

**Prevalence and Antimicrobial Resistance Pattern of Bacterial Isolates in a Tertiary Care Hospital of Assam, India**

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**Abstract**

**Background:** Antimicrobial resistance (AMR) has emerged as a major public health problem all over the world. Infections caused by resistant microbes fail to respond to treatment, resulting in prolonged illness and greater risk of death. India is among the nations with the highest burden of bacterial infections. The crude mortality from infectious diseases in India today is 417 per 100,000 persons. The present study is undertaken with the object of find the prevalence of common organism isolated and drug resistance pattern in the isolates in a tertiary care hospital in Assam.

**Methods:** A retrospective cross sectional study of non repeated samples of patients attending OPD and those admitted in different clinical departments. Antimicrobial susceptibility testing was performed using Kirby-Bauer's disc diffusion method. MAR index of > 0.2 indicates that an organism must have originated from an environment where antibiotics are often used. Data analysis was done by using Microsoft excel 2007 and SPSS Statistical software package 16.0.

**Results:** Out of 556 samples, 319 (57.4%) showed significant growth of organisms. most common organisms were *staphylococcus aureas* (38.9%). Amoxiclav, cephalosporin, flouroquinolone resistance demonstrated by all organisms. Comparatively less resistance to

aminoglycosides, nitrofurantoin and 100% sensitive to Linezolid and Imipenem detected. Highest MAR index (>0.2) exhibited by E.Coli (80%).

**Conclusion:** The fact that antibiotic use is increasing is not, itself, indicative of a problem, but evidence from studies of prescribing patterns suggests that antibiotics are often used in inappropriate ways. There is need of continuous AMR surveillance, effective infection control practices and adopt a suitable hospital antibiotic policy.

**Keyword:** Antibiotic resistance, MAR index, Tertiary care hospital

**Introduction**

Antimicrobial resistance (AMR) has emerged as a major public health problem all over the world. Infections caused by resistant microbes fail to respond to treatment, resulting in prolonged illness and greater risk of death. Although antimicrobial resistance is a natural phenomenon, it is being propagated by misuse of antimicrobial medicines, inadequate or inexistent programmes for infection prevention and control (IPC), poor-quality medicines, weak laboratory capacity, inadequate surveillance and insufficient regulation of the use of antimicrobial medicines<sup>[1]</sup>.

Several intrinsic factors such as point mutation, gene amplification and extrinsic factors like horizontal transfer of resistant gene between bacteria within and across

species by transposons, integrins or plasmids have been postulated for the development of resistance, which cannot be reduced once developed even by restricting the antibiotic usage. Social factors such as demographic changes, deficient hygienic practices and overcrowding have been enumerated for the emergence of AMR<sup>[2]</sup>

The rise in resistance not only impedes the ability to treat bacterial infections in humans and animals but has broader societal and economic effects that ultimately threaten achievement of the Sustainable Development Goals. This situation requires urgent, coordinated action at global, regional and national levels<sup>[3,4]</sup>.

India is among the nations with the highest burden of bacterial infections. The crude mortality from infectious diseases in India today is 417 per 100,000 persons. Consequently, the impact of AMR is likely to be higher in the Indian setting. The emergence of resistance is not only limited to the older and more frequently used classes of drugs but there has also been a rapid increase in resistance to the newer and more expensive drugs, like carbapenems<sup>[5]</sup>.

The emergence of multiresistant strains and pan-resistant strains of these organisms can even cause a sudden outbreak of infection in a clinical unit. High prevalence of multidrug resistance indicates a serious need for surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens<sup>[6]</sup>. Multiple antibiotic resistance (MAR) indexing has been shown to be a cost-effective and valid method of bacteria source tracking<sup>[7]</sup>. The emergence of MAR pathogenic strains will indicate the possible nosocomial infection in the hospital environment [8]. Taking all these in to consideration, the present study was undertaken with the following study objectives. The present findings will help in proper usage of antibiotics and even in preventing nosocomial infections.

### **Objective of the study**

1. To study the prevalence of common organism isolated in a tertiary care hospital in south Assam
2. To study the drug resistance pattern in the isolates so as to help in developing a proper antibiotic policy.

### **Material and methods**

**Type of study:** A retrospective hospital-based cross sectional study

**Study population** The study was conducted in the microbiology department of Silchar Medical College & Hospital. The study subjects included are the non repeated samples of patients attending OPD and also admitted in different clinical departments.

**Time period:** May to August 2017.

Inclusion criteria:

- i. Common samples such Pus, Urine, Blood Culture, Sputum are included.
- ii. The organism considered for analysis which are more than 30 numbers of isolates.
- iii. The commonly performed antibiotics susceptibility test are considered.

### **Identification and antimicrobial susceptibility testing**

The various samples received in bacteriology section were inoculated on blood agar and MacConkey agar and incubated at 37°C for 18-24hours (overnight incubation) as per standard operative guidelines (CLSI Clinical laboratory standard institute). Bacteria identified by culture, biochemical and morphological characteristics. Hi-media kits was used to identify different organisms. Antimicrobial susceptibility testing was performed using Kirby-Bauer's disc diffusion method using Mueller-Hinton Agar plates. Hi media antimicrobial disks were used for testing sensitivity. Values obtained were interpreted according to the Clinical and Laboratory standards Institute (CLSI) into resistant, intermediate and sensitive categories reported in the form diameter of

inhibition zone and compared with standard charts obtained from Himedia Labs, Mumbai .

MAR is a tool that gives an indirect suggestion of the probable source(s) of an organism. MAR index of  $> 0.2$  indicates that an organism must have originated from an environment where antibiotics are often used.

The MAR index, when applied to a single isolate, is defined as  $a/b$ , where  $a$  represents the number of antibiotics to which the isolate was resistant, and  $b$  represents the number of antibiotics to which the isolate was exposed.

If indexing is applied to a sample from which several isolates were taken, the index of the sample would be  $a/(b * c)$ , where  $a$  is the aggregate antibiotic resistance score of all isolates from the sample,  $b$  is the number of antibiotics, and  $c$  is the number of isolates from the sample<sup>[7]</sup>.

Multidrug-resistant (MDR) strains i.e. strains showing resistance to at least two of the following group of antibiotics fluoroquinolone, aminoglycosides, and cephalosporins.

Data analysis was done by using Microsoft excel 2007 and SPSS Statistical software package 16.0. Percentage value was used to show the proportions and presented in table and graphs.

### Results and observations

Out of 556 samples, 319 (57.4%) showed significant growth of organisms and remaining samples 13.1% show MBG or Normal flora; 29.5% samples had no organisms grown.

A total of 319 isolates recovered from various samples of patients admitted in clinical departments, out of which most common organisms were *staphylococcus aureas* (38.9%), of which predominantly was from urine 46.5%, blood 46.0% ,Pus(36.1%),followed in order by *Pseudomonas*(19.4%) isolated from sputum(28.0%), Pus(24.7%) and blood (22.0%); *Klebsiela*

*species*(20.7%) most commonly identified from Sputum(52.0%),Pus(20.9%) and Blood(16.0%);*E.Coli*(12.5%) majority were from specimens Urine(15.1%),pus(13.3%) and blood(10.0%);*proteus*(2.2%) isolated from pus(3.2%) and urine(2.3%);*candida*(3.4%) detected in Urine(9.3%) and Blood(6.0%).*Enterococcus*(1.9%) and *Acenotobacter*(0.9%) isolated from Urine(7.0%) and Pus(1.9%) respectively.[Table-1] The MRSA isolated was 37.1% & MSSA 62.9%,

### Antibiotic susceptibility

Antibiotic susceptibility done for 292 isolates which were adequate in numbers( $> 30$ ).

Overall MRSA isolate were 43.9% resistant,4.8% intermediate and 51.3% sensitive while MSSA isolate were 30.5% resistant,3.6% intermediate and 65.9% sensitive to different antibiotics. Highest resistance was observed with Cefpodoxime and least with nitrofurantoin for MRSA and MSSA. Amongst all the organisms, significantly highest resistance was observed with Ofloxacin(87.5%) for MRSA followed by E.Coli(81.3%).Linezolid was found to be 100% sensitive for MRSA and MSSA.

*Pseudomonas* show comparatively higher resistance than with other organisms to all groups of antibiotics. All gram negative bacteria show higher resistance with Amikacin, Levofloxacin & cephalosporins. E Coli demonstrated significantly highest resistance with Ciprofloxacin(87.5%) and PSE with Cefuroxime(86.7%). PSE, in addition present resistance with Piperacillin+tazobactam (21.2%) and Ceftazidime (34.5%). Imipenem was 100% sensitive to all gram negative organisms.[Table-2]

Out of the total number of isolates of *Staphylococcus aureas* (124), *E.Coli* (40) , *Klebsiella* (66) and *Pseudomonas*(62) ,61.3%,80.0%,77.3%, and 77.4% respectively were found to be Multiple Antibiotic

Resistance (MAR) index  $>0.2$ . [Table-3] The MDR detected in organisms in the  $MAR > 0.2$  group was *Staphylococcus aureus* (59.2%), *E. Coli* (68.8%), *Klebsiella* (64.7%) and *Pseudomonas* (62.5%). MAR index of the different organism samples are as *Pseudomonas* 0.35, *Klebsiella* 0.38 *E. Coli* 0.4 *staphylococcus aureus* 0.3 respectively.

### Discussion

*Staphylococcus aureus* is a common component of skin flora, and 30% to 50% of healthy adults are colonized with it at any given time [26] and it has been recognized as one of the most devastating persistent human pathogen that contributes toward hospital infection worldwide [24].

The present study observed *staphylococcus aureus* (38.9%) as the most common organism isolated which is conformity with another study conducted in Raipur, Chattisgarh where The prevalence of *Staphylococcus aureus* in the culture positive samples was found 36.3% [8].

In contrast with the present findings, other study from south India where *E. Coli* was the most common organism [9].

Similar with present prevalence of *E. Coli* (12.8%), a lower 8% of hospital acquired *E. Coli* infection has been reported from a study conducted in the intensive care units (ICUs) at AIIMS, Delhi in 2001 [10]. The proportion of *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Enterococci* isolates as found in our study were similar with other studies [9,11] except for *Pseudomonas* which is reported as low 8% from south india study [11].

In a retrospective study conducted in SMCH [12] during the period from January 2014 to June 2015 the prevalence of *staphylococci* strains detected by standard tests were 15% (724/4823) which is much lower than present finding.

Similar prevalence of MRSA and MSSA has been reported from other studies [12,13]

(MRSA) were 311 (42.96%). MSSA 413 (57.04%) in the same study [12], and also by other studies tertiary care hospital MSSA (51.63%) done in Pondicherry [13]. and MRSA (34.8%) MSSA (65.2%) from Raipur [8]. However, lower prevalence of MRSA and MSSA observed in other study 13.6% and 20.8% respectively [9].

### Antibiotic Resistance Pattern

#### *Staphylococcus aureus*

Similar resistance was observed with antibiotics Cefuroxime, Gentamicin, Levofloxacin, Nitrofurantoin. However, significant increase has been observed with increasing development of resistance with Amikacin, Ciprofloxacin, Amoxiclav.

Consistent with the present finding 100% sensitivity has been reported to linezolid by another study [14,15].

Similar to the present finding other studies have reported 40-48% resistance to ciprofloxacin [16,17,18]. However, MRSA isolate shown higher resistance to antibiotics such as gentamycin (63.3%), nitrofurantoin (80.7%), ciprofloxacin (63.3%) comparing to the present finding [8].

In a study conducted in a tertiary care hospital in New Delhi, maximum resistance was noted to penicillin (89.2%) which is similar with our result (90% resistant to amoxiclav).

Among the fluoroquinolones, maximum resistance in MRSA was seen to ciprofloxacin (92.5%) whereas a lower resistance is noted with MRSA (44.4%) and still lower with MSSA (30.3%) in the present study. However, similar ofloxacin (80.4%) and little lower levofloxacin (49.5%) corroborating with our study finding of 87.5% and 30% respectively for these two antibiotics [19].

#### *Pseudomonas*

High resistance was observed against third generation cephalosporins in our study, which is similar to the reports given by Ganguly et al. of India National working group-

India in its situation analysis performed at Delhi, Vellore and Mumbai in 2011<sup>[10]</sup>. Similar Cefixime resistance reported by other author(76.5%). However, in contrast with present study higher Ceftazidime(79.4%),Pipercillin(44.1%) and lower resistance to Cefuroxime(11.8%), Ofloxacin(17.7), Gentamycin(5.9%), Amikacin(14.7%)<sup>[11]</sup>. The MDR against NFGNB (pseudomonas,acinetobacter) reported from study conducted in south India reported higher of 85% compared with our study(62.5%)<sup>24</sup>.

In contrast to present observation of 77.4%, nearly all isolates 31(91.2%) had Multiple Antibiotic Resistance (MAR) index that was higher 0.2 and MAR index of the Pseudomonas sample is 0.4<sup>[11]</sup> similar to our finding of 0.35.

### **Klebsiella**

Similar resistance pattern has been reported from a study from Assam Medical College, Assam. However, in the present study lower resistance is seen with gentamicin, amikacin and ofloxacin<sup>[20]</sup>. Least resistance was exhibited to gentamycin, amikacin and nalidixic acid mimicking the results of many studies<sup>[10,21,22]</sup>.

The proportion of isolates with Multiple Antibiotic Resistance (MAR) index greater than 0.2 was 66.7%<sup>[11]</sup> and which is lower than the present study(77.3%). The MAR index of the Klebsiella sample was 0.4 in the same study comparable to our finding of 0.38.

### **E Coli**

Highest resistance exhibited by Cefpodoxime, Fluroquinolone followed by amoxiclav which is contrast with findings from other study highest resistance by E. coli was noted against ampicillin/amoxicillin followed by fluoroquinolones, cotrimoxazole, third generation cephalosporins<sup>[9]</sup>. Resistance to nitrofurantoin was less, which is in accordance with the report from community based pilot study by WHO in

Vellore, Tamil Nadu. Similar higher sensitivity of 68% to amikacin reported prospective study performed in a tertiary care hospital in Pondicherry during 2011[22].

### **Conclusion**

Antibiotic use has been increasing steadily in recent years. Between 2005 and 2009, the units of antibiotics sold increased by about 40 per cent. Increased sales of cephalosporins were particularly striking. The fact that antibiotic use is increasing is not, itself, indicative of a problem, but evidence from studies of prescribing patterns suggests that antibiotics are often used in inappropriate ways. Empiric treatment in a setting that lacks information about the patterns of bacterial disease and their antibiotic susceptibilities is thus viewed as a major driver of resistance [23]. The pattern of antibiotic resistance in the current set up need continuous surveillance, effective infection control practices and to adopt suitable hospital antibiotic policy to tackle the scenario and join international efforts to control the menace.

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**Table-1 organisms isolated from different samples**

	Staph	PSE	Klebsiella	E.Coli	Proteus	Candida	Enterococcus	Acinetobacter
Pus (n=158)	57 (36.1)	39 (24.7)	33 (20.9)	21 (13.3)	5 (3.2)			3 (1.9)
Blood (n=50)	23 (46.0)	11 (22.0)	8 (16.0)	5 (10.0)		3 (6.0)		
Urine (n=86)	40 (46.5)	5 (5.8)	12 (14.0)	13 (15.1)	2 (2.3)	8 (9.3)	6 (7.0)	
Sputum (n=25)	4 (16.0)	7 (28.0)	13 (52.0)	1 (4.0)				
Total (n=319)	124 (38.9)	62 (19.4)	47 (20.7)	40 (12.5)	7 (2.2)	11 (3.4)	6 (1.9)	3 (0.9)

**Table-2 Resistance pattern of different organism to antibiotics**

Antibiotic	MRSA	MSSA	PSE	Klebsiella	E.Coli
Amikacin	20	3.2	34.7	28	25.7
Amoxiclav	90	71.1	88.9	87.5	80.8
Cefixime	91.2	68.5	76.3	69.2	59.1
Cefpodoxime	100	88.9	100	93.3	89.5
Ceftazidime			34.5		
Ceftriaxone			67.9	66.7	65.7
Cefuroxime	42.9	0	86.7	70.4	66.7
Ciprofloxacin	44.4	30.3		50	87.5
Gentamycin	30	0		29.4	
Imipenem			0	0	0
Levofloxacin	30	21.7	45.5	39.3	50
Linezolid	0	0			
Nitrofurantoin	8.7	6.3			18.2
Ofloxacin	87.5	53.6	51.7	21.1	81.3
Piperacillin+tazobactam			21.2		

**Table-3 Multiple antibiotic resistance indices for different isolates**

MAR	Staph(n=124)	E.Coli(n=40)	Klebsiella(n=66)	Pseudomonas(n=62)
0	17(13.7)	6(15)	4(6.1)	7(11.3)
0.1	31(25)	2(5)	11(16.7)	7(11.3)
0.2	11(8.9)	3(7.5)	6(9.1)	8(12.9)
0.4	30(24.2)	1(2.5)	4(6.1)	4(6.5)
0.5	3(2.4)	6(15)	8(12.1)	6(9.7)
0.6	23(18.5)	2(5)	6(9.1)	10(16.1)
0.7	2(1.6)	12(30)	18(27.3)	4(6.5)
0.8	7(5.6)	7(17.5)	9(13.6)	15(24.2)
0.9	0	1(2.5)	0	1(1.6)
1	0	0	0	0