Treatment Protocol of Ventilator-Associated Pneumonia based on Microbial Susceptibility in Pediatric Intensive Care Unit, Isfahan, Iran

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Abstract

Background

Choosing a unique empiric treatment for ventilator associated pneumonia (VAP) can be challenging. We aimed to determine the antimicrobial susceptibility pattern of Intensive Care Unit (ICU) of the only referral pediatric hospital in Isfahan in order to design the optimal empiric treatment protocol.

Materials and Methods: In this cross-sectional study 343 isolates were detected from 243 pediatric patients, from August 2017 to December 2018 in Imam Hossein Hospital, Isfahan, Iran. In suspicious cases of VAP, sampling was performed via non-Bronchoscopic Bronchoalveolar Lavage (NB-BAL). Microbial susceptibility and resistance were assessed. The treatment protocol of VAP was prepared based on existing guidelines.

Results: Out of 343 isolates 42 (12.2%) of the positive cultures were *Candida albicans* and 301 (87.8%) were bacterial isolates. Gram-negative bacteria were the most common organisms with the cumulative percentage of 62.9% of bacterial isolates. When tested with oxacillin, 61.5% of *Staphylococcus aureus* were MSSA and 38.5% were MRSA. The mentioned common gram-negative organisms had more than 25% resistance to at least one antibiotic from three or more antibiotic classes. However, *P. aeruginosa* showed below 20% resistance to majority of antibiotics. Twenty-seven (11.1%) of patients had VAP, 25 (92.6%) of whom were gram-negative infections.

Conclusion: The limited time period and sample size without any follow-up, made it impossible to define an effective treatment protocol. We defined our antibiogram in accordance with the existing standard guidelines and we designed a local protocol. An effective antibiotic against MRSA should be used in the empiric treatment of VAP. Also, in presence or absence of multidrug-resistant (MDR) pathogen risk-factors, it is necessary to use two effective antipseudomonal antibiotics from different antibiotic classes.

Key Words: Microbial Sensitivity Test, Pediatric, Pneumonia, Ventilator-Associated.

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1- INTRODUCTION

Hospital-acquired pneumonia (HAP) is a lung parenchyma infection caused by nosocomial pathogens (1), which develops in patients hospitalized for more than 48 hours (1, 2). Ventilator-associated pneumonia (VAP) is a type of HAP that occurs in intensive care unit (ICU) patients under mechanical ventilation for more than 48 hours (1-4). Pneumonia associated with mechanical ventilation is the second most prevalent nosocomial infection (5). A systematic review of VAP from 1947-2010 that was performed among pediatric patients identified *Pseudomonas aeroginosa* followed by *Staphylococcus aureus* as the predominant microorganisms causing pediatric VAP (6).

The 2016 HAP/VAP guidelines recommend that in order to minimize patients' exposure to unnecessary antibiotics and to reduce microbial resistance, the antibiogram data should be prepared to reduce the unnecessary usage of two antipseudomonal and anti-methicillin-resistant *Staphylococcus aureus* (MRSA) antibiotics in empiric therapy (7). Hence, each hospital must deliver the best antibiotic choice for each organism to its clinicians by determining the prevalence of bacteria causing hospital infections and their local antibiogram (7).

For the purpose of antibiogram preparation, utilizing antimicrobial susceptibility profiles from homogenous patients, e.g. ICU patients, may improve the specificity, sensitivity (8, 9), and the accuracy of antimicrobial stewardship program (ASP) assessment and outcome relationship (10-12). HAP/VAP guidelines recommend that all hospitals determine their local antibiograms regularly, especially those specific to their ICU patients (6). They also recommend that empiric treatment of VAP should be designed in accordance with the local distribution of organisms and their susceptibilities (7, 13-16).

It is documented that the samples obtained through non-bronchoscopic bronchoalveolar lavage (NBBAL) by a pediatric ICU fellow in the suspected cases of VAP are similar to those taken by Bronchoscopic Bronchoalveolar Lavage (BAL) with high sensitivity and specificity rates (17-22). Organism growth in such samples is highly indicative of lower airway infection. Currently, in most academic ICUs of Iran including our center, an educational hospital affiliated with Isfahan University of Medical Sciences, Iran, and the only referral pediatric hospital of the province, the prevalence rates of local pathogens and microbial resistance are unclear. In most cases, the highest antibiotic coverage is chosen for patients, which not only imposes high expenses on patients and the health care system, but also uncontrollably increases microbial resistance. Considering the importance of antibiogram determination in each hospital and its associated ICU, in order to design an empiric antibiotic therapy, and due to the lack of specific therapeutic protocols in our center, we sought to design this study in order to determine the PICU antibiogram in Imam Hossein Hospital, Isfahan, Iran, and to explain the protocol for the treatment of VAP in this center.

2- MATERIALS AND METHODS

2-1. Study design and population

This cross-sectional study was conducted from August 2017 to December 2018 in Imam Hossein Hospital of Isfahan city, Iran, which is the only pediatric referral hospital of the province. Data gathered from 243 pediatric patients who were hospitalized for more than 48 hours in sites other than the emergency wards and had positive cultures. Based on Clinical and Laboratory Standard Institute (CLSI), a minimum of 30 isolates per anatomical site of infection or each

hospital unit was required to be included in the analysis of antibiogram (23).

2-2. Methods

In this study, all isolates were collected throughout a 16-month period from all hospital units, except for emergency ward after 48 hours of hospitalization or more. The microorganisms isolated after 48 hours of hospital admission were considered as nosocomial pathogens. Screening isolates were not included in this sample. The samples were obtained from suspected sites of infection in accordance with the clinical and paraclinical manifestations of the patients (24).

2-3. Laboratory measurements

Samples were isolated from blood (via BD BACTEC™ or standard disk diffusion blood culture), urine (via suprapubic sampling), respiratory secretions (NBBAL), and other sites in patients suspected to have infections such as sepsis, urinary tract infections (UTI), respiratory infections, VAP, peritonitis and wound infections. Microbial susceptibility testing was performed by disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) guideline (23, 24). In accordance with the latest CLSI guideline intermediate-resistant specimens were not reported as susceptible, and the cumulative antibiogram demonstrated the susceptibility percentage profiles (25). Because it was possible to study all suspected cases, the study was conducted as a census and it was not necessary to estimate a sample size. Patients' information and data from the positive cultures were collected into a specifically-designed form. Cases of VAP were collected in separate forms.

2-4. Intervention

In cases with the assumptive diagnosis of VAP, sampling was performed by a fellowship of PICU via non-

Bronchoscopic Bronchoalveolar Lavage (NB-BAL). According to Infectious Diseases Society of America (IDSA) (7), and American Thoracic Society (ATS) guidelines (26), cases that had been intubated for more than 48 hours, had infiltrations on chest X-ray, and had at least one of the clinical signs of a new-onset fever, leukopenia, leukocytosis or purulent respiratory secretions were considered as the suspicious cases of VAP. Patients who were intubated and had hemodynamic instability, worsening of blood gases or a decrease in oxygen saturations were also considered as the suspicious cases of VAP. Furthermore, NBBAL was performed and the associated samples were sent for culture and analysis (26, 27). NBBAL was performed in accordance with previous successful studies (5, 28). Distal bronchial samples were obtained from each patient according to the following technique: for a few minutes, the patients' lungs were preoxygenated with 100% oxygen before disconnection of the ventilator.

A sterile catheter was inserted through the endotracheal tube and advanced as far as possible. Then a second catheter was passed through it (28). Next 1 ml/kg normal saline was injected to the bronchus by the second catheter and then 1-3 cc was suctioned by Falcon tube and sent for bacteriologic examination, culture, and analysis. Samples were taken by one catheter in cases with ET size less than 4.5 mm or acute respiratory distress syndrome (ARDS) that needed high positive end-expiratory pressure (PEEP). Based on the same guideline, BAL samples can be reported in quantifiable values (colony counts), or can be declared semi-quantitatively (i.e. mild, moderate, or heavy growth) (7). We used the semi-quantitative method. Urine samples were obtained through suprapubic urine bladder drainage in patients less than one-year-old. In patients older than 12 months, samples

were obtained from urinary catheters. Gram-negative (bacilli) were differentiated with help of tests such as triple sugar iron (TSI) agar, Sulfur Indole Motility Media (SIM), urea, citrate, phenylalanine deaminase (PAD) test, and lysine and oxidase test. According to Clinical and Laboratory Standard Institute (CLSI) (2017), microbiology determination was performed to assess colistin susceptibility. According to CLSI (2017), cefoxitin and oxacillin are considered as surrogate agents for Staphylococcus. This means that the specimens resistant to oxacillin are resistant to cefoxitin and vice versa.

2-5. Ethical consideration

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all patients' caregivers for the purpose of publication, with the assurance of confidentiality of personal data.

2-6. Inclusion and exclusion criteria

Pediatric patients who were hospitalized for more than 48 hours in sites other than the emergency wards and had positive cultures were included in this study. Based on Clinical and Laboratory Standard Institute (CLSI), a minimum of 30 isolates per anatomical site of infection or each hospital unit was required to be included in the analysis of antibiogram. Patients were excluded if they did not meet the selection criteria above (23).

2-7. Data Analysis

To determine the frequency distribution of organisms isolated from PICU patients more than 48 hours after admission and their rate of resistance, descriptive statistics including numbers and percentages were used. For data analysis, independent samples t-test and Chi-square

were used where applicable. This study was not undertaken for any screening purposes, and specimens were obtained from suspected infectious sites and from patients with a clinical suspicion of infection. Hence, no suspected VAP cases were evaluated or reported. Statistical Package for Social Sciences version 22.0 was used for the means of data analysis. After preparing the results of the microbial susceptibility tests, and calculating the percentage and frequency of microbial resistance, the treatment protocol of VAP was prepared based on the existing validated guidelines for VAP (1, 7). Data were analyzed using one-way ANOVA, logistic regression, and Chi-square tests. The level of significance for all tests (type 1 error) was considered 0.05. Before going through the analysis we checked the normality of the data and for the data with non-normal distributions, non-parametric tests were used.

3- RESULTS

Overall, 343 isolates were detected from 243 patients (49.4% girls) suspected of hospital-acquired infection. Age was distributed with 59.9% < 1 year-old, 24.8% between 1-5 year-old, 9.9% with 5-12 years of age, and 5.4% >12 years old. The most isolates were gathered from PICU (44.9%), followed by (14.3%) from NICU, and (14%) from Pediatric Nephrology and Neurology wards (**Table. 1**). Moreover, 165(48.1%) isolates were detected 2-7 days after hospital or ICU admission, 149(43.4%) isolates within 7-30 days into their hospital/ICU admission, and 29(8.5%) isolates were detected with > 30 days after hospital or ICU length of stay. It is worth mentioning that 42 out of 343(12.2%) positive cultures were *Candida albicans* and 301 out of 343(87.8%) were bacterial isolates. Gram-negative organisms were the most frequent, with 34(9.9%) *Acinetobacter baumannii* isolates, 34(9.9%) *Klebsiella* isolates, 32(9.3%) *Enterobacter* isolates,

31(9%) *Pseudomonas aeruginosa* isolates, 31(9%) *Escherichia coli* isolates, 25(7.3%) *Serratia* isolates, 7(21%) *Citrobacter* isolates and 2(0.6%) *Proteus* isolates, with the cumulative percentage of 57% of all isolates and constituting 65.1% of bacterial isolates. Gram-positive isolates were less frequent than Gram-negative ones and included 17(5%) *Staphylococcus aureus* isolates, 60(17.5%) *Staphylococcus epidermidis* isolates, 7(2%) *Staphylococcus haemolyticus* isolates, 3(0.9%) *Staphylococcus saprophyticus* isolates, 15(4.4%) *Enterococcus* spp., and 3(0.9%) *viridans* streptococci (from blood) with cumulative percentage of 30.7% of all isolates (**Figure. 1**).

Table-1: Isolated strains obtained from different hospital wards (n=343).

Hospital wards	Number (%)
PICU	154 (44.9%)
NICU	49 (14.3%)
Pediatric Nephrology and Neurology wards	48 (14%)
Pediatric Respiratory and Gastroenterology wards	38 (11%)
NICU of Surgery ward	32 (9.3%)
Pediatric Surgery ward	15 (4.4%)
Pediatric Infectious Diseases ward	5 (1.5%)
Dialysis ward	2 (0.6%)
Total	343(100%)

PICU: pediatric intensive care unit, NICU: neonatal intensive care unit.
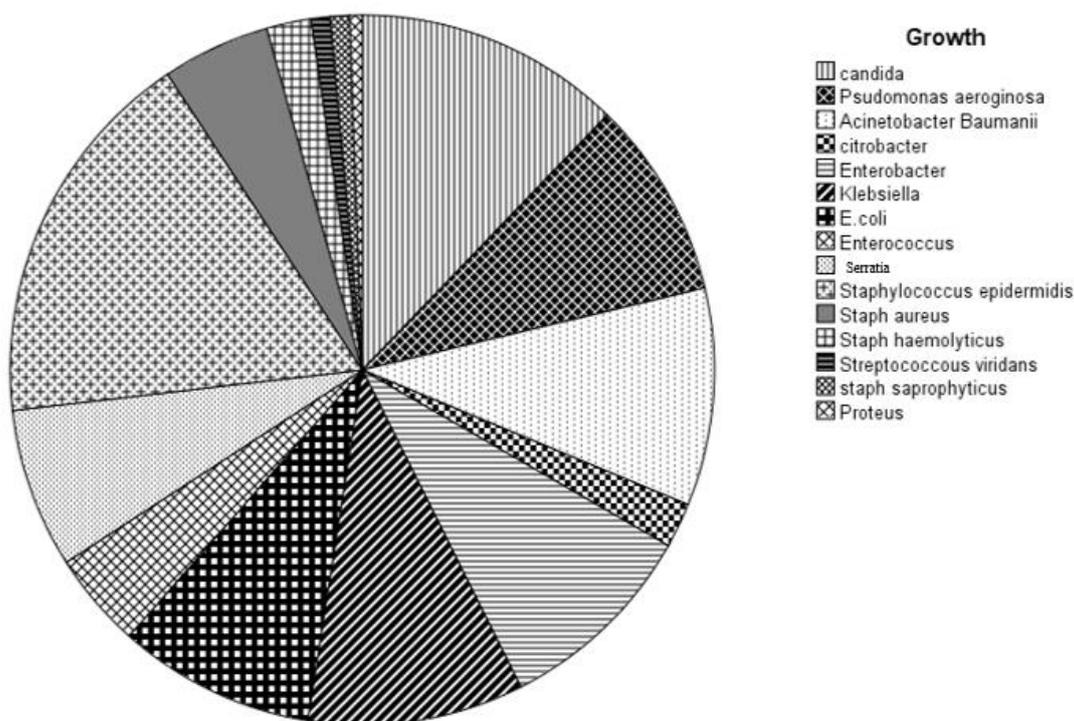

Fig.1: Isolated microorganisms.

In addition, 154(44.9%) isolates were detected from blood, 89(25.9%) from urine, 30(8.7%) from BAL specimen, 6(1.7%) from respiratory secretion, 12(3.5%) from wound, 10(2.9%) from peritoneal fluid, 22(6.4%) from eye discharge, 8(2.3%) from throat culture and 5(1.5%) from cerebrospinal fluid shunt. Results of cultures reported in exact accordance with CLSI 2017 cumulative antibiogram protocols are presented in **Tables 2, 3**. Pseudomonas had no resistance against amikacin but had 14.3% resistance to gentamicin. It seems that Pseudomonas aeruginosa isolates were the most sensitive Gram-negative organisms to most antibiotic classes. Acinetobacter isolates were sensitive to colistin in 12 cases, resistant to colistin in 2 cases and 20 cases were not reported.

According to CLSI (2017), colistin is within the group O (other) of the antimicrobial agents that are needed to be checked for VAP (i.e. not primarily/routinely checked), and is only assessed when there is resistance to other agents. Therefore 20 cases of Acinetobacter isolates were not evaluated for colistin susceptibility. Among *Enterobacter* isolates, sensitivity rates to meropenem and imipenem were 66.6% and 50%, respectively. For both *Enterobacter* and *Klebsiella* isolates, meropenem was less tested and reported than imipenem. Among the 15 *Enterococcus* isolates, three cases were sensitive to vancomycin, seven cases were resistant and results of vancomycin testing of five cases were not reported. In total, 7 out of 10 cases of vancomycin-tested *Enterococcus* isolates were vancomycin-resistant (VRE). Among *Staphylococcus aureus* isolates, eight cases were sensitive to oxacillin (cefoxitin), five cases were resistant and four cases were not reported. Among oxacillin-tested *Staphylococcus aureus* isolates, 61.5% were methicillin-sensitive *Staphylococcus aureus* (MSSA), and 38.5% were MRSA. Based on The American Thoracic Society and the Infectious Diseases Society of America (ATS-IDSA) guidelines, 27 patients had VAP with positive BAL culture, all of whom were in PICU. The frequency of isolates in BAL specimen was as follows: *Candida albicans* 1 case (3.7%), *Pseudomonas aeruginosa* 9 cases (33.3%), *Acinetobacter* 10 cases (37%), *Klebsiella* 3 cases (11.1%), *Serratia* 3 cases (11.1%), and *Staphylococcus aureus* 1 case (3.7%). Gram-negative organisms were the most frequent cause of VAP with cumulative percentage of 92.6%.

Only one *Staphylococcus aureus* isolate in BAL specimen was sensitive to cotrimoxazole and vancomycin and resistant to amikacin, clindamycin, oxacillin, penicillin, and erythromycin. Mean (SD) of hospital stay before VAP was 2.8 (0.62) days. Mean (SD) of ICU stay before VAP was 2.7 (0.64) days with minimum and maximum of 2 and 4 days, respectively. The mean (SD) for days of mechanical ventilation before VAP was 2.6 (0.62) with the minimum, and maximum of 2 and 4 days, respectively. Finally, 55.6% of VAP cases occurred during 7-30 days within their ICU stay, and 48.1% occurred 7-30 days into the initiation of mechanical ventilation and intubation.

Table-2: Antimicrobial susceptibility determinations of gram negative organisms.

Organisms		B-lactams								Aminoglycosides		FQs	Other categories	
Gram negative organism	No. strains	Ampicillin	Cefazolin	Cefotaxime	Ceftazidime	Meropenem	Imipenem	Piperacillin-tazobactam	Ampicillin sulbactam	Amikacin	Gentamicin	Ciprofloxacin	Trimethoprimsulfa methoxazole	Colistin
Acinetobacter. B	34	R	—	7.4%	31.8%	13.3%	13.7%	42.8%	R	21.7%	54.5%	33.3%	14.2%	85.7%
Klebsiella	34	R	—	7.1%	7.6%	16.6%	45.8%	68.1%	R	38.7%	33.3%	38.4%	46.6%	75%
Enterobacter	32	—	R	11.1%	27.2%	66.6%	50%	70.8%	R	60%	83.3%	66.6%	36.3%	—
Pseudomonas. A	31	—	—	R	86.3%	81.8%	86.3%	91.3%	—	100%	85.7%	92.8%	R	—
E.coli	31	R	—	3.7%	11.1%	100%	68.1%	85%	R	77.2%	42.8%	50%	41.6%	100%
Serratia	25	R	R	4.7%	11.1%	57.1%	52.9%	46.1%	R	4.7%	37.5%	77.7%	87.5%	100%
Citrobacter	7	R	R	50%	25%	14.2%	40%	60%	—	75%	100%	100%	66.6%	—
Proteus	2	—	—	50%	R	—	50%	100%	—	100%	100%	R	—	—

FQ: Fluoroquinolone; R, intrinsic resistance. S% for each organism/antimicrobial combination was generated by including the first isolate of that organism encountered on a given patient. Drug not tested or drug not indicated.

Table-3: Antimicrobial susceptibility determinations of gram positive organisms.

Gram positive organisms	No. strains	Ampicillin	Penicillin	Cefotaxime	Clindamycin	Erythromycin	Tetracycline	Amikacin	Rifampin	Oxacillin	Vancomycin	linezolid	Trimethoprimsulfa methoxazole
Enterococcus species	15	25%	22.2%	R	R	R	11.1%	14.2%	—	—	30%	87.5%	100%
Staphylococcus Aureus	17	—	10%	R	R	R	75%	72.7%	75%	61.5%	50%	—	77.7%
Staphylococcus Coagulase-negative	70	—	19%	R	11.2%	8.9%	100%	61.7%	90%	21.4%	79.5%	100%	47.5%
Viridans group streptococci	3	100%	100%	R	100%	R	—	—	—	—	50%	100%	—

R: intrinsic resistance. S% for each organism/antimicrobial combination was generated by including the first isolate of that organism encountered on a given patient. Drug not tested or drug not indicated.

4- DISCUSSION

Considering the importance of local antibiogram determination in empiric therapy, in the current study, the sensitivity and resistance of all isolates were detected in children who were admitted to PICU for more than 48 hours. This was performed by the examination of the suspicious site of infection and explained based on CLSI (2017) cumulative antibiogram protocol for the purpose of explanation of VAP treatment protocol. Gram-negative organisms including *Acinetobacter*, *Klebsiella*, *Enterobacter*, *Pseudomonas aeruginosa*, *E. coli*, *Serratia*, and *Citrobacter* had the highest prevalence, respectively. Subsequently, there were Gram-positive organisms including *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus* spp., and *viridans* streptococci.

Coagulase-negative Staphylococci are considered as contaminants unless proven otherwise throughout a confirmed infectious site. We, on the other hand, merely reported the positive results, but did not take those results into account in the case of treatment decision. No true episode of infections was detected, hence it did not affect our stewardship program. The majority of positive cultures were from blood (peripheral or central line sample), which suggests the high prevalence of sepsis as a nosocomial infection, and it is indicative of the importance of the methods of preventing infection transmissions such as hand hygiene with alcohol-based hand rub or soap (29). Most cases of nosocomial infection were detected in PICU that may indicate an increased risk of nosocomial infection in ICU patients due to longer hospitalization time, invasive procedures, critical illness, sedation, and mechanical ventilation. Among BAL cultures, one case grew *Candida albicans*. Nosocomial fungal pneumonia may occur in neutropenic or immunocompromised patients (30-33). The only case of fungal VAP in our study was a chronic renal failure patient, who was under hemodialysis and was considered to be immunocompromised. This finding shows that in immunocompromised patients, clinicians should consider the possibility of fungal infection. Most VAP cases (25 out of 27 cases [92.6%]), were infected with Gram-negative organisms, which included *Pseudomonas*, *Acinetobacter*, *Klebsiella*, and *Serratia*. One case was reported to be MRSA, a Gram-positive organism. These results were perfectly consistent with the ATS-IDSA (Society of America and the American. Thoracic Society) HAP-VAP guideline.

Aerobic Gram-negative bacilli, for example, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia*, and *Acinetobacter* spp., are common pathogens of HAP and VAP (26). In a study by El-solh et al. that was performed in elderly patients with severe pneumonia, *Staphylococcus aureus* (9%), and enteric Gram-negative rods (15%) were the most frequent causes of nursing home-acquired pneumonia (34). The types of isolated organisms and their prevalence were completely consistent with our study findings. A study in Lahore General Hospital in 2018 showed that out of 445 samples of tracheal secretions the most common bacterium was *Klebsiella pneumonia* and the highest susceptibility trend was seen with combination drugs such as piperacillin-tazobactam among Gram-negative bacteria (35), but our results showed most sensitivity to colistin in Gram-negative organisms. Richards studied ICU patients in the United States and showed that infections due to Gram-positive organisms such as *Staphylococcus aureus*, particularly MRSA, are rapidly increasing in the United States (26, 36). *Staphylococcus aureus* pneumonia is common in head trauma, diabetes mellitus, and ICU patients (37). In this study, only

one of the *Staphylococcus aureus* isolates from bronchoalveolar lavage (BAL) specimens was methicillin-resistant Staphylococcus aureus (MRSA), which is consistent with the findings of Richards et al. (36). In one case of MRSA VAP, the patient had a history of more than 30 days of ICU stay, tracheostomy tube placement, mechanical ventilation and consumption history of a broad spectrum of antibiotics (37). In hospitalized patients, especially in ICU patients, the rates of nosocomial pneumonia by multi-drug resistant (MDR) organisms have increased significantly. Habibian et al.'s study that was conducted during 12 months from 2013 to 2014 demonstrated *Klebsiella* and *Pseudomonas aeruginosa* as the most prevalent organisms. Resistance to ceftazoxim, ciprofloxacin and carbenicillin was changed from 62.5% to 19%, 100% to 88%, 55% to 71%, respectively, in the second half of their study (38).

The organisms that were isolated and the extent of their resistance were consistent with our results. Puzniak et al.'s study which was conducted in 2019 in the United States, revealed that commonly-used antipseudomonal drugs, alone or in combination, do not achieve 95% coverage against *Pseudomonas aeruginosa* isolates of hospitals, suggesting that new drugs are needed to attain this goal. They argued that local institutional use of combination antibiograms optimizes empiric therapy of difficult-to-treat pathogens (39). However, our findings show that among Gram-negative organisms *Pseudomonas aeruginosa* is the most sensitive one, but other ones are highly resistant to most antibiotic classes and new drugs are needed to attain sufficient coverage. In our study, the most common Gram-negative organisms had a higher than25% resistance to at least one antibiotic from three or more antibiotic classes. However, *Pseudomonas aeruginosa* showed resistance below 20% to most antibiotics

and had higher sensitivity than other Gram-negative organisms. The 2017 European Respiratory Society (ERS) guideline has recommended to start combination antibiotic therapy including coverage of Gram-negative isolates and MRSA for high-risk HAP/VAP patients (1). In empiric therapy, broad-spectrum antibiotics for *Pseudomonas aeroginosa* and extended-spectrum β-lactamase-producing isolates are recommended in case of high rate of *Acinetobacter* in the unit, septic shock at the onset of HAP/VAP, hospitals with high rate of MDR pathogens and patients at risk for resistant pathogens. Resistant isolates risk factors include units with high rate of MDR isolates, defined as isolates that are resistant to at least one agent from three or more classes of antibiotics, previous antibiotic use, and hospital stay for more than five days, and prior colonization with MDR pathogens. The rate of resistant organisms varies among different units and hospitals. However, in local microbiological data, the prevalence of resistant pathogens more than 25% is considered as MDR high-risk situation (1).

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considered as MDR high-risk situation (1). ATS, IDSA HAP/VAP guideline recommends that in patients with suspected VAP, empiric regimens should include *Staphylococcus aureus* and other Gram-negative organisms' coverage. In empiric treatment of suspicious cases of VAP, MRSA coverage is suggested in any of the following conditions: antimicrobial resistance risk factors (septic shock at the onset of VAP, ARDS before VAP, antibiotic use within 90 days, hospitalization for ≥5 days before VAP, or acute renal replacement therapy preceding VAP), patients being treated in units with MRSA prevalence of more than 10-20%, and patients in units with unknown prevalence of MRSA. Otherwise, an antibiotic effective against MSSA is sufficient (7). In cases of MRSA coverage, the guideline recommends linezolid or vancomycin use.

According to the current findings, in our center 38.5% of *Staphylococcus aureus* isolates were MRSA, so in this center, the empiric regimen in suspected VAP patients must include vancomycin or linezolid antibiotics. The same guideline suggests prescribing two active agents against Gram-negative isolates from two different classes in any of the following conditions: a risk factor for MDR pathogens as described earlier, units with > 10% Gram-negative organisms resistant to an antibiotic considered for monotherapy, and in ICUs with unknown local microbial susceptibility rate (7). The goal of this empiric therapy is to ensure that patients receive ≥ 95% effective antibiotics against the pathogens. Each individual ICU can modify these thresholds to its circumstances. According to the two main guidelines and current results, in our hospital, two anti-pseudomonal antibiotics should be used in empiric therapy of VAP in either the presence or absence of MDR risk-factors due to high resistance rate of Gram-negative organisms other than

Pseudomonas and high prevalence of other Gram-negative isolates such as *Acinetobacter* and *Klebsiella*. Antifungal treatment may also be considered if the patient is immunocompromised, has any risk factors of invasive fungal infection or is strongly suspected by the clinician (for instance, has a candida score >3). Risk factors of an invasive fungal infection include central line catheter in ICU, total parenteral nutrition and acute renal failure, especially if hemodialysis is required (40, 41).

4-1. Study Limitations

The limitation of this study is the small sample size and the short period of time for sample collection. Collecting samples over a longer period will yield more reliable results. VAP information can be collected and analyzed to determine the predictive value of each clinical sign and symptom and laboratory findings such as fever, leukocytosis, leukopenia, worsening of blood gas and oxygenation.

5- CONCLUSION

The current study showed that Gram-negative bacteria were the most common organisms with the cumulative percentage of 62.9% of bacterial isolates. Common gram-negative organisms had greater than 25% resistance to at least one antibiotic from three or more antibiotic classes. However, *P. aeruginosa* showed resistance of less than 20% to the majority of antibiotics. When tested with oxacillin, 61.5% of *Staphylococcus aureus* were MSSA and 38.5% were MRSA. 27 (11.1%) of patients had VAP, 25 (92.6%) of which were gram-negative infections. According to the results of our center's antibiogram, an effective antibiotic against MRSA should be used in the empiric treatment of VAP. Also, in the presence or absence of MDR pathogens risk-factors as explained earlier, it is necessary to use two effective antipseudomonal antibiotics from different antibiotic classes. Once the

culture and its antibiogram are prepared, the antibiotic can be changed to a sensitive one or step down therapy. We defined our antibiogram in accordance with the existing standard guidelines and based on the antibiograms we designed a local protocol. The limited time period and sample size without any follow-up, made it impossible for our study to define an effective treatment protocol. However, this study designed a modified version of the standard protocols that will definitely help further studies modify and confirm the ultimate treatment protocol for VAP in PICUs in our region. We recommend performing further studies with larger sample sizes and longer recruitment window to determine the local antibiogram of each unit, which can provide more precise results. Choosing correct empiric antibiotic treatment is also influenced by microbial resistance and in the first step a cohort study is needed to determine risk factors of MDR infections. Moreover, future similar studies should determine minimum inhibitory concentration (MIC) of the drugs in order to help decide which antibiotics to choose for the purpose of combination therapy.

6- ACKNOWLEDGMENT

7- CONFLICT OF INTEREST: None.

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