MICROBIOLOGICAL ORGANISMS AND THEIR ANTIMICROBIAL SENSITIVITY CAUSING VENTILATOR ASSOCIATED PNEUMONIA (VAP)

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ABSTRACT

Objective: To determine the frequency of different causative bacteriological organisms and their antibiotic sensitivity from Endotracheal Aspirate (EA) of patients suffering from Ventilator Associated Pneumonia (VAP).
Study Design: Prospective cross sectional study.
Place and Duration of Study: Intensive Care Unit (ICU), Combined Military Hospital (CMH) Lahore, from May 2013 to Nov 2013.
Material and Methods: A total of 180 cases of VAP, fulfilling the ınclusion criteria and admitted in the ICU, were included in the study using the non-probability consective sampling technique. A written informed consent was obtained from the family. All these patients underwent endotracheal aspirate for microscopy and culture. Antibiotic sensitivity was determined using standard antibiotics regimens.
Results: Out of 180 patients, 165 (91.7%) were culture positive while 15 (8.3%) were culture negative. Gram-negative bacilli accounted for about 70% of all isolates. The most common organism isolated was Pseudomonas aeruginosa 25% (n=45) followed by MRSA 18.9% (n=34), *Klebsiella* 15.6% (n=28), Actinobacter spp 13.3% (n=24), *E.coli* 11.7% (n=21) and *Citrobacter spp* 4.4% (n=8). Carbapenem was the most sensitive drug that was seen in our setup but still 43.9% of the isolates showed resistance against it and resistance was noted still higher with Actinobacter spp, where 83% isolates were resistant. Quinolones showed resistance in 100% of the isolates of Actinobacter, MRSA and Citrobacter. While more than 50% strains of Pseudomonas, *E.coli* and *Klebsiella* were also resistant to quinolones. Cephalosporins showed excellent sensitivity towards gram negative bacteria which included Citrobacter (100% sensitive) and *E.coli* (80% sensitive). Polymxins showed more than 50% sensitivity to Pseudomonas aeruginosa, Actinobacter, *E. coli* and *Klebsiella*.
Conclusion: VAP remains a very important hospital-acquired infection. The most prevalent etiological organism in our study was Pseudomonas aeruginosa and the most effective antibiotics were carbapenems.

Keywords: Bacteria, Drug sensitivity, Resistant organism, Ventilator associated pneumonia.

INTRODUCTION

Ventilator Associated Pneumonia (VAP) is the most common device-associated nosocomial infection acquired among patients who are Mechanically Ventilated (MV) in the Intensive Care Unit (ICU). The International Nosocomial Infection Control Consortium (INICC) data suggests a VAP incidence as high as 13.6/ 1000 Mechanical Ventilation (MV) days. The occurrence of VAP in Asian countries is much higher, and ranges from 3.5 to 46 infections/1000 MV days[1]. Various studies have shown this incidence ranges from 20 to 26% in Pakistan[2]. The diagnosis of VAP requires assessment of various clinical criteria which includes; presence of fever, increased WBC, persistent or new x-ray infiltrates, purulent bronchial secretions and impaired oxygenation. The bacteriological investigation of the respiratory samples appears to be of extreme usefulness. Endotracheal Aspi-rate (EA) is used frequently as a diagnostic method in intubated patients with suspicion of pulmonary infection, because of its simplicity and minimal required training. Despite advances in antimicrobial therapy, better supportive care modalities and use of a wide range of preventive measures, VAP continues to be an important cause of morbidity, longer stay

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and mortality in ICU. The mortality associated with VAP ranges from 24–76%, and is even higher among critically ill patients[3].

In most Western hospital ICU's, gram positive organisms, especially Methicillin-Resistant Staphylococcus Aureus (MRSA), are the commonest pathogens[4]. This is in contrast to most of Asian developing countries where the most common pathogens are gram negative organisms[5]. A Pakistani study showed that the common pathogens causing VAP were Pseudomonas aeruginosa (63%), Acinetobacter lwoffii (22%) and Staphylococcus aureus (33%). The prevalence of antimicrobial resistance among VAP pathogens is steadily increasing. Staphylococcus resistance to methicillin has increased to 11% and Klebsiella resistance to third-generation Cephalosporins has increased to 47%. Pseudomonas resistance to imipenem, fluoroquinolones, and third-generation Cephalosporins has increased to 15, 9, and 20% respectively[3]. VAP patients receiving partially or totally inappro-priate therapy are at significantly high risk for death. A delay of >2 days in administering the first dose of appropriate antibiotic therapy significantly prolongs the duration of ventilation. Early administration of appropriate antibiotic therapy, based on the antibiogram of the VAP pathogens identified by quantitative/qualitative culture of endotracheal aspirate, can lead to an improved outcome of patients. As the causative organisms as well as antibiotic sensitivity spectrum vary from hospital to hospital, the current study will provide the information about the frequency of different microorganisms causing VAP and their antibiotic sensitivity patterns in our hospital.

MATERIAL AND METHODS

This prospective cross sectional study was carried out from 23rd May 2013 to 23rd Nov 2013 at the ICU of CMH Lahore, which is a tertiary care hospital. A total of 180 cases fulfilling the inclusion criteria were included in the study through non-probability consecutive sampling technique. WHO calculator was used to calculate sample size keeping confidence interval 95%, absolute precision 5% and anticipated population proportion 45%. The patients aged between 15-60 years who developed clinical or radiological signs of pneumonia after being on ventilator for at least 48 hours were studied. Patients who already had chest infections before being ventilated, those who had lobectomy/pneumonectomy, brain dead patients and those who were on two or more antibiotic before intubation were excluded from the study. Permission was obtained from "Hospital Ethical Committee". A written informed consent was obtained from the patients before being ventilated if possible or the families wherever applicable. The patients were sedated by using inj. thiopentone sodium or inj. midazolam or narcotics like inj. morphine or inj. Pethidine while placing on the ventilator. Endotracheal Intubation was achieved by using either depolarizing neuromuscular blockers like inj. suxamethonium or nondepolarizing agents like inj. pancuronium or inj. atracurium. A disposable cuffed endotracheal tube made up of polyvinyl chloride was used. Patients were placed on Adult Star Ventilator (Infrasonic Inc. San Diego California USA). Demographic characteristics (name, age, sex, residence, contact number) for each patient were recorded. A detailed history was obtained including any pre-existing lung diseases. The diagnosis of pneumonia was suspected when the patients showed at least three of these features; fever (temperature >38.5°C), purulent tracheobronchial secretions, leukocytosis (>12000/mm^3), leukopenia (4000/mm^3) and new, progressive or persistent (24 hours.) infiltrate on the chest radiograph. All patients with clinical diagnosis of VAP underwent Endotracheal Aspirate (EA) to ascertain which organisms were causing VAP and their antibiotic sensitivities was also determined. Endotracheal aspirate (EA) was performed by advancing a 22-inch Ramson's 12 F suction catheter through endotracheal tube with syringe and injecting 10 ml of normal saline for <1 min until resistance was met and then applying suction. The EA was collected in a sterile container and transported within two

hours for culture. Gram stain preparations were made from all aspirate samples. Samples were inoculated onto 5% blood agar, MacConkey agar, which were reconstituted according to the manufacturer's specifications, and sterilized at 121°C for 15 minutes. The plates were incubated at 37°C for 18-24 hours and were read the following day. Isolated colonies were subjected to Gram staining and biochemical tests for identification. Identification was performed according to standard biochemical tests. Antimicrobial susceptibility test was done using Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) 2011 guidelines. Small wafers containing antibiotics were placed onto a plate upon which bacteria were growing. If the bacteria were sensitive to the antibiotic, a clear ring, or zone of inhibition, was seen around the wafer indicating poor growth. All the VAP patients were treated by empirical treatment till drug sensitivity of the required organism was known and the treatment was altered thereafter. Data was analyzed using SPSS version 10. Frequencies and percentages were calculated for qualitative variables like gender, microorganisms and their drug sensitivity. Mean and standard deviation were calculated for the quantitative variable like age.

RESULTS

A total of 180 patients of VAP were studied. Out of 180 patients 165 (91.7%) were culture positive while 15 (8.3%) were culture negative. There were 114 (63.3%) male while 66 (36.7%) were female patients. The mean age of patients was 50.23 years (SD 17.343). The most common organism isolated was Pseudomonas aeruginosa n=45 (25%) followed by MRSA n=34 (18.9%), Actinobacter spp. n=24 (13.3%), Klebsiella n=28 (15.6%), *E.coli* n=21 (11.7%) and Citrobacter spp n=8 (4.4%). Results for antimicrobial resistance in major bacterial isolates revealed; In Pseudomonas resistance to Cephalosporins, polymxins, aminoglycosides, quinolones, penicillin and tetracycline was 66.7%, 55.6%, 55.6%, 66.7%, 66.7% and 88.9% respectively, while 100% strains were resistant to glycyclines, linezolid, vancomycin, macrolides and lincosamide. In *S.aureus* resistance to aminoglycosides, linezolid, tetracyclines, macrolides and licosamide was 55.9%, 26.5%, 73.5%, 55.9% and 55.9% respectively while 100% were resistant to Cephalosporins, Polymyxins, Quinolones, Penicillin, Carbapenems and Glycyclines. No vancomycin resistance was observed. In *Klebsiella spp*. resistance to cephalosporin, polymyxins, aminoglycosides, quinolones, glycylines and penicillin was 42.9%, 14.3%, 57.1%, 57.1%, 57.1% and 42.9% respectively while 100% were resistant to linezolid, tetracyclines, vancomycin, macrolides and lincosamide. In Actinobacter spp resistance to cephalosporin (cefoperazone), polymyxin, glycyclines and penicillin was 62.5%, 37.5%, 79.2% and 83.3% respectively while 100% were resistant to aminoglycosides, quinolones, linezolid, tetracycline, macrolides and lincosa-mide. In *E.coli* spp resistance to carbapenems, cephalosporins, glycyclines, aminoglycosides, quinolones, penicillins and tetracycline was 23.8%, 19%, 19%, 42.9%, 61.9%, 61.9% and 61.9% while all isolates were resistant to linezolid, vancomycin, macrolides and lincosamide. In Citrobacter spp resistance to polymyxin, aminoglycoside, quinolones, linezolid, vancomycin, macrolides, licosamide was 100% while 50% isolates were resistant to penicillin. The antibiotic sensitivity patterns are presented in table.

DISCUSSION

VAP is a nosocomial infection associated with high mortality rates and diverse groups of etiological microorganisms. These bacteria are usually resistant to many of the routine antibiotics available. Ventilator associated pneumonia not only increases the duration of ICU stay but also increases the burden in the term of cost of treatment. Centers for Disease Control and Prevention data report an incidence of VAP of 0.0-5.8/1000 ventilator days in the ICUs of various hospitals. However, the incidence of VAP reported in literature is as high as 58%[6]. Every day that patients spend in the

ICU and on MV increases the risk of infection. Factors facilitating infection include underlying diseases, comorbid factors, malnutrition, nasogastric tube use, gastroesophageal reflux, sedation, invasive procedures to the respiratory system and aspiration of contaminated secretions accumulating on the endotracheal cuff[7]. The mortality rate of VAP ranges from 24 to 50% in

Table: Drug sensitivity pattern of different micro-organisms in the study population.

Pathogen	Antibiotics	Sensitivity n (%)	Resistance n (%)
Pseudomonas Aeruginosa	Cabapenems	45 (100%)	
	Cephalosporins	15 (33%)	30 (66.7%)
	Polymyxin B	20 (44.4%)	25 (55.6%)
	Aminoglycosides	15 (33.3%)	25 (55.6%)
	Quinolones	15 (33.3%)	30 (66.7%)
	Glycyclines	-	45 (100%)
	Linezolid	-	45 (100%)
	Penicillin	15 (33.3%)	30 (66.7%)
	Tetracycline	5 (11.1%)	40 (88.9%)
	Vancomycin	-	45 (100%)
	Macrolides	-	45 (100%)
	Lincosamide	-	45 (100%)
Staphylococcus Aureus	Cabapenems	-	34 (100%)
	Cephalosporins	-	34 (100%)
	Polymyxin B	-	34 (100%)
	Aminoglycosides	15 (44.1%)	19 (55.9%)
	Quinolones	-	34 (100%)
	Glycyclines	-	34 (100%)
	Linezolid	25 (73.5%)	9 (26.5%)
	Penicillin		34 (100%)
	Tetracycline	9 (26.5%)	25 (73.5%)
	Vancomycin	34 (100%)	
	Macrolides	15 (44.1%)	19 (55.9%)
	Lincosamide	15 (44.1%)	19 (55.9%)
Klebsiella Spp.	Cabapenems	28 (100%)	
	Cephalosporins	4 (14.3%) Cefotaxime, 12 (42.9%) cefipime	12 (42.9%)
	Polymyxin B	24 (85.7%)	4 (14.3%)
	Aminoglycosides	12 (42.9%)	16 (57.1%)
	Quinolones	12 (42.9%)	16 (57.1%)
	Glycyclines	12 (42.9%)	16 (57.1%)
	Linezolid	-	28 (100%)
	Penicillin	16 (57.1%)	12 (42.9%)
	Tetracycline	-	28 (100%)
	Vancomycin	-	28 (100%)
	Macrolides	-	28 (100%)
	Lincosamide	-	28 (100%)
Actinobacter Spp.	Cabapenems	4 (16.7%)	83.3%
	Cephalosporins	4 (16.7%) cefipime, 9 (37.5%) cefoperazone	20 (83.3%)
	Polymyxin B	15 (62.5%)	9 (37.5%)
	Aminoglycosides	-	24 (100%)
	Quinolones	-	24 (100%)
	Glycyclines	5 (20.8%)	19 (79.2%),
	Linezolid	-	24 (100%)
	Penicillin	4 (16.7%)	20 (83.3%).
	Tetracycline	-	24 (100%)
	Vancomycin	-	
	Macrolides	-	100%
	Lincosamide	-	100%
Enterobactereace	Cabapenems	16 (76.2%)	5 (23.8%)
	Cephalosporin	17 (80.9%)	4 (19%)
	Polymyxin B	17 (81%) poly B, 4 (19%)poly E	
	Aminoglycosides	12 (57.1%)	9 (42.9%)
	Quinolones	8 (38.1%)	13 (61.9%)
	Glycyclines	17 (81%)	4 (19%)
	Linezolid	-	21 (100%)
	Penicillin	8 (38.1%)	13 (61.9%)
	Tetracycline	8 (38.1%)	13 (61.9%)
	Vancomycin	-	21 (100%)
	Macrolides	-	21 (100%)
	Lincosamide	-	21 (100%)

various studies. A study by Ali et al showed 30-day mortality was 23.6%[8]. The microbiological diagnosis of VAP can be reached by invasive methods, such as fiber optic Bronchoscopic protected Specimen Brush (PSB) and Broncho Alveolar Lavage (BAL), or by noninvasive methods, such as endotracheal aspiration (EA). The former methods demand expert personnel, have potential complications, and are not promptly available; the latter methods can be readily performed, being also cost-effective and less invasive[9]. Authoritative guidelines state that diagnostic sensitivity of EA and BAL is in accordance[10]. Etiological agents widely differ according to the population of the patients in ICU, duration of hospital stay and prior antimicrobial therapy. The common organism causing VAP in our study were Pseudomonas aeruginosa (25%), staphylococcus aureus (18.9%) Klebsiella spp (15.6%), Actinobacter spp (13.3%), E.coli (11.7%) and Citrobacter (4.4%). So the majority of VAP was caused by gram negative organism as documented in most of the other Asian studies[5] in contrast to the Western countries where *S.aureus* is the most prevalent organism. A recent report presented by a panel of experts from ten Asian countries suggested that the prevalence of MDR pathogens is rising in Asian countries, and Actionbacter Baumannicalcoaceticus complex is emerging as a major pathogen in most of these ICUs[5]. MDR pathogens are referred to bacteria such as Pseudomonas species, Acinetobacter species, MRSA, and enteric Gram-negative bacilli expressing Extended Spectrum Beta Lactamase (ESBL) and AmpC β-lactamases and characteristically, displaying high levels of antibiotic resistance[11].

The aim of our study was to reemphasize the importance of identifying different micro-organisms and their culture sensitivity, which cause VAP, by EA in order to design the most effective empirical treatment in our setting. We found that the gram-negative bacilli accounted for 70% of all isolates causing VAP. Notably, 100% of Staphylococcus aureus infections were caused by methicillin-resistant strains, 51% of Enterobacteriaceae isolates were resistant to ceftriaxone, and 59% of Pseudomonas aeruginosa isolates were resistant to quinolones. Cephalosporins showed activity against gram negative bacteria which included some Enterobactereace (83.3%) and Citrobacter (100%) species. Eighty percent isolates of Enterobactereace were sensitive to 3rd and 4th generation cephalosporins. Aminoglycosides were not effective at all against Actinobacter and Citrobacter with a resistance of 100%, while only 44% isolates of pseudomonas aeruginosa were sensitive to these drugs. Quinolones showed resistance in 100% of the isolates of Actinobacter, Methicillin Resistant Staphylococcus Aureus (MRSA) and Citrobacter. While more than 50% strains of Pseudomonas, *E. coli* and *Klebsiella* were resistant to quinolones (Levofloxacin, ciprofloxacin, moxifloxacin). Hence, quinolones should not be used empirically in our ICU setup due to on-growing resistance of organisms. Carbapenems were found to be the most effective drug, still 43.9% of overall isolates showed resistance against Carbapenems. All isolates of *P. aeruginosa, K. pneumonia* and *Citrobacter* were sensitive to Carbapenems. The emergence of the resistance to Carbapenems was noted especially in Actinobacter (83.3%) and *Escherichia coli* (23.8%). This might be due to a carbapenemase producing strains. The commonest pathogen in our study, Pseudomonas aeruginosa, was resistant to the commonly used beta-lactam antibiotics, with more than 66% (30) isolates resistant to all generations of cephalosporin, while 33% isolates being sensitive only to cefipime and 44.4% isolates were sensitive to polymyxins. All 34 (100%) isolates of *S. aureus* were resistant to penicillin, indicating the high prevalence of MRSA as a cause of VAP in our setting. All MRSA were sensitive to vancomycin and only 25 (73.5%) to linezolid. Twelve isolates of *K. pneumoniae* out of 28 (42.9%) were resistant to ceftriaxone and ceftazidime, suggesting a high prevalence of Extended-Spectrum Beta-Lactamase (ESBL) producing organism. Twelve isolates (42.9%) were sensitive to gatifloxacin,

tigeycycline and aminoglycoside, while 28 isolates (100%) were sensitive to Carbapenem. Only 4 isolates (16.7%) of Actinobacter were sensitive to Carbapenems. However, 15 isolates (62.5%) were sensitive to polymyxins. To summarize, the most effective drug therapy for *P.aueruginosa, Staphylococcus aureus*, *Klebsiella spp* and *Actinobacter spp* were Carbapenems, vancomycin and polymxins respectively. *P.aeruginosa* showed high resistance against Cephalosporins, aminoglycosides and quinolones. Most of the Actinobacter spp were resistant to Carbapenems and sensitive to Polymyxins while other gram negative isolates (*Klebsiella spp and E.coli spp*) showed sensitivity to Carbapenems and resistance to aminoglyco-sides and quinolones.

Our study results are comparable to various international and national studies. The minor variations can be attributed to the different patient populations studied, the method used to obtain and analyze specimens and the clinical criteria used for VAP. Besides, pathogens associated with VAP have been shown to vary among different hospitals. A study carried out by Kumar et al in Pakistan showed that commonest organism causing VAP in their hospital was Pseudomonas Aeruginosa n=29 (63.5%), the figure which is quite high as compared to our study (25%). Other common organisms in their study, in the order of frequency, were *S.aureus* (33.3%), Actinobacter spp (22.6%), Entero-bacteriaceae (21.4%) and streptococcal pneumoniae (13%). Fifty two percent of the Actinobacter spp were multidrug resistant in their study[12]. Gram negative bacilli were isolated from 77% specimens which is 70% in our study population. Ceftazidime, ciprofloxacin, and amikacin resistance rate were higher against Acinetobacter and *P.aeruginosa* and Carbapenem was the most sensitive drug[13]. A Turkish study performed by Karatas et al showed showed Gram negative bacteria were isolated at a level of 78.9% from endotracheal material from patients with VAP, Gram positive bacteria at 19.4% and fungi at 1.7%. Polymicrobial growth was determined in 4.2% of VAPs. The five most common causes of VAP were Acinetobacter species (31.0%), Pseudomonas aeruginosa (27.6%), Staphylococcus aureus (15.1%), Klebsiella species (6.5%) and *Escherichia coli* (5.6%)[14]. An Indian study by Mathai et al found, the most common organisms grown were Acinetobacter (58 isolates, 53.2%), Klebsiella (17 isolates, 15.6%), Pseudomonas (14 isolates, 12.8%), and Escherichia coli (nine isolates, 8.25%). Many of the isolated organisms exhibited resistance to the commonly used antibiotics and 26 (27.3%) patients were found to be infected with MDR organisms. There were a high proportion of Extended Spectrum β Lactamases (ESBL) producing strains among Klebsiella species (13 isolates, 76.5%) and *E. coli* (5 isolates, 55.55%) strains. While all strains of Acinetobacter were MDR organisms, 25 of these isolates (43.1%) were resistant to the Carbapenem group of antibiotics. A significant number of Klebsiella (12 isolates, 70%) and Pseudomonas (four isolates, 28.5%) isolates also demonstrated resistance to Carbapenems[1].

There are few limitations to our study. Firstly, endotracheal aspirate is an operator dependent procedure so error in collection and further contamination of that specimen during transport to the laboratory can occur. Secondly due to expensive newer antibiotics it was not possible to use them in all our VAP patients. Local antibiograms in every institute should be reviewed when empirical therapy for VAP is being selected. Antibiotics should be further adjusted on the basis of culture results. The first antibiotic regimen should be optimized, because inappropriate initial therapy is associated with worsened outcomes, even if the regimen is subsequently changed on the basis of the microbiologic results. The incidence of VAP caused by MDR organisms is on the rise. A study with even larger sample size and further controlled trials are required to elucidate the findings of our study and to build an antibiogram for various hospitals.

CONCLUSION

Pseudomonas, Actinobacter and Staphylococcus aureus are the commonest etiological agents of VAP. Carbapenems, poly-mixins, vancomycin and in some cases cephalosporin seem reasonable effective anti-biotics against these organisms.

RECOMMENDATION

Local epide-miological data should be collected at all centers, as this information will guide the initial empirical antibiotic therapy, will prevent the development of more resistant strains and thereby will reduce the morbidity and mortality associated with VAP.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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