INTRODUCTION

The intensive care unit (ICU) has an extremely vulnerable population of all hospitalized patients because of their reduced host defences, and multiple invasive procedures like intubation, mechanical ventilation, vascular access etc., which may allow pathogenic and opportunistic microbes to flourish.1 Inspite of more strict hygiene protocols in ICU than in any other part of the hospital, many patients especially the immunocompromised ones, acquire infection.2 The prevalence of infections acquired in ICUs is quite high in developing countries, varying between 4.4% and 88.9%, than in developed countries. Device-associated infection rates are also high in developing countries especially ventilator-associated pneumonia (VAP) followed by Klebsiella pneumoniae (n=71, 16%) susceptible to tigecycline and meropenem. Others were Pseudomonas aeruginosa, Escherichia coli, Coagulase Negative Staphylococcus, Staphylococcus aureus, Enterococcus spp., Streptococcus spp., Klebsiella oxytica, Stenotrophomonas maltophilia, and Candida spp.

The intensive care unit (ICU) has an extremely vulnerable population of all hospitalized patients because of their reduced host defences, and multiple invasive procedures like intubation, mechanical ventilation, vascular access etc., which may allow pathogenic and opportunistic microbes to flourish.1 Inspite of more strict hygiene protocols in ICU than in any other part of the hospital, many patients especially the immunocompromised ones, acquire infection.2 The prevalence of infections acquired in ICUs is quite high in developing countries, varying between 4.4% and 88.9%, than in developed countries. Device-associated infection rates are also high in developing countries especially ventilator-associated pneumonia (VAP) followed by central venous catheter-related bloodstream infections (CRBSI).3,4 The ongoing emergence of "multidrug" antimicrobial resistance (MDR) in various pathogens is not a major threat.5 Antimicrobial resistance in ICU is higher than any other unit of the hospital.6 Serious infections caused by these multidrug resistant organisms become difficult to treat causing problems for clinicians.7 Overuse or misuse of antimicrobial agents along with recent or prolonged exposure to antimicrobial therapy has been shown to be a risk factor for acquisition of MDR pathogens.8,9 Bacteria can develop resistance to most antimicrobials by either producing enzymes that destroy the integrity of the antibiotic or mutations at the binding site preventing the attachment of antimicrobial. They can also down regulate their outer membrane proteins; hence, not allowing antimicrobials to enter or simply push them out by their efflux pumps. Multidrug resistant strains may acquire several mechanisms at the same time.10

The treatment of patients with serious infections must be prompt and correct. Numerous studies have demonstrated that mortality risk is significantly increased when the initial antibiotic regimen does not adequately cover the infecting pathogen. Selecting such a regimen is complicated by the increasing prevalence of resistance to commonly used antimicrobials.11

Emergence of resistance in ICU demands increased awareness of MDR pathogens by physicians. The medical fraternity needs to understand that antimicrobials constitute a precious and finite resource. Unless conscious efforts are bing made to contain the menace of drug resistance, MDR organisms untreatable by every known antibiotic may emerge, reversing the

ABSTRACT

Objective: To determine the frequency and antibiogram of pathogens in an intensive care unit (ICU).

Study Design: Cross-sectional, observational study.

Place and Duration of Study: Department of Microbiology, Army Medical College, National University of Science and Technology, Islamabad, from January 2013 to January 2014.

Methodology: Clinical samples, received from patients admitted in ICU, were inoculated on various medias like blood agar, chocolate agar, MacConkey agar and urine samples on CLED. These were then incubated at 37°C for 24 hours. Isolates were identified by colony morphology, Gram reaction, catalase test, oxidase test. Species identification in case of Gram Negative Rods was done by using API 20E (BioMérieux). Antibiotic susceptibility was done by using modified Kirby-Bauer disc diffusion technique. Bacterial isolates were prepared and inoculated on Mueller-Hinton agar plates followed by application of various antibiotic disc (Oxoid, UK) as per manufacturer's instructions. The plates were then incubated at 37°C aerobically for 18 - 24 hours. Zone diameters were measured and interpreted as sensitive and resistant, according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Out of the 367 positive cultures, 116 (31.08%) were Acinetobacter baumannii susceptible to minocycline and tigecycline followed by Klebsiella pneumoniae (n=71, 16%) susceptible to tigecycline and meropenem. Others were Pseudomonas aeruginosa, Escherichia coli, Coagulase Negative Staphylococcus, Staphylococcus aureus, Enterococcus spp., Streptococcus spp., Klebsiella oxytica, Stenotrophomonas maltophilia, and Candida spp.

Conclusion: Acinetobacter baumannii was the most frequently isolated pathogen. Most of the cultures yielding pathogens were from respiratory tract samples. Gram negative isolates were multidrug resistant but most were tigecycline and susceptible to meropenem.

Key Words: ICU pathogens. Minimum inhibitory concentration. Multidrug-resistant bacteria.
medical progress made by mankind and throwing us back to the pre-antibiotic era. The collective findings of the studies suggest that Gram-negative bacterial resistance increases the burden in the ICU as measured by mortality, length of stay, and costs.12,13 The prevalence of pathogens varies from region to region. Awareness of local trends can help clinicians to decide empirical treatment ensuring a better outcome.

The purpose of this study was to determine the frequency and antibiogram of pathogens in an ICU.

**METHODOLOGY**

This cross-sectional study was done in Department of Microbiology, Army Medical College, Rawalpindi, and National Institute of Science and Technology, Islamabad, from January 2013 to January 2014. From the ICU of Military Hospital, Rawalpindi, 526 samples were collected by non-probability convenience sampling. All isolates from clinical specimens of admitted patients in ICU were included, however, duplicate samples from same patient were excluded from the study.

Clinical samples like blood, pus, catheter tip, bronchoalveolar lavage, central venous line received were inoculated on blood, chocolate and MacConkey agar. Urine samples were inoculated on CLED. These were then incubated at 37°C for 24 hours. Isolates were identified by their colony morphology, Gram reaction, catalase test, and oxidase test. Coagulase test was done for identification of *Staphylococcus (S.) aureus*. Species identification of Gram negative bacilli was done by using API 20 E (BioMérieux). Methicillin resistant *S. aureus* was detected by using cefoxitin disc (30 µg). For the identification of vancomycin resistant enterococcus VRE vancomycin, E-strip was used.

Bacterial suspensions of isolates equivalent to 0.5 McFarland’s turbidity standard were prepared and inoculated on Mueller-Hinton agar plates. It was followed by application of various antibiotic disc (Oxoid, UK) as per manufacturer's instructions. The plates were then incubated at 37°C aerobically for 24 hours. Zone diameters for each antibiotic was measured and interpreted as susceptible or resistant, according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Statistical analysis of data was done by using Statistical Package for Social Sciences version 21 (SPSS 21). Frequencies and percentages were calculated for qualitative data like susceptibility, resistance, and frequency.

**RESULTS**

A total of 526 samples were received from a total of 257 admitted patients over a period of one year. Out of 257 patients, 156 (60.7%) were males and 101 (39.29%) females with age ranging from 16 to 80 years.

Most of the positive cultures were from respiratory tract samples (n=238, 64.32%) followed by blood (n=78, 21.08%). The others were from urinary tract, pus, cerebrospinal fluid, stool, genital tract, tissue and ascitic fluid. Among the total positive cultures, Gram negative rods were the most frequently isolated (n=304, 82.16%) followed by Gram positive cocci (n=60, 16.21%) and Candida (n=6, 1.62%).

From a total of 370 positive cultures, most commonly isolated pathogen was *Acinetobacter baumannii* followed by *Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli, Coagulase Negative Staphylococcus (CONs), Enterococcus spp., Enterobacter spp. Staphylococcus aureus, Candida spp., Streptococcus spp., Klebsiella oxytoca, Stenotrophomonas maltophilia, Providentia rettgeri and Serratia marcesences*. Comprehensive breakup of various isolated microbes are shown in Table I.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Respiratory system</th>
<th>Blood</th>
<th>Urinary system</th>
<th>Pus</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>89</td>
<td>20</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>115</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>53</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>71</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>44</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>16</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Coagulase Negative Staphylococcus</td>
<td>9</td>
<td>21</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>32</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>9</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Others</td>
<td>15</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>238 (64.32%)</td>
<td>78 (21.08%)</td>
<td>25 (6.75%)</td>
<td>19 (5.13%)</td>
<td>10</td>
<td>370</td>
</tr>
</tbody>
</table>

**Table II:** Percentages of susceptibility of most prevalent microorganisms to various antimicrobial.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Amikacin (%)</th>
<th>Vanillic acid (%)</th>
<th>Gentamicin (%)</th>
<th>Ceftiraxone (%)</th>
<th>Ceftriaxone (%)</th>
<th>Tigecycline (%)</th>
<th>Obitam (%)</th>
<th>Meropenem (%)</th>
<th>Ulbactam (%)</th>
<th>Colistin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>-</td>
<td>8.62</td>
<td>31.03</td>
<td>27.58</td>
<td>15.51</td>
<td>8.62</td>
<td>86.20</td>
<td>89.65</td>
<td>17.24</td>
<td>13.79</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>29.57</td>
<td>5.63</td>
<td>45.07</td>
<td>22.53</td>
<td>22.53</td>
<td>15.49</td>
<td>60.56</td>
<td>85.91</td>
<td>42.25</td>
<td>71.83</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>77.4</td>
<td>-</td>
<td>72.58</td>
<td>83.87</td>
<td>85.48</td>
<td>86.70</td>
<td>-</td>
<td>-</td>
<td>95.16</td>
<td>85.48</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>83.87</td>
<td>3.22</td>
<td>25.90</td>
<td>32.25</td>
<td>6.45</td>
<td>3.22</td>
<td>-</td>
<td>80.64</td>
<td>58.66</td>
<td>98.77</td>
</tr>
</tbody>
</table>

The most prevalent pathogen Acinetobacter baumannii showed low susceptibility against most of the currently used antimicrobials except for minocycline (86.20%) and tigecycline (89.65%). Similarly Klebsiella pneumoniae except for tigecycline (85.91%) and meropenem (71.83%) showed low susceptibility to other antimicrobials. Escherichia coli showed better susceptibility to meropenem (96.77), amikacin (83.87%), and tigecycline (80.64%). Pseudomonas aeruginosa was susceptible to most of the antimicrobials tested. Comprehensive review of susceptibility pattern of most prevalent gram negative bacilli is shown in Table II.

Vancomycin and linezolid were the most effective antimicrobials for Gram positive cocci. Only one isolate of S. aureus was resistant to cefoxitin, giving the prevalence of MRSA being 12.86%. While prevalence of VRE was 17.6% from all the isolated enterococci.

**DISCUSSION**

In this present study, the most frequently isolated pathogen was Acinetobacter baumannii, which was different from other studies. Acinetobacter baumannii can survive under a wide range of environmental conditions which makes it difficult to control and treat. It can persist on surfaces for a long period of time and is highly antimicrobial resistant which makes it a frequent cause of outbreak in healthcare settings. In Iran, Pseudomonas aeruginosa was the most frequently isolated pathogen, whereas in Canada it was methicillin sensitive S. aureus (MSSA). E. coli was the most prevalent isolated pathogen in India.

Acinetobacter baumannii was most frequently isolated from respiratory tract samples and was found to be resistant to most of the currently used antimicrobials. In a study done in Shiraz, Iran, Acinetobacter baumannii was sensitive to meropenem (88.9%) and imipenem (83.3%); whereas in this study, susceptibility was quite low to meropenem (13.79%). Multidrug resistant Acinetobacter baumannii was also isolated in India as in this study, showing susceptibility to Polymixin B only.

Klebsiella pneumoniae was mainly isolated from respiratory tract samples in this study, whereas in a study done in India, majority were isolated from urine samples and showed susceptibility to amikacin (62%) and to sparfloxacin (50%); whereas Klebsiella pneumoniae isolated in this study was only (29.9%) susceptible to amikacin and it was highly susceptible to tigecycline (89.9%) and meropenem (71.87%), thus making them a good treatment option. In contrast to this study, Klebsiella pneumoniae isolated from ICU in Canada showed susceptibility to most of the antimicrobials tested.

Pseudomonas aeruginosa isolated in this study was mostly sensitive to the antimicrobial tested. Similarly, isolates from ICUs in Canada were also sensitive giving clinicians a wide range of antimicrobials to choose from. In contrast to this study, Pseudomonas was highly resistant showing maximum susceptibility to ceftazidime (43%) in India. Similarly, highly resistant strains of Pseudomonas aeruginosa were isolated from Shiraz, Iran.

In this study, Escherichia coli was isolated mainly from respiratory system followed by urinary system while in other studies it was mainly isolated from urinary system, thus making it the primary cause of urinary tract infection. In this study, E. coli was found to be quite resistant to antimicrobials tested. It showed lowest susceptibility to ciprofloxacin (6.45%) and to ceftriaxone (3.22%). Though it showed good susceptibility to meropenem (96.77%), which was similar to the study done in Iran where susceptibility to meropenem (87.7%) was also high. In India, E. coli showed quite resistant pattern but was sensitive to amikacin (70%), as in this study where E. coli showed good susceptibility to amikacin (83.37%) and tigecycline (80.64%).

As for Gram positive cocci linezolid and vancomycin were the most effective antimicrobials showing 100% susceptibility. For enterococcus co-trimoxazole also proved to be effective. For S. aureus, clindamycin and minocycline also proved to be good options. CONS were found to be highly sensitive to all antimicrobials tested.

**CONCLUSION**

Acinetobacter baumannii was the most frequently isolated pathogen. Most of the isolated pathogens were from respiratory tract samples. Most of the Gram negative bacilli isolated were multidrug resistant but among the tested antimicrobials, tigecycline and meropenem were found to be a good treatment options.

**REFERENCES**


