

Bacteriological Profile and Antimicrobial Susceptibility Pattern of Clinical Specimen at a Tertiary Care Centre, Rajasthan

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Abstract

Original Research Article

Abstract: Background: The prevalence 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:** The Aims of this study includes Isolation and identification of pathogens from various clinical samples and their antibiotic susceptibilities patterns in a tertiary care centre, Rajasthan. **Materials and Methods:** This retrospective study was conducted in Department of Microbiology. The records of bacteriology section were compiled for a period of 12 months from October 2016 to September 2017. The results were consolidated for types of clinical samples, organisms isolated and their susceptibility patterns. **Results:** A Total of 352 samples were received in Department of Microbiology over a period of one year from October 2016 to September 2017. Out of 352 samples 129 (36.64%) were positive on culture. In this study *E. coli*, 27(20.9%) was the predominant isolate followed by *Pseudomonas spp*, 23(17.8%) and *Staphylococcus aureus*, 20(15.5 %). The susceptibility pattern in *E. coli* to Ceftazidime, Gentamicin and Meropenem were 81.4%, 59.2% and 96.2% respectively and in *Pseudomonas* to Piperacillin/Tazobactam and Meropenem both were 87.0%. Among *S. aureus*, 50.0% strains were Methicillin resistant. **Conclusions:** Gram negative bacteria still remain the predominant causes in most of the clinical infections in health care settings. *E coli* were 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*, *Pseudomonas*, Antimicrobial Resistance.

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INTRODUCTION

Antibiotic resistance is a serious problem that has the potential to drag the world into pre-antibiotic era. Bacterial infections remain a major cause of morbidity and mortality in patients [1]. Inappropriate and irrational use of antibiotics has led to increasing resistance in commonly isolated gram positive and gram negative organisms [2]. Empirical treatment with ineffective antibiotics prescribed by physicians and poor patient adherence to antibiotic regimens could eventually lead to mutation and drug resistance [3]. Environmental exposure also leads to resistance to antibiotics used as growth enhancers in animal foods [4]. 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 [5]. Antibiotic susceptibility testing in laboratories according to standard protocol is one of the effective interventions to promote rational antibiotic use [5]. 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.

MATERIAL AND METHOD

This retrospective study was conducted in Department of Microbiology. The records of

bacteriology section were reviewed for a period of 12 months from October 2016 to September 2017. Microbiological samples like urine, pus, sputum, blood, sterile fluids, etc. were collected as per hospital sample collection protocol. All samples were processed as per standard microbiology laboratory operating procedures. Final identification was done by colony characteristics, Gram's staining, motility testing (hanging drop preparation) and routine biochemical test (Catalase, coagulase, indole, methyl red, citrate, urease, Triple sugar iron, PPA, and oxidase testing).

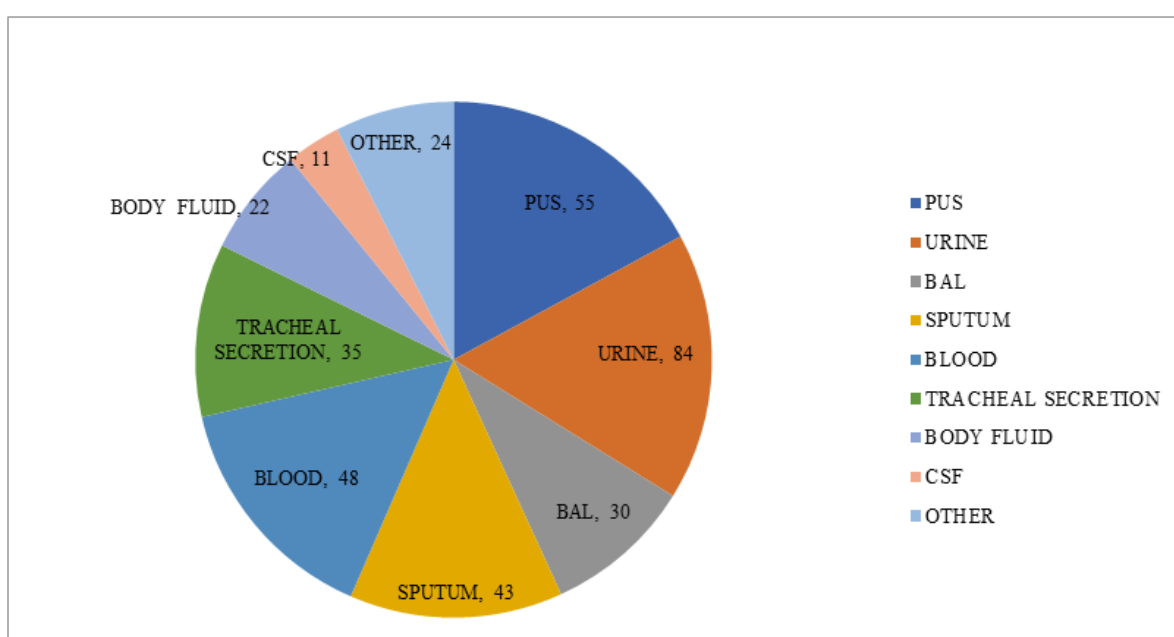
The antibiotic susceptibility for isolated pathogens was performed on Muller Hinton agar (Himedia) by Kirby-Bauer disk diffusion method and interpreted according to the Clinical Laboratory Standards Institute breakpoint values (M100-S26).

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

RESULT

A total of 352 samples were received and analysed in Department of Microbiology over a period of 12 months from October 2016 to September 2017. Out of these 84 (23.86%) urine, 55 pus (15.62), 48(13.64%) blood, 43(8.52%) sputum, 35(9.94) Tracheal secretion, 30(8.52%) BAL fluid, 22(6.52%) sterile body fluids from various sites, 11(3.12%) CSF, and 24(6.81%) Another sample.

Detail of Distribution of various clinical specimens is given in Graph-1.



Graph 1: Distribution of various clinical specimens

Out of 352, 129(36.65%) samples were positive for bacterial growth. It was found that cultures of 34(26.36%) Urine, 31(24.03) Pus, 07(5.43%) Blood, 15(11.63%) Sputum, 20(15.50%) Tracheal Secretion,

12(9.30%) BAL fluid, 04(3.10%) Sterile Body Fluids from various sites, 01(0.76%) CSF and 05(3.88%) Other samples were positive (Table-1).

Table-1: Number of positive samples

Specimen	Number of Positive samples
Urine	34(26.36%)
Pus	31(24.03%)
Blood	07(5.43%)
Sputum	15(11.63%)
Tracheal Secretion	20(15.50%)
BAL fluid	12(9.30%)
Other Sterile Body Fluids	04(3.10%)
CSF	01(0.76%)
Other samples	05(3.88%)
Total	129

Total 61.24% (79/129) of infections were caused by gram-negative and 38.76% (50/129) by gram-positive isolates. Out of total 129 bacterial isolates positive, 27(20.93%) were *E. coli*, 23(17.83%) *Pseudomonas aeruginosa*, 20(15.50%) *Staphylococcus*

aureus, 20(15.50%) Coagulase negative *Staphylococcus spp*, 16(12.40%) *Klebsiella spp*, 8(6.20%) *Enterococcus spp*, 6(4.65%) *Acinetobacter spp*, 6(4.65%) *Citrobacter spp*, 2(1.55%) *Streptococcus pyogenes*, 1(0.76%) *Salmonella enterica* (Table-2).

Table-2: Isolates from various clinical specimens

Organism	Number
<i>E. coli</i>	27
<i>Klebsiella spp</i>	16
<i>Citrobacter spp</i>	6
<i>Acinetobacter spp</i>	6
<i>Pseudomonas spp</i>	23
<i>Staphylococcus aureus</i>	20
CONS	20
<i>Streptococcus pyogenes</i>	2
<i>Enterococcus spp</i>	8
<i>Salmonella enterica</i>	1
Total	129

E. coli 20.93% was the most frequent organism encountered in urinary tract infections and highest number of *Pseudomonas* infection was found in

pus followed by tracheal aspirates. Distribution of various isolates in different clinical specimens was given in Table-3.

Table-3: Distribution of various isolates in different clinical specimens

Organisme	Pus	Urine	Bal	Blood	TS	Sputum	Body fluid	CSF	Other	Total
<i>E. coli</i>	6	18	-	-	-	1	-	-	2	27
<i>Klebsiella spp</i>	2	1	1	-	4	5	1	-	2	16
<i>Citrobacter spp</i>	1	2	-	1	-	2	-	-	-	6
<i>Acinetobacter spp</i>	-	-	-	-	5	-	-	1	-	6
<i>Pseudomonas spp</i>	7	2	3	2	4	2	3	-	-	23
<i>S. aureus</i>	6	4	3	1	2	3	-	-	1	20
CONS	8	-	4	2	4	2	-	-	-	20
<i>S. pyogenes</i>	1	-	-	-	1	-	-	-	-	2
<i>Enterococcus spp</i>	-	7	1	-	-	-	-	-	-	8
<i>Salmonella enterica</i>	-	-	-	1	-	-	-	-	-	1

Among *E. coli*, 81.4% (22/27) of isolates showed sensitive to third generation Cephalosporins (Ceftazidime) while 22.2% (6/27) of *E. coli* strains were sensitive to Amoxicillin Clavulanic acid. 96.20% (26/27) of *E. coli* were sensitive to Meropenem. *Pseudomonas spp* (17.83%) was the next frequently isolates in our study in which was sensitive to Piperacillin / Tazobactam, Ceftazidime, Meropenem 87.0%) 20/23, (43.5%) 10/23, (87.0%) 20/23 were sensitive to respectively. *Staphylococcus aureus* (15.50%) was the next frequently isolated organism in which 50.0% (10/20) were Methicillin sensitive.

The sensitive pattern in *Klebsiella spp* were 62.5%, 25.5 % and 93.8% to Ceftazidime, Amoxicillin-Clavulanic acid and Meropenem respectively. Among all isolates of Enterobacteriaceae most of the isolates found to be sensitive to Meropenem. Among *Enterococcus spp*, (87.5%) isolates were sensitive to Vancomycin and Linezolid. Out of 2 isolates of *Streptococcus pyogenes* 1 isolate showed resistance to Penicillin. The antibiotic sensitivity patterns of the bacteria are shown in Table 4 and Table-5.

Table-4: Susceptibility pattern of Gram-negative bacterial isolates

1. Organism	Ampicillin	AmoxI-Clav	Piperacillin/ Tazobactam	Cefotaxime	Ceftazidime	Cefepime	Meropenem	Ofloxacin	Gentamicin	Nitrofurantoin
<i>E. coli</i> (n=27)	4 (14.8)	6 (22.2)	20 (74.07)	8 (29.6)	22 (81.4)	8 (29.6)	26 (96.2)	14 (51.8)	16 (59.2)	16/18 (88.9)
<i>Klebsiella spp.</i> (n=16)	2 (12.5)	4 (25.5)	12 (75.0)	10 (62.5)	10 (62.5)	6 (37.5)	15 (93.8)	11 (68.8)	10 (62.5)	0/1 (0.0)
<i>Citrobacter spp.</i> (n=6)	-	-	5 (83.3)	2 (33.3)	2 (33.3)	2 (33.3)	6 (100)	2 (33.3)	2 (33.3)	-
<i>Acinetobacter spp.</i> (n=6)	-	-	3 (50.0)	2 (33.3)	1 16.7	1 16.7	6 (100)	2 (33.3)	2 (33.3)	-
<i>Pseudomonas spp.</i> (n=23)	-	-	20 (87.0)	-	10 (43.5)	3 (13.0)	20 (87.0)	8 (34.8)	10 (43.5)	12 (52.2)

Table-5: Susceptibility pattern of Gram-Positive bacterial isolates

2. Organism	Ampicillin	Penicillin	Clindamycin	Erythromycin	Cefoxitin	Co-trimoxazole	Gentamicin	Vancomycin	Linezolid	Norfloracin (FOR URINE)	Ofloxacin
<i>S. aureus</i> (n=20)	2 (10)	1 (5)	15 (75)	16 (80)	10 (50)	11 (55)	12 (60)	19 (95)	20 (100)	2/4 (50)	14 (70)
CONS (n=20)	5 (25)	2 (10)	16 (80)	16 (80)	05 (25)	12 (60)	14 (70)	20 (100)	20 (100)	-	16 (80)
<i>S. pyogens</i> (n=2)	1 (50)	1 (50)	2 (100)	2 (100)	-	1 (50)	-	2 (100)	2 (100)	-	1 (50)
<i>Enterococcus spp</i> (n=8)	1 (12.5)	1 (12.5)	-	4 (50)	-	-	-	7 (87.5)	7 (87.5)	2/7 (28.6)	3 (37.5)

DISCUSSION

Bacterial infections are a major threat to human health. In this study we demonstrated that gram negative bacterial infections were most predominant infections (61.24%) at our hospital while gram positive infections were only 38.76%. Similar findings are quoted by Shekhar *et al.*, (2014) in their study where prevalence of Gram-negative bacteria was more than Gram-positive bacteria [6]. Ghanshani R *et al.*, (2015), also observed similar findings and resistance to antibiotics was high [7]. Another study by Ghosh *et al.*, (2009) from a teaching hospital in West Bengal recorded similar findings [8]. In contrast to our study Mundhada *et al.*, (2015) recorded that isolation rate of gram-negative bacteria was much higher [9].

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 [10]. These trends reflected both an absolute and a proportionate increase in gram-positive

infections. Studies have previously shown that bacteremia due to MRSA in hospitals, increased from 11.7% in 1990 to 39.2% in 1998 [11]. But gradually infections caused by MDR gram-negative bacilli have become a growing problem, with a decline in the proportion of MRSA bacteremia [12]. 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 [11]. Amongst gram negative bacteria, *E. coli* (20.93%) and *Pseudomonas* (17.83%) was the most frequently isolated organism followed by *S. aureus* (15.50%) and *Enterococcus spp* (6.20%). Several authors have postulated similar observations [13-15]. 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* are the most frequent organism encountered in urinary tract infections [16]. Highest number of *Pseudomonas* infection was found in pus followed by

tracheal aspirates. Similar results had been obtained in different studies in India reported by Chander *et al.*, [17], Mohanasoundaram *et al.*, [18] and Arora *et al.*, [19] respectively. However Gram-negative bacteria also cause infections including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis in healthcare settings [16]. *Klebsiella spp*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* were other important gram-negative bacteria isolated from a variety of clinical samples in our study.

S. aureus (15.50%) was the next frequent organism isolated in our study. Among *S. aureus* strains 50.0% were found to be Methicillin sensitive while 50.0% 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%) [20]. Some recent studies have summarized that MSSA infection still remains more prevalent despite the increasing incidence of MRSA infections [21, 22]. 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 [21, 23]. The practice of assuming all cases of MRSA and treatment with Vancomycin should not be encouraged [24]. Distinguishing between MRSA and MSSA infections would assure that patients receive optimal treatment.

Our study showed that 81.40% of *E. coli*, 62.5% of *Klebsiella spp*, 17.83% of *Pseudomonas spp*, and 16.7% of *Acinetobacter spp*. and 33.3% of *Citrobacter spp*. were sensitive to third generation Cephalosporins (Ceftazidime). 22.2% of *E. coli* and 25.5% of *Klebsiella spp* were sensitive to Amoxicillin Clavulanic acid. In addition, 50% of *Acinetobacter* strains and 87.0% of *Pseudomonas spp* were sensitive to Piperacillin-Tazobactam. Among strains resistant to Meropenem, further mention method 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 hospital.

Our study observed that gram negative bacteria are acquiring resistance to multiple drugs and are acquiring resistant to most available antibiotics which are a great concern. It is therefore emphasized that periodic antibiograms of every hospital should be done so that trends of antimicrobial resistance in a given area can be easily monitored. Physician 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 [25].

CONCLUSION

This study concluded that gram negative bacteria still remain the predominant causes in most of the clinical infections in health care settings. *E. coli* and *Pseudomonas spp* is the predominant organism in most of the cases. Among gram positive organism's MRSA is slowly expanding. It is recommended that routine antimicrobial susceptibility testing is necessary 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 or regular antimicrobial susceptibility monitoring is essential which helps and guides the physicians to prescribe the right combinations of antimicrobials to limit and prevent the emergence of multi-drug resistant.

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