

## Bacteriological profile and antimicrobial susceptibility pattern of clinical samples at a tertiary care centre

Monika Rajani<sup>1,\*</sup>, Malay Banerjee<sup>2</sup>

<sup>1</sup>Assistant Professor, <sup>2</sup>Professor & Head, Dept. of Microbiology, Career Institute of Medical Sciences & Hospital, Lucknow, Uttar Pradesh

**\*Corresponding Author:**

Email: drmrjani@rediffmail.com

### Abstract

**Background:** The occurrence of bacterial infections is a major cause of morbidity and mortality in patients. Inappropriate and irrational use of antibiotics has led to increasing resistance in commonly isolated gram positive and gram negative organisms. Antimicrobial resistance is a matter of concern as it compromises the management of infectious diseases and increases the cost of health care as well.

**Aims and Objectives:** This study was undertaken to document the common organisms isolated in patients in a variety of clinical conditions encountered and describe their antibiotic susceptibilities.

**Materials and Methods:** This retrospective study was conducted in Department of Microbiology at Career Institute of Medical Sciences and Hospital. The records of bacteriology section were compiled for a period of 6 months from January 2016 to June 2016. The results were consolidated for types of clinical samples, organisms isolated and their susceptibility patterns.

**Results:** Total 1121 samples were received in Department of Microbiology over a period of six months from January 2016 to June 2016 and 283(25.2%) were positive on culture. E coli, 38 (13.4%) was the predominant isolate followed by Staphylococcus aureus, 31(10.9%). The resistance pattern in E coli to Ceftazidime, Amoxicillin clavulanic acid and Imepenem was 30.3%, 22.9% and 14.7% respectively. Among S. aureus, 15.8% strains were Methicillin resistant.

**Conclusions:** Gram negative bacteria still remain the predominant causes in most of the clinical infections in health care settings with E coli being the most common organism in most of the cases. Antimicrobial resistance is a major challenge and antibiotics need to be tested and prescribed according to standard guidelines. Local anti-biograms should be available periodically to help clinicians guide on antibiotic prescribing.

**Keywords:** E coli, S. Aureus, Antimicrobial Resistance.

### Introduction

Bacterial infections remain a major cause of morbidity and mortality in patients.<sup>(1)</sup> This problem is further exaggerated by the trends of rising antibiotic resistance even in commonly implicated organisms all over the world.<sup>(1)</sup> Inappropriate and irrational use of antibiotics has led to increasing resistance in commonly isolated gram positive and gram negative organisms.<sup>(2)</sup> Empirical treatment with ineffective antibiotics prescribed by physicians and poor patient adherence to antibiotic regimens could eventually lead to mutation and drug resistance.<sup>(3)</sup> Environmental exposure also leads to resistance to antibiotics used as growth enhancers in animal foods.<sup>(4)</sup> Antimicrobial resistance is a matter of concern as it compromises the management of infectious diseases and increases the cost of health care delivery.

Rational use of antibiotics in hospitals significantly contribute in reducing social and financial burden.<sup>(5)</sup> Antibiotic susceptibility testing in laboratories according to standard protocol is one of the effective interventions to promote rational antibiotic use.<sup>(5)</sup> It helps in monitoring trends of resistance patterns to particular antibiotics in different isolates thus serving as an effective epidemiological tool.

It is possible that antibiotic susceptibility patterns in different bacteria may differ from one hospital to another

and may also exhibit diversities in different geographical areas. Thus this study was undertaken to document the common organisms isolated in patients in a variety of clinical conditions encountered and describe their antibiotic susceptibilities.

### Materials and Methods

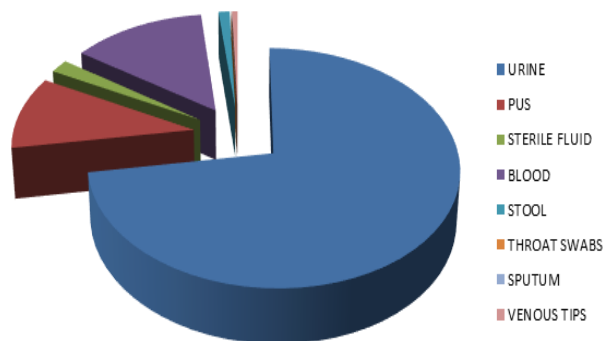
This retrospective study was conducted in Department of Microbiology at Career Institute of Medical Sciences and Hospital, a tertiary care centre in North India. The records of bacteriology section were reviewed for a period of 6 months from January 2016 to June 2016. Microbiological samples namely urine, pus, exudates, sputum, blood, sterile fluids, stool were collected as per hospital sample collection protocol. All samples were processed as per standard microbiology laboratory operating procedures. Isolates were identified up to species level by gram staining, colony morphology and routine biochemical tests. Methicillin resistance in Staphylococcus spp was tested by ceftoxitin (30ug) disc diffusion method.

The antibiotic susceptibility for isolated pathogens was performed on Muller Hinton agar (Himedia) by Kirby-Bauer disk diffusion method and interpreted according to the Clinical Laboratory Standards institute breakpoint values (M100-S25, January 2015).

*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for Quality control.

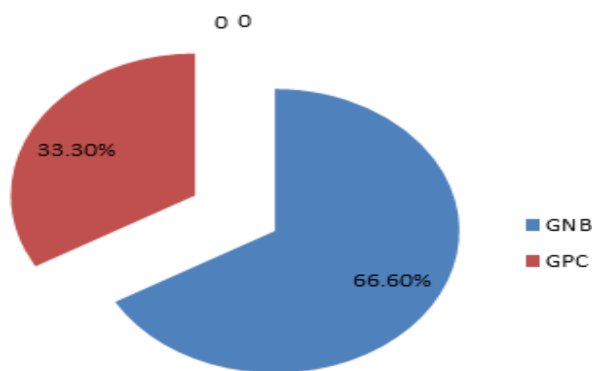
**Results**

A total of 1121 samples were received in Department of Microbiology over a period of six months from January 2016 to June 2016. The samples comprised of 721 (64.3%) urine, 102 pus (9.09%), 136(12.1%) blood, 12(1.01%) stool, 14(1.2%) throat swabs, 10(0.8%) sputum, 6(0.5%) peripheral venous tips and 20(1.7%) sterile fluids from various sites.



**Fig. 1: Distribution of clinical samples**

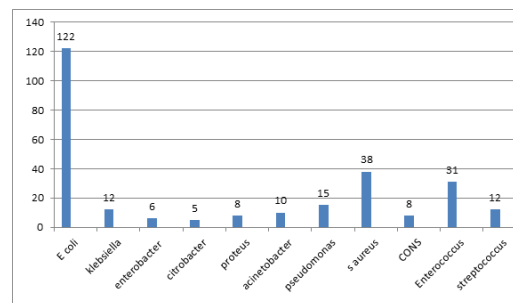
Out of 1121 samples processed, 283 (25.2%) yielded clinically significant growth which included 267 bacterial and 16 yeast isolates. Also, 66.6% (178/267) of infections were caused by gram-negative and 33.3% (89/267) by gram-positive isolates.



**Fig. 2: Frequency of gram negative and gram positive isolates**

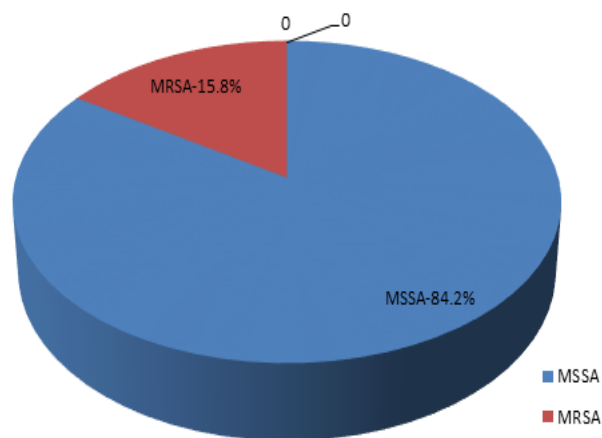
Out of total 267 bacterial isolates positive, 122(43.1%) were *E coli*, 38(13.4%) *Staphylococcus aureus*, 31(10.9%) *Enterococcus faecalis*, 15(5.3%) *Pseudomonas aeruginosa*, 12(4.2%) *Klebsiella pneumoniae*, 10(3.5%) *Acinetobacter baumannii*, 8(2.8%) *Coagulase negative Staphylococcus spp*, 5(1.7%) *Citrobacter koseri*, 6(2.1%) *Enterobacter*

*aerogenes*, 4(1.4%) *Proteus vulgaris* and 4(1.4%) *Proteus mirabilis*.



**Fig. 3: Distribution of Isolates**

*E coli* (43.1%) was the most frequently isolated organism in our study. Among *E coli*, 30.3%(37/122) of isolates showed resistance to third generation Cephalosporins while 22.9%(28/122) of *E coli* strains were resistant to Amoxicillin Clavulanic acid. 14.7%(18/122) of *E coli* were resistant to Imepenem. *Staphylococcus aureus* (13.4%) was the next frequently isolated organism in which 15.8%(6/38) strains were Methicillin resistant while 84.2% (32/38) were Methicillin sensitive.



**Fig. 4: Frequency of MSSA and MRSA among S. aureus isolates**

The resistance pattern in *Klebsiella pneumoniae* was 41.6%(5/12), 33.3%(4/12) and 50%(6/12) to Ceftazidime, Amoxicillin Clavulanic acid and Imepenem respectively. Among all isolates of enterobacteriaceae none were found to be resistant to Imepenem. In case of *Enterococcus faecalis*, 6.4% (2/31) isolates were resistant to Vancomycin but none were resistant to Linezolid. Out of 12 isolates of *Streptococcus pyogenes* identified none showed resistance to Penicillin. The antibiotic sensitivity patterns of the bacteria are shown in Table 1 and Table 2.

**Table 1: Antibiotic resistance pattern among gram negative isolates**

Isolates (n=178)	I	NX	NIT	OF	LEVO	PT	AC	A	G	AK	CX	CA	CL
Escherichia coli (122)	18 (14.7)	15 (12.2)	8 (6.5)	42 (34.4)	12 (9.8)	-	28 (22.9)	41 (33.6)	8 (6.5)	7 (5.7)	46 (37.7)	37 (30.3)	0 (0)
Klebsiella pneumonia (12)	6 (50)	1 (8.3)	4 (33.3)	3 (25)	0 (0)	-	4 (33.3)	8 (66.6)	2 (16.6)	2 (16.6)	3 (25)	5 (41.6)	0 (0)
Pseudomonas aeruginosa (15)	6 (40)	4 (26.6)	4 (26.6)	3 (20)	1 (6.6)	5 (33.3)	-	-	2 (13.3)	-	7 (46.6)	7 (46.6)	0 (0)
Acinetobacter baumannii (10)	6 (60)	-	-	5 (50)	0 (0)	5 (50)	-	8 (80)	-	2 (20)	4 (40)	6 (60)	0 (0)
Citrobacter koseri (5)	2 (40)	2 (40)	1 (20)	1 (20)	0 (0)	1 (20)	-	3 (60)	-	1 (20)		3 (60)	0 (0)
Enterobacter aerogenes (6)	2 (33.3)	-	1 (16.6)	2 (33.3)	1 (16.6)	-	1 (16.6)	1 (16.6)	-	1 (16.6)	1 (16.6)	2 (33.3)	0 (0)
Proteus spp (8)	0 (0)	0 (0)	-	2 (25)	-	-	2 (25)	4 (50)	-	3 (37.5)	2 (25)	2 (25)	-

A-Ampicillin, AC-Amoxiclav, CA-ceftazidime, OF-Ofloxacin, G-Gentamicin, AK-Amikacin, NIT-Nitrofurantoin, NX-Norfloxacin, I-Imipenem, LEVO-Levofloxacin, PT-Pipereacillin tazobactam, CL-Colistin, CX-Cefixime.

**Table 2: Antibiotic resistance pattern among gram positive isolates**

Isolates (89)	NX	Nit	Of	Levo	A	P	G	AK	VA	E	CD
MSSA (32)	-	1 (3.1)	10 (31.2)	4 (12.5)	-	23 (71.8)	4 (12.5)	-	-	8 (25)	1 (3.1)
MRSA(6)	-	-	2 (33.3)	-	-	6 (100)	2 (33.3)	-	-	2 (33.3)	0 (0)
CONS(8)	-	-	-	3 (37.5)	-	2 (25)	-	-	-	1 (12.5)	0 (0)
Enterococcus SPP(31)	8 (25.8)	4 (12.9)	4 (12.9)	-	5 (16.1)	6 (19.3)	-	4 (12.9)	2 (6.4)	4 (12.9)	-
Strep pyogenes (12)	-	-	-	-	-	0 (0)	-	-	-	-	-

P-Penicillin, E-erythromycin, CD-Clindamycin, A-Ampicillin, VA-Vancomycin, NX-Norfloxacin, NIT-Nitrofurantoin, G-Gentamicin, LEVO-Levofloxacin, OF-Ofloxacin.

## Discussion

Bacterial infections are a major threat to human health. Our study demonstrated that gram negative bacterial infections were most predominant infections (66.6%) at our setup while gram positive infections were only 33.3%. Similar findings are quoted by Shekhar et al(2014) in their study where prevalence of Gram-negative bacteria was more than Gram-positive bacteria.<sup>(6)</sup> Ghanshani R et al(2015), also observed similar findings and resistance to antibiotics was high.<sup>(7)</sup> Another study by Ghosh et al (2009) from a teaching hospital in West Bengal recorded similar findings.<sup>(8)</sup> Mundhada et al(2015) evaluated the bacterial and fungal profile of burn wound and recorded that isolation rate of gram negative bacteria was much higher.<sup>(9)</sup>

In the studies conducted in mid 1990's gram-positive bacteria, particularly *S. aureus* and vancomycin resistant enterococcus faecium emerged as major pathogens in abdominal and surgical site infections.<sup>(10)</sup> these trends reflected both an absolute and a proportionate increase in grampositive infections. Studies have previously shown that bacteremias due to mrsa in hospitals, increased from 11.7% in 1990 to 39.2% in 1998.<sup>(11)</sup> But gradually infections caused by MDR gram-negative bacilli have become a growing problem, with a decline in the proportion of MRSA bacteremias.<sup>(12)</sup> This transition has resulted from a number of practices that have since been implemented, which includes surveillance cultures of on admission to detect *S. aureus* colonization. Also contact and isolation precautions for those colonized with *S. aureus*, and the use of alcohol-based hand-washing gels have been very helpful in combating MRSA<sup>(11)</sup> Amongst gram negative bacteria, *E Coli* (43.1%) was the most frequently isolated organism followed by *S. aureus* (13.1%) and *Enterococcus* spp(10.9%). Several authors have postulated similar observations.<sup>(13,14,15)</sup> Our Findings can be explained by the fact that urinary tract infection was the most common clinical condition for which samples were received in our laboratory. *E coli* is the most frequent organism encountered in urinary tract infections.<sup>(16)</sup> However Gram-negative bacteria also cause infections including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis in healthcare settings.<sup>(16)</sup> *Klebsiella pneumoniae*, *Proteus* spp, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* were other important gram negative bacteria isolated from a variety of clinical samples in our study.

*S. aureus* (13.4%) was the next frequent organism isolated in our study. Among *S. aureus* strains 84.2% were found to be Methicillin sensitive while 15.8% were Methicillin resistant. Similar observations were made by Prasanth V Venniyil (2016) in a study of community-associated methicillin-resistant *Staphylococcus aureus* in patients with pyoderma who recorded the frequency of Methicillin sensitive *Staphylococcus aureus* (MSSA) much higher (78.12%) than Methicillin

resistant *Staphylococcus aureus*(21.98%).<sup>(17)</sup> Some recent studies have summarized that MSSA infection still remains more prevalent despite the increasing incidence of MRSA infections.<sup>(18,19)</sup> It is suggested by various authors that as most *S. aureus* infections are attributable to MSSA, clinicians should be encouraged to obtain cultures from soft tissue infections before prescribing antimicrobial therapy.<sup>(18,20)</sup> The practice of assuming all cases of MRSA and treatment with Vancomycin should not be encouraged.<sup>(21)</sup> Distinguishing between MRSA and MSSA infections would assure that patients receive optimal treatment.

Our study postulated that 30.3% of *E coli*, 41.6% of *Klebsiella* spp, 46.6% of *Pseudomonas* spp, and 60% of *Acinetobacter* spp and *Citrobacter* spp were resistant to third generation Cephalosporins. 22.9% of *E coli* and 33.3% of *Klebsiella* spp were resistant to Amoxicillin Clavulanic acid. In addition, 50% of *Acinetobacter* strains and 33.3% of *Pseudomonas* spp were resistant to Piperacillin Tazobactam. Among strains resistant to Imepenem also further testing with colistin was performed and none of the strains were resistant to Colistin. On the other hand Quinolones and Aminoglycosides still maintained a high level of susceptibility in our set up.

Our study observed that gram negative bacteria are acquiring resistance to multiple drugs and are increasingly resistant to most available antibiotics which is a matter of concern. It is therefore emphasized that periodic antibiograms of every hospital should be released so that trends of antimicrobial resistance in a given area can be easily monitored. Clinicians should be aware of their local antimicrobial resistance patterns in order to be more efficient in dealing with bacterial infections and to prevent the spread of drug resistant bacteria. Existing data from various studies show that there is an association between antimicrobial resistance with increase in mortality, morbidity, length of hospital stay and cost of health care.<sup>(22)</sup> The challenge of antimicrobial resistance in developing countries is huge and appropriate infection control measures are also needed to combat the menace.<sup>(23,24)</sup>

## Conclusion

Our study concluded that gram negative bacteria still remain the predominant causes in most of the clinical infections in health care settings. *E coli* is the predominant organism in most of the cases. Among gram positive organisms MRSA is slowly expanding. But as prevalence of MSSA still remains high it is recommended that proper antimicrobial susceptibility testing is warranted before starting a patient on Vancomycin. Antimicrobial resistance is a major challenge and antibiotics need to be tested and prescribed according to standard guidelines. Local anti-biograms should be available periodically to help clinicians guide on antibiotic prescribing.

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