Susceptibility pattern of tracheal tube isolates from Intensive Care Unit of Fauji Foundation Hospital Rawalpindi

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Abstract

Objective: To determine the prevalence of resistant pathogens and their antimicrobial susceptibility pattern in an intensive care unit.

Method: The cross-sectional observational study was conducted at Foundation Hospital, Rawalpindi, Pakistan, from May to September 2016, and comprised tracheal tubes which were collected in sputum culture bottles from patients with clinical findings of ventilator-associated pneumonia. The tubes were cultured to locate the resistant pathogens.

Result: A total of 113 different strains of bacteria were isolated from 80 patients. The main isolated bacteria was *acinetobacter baumannii* 45(39.8%) followed by *klebsiella pneumonia* 14(12.3%) and methicillin-resistant *staphylococcus aureus* 13(11.5%). Polymyxin B was the most appropriate drug for treating patients infected with *acinetobacter baumannii* with a sensitivity of 64% while vancomycin and linezolid had 100% sensitivity for methicillin-resistant *staphylococcus aureus*.

Conclusion: *Acinetobacter baumannii* was the most prevalent strain in tracheal tubes and polymyxin B was the most effective medicine.

Keywords: Nosocomial infections, Tracheal tubes, Antimicrobial resistance.
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Introduction

A number of host defence mechanisms protects the trachea and lungs from bacterial infection. These host defences not only filter and humidify air but also trap the invaders and then clear the airway with the aid of coughing, mucous and cilia. Furthermore, humoral and cellular immune mechanisms also play their active role in eradicating infectious agents.[1,2] However, in critically ill patients, host defences may be impaired and aid in establishing infection.[3] Moreover, in these patients the endotracheal tube (ETT) not only impairs mucocilliary clearance and cough reflex, but also facilitates bacteria entry into the lower respiratory tract by permitting leakage of secretions around the ETT cuff. It also prevents the exit of bacteria from the lower airway, creating a need for manual tracheobroncheal suctioning. Nonetheless, suctioning can further upsurge the risk of embolisation of biofilm mass associated with ETT.[4-6] In addition, ETT insertion could cause injury and infection of the lower respiratory tract.[7,8]

Ventilator-associated pneumonia (VAP) is the inflammation to lung parenchyma in any inpatient on mechanical ventilator for more than 2 days. VAP is the most common and most fatal type of hospital-acquired pneumonia (HAP). It needs to be subjected to prompt diagnosis and apposite treatment.[9] The time of onset of pneumonia is an important risk factor for specific pathogens and outcome in patients with VAP. Early onset VAP, defined as occurring within the first 4 days of hospitalisation, is usually caused by antibiotic-sensitive bacteria, which means community-acquired bacteria, whereas the late onset VAP, more than 5 days, is associated with increased mortality in patients.[10]

The emergence of multi-drug resistant (MDR) pathogens has invalidated all the available treatment and principally the MDR pathogens in intensive care unit (ICU) patient is a major cause of morbidity and mortality. MDR biofilm-forming pathogens are particularly more notorious in a variety of device-related infections.[11] The microbes residing in biofilms are almost 1000 times more resistant than the planktonic microbes and are not only protected

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from the effect of antibiotics but also from host defence mechanisms, making treatment not only difficult, but, in most cases, requiring device withdrawal.[12]

Bacterial biofilm has been observed universally on the surface of ETT in mechanically ventilated patients.[13] Some data shows a good concordance between bacterial colonisation of the airway and microbial findings in the biofilm. Even the same microorganisms causing VAP could be found in the ETT biofilm, leading to the potential implication of biofilm in the genesis of VAP. Pathogens associated with VAP are the main cause of treatment failure and relapse of infection.[14]

The current study was planned to identify the bacterial pathogens causing VAP in an ICU setting and to ascertain the antibiotic profile.

Material and Methods

The cross-sectional observational study was conducted between May and September 2016 at the Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, Pakistan, and comprised ETT from ICU patients on ventilator support for 48 hours. Repeated samples from the same patient were excluded.

The tubes were 2-5cm in length. Isolates of count >103 colony-forming unit (CFU)/mL were isolated using dilution method. They were characterised and antibiogram was determined using standard antibiotics regime.

The antibiotics used were polymyxin B (PB 300mg), doxycycline (DO 30mg), gentamicin (CN 10mg), amikacin (AK 30mg), imipenem (IMP 10mg), ciprofloxacin (CIP 5mg), meropenem (MEM 10mg), trimethoprim/ sulmethoxazole (SXT 25mg), sulzone (SCF), minocycline (MH 30mg), chloramphenicol (C 30mg), vancomycin (VA 30mg), linezolid (LZD 30mg), erythromycin (E 15mg), ceftazidime (CAZ 30mg), and aztreonam (ATM 30mg).

and the ETTs were mixed with sterilised distilled water and 0.01 mL of sample solution was inoculated on blood agar and MacConkey agar. These petri plates were then incubated for 48 hours at 37^{O}C. Samples producing growth of >103CFU/mL were considered significant.

Bacterial colonies were further identified by biochemical testing. Coagulase testing was done to confirm staphylococcus aureus. Whereas gram-negative microbes were identified by oxidase, bile esculin hydrolysis and analytical profile index (API) 20E. Methicillin-resistant *staphylococcus aureus* (MRSA) was identified by catalase, coagulase and deoxyribonuclease (DNase test) using cefoxitin disc (30µg).

Inoculum's turbidity was compared with 0.5 McFarland and spread evenly over Mueller-Hinton agar plate. Antibiotic discs were applied as per the manufacturer's instructions. For vancomycin, minimum inhibitory concentration (MIC) was taken by using E strips. For polymyxin to use on gram-negative rods (GNR) also, E strips were employed. The plates were then incubated at 37^{O}C for 24 hours. Zones of inhibitions were measured and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2017.[15]

Results

A total of 113 different strains of bacteria were isolated from 80 patients. The main isolated bacteria was *acinetobacter baumannii* 45(39.8%) followed by *klebsiella pneumonia* 14(12.3%) and MRSA 13(11.5%) (Table 1).

PB was the most appropriate drug for treating patients infected with *acinetobacter baumannii* with a sensitivity of 64% while VA and LZD had 100% sensitivity for MRSA (Table 2).

Discussion

The current study was done to determine the prevalence of MDR pathogens in ETT. VAP remains the leading cause of death in patients with ICU-acquired infections associated with an attributable mortality of around 30%. Increasing antimicrobial resistance in patients with VAP challenges intensivists to search for alternative therapeutic options.[14]

Table-1: Different microorganisms isolated from endotracheal tube.

Microorganism	n (%)
Acinetobacter species	45 (39.8)
Klebsiella pneumonia	14 (12.3)
MRSA	13 (11.5)
Pseudomonas species	10 (8.8)
Pantoeaagglomerans	8 (7)
Escherichia coli	7 (6.1)
Enterococcus faecalis	5 (4.4)
Proteus mirabilis	3 (2.6)
Burkholderiacepacia	3 (2.6)
Stenotrophomonas	2 (1.7)
Escherichia vulneris	1 (0.8)
Providenciastuartii	1 (0.8)
Serratiamarcescens	1 (0.8)

MRSA: Methicillin-resistant staphylococcus aureus

Table-2: Sensitivity for drugs against various microbes.

Antibiotic	Acinetobacter n=45	Klebsiella pneumonia n=14	MRSA n=13	Pseudomonas speceies n=10	Pantoeaagglomerans n=8
Polymyxin B (PB 300)	64.4	28.5	-	40	37.5
Doxycycline (DO 30)	44.4	21.4	53.8	-	12.5
Gentamycin (CN 30)	22.2	42.8	-	30	12.5
Amikacin (AK 30)	20	42.8	-	40	25
Imipenem (IMP 10)	13.3	50	-	40	25
Ciprofloxacin (CIP 5)	11.1	14.2	-	40	25
Meropenem (MEM 10)	11.1	50	-	40	25
Trimethoprim/sulfamethoxazole (SXT 25)	8.8	14.2	30.7	-	12.5
Sulzone (SCF)	6.6	7.1	-	40	12.5
Minocycline (MH 30)	6.6	14.2	-	-	-
Chloramphenicol (C 30)	-	-	46,1	-	-
Vancomycin (VA 30)	-	-	100	-	-
Linezolid (LZD 30)	-	-	100	-	-
Erythromycin (E 15)	-	-	15.3	-	-
CEFTAZIDIME (CAZ 30)	-	-	-	20.0	-
Aztreonam (ATM 300	-	-	-	20.0	-

MRSA: Methicillin-resistant staphylococcus aureus

Acinetobacter (A.) species were found to be the most resistant pathogens prevailing in our ICU setup, followed by *klebsiella* (K.) species, MRSA and Pseudomonas (P.) species (spp.) bacteria.

A. baumannii has become endemic in hospitals due to its versatile genetic machinery, which allows it to quickly evolve resistance factors, and to its remarkable ability to tolerate harsh environments. Infections and outbreaks caused by MDR-AB are prevalent and have been reported worldwide over the past 20 or more years.[16] A. species were also the most prevalent in a previous study.[17]

The growing epidemic of infections in the ICU caused by MDR pathogens has led clinicians to reconsider prescribing pPB and colistin (polymyxin E) drugs that were removed from use in the past because of their neuro- and nephro-toxicity.[17]

An earlier study evaluated the efficacy of PB in MDR pathogens when there is very narrow choice.[18] In case of *A. baumannii*, 64% of the cultures were susceptible to PB and 44% against DO, 50% of K. species were sensitive to MEM. VA was found to be 100% sensitive for MRSA. For *pseudomonas*, multiple antibiotics, like PB, AK, IMP, CIP, MEM and SCF displayed 40% sensitivity. For *Pantoeaagglomerans*, PB was the most effective drug (37%).

In a previous study, incidence of VAP was found to be 44.2% among the mechanically ventilated patients and *K. pneumoniae* (34%) was the most common pathogen isolated, followed by *P. aeruginosa* (20%). Among them, most of the *K. pneumoniae* and *P. aeruginosa* isolates were resistant to penicillins, cephalosporins, fluoroquinolones, but were sensitive to piperacillin/tazobactum, cefaperazone/sulbactum, and carbapenems. All isolates were sensitive to AK.[19] However, in the present study *K. pneumoniae* displayed 42% sensitivity to AK and *pseudomonas* was 40% sensitive.

Conclusion

PB was found to be the most appropriate drug for treating patients with *A. baumannii*. DO was another good option As *K. pneumoniae* revealed better sensitivity to carbapenems and aminoglycosides. For MRSA, glycopepetides was the drug of choice. *P. aeruginosa* showed equal susceptibility to carbapenem, aminoglycosides and polymyxins.

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References

1. Niederman MS. The clinical diagnosis of ventilator-associated pneumonia. Respir Care 2005; 50: 788-96.
2. Joseph NM, Sistla S, Dutta TK, Badhe AS, Parija SC. Ventilator-associated pneumonia: a review. Eur J Intern Med 2010; 21: 360-8.
3. Cook DJ, Walter SD, Cook RJ, Griffith LE, Guyatt GH, Leasa D, et al. Incidence of and risk factors for ventilator-associated pneumonia in critically ill patients. Ann Intern Med 1998; 129: 433-40.

4. Bauer TT, Torres A, Ferrer R, Heyer CM, Schultze-Werninghaus C, Rasche K. Biofilm formation in endotracheal tubes. Association between pneumonia and the persistence of pathogens. Monaldi Arch Chest Dis 2002; 57: 84-7.
5. Inglis TJ, Millar MR, Jones JG, Robinson DA. Tracheal tube biofilm as a source of bacterial colonization of the lung. J Clin Microbiol 1989; 27: 2014-8.
6. Grgurich PE, Hudcova J, Lei Y, Sarwar A, Craven DE. Management and prevention of ventilator-associated pneumonia caused by multidrug-resistant pathogens. ?Expert Rev Respir Med 2012; 6: 533-55.
7. Rello J, Sonora R, Jubert P, Artigas A, Rué M, Vallés J. Pneumonia in intubated patients: role of respiratory airway care. Am J Respir Crit Care Med 1996; 154: 111-5.
8. Craven DE, Steger KA. Epidemiology of nosocomial pneumonia. Chest 1995;108:1S-16S.
9. Gil-Perotin S, Ramirez P, Marti V, Sahuquillo JM, Gonzalez E, Calleja I, et al. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept. Crit Care 2012; 16: R93.
10. Patel S. Antimicrobial Therapy for Hospital-Acquired Pneumonia. US Pharm 2016; 41: HS11-5.
11. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001; 358: 135-8.
12. Donlan RM. New approaches for the characterization of prosthetic joint biofilms. Clin Orthop Relat Res 2005; 437: 12-9.
13. Zur KB, Mandell DL, Gordon RE, Holzman I, Rothschild MA. Electron microscopic analysis of biofilm on endotracheal tubes removed from intubated neonates. Otolaryngol Head Neck Surg 2004; 130: 407-17.
14. Michalopoulos A, Fotakis D, Virtzili S, Vletsas C, Raftopoulou S, Mastora Z, et al. Aerosolized colistin as adjunctive treatment of ventilator-associated pneumonia due to multidrug-resistant Gram-negative bacteria: a prospective study. Respir Med 2008; 102: 407-12.
15. Patel JB, editor. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute; 2017.
16. Johnson JK, Robinson GL, Zhao L, Harris AD, Stine OC, Thom KA. Comparison of molecular typing methods for the analyses of Acinetobacterbaumannii from ICU patients. Diagn Microbiol Infect Dis2 016; 86: 345-50.
17. Jakribettu RP, Boloor R. Characterisation of aerobic bacteria isolated from endotracheal aspirate in adult patients suspected ventilator associated pneumonia in a tertiary care center in Mangalore. Saudi J Anaesth 2012; 6: 115-9.
18. Pastewski AA, Caruso P, Parris AR, Dizon R, Kopec R, Sharma S, et al. Parenteral polymyxin B use in patients with multidrug-resistant gram-negative bacteremia and urinary tract infections: a retrospective case series. Ann Pharmacother 2008; 42: 1177-87.
19. Kofteridis DP, Alexopoulou C, Valachis A, Maraki S, Dimopoulou D, Georgopoulos D, et al. Aerosolized plus intravenous colistin versus intravenous colistin alone for the treatment of ventilator-associated pneumonia: a matched case-control study. Clin Infect Dis 2010; 51:1238-44.