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Original article

The role of bacterial colonization of ventilator circuit in development of ventilator associated pneumonia in ICU of Medical Center Hospital in Tripoli, Libya

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ABSTRACT

Introduction: In mechanically ventilated patients, ventilator-associated pneumonia (VAP) is a major cause of prolonged hospitalization with increased morbidity and mortality. There is a lack of studies on the relationship between bacterial colonization of the ventilator circuit (VC) and VAP. This study aimed to investigate the role of bacterial colonization of VC in the development of VAP and identify antibiotic susceptibility trends for isolated strains.

Methods: A prospective study of the bacterial culture has been performed between February 2021 to March 2021 on a total of 100 mechanically ventilated patients, (n =50) samples have been obtained from patient's lower respiratory tract (LRT) and (n =50) were taken from mechanical ventilator equipment VC. Paired samples of bacteria isolated from VC and LRT, where VC was colonized before LRT.

Results: A total of 58 samples were cultured positively, while 42 specimens showed negative bacterial growth. However, there was no substantial difference in comparing between the bacterial colonization of the ventilator system and the patient samples. Most isolated organisms were gram-negative bacteria which were found in the ventilator circuit with 26 (68.4%), and 14 (70%) in patient's LRT. Gram-positive was detected in 12 (31.6%) and 6 (30%) of the ventilator circuit, and patient's LRT, respectively. The predominant bacterial type was *Acinetobacter baumannii* organism at the VC with 10 (26.3%) and LRT at 4 (20%) followed *by Klebsiella pneumoniae* (8 (21.1%) in VC and 4 (20%) in LRT). Moreover, *A. baumannii* showed a full resistance to amoxicillin and the first generation of cephalosporins, while the other bacterial types were resistant to the most antibiotics used in this research.

Conclusions: Bacterial colonization of ventilator circuit VC is a significant cause of VAP development in mechanically ventilated patients. Preventive strategies for the early detection and decontamination of contaminated VC can play a crucial role in ventilator-associated pneumonia prevention.

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1. INTRODUCTION

The infections acquired by the intensive care unit are a major cause of worldwide morbidity and death. [1]. Patients of the intensive care units (ICUs) are more defenseless against different nosocomial diseases because of their basic diseases and introduction to different invasive medical devices [2]. Critical patients admitted to ICUs are always at increased risk of developing different antibiotic-resistant organism infections [3]. The incidence of ICU-acquired infections is notably greater in developing countries than in industrialized countries, various between 4.4% and 88.9% [2]. In fact, in developing countries, the rate of device-related infections, especially ventilator-associated pneumonia accompanied by central venous catheter-related infections of the bloodstream occurs at a higher level than in the nations of Europe and the United States [4].

In clinical practice, patients with acute respiratory failure or severe diseases must use ventilators for life support [2, 4]. The American Association for Respiratory care clinical practice guidelines states that the ventilator system used for critically ill patients does not need to be changed daily for infection control [5]. Furthermore, pneumonia may develop following contamination of respiratory therapy equipment, such as the ventilator circuit (VC). However, the maximum time until which a system can be continually used safely remains unknown. The correlation between ventilator-associated pneumonia (VAP) and heated or unheated humidifiers, the type of humidifier, humidifier water refill method, and removal of condensate water from the system is still uncertain. Currently, research into ventilator system contamination is limited in Libya.

VC contamination frequently occurs after ventilation and may raise the risk of pneumonia in mechanically ventilated patients [6]. After ventilation, different parts of both reused and disposable VCs have been reported to be colonized [7]. Besides, VC-isolated bacteria are highly similar to those of lower respiratory tract (LRT) patients [11]. Hospital-acquired pneumonia (HAP), Nosocomial pneumonia (NP), and VAP are significant causes of morbidity and mortality in hospitalized patients despite advances in antimicrobial therapy and better supportive care modalities [8, 9].

Aerobic, gram-negative bacilli, such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter* species, are common pathogens causing NP [7]. Thus, VC is an essential pathogens source that can cause infection if aspirated by patients [10]. The selection of antimicrobial agents active against the microorganisms associated with NP seemed to be an important determinant of hospital mortality [11, 12] Appropriate antimicrobial therapy when initiated early, was shown to reduce mortality among critically ill patients with NP [11, 13-15]. More rapid identification of infected patients and accurate selection of antimicrobial agents represent important goals. This study aims to determine the distribution of common microorganisms that are involved in the contamination of ventilator systems, and its association with development of VAP in addition to antibiotic susceptibility patterns of isolated strains.

2. MATERIALS AND METHODS

This prospective study was performed from February 2021 to March 2021. Mechanically ventilated adult patients admitted to the Intensive Care Unit of Medical Center Hospital in Tripoli city Libya were enrolled. The major diagnoses of the patients on admission were hospitalized at general medical wards. Patients were excluded if ventilated more than 48 hours at admission to the ICU. In the study we recruited 50 swabs from ventilator machines were taken on day 2 after receiving mechanical ventilation (MV) and the other 50 samples of the patient's LRT were collected at day 5 after MV. Furthermore, Endotracheal Aspiration (ETA) was used to obtain LRT samples which is a common procedure within intensive care units is the suctioning of respiratory secretions in patients for bacterial culture. Clinical characteristics liked radiography was taken to verify that selected patients are non-VAP patients.

Approval for the study was given by the appropriate ethics committees. A definite diagnosis of VAP was confirmed by positive culture of ETA ($>10^5$ CFU/mL) [16]. Cultures with lower colony count were considered as colonization or contamination.

The strains were isolated and cultured according to the operating rules of clinical examination in the Tripoli Medical Hospitals.

Specimens were analyzed at microbiology facilities of infection control laboratory and Al-Farabi private laboratory in Tripoli. The samples were immediately inoculated under aseptic conditions into nutrient agar plates and aseptically subculture on the specific medium including MacConkey agar, mannitol salt agar, and blood agar plates, and were incubated at 37°C for 24 hours, and then gram staining of bacterial colonies was performed. Isolation and identification of different bacterial strains of positive cultures were performed using API20E and conventional biochemical tests including (Catalase, Coagulase, oxidase, indole, urease). Furthermore, after identifying the type of bacteria, the resistance and sensitivity pattern of bacterial types were identified and screened by using anti-microbial susceptibility testing [17, 18]. Muller–Hinton agar was used to investigate the susceptibility of studied isolated by using the following antibiotics Amikacin (AN), Amoxicillin (AMC), Aztreonam (ATM), Cephalexin (CEP), Ciprofloxacin (CIP), Ceftriaxone (CRO), Gentamycin (GN), Cefoxitin (FOX), Imipenem (IPM), Levofloxacin (LVX), Meropenem (MEM), Sulfamethoxazole (SMZ), Tobramycin (TM), Vancomycin (VA). Once the data was collected, it was entered into an Excel sheet for statistical analysis.

3. RESULTS

Out of the 100 specimens divided equally between ventilator circuits and lower respiratory tract patients were analyzed. Only 58 samples were culture positively. This number of isolated strains was detected among VC after 1 day of MV and patient's LRT after 5 days from MV (38 (65.5%), 20 (34.5%)) respectively, whereas the remaining samples showed no microbial growth after the incubation period. The most common organism isolated was gram-negative from the ventilators (26, 68.4%) and mechanically ventilated patient's LRT were 14 (70%). Other isolates were Gram-positive from the patient's LRT were 6 (30%) and from Ventilator circuits were 12 (31.6%) as shown in Table 1.

Table 1. Distribution of bacteria types of isolates

Type of isolates	Ventilator machine (Circuit-Suction) n=38 (65.5%)	Patients (Lower Respiratory tract) n= 20 (34.5%)
Gram positive	12(31.6%)	6(30%)
Gram negative	26 (68.4%)	14 (70%)

Moreover, bacteria isolated from VC are at high level of similarity with those from patient's LRT. Amongst the bacterial isolates *Acinetobacter baumannii* was the most frequent organism at the VC and LRT with 10 (26.3%) and 4 (20%) respectively, followed by *Klebsiella pneumonia* (8 (21.1%)) in ventilator machine and patients' samples 4 (20%). *Staphylococcus aureus* accounting was 6 (15.9%) in VC and 4 (20%) in patient's LRT, whereas the last

Table 2. Organisms isolated from ventilator circuits and patients' lowe respiratory tracts			
Species of isolates	**Number of total isolated n=58**	**Ventilator machine (Circuit-Suction) n=38 (65.5%)**	**Patients (Lower respiratory tract) n=20 (34.5%)**
A. Baumannii	14 (24.1%)	10 (26.3%)	4 (20%)
K. pneumoniae	12 (20.7%)	8 (21.1%)	4 (20%)
S. aureus	10 (17.2%)	6 (15.9%)	4 (20%)
S. epidermidis	8 (13.8%)	6 (15.8%)	2 (10%)
P. aeruginosa	6 (10.4%)	4 (10.5%)	2 (10%)
S. marcescens	6 (10.4%)	4 (10.5%)	2 (10%)
E. coli	2 (3.4%)	0 (0%)	2 (10%)

isolated species recorded with the lowest percentage, were *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Serratia marcescens*. *Escherichia coli* were only found with a small percentage with patient's LRT as illustrated in Table 2.

Most bacterial types that isolated in this research were resistant to the most antibiotic types, *Acinetobacter baumannii* showed a full resistance to AMC, GN, and the first generation of cephalosporins (CEP), and it was intermediated to the third generation of cephalosporins (CRO), On the other hand, *Klebsiella pneumoniae* was also a fully resistant to GN and most other types of antibiotics, *Staphylococcus epidermidis* was sensitive to GN, *Staphylococcus aureus* was a fully resistant to FOX, GN and susceptible to VA. *Pseudomona aeruginosa* was also resistant to all types of antibiotics except GN which it was slightly sensitive to these types of antibiotic disk, *E. coli* and *Serratia marcescens* were sensitive to most antibiotic disks except AMC which were resistant to this type of antibiotic as indicated in Table 3.

4. DISCUSSION

A few studies have to date assessed bacterial contamination of ventilator systems for critical patients. In this research, the most common organisms isolated from VC were gram-negative bacteria meanwhile *A. baumannii* organism was higher in VC than LRT in patients. This finding is similar to other previous studies, which mentioned that gram-negative bacteria have also been reported to mainly colonize VC [19]. These results may suggest that gram-negative bacteria from VC may contribute meaningfully to the formation of VAP. Gramm negative bacteria, which are frequently isolated from vital healthcare environments and patients, could play a significant role in VC contamination [20, 21]. Health-care worker's hands are frequently contaminated with gram-negative bacteria and possibly lead to VC contamination during manipulation [22]. *S. aureus*, one of the most common VAP pathogens, was also frequently isolated from VC [8, 23] whereas *E. coli* was the lowest isolated bacteria. However, this result was

Table 3. Antibiotic resistance pattern of isolated bacteria from ventilator circuits and patients							
Antibiotic	**Bacterial isolates n (%)**						
	***A. baumannii* (n=14)**	***K. pneumoniae* (n=12)**	**MRSA (n=10)**	***S. epidermidis* (n=8)**	***P. aeruginosa* (n=8)**	***S. marcescens* (n=6)**	***E. coli* (n=2)**
Amikacin	10 (71.4%)	8 (66.7%)	10 (100%)	8 (100%)	6 (100%)	2 (33.3%)	2 (100%)
Amoxicillin	14 (100%)	12 (100%)	6 (60%)	8 (100%)	6 (100%)	4 (66.7%)	2 (100%)
Aztreonam	14 (100%)	12 (100%)	10 (100%)	6 (75%)	4 (66.7%)	0 (0%)	0 (0%)
Cephalexin	12 (85.7%)	10 (83.3%)	10 (100%)	6 (75%)	4 (66.7%)	2 (33.3%)	0 (0%)
Ciprofloxacin	14 (100%)	12 (100%)	10 (100%)	8 (100%)	0 (0%)	4 (66.7%)	2 (100%)
Ceftriaxone	4 (28.6%)	12 (100%)	10 (100%)	8 (100%)	0 (0%)	6 (100%)	2 (100%)
Gentamycin	14 (100%)	12 (100%)	4 (40%)	4 (50%)	2 (33.3%)	2 (33.3%)	0 (0%)
Cefoxitin	12 (85.7%)	10 (83.3%)	10 (100%)	8 (100%)	4 (66.7%)	2 (33.3%)	0 (0%)
Imipenem	14 (100%)	8 (66.7%)	10 (100%)	8 (100%)	6 (100%)	4 (66.7%)	0 (0%)
Levofloxacin	14 (100%)	12 (100%)	10 (100%)	8 (100%)	6 (100%)	2 (33.3%)	0 (0%)
Meropenem	10 (71.4%)	12 (100%)	10 (100%)	8 (100%)	4 (66.7%)	2 (33.3%)	0 (0%)
Sulfamethoxazole	10 (71.4%)	12 (100%)	10 (100%)	8 (100%)	4 (66.7%)	2 (33.3%)	0 (0%)
Tobramycin	12 (85.7%)	12 (100%)	10 (100%)	8 (100%)	2 (33.3%)	0 (0%)	0 (0%)
Vancomycin	14 (100%)	12 (100%)	6 (60%)	8 (100%)	2 (33.3%)	0 (0%)	0 (0%)

MRSA: Methicillin-resistant Staphylococcus aureus.

completely different in a report from Iran 2016, *E. coli* was the most common pathogen found in respiratory tracts of ICU patients [7].

However, bacterial colonization of VC was detected at day 1, while LRT was detected until day 5, which made VC contaminated by patient's secretion less likely in this case. Clinical characteristics of patients included in this study demonstrated the patients were not hospitalized at the same time. This indicated that organism may survive for a period of time in ICU environment and disseminated to other patients through VC. Several studies have also reported outbreaks of VAP caused by pathogens from VC [26, 27].

The purpose of all intensive care units was to minimize antimicrobial resistance inside the ICUs since the result was improved and the overall cost and the time of ICU stay decreased. Severe use of antibiotics contributes to the appearance of multi-resistant microorganisms in the environment of the ICU. The current study showed a high prevalence of antibiotic-resistant organisms. *P. aeruginosa* was fully resistant to amikacin (100%), imipenem (100%), levofloxacin (100%), and cephalexin (66%). while Ceftriaxone was the most effective antibiotic against *P. aeruginosa* in Tripoli hospitals. This result was quite similar to another study that represented that *P. aeruginosa* was also a multidrug-resistant bacteria type [24]. Their *P. aeruginosa* isolates showed high resistance to cephalexin (95.3%), levofloxacin (63.9%), and ceftriaxone (60.9%). but Amikacin was the most effective antibiotic against *P. aeruginosa* followed by imipenem. Our finding showed that *S. epidermidis* were fully resist nt to vancomycin unlike, previous work in Iran, which reported that all cases of 90% *S. epidermidis* were sensitive to vancomycin [25].

From this study, we concluded that gram-negative bacteria had a multi-drug resistant complication as well as gram-positive. This finding is most consistent with many of the previous studies which determined a multi-drug resistant bacterium to most antibiotic types and that probably related to misuse of antibiotics.

There are many limitations to our research as well. First, the sample size is relatively small and the time is short. Second, local ICU conditions and infection management strategies may have a direct effect on the outcomes of VC bacterial colonization.

5. CONCLUSIONS

This study concluded that bacterial colonization of VC is a major source of causing VAP pathogens in mechanically ventilated patients. However, the incidence of VAP can be reduced by infection management strategies to reduce VC bacterial colonization and transmission. Further studies are needed to reduce VC contamination to decrease VAP in mechanically ventilated patients.

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