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ORIGINAL ARTICLE

Hospital-acquired pneumonia in critically ill children: Incidence, risk factors, outcome and diagnosis with insight on the novel diagnostic technique of multiplex polymerase chain reaction

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

[a] *Department of Pediatrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[b] *Department of Microbiology, Ain Shams University, Cairo, Egypt*

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Abstract Hospital-acquired pneumonia (HAP) is the most frequent hospital-acquired infection in critically ill patients. National Nosocomial Infections Surveillance (NNIS) system reported that HAP accounts for as much as 31% of all nosocomial infections acquired in medical intensive care units (ICU). The increasing incidence of infections caused by antibiotic-resistant pathogens contributes to a high mortality rate, longer ICU stay and higher costs. In this study, we aimed to identify the incidence of HAP, the associated risk factors, and its effect on outcome. We evaluated as well the usefulness of multiplex polymerase chain reaction (m-PCR) as a novel tool for emergency diagnosis of HAP.

We examined all consecutive admissions to Pediatric ICU from February 2010 to August 2010. Patients were diagnosed to have HAP when their Clinical Pulmonary Infection Score (CPIS) index was more than 6. Blood and endotracheal aspirate (ETA) were tested for bacterial pathogens by microbiological cultures and multiplex PCR simultaneously for all enrolled patients.

Twenty-five patients out of 90 admissions (27.7%) developed HAP during the observation period, with incidence rate of 13 per 1000 patient-days and overall mortality of 56%. Gastro-esophageal

* Corresponding author. Address: Abbas El-Akad Street, Nasr City, Cairo. Egypt. Tel.: +20 1000837437.
E-mail address: mervatgmansour@gmail.com (M.G.E. Mansour).

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reflux disease (GERD), mechanical ventilation (MV), endotracheal re-intubation and sedation were the main recorded risk factors for HAP. ETA had better diagnostic yield than blood specimens for the diagnosis of HAP. Multiplex-PCR showed better sensitivity and positive predictive value than bacterial culture for etiological diagnosis of HAP. *Acinetobacter* and *Klebsiella pneumoniae* were the most common identified pathogens. In conclusion, hospital-acquired pneumonia adversely affects patients outcome in our setting, for which we should manipulate the identified modifiable risk factors. Moreover, m-PCR permits simultaneous detection of several bacterial pathogens in a single reaction which can optimize the emergency diagnosis of HAP and can improve etiology-directed clinical management of bacterial pneumonia.

1. Introduction

Several reports published recently confirm that hospital-acquired pneumonia (HAP) remains to be a major medical problem in most European countries, and in the United States despite the advances in the quality of patient care, availability of effective antibiotics, complex technological diagnostic facilities and awareness in infection control measures [1]. Its occurrence represents additional cost, morbidity and most importantly, mortality among patients hospitalized initially for other reasons. The reported frequency varies with the definition, type of ICU, patients' population, and antibiotic policies [2].

Etiologic diagnosis of HAP 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, in less than one hour directly from patient specimens making it very specific and time-saving for diagnosis [3,4].

The Egyptian data on HAP are lacking. The aim of this study was to determine the incidence of hospital-acquired pneumonia, to identify risk factors associated with its development, and describe its effect on hospital stay and mortality in critically ill patients. Also in our study, we aimed to evaluate the usefulness of m-PCR as an emergency tool for the diagnosis of HAP compared to bacterial culture.

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 medical ICU with 10 beds and average occupancy rate of 80% during the time of the study.

2.2. Patients' data acquisition

Demographic variables such as gender, age, underlying disease together with the patients' clinical data, degree of critical illness by PELOD score [5], risk factors of pneumonia, length of PICU stay, duration of mechanical ventilation, and antibiotic regimen were collected from patients' records after getting the parents' consent and the approval of ethical committee. Data collection began within 24 h from the time of admission to the ICU and continued until the diagnosis of HAP was made or until the third day after the transfer out of the PICU.

2.3. Patients' enrollment

All admitted patients were observed daily for the diagnosis of HAP according to The Clinical Pulmonary Infection Score (CPIS). The method of establishing the diagnosis of HAP 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 hospital-acquired pneumonia. The Centers for Disease Control and Prevention and the National Healthcare Safety Network have developed

Box 1. Centers for Disease Control and Prevention criteria for nosocomial pneumonia (adult)

Radiology

Two or more serial chest radiographs[a] with at least one of the following:

- New or progressive and persistent infiltrate
- Consolidation
- Cavitation

Signs/symptoms/laboratory

At least one of the following:

- Fever (> 38 °C or > 100.4 °F) with no other recognized cause
- Leukopenia (< 4000 white blood cell count per microliter [WBC/μL] or leukocytosis (> 12,000 WBC/μl)
- For adults 70 years old or older, mental status changes with no other recognized cause

And at least two of the following:

- New onset of purulent sputum, or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements
- New-onset or worsening cough, or dyspnea, or tachycardia
- Rales or bronchial breath sounds
- Worsening gas exchange (PaO_2/fraction of inspired oxygen [FiO_2] ⩽240), increased oxygen requirements, or increased ventilation demand

[a]In patients with no underlying pulmonary or cardiac disease, one definitive radiograph is acceptable.

From Centers for Disease Control and Prevention. Available at: www.cdc.gov/ncidod/hop/nnis/members/pneumonia/final/pneumoniacriteriav1. Accessed May 1, 2008. Adapted from [6].

criteria for the diagnosis of nosocomial pneumonia, taking into account clinical factors, such as fever and leukocytosis, as well as radiological criteria, including persistent new findings on chest radiograph (Box 1) [6].

We used the Clinical Pulmonary Infection Score (CPIS) to help quantify clinical findings and represent a ''weighted approach'' to the clinical diagnosis of HAP. This scoring system includes both clinical and radiological factors that increase the likelihood of the presence of nosocomial pneumonia. 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 pneumonia (Table 1) [7].

2.4. Microbiological assessment

2.4.1. Clinical specimens

Both endotracheal aspirate (ETA) and blood samples were 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 disk diffusion method according to the National Committee for clinical Laboratory standards (NCCLS) Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disc susceptibility test: Approved standard-Ninth edition (200).

2.4.3. Multiplex PCR

Collected specimens were assessed for seven bacterial agents; that are considered serious causative pathogens for HAP 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 seven organisms). *DNA extraction:* DNA was extracted from the samples using MagNA pure Compact Nucleic Acid Isolation Kit I (Cat. No. o3730964001); 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, Chlamydiapneumoniae, Legionella pneumophilia, Staphylococcus aureus,* and *Streptococcus pneumoniae* were selected according to Kumar et al. [8]. Primers for *Klebsiella pneumoniae* were made according to Kurupati et al. [9]. Primers for *Pseudomonas aeruginosa* were chosen according to Qin et al. [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 or HAP in pediatric patients; therefore, we used a clinical definition of HAP based on 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 gold standard for the diagnosis of HAP (CPIS $\geqslant$ 6 is the cut-off for diagnosis of HAP) [7].

Sensitivity of culture or m-PCR represents the proportion of patients with HAP who were test positive. *Specificity* is

Table 1 Modified Clinical Pulmonary Infection Score.

Measurement	Points		
	0	1	2
Temperature (°C)	36.5–38.4	38.5–38.9	$\leqslant$36.4 or $\geqslant$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		$\leqslant$240 and no acute respiratory distress syndrome

Adapted from Swoboda et al. [7].

Table 2 Characteristics of patients and comparison of patients who developed and did not develop hospital-associated pneumonia (HAP).

Characteristics	All patients (n = 90)	Patients with HAP (n = 25)	Patients without HAP (n = 65)	p-Value
Patients-related characteristic				
Mean age ± SD, months	21 ± 18.7	22.5 ± 19.8	23.6 ± 18	0.61
Male sex, n (%)	44 (49%)	19 (76%)	25 (38%)	<0.01
Median PELOD Score (interquartile range)	24 (9–39)	25 (10–42)	23 (9–39)	0.71
Admission diagnosis, n (%)				
Pulmonary diseases	11 (12%)	3 (12%)	8 (13%)	0.96
Cardiac diseases	13 (14%)	3 (12%)	10 (15%)	0.69
Neurologic diseases	43 (48%)	13 (52%)	30 (46%)	0.61
Acute gastrointestinal infections	9 (1%)	3 (12%)	6 (9%)	0.69
Postoperative	14 (16%)	3 (12%)	11 (17%)	0.67
Outcome				
Length of PICU stay, days; mean ± SD	34 ± 47	44 ± 45	22 ± 34	<0.05
Length of MV, days; mean ± SD	30 ± 35	39 ± 43	22 ± 28	<0.01
Predicted mortality rate %, (range)	34% (1–99)	34% (1–90)	36% (2–98)	–
Actual mortality, n (%)	26 (29%)	11 (44%)	15 (23%)	0.04
Risk factors for HAP, n (%)				
Mechanical ventilation	32 (36%)	19 (76%)	13 (20%)	<0.01
Re-intubation	13 (14%)	9 (36%)	4 (6%)	<0.001
Sedation	26 (29%)	13 (52%)	13 (20%)	<0.01
Nasogastric feeding	32 (36%)	17 (68%)	15 (23%)	<0.001
Central venous Catheter	46 (51%)	11 (44%)	35 (54%)	0.41
Gastro-esophageal reflux (GERD)	43 (48%)	21 (84%)	22 (34%)	<0.001
Anti-reflux drugs (H_2 Blockers)	58 (64%)	23 (92%)	35 (54%)	<0.001
VAP*	–	19 (76%)	–	–
Non-VAP		6 (24%)		
Rate of HAP, n per 1000 patient-days	–	13	–	–

* VAP = ventilator-associated pneumonia.

the proportion of patients without HAP who were test negative. *Predictive value of a positive test* (*PPV*) is the proportion of patients with positive tests who have disease. *Predictive value of a negative test* (*NPV*) is the proportion of patients with negative tests who do not have disease.

3. Results

3.1. Patients' cohort characteristics

Ninety patients were admitted to the PICU during the study period with mean age 21 ± 18.7 months, and males represented 49% of them. Their median PELOD score was 24 with predicted mortality rate of 34%. Almost half of admitted cases were admitted due to neurological emergency (coma and acute flaccid paralysis). Twenty-five patients developed HAP after 72 h of admission according to CPIS score. Nineteen patients out of 25 had ventilator-associated pneumonia (VAP) (76%). The recorded rate of HAP during the period of study was 13 cases per 1000 patient-days. Forty-four percent of patients with HAP were less than 12 months, and 76% of them were males. The distribution of the primary cause of PICU admission did not differ significantly between patients who acquired HAP and those who did not (Table 2).

3.2. Hospital-acquired pneumonia and its risk factors

Univariate analysis of different risk factors for the development of HAP in our study revealed that mechanical ventilation (OR: 10; 95% CI: 3.2–30; $p < 0.001$), re-intubation (OR: 8.7; 95% CI: 2.3–31.5; $p < 0.001$), sedation by benzodiazipins and opioids (OR: 4.3; 95% CI: 1.6–11.7; $p < 0.01$), nasogastric feeding (OR: 7.1; 95% CI: 2.6–19.6; $p < 0.001$), gastro-esophageal reflux (OR: 10.3; 95% CI: 3.1–33.6; $p < 0.001$), and H_2 Blockers (OR: 9.6; 95% CI: 2.14–45.3; $p < 0.001$) were significant risk factors for acquiring HAP (Table 3).

3.3. Clinical outcomes of hospital-associated pneumonia

Length of PICU stay was doubled in patients who acquired HAP compared to patients without HAP ($p < 0.05$). Duration of mechanical ventilation was significantly longer in patients with HAP ($p < 0.01$). HAP lead to 10% increment in the actual mortality rate above the predicted rate for the affected patients with significant higher mortality than patients without HAP (Table 2).

3.4. Etiologic diagnosis of HAP by multiplex-PCR and bacterial culture

In our study, multiplex-PCR had a better diagnostic yield for HAP than bacterial culture (sensitivity 76%, specificity 97%, PPV 90%, NPV 93% vs. 24%, 92%, 55%, 79%, respectively, for culture).

Acinetobacter was the most frequent organism isolated by culture followed by *K. pneumoniae* and *P. aeruginosa* (Table 4). Multiplex-PCR significantly increased the diagnostic yield

Table 3 Risk factors for hospital-associated pneumonia.

Risk factor	Odds ratio (OR)	95% CI*	*p*-Value
Mechanical ventilation	10	3.2–30	< 0.001
Re-intubation	8.7	2.3–31.5	< 0.001
Sedation	4.3	1.6–11.7	< 0.01
Nasogastric feeding	7.1	2.6–19.6	< 0.001
Central venous catheter (CVC)	0.67	0.27–1.7	0.4
GERD	10.3	3.1–33.6	< 0.001
H_2 blockers	9.6	2.14–45.3	< 0.001

* 95% CI = 95% Confidence Interval.

Table 4 Summary of m-PCR and bacterial culture results in endotracheal aspirate (ETA) and blood samples.

Variable	ETA (n = 25)			Blood samples (n = 25)		
	m-PCR	Culture	*p*	m-PCR	Culture	*p*
Positive cases, *n* (%)	19 (76%)	6 (24%)	< 0.001	17 (68%)	2 (8%)	< 0.001
Streptococcus pneumoniae	2 (8%)	–	< 0.16	–	–	< 1.00
Methicillin-resistant *Staphylococcus* (MRSA)	6 (24%)	–	< 0.01	2 (8%)	–	< 0.16
Klebsiella pneumoniae	10 (40%)	5 (20%)	0.03	10 (40%)	1 (4%)	< 0.003
Mycoplasma pneumoniae	6 (24%)	–	< 0.01	5 (20%)	–	0.03
Chlamydia pneumoniae	1 (4%)	–	0.3	1 (4%)	–	0.32
Pseudomonas aeruginosa	–	2 (8%)	0.16	–	1 (4%)	0.32
Legionella pneumophilia	–	–		–	–	
Acinetobacter#	–	7 (28%)	–	–	4 (16%)	–
Acinetobacter# nd MRSA	–	–	–	–	1 (4%)	–
Klebsiella pneumoniae and *Pseudomonas aeruginosa*	–	1 (4%)	–	–	–	–

Acinetobacter is not involved in the m-PCR panel.

of MRSA, *K. pneumonia*, *Streptococcus pneumoniae* and *M. pneumoniae*. Polymicrobial pneumonia was diagnosed in two patients, where a combined growth of MRSA and *Acinetobacter* was present in one patient and combined growth of *P. aeruginosa* and *K.* pneumonia in another patient.

4. Discussion

Both developed and resource-poor countries are faced with the burden of health care-associated infections. In a World Health Organization (WHO) cooperative study involving 55 hospitals in 14 countries from four WHO regions, about 8.7% of hospitalized patients had nosocomial infections; among and represents the second most common nosocomial infection is HAP [11]. The frequency of hospital-acquired pneumonia in our PICU during study period was 13 per 1000 patient-days with associated mortality rate of 56% with predominance of Gram-negative pathogens; *K.* pneumoniae was diagnosed in 40%, *Acinetobacter* in 28% with MRSA in 24%. A 6-year surveillance study from 2002 to 2007 involving intensive care units in Latin America, Asia, Africa, and Europe, using Center for Disease Control's NNIS definitions, revealed higher rates of HAP and VAP than those of comparable United States ICUs. The survey also reported higher frequencies of mortalities associated with MRSA, Enterobacter species, and *P. aeruginosa* [12]. Patra et al. [13] reported that VAP constituted 76% of patients with hospital-acquired pneumonia and represented the most frequent nosocomial infection in intensive care units (80%) with an overall mortality of nosocomial pneumonia reaching 60%; all were secondary to Gram-negative infections with *Pseudomonas* contributing to 57.1% of deaths followed by *Klebsiella*, *Escherichia* coli and *Acinetobacter*. The increasing incidence of infections caused by antibiotic-resistant pathogens contributes to the emerging seriousness of these infections with expected higher mortality rate. Numerous studies have demonstrated that severe underlying illness predisposes patients in the ICU to the development of pneumonia and contribute to the associated high mortality rates [14].

In our study; the mean length of stay was doubled following HAP together with 50% increment in the mean duration of mechanical ventilation days. In another study, HAP lengthens the hospital stay by 7–9 days and is associated with a higher cost of medical care [14]. Fifty-two percent of enrolled patients with HAP in our study were admitted primarily to the PICU due to neurological emergencies (e.g. acute flaccid paralysis with rapid progression to involve respiratory muscles, status epileptics and coma). This group of patients had major neuro-critical dependency during their stay due to muscle paralysis. In Chiang Mai University Hospital, a tertiary care center, the most common indication for PICU admission was respiratory failure [15], while in a tertiary teaching hospital in southern Brazil, bronchiolitis was reported to be the most frequent cause for PICU admission (77.6%) [16]. It is noted in our study that 44% of patients acquired HAP were less than 12 months and this may be explained by the vulnerability of this age group to life-threatening diseases. Seventy-six percent of them were males while females represent 24%, which raised the question of whether males are more susceptible to progression of illness and more vulnerable to critical illness. The same finding was noted by a study done by Khaled Amro [17], in a referral hospital of South Jordan where 34.4% of enrolled patients

with nosocomial pneumonia were less than one year age. Boys represent 57% of total number of patients enrolled by Srinivasan et al. [18] in a tertiary pediatric care center. Another study done by Martinbiancho et al. [19] also recorded a higher incidence of HAP among (males 63%).

Many risk factors may favor or contribute to the development of nosocomial pneumonia. In our study, gastro-esophageal reflux disease (GERD) and treatment with H_2 Blockers, mechanical ventilation, nasogastric tube feeding, and use of benzodiazepines and opioids as sedative drugs, and re-intubation were significant risk factors for HAP. The primary route through which organisms enter the lower airways is via aspiration of colonized oropharyngeal secretions [19]. Micro-aspiration plays a central role in the pathogenesis of nosocomial pneumonia among critically ill patients especially those with nasogastric feeding and on mechanical ventilation (MV). The oropharynx of hospitalized patients becomes colonized with aerobic gram-negative bacteria within few days of admission. Therefore, nosocomial pneumonia is caused predominantly by the Gram-negative bacilli [20]. Within 12 h of 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 [21]. Impaired muco-ciliary clearance with mucosal injury and glottic dysfunction associated with prolonged intubation further aggravates the risk of VAP with re-intubation [12]. Daily interruption of sedative infusions in critically ill patients receiving mechanical ventilation decreases the duration of mechanical ventilation and reduces the length of ICU stay. Consequently, this practice can be considered worthy for reducing VAP risk and its occurrence [21–22].

The causative organisms vary according to the patients' medical condition, demographics, methods of diagnosis, the durations of hospital and ICU stays, the antibiotic policy and the prior exposure to antimicrobials. All these factors significantly influence the distribution patterns of etiologic agents from one ICU to another [23]. The most frequent organisms detected in our study were *K. pneumoniae* (40%), *Acinetobacter* (28%) and followed by *M. pneumoniae* (24%) and MRSA (24%), while the least common isolates were *Streptococcus pneumoniae* (8%) and *C. pneumoniae* (4%).

Acinetobacter baumannii is now recognized to be capable of causing life-threatening infections including pneumonia [24]. This appears to be due to their ability to survive on health-care workers' hands and environmental surfaces and their intrinsic resistance to many common antibiotics, rather than any intrinsic virulence factors [25]. Antibiotic therapy and critical illness can suppress the normal bacterial flora and lead to an overgrowth of Enterobacteriaceae like *K. pneumoniae* in the respiratory tracts. The most concerning is the acquisition of extended-spectrum β-lactamases that render the bacteria resistant to penicillin and cephalosporin antibiotics [26]. Extended spectrum β-lactamase (ESBL) producing *K. pneumoniae* may cause serious nosocomial infections especially in critically ill patients. Numerous outbreaks have been described with ICU-acquired ESBL-producing *K. pneumoniae* where infection increased from a baseline rate of 0.44 cases per 1000 patient-days to 6.86 cases per 1000 patient-days [26].

Streptococcus pneumoniae was not a common finding in our study because it predominates in the early days after intubation and is rapidly cleared after beginning antibiotic therapy as the isolates remain susceptible to achievable concentrations of traditional lactam antibiotics [27]. MRSA was detected as a causative agent for HAP in 24% of cases in our study by m-PCR. *Staphylococcus aureus* (both Methicillin sensitive and resistant strains), constitutes the most frequently isolated pathogen in the ICU. The incidence of MRSA as a cause of VAP was 12–15%, but increased to approximately 30% in patients with prolonged mechanical ventilation and prior antibiotic therapy [28]. Methicillin resistance in *Staphylococcus aureus* has the particularity to add multiple antimicrobial resistances up to 80% of macrolide resistance and 90% of quinolone resistance [29]. Those strains with mecA are resistant to all commercially available β-lactams, with considerable country-to-country variability [29]. Traditionally, we are not used to find *M. pneumoniae* in our bacteriological cultures as a causative pathogen of HAP, but in this study it was detected in 24% of patients and diagnosed only by m-PCR. Since that, we considered *M. pneumoniae* in the etiologic diagnosis of HAP in critically ill patients who develop nosocomial pneumonia within the first week after intubation [12,28,29], and our therapeutic regimen should include these findings. Altogether with VAP task forces, the emergence of resistance is a concern for intensive care specialists worldwide thus it is imperative for investigators from different countries and regions to exchange precise and updated epidemiological data on the encountered HAP and VAP.

In our study, m-PCR optimized the diagnosis and management of hospital-acquired pneumonia over bacterial culture. The use of PCR leads to an increase in diagnostic sensitivity, especially in micro-organisms that cannot be easily cultured and in case of a low burden of micro-organisms with previous antibiotic therapy [30]. This is in agreement with Strålin et al. [31], who recommended multiplex PCR to be a useful etiological diagnostic tool in lower respiratory tract infection patients, particularly in those treated with antibiotics. The same was suggested early by Hendolin et al. [32] where multiplex PCR method improved the rapid and simultaneous detection rate of four bacterial pathogens in upper respiratory tract infections significantly compared to that of the conventional culture method.

5. Conclusion

Our study is one of the few prospective observational studies in critically ill children describing the risk factors and outcomes of hospital-acquired pneumonia. We found that gastro-esophageal reflux, treatment with H_2 blockers, mechanical ventilation, nasogastric feeding, use of sedative drugs and re-intubation are significant risk factors for HAP in our setting. These findings need to be confirmed in larger, multicenter studies to clarify risk factors and the impact of prevention strategies. Our study shows that HAP negatively impacts clinical and economic outcomes in critically ill pediatric patients by prolonging the length of mechanical ventilation and ICU stay and may increase total hospital charges and absolute hospital mortality rate. Moreover, m-PCR optimized the emergency diagnosis of hospital-acquired pneumonia over bacterial culture, especially in micro-organisms that cannot be easily cultured. Though the bacterial pattern of HAP in our study may differ from other countries, it is imperative for investigators from different regions to exchange precise and updated epidemiological data about HAP concerns.

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References

[1] Bartlett JG. Hospital-acquired pneumonia: the Merck Manual for healthcare professionals. 2008. <http://www.merck.com/mmpe/sec05/ch052/ch052c.html>.
[2] Alp E, Güven M, Yıldız O, Aygen B, Voss A, Doganay M. Incidence, risk factors and mortality of nosocomial pneumonia in Intensive Care Units: a prospective study. Ann Clin Microbiol Antimicrob 2004;3:17.
[3] Dodek P, Keenan S, Cook D, Heyland D, Jacka M, Hand L. For the Canadian Critical Care Trials Group and the Canadian Critical Care Society. Evidence-based clinical practice guideline for the prevention of ventilator-associated pneumonia. Ann Intern Med 2004;141:305–13.
[4] Lisboa T, Rello J. Diagnosis of ventilator-associated pneumonia: is there a gold standard and a simple approach? Curr Opin Infect Dis 2008;21(2):174–8.
[5] Leteurtre S, Martinot A, Duhamel A, et al. Validation of the pediatric logistic organ dysfunction (PELOD) score: prospective, observational, multicentre study. Lancet 2003;362:192–7.
[6] Alicia NK, Pamela AL. Hospital-acquired pneumonia: pathophysiology, diagnosis, and treatment. Surg Clin North Am 2009;89:439–61.
[7] Swoboda SM, Dixon T, Lipsett PA. Can the clinical pulmonary infection score impact ICU antibiotic days? Surg Infect (Larchmt) 2006;7:331–9.
[8] Kumar S, Wang L, Fan J, Kraft A, Bose ME, Tiwari S, et al. Detection of 11 common viral and bacterial pathogens causing community-acquired pneumonia or sepsis in asymptomatic patients by using a multiplex reverse transcription-PCR assay with manual (enzyme hybridization) or automated (electronic microarray) detection. J Clin Microbiol 2008;46(9):3063–72.
[9] Kurupati P, Chow C, Kumarasinghe G, Poh CL. Rapid detection of *Klebsiella pneumoniae* from blood culture bottles by real-time PCR. J Clin Microbiol 2004;42(3):1337–40.
[10] Qin X, Emerson J, Stapp J, Stapp L, Abe P, Burns JL. Use of real-time PCR with multiple targets to identify *Pseudomonas aeruginosa* and other non-fermenting Gram-negative bacilli from patients with cystic fibrosis. J Clin Microbiol 2003;1(9):4312–7.
[11] Rosenthal VD, Maki DG, Mehta A, Alvarez-Moreno C, Leblebicioglu H, Higuera F. International Nosocomial Infection Control Consortium report, data summary for 2002–2007. Am J Infect Control 2008;36(9):627–37.
[12] Chawla R. Epidemiology, etiology, and diagnosis of hospital-acquired pneumonia and ventilator-associated pneumonia in Asian countries. Am J Infect Control 2008;36(4):S93–S100.
[13] Patra PK, Jayashree M, Singhi S, Ray P, Saxena AK. Nosocomial pneumonia in a pediatric intensive care unit. Indian Pediatr 2007;44:511–8.
[14] Apfalter P, Stoiser B, Barousch W, Nehr M, Kramer L, Burgmann H. Community-acquired bacteria frequently detected by means of quantitative polymerase chain reaction in nosocomial early-onset ventilator-associated pneumonia. Crit Care Med 2005;33(7):1492–8.
[15] Osenthal VD, Maki DG, Mehta A, Alvarez-Moreno C, Leblebicioglu H, Higuera F. International Nosocomial Infection Control Consortium report, data summary for 2002–2007, issued January 2008. Am J Infect Control 2008;36(9):627–37.
[16] Nithiwathanapong C, Reungrongrat S, Ukarapol N. Prevalence and risk factors of stress-induced gastrointestinal bleeding in critically ill children. World J Gastroenterol 2005;11(43):6839–42.
[17] Khaled Amro. Re-intubation increases ventilator-associated pneumonia in pediatric intensive care unit patients. RMJ 2008;33(2):145–9.
[18] Srinivasan R, Asselin J, Gildengorin G, Kronish JW, Flori HR. A prospective study of ventilator-associated pneumonia in children. Pediatrics 2009;123(4):1108–15.
[19] Martinbiancho JK, Carvalho PRA, Trotta EDA, Schweiger AP, Rau R, Moreira LB. Evidence of safety of chloral hydrate for prolonged sedation in PICU in a tertiary teaching hospital in southern Brazil. Eur J Clin Pharmacol 2009;65(12):1253–8.
[20] Cunha BA. Legionnaires' disease: clinical differentiation from typical and other atypical pneumonias. Infect Dis Clin North Am 2010;24(1):73–105.
[21] Sharma H, Singh D, Pooni P, Mohan U. A study of profile of ventilator-associated pneumonia in children in Punjab. J Trop Pediatr 2009;55(6):393–5.
[22] Augustyn B. Ventilator-associated pneumonia risk factors and prevention. Crit Care Nurse 2007;27:32–9.
[23] Sierra R, Benítez E, León C, Rello J. Prevention and diagnosis of ventilator-associated pneumonia. A survey on current practices in southern Spanish ICUs. Chest 2005;128:1667–73.
[24] Alp E, Voss A. Ventilator associated pneumonia and infection control. Ann Clin Microbiol Antimicrob 2006;5(7).
[25] Lone R, Shah A, Kadri SM, Lone S, Faisal S. Nosocomial multi-drug-resistant acinetobacter infections – clinical findings, risk factors and demographic characteristics. Bangladesh J Med Microbiol 2009;3(1):34–8.
[26] Laurent C, Rodriguez-Villalobos H, Rost F, Strale H, Vincent JL, Deplano A, et al. Intensive care unit outbreak of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* controlled by cohorting patients and reinforcing infection control measures. Infect Control Hosp Epidemiol 2008;29:517–52.
[27] Park DR. The microbiology of ventilator-associated pneumonia. Respir Care 2005;50(6):742–63.
[28] Weigelt J, Itani K, Stevens D, Lau W, Dryden M, Knirsch C. Linezolid versus vancomycin in treatment of complicated skin and soft tissue infections. Antimicrob Agents Chemother 2005;49:2260–6.
[29] Torres A, Ewig S, Lode H, Carlet J. Defining, treating and preventing hospital acquired pneumonia: European perspective. Intensive Care Med 2009;35:9–29.
[30] American Thoracic Society Documents. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 2005;171:388–416.
[31] Strålin K, Korsgaard J, Olcén P. Evaluation of a multiplex PCR for bacterial pathogens applied to bronchoalveolar lavage. ERJ 2006;28(3):568–75.
[32] Hendolin PH, Markkanen A, Ylikoski J, Wahlfors J. Use of multiplex PCR for simultaneous detection of four bacterial species in middle ear effusions. J Clin Microbiol 1997;35(11):2854–8.