Original article

A survey on microorganisms and their sensitivity by E-Test in ventilator-associated pneumonia at Toxicological-Intensive Care Unit of Loghman-Hakim Hospital

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Abstract. *Introduction:* Ventilator associated pneumonia (VAP) is the most common nosocomial infection at ICUs, with high mortality and morbidity. The diagnostic method for VAP is based on the combination of clinical, radiological, and microbiological criteria. Lower respiratory tract culture results are useful to confirm the etiology of VAP and adjusted antibiotics. Endotracheal aspiration (EA) is the simplest noninvasive technique for performing lower respiratory tract culture, with high sensitivity and moderately high specificity. The aim of this survey was to evaluate the quantitative cultures of endotracheal aspirates in VAP patients and the sensitivity patterns of microorganisms through E-test. *Method:* Among 582 ICU admitted patients who were under mechanical ventilation for more than 48 hours, 72 suspected patients of VAP were prospectively evaluated during a 10 month period. Evaluation of our ICU standards by APACHE III scoring, and GCS were carried out on the first day of admission in all patients. Quantitative cultures of EA were performed on all 72 patients. Antibiotic resistance pattern of isolated pathogens was defined by E-test. *Results:* VAP was confirmed in 46 out of 72 cases (50, 69.4% males and 22, 30.6% females - mean age was 33±12 years) through quantitative cultures of EA samples. The probable incidence of VAP was 7.9% (per ventilated patients ≥48 hours). The mean APACHE III score was 31.28±16. GCS in most of the patients was between 8 and 12. Staphylococcus aureus was the most frequently isolated organism (58.7%), with high sensitivity to Amikacin, Ciprofloxacin, and Teicoplanin (>92%); Pseudomonas aeruginosa was the second most frequent organism (17.4 percent); Acinetobacter isolates were potentially drug resistant, and only Amikacin was effective. *Conclusion:* Tracheal aspirates in combination with clinical findings show important roles in the management of VAP and decrease inappropriate antimicrobial therapy. S. aureus is the main agent leading to VAP in the TICU of the Loghman Hakim Hospital. (www.actabiomedica.it)

Key words: ventilator associated pneumonia, endotracheal aspiration, micro organisms, E-test

Introduction

Ventilator associated pneumonia (VAP) is the most common nosocomial infection in Intensive Care Units (ICU), and results in high mortality and morbidity. It is defined as parenchymal lung infection that occurs after the first 48 hours of mechanical ventilation (1-2).

In different investigations, the incidence of VAP varies widely from 7 to 70 percent according to the

studied population, the definition, and the type of hospital or ICU (3-9).

No "gold-standard "diagnostic method for VAP is available, but according to the center for disease and control (CDC) definition it is based on the combination of clinical, radiological, and microbiological criteria (1).

In order to decrease mortality rate, initial empiric antimicrobial therapy should be started when VAP is suspected. Lower respiratory tract culture results are useful in confirming the etiology of VAP and adjusting antibiotics. It is notable that changing therapy based on the culture results leads to a reduced consumption of antibiotics (10). The methods of lower respiratory tract culture included endotracheal aspirate, bronchoalveolar lavage, or protected specimen brush (11).

Endotracheal aspiration (EA) is the simplest noninvasive technique. EA cultures have high sensitivity and moderately high specificity.

Despite newer bronchoscopic methods for diagnosing VAP, many physicians continue to use EA in diagnosing VAP (12).

Patients' demographics in the ICU, methods of diagnosis, antibiotic policy, and duration of hospital and ICU stay are the reasons for causative organism variations in VAP.

In the National Nosocomial Infection Surveillance System (NNIS) report, Staphylococcus aureus (S. aureus) was the most frequent organism (1).

The aim of this survey was to evaluate the quantitative cultures of endotracheal aspirates in VAP patients and the sensitivity patterns of micro organisms through E-test.

Materials and methods

Study Design and protocol

This prospective study was performed during a 9 month period from May 2007 to February 2008, in the Toxicological intensive care unit (TICU) at the Loghman Hakim Hospital Poison Center (LHHPC) - a unique referral care center of poisoning in Tehran/ Iran. This center estimated nearly 20000 poisoned patients every year. Daily turn over in this center is 80-100 patients.

The study protocol with code number 48-1.20.2008 was approved by the research ethics committee of the Shaheed Beheshty University, M.SC.

During the study period, patients under mechanical ventilation were enrolled based on the following inclusion criteria: age older than 17 years, at least 48 hours of mechanical ventilation with the clinical suspicion of ventilator-associated pneumonia. Patients with AIDS, lung cancer, COPD, and patients who received antibiotics before 24 hours prior to admission were excluded.

The mean Acute Physiology and Chronic Health Evaluation III (APACHE III) score was calculated on the first day of admission in all patients. This standardized evaluation, included 4 criteria: Age/Chronic health evaluation, Acid-base, Vital signs/laboratory, and neurological abnormalities.

Patients were regularly followed by an infectious disease specialist and a well trained ICU nurse. Age, sex, mental status by GCS, and type of poisoning were collected at baseline. Endotracheal aspirate samples were performed on the basis of the standard procedure described in the specimen collection section, in each patient.

Diagnosis of VAP

For the diagnosis of VAP, we checked for persistent or progressive radiographical infiltration and at least two of the following criteria: 1) temperature higher than 38˚C or lower than 35˚C; 2) leukocyte count higher than 10000/μL or lower than 4000/μL; 3) presence of new purulent respiratory secretion or any changes in sputum; 4) positive blood cultures or pleural effusion cultures; 5) detection of râles or dullness on chest examination; 6) at least 10% decrease in arterial PO_2 (13).

Specimen collection and microbiological processing

All patients underwent non-protected endotracheal aspiration (NPEA) with a 12 F suction catheter gently guided through the endotracheal tube for approximately 24 cm. Gentle aspiration was then performed without instilling saline. After the catheter

was withdrawn, approximately 2-5 ml saline was injected with a sterile syringe to flush the exudate into a sterile container for collection and the specimens were immediately sent to the laboratory for microbiological processing.

All specimens were mechanically liquefied and homogenized by mixing with vortex for 1 minute and then centrifuged for 10 minutes. All samples were gram stained for the assessment of the type of putative bacteria and the evaluation of intracellularity and serially diluted in 0.9% sterile saline solution with final dilution of 10^{-2}, 10^{-3} and 10^{-4}. Specimens were then plotted on 5% sheep blood agar and MacConkey agar. The plates were incubated over night at 37°C. After preliminary characterization of the isolated bacteria by gram stain and colony morphology, species identification was carried out and evaluation of antimicrobial susceptibility by E-Test method (AB BIODISK, Sweden/HIMEDIA, India) for the 10 antibiotics most commonly used in treatment of ventilator-associated pneumonia was performed.

The assessed antibiotics were as follows: Meropenem, Vancomycin, Amikacin, Ceftriaxone, Clindamycin, Teicoplanin, Ciprofloxacin, Cefepime and piperacillin/Tazobactam.

Statistical analysis

The statistical package for social sciences (SPSS version 11.5) was used to perform statistical calculations. According to the study criteria, data of the patients were analyzed through appropriate statistical tests, such as χ^2 and t-test. The alpha level of significance was set at 0.05. The diagnostic threshold for NPTA was 10^5 cfu/mL. Detection of ≥5% of neutrophils or macrophages with intracellular organisms on a gram stain of a smear of centrifuged specimen was also diagnostic of VAP.

Results

Among 582 ICU admitted patients under mechanical ventilation for more than 48 hours, 72 suspected patients of VAP 50, 69.4%, males and 22, 30.6%, females; mean age 33 years, range, 13-78, SD=12) were prospectively evaluated during a 10 month period. The incidence of VAP was 8%.

GCS in most of the patients (n=50, 70%) was between 8 and 12, seven patients were in deep coma, and in the others (20%) it was above 13.

APACHE III score is 0-299, and our scoring was 31.28±16.

The reasons for ICU admission were poisoning with antidepressant tablets in 30 cases (41.2%), sedative tablets in 18 cases (25%), opioids in 18 cases (25%), organ phosphorus toxins in 3 cases (4.8%), analgesic tablets in 2 cases (2.7%), and antihypertensive tablets in 1 case (1.3%).

Paraclinical findings

WBC count in 45 patients (63.9%) was reported as 12000/μL to 25000/μL, and left shift was detected in 27 cases (37.5%).

Forty six out of 72 tracheal cultures were positive.

Fifteen specimens revealed intracellular bacteria in ≥5% of neutrophils and macrophages on gram stain.

The analysis of the quantitative culture for the 46 patients is summarized in Table 1. The most frequently isolated organism was S. aureus and Pseudomonas aeruginosa (p. aeruginosa).

Early onset VAP (≤5 days) was reported in 43 patients, and late onset VAP (>5 days) in 3 patients.

S. aureus was detected in late onset VAPs.

The patterns of pathogen susceptibility to antimicrobials and protocol of the antimicrobials prescription in our ICU may be seen in table 2 and 3.

Most of the VAP cases (78.3%) were cured, eight patients (17.4 %) died and two patients were admitted to the private general hospital.

Table 1. Microorganisms that were detected in quantitative cultures

Microorganism	No. (%)
Staphylococcus aureus	27 (58.7)
Pseudomonas aeruginosa	8 (17.4)
Klebsiella pneumoniae	5 (10.9)
Sterptococcus pneumoniae	4 (8.7)
Acinetobacter	2 (4.3)
Total	46 (100)

Table 2. The pattern of pathogen susceptibility to antimicrobials

Microorganisms / Antibiotic	Acinetobacter (%)			Sterptococcus pneumoniae (%)			Klebsiella (%)			Pseudomonas aeruginosa (%)			Staphylococcus aureus (%)		
	Res	Inter	Sen	Res	Inter	Sen	Res	Inter	Sen	Res	Inter	Sen	Res	Inter	Sen
Amikacin	100	-	-	100	-	-	100	-	-	87.5	-	12.5	96.3	3.7	-
Cefepime	-	-	100	-	25	75	20	40	40	25	-	75	59.3	-	40.7
Cefotaxime	-	-	100	75	-	25	60	-	40	25	-	75	88.9	3.7	7.4
Ceftazidime	-	50	50	50	25	25	40	20	40	87.5	-	12.5	22.2	40.7	37
Ceftriaxone	-	-	100	100	-	-	60	-	40	12.5	-	87.5	88.9	3.7	7.4
Ciprofloxacin	50	-	50	75	-	25	80	-	20	62.5	-	37.5	96.3	-	3.7
Clindamycin	-	-	100	50	50	-	20	20	60	-	-	100	88.9	-	11.1
Imipenem	-	-	100	100	-	-	60	20	20	37.5	-	62.5	40.7	3.7	55.6
Teicoplanin	×	×	×	×	×	×	-	-	100	-	-	100	92.3	-	7.7
Piperacillin/Tazobactam	-	-	100	100	-	-	100	-	-	50	-	50	85.2	3.7	11.1
Vancomycin	-	-	100	75	-	25	40	-	60	-	-	100	88.9	11.1	-

Res: Resistance, Inter: Intermediate, Sen: Sensitive, ×: Not available

Table 3. Protocol of the antimicrobial prescription in Loghman Hakim Hospital TICU

Antibiotic	Patients with NPTA and negative tracheal culture n (%)	Patients with NPTA and positive tracheal culture n (%)
Meropenem+Vancomycin+amykacin	11 (42.3)	33 (71.7)
Ceftiaxone+Clindamycin	8 (30.8)	4 (8.7)
Teicoplanin	0	3 (6.5)
Ciprofloxacin+Meropenem+Vankomycin	1 (3.8)	2 (4.3)
Cefepime+Clindamycin	6 (23.1)	4 (8.7)
Total	26 (100)	46 (100)

Discussion

VAP in ICUs causes poor outcome and morbidity with a mortality rate ranging from 25-50 percent. Therefore early diagnosis and empiric antibiotic therapy is necessary. It is estimated that late diagnosis has been associated with increased mortality for more resistant microorganisms (14-16).

According to our study, the incidence of VAP was 8 % (per ventilated patients), while the incidence density is reported to range from 13 to 51 per 1000 ventilator days (1, 17).

The European Prevalence of Infection in the Intensive Care study, showed that VAP was the most frequent infection acquired in the ICU (about 45% of all infections in European ICUs) (18).

This difference is due to the type of ICU, reason for admission (over dose toxicity) and faster turn over of patients.

With reference to the type of our TICU, the mean age was 33.3±12; this young adult patient age is completely predictable, in comparison with other studies which showed a mean age higher than 45 years (20-22).

Gender variety in our study was statistically significant, 50 (69.4%) males/22(30.6%) females ($p<0.005$). According to the published data this difference was prominent (22).

The mean APACHE III score was 31.28±16.

Most studies used the APACHE II for their ICU evaluation: it takes into account two groups of patiens: VAP and non VAP patients. According to Shalini et al,

APACHE II in VAP cases was 13.6±6, and 11.0±5 in the non VAP group (19).

Aybar Türko lu showed no difference between VAP and non VAP patients. In another study, the same data were reported (19 VAP cases and 20 non VAP cases) (13, 20).

Patient mental status according to GCS score was 8 to 12 in 70% and lower than 8 in 10%.

On the contrary, Erbay reported a GCS <9 in most cases (62.2%) (21).

The EA method with intracellular technique showed a high sensitivity which is compatible with other procedures such as BAL and PSB, but with different specificity (12, 23).

In our study the most frequent isolated microorganisms were S. aureus (58.7%) and P. aeruginosa (17.4%). It is notable that P. aeruginosa (the most predominant microorganism in most ICUs), in our ICU was detected in only 8 patients and Acinetobacter was detected in only 2 cases (12, 24-26).

The responsible pathogens for VAP are different and depend on the duration of mechanical ventilation, prior antibiotics exposure , severity of the disease, underlying diseases and the length of ICU stay. In recent years, resistant microorganisms have developed in different types of ICUs (10, 24-26).

The use of broad spectrum antibiotics in our ICU is one of the major problems due to the short period of stay.

Erbay et al reported that the most frequent isolated microorganisms were methicillin resistant S.aureus (30.4%), P. aeruginosa (21.4%), and Acinetobacter (12.5%).

In our study S. aureus was predominant in drug and opioid users (25%) (27).

In most published articles gram negative bacteria have been reported as the major responsible pathogen (10, 19, 24-26).

In table 2 S. aureus is shown as sensitive to Amikacin and Ciprofloxacin (96.3%), and Teicoplanin 92.3%. Cefepime (as forth generation cephalosporin) (59.3%) and in others like Vancomycin, Ceftriaxone, cefotaxim and Clindamycin, this sensitivity was 89%. Michel R. showed that Staph was sensitive to Oxacillin in 52% (28).

P. aeroginosa as second pathogen was sensitive to Amikacin and Ceftazidim (87.5%) while for two stronger antibiotics like Piperacillin/Tazobactam was 50% and Ciprofloxacin 62.5% but in one of anti VAP agent like Imipenem it was more than 60% resistant. Similarly, in Erben et al reports, Amikacin was 84% effective, but on the contrary they found that P. aeroginosa was 62% resistant to Ciprofloxacin, 50% to Ceftazidime and 32% to Imipenem (10).

Rajasekhar et al reported that pseudomonas was completely resistant to Imipenem, Meropenem, Cefoperazone and sulbactum (29).

The rate of Imipenem resistance of P. aeroginosa ranges from 14.3% to 80% among different ICUs (30).

In the current study, Acinetobacter was the most resistant isolated pathogen, and showed a 100% sensitivity to Amikacin and a 50% sensitivity to Ciprofloxacin.

Rajasekhar et al reported that Acinetobacter was resistant to Piperacillin Tazobactum, Timentin, Gentamicin, Ampicillin, Imipenem, Meropenem, and sensitive to Cefoperazone sulbactum, Imipenem, and Meropenem (29).

According to recent surveys, Acinetobacter are the most important pathogens, due to their resistance to commonly used antimicrobial agents. In recent years, resistance to Carbapenems (one of the most active agents against Acinetobacter) has increased (10, 26).

In our experience, all isolated K. pneumoniae were susceptible to Amikacin, Tazocine and 80% to Ciprofloxacin, wich is compatible to what was reported by Erben et al.

K. pneumoniae has a 40-60% sensitivity to Cefotaxime, Ceftazidime, Ceftriaxone, Vancomycin and Imipenem and no response to Targocide. On the contrary, Erben et al reported that only 25% of K. pneumoniae were sensitive to Ceftazidime and all of them were susceptible to Imipenem (10).

According to Rajasekhar's findings, Imipenem, Meropenem, Cefoperazone sulbactum were not effective, but Ampicillin, Quinolones, Ticarcillin were 100% effective.

We can conclud that Tracheal aspirates in combination with clinical findings have an important role in the management of VAP and decrease inappropriate

antimicrobial agent therapy. S. aureus is the main agent of VAPs in the TICU of the Loghman Hakim Hospital.

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