Profile and antibiotic-resistance pattern of bacteria isolated from endotracheal secretions of mechanically ventilated patients at a tertiary care hospital

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Abstract:

BACKGROUND: Critically ill patients on mechanical-ventilation are always at a higher risk of acquiring ventilator-associated respiratory infections. The current study was intended to determine the antibiotic-resistance pattern of bacteria recovered from the endotracheal (ET) specimens of ventilated patients.

MATERIALS AND METHODS: This was a single-centered, retrospective study carried out in a 400-bed tertiary care hospital in Oman. The data of profile and antibiotic resistance pattern of bacterial isolates recovered from ET aspirates of ventilated patients during the period from January 2017 to August 2019 were retrieved from hospital database. The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 22 (IBM, Armonk, New York, USA). Descriptive statistics were applied to find the frequencies and percentages. Charts and tables were constructed.

RESULTS: In total, 201 bacterial isolates recovered from ET secretions of 154 ventilated patients were studied. The rate of isolation was predominant among males (65.6%) and in elderly people (50%). Gram-negative bacilli (GNB) were predominantly (88.6%) isolated. Acinetobacter baumannii (31.3%) was the most common isolate and 86% of them were multidrug-resistant strains. Klebsiella pneumoniae (23.9%) and Pseudomonas aeruginosa (22.9%) were the other common GNB, whereas Staphylococcus aureus was the most frequently isolated Gram-positive bacteria. Gentamicin showed good in vitro activity against S. aureus and all the GNB except A. baumannii reflecting good choice for empirical therapy.

CONCLUSION: Gram-negative bacteria were the predominant isolates in ET secretions of ventilated patients. There was an alarmingly high rate of antimicrobial resistance among GNB. A rational use of antibiotics, regular monitoring of antibiotic resistance and use of right combination of drugs, in addition to refining of existing infection control practices are critical to control the emergence of drug-resistant strains.

Keywords: Acinetobacter, critical care, drug resistance, mechanical ventilation, respiratory infections

Introduction

Hospital-associated infections and antibiotic resistance are the important global public health concerns.¹⁻⁴ Critically ill patients admitted to intensive care units (ICUs) are at higher risk of acquiring nosocomial infections with drug-resistant organisms.¹⁻⁴ This is due to the fact of their immunocompromised profile, serious illness, use of invasive devices, prolonged...
length of hospital stay, and overuse of broad-spectrum antibiotics.\textsuperscript{[5,8]} The indiscriminate use of antibiotics further drives the emergence of the multidrug-resistant (MDR) superbugs, and this creates a major hurdle in treating critically ill patients of ICUs.\textsuperscript{[6,7]}

The intubation for mechanical ventilation is one of the life-saving practices employed for patients admitted to ICUs. However, it is associated with a greater risk of acquiring lower respiratory tract infections (LRTIs) including pneumonia (ventilator-associated pneumonia [VAP]) among ventilated patients.\textsuperscript{[5,8]} Ventilator-associated pneumonia accounts for 15% of nosocomial infections and is the second most common cause of hospital-acquired infection with a high rate of morbidity and mortality.\textsuperscript{[9]} The mortality among these patients is on the rise due to the advent and constant increase in the number of MDR pathogens such as MDR Gram-negative rods including extended-spectrum beta-lactamase (ESBL) producers, carbapenem, and colistin-resistant organisms, and methicillin-resistant \textit{Staphylococcus aureus} (MRSA).\textsuperscript{[9]} Concerning pathogens associated with LRTI, Gram-negative rods such as \textit{Acinetobacter spp.}, \textit{Pseudomonas aeruginosa}, and \textit{Klebsiella pneumoniae} are predominant, whereas Gram-positive bacteria such as \textit{S. aureus} including MRSA are comparatively less common.\textsuperscript{[5,6,9]} \textit{Enterobacter spp.}, \textit{Proteus spp.}, \textit{Providencia spp.}, \textit{Stenotrophomonas maltophilia}, and \textit{Enterococcus spp.} are other less commonly associated etiological agents.\textsuperscript{[5,6,9]} Apart from this, ventilated patients develop infections of multi-bacterial etiology, and these factors further complicate the therapy due to wide variation in the antibiotic-susceptibility patterns of involved pathogens.\textsuperscript{[5,8]} Furthermore, these bacteria are prevalent in the hospital environment, capable of surviving in the dry and humid surfaces such as ventilators and heart-lung machines and have the ability to develop resistance to most of the commonly used antibiotics in the ICU settings such as beta-lactams, fluoroquinolones, cephalosporins, aminoglycosides, carbapenems, and trimethoprim-sulfamethoxazole (TMX-SMX).\textsuperscript{[6,10]} This would further impede in the selection of appropriate empirical therapy.

The etiology of LRTI among ICU patients may vary from one country to another and within the country from one region to another according to the differences in ICU settings, the severity of the patients’ illness, use of antibiotics, and infection control practices.\textsuperscript{[5]} Therefore, it is imperative for local physicians to have accurate knowledge about the bacterial profile of the local hospital ICU settings and also their antibiotic susceptibility patterns.\textsuperscript{[11]} This would further help clinicians to select the most appropriate antibiotics for the empirical therapy of critically ill patients of ICUs. On a thorough literature search, the authors could not find any such studies in the North-Batinah region of Oman. Hence, the current study was aimed to determine the bacterial profile and also antibiotic-resistance patterns of the bacteria isolated from the ET secretions of the ventilated patients.

\textbf{Materials and Methods}

\textbf{Study design}
This descriptive, cross-sectional study was carried out at a 400-bed tertiary care ministry hospital in the North-Batinah region of Oman. The data of bacterial isolates recovered from endotracheal (ET) secretions of the ventilated patients during the period from January 2017 to August 2019 were retrieved systematically from the Al-Shifa computerized system and microbiology laboratory. The data were analyzed for patients’ sociodemographic characteristics, bacterial profile, and their antibiotic resistance patterns. All patients’ data were anonymized and maintained with high confidentiality.

\textbf{Inclusion criteria}
All the specimens with positive bacterial growth were included in the study.

\textbf{Exclusion criteria}
All repeat isolates and the specimens with the growth of candida species were excluded from the study.

\textbf{Ethical consideration}
The study was approved by the Research and Ethical Committee, Ministry of Health, Oman (MH/DHGS/NBG/1923208774/2019).

\textbf{Data collection and sample processing procedure}
\textit{Bacterial identification and antibiotic susceptibility testing}
ET secretions sent to the microbiology laboratory were screened by Gram stain and cultured by plating on MacConkey agar and blood agar and incubated at 37°C in ambient air. The isolates were identified up to the species level by the standard microbiological methods and the automated VITEK 2 system (Bio-Merieux, France) as recommended by the Clinical Laboratory Standards Institute (CLSI).\textsuperscript{[12]} Antimicrobial susceptibility testing was performed by Kirby–Bauer’s disc-diffusion method on Mueller-Hinton agar using Oxoid antibiotic discs as recommended by the CLSI.\textsuperscript{[12]} Colistin resistance of Gram-negative organisms was determined by Epsilometer (E) test. The antibiotic panels used include gentamicin (10 µg), clindamycin (2 µg), linezolid (30 µg), erythromycin (15 µg), ampicillin (10 µg), TMX-SMX (1.25/23.75 µg), ciprofloxacin (5 µg), amoxicillin-clavulanic acid (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), amikacin (10 µg), doxycycline (30 µg), vancomycin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), cefazidime (30 µg), and colistin (10 µg) as recommended
by the CLSI. Quality control was performed using Escherichia coli ATCC 25922, P. aeruginosa ATCC 27853, and S. aureus ATCC 29213. The antibiotic susceptibility report of each isolate was interpreted as sensitive, intermediate, or resistance as per the CLSI guidelines. The organisms that have acquired nonsusceptibility to at least one antimicrobial agent in three or more classes of antimicrobial agents were termed as MDR pathogens. Further, MDR organisms (MDROs) were categorized as MRSA, ESBL producers, and carbapenem-resistant Enterobacteriaceae (CRE).

**Identification of multidrug-resistant organisms**

The organisms that have acquired nonsusceptibility to at least one antimicrobial agent in three or more classes of antimicrobial agents were termed as MDR pathogens. Further, MDR organisms (MDROs) were categorized as MRSA, ESBL producers, and carbapenem-resistant Enterobacteriaceae (CRE).

**Staphylococcus species** were tested for methicillin resistance by using cefoxitin disc (30 µg). Inhibition zone ≤21 mm with cefoxitin disc was reported as methicillin resistant, and a zone diameter of ≥22 mm was considered sensitive according to the CLSI guidelines. Gram-negative bacilli were further tested for the production of ESBL by a double-disc diffusion method using ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg). An increase in diameter of ≥5 mm with ceftazidime plus clavulanic acid as compared to ceftazidime disc alone was considered positive for ESBL production. The resistance of Enterobacteriaceae to imipenem and meropenem (carbapenems) was referred to as CRE.

**Data analysis**

The data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) software version 22 (IBM, Armonk, New York, USA). Descriptive statistics were applied to find the frequencies and percentages. Charts and tables were constructed.

**Results**

In total, 201 isolates recovered from 154 ET secretion samples were studied. Table 1 depicts the sociodemographic and culture characteristics of isolates. The frequency of isolation was predominant among males (65.6%) compared to females (34.4%). A large number of samples (118/154, 76.6%) showed monomicrobial growth, whereas the remaining samples (36/154, 23.4%) showed polymicrobial growth of two or more types of bacteria. The rate of isolation was the highest among patients aged more than 60 years (50%). Gram-negative organisms were the predominant isolates (88.6%) compared to Gram-positive organisms (11.4%). Among Gram-negative rods, A. baumannii (31.3%) was the most frequently isolated organism followed by K. pneumoniae (23.9%) and P. aeruginosa (22.9%). Other Gram-negative isolates such as E. coli, S. maltophilia, Proteus spp., Enterobacter spp., and Providencia spp. are less frequently isolated. S. aureus including MRSA was the most frequently isolated Gram-positive organism (8.8%). Tables 2 and 3 depict an antibiotic-resistant pattern of Gram-negative and Gram-positive organisms, respectively. It can
be observed that antibiotic resistance to the tested antibiotics varied between 0% and 100% in both Gram-negative and Gram-positive organisms.

**Discussion**

LRTIs among critically ill ICU patients are important nosocomial infections, occurring in 7%–40% of ventilated patients.[4] These result in high morbidity, prolonged length of hospital stay, increased health-care expenses, and increased mortality among intensive care patients. Risk of acquiring these infections is directly related to the duration of exposure to ventilators.[4] Ventilator-associated LRTIs including VAP occur due to the interplay of multiple factors such as impaired host defense, easy access of pathogens to lower respiratory tract, and the virulence nature of the pathogen.[6,14] In addition, constantly increasing population of drug-resistant pathogens is impending a serious situation. This implies the need for continuous monitoring of antimicrobial resistance, updated regulations on antibiotic policy, cautious use of antibiotics, and also refining the hospital infection control practices to prevent further aggravation of anti-microbial resistance especially in the ICUs where infection and antimicrobial consumption are significantly higher.[4]

The present study reviewed the bacterial profile and their antibiotic-resistant patterns isolated from ET secretions of the mechanically ventilated patients, and perhaps, it is the first study in North-Batinah region of Oman. In our study, the percentage of positive growth was 67.5% and the frequency of isolation was predominant among males (65.6%). Furthermore, the highest positive growth (50%) was seen in the ET specimens obtained from the elderly people aged more than 60 years. These bacteria that colonize initially may later result in LRTIs. In a study conducted by Malik et al. in Pakistan, the percentage of positive growth was reported to be 83%.[5] Another study conducted by Jamil et al. in Bangladesh,
the rate of positive growth was 93.8%. In comparison, a relatively low rate of positive growth was observed in our study, and this could be due to the fact of better infection control practices in ICU settings of our hospital.

The National Nosocomial Infections Surveillance of CDC of the United States of America reports that aerobic Gram-negative rods are associated with 60% of hospital-acquired pneumonia. In our study, Gram-negative bacilli were isolated at a significantly higher rate (88.6%) as compared to Gram-positive bacteria (11.4%) from ET specimens. This finding was consistent with the reports of Veena Kumari et al., Chandra et al., and Gupta et al. which showed the rate of isolation of Gram-negative bacteria to be 92%, 85%, and 86%, respectively. Advances and increased application of medical and surgical interventions including ICU procedures contribute to the increased occurrence of nosocomial infections in ICU patients. Among Gram-negative bacilli, A. baumannii (31.3%) was found to be the most common isolate in our study followed by K. pneumoniae (23.9%) and P. aeruginosa (22.9%). In a similar study by Deepthi Gupta et al., enteric Gram-negative aerobic rods were found to be the most frequent isolates with K. pneumoniae being the most common species followed by nonfermentative Gram-negative bacilli such as A. baumannii and P. aeruginosa. Malik et al. and Chandra et al. have also reported K. pneumoniae as the most common isolate in lower respiratory tract specimens of the ventilated patients. However, similar to our study, George and Sequiera have reported A. baumannii (37.5%) as the most common isolate followed by P. aeruginosa (21.8%) and K. pneumoniae (15.6%). These findings suggest that Gram-negative bacteria were more frequently associated with nosocomial infections, especially in critically ill patients and are difficult to treat because of their high drug resistance nature. In addition, these bacteria, especially Acinetobacter and Pseudomonas have the ability to survive in humid and dry conditions and also have the ability to colonize equipment such as ventilators and heart–lung machines. This warrants for strict measures to control the dissemination of Gram-negative bacilli, especially in the ICU settings.

The maximum use of antibiotics in ICU patients drives the emergence of MDR strains. Therefore, the primary goal in all ICUs is to reduce the antimicrobial resistance and thereby improve the patient’s outcome of illness in terms of reducing health-care expenses, length of ICU stay, and mortality. In our study, MDR among the Gram-negative isolates of ET secretions was alarmingly high (Table 4). The most common MDRO Gram-negative bacteria in our study [Table 4] were A. baumannii (85.7%), K. pneumoniae (73%), E. coli (67%), and P. aeruginosa (24%). Among MDROs, ESBL production was observed maximally among E. coli (3/4) and K. pneumoniae (6/35), while carbapenem resistance (CRE) was predominantly noticed among K. pneumoniae (23/35) in Enterobacteriaceae family. These findings were consistent with the report of Malik et al.

Acinetobacter spp. is emerging as a major MDR pathogen in nosocomial infections especially in critically ill patients. In the present study, more than 80% A. baumannii showed resistance to ciprofloxacin, aminoglycosides (gentamicin and amikacin), cephalosporins, piperacillin-tazobactam, carbapenems (imipenem and meropenem), and TMX-SMX. Among aminoglycosides, Acinetobacter spp. were more resistant to gentamicin (91.8%) compared to amikacin (82%). However, all Acinetobacter spp. were susceptible to colistin. Similar high-level resistance of A. baumannii to these drugs and the highest sensitivity to colistin (98.6%) was reported by Jamil et al. in Bangladesh. This alarmingly low susceptibility of A. baumannii to most of these drugs, and the rapid emergence of MDR clones was probably due to overuse in critically ill patients and persistence in ICU settings.

In our study, K. pneumoniae showed uniformly high-level resistance to most of the tested antibiotics (60%–100%) except for gentamicin (38.3%) and colistin (14.3%). In contrast,

**Table 4: Multidrug-resistant organisms isolated from endotracheal secretions**

| Organisms                  | Non-MDR isolates, n (%) | MDR isolates, n (%) |
|----------------------------|-------------------------|--------------------|
|                            | Total MDR | ESBL   | CRE  |
| Acinetobacter baumannii    | 9 (14)     | 54 (86) | -    |
| Klebsiella pneumoniae      | 13 (27)    | 35 (73) | 6 (17) | 23 (66) |
| Pseudomonas aeruginosa     | 35 (76)    | 11 (24) | 4 (67) | 3 (75) | 0 |
| Escherichia coli           | 2 (33)     | 1 (33)  | 0     |
| Proteus spp.               | 2 (67)     | 1 (50)  | 0     | 0 |
| Providencia spp.           | 1 (50)     | 1 (50)  | 0     | 0 |
| Enterobacter spp.          | 3 (75)     | 1 (25)  | 0     | 0 |
| Staphylococcus aureus      | 8 (47)     | 9 (53)  | -     |
| Enterococcus spp.          | 1 (50)     | 1 (50)  | -     |
| Others                     | 14         | -      | -     |
| Total                      | 88 (44)    | 113 (56) |

MDR=Multi-drug resistant, ESBL=Extended-spectrum beta-lactamase, CRE=Carbapenem-resistant Enterobacteriaceae
P. aeruginosa showed relatively good susceptibility toward ciprofloxacin (76%), gentamicin (69%), amikacin (71%), ceftazidime (68%), and piperacillin-tazobactam (67%). P. aeruginosa showed 100% susceptibility to colistin in our study similar to the report of Malik et al.\[5\] However, decreased susceptibility of P. aeruginosa to carbapenems (<40%) and TMP-SMX (25%) was noted. These findings indicate that the Gram-negative bacteria have become increasingly resistant to multiple drugs which may be due to the facts of cross-infections and other factors such as overuse of antibiotics. Therefore, it emphasizes the regular characterization of bacteria and their resistance pattern for standardizing the most appropriate empirical therapy to control the drug-resistant strains.

In contrast, other Gram-negative bacteria belonging to Enterobacteriaceae such as E. coli, Enterobacter spp., Proteus spp., and Providencia spp. have shown good susceptibility to quinolones, aminoglycosides, carbapenems, TMX-SMX, and piperacillin-tazobactam, but there was an alarming rise in the resistance to amoxicillin-clavulanic acid, ampicillin, and cephalosporins. These findings were in accordance with other studies conducted elsewhere.\[6,15–17\] All S. maltophilia strains showed susceptibility toward TMP-SMX in our study. Similar studies reported the high rate of susceptibility of S. maltophilia toward TMP-SMX, making it one of the drug of choice to treat the associated infections.\[20–21\]

In the present study, 53% of S. aureus were MRSA. Regarding antibiotic susceptibility among Gram-positive cocci, resistance percentage of all species except Enterococcus spp. to gentamicin, ciprofloxacin, TMX-SMX, and erythromycin ranged between 0–25 percent. All S. aureus showed 100% susceptibility to cephradine, linezolid, and vancomycin while Enterococcus spp. showed 100% susceptibility to vancomycin. These results were in-line with the reports of Chandra et al. and Gupta et al.\[16,17\]

**Limitation of the study**

The present study was limited to a single tertiary care hospital and the bacterial profile reflects the local environmental settings. Therefore, the findings of our study cannot be generalized. More such multi-center studies may provide robust information, and it would help in making generalized recommendations.

**Conclusion**

In conclusion, we found the predominance of Gram-negative bacteria (GNB) in ET secretions with A. baumannii being the most common isolate. One of the grave concerns associated with nosocomial infections in ICU settings are the upsurge in MDR respiratory pathogens which are difficult to treat. Therefore, combined approach of strict antibiotic policy, judicious use of antibiotics, and refined infection containment procedures are indispensable to overcome antibiotic resistance and to maintain a low level of resistant organisms. In addition, there is also a need for prioritization of molecular diagnostic techniques for the most accurate and rapid identification of pathogens and improved management of severe infections caused by drug-resistant pathogens.

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**Conflicts of interest**

There are no conflicts of interest.

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