Characterization of pathogens involved in ventilator associated pneumonia in surgical and medical intensive care units - A single center experience

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Abstract: In the present study 60 samples were collected from lower respiratory tract of patients suffering from Ventilator Associated Pneumonia (VAP) admitted in surgical and medical Intensive Care Units (ICUs) of Khyber Teaching Hospital, Peshawar, Pakistan. Recovered pathogens were characterized and their susceptibility pattern against commonly used antibacterial agents investigated. Most frequent bacterial pathogen found was methicillin- resistant *Staphylococcus aureus* (MRSA) (40%) followed by members of Enterobacteriaceae (22%; of which *Escherichia coli* (50%), *Klebsiella pneumonia* (30%), *Enterobacter cloacae* (10%) and *Citrobacter freundii* (10%), *Pseudomonas aeruginosa* 20% and *Acinetobacter baumannii* 18%. Majority of the specimens yielded polymicrobial growth (85.75% polymicrobial growth compared to 14.25% specimens yielding monomicrobial growth). The susceptibility pattern showed that *A. baumannii* was the most resistant bacterial pathogen. Based on the results of susceptibility pattern obtained in the present study, combination of linezolid with meropenem and colistin has been found to be the best combination option for empirical therapy for VAP pathogens in this region.

Keywords: Empirical therapy, Ventilator Associated Pneumonia (VAP), MRSA, *Pseudomonas aeruginosa.*

INTRODUCTION

Hospital Acquired/associated Infections (HAIs) including pneumonia usually develop within 48 hours or more, of admission into a hospital. The most significant risk factor of Hospital Acquired Pneumonia (HAP) is ventilator, and is the second most frequently occurring HAI (Alvarez, 2001). Ventilator bypasses host defense system hence access of bacteria to lower respiratory tract is facilitated (Dezfulian *et al*., 2005). Biofilms also develop on the inert surfaces of ventilators, shed heavy load of bacteria that are aspirated to the lower respiratory tract. Mechanical abrasion, irritation of the respiratory mucosa, impairment of normal laryngeal functions and increase sedation lead to high risk of aspiration of upper respiratory tract secretions. Postoperative (inhalational) pneumonia develops by aspiration of gastric contents containing bacterial flora of the oropharynx. Besides this, penetrating wounds, a high Glasgow Coma Scale score, spinal cord injury, a high Injury Severity Score, number of blood units transfused in the resuscitation room and the place of initial intubation are some of other contributing factors responsible for VAP (American Thoracic Society, 2005). Lower respiratory tract infections are at the top of the mortality list in HAIs, associated with invasive medical devices and surgical procedures (Andrew *et al*., 2006).

Recent emergence of Multi Drug Resistant (MDR) pathogens causing VAP is a challenge for the proper antimicrobial treatment (Gonzalez-Villoria and Valverde-Garduno, 2016; Lollar *et al*., 2016). Sixty percent of HAIs of pneumonia are caused by aerobic gram negative rods, majority are members of Enterobacteriaceae or *Pseudomonas* species (Andrew *et al*., 2006). Prognosis with *Pseudomonas* infection is worse than gram positive bacterial infection in VAP (Arozullah *et al*., 2001). MDR organisms including *Pseudomonas* spp., *Acinetobacter baumannii*, Extended Spectrum β-Lactamases (ESBL) and carbapenemase producing bacteria are increasing worldwide (Giuffrè *et al*., 2016; Potron *et al*., 2013). Many studies report the emergence of new polymyxin resistant strains of *A. baumannii* in ICUs (Perez *et al*., 2007) which can infect any body site including lower respiratory tract in nosocomial pneumonia with increasing ratio (Bonten *et al*., 2004). Studies carried out in 13 different European countries indicate that the most frequently isolated bacteria (39%) from the lower respiratory tract samples are *Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp., *Citrobacter* spp. and *E. coli*. Other micro-organisms are less frequently isolated from VAP as Fungi (1%), *Streptococcus pneumoniae* (4%), Coagulase negative *Staphylococci* (1%), *Neisseria* spp (3%), *Stenotrophomonas* (2%) and anaerobes (1%) (Chastre and Fagon, 2002).

VAP increases morbidity, mortality and economic cost (Croce, 2003). Lower respiratory tract bacterial infections in ICUs account for 10-25% of all admitted patients

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resulting in mortality of 22-71% (American Thoracic Society, 2005). Identification, susceptibility patterns of pathogens at the time of initial treatment of Lower Respiratory Tract Infections (LRTIs) make early empirical therapy a challenge (Daniels, 2008). Although The American Thoracic Society, Infectious Diseases Society of America (2005) and other organizations recommend empirical monotherapy with acylaminopenicillins + β-lactamase inhibitors, 3rd generation cephalosporins, quinolones, carbapenems and various other combination regimes for VAP (Kalil *et al*., 2016); however, no consensus gold standard therapy is available. Such inappropriate use of antibiotics for the treatment of VAP in ICUs promote emergence of drug resistance in bacteria (El-Solh, 2001). Further studies in this regard are required to improve the quality of combinational empirical therapy in VAP.

MATERIALS AND METHODS

Sputum samples and tracheal secretions were collected from patients admitted in surgical and medical ICUs of Khyber Teaching Hospital, Peshawar, Pakistan from Jan 2013 to Dec 2013.

Inclusion criteria

All patients regardless of age, gender with ventilator support for ≥ 48 hours were included in the present study. All lower respiratory tract samples collected from these patients were included in the study if they showed significant pus and squamous epithelial cells ratio. A ratio of 25:1 was considered significant for sputum samples and 10:1 for tracheal secretions at low power microscopic fields.

Exclusion criteria

All other samples that failed to fulfill the significant leukocytes/squamous epithelial cells ratio were excluded from the study. Patients with “Atypical pneumonia” viral pneumonia were also excluded from the present investigation.

Negative/comparative Non-ventilator associated control patients

Ten patients, diagnosed clinically, suffering from lower respiratory tract bacterial infection both from surgical and medical ICUs were selected as negative control. These patients were not supported with ventilator. Samples obtained from these patients were processed as mentioned above.

Ventilator associated pneumonia (VAP) criteria

Following criteria were followed for the definition of VAP

1. Purulent and copious nature of respiratory secretions e.g. abundant tracheal secretions and sputum
2. Temperature > 38^{o}C
3. Increased consumption of pulmonary oxygen
4. Leukocyte count >11,000/mm^3 or <11,000/mm^3 with band forms
5. Diffuse or localized lung infiltrate on chest X-rays
6. Positive culture results of lower respiratory tract samples e.g. colony count ≥ 10^4 CFU/ml

Patient population

A total of 60 patients supported with ventilator, including 40 male and 20 female were included in this study. Forty patients were from Surgical ICU, including 27 male and 13 female. Twenty patients were from Medical ICU, including 13 male and 7 female. Ten patients without ventilator support were also included in this study as negative/comparative controls.

Sample population

A total of 70 samples (50 tracheal secretions and 20 sputum) were collected (surgical ICU=50 samples, medical ICU=20 samples). Out of 50 samples from surgical ICU, 38 were tracheal secretions (30 cases + 08 control) and 12 were sputum samples (08 cases + 04 control). Out of 20 samples from medical ICU, 12 samples were tracheal secretions and 8 were sputum samples.

Microbiological evaluation and diagnosis

All the samples were collected using standard protocols. Direct microscopy using 40x objective was used to determine significant leukocytes / squamous epithelial cells ratio criterion mentioned above.

Antibiotic susceptibility testing

Antibiotic susceptibility was performed as described by Bauer *et al.,* (1996) according to Clinical and Laboratory Standards Institute (CLSI). For determination of ESBL, a β-lactam and a β- lactamase inhibitor combination (Co-amoxiclave) discs were applied in the centre of the culture lawn. For the determination of MRSA, 2nd generation cephalosporin; cefoxitin (Fox) disc was applied on the *S. aureus* culture lawn. The growth resistant to cefoxitin was considered to be an MRSA. Muller Hinton Agar as recommended by CLSI was used for antibiotic susceptibility testing. Other media used in this study were MacConkey agar, Blood Agar, Chocolate Agar, Mannitol Salt Agar, DNAse Agar and an anaerobic culturing medium the “Wilkins Chalgrins”. “API 10 S” (Biomerieux) strips were used for biochemical identification. The following bacterial strains were used as reference strains in the study:

E.coli ATCC 25922
P. aeruginosa ATCC 27853
S. aureus ATCC 25923

Mathematical model for calculation of VAP [Clinical Pulmonary infection score (CPIS)]

The CPIS (with 6 variables) was used in this study for the calculation of VAP and lower respiratory tract infections. The 6 variables were fever, chest radiographs, Complete Blood Counts (CBC), ratio of partial pressure of carbon dioxide and oxygen, consistency of lower respiratory tract samples and microbiological culture results. Each variable was assigned values of 0, 1 and 2, depending on the degree of severity. These values were calculated and the score ≥ 6 was considered as significant.

RESULTS

A total of 50 VAP cases were diagnosed based on microbiological and clinical (CPIS) criteria. Main characteristics and variables of these patients are given in the table 1. Most of the MRSA positive patients were supported with ventilator for >72 hours. Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* positive patients had ventilator support of ≥48 hours (Table 2). Only one MRSA, 2 Enterobacteriaceae and one *Pseudomonas* positive patients had ventilator support for <48 hours. All of the tracheostomized patients showed growth of *E. coli*, indicating that tracheostomy procedure may be a more significant risk factor for the development of VAP caused by *E. coli*.

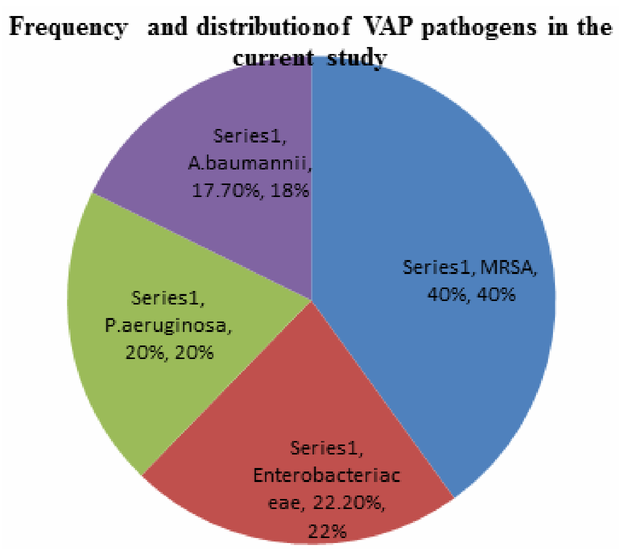

Fig. 1: Frequency and distribution of pathogen associated with VAP in this study

Microbial aetiology of VAP and non VAP patients

Sixty two bacterial isolates were recovered from 60 admitted VAP patients. The most frequent bacterial pathogen recovered was *S. aureus* (22 MRSA and 4 MSSA) followed by Enterobacteriaceae 14 (06 *E. coli*, 04 *K. pneumoniae*, 03 *E. cloacae* and 1*C. freundii*), *P. aeruginosa*, 11 and *A. baumannii* 09 were obtained. In three cases from surgical ICU, *Candida albicans* was isolated. Two isolates of *Stenotrophomonas maltophilia* was obtained from tracheal secretions of patients admitted in medical ICU. No anaerobic bacteria were isolated from any sample in the present study. The tracheal secretions and sputum in most of the cases were highly purulent with polymorphs neutrophils in abundance, almost 20-25/hpf (high power field). The purulent and copious nature of secretions was higher in *A. baumannii* positive cases.

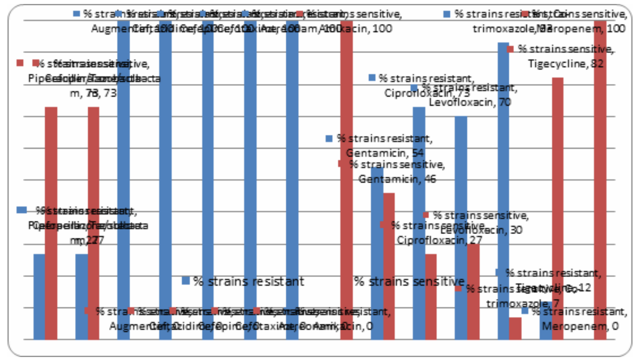

Fig. 2: Antimicrobial susceptibility pattern of members of Enterobacteriaceae n=14

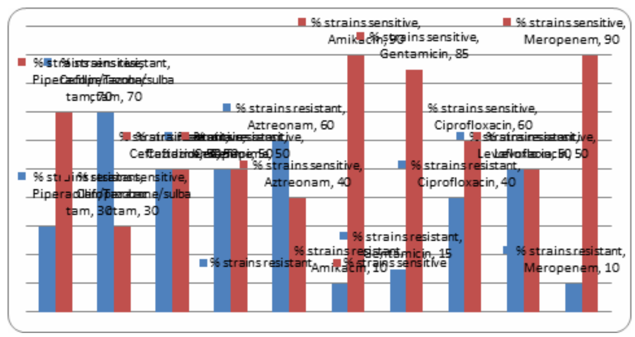

Fig. 3: Antimicrobial susceptibility pattern of members of *P.aeruginosa* n=11

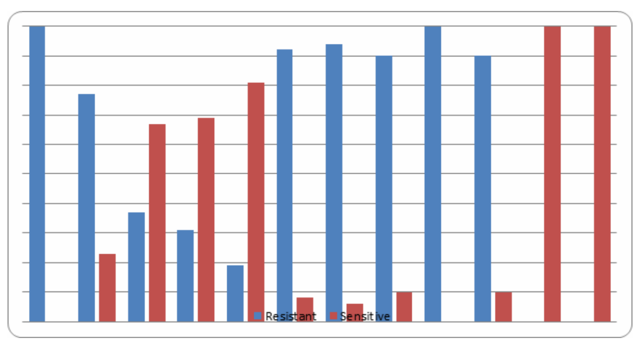

Fig. 4: Antimicrobial susceptibility pattern of MRSA n=22

Microbial aetiology from non-VAP cases was quite different from VAP cases. Samples from 02 patients failed to culture any bacterial pathogen. *E. coli* was the predominant isolate in most of the remaining cases followed by *Pseudomonas* spp. Only in one non-VAP case from medical ICU, a fungus was isolated. No MRSA were recovered from non-VAP cases. This highly signifies one strong conclusion that MRSA is the leading cause of ventilator associated lower respiratory tract bacterial infections or VAP in this study.

Table 1: Characteristics of the patients with Ventilator Associated Pneumonia (VAP)

General characteristics	Values (% & Average)
Age (Years)	Average 40
Male	
Female	
Smoking	No history
Alcohol abuse	No history
Co-morbidities	
COPD	20%
ARDS	16%
Asthma	05%
Diabetes mellitus	10%
Clinical & Laboratory findings	
Fever	80%
101^o - 102^o	10%
101^o - 103^o	Average: 16-18 × 10^9/l.
CBC (normal range: 04-11×10^9/l)	
Radiological findings (Chest X – rays)	75 %
*Case 1: Consolidation or diffused infiltration	25 %
Haziness	25 %
** Case 2: Diffused infiltration	80 %
***Case 3: Marked infiltration	
Surgical procedures and Trauma	75%
RTA	05 – 10%
liver cirrhosis, bomb blast injuries, respiratory acidosis, severe sepsis and septic shock, organic poisoning, laparotomy, craniectomy,	
Multiple fractures	95% of RTA
skull and cranial injuries	30–35% of multiple fractures
cerebral edema	80 % of multiple fractures
Spinal cord injury	05 % of multiple fractures
subdural hemorrhage	05 % of multiple fractures
****Prescribed Antibiotics	
2^{nd} generation cephalosporins (Ceftriaxone & Cefotaxime)	60 %
Metronidazole	58 %
Carbapenemes	35%
Quinolones	10 %

*MRSA cases, **Enterobacteriaceae & *P. aeruginosa* cases, ****A. baumannii* cases, ****Empirical therapy antibiotics clinically prescribed in ICUs, RTA (Road traffic accidents), COPD (Chronic obstructive pulmonary disease), ARDS (Acute respiratory distress syndrome), CBC (Complete blood count)

Table 2: VAP cases with duration of ventilator support

Total Patients(n)=*70	Ventilator +ve(n=60)	Duration of ventilator				
S. aureus (n=26)		<48hrs	≥48hrs	>72hrs	≥120hrs	≥168hrs
MRSA (n=22)	20	01	03	10	05	01
MSA (n=04)	[1]03	-	-	03	-	-
Enterobacteriaceae (n=14)	[2]12	-	07	01	02	02
P. aeruginosa (n=11)	10	-	07	-	02	01
A. baumannii (n=09)	08	-	05	02	01	-

*Ten Non-ventilator associated control cases are not shown, [1] Tracheostomy was done for one of the ventilator positive patients[2], 04 patients were tracheostomized. All of the tracheostomized patients showed growth of *E. coli*, indicated that tracheostomy procedure may be a more significant risk factor for development of VAP, caused by *E. coli*.

Table 3: Polymcirobial and Monomicrobial distribution of VAP pathogens (%)

Microorganisms	Monomicrobial	Polymicrobial	Two pathogens	Three pathogens
Staphylococcus aureus (MRSA)	15	86	75	11
Pseudomonas eruginosa	28	72	70	2
Enterobacteriaceae	15	85	60	25
Acinetobacter baumannii	0	100	100	0
MRSA + *P. aeruginosa*	-	75	74	1
MRSA + *A. baumannii*	-	95	95	0
MRSA + *Enterobacteriaceae*	-	58	40	18

MRSA is not obtained or shown to be the most significant cause of VAP in such higher frequencies in any other previous study. Our results are totally different from the previous studies that considered Enterobacteriaceae or *Pseudomonas* to be the most frequent bacterial pathogens of VAP. The current study on one side indicates that MRSA is the most frequent cause of VAP, especially in this part of the globe and on the other side recommends for the proper modification of the present empirical therapy for VAP patients based on the obtained susceptibility.

Derivation/diagnosis of VAP by CPIS

All of the MRSA, MSSA, Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* positive samples significantly correlated with all other variables of the CPIS statistical calculation. Thirty percent MRSA, 32% Enterobacteriaceae, 27% *P. aeruginosa* and 22% *A. baumannii* got score of ≥6. Fifty four percent MRSA, 51% Enterobacteriaceae, 61% *P. aeruginosa* and 76% *A. baumannii* got ≥7 score. Only 15% MRSA, 16% Enterobacteriaceae, 11% *P. aeruginosa* and 2% *A. baumannii* cases failed to fulfill the CPIS mathematical calculations criteria in this study. Tracheal secretions gave more significant results than sputum samples indicating that tracheal secretions are more appropriate samples to diagnose VAP. Out of 50 total VAP cases, 45 were confirmed by CPIS calculations, while 5 cases failed to fulfill the CPIS statistical criteria. Using CPIS as the basic mathematical tool for calculation of VAP, the highest VAP was calculated for MRSA [18 (40%)] followed by Enterobacteriaceae [10 (22.2%)], *P. aeruginosa* [9 (20 %)] and *A. baumannii* [8 (17.7 %)] (fig. 1). It is concluded that CPIS is the most reliable mathematical tool for calculation and final diagnosis of VAP.

Polymicrobial nature of VAP

Out of the total 22 MRSA recovered from specimens of patients in surgical and medical ICUs, 19 were isolated in combination with another type of bacterial pathogen indicating a polymicrobial nature of infection. Out of the total 18 MRSA confirmed VAP cases; 86% were associated with other types of bacteria isolated from the same lower respiratory tract samples. Fifteen percent MRSA were isolated singly. Out of the total 11 *P. aeruginosa* isolates from both ICUs, 08 were recovered in combination with other bacterial pathogen. In only 03 cases, it was isolated as single pathogen. Similarly, out of 09 *P. aeruginosa* VAP cases, 72% were isolated in combination with other types of bacterial pathogens and 28% were isolated singly. Out of the total 14 members of Enterobacteriaceae isolated both from ICUs, 12 were isolated in combination with other types of bacteria. Out of 11 total Enterobacteriaceae VAP cases, 85% were associated with a second type of bacterial pathogen isolated from VAP cases. The most interesting results of the polymicrobial nature of ventilator associated lower respiratory tract infections was *A. baumannii* infections. All of *A. baumannii* isolates (100%) were isolated in combination with other types of bacterial pathogens and not a single *A. baumannii* was isolated alone (table 3).

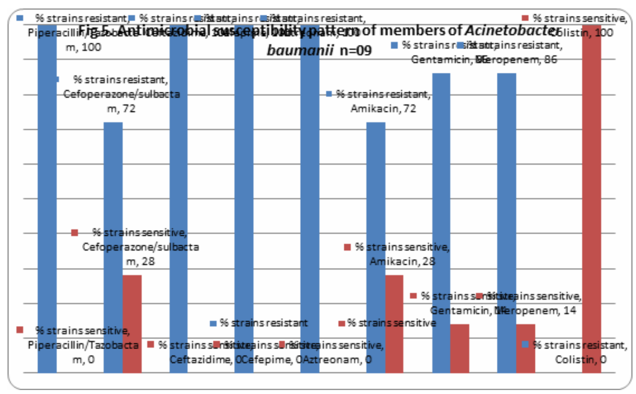

Fig. 5: Antimicrobial susceptibility pattern of members of *Acinetobacter baumanii* n=09

Antimicrobial susceptibility of VAP pathogens

MDR strains were involved more in VAP cases as compared to control cases in this study (figs. 2, 3, 4 and 5). To the best of our knowledge, such high ratio of resistance to particular antimicrobial agents in this study is not reported previously. Resistance behavior of MDR bacterial pathogens involved in VAP in the current study is discussed below.

DISCUSSION

The present study investigates the frequency of microorganisms associated with VAP in ICUs and focuses on pathogen's susceptibility pattern against commonly used antibacterial drugs and derivation of an anti-biogram for a proper local empirical therapy. Bacterial pathogens responsible for VAP are mainly gram negative bacilli;

however, infections due to gram positive cocci e.g. MRSA have been rapidly emerging (Fredkin, 2001; Haque *et al*, 2010). Results of this study indicate that MRSA is the most frequent bacterial pathogen responsible for VAP in patients admitted in ICUs in this part (fig. 1). Aetiological agents from different studies indicate that microorganisms responsible for early onset VAP are mainly *H. influenzae*, *S. pneumoniae*, MSSA and drug sensitive Enterobacteriaceae strains. MDR pathogens like *P. aeruginosa*, *A. baumannii* and MRSA are mainly isolated from late onset VAP and are associated with increased mortality (Koulenti *et al*., 2016; Krishnamurthy *et al*., 2013). Another study revealed that aerobic gram negative rods were responsible for 79% of VAP cases and gram positive cocci were only isolated from 21% of VAP cases (Hira *et al*., 2002). In a comparative study it was found that the most frequent pathogen was non-lactose fermenting gram negative bacillus; *P. aeruginosa* followed by MRSA and *Enterobacter* spp. (Gaynes *et al*., 2005). A recent study on the incidence of VAP reported MRSA in the list of pathogens; however, the most frequent VAP pathogens were non-fermenting aerobic gram negative bacilli (Duszyńska *et al*., 2015). With respect to the type of pathogens, these studies correlate with our findings; however, frequency and distribution of VAP pathogens in our study indicate that MRSA is the leading cause of VAP in this study (fig. 1). Using CPIS as a basic tool for calculation of VAP, MRSA VAP was calculated as high as 40% in our study. MRSA VAP detected in our study is not found in such high frequencies in previous reports. However, a recent increase in MRSA infections in chronically ill patients with prolonged ventilator support has also resulted in increased incidence of pneumonia caused by strains of MRSA (Rubenstein *et al*., 2008; Gagneja *et al*., 2011). Some studies have concluded that anaerobic bacteria are equally involved in VAP. However, in a large scale study no anaerobic bacteria were isolated from 185 suspected VAP episodes (Ibrahim *et al*., 2000). A study, specially designed for the incidence of VAP pathogens, no anaerobic bacteria were found (Dey and Bairy, 2007) which supports our findings.

The most interesting results obtained in the present study revealed a very high percentage (86%) of the polymicrobial nature of VAP. *A. baumannii* was never isolated singly and always was associated with another type of bacterial pathogen. The highest percentage was obtained for association of *Acinetobacter* VAP with MRSA VAP except one case. As in almost all VAP cases of *A. baumannii*, it was associated with MRSA, indicates that *A. baumannii* is a highly opportunistic bacterial pathogen and MRSA infection makes conditions highly favorable for secondary bacterial infections, especially for *A. baumannii*. Studies have shown the impact and association of *S. aureus* with pathogenesis in polymicrobial infections. This impact is either antagonistic or mutualistic/synergistic depend on the type of micro-organism associated in polymicrobial infections with *S. aureus*. *S. aureus* is competitive in nature during polymicrobial infections and only few interactions are cooperative. In cooperative interaction between *S. aureus* and *H. influenza*, *S. aureus* induces the lysis of Red Blood Cells (RBC). Hemin and NAD are released from the lysed RBCs which act as nutrient and support the growth of *H. influenzae*. *S. aureus* also helps influenza virus and induces pathogenesis. Host sialic acid receptors are cleaved by Staphylococcal proteases which increases the infectivity of influenza virus by release of the virus from the surface of the host cells (Nair *et al*., 2014). Advanced molecular studies are required to investigate the cooperative nature of *S. aureus* with opportunistic pathogens as *A. baumannii* in polymicrobial infections. The polymicrobial nature highly signifies proper selection of antibiotics for empirical therapy of VAP in ICUs.

Early effective empirical therapy reduces mortality rate significantly. Inadequate therapy is associated with high mortality rate of up to 91%. In almost 60% cases, a 2nd generation cephalosporin "ceftriaxone" was used in empirical therapy. In this study, recovered MDR pathogens were resistant to all β-lactams including ceftriaxone. Ceftriaxone is not a drug of choice against *P. aeruginosa* and most of the isolates in this study were resistant to cephalosporins. Likewise, all Enterobacteriaceae (*E. coli*, *K. pneumoniae*, *E. cloacae*, and *C. freundii*) and *A. baumannii* were absolutely resistant (100%) to cefepime and ceftazidime (fig. 2). *P. aeruginosa* isolated from VAP cases in this study were resistant to cefepime and ceftazidime (fig. 3). Therefore, use of cephalosporins against VAP should not be considered as drugs of choice. As per guidelines of American Thoracic Society (2005) antipseudomonal fluoroquinolones should also be included for patients at risk for VAP caused by multidrug-resistant organisms; however, resistance of VAP pathogens to fluoroquinolones was calculated to be very high in the current study (figs. 2 & 3). So the use of fluoroquinolones in ICUs was also inappropriate to treat patients at risk of VAP caused by multidrug-resistant organisms. In case of aminoglycosides, although very little resistance was observed in *P. aeruginosa* and Enterobacteriaceae; however, MRSA (fig. 4) and *A. baumannii* were highly resistant to aminoglycosides in the current study (fig. 5). Significant activity of amikacin was observed against *P. aeruginosa* and Enterobacteriaceae; however, the use of amikacin in combination therapy is highly associated with nephrotoxicity. Metronidazole is also prescribed in combination with ceftriaxone; however, anaerobic bacteria were not shown to be the causative agents of VAP in this study. Very little resistance was noted in Enterobacteriaceae and *P. aeruginosa* for carbapenems (figs. 2 & 3). MRSA and *A. baumannii* isolated in this study were absolutely resistant to carbapenems; therefore no other anti *Acinetobacter* or anti-MRSA antibiotics were included in the combination therapy along with carbapenems (figs. 4 & 5). Based on the current susceptibility pattern it is concluded that carbapenems /meropenem in an appropriate combination with other antibiotics is the right choice for an empirical therapy in this region. As almost all bacterial infections of the lower respiratory tract in the present study were of polymicrobial nature, therefore, empirical monotherapy is highly discouraged in the current study.

Most of the microorganisms responsible for VAP isolated in the current study were MDR pathogens. In the present study, the antibiogram for Enterobacteriaceae indicates that this family is mostly resistant to commonly prescribed antibiotics in ICUs. Most of the members of Enterobacteriaceae isolated in the current study were extended Spectrum Beta-lactamase (ESBL) producers. A total of 73% of Enterobacteriaceae isolates from VAP cases were ESBL producers and 23% were non ESBL, therefore, penicillins, cephalosporins and monobactam were also excluded from empirical therapy regimes. Meropenem, imipenem, amikacin and tigecycline were found in the sensitive ranges (fig. 2). Meropenem / imipenem were preferred over tigecycline, because of the absolute sensitive range of the carbapenems. The antibiogram determined for *P. aeruginosa* VAP cases in our study showed that not even a single strain of this bacterium was absolutely sensitive to any class of antibiotics (fig. 3). Meropenem and imipenem were the only options against VAP caused by *P. aeruginosa*. The worse *in vitro* antibiogram recorded for *A. baumannii* was resistance to all of the antibiotics prescribed in the empirical therapies in ICUs except colistin (fig. 5).

The antibiogram derived for MRSA VAP cases showed resistance to penicillins, cephalosporins, fluoroquinolones and aminoglycosides however 100% of these isolates were sensitive to vancomycin and linezolid (fig. 4). For patients with early onset VAP without prior antibiotics treatment, a monotherapy with second generation cephalosporins such as cefomandole, cefotetan and cefuroxime or a third generation cephalosporins such as cefotaxime and ceftriaxone or augmentin is recommended. In case of prolonged mechanical ventilation and prior antibiotics treatment; combination of aminoglycosides or ciprofloxacin with piperacillin-tazobactam or imipenem with vancomicin is recommended. In fact mortality is high with inappropriate antibiotics in the early 48 hours of VAP, therefore the use of combination therapy is highly recommended for VAP as its aetiology is almost always polymicrobial. The resistance behavior of polymicrobial VAP pathogens in our study indicated that antibiotics should always be prescribed in combination in empirical therapy regimes, because none of the member of polymicrobial VAP was simultaneously sensitive to a single class of antibiotics. On the basis of results obtained in this study, the following combinations of antibiotics are recommended in VAP patients in ICUs.

1- Vancomicin, meropenem or imipenem and colistin.
2- Linezolid, meropenem or tigecycline and colistin.
3- Doxycycline, meropenem and colistin.
4- Rifampicin, tigecycline or meropenem and colistin.

These combinations were further adjusted and an optimum triple combination was derived for empirical therapy. Although imipenem is an effective bactericidal agent against Enterobacteriaceae and *P. aeruginosa*; however, it has certain limitations. The efficacy of meropenem was nicely described in a comparative study of meropenem monotherapy versus conventional combination therapy of ceftazidime and amikacin in combating VAP (Iregui, 2002). However, we suggest that meropenem should always be prescribed in combination therapy, because VAP cases in our study was of polymicrobial nature and MDR pathogens including MRSA and *A. baumannii* were highly resistant to carbapenems (meropenem and imipenem) and tigecycline (fig. 4 & fig. 5). A recent study indicated that tigecycline was highly active *in vitro* against carbapenem-resistant *A. baumannii* (Cakirlar *et al*., 2015); but *A. baumannii* isolated from confirmed VAP cases in our study were highly resistant to tigecycline. There is no prospective clinical trial to guide therapy for multi drug resistant *A. baumannii*, however, intravenous colistin treatment of pneumonia caused by MDR *P. aeruginosa* and *A. baumannii* have been shown to be associated with acceptable clinical outcomes (Khilnani *et al*., 2011; Jakribettu and Boloor, 2012). As no resistance was observed against colistin by *A. baumannii* and *P. aeruginosa* isolated in the current study that is why this antimicrobial agent is included in the empirical therapy regimes. Vancomycin is an effective agent to treat MRSA and its sensitivity results were highly significant in our study; however, the use of vancomicin in combating VAP is questionable (Kollef *et al*., 2004). Therefore, linezolid replaced vancomycin from empirical therapy regimes derived in the current study. The optimum triple combinations of antibiotics derived in our study came out to be linezolid with meropenem and colistin.

Recommendations and suggestions

The triple combination antimicrobial empirical therapy derived in the current study is suitable for the area where this study was conducted. Patients targeted under this study significantly belong to a vast area, where they rely on the limited available tertiary care hospital settings in a developing country like Pakistan. We also recommend our findings for areas where infection control protocols are ignored not implemented properly. We suggest that our findings may be followed as a guide in the tertiary care settings of developing areas and such studies should be conducted periodically to check the type of pathogens involved in VAP and their frequency of resistance. Although the current study covers a significant patient population; however, antimicrobial empirical therapy derived in this study should carefully be followed in other areas/ICUs/Hospitals. Socioeconomic status of the population, patient's characteristics (table 1) and degree of following and adopting strict infection control protocols in ICUs should be considered before applying these findings to other areas. We strongly recommend such studies to be conducted in other areas of developing countries to screen out bacterial pathogens involved in VAP and derive area specific antimicrobial empirical therapy for such life threatening infections that will significantly decrease morbidity, mortality and cost.

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