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Role of bacteriological investigation of endotracheal aspirate in diagnosis of ventilator-associated pneumonia

Enas A. Daef [1], Entsar H. Ahmed[1*], M.Sh. Badawy[2], Hend M. Omar[3], Hamdy A. Mohamdien[3] and Esam A. Moktar[4]

[1]Department of Microbiology and Immunology, Assiut University, Egypt.
[2]Department of Chest, South Valley University, Egypt.
[3]Department of Chest, Sohag University, Egypt.
[4]Department of Cardiothoracic, Sohag University, Egypt.

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Ventilator associated pneumonia (VAP) is a common complication of ventilatory support for patients with acute respiratory failure, currently related to high mortality rate. Therefore, this complication of mechanical ventilation requires a prompt diagnosis and adequate antibiotic treatment. The study aimed to investigate the role of endotracheal aspirate (ETA) surveillance cultures in identifying the aetiology of VAP earlier. The study was conducted over a period of 12 months and included 152 patients under mechanical ventilation for >48 h from different ICUs of Assiut University Hospitals. Quantitative cultures of ETA at threshold of 10^5cfu/ml were performed. The organisms were primarily identified by colony morphology, microscopy of Gram stain and standard biochemical tests. The antibiotic resistance pattern of the isolated bacteria was determined by Kirby-Bauer disk diffusion method. VAP was suspected in 92/152 patients (60.53%). Microbiological support for VAP was obtained by ETA in 90 patients. Positive cultures occurred in 88 patients, the infection was polymicrobial in 50 (54.34%) of cultures. Major isolated pathogenic bacteria were gram negative (54.79%); *Klebsiella sp*ecies was the commonest organism (23.29 %). Gram positive bacteria were detected in 42.47% of the cultures; methicillin resistant *Staphylococcus aureus* (MRSA) was the predominant organism (24.40%). Gram negative bacteria showed high resistance to penicillins, cephalosporins and quinolones, the least resistance was to imipenem. Mortality was higher in VAP group (47.8%) than non VAP (30%). It is indicated that quantitative cultures of ETA is a useful method for early diagnosis of VAP with subsequent proper selection of adequate therapy.

Key words: Endotracheal aspirate, quantitative cultures, ventilator-associated pneumonia.

INTRODUCTION

Ventilator associated pneumonia (VAP) is defined as infection of lung parenchyma which develops 48 h or more after mechanical ventilation and not present or incubating at the time of initiation of mechanical

ventilation (Ali et al., 2015).

VAP is associated with prolonged mechanical ventilation and increased hospital costs; mortality ranges from 17 to 50% (Yang et al., 2009). Inadequate antibiotic treatment has led to an increase in the incidence of the multi-drug resistant (MDR) strains of pathogen (American Thoracic Society, ATS 2005; Golia, 2013). Early appropriate antibiotic therapy is associated with better outcomes including reduction in mortality (Muscedere et al., 2012). The diagnosis of VAP remains challenging and there is a lack of diagnostic standardization. One of the major stumbling blocks to improving diagnosis of VAP is that there is no diagnostic gold for diagnostic techniques against which to compare (Guidelines for the management of adult with VAP, 2005).

This has paved the way for less invasive tests such as endotracheal aspirates (ETA) and quantitative ETA cultures with a threshold of 10^5 to 10^6 bacteria per milliliter of exudates that is considered as optimal for the microbiological confirmation of VAP (Nair et al., 2008). Routine endotracheal aspirate cultures of critically ill patients in intensive care units (ICUs) may be predictive of patients who are at high risk of invasive disease, and may guide the selection of appropriate empirical therapy based on the predominant pathogens identified in these cultures in the event of the development of VAP (Joseph et al., 2010). An ETA sample is more readily obtainable from mechanically ventilated patients, and is more frequently a component of microbiological surveillance (Brusselaers et al., 2013).

The American thoracic society (ATS) guidelines recommended quantitative/ semi quantitative culture of ETA or bronchoscopic aspirates from the infected lung segments for the diagnosis of VAP (Rajasekhar et al., 2006). The majority of VAP guidelines recommend the use of ETA or bronchoalveolar lavage fluid (BALF) analysis to diagnose VAP. These guidelines thereby suggest that the results of ETA and BALF analysis are in accordance (Muscedere et al., 2008; Raoof and Baumann, 2014).

The present study is undertaken to bacteriologically confirm the clinical diagnosis of VAP using quantitative culture of endotracheal aspirate.

MATERIALS AND METHODS

A prospective, single-center, observational, clinical study enrolling 152 mechanically ventilated patients selected from different intensive care units (ICUs) of Assiut University Hospitals, Egypt; including medical ICU, respiratory ICU and trauma ICU over a 12 months period from July 2013 to July 2014. The study was approved by faculty research ethics committee. Informed written consent was obtained from a close relative of all subjects.

Study population

Inclusion criteria

Patients were eligible for the study if placed on mechanical ventilation (MV) for ≥48 h.

Exclusion criteria

All patients with clinical and radiological signs suggestive of pneumonia or acute respiratory distress syndrome (ARDS) secondary to pneumonia on admission were excluded. For each patient, the following data are collected: age, gender, admission diagnosis, date and duration of mechanical ventilation.

The enrolled patients were carefully followed up for signs of VAP. This included apart from clinical examination, regular recording of body temperature, observance of tracheal aspirate appearance, leukocyte count and chest radiograph. The diagnosis of VAP was based on the American College of Chest Physicians criteria and was defined as the occurance of new or progressive pulmonary infilterates on chest X-rays along with the presence of at least two of the following criteria: (a) fever ≥38°C, (b) leukocytosis ≥ 10,000 cells/mm^3, or leukopenia≤4,000 cells/mm^3 (c), purulent tracheal secretions (AST, 2005). Patients with clinical suspicion of VAP (based on the above criteria) underwent endotracheal aspiration (ETA).

Procedure of ETA

Endotracheal aspirate (≥1 ml) was collected under aseptic precaution after 48 h of intubation whenever patient was suspected to have developed VAP. The ETA was collected using a 22-inch, 12 F suction catheters, which was gently introduced through the endotracheal tube for a distance of approximately 25 to 26 cm. Chest vibration or percussion for 10 min was used to increase the retrieved volume (1 ml) in case the patient produced very little secretions. Only one ETA sample was collected from each patient and was immediately taken to the laboratory for processing. The aspirate specimens showing the presence of <10 squamous epithelial cells per low power field or organisms seen under oil immersion in the entire field on Gram stain by direct microscopy were included in the study (Forbes et al.,2007).

Method of quantitative analysis

Quantitative analysis of ETA was done according to gram stain smear interpretation. Depending on the number of organisms seen on direct smear, the clinical sample was diluted in 1 in 100 or 1 in 1000 and subsequently 10 µl of diluted sample was uniformly inoculated on to blood agar, chocolate agar and McConkey agar. If no organism was seen on direct smear, an undiluted sample was inoculated on the agar plates. After overnight incubation, the

*Corresponding author. E-mail: entsarhamed1973@yahoo.com.

Table 1. Monomicrobial and polymicrobial growth in VAP.

Type of infection	Number of percentage (%)
Monomicrobial	38 (41.30)
Polymicrobial	50 (54.34)

number of colonies were counted on each plate and multiplied by the appropriate dilution factor to express the colony count as cfu/ml. Samples with large mucus plugs were liquefied and homogenized by vortexing for one minute with glass beads followed by centrifuging at 3000 rotations per minute (rpm) for 10 min. The cfu/ml considered as significant helps discriminate colonization from infection, with thresholds of $>10^5$ cfu /ml being suggestive of infection rather than colonization (Nair et al., 2008). Organisms were primarily identified by colony morphology, microscopy of Gram stain and standard biochemical tests (Harvey et al., 2012). The antibiotic resistance patterns of the isolated microorganisms were determined by Kirby-Bauer disk diffusion method using commercially available discs (HiMedia, Mumbai, India) and interpreted as recommended by Clinical Laboratory Standard Institute (CLSI, 2010). Resistance to three or more classes of antibiotics was recorded as multidrug resistance (MDR).

Statistical analysis

Date entry and analysis were done using Statistical Package for Social Science Statistical Package for Social Science version 14 (SPSS). Results were presented as number, percentage, mean, standard deviation for quantitave variables and number (percentage) for qualitative variables. Difference in proportion was assessed by using Chi-square test. P. value was considered statistically significant when $P < 0.05$.

RESULTS

During the 12 months study period, 152 mechanically ventilated patients for > 48 h were enrolled. Their ages ranges from 18 to 79 years with a mean age of 54.15±15 years,100 (65.79%) were males and 52 (34.21%) were females. VAP was diagnosed clinically and rardiologically in 92 (60.53%) patients. Quantitative culture of ETA was done in 90 patients and positive culture occurred in 88 patients yielding 146 isolates. Fifty (54.34%) of the positive cultures were polymicrobial and 38(41.30%) were monomicrobial (Table 1). Major pathogenic isolated bacteria were gram negative (54.79%), among this group *Klebsiella* species,was the predominant pathogen (23.29%) followed by *Escherichia coli* (*E. coli*) (8.22%),then *Acinetobacter* and *Proteus* species (6.85%) for each. Gram positive bacteria were detected in (42.47%) of the cultures, methicillin resistant *Staphylococcus aureus* (MRSA) was the major organism (27.4%) (Table 2).

Gram-positive isolates in the present study were highly resistant to penicillins, cephalosporins and macrolides while lower resistance was detected to chloramphenicol, tetracyclines, linezolid and vancomycin (Table 3). Gram negative bacteria showed high resistance to many groups of antimicrobials as penicillins (except for *E.coli* that showed 0% resistance to piperacillin –tazobactam), cephalosporins and quinolones (50-100%), and the least resistance was reported to imipenem and meropenem (0 to 50%) (Table 4). Mortlity rate was higher in VAP group (47.8%) than non VAP group (30%) (Table 5).

DISCUSSION

VAP is a common complication of mechanical ventilation (MV), with a significant mortality rate, especially when associated with potentially antibiotic-resistant microorganisms (Melsen et al., 2009).

VAP is one of the most important causes of mortality in patients treated with invasive mechanical ventilation (IMV) in intensive care unit (ICU). Microbiological examinations are required as clinical and radiological findings are usually insufficient in the diagnosis (Gurgun et al., 2013). The microbiological diagnosis of VAP can be reached by invasive methods, such as fiberoptic bronchoscopic protected specimen brush (PSB) and brochoalveolar lavage (BAL), or by non-invasive methods, such as endotracheal aspiration (EA). The latter methods can be readily performed, being also cost effective and less invasive. Ideally, both specimens can be quantitavely cultured, aiming at reducing inappropriate treatment and the selection of multi resistant organisms (Corrêa et al., 2014). Quantitative EA culture is a useful noninvasive tool for the diagnosis of pneumonia pathogens in critically ill patients.

Additionally, the results of quantitative EA cultures were comparable to the results of using invasive methods and were helpful in limiting the prescription of broad-spectrum antibiotics (Yagmurdur et al., 2016).

This study was conducted from July 2013 to July 2014 and included 152 mechanically ventilated adult patients for≥ 48h admitted to different ICUs in Assuit University Hospitals.

VAP was suspected clinically and radiologically in 92 patients (60.53%). This percentage is higher than the reported by Francois et al., 2013 as VAP was detected in 9 to 40 % of intubated patients. VAP was confirmed by quantitative culture of ETA which was done in 90 patients and yield positive result in 88 patients giving 146 isolates as the infection was polymicrobial in almost 54% of VAP patients. In a study by Ahmed et al. (2014) among 48 VAP cases 32 (66.67%) were monomicrbial and 16 (33.33%) were polymicrobial. Charles et al. (2013) reported that 72.2% of VAP patients had monomicrobial and 27.8% had polymicrobial infection.

Gram negative organisms had been recovered in 80 (54.79%) isolates while gram positive organisms were

Table 2. Aetiological agents of VAP.

Individual Isolated organism	Number of percentage (%)
Total No. of isolates	146
Gram negative organisms	80 (54.79)
Klebsiella *sp.*	34 (23.29)
E. coli	12 (8.22)
Acinetobacter *sp.*	10 (6.85)
Proteus *sp.*	10 (6.85)
Heamophilus *sp.*	4 (2.74)
Pseudomonas *sp.*	4 (2.74)
Burkholderia *sp.*	2 (1.37)
Enterobacter	4 (2.74)
Gram positive organims	62 (42.47)
MRSA	40 (27.40)
MSSA	12 (8.22)
Enterococci	10 (6.85)
Candida *sp.*	4 (2.74)

MRSA: methicillin resistant *S. aureus;* MSSA:methicillin sensitive *S. aureus.*

Table 3. Antibiotic susceptibility pattern of Gram positive bacteria.

Variable	Percentage of resistant gram positive bacteria		
Antibiotics	MRSA	MSSA	Enterococci
Penicillins			
Penicillin G	100	100	100
Extended penicillins			
Amoxicillin	100	100	100
Ampicillin	97.6	100	100
Cloxacillin	97.4	40	100
Pencillins+beta lactam inhibitors			
Amoxicillin/clavulonic acid	100	100	80
Cephalosporins			
Cefazoline	89.6	66.7	100
Ceftriaxone	100	100	77
Quinolones			
Ciprofloxacin	97.1	66.7	100
Lomifloxacin	92.5	66.7	100
Norfloxacin	90	50	91.7
Aminoglycosides			
Amikacin	64.7	100	33.3
Gentamycin	100	83.3	81.8
Glycopeptides			
Vancomycin	33.3	10.9	40
Ticoplanin	66.7	38.8	43.8

Table 3. Contd.

Lincosamides			
Clindamycin	74.5	66.7	100
Macrolides			
Azithromycin	85	100	100
Erythromycin	73.3	66.7	84.6
Others			
Chloramphenicol	44.4	33.3	46.2
Tetracyclin	67.3	66.7	58.3
Oxytetracyclin	100	80	66.7
Trimethoprim/Sulphamethoxazole	100	55	66.7
Linezolid	11.8	0	33.3

MRSA: methicillin resistant *S. aureus;* MSSA:methicillin sensitive *S. aureus.*

responsible for 42.47% of the isolates. In agreement with the current study**,** Arabi et al. (2008) reported the range for gram negative bacilli and gram positive cocci as 41 to 92% and 9 to 52%, respectively. In this work, *Klebsiella sp.* was the most common gram negative bacteria (23.29%), followed by *E. coli* (8.22%). This was in accordance with Lagamayo (2008) who reported that both organisms have been responsible for nosocomial outbreaks of infection.

Acinetobacter sp. was detected in 6.85% of pathogens. This is in agreement with the world wide surveillance system that reported *Acinetobacter baumannii* to cause 7% of isolates in hospital acquired pneumonia and VAP (Jones, 2010**).** Nosocomial *A. baumannii infection* is commonly acquired through cross-transmission because of its propensity to survive in the hospital environment. Data from published studies have shown that *A. baumannii* can survive for long periods of time on inanimate surfaces (Borer et al., 2005). Daef et al. (2014) recorded an outbreak caused by 51 strains of multi-drug resistant *Acinetobacter baumannii*. Mokhless et al. (2010) used ETA as a diagnostic sample but he reported different frequencies for causative organisms; *Acinetobacter sp.* 51.5%, *P. aeruginosa* 18.2% and *Klebsiella sp.* 15.1%.

MRSA was identified in 27.40% of isolates. This is in agreement with Lee et al. (2013) who identified MRSA (27.9%) in a multicenter study. The microbiological spectrum in this study was in accordance to that had been reached by Daef and Elsherbiny **(**2012) in which the most frequently isolated microorganisms were gram negative bacteria (54.2%) amongest which, *Klebsiella sp.* was the most common while gram positive bacteria accounted for 45.8% with MRSA being the predominant (23.6%). C*andida sp.* was detected in 2.74% of isolates. In many studies of VAP in developing countries *Candida sp.* accounted for 0 to 7% of VAP episodes (Arabi et al., 2008).

The present study showed that gram negative bacteria had high resistance to many groups of antimicrobials as penicillins (except for *E.coli* that had 0% of resistance to piperacillin –tazobactam), cephalosporins and quinolones (50 to 100%). In agreement with this, Ashour and El-Sharif (2009)**,** reported high resistance to many groups of antibiotics in Egypt.

Extended spectrum beta-lactamases (ESBLs) are defined as enzymes produced by certain bacteria that are able to hydrolyze extended spectrum cephalosporin. They are therefore effective against beta-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime and oxyiminomonobactam (Ghafourian et al., 2015). In recent years, ESBL production in *Enterobacteriaceae*, particularly *Escherichia coli*, has significantly increased in several countries (Eckert et al**.,** 2004**).** Regarding the current study, all gram negative isolated pathogens had high resistance to cefotaxime, ceftazidime and ceftriaxone. These results suggest a high prevalence of extended spectrum B lactamase producing strains. Similar results found by Mukhopadhya et al. (2010) as all the enterobacterial isolates in their study were ESBL producing.

Looking at individual organisms, *Acinetobacter s*howed 100% resistance to cephalosporins but much lower resistance to imipenem (42.9%) while all isolates were sensitive to tigecyclin and azithromycin (0 % resistance). Varun et al., 2012 found that (100%) of isolates of *Acinetobacter baumannii* were multidrug resistant (MDR) that is, resistant to three or more class of antibiotics.

E. coli resistance to cephalosporins ranged from 66 to 100% and to quinolones from 75 to 80%, this is in accordance with Karlowsky et al. (2006) who reported that *E. coli* isolates showed high resistance to

Table 4. Antibiotic susceptibility pattern of Gram negative bacteria.

Antibiotics	Percentage of resistant gram negative bacteria				
	Klebsiella sp.	***E.coli***	**Acinetobacter sp.**	**Enterobacter**	**Pseudomonas *sp.***
Extended penicillins					
Ampicillin	100	100	100	100	100
Piperacillin / Tazobactam	80	0	70	75	75
Penicillins+ beta lactam inhibitors					
Amoxicillin/calvulonic acid	100	93.3	64.3	100	76.2
Cephalosporins					
Cefazoline	98.4	100	100	83.3	100
Cefaclor	98.1	100	100	100	100
Cefoperazone	90.9	92.9	100	100	93.3
Ceftriaxone	86.7	88.9	100	80	84.6
Cefotaxtime	80	50	65	80	100
Ceftazidime	100	70	100	75	100
Cefixime	100	66	100	50	100
Cefopodoxime	100	66.7	100	100	60
Quinolones					
Lomifloxacin	74.2	80	90	83.3	72.2
Ciprofloxacin	67.9	76.9	90	83.3	72.2
Norfloxacin	80	80	85.7	83.3	81.3
Levofloxacin	58.1	75	64.3	80	70
Glycopeptides					
Amikacin	47.2	46.7	45.5	50	65
Macrolides					
Azithromycin	66.7	100	0	40	100
Others					
Imipenem	19	0	42.9	50	24
Tobramycin	81	87.5	90	80	77.8
Aztreonam	91.3	100	71.4	80	76.2
Chloramphenicol	61.6	45.5	83.3	0	72.7
Nalidixic Acid	73.5	66.7	100	100	85.7
Oxytetracyclin	90.2	100	71.4	100	100
Trimethoprim/ Sulphamethoxazole	85.7	75	66.7	83.3	90
Tigecyclin	48.1	100	0	40	66.7
Meropenem	23.7	0	50	0	32

Table 5. Outcomes of the study population.

Variable	VAP No. (92)	Non (VAP) No. (60)	
Death	44 (47.8%)	18 (30%)	*P* value 0.03*
Discharge	48 (52.2%)	42 (70%)	

*P < 0.05 is statistically significant.

cephalosporins and quinolones.Pseudomonas *sp.* was resistant to piperacillin- tazobactam (75%), ceftazidime (100%), ciprofloxacin (72.2%), amikacin (65%) and imipenem (24%). In comparison, Varun et al. (2012) reported the following resistance rates for *Pseudomonas*, piperacillin-tazobactam (23.53%), ceftazidime (35.29%), ciprofloxacin and amikacin (82.35%) and imipenem (47.06%).

Gram-positive isolates in the present study were highly resistant to penicillins, cephalosporins and quinolones. These antibiotics are commonly prescribed empirically in the ICUs. Lower resistance was detected to chloramphenicol and tetracyclines which may reflect the reduction in their use. Some studies noticed a positive correlation between resistance rates of hospital isolates and the utilization rates of ciprofloxacin, cephalosporin, carbapenems, piperacillin/tazobactam, or all of these (Willemsen et al., 2009). Resistance of gram positive organisms to macrolides (azithromycin and erythromycin) was 50 to 100%. Ahmed et al. (2011) reported the resistance of gram positive bacteria to macrolides were 64.3 and 66.4%.

The high rates of antimicrobial resistance identified in the present study is similar to what has been found by Daef and Elsherbiny (2012). They reported gram negative bacteria with high resistance (50 to 100%) to many groups of antimicrobials, as penicillins, cephalosporins, quinolones and aminoglycosides. Mortality was higher in VAP patients (47.8%) than non VAP and this is in agreement with Gupta et al. (2011) who reported mortality in 46.67% of VAP patients.

Conclusion

VAP is a common and serious hospital aquired infection. The bacteriological approach for the management of VAP helps choosing the appropriate antibiotics. This study showed that quantitatve culture of ETA is helpful for early diagnosis and management of VAP.

Conflicts of interest

The authors have not declared any conflict of interest

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