Original Article

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Microbiology Section

Bacteriological Spectrum and Antimicrobial Resistance Pattern in A Multidisciplinary Intensive Care Unit

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ABSTRACT

Introduction: In Intensive Care Units, the rate of infection due to multidrug resistant pathogens is high and accounts for increase in duration of hospital stay, mortality and morbidity and cost incurred to the patient as well as the hospital. The pathogens responsible for infection vary greatly from place to place.

Aim: To identify the spectrum of bacterial pathogens and their anti-microbial resistance pattern in a multi-disciplinary Intensive Care Unit (ICU).

Materials and Methods: A retrospective study was conducted in the Department of Microbiology from May 2013 – April 2014. All clinical samples received in the microbiology lab from the intensive care unit which were positive by culture were included in this study. They were processed according to standard microbiological methods. Antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method and the results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta Lactamase (ESBL) was also done as per CLSI guidelines.

Results: A total of 186 samples were processed. The most frequent isolate was *Acinetobacter* species (21%). Endotracheal (ET) aspirate (45.2%) was the most common source for these isolates. Methicillin resistance was detected in 25% of the *Staphylococcus* isolates. Of the 88 Enterobacteriaceae isolates, 30% were found to be Extended Spectrum β-Lactamase (ESBL) producers. *Klebsiella* species accounted for 40.5% of the ESBL producers. The resistance rates to antimicrobials were higher for *Acinetobacter* species including 41% resistance to imipenem.

Conclusion: *Acinetobacter* species was the most frequently isolated organism which showed higher resistance pattern. Multidrug resistant organisms are on the rise and strict measures are required to control infections due to these organisms.

Keywords: Antimicrobial susceptibility, ICU infections, Multidrug resistance

INTRODUCTION

Patients admitted in the intensive care units (ICUs) in hospitals are critically ill and most vulnerable to infections [1]. An international study of infections in ICU's conducted in 2007 showed that patients who had longer ICU stays had higher rates of infection [2]. The higher rates of infections are mainly due to therapeutic interventions such as indwelling catheters, immunosuppressive therapy and irrational use of broad-spectrum antibiotics [3].

Among the hospitalised patients, multi-drug resistant infections are one of the leading causes of mortality and morbidity, accounting for major burden to public health system worldwide [3]. In the past, Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococci* (VRE) had gained importance; but now a days infections due to gram negative organisms are becoming a greater problem in health care facilities. Infectious Diseases Society of America (IDSA) addresses three categories of gram negative bacilli namely Extended Spectrum β-Lactamase (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae*, multi-drug resistant *Pseudomonas* species and carbapenem resistant *Acinetobacter* species as high priority bacterial pathogens [4].

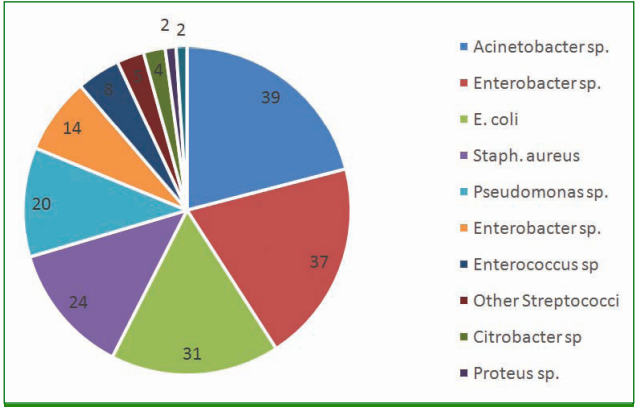
The spectrum of organisms resulting in infections and their antimicrobial resistance pattern, varies widely from one country to another and also from one hospital to another. It even varies among different ICUs in the same hospital [5]. This study was undertaken, to identify different pathogens causing infections and their antimicrobial resistant pattern in our ICU.

MATERIALS AND METHODS

A retrospective study was carried out between the period of May 2013 – April 2014 in the Department of Microbiology at Sri Devaraj Urs Medical College and R.L Jalappa Hospital, Kolar, after obtaining ethical clearance from the institutions ethics committee. Laboratory data of specimens such as endotracheal (ET) aspirate, urine, pus, sputum, blood and body fluids collected from patients admitted in a multi-disciplinary Intensive Care Unit (ICU) at R.L Jalappa Hospital, Kolar were analysed. A total of 186 samples collected from 175 patients

Organism	ET aspirate n=84 (%)	Pus n=29 (%)	Blood n=29 (%)	Sputum n=18 (%)	Urine n=17 (%)	Body Fluids n=9 (%)	Total n=186 (%)
Acinetobacter sp.	26 (31)	3 (10.3)	2 (6.9)	6 (33.3)	2 (11.8)	-	39 (20.9)
*Klebsiella*sp.	17 (20.2)	5 (17.2)	2 (6.9)	6 (33.3)	4 (23.5)	3 (33.3)	37 (19.9)
E. coli	7 (8.3)	9 (31)	5 (17.2)	-	6 (35.3)	4 (44.4)	31 (16.7)
Staphylococcus aureus	7 (8.3)	6 (20.7)	9 (31)	1 (5.6)	-	1 (11.1)	24 (12.9)
Pseudomonas sp.	15 (17.9)	2 (6.9)	1 (3.4)	2 (11.1)	-	-	20 (10.8)
Enterobacter sp.	7 (8.3)	-	4 (13.8)	1 (5.6)	2 (11.8)	-	14 (7.5)
Enterococcus sp	-	1 (3.4)	4 (13.8)	-	2 (11.8)	1 (11.1)	8 (4.3)
Other *Streptococci*	2 (2.4)	-	1 (3.4)	2 (11.1)	-	-	5 (2.7)
Citrobacter sp	3 (3.6)	1 (3.4)	-	-	-	-	4 (2.2)
Proteus sp.	-	1 (3.4)	-	-	1 (5.9)	-	2 (1.1)
B-Hemolytic *Streptococci*	-	1 (3.4)	1 (3.4)	-	-	-	2 (1.1)
Total	84 (45.2)	29 (15.6)	29 (15.6)	18 (9.7)	17 (9.1)	9 (4.8)	186

[Table/Fig-1]: Pattern of organisms isolated from different samples



[Table/Fig-2]: Distribution of isolates

admitted in this multidisciplinary ICU which yielded growth were included in the study. This difference in patients and sample size was because different types of samples were received from the same patient in 10 cases. If the same sample was received from one patient twice and yielded growth of same organism, it was considered as one sample. Samples which were reported as no growth and samples from other wards and ICU's such as Paediatric ICU and Neonatal ICU were not included in the study.

All samples were inoculated on blood agar, MacConkey's agar and Chocolate agar. Urine was inoculated by 'standard loop technique' [6] using disposable plastic flexiloop (Hi-Flexiloop, HiMedia, Mumbai). ET aspirate samples were processed as per Swetha K et al., [7]. All the inoculated samples were incubated overnight at 37°C. Identification of the organism was done based on colony characteristics, morphology on gram staining and standard biochemical reactions such as triple sugar iron test, Citrate utilization test (Simmon's), Urease hydrolysis test (Christensen's), Mannitol motility test, Indole test and Lysine Iron agar [8].

Antibiotic Tested	*S. aureus* n=24 (%)	*Enterococcus* species n=8 (%)
Penicillin	2 (8.3)	1 (12.5)
Erythromycin	14 (58.3)	NT
Clindamycin	21 (87.5)	NT
Cefoxitin	18 (75)	NT
Trimethoprim-Sulphamethoxazole	19 (79.2)	NT
Linezolid	24 (100)	8 (100)
Vancomycin	24 (100)	8 (100)
Tetracycline	24 (100)	NT
Chloramphenicol	23 (95.8)	NT
Gentamin*	22 (91.7)	2 (25)
Ciprofloxacin	10 (41.7)	NT

[Table/Fig-3]: Antibiotic sensitivity pattern of gram positive isolates
NT = not tested
**Enterococcus* species were tested with high level gentamicin

Antimicrobial susceptibility testing was done by modified Kirby Bauer's disc diffusion method and the results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. Screening for Methicillin resistance in *Staphylococcus aureus* isolates was done using cefoxitin (30 µg) disc. Screening and confirmatory tests for detection of Extended spectrum β-lactamase (ESBL) in Enterobacteriaceae isolates was also done according to CLSI guidelines [9].

RESULTS

During the 12 month study period, the most frequent sample was ET aspirate (45.2%) followed by blood (15.6%), pus (15.6%), sputum (9.7%), urine (9.1%) and peritoneal fluid (4.8%) [Table/Fig-1].The clinical conditions associated with these patients included lower respiratory tract infections

Anti-microbials Tested	*Klebsiella* sp. n=37 (%)	*E. coli* n=31 (%)	*Enterobacter* sp. n=14 (%)	*Citrobacter* sp. n=4 (%)	*Proteus* sp. n=2 (%)	*Pseudomonas* sp. n=20 (%)	*Acinetobacter* sp. n=39 (%)
Ampicillin	0 (0)	2 (6.5)	0 (0)	0 (0)	0 (0)	NT	NT
Gentamicin	18 (48.6)	23 (74.2)	11 (78.6)	3 (75)	1 (50)	12 (60)	17 (43.6)
Amikacin	18 (48.6)	30 (96.8)	11 (78.6)	3 (75)	1 (50)	14 (70)	20 (51.3)
Cefotaxime	6 (16.2)	4 (12.9)	0 (0)	0 (0)	1 (50)	NT	NT
Ceftazidime	5 (13.5)	4 (12.9)	0 (0)	0 (0)	1 (50)	14 (70)	8 (20.5)
Piperacillin	4 (10.8)	4 (12.9)	0 (0)	0 (0)	1 (50)	16 (80)	5 (12.8)
Piperacillin/Tazobactam	18 (48.6)	16 (61.6)	5 (35.7)	0 (0)	1 (50)	19 (95)	18 (46.2)
Ciprofloxacin	11 (29.7)	4 (12.9)	7 (50)	2 (50)	1 (50)	12 (60)	9 (23.1)
Levofloxacin	27 (73)	13 (41.9)	6 (42.9)	2 (50)	1 (50)	12 (60)	19 (48.7)
Imipenem	37 (100)	31 (100)	13 (92.9)	4 (100)	2 (100)	19 (95)	23 (59)
Chloramphenicol	33 (89.2)	26 (83.9)	9 (64.3)	2 (50)	1 (50)	NT	NT
Trimethoprim-Sulphamethoxazole	12 (32.4)	12 (38.7)	6 (42.9)	0 (0)	1 (50)	NT	10 (25.6)
For urinary isolates [9]							
Additional anti-microbials tested	***Klebsiella* sp. n=6 (%)**	***E. coli* n=0**	***Enterobacter* sp. n=4 (%)**	***Citrobacter* sp. n=2 (%)**	***Proteus* sp. n=0**	***Pseudomonas* sp. n=0**	***Acinetobacter* sp. n=0**
Nitrofurantoin	5 (83.3)	-	4 (100)	2 (100)	-	-	-
Norfloxacin	1 (16.7)	-	0 (0)	0 (0)	-	-	-

[Table/Fig-4]: Antibiotic resistance pattern of gram negative isolates
NT = not tested

(54.8%), skin and soft tissue infections (15.6%), septicaemia (15.6%) and Urinary Tract Infections (9.1%).

From these samples, 186 organisms were isolated. Out of these, 39 (21%) isolates were gram positive cocci and 147 (79%) isolates were gram negative bacilli. The most common isolate among gram positive cocci was *Staphylococcus aureus* (12.9%), while among gram negative bacilli it was *Acinetobacter* species (21%). The distribution of organisms isolated in this study was depicted in [Table/Fig-2].

Acinetobacter sp was the most common organism isolated from sputum and ET secretions (31.4%) followed by *Klebsiella* spp (22.6%). In peritoneal fluid, urine and pus the most frequent isolate was *E. coli* (44.4%, 35.3%, 31%), whereas in blood it was *Staphylococcus aureus* (31%) [Table/Fig-1].

The antimicrobial sensitivity pattern of these isolates is depicted in [Tables/Fig-3,4] .Least effective antibiotic among gram positive organisms was Penicillin. Only 9.7% of the isolates were sensitive to penicillin. Among *Staphylococci*, 8.4% isolates were sensitive to penicillin followed by 41.7% to Ciprofloxacin. 25% of the staphylococcal isolates were Methicillin resistant (MRSA). Among the enterococcal isolates, 12.5% were sensitive to penicillin followed by 25% to gentamicin. All the gram positive isolates were sensitive to vancomycin and linezolid.

Among the gram negative organisms, isolates belonging to family Enterobacteriaceae showed maximum sensitivity to Imipenem (98.9%) followed by Chloramphenicol (80.7%) and Amikacin (71.6%). Least effective drugs were ampicillin (2.3%), piperacillin (10.4%) and cefotaxime (12.5%). Extended Spectrum Beta Lactamase (ESBL) producers were seen in 30% of the isolates belonging to family Enterobacteriaceae and the highest number was seen in *Klebsiella* species (40.5%). Among the non-fermenting gram negative isolates, sensitivity to imipenem was 71.2% followed by piperacillin/tazobactam (62.7%) and amikacin (57.6%). *Acinetobacter* showed least sensitivity to antibiotics. Its sensitivity ranged from 12.8% for piperacillin to 59% for imipenem [Table/Fig-4].

DISCUSSION

In the present study, we noted that 45.4% of the Infections were seen in patients who received mechanical ventilation through endotracheal tubes or tracheostomy, 16.7% were blood stream infections and 8.1% were catheter associated urinary tract infections. This indicates that infections in the ICU are usually associated with invasive devices. Hence, practicing strict aseptic precautions and effective disinfection is of utmost importance during device insertion.

Infections due to gram negative bacteria are becoming a great problem in health care facilities and ICUs. MA Khan reports that 85% of the infections in their ICU were due to Gram negative organisms [10]. Our study showed 79% of the infections were due to gram negative organisms. The situation is further complicated due to emergence of multi

drug resistance among gram negative organisms such as ESBL producing *Klebsiella* and *Escherichia coli*, multidrug resistant *Pseudomonas* species and carbapenem resistant *Acinetobacter* species [11].

Extended spectrum β-lactamase (ESBL) producing organisms have been increasing since 2005. In the present study, 30% of the isolates belonging to family Enterobacteriaceae were ESBL producers and the highest number was seen in *Klebsiella* species (40.5%). The incidence of ESBL in our ICU was relatively less. This finding is supported by a multicentric study across Karnataka by Rao S et al., who also reports lower prevalence of ESBL in this region (47%) when compared to 83.5% in Bellary and 63.5% in Dharwad and Mangalore [12]. Among the gram positive cocci, we did not isolate any Vancomycin Resistant *Enterococci* (VRE), but Methicillin resistance was seen in 25% of the Staphylococcal isolates, which is comparable to the prevalence of MRSA in different parts of the country ranging from 30 - 85% [13,14].

In the recent years, *Acinetobacter* species has emerged as an important pathogen. The frequency of isolation has increased from 2 - 4% to 10 - 30% of all infections in the ICU in the last 15 years [15]. Gupta N et al., reported maximum isolation rate of *Acinetobacter* species from the ICU. They also observed a high level of antimicrobial resistance among *Acinetobacter* isolates [16]. In our ICU, the most frequent isolate was *Acinetobacter* species accounting for 21% of the total isolates. *Acinetobacter* species are intrinsically resistant to certain antibiotics such as amoxicillin, amoxicillin-clavulanic acid, cefazoline, ertapenem, trimethoprim and fosfomycin [17]. It also has an extraordinary ability to develop multiple resistance mechanisms against several antibiotics including cephalosporins, aminoglycosides, carbepenems and quinolones [15]. A previous study in this hospital in 2010 reported a resistance of 29.4% to imipenem [18], while in the present study, the resistance of *Acinetobacter* species to Imipenem was found to be 41%.This trend of increasing resistance to imipenem in our study is an imminent threat and a cause for concern.

This study has given us a representation of the major pathogens prevalent in our ICU and their anti-microbial sensitivity pattern. This will aid the physicians to institute appropriate empirical therapy and avoid indiscriminate use of antibiotics. It also helped us in formulating the antibiotic policy.

LIMITATION

As it was a retrospective study, we were unable to follow up and monitor patient outcome.

CONCLUSION

Patients admitted in Intensive Care Units are more susceptible to infections. Emergence of multidrug resistant organisms has worsened this problem as such infections are difficult to treat. Reduction of infections due to multidrug resistant organisms is a goal of all ICU's. Judicious use of anti-microbials, strict adherence to the antibiotic policy and infection control practices, implementation and practice of antibiotic stewardship programmes are necessary measures to reduce infections and spread of multidrug resistant organisms.

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