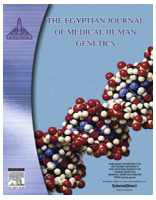
The Egyptian Journal of Medical Human Genetics xxx (2017) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com



Original article

Multiplex polymerase chain reaction: Could change diagnosis of Ventilator-associated pneumonia in pediatric critical care units to the fast track?

Mervat Gamal Eldin Mansour [a,*], Sherin Albendary [b]

[a] Pediatric Department, Ain Shams University, Egypt
[b] Microbiology Department, Ain Shams University, Egypt

ARTICLE INFO

Article history:
Received 29 July 2017
Accepted 22 August 2017
Available online xxxx

Keywords:
m-PCR
Ventilator-associated pneumonia
Bacterial diagnosis
Turnaround time

ABSTRACT

Background: Ventilator-associated pneumonia (VAP) is a frequent hospital-acquired infection in critically ill children. The increasing incidence of infections by antibiotic-resistant pathogens adds significantly to the cost of hospital care and to the length of hospital stays. Besides clinical prerequisites for presumptive diagnosis of VAP, rapid identification of the causative pathogen is essential for appropriate treatment.
Aim of study: To identify the causative bacterial pathogens of VAP by both conventional microbiological cultures and multiplex reverse transcriptase reaction (m-PCR) methods with assessment of turnaround time for both diagnostic modalities together with their diagnostic accuracy.
Methods: Patients were diagnosed to have VAP when their Clinical Pulmonary Infection Score (CPIS) index was more than 6. Endotracheal aspirate was subjected to both microbiological cultures and multiplex PCR for bacterial pathogens.
Results: Multiplex-PCR showed better sensitivity and positive predictive value than bacterial culture for etiological diagnosis of VAP. *Acinetobacter* and *Klebsiella pneumoniae* were the most common identified pathogens. Mean turnaround times were 6 h for multiplex PCR and 72 h for conventional microbiology. Significant shorter turnaround time was recorded with m PCR compared to microbiological culture.
Conclusion: Multiplex-PCR permits simultaneous detection of several bacterial pathogens in a single reaction with best turnaround time that permit optimization of emergency diagnosis of VAP and subsequently improve early management of selective bacterial pathogens.

1. Introduction

Within twelve hours of endotracheal intubation; a biofilm is formed around the endotracheal tube which contains large amounts of bacteria that can be disseminated into the lungs by ventilator-induced breaths. This biofilm may become dislodged during suctioning, or repositioning of the endotracheal tube [1]. Impaired muco-ciliary clearance with mucosal injury and glottis dysfunction associated with prolonged intubation further aggravates the risk of VAP with re-intubation [2]. Daily interruption of sedative infusions in critically ill patients receiving mechanical ventilation decreases the duration of mechanical ventilation and reduces the length of intensive care unit (ICU) stay. Consequently, this practice can be considered worthy for reducing VAP risk and its occurrence [3,4].

Etiologic diagnosis of Ventilator-associated pneumonia is considered a microbiological emergency because of its impact on disease associated morbidity and mortality and antibiotic management, so rapid diagnostic information is clearly more beneficial to patients than more complete but delayed information. Multiplex-PCR is a universal technique making it possible to identify more than one micro-organism, from single patient' specimen [3,4].

The aim of this study was to identify the causative bacterial pathogens of VAP by both conventional microbiological cultures and multiplex reverse transcriptase reaction (m-PCR) methods with assessment of turnaround time for both diagnostic modalities together with their diagnostic accuracy.

2. Patients and methods

2.1. Study setting

This study was conducted in pediatric intensive care unit (PICU) of Ain Shams University Hospital, which is a multidisciplinary

Peer review under responsibility of Ain Shams University.
* Corresponding author.
E-mail address: mervatgmansour@gmail.com (M.G.E. Mansour).

https://doi.org/10.1016/j.ejmhg.2017.08.012
1110-8630/

medical ICU with 10 beds and average occupancy rate of 100% during time of the study.

2.2. Patients' data acquisition

Demographic variables such as gender, age, underlying disease together with the patients' clinical data, and degree of critical illness by Pediatric Logistic Organ Dysfunction (PELOD) score [5], length of PICU stay, duration of mechanical ventilation, and antibiotic regimen were collected from patients' records after getting the care 'caregivers' consent and the approval of ethical committee of Ain Shams University. The work has been carried out in accordance with the Code of the World Medical Association (Declaration of Helsinki) for experiments in humans. Data collection began within 24 h from the time of admission to the PICU.

2.3. Patients' enrollment

All admitted patients were observed daily for the diagnosis of VAP. The method of establishing the diagnosis of VAP remains controversial and no method has emerged as the gold standard. For these reasons, clinical guidelines are available to aid in decision making about acquisition of ventilator-associated pneumonia [5,6]. We used the Clinical Pulmonary Infection Score (CPIS) to help quantify clinical findings and represents a "weighted approach" to the clinical diagnosis of VAP. This scoring system includes both clinical and radiological factors that increase the likelihood of the presence of VAP. Point values are assigned to each criteria and a sum is calculated. Traditionally, a threshold score of more than six has been used to diagnose VAP (Table 1) [5,6].

2.4. Microbiological assessment

2.4.1. Clinical specimens

Endotracheal aspirate (ETA) was screened from enrolled patients. These specimens were tested against important and common bacterial pathogens by both bacterial culture and multiplex Polymerase Chain Reaction (m-PCR).

2.4.2. Bacterial cultures

Specimens were obtained and sent to the laboratory within one hour of collection. They were decontaminated and centrifuged before inoculation. Inoculation was on Blood agar and Chocolate agar for blood specimens and further on McConkey agar for ETA. Specimens were incubated for one week and observed for colonies every day in ETA and every 48 h in blood specimens. Colonies were identified by gram stain and biochemical profile. Infection was defined by semi-quantitative count of more than 10^5 CFU/ml. Antibiotic sensitivity test was done for positive isolates using disc diffusion method according to National Committee for clinical Laboratory standards (NCCLS) [7]. Isolated bacterial agents are *Streptococcus pneumoniae*, Methicillin-resistant *Staphylococcus* (MRSA), *Klebsiella pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Pseudomonas aerogenosa*, *Legionella pneumophilia* and *Acinetobacter*.

2.4.3. Multiplex PCR

Collected specimens were assessed for seven bacterial agents; that are considered serious causative pathogens for VAP worldwide. These organisms are sharing some physical properties during their processing for PCR (*Acinetobacter* was not involved in the panel because it has different incubation temperature than the selected primers for the other 7 organisms). *DNA extraction:* DNA was extracted from the samples using MagNA pure Compact Nucleic Acid Isolation Kit I (Cat. No. 03730964001); supplied by (Roche, Germany). *Amplification by PCR:* This was done by Light Cycler-DNA Amplification Kit SYBR Green I (Cat. No. 2015137). The kit used Light Cycler 2.0 System (Roche, Germany). *Primers:* Primers were non-labeled forward primers and biotin-labeled reverse primers with horseradish peroxidase – labeled probes. Primers for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Legionella pneumophilia*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were selected according to Kumar et al. [7]. Primers for *Klebsiella pneumoniae* were made according to Kurupati et al. [8]. Primers for *Pseudomonas aerogenosa* were chosen according to Qin et al. [9].

2.4.4. Turnaround time (TAT)

We calculated **brain to brain TAT (Total TAT)** as outlined by Lundberg including the total testing cycle as a series of nine steps: ordering, collection, identification, transportation, preparation, analysis, reporting, interpretation and taking a decision by the ordering physician. We used the term **Laboratory TAT** as the time elapsed from physician request of the laboratory test till a report is available by the laboratory [10].

2.5. Statistical analysis

Analysis of data was done by IBM computer using SPSS 12-USA. Description of quantitative variables is expressed as mean and standard deviation (SD). Description of qualitative variables is expressed as number and percentage. The Wilcoxon's signed-rank test compared the difference between culture and PCR in different study specimens. There is no gold standard for the diagnosis of VAP in pediatric patients; therefore, we used a Clinical Pulmonary Infection Score of NNIS (National Nosocomial Infections Surveillance) age-specific guidelines. Sensitivity and Specificity of m-PCR and bacterial culture were referred to CPIS as the clinical

Table 1
Modified Clinical Pulmonary Infection Score.

Measurement	Points		
	0	1	2
Temperature (°C)	36.5–38.4	38.5–38.9	≤36.4 or ≥39
Peripheral white blood cell count	4000–11,000	<4000 or >11,000 (>50% bands: add 1 extra point)	
Tracheal secretions	None	Nonpurulent	Purulent
Chest radiograph	No infiltrate	Diffuse or patchy infiltrate	Localized infiltrate
Progression of infiltrate from prior radiographs	None		Progression (acute respiratory distress syndrome or congestive heart failure thought unlikely)
Culture of endotracheal tube suction	No growth/light growth	Heavy growth (some bacteria on gram stain: add 1 extra point	
Oxygenation (Pao_2/fraction of inspired oxygen [Fio_2])	>240 or acute respiratory distress syndrome		≤240 and no acute respiratory distress syndrome

Adapted from Alicia et al. [5] and Swoboda et al. [6].

Please cite this article in press as: Mansour MGE, Albendary S. Egypt J Med Hum Genet (2017), https://doi.org/10.1016/j.ejmhg.2017.08.012