelines of 2020.

For Gram positive organisms, the antibiotics that were tested and reported are Penicillin (PEN-10units), Ampicillin (AMP10ug), Cefoxitin (CX30ug), Gentamicin (GEN10ug), High Level Gentamicin (HLG 30ug), Ciprofloxacin (CIP5ug), Levofloxacin (LE5ug), Moxifloxacin (MOX5ug), Ofloxacin (OF5ug), Cotrimoxazole (COT1.25/23.75ug), Clindamycin (CD2ug), Azithromycin (AZM15ug), Erythromycin (E15ug), Nitrofurantoin (NIT300ug), Linezolid (LZ30ug), Vancomycin (VANE-strip), Teicoplanin (TEI30ug), Chloramphenicol (C 30ug), Tetracycline (TE 30ug).

The antibiotics tested and reported in the panel of Gram negatives were Ampicillin (AMP10 ug), Amoxicillin Clavulanic acid (AMC 20/10ug), Ampicillin sulbactam (A/S10/10ug), Ticarcillin clavulanic acid (TCC 75/10ug), Piperacillin Tazobactam (PIT100/10ug), Ceftazidime (CAZ30ug), Ceftriaxone (CTR30ug), Cefepime (CPM30ug), Aztreonam (AT30ug), Meropenem (MRP10ug), Gentamicin (GEN10ug), Tobramycin (TOB10ug), Ciprofloxacin (CIP5ug), Ofloxacin (OF5ug), Cotrimoxazole (COT1.25/23.75ug), Fosfomycin (FOS200ug), Nitrofurantoin (NIT 300ug), Chloramphenicol (C 30ug), Tetracycline (TE30ug). All antibiotics were procured from HiMedia Labs (Mumbai, India). The data was then retrospectively analyzed to obtain the antibiogram picture.

## 3. Results

The total number of culture positive samples received during the study period was n=1143. Samplewise break up of isolates showed Blood (n=246;21.5%), Urine

(n=333;29.1%), Pus (n=348;30.4%), Endotracheal aspirate (n=54;4.7%), Miscellaneous samples (n=162;14.1%). Gram positive organisms amongst these were n = 462(40.4%), whilst Gram negative organisms were n= 681 (59.6%). Organism wise distribution of the isolates showed *Escherichia coli*[n= 261(22.8%)] as the most frequently isolated organism, followed by *Staphylococcus aureus* n=162(14.2%), Other Staphylococcal sp n= 147(12.8%), Enterococcus sp n =144(12.6%), *Klebsiellapneumoniae* n=108(9.4%), *Pseudomonassp* n= 84(7.4%), *Acinetobacter baumannii calcoaceticus* complex n=84(7.4%) & miscellaneous organisms accounted for n= 153(13.4%) of the total isolates. The Miscellaneous organisms comprised of *Proteus*sp, *Citrobacter*sp, *Enterobacter*sp, *Salmonella*sp, *Morganella*sp being the prominent few amongst the Gram negatives. Gram positive isolates in the Miscellaneous category included *Streptococcus pneumoniae*, *Streptococcuspyogenes*, *Streptococcusagalactiae* & *Micrococcus*sp. Frequency distribution of the organisms is given as shown in Table 1. Isolate listing based on the sample from which they have been isolated is depicted in Table 2.

Antibiotic susceptibility testing was performed in accordance with CLSI 2020 guidelines and the non susceptibility percentage obtained for Gram Negative Bacteria has been depicted in Table 3.

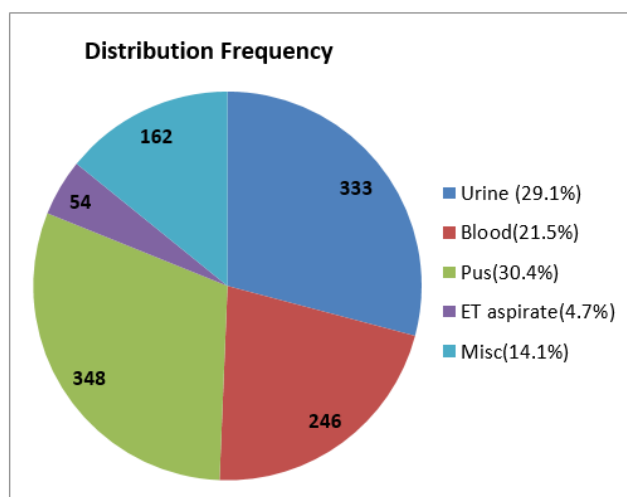


Fig. 1:

Extended spectrum beta lactamase (ESBL) prevalence in *Escherichia coli* was seen in n=166(63.6%), *K.pneumoniae*n=89(82.4%), *Acinetobacter*sp n=71(84.5%) & *Pseudomonas* sp n= 59(70.2%). Methicillin resistant *Staphylococcus aureus* was found to account for 54% of the total *S.aureus*isolates. Whilst Methicillin Resistant Other *Staphylococcal* sp (MR-CoNS) was seen in 58% of the total isolates identified as Other *Staphylococcal* sp. Inducible Clindamycin resistance, (D-test) was positive

in n=111 (35.9%) of the total isolates belonging to genus *Staphylococcus*.

#### 4. Discussion

Periodic assessment of antibiogram data is one of the key steps in understanding the antibiotic resistance burden that the hospital has to deal with in day to day practice. Clinical microbiology laboratory must have a robust system in place to document its findings and regularly present the same to clinicians to ensure better health care delivery. The role of a clinical microbiologist extends beyond accurate reporting of samples that come for culture & sensitivity testing. The reports must also act as a ready reference for better understanding of terms routinely reported viz; MRSA, VRE, ESBL, CRE, CRAB, D test (Inducible Clindamycin resistance) etc. Over a period of time, the laboratory must be able to formulate a hospital based empiric antibiotic policy which may serve as the guiding principle for providing rational treatment across all specialities. The dynamic nature of resistance mechanisms necessitates regular tabulation & presentation of this data during infection control meetings or discussions, which in many ways will be unique to the given geographical niche. This activity also helps in various world- wide bodies to formulate empiric treatment guidelines.<sup>4,5</sup> Our study was aimed at bridging the gap that existed for the local antibiogram data for Eastern Uttar Pradesh, which happens to be one of the most populated areas and carries a high infectious diseases burden. The study also assumes significance because this region also witnesses a significant number of patients resorting to either over the counter medications, or frequent drug prescriptions by unauthorized people to prescribe antibiotics. By the time a patient presents to tertiary care centre to seek medical help he/she has been exposed to various classes of antibiotics which ultimately adds to the drug resistance and may lead to treatment failures. In our analysis Gram negative organisms were found to be the predominant bacteria accounting for almost 60% of all culture positive samples. This finding is in conformity with that reported by Abebe et al. They reported Gram-negative bacteria as the more frequently isolated organism from different clinical samples.<sup>6</sup> Based on the sample type and isolated organisms, the presentation commensurates with various other studies.<sup>7,8</sup> For Blood cultures, the most common isolate was Other Staphylococcal spCoNS (46.3%) obtained from paired blood samples, followed by *Enterococcus* sp(17%).<sup>9–11</sup> Gram negative sepsis accounted for 24% of all blood cultures. Amongst urine samples *Escherichia coli* was the most common organism (53%). Various published data suggest a higher prevalence of *E.coli* ranging from 60 – 85% of all urine isolates.<sup>12–16</sup>

For Pus aspirates/swabs that were received, *Staphylococcus aureus* alone accounted for 33% of the

**Table 1:** Frequency distribution of isolates

Sample Type	Total isolate	S.aureus	Other Staphylococcus	Enterococcus species	Escherichia Coli	Klebsiella species	Pseudomonas species	Acinetobacter Baumannii	Other
Blood	246	24	114	42	12	12	03	09	30
Urine	333	03	03	90	174	15	15	09	24
Pus	348	114	15	00	63	30	48	12	66
Tracheal Aspirate	54	00	00	00	00	21	03	30	00
Miscellaneous	162	21	15	12	12	30	15	24	33
<b>Total</b>	<b>1143</b>	<b>162</b>	<b>147</b>	<b>144</b>	<b>261</b>	<b>108</b>	<b>84</b>	<b>84</b>	<b>153</b>

**Table 2:** Frequency distribution of organisms with their sample type

S. No	Antibiotic	<i>E.coli</i>	<i>K.pneumoniae</i>	<i>Acinetobacter</i> sp	<i>Paeruginosa</i>
1	Ampicillin	96%	-IR-	IR	IR
2	Ampicillin sulbactam	63%	78%	20%	IR
3	Amoxicillin Clavulanic acid	88%	72%	IR	IR
4	Piperacillin Tazobactam	48%	77%	88%	36%
5	Ceftazidime	80%	93%	86%	41%
6	Ceftriaxone	88%	81%	91%	IR
7	Cefepime	82%	78%	90%	44%
8	Aztreonam	74%	77%	IR	34%
9	Meropenem	36%	63%	81%	64%
10	Gentamicin	31%	60%	84%	39%
11	Tobramycin	34%	58%	79%	32%
12	Ciprofloxacin	94%	89%	88%	49%
13	Ofloxacin	92%	85%	-	44%
14	Cotrimoxazole	41%	77%	80%	IR
15	Fosfomycin	1.5%	-NR-	IR	-
16	Nitrofurantoin	16%	35%	-	-
17	Chloramphenicol	19%	60%	IR	IR
18	Tetracycline	60%	44%	62%	IR

**Table 3:** Nonsusceptibility percentages profile for Gram negative organisms from all specimens

S. No.	Antibiotic	<i>Staphylococcus</i> sp	<i>Enterococcus</i> sp
1	Penicillin	100%	80%
2	Ampicillin	-	64%
3	Cefoxitin	55%	-
4	Gentamicin	18%	IR
5	High Level Gentamicin	-	82%
6	Levofloxacin	80%	96%
7	Moxifloxacin	71%	79%
8	Ofloxacin	92%	•
9	Cotrimoxazole	41%	IR
10	Clindamycin	60%	IR
11	Azithromycin	61%	-
12	Erythromycin	82%	91%
13	Linezolid	< 0.1%	7.5%
14	Vancomycin	0%	4%
15	Teicoplanin	< 1%	9%
16	Nitrofurantoin	15%	42%
17	Chloramphenicol	6%	24%
18	Tetracycline	11%	56%

NR- Not Reported, IR – Intrinsic Resistance

total number of isolates various authors have reported similar findings.<sup>17–19</sup>

Gram negative organisms from Pus were seen more commonly in patients who had undergone GI surgeries or Burn Patients, this commensurated with the findings of Jauhari et al., Karki B et al., D'Avignon LC et al.<sup>20–22</sup> Tracheal aspirates obtained from ventilated patients exclusively showed Gram negative bacterial predominance with *Acinetobacter baumannii calcoaceticus* complex (55.5%), *Klebsiellapneumoniae* (38.8%) & *Pseudomonas* sp (5.5%) as the major offending microorganisms. This commensurate with the findings of other published studies from India.<sup>23,24</sup> A very high degree of resistance was observed in Gram negative bacterial infections to almost all classes of antibiotics as depicted in Table 3. For *E.coli* more than 90% resistance was observed for Ampicillin, Beta lactam combination agents resistance ranged from 48%-88%, 3<sup>rd</sup> generation Cephalosporins also showed high resistance with upto 80% of the isolates resistant to the more frequently prescribed cephalosporins viz; Ceftriaxone/Cefotaxim, Cefepime & Ceftazidime, Fluoroquinolones (Ciprofloxacin & Ofloxacin) were resistant in almost 90% of isolates. Prevalence of Carbapenem resistant was around 36%. While ESBLs also showed an alarmingly high number as described earlier. Aminoglycoside resistance was found to be roughly around 30%. Nitrofurantoin, Chloramphenicol were few drugs that exhibited < 20% resistance. For urinary isolates Fosfomycin resistance was found to be 1.5%. This could also be attributed to the drug being less commonly abused /misused in the present scenario. These findings tends to denote similar frequencies from across the country data that are available. Batra, et al reported that resistance amongst uropathogens for ampicillin and amoxicillin/clavulanate to be more than 90% and for cotrimoxazole and fluoroquinolones was nearly 60–70%.<sup>25</sup>

For Gram positive organisms, MRSA prevalence in clinical samples was found to be 54%, whilst MR-Other *Staphylococcal* sp had a slightly higher prevalence at 58% our result is in conformity with the study done by Anupurba et al. and Tiwari et al., who reported MRSA prevalence of 54.85% and 59.3% respectively.<sup>26,27</sup>

However, some of the authors have observed a lower prevalence of MRSA in their study but all of them reported increasing trend of MRSA prevalence in their study.<sup>28,29</sup> Our study found significantly higher prevalence of Inducible Clindamycin resistance (D test positive isolates) to be 35.9%, which is alarming as it renders Macrolides, Lincosamides & Streptogramins as a non viable option. Published data from studies of Abhishek Debnath et.al, Thapa et al., Goudarzi M et al., Kishk et al., Pratibha et al. seem to suggest an increasing trend of this type of inducible resistance.<sup>30–34</sup>

## 5. Conclusion

The need to highlight these alarm signals of trends of growing antibiotic resistance is vital to survival of human race. With limited drug developments in the pipeline and longer duration of drug development, it is imperative that the available drugs are used conservatively, yet rationally judiciously based on antibiogram pattern to ensure maximum benefit to all. Shifting our focus from Cephalosporins and beta lactam antibiotics, we also need to look into antibiotics that are lesser used and have been on holiday. Empiric management alone, only worsens our case against the fight in antibiotic resistance and the practice of evidence based medicine must be inculcated in every health care professional. A step taken in the right direction will surely serve as a guide for the mankind that is yet to grace our Planet.

## 6. Limitations

Our study focussed on the local antibiotic resistance burden and may not be representative of the resistance pattern prevailing throughout the country. The clinical presentations and correlation with various other factors viz; duration of hospital stay, cross infections were beyond the scope of this particular study and also the mechanisms of resistance at a genetic/molecular level were not accounted for in this study.

## 7. Source of Funding

Nil.

## 8. Conflicts of Interest

The authors declare no conflict of interest.

## 9. Author's Contribution

AS conceptualized the study and guided the entire process of manuscript writing, MR supervised the study & performed manuscript writing, RKS did the data analysis and result compilation & MKS had performed the bench work to enable this study.

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