Isolation and Antimicrobial Susceptibility Profile of Microorganisms Isolated from Ventilator Associated Pneumonia Patients

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Abstract

Objectives: Ventilator-associated pneumonia (VAP) is the second most prevalent nosocomial infectivity in ICU and most frequent among mechanically ventilated patients. VAP is divided in the two groups based upon mechanical ventilation duration, early and late onset VAP. The most common pathogens in Pakistan were Acinetobacter lwoffi (22%), *Staphylococcus aureus* (33%) and *Pseudomonas aeruginosa* (63%). Improper antibiotic therapy has increased the frequency of MDR strains of the pathogens, further aggravating threat posed by disease. The objectives of the study were to identify the organisms up to species level and to determine the Minimum Inhibitory Concentration (MIC) of the isolated organisms by using VITEK.

Methods: It was a descriptive study in which 74 patients having ventilator associated pneumonia i.e., patients who developed pneumonia after 48 hours of stay in intensive care unit of hospital were included. Collected Tracheobronchial secretions were sent to the pathology laboratory as soon as for culture and sensitivity. After initial identification of isolates, final identification/confirmation was done by Vitek 2-compact system.

Results: Among 74 patients, 51.0% were males and 54.0% were 51-60 years old. Major cause of ICU admission was head trauma and stroke. Among 72 patients, *klebsiella pneumonia* was identified in 37.0% patients, *pseudomonas aeruginosa* in 28.0% patients, *acinetobacter baumannii* in 21.0% patients and *Escherichia coli* was identified in 14.0% patients. *Klebsiella pneumoniae* was 100% resistant to piperacillin. *Pseudomonas aeruginosa* were 80% resistant to Ampicillin/salbactem. *Acinetobacter baumanii* was 100% resistant to ampicillin/salbactem, piperacillin, cefuroxime axetil, cefixime, ceftriaxone cefepime, aztreonam, meropenem, levofloxacin, moxifloxacin, trimethoprim. Similarly, *E. coli* was 60.0% resistant to ampicillin/ salbactem, piperacillin, cefuroxime, cefuroxime axetil, cefixime, ceftriaxone, cefepime, aztreonam, meropenem, levofloxacin, moxifloxacin and chloramphenicol.

Conclusion: A very high prevalence of multi drug resistance organisms are noted among ventilator associated pneumonia at a teaching hospital in Pakistan.

Keywords: Ventilator-associated pneumonia; Hospital-acquired pneumonia; Multi-drug resistance

Introduction

Ventilator-associated pneumonia is a significant form of HAP (hospital-acquired pneumonia). It causes development of infection of lung parenchyma after patients had experienced intubation for over 48 hours and got the mechanical ventilation for over 48 hours or the tracheostomy [1,2]. Ventilator associated pneumonia (VAP) is divided in two groups based upon mechanical ventilation duration: early onset VAP (taking place after 2 to 4 days) and late onset VAP (taking place after the day five) [3].

Ventilator associated pneumonia mostly investigated in majority of the parts of world. As per SENTRY antimicrobial observation program carried out in United States, South America and Europe, the most prevalent contributory pathogen taken all the regions together is *Staphylococcus aureus* (20 percent), Acinetobacter genus (14 percent) and *Pseudomonas aeruginosa* (27 percent) [4]. The widespread pathogens in Pakistan were *Acinetobacter lwoffi* (22 percent), *Staphylococcus aureus* (33 percent) and *Pseudomonas aeruginosa* (63 percent) [5]. The VAP etiologic agents broadly vary as per population of the patients in ICU, hospital stay period and previous antimicrobial treatment [6,7].

Improper antibiotic therapy has increased the frequency of MDR (Multidrug Resistance) strains of the pathogens, further aggravating threat posed by disease [8]. Due to these organisms VAP is generally recognized like late-onset VAP [6].

Normally, bacteria leading to early-onset ventilator-associated pneumonia comprise *Streptococcus pneumoniae* (in addition to other streptococcus genus), MSSA (Methicillin-sensitive *Staphylococcus aureus*, antibiotic-sensitive Entericgram-negative bacilli, Hemophilus influenzae, *Escherichia coli*, Enterobacter genus, Proteus species, Serratia marcescens and *Klebsiella pneumonia*, [9]. Late-onset VAP culprits are generally multidrug resistant bacteria, for example MRSA (Methicillin-resistant *S. aureus*), *Pseudomonas aeruginosa*, Acinetobacter and ESBL (extended-spectrum beta-lactamase) producing bacteria [10].

Diagnosis of ventilator associated pneumonia is founded on the combination of radiological, bacteriological and clinical criteria [11]. The introduction of Vitek technology will not only identify the organisms upto species level with great precision and accuracy but will perform antimicrobial susceptibility upto minimum inhibitory concentration level, its usage in diagnostic microbiology will not only save the time but also help treat the critical patients in time. This instrument has the ability to perform identification and susceptibility in minimum of 8-10 hours and maximum of 18 hours. According to CLSI 2016 guidelines, most of the drugs/antibiotics should be tested for MIC.

Several studies have been carried out in Pakistan and worldwide regarding microbiological and susceptibility profile of microorganism in ventilator associated pneumonia, but no study was undertaken in Southern Punjab. Therefore, it is pertinent to conduct a study in the hospitals of southern Punjab namely Nishtar Hospital Multan, Sheikh Zayed Hospital Rahim Yar Khan and Bahawal Victoria Hospital about this topic. The results of the study will help policymakers and health planners for better planning.

Materials and Methods

It was a descriptive study in which 74 patients having ventilator associated pneumonia i.e., patients who developed pneumonia after 48 hours of stay in intensive care unit of hospital were included. Convenience sampling technique was used for the selection of patients. Collected Tracheobronchial secretions were sent to the pathology laboratory as soon as for culture and sensitivity. After initial identification of isolates, final identification/confirmation was done by Vitek 2-compact system. The Vitek 2 compact system is a fully automated system for identification and antimicrobial sensitivity on MIC basis. Minimum inhibitory concentration of the following antibiotics was done by using Vitek system, the antibiotics were as follow; ampicillin sulbactam, ticarcillin/clavulanic acid, piperacillin, cefuroxime, cefixime, ceftriaxone, cefepime, aztreonam, meropenem, levofloxacin, moxifloxacin, tetracycline, chloramphenicol, colistin and trimethoprim. The MIC, of all the antibiotics were interpreted by the system according to CLSI guidelines.

Results

Among 74 patients, 38 (51.0%) were males while 36 (49.0%) were female patients (**Figure 1**). Of these 74 patients, 14

(19.0%) were less than 20 years old, 9(12.0%) were 21-30 years old and 11 (15.0%) patients were 31-50 years old while 40 (54.0%) patients were 51-60 years old (**Figure 2**). 30 patients (40.0%) had head trauma, 21 (28.0%) stroke, 13 (18.0%) DIC, 5 (7.0%) ARDS (Acute respiratory distress syndrome) and 5 (7.0%) patients had other problems (**Figure 3**). Among 3 patients who died due to ventilator associated pneumonia, 2 (20.0%) were died with history of late diagnosis and 1 (10.0%) died with history of timely diagnosis (**Figure 4**). Among 72 patients, *klebsiella pneumonia* was identified in 27(37.0%) patients, *pseudomonas aeruginosa* in 20 (28.0%) patients, *acinetobacter baumannii* in 15 (21.0%) patients and *Escherichia coli* was identified in 10 (14.0%) patients (**Table 1**). Out of 27 *klebsiella pneumoniae*, 100% (27) were resistant to piperacillin, 70% (19) were resistant to levofloxacin, moxifloxacin, chloramphenicol, 55% (15) were resistant to ceftriaxone, trimethoprim, 52% (14) were resistant to cefuroxime, 41% (11) were resistant to cefuroxime axetil Ampicillin/sulbactem, 40% (11) were resistant cefepime, aztreonam, and 37% (10) were resistant to cefixime. *Klebsiella pneumoniae* were sensitive to meropenem, minocycline, tetracycline, tigecycline and colistin (**Table 2**).
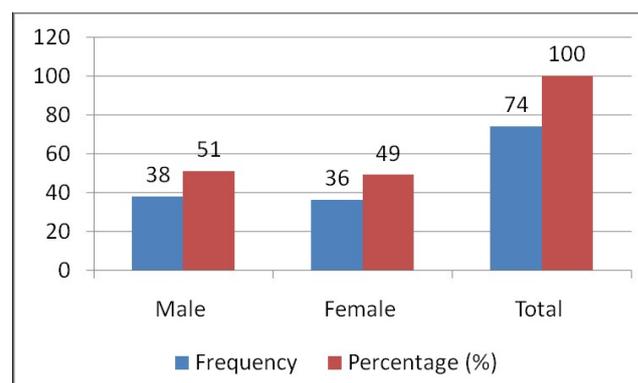

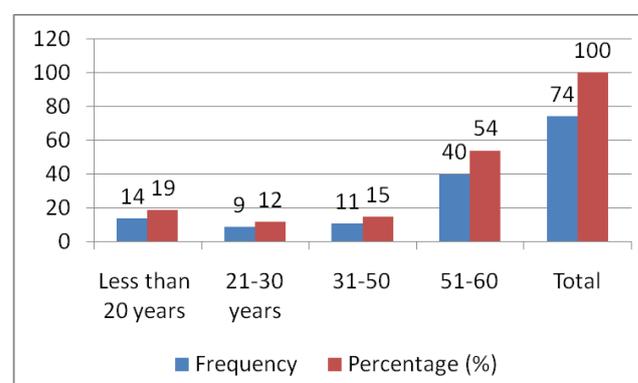**Figure 1** Distribution of patients according to gender.

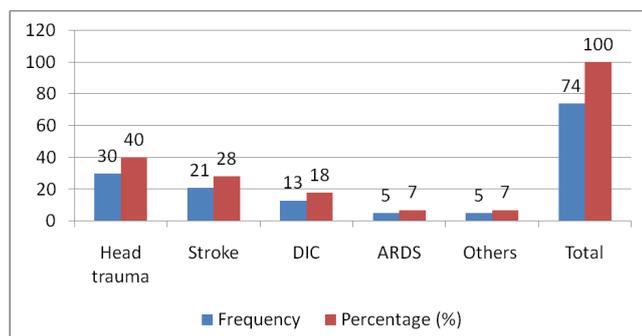**Figure 2** Distribution of patients according to age.

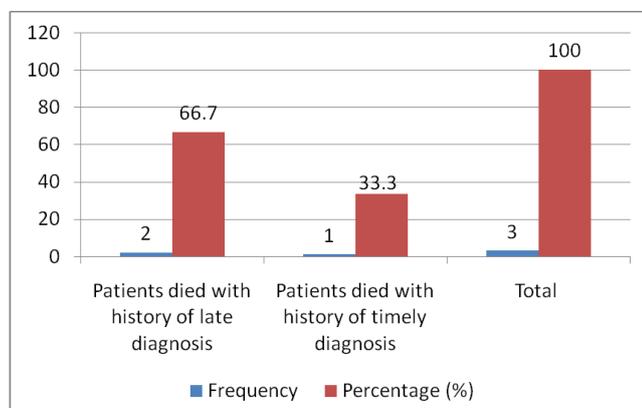**Figure 3** Distribution of patients according to primary cause of ICU admission.

Figure 4 Distribution of patients according to mortality rate due to VAP.

Table 1 Identification of VAP organisms.

Organism	Frequency	Percentage (%)
Klebsiella pneumoniae	27	37
Pseudomonas aeruginosa	20	28
Acinetobacter baumannii	15	21
Escherichia coli	10	14
Total	72	100

Table 2 Antibiotics resistance pattern of organisms isolated.

	Klebsiella pneumoniae	*Pseudomonas aeruginosa*	*Acinetobacter baumanii*	*Escherichia coli*
	n=27	n=20	n=15	n=10
Ampicillin/Sulbactam	41% (11)	80% (16)	100% (15)	60% (6)
Ticarcillin/Clavulanic Acid	-	20% (4)	-	-
Piperacillin	100% (27)	20% (4)	100% (15)	60% (6)
Cefuroxime	52% (14)	65% (13)	100% (15)	60% (6)
Cefuroxime Axetil	41% (11)	65% (13)	100% (15)	60% (6)
Cefixime	37% (10)	65% (13)	100% (15)	60% (6)
Ceftriaxone	55% (15)	65% (13)	100% (15)	60% (6)
Cefepime	40% (11)	35% (7)	100% (15)	60% (6)
Aztreonam	40% (11)	-	100% (15)	60% (6)
Meropenem	0% (0)	20% (4)	100% (15)	60% (6)
Levofloxacin	70% (19)	0% (0)	100% (15)	60% (6)
Moxifloxacin	70% (19)	30% (6)	100% (15)	60% (6)
Minocycline	0% (0)	65% (13)	100% (15)	0% (0)
Tetracycline	0% (0)	65% (13)	100% (15)	40% (4)
Tigecycline	0% (0)	65% (13)	0% (0)	0% (0)
Chloramphenicol	70% (19)	65% (13)	100% (15)	60% (6)
Colistin	0% (0)	0% (0)	0% (0)	0% (0)
Trimethoprim	55% (15)	65% (13)	100% (15)	40% (4)

Out of 20 *Pseudomonas aeruginosa* 80% (16) were resistant to Ampicillin/salbactem, 65% (13) were resistant to cefuroxime, cefuroxime axetil, cedixime, ceftriaxone, minocycline, tetracycline, tigecycline chloramphenicol, trimethoprim 35% (7) were resistant to cefepime, 30% (6) were intermediate resistant to moxifloxacin, and 20% (4) were resistant to meropenem, piperacillin, ticarcillin/clavulanic acid.

Acinetobacter baumanii were 100% (15) resistant to ampicillin/salbactem, piperacillin, cefuroxime axetil, cefixime, ceftriaxone cefepime, aztreonam, meropenem, levofloxacin, moxifloxacin, trimethoprim, and 100% intermediate resistant to minocycline. This is sensitive to Colistin and tigecycline.

E. coli were 60% (6) were resistant to ampicillin/salbactem, piperacillin, cefuroxime, cefuroxime axetil, cefixime, ceftriaxone, cefepime, aztreonam, meropenem, levofloxacin, moxifloxacin, and chloramphenicol, 40% (4) were resistant to tetracycline, trimethoprim, *E. coli* was sensitive to Minocycline and colistin.

Discussion

Present study was carried out at Department of Microbiology, University of Health Sciences, Lahore regarding isolation and antimicrobial susceptibility profile of microorganisms isolated from ventilator associated pneumonia patients. To acquire appropriate outcomes, total 74 patients were included in the study and samples were collected from the patients who developed pneumonia after 48 hours of stay in intensive care unit of the hospital.

Study disclosed that more than half (51.0%) of the patients were male and 49.0% were female patients. The findings of a similar study carried out by Kumar and coworkers are almost comparable with our study who reported that more than half

(64.2%) of the patients were male and 35.8% were female patients [5].

Age is a leading factor that plays a significant role in an individual life because with increasing age resistance power is reduced and elderly people are more susceptible to numerous infections. It is important to mention that most of the patients (54.0%) were 51-60 years old followed by 31-50 years old (15.0%) and 21-30 years old (12.0%) while 19.0% patients were less than 20 years old. The results of our study are comparable with the study undertaken by Nkirote who elucidated that majority of the patients (50.1%) were 51-60 years old, followed by 31-50 years old (37.3%), 21-30 years old (6.30%) and less than 20 years old (6.3%) [12].

During study initial cause of ICU admission was also assessed, study disclosed that major proportion (40.0%) of the patients had head trauma, 28.0% had stroke, 18.0% had disseminated intravascular coagulation and 7.0% had acute respiratory distress syndrome while 7.0% patients were admitted in the health care facility due to other health problems. In a study Charles and associates indicated that 22.2% patients were admitted due to respiratory diseases, 22.2% due to cardiology diseases and 5.6% patients admitted in the hospital due to trauma [13].

When the mortality rate among patients was evaluated, study showed there were three mortalities. Among these patients, 66.7% died with history of late diagnosis while 33.3% died with history of timely diagnosis. The findings of our study are consistent with a study conducted by Badawy and colleagues who reported that 59.0% patients died with history of late diagnosis while 31.0% died with history of timely diagnosis [14].

It was found during study that among 72 patients, *klebsiella pneumonia* was identified in majority (37.0%) of patients, *pseudomonas aeruginosa* in 28.0% patients, *acinetobacter baumannii* in 21.0% patients and *Escherichia coli* was identified in 14.0% patients. While findings of the study done by Turković and collaborators demonstrated that *Pseudomonas aeruginosa* was identified in most of the patients (19.0%), *Acinetobacter baumannii* was identified in 13.6% patients, *Escherichia coli* in 8.1% patients and *Klebsiella pneumonia* was identified in 8.1% patients. Another study carried out in India by Kant and fellows, highlighted that *Acinetobacter baumannii* was identified among 25.4% patients, *Pseudomonas aeruginosa* among 17.9% patients, *Klebsiella pneumonia* among 10.4% patients and *Escherichia coli* among 8.9% patients [15,16].

Study revealed that among 27 *klebsiella pneumoniae* organism, 100.0% were resistant to piperacillin, 70% were resistant to levofloxacin, moxifloxacin, chloramphenicol, 55% were resistant to ceftriaxone, trimethoprim, 52% were resistant to cefuroxime, 41% were resistant to cefuroxime axetil Ampicillin/sulbactam, 40% were resistant to cefepime, aztreonam, and 37% organism were resistant to cefixime while *Klebsiella pneumoniae* were sensitive to meropenem, minocycline, tetracycline, tigecycline, colistin. The findings of the study undertaken by Ahmed and companions also elucidated that *Klebsiella pneumoniae* were 100.0% resistant to piperacillin, 30.0% resistant to ceftriaxone, Ampicillin and 44.4% to tigecycline. A recent study undertaken by Djordjevic et al. highlighted 62.8% resistant to meropenem [8,17].

Study disclosed that among 20 *pseudomonas aeruginosa*, 80% were resistant to Ampicillin/salbactem, 65% were resistant to cefuroxime, cefuroxime axetil, cedixime, ceftriaxone, minocycline, tetracycline, tigecycline chloramphenicol, trimethoprim, 35% were resistant to cefepime, 30% were intermediate resistant to moxifloxacin and 20% were resistant to meropenem, piperacillin, ticarcillin/clavulanic acid. In a study conducted by Turković and collaborators indicated that *pseudomonas aeruginosa* were 9.0% resistant to meropenem and 4.0% resistant to piperacillin [15].

It was found during study that *Acinetobacter baumanii* were 100% resistant to ampicillin/salbactem, piperacillin, cefuroxime axetil, cefixime, ceftriaxone cefepime, aztreonam, meropenem, levofloxacin, moxifloxacin, trimethoprim and 100% intermediate resistant to minocycline while sensitive to colistin and tigecycline. The findings of the study carried out by Turković and collaborators highlighted that *Acinetobacter baumanii* were 8.0% resistant to ampicillin/salbactem and 63% resistant to meropenem [15]. A study done by Sarkar et al. showed that *Acinetobacter baumanii* were 30% sensitive to tigecycline [18].

Study further disclosed that *E. coli* were 60.0% resistant to ampicillin/salbactem, piperacillin, cefuroxime, cefuroxime axetil, cefixime, ceftriaxone, cefepime, aztreonam, meropenem, levofloxacin, moxifloxacin and chloramphenicol, 40% resistant to tetracycline, trimethoprim while sensitive to minocycline and colistin. A similar study carried out by Golia et al. highlighted that *E. coli* were 100% resistant to ampicillin and aztreonam [6].

Conclusion

Major cause of ICU admission was head trauma and stroke, and a very high prevalence of multi drug resistance ventilator associated pneumonia was observed at a teaching hospital in Pakistan.

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