LETTERS TO THE EDITOR

Detection and Quantitation of the Etiologic Agents of Ventilator-Associated Pneumonia in Endotracheal Tube Aspirates From Patients in Iran

TO THE EDITOR—Pneumonia is the second most common nosocomial infection, accounting for approximately 18% of such infections. The mortality rate associated with nosocomial pneumonia can be decreased from 91.6% to approximately 30.5% if appropriate treatment is instituted. Critically ill patients who require mechanical ventilation support are at especially high risk of developing ventilator-associated pneumonia (VAP), which has an estimated annual incidence of 1%-25% among hospitalized patients. Longer hospital stay, mortality, and morbidity are negative outcomes associated with VAP. The risk of pneumonia has been reported to be 6.5% among patients who receive ventilatory support for 10 days; this rate increases to 28% among patients who receive ventilatory support for 30 days.[1-3]

The most common noninvasive sampling technique for the diagnosis of VAP is endotracheal aspiration. Two invasive sampling methods are also common: protected-specimen brush sampling and bronchoalveolar lavage. Endotracheal aspiration is less expensive, compared with bronchoalveolar lavage or protected-specimen brush sampling. The threshold currently used for the diagnosis of pneumonia is 10^4 to 10^5 colony-forming units (cfu) of a pathogen per milliliter. This study sought to determine the different types and prevalences of organisms responsible for nosocomial VAP at our hospital in Iran, quantify the number of organisms isolated from endotracheal aspiration samples, and clarify the isolates' susceptibility patterns to common antibiotics.

All patients included in the study had a length hospital stay of more than 2 weeks. These patients had been transferred to the intensive care unit for different reasons and had undergone tracheotomy because they had difficulty breathing. The research was performed in Milad Hospital (Tehran, Iran). Milad Hospital has 1,000 beds, 120 of which are in the intensive care units. A total of 249 endotracheal aspiration samples were collected during 4 months from July 2001 to November 2002.

Quantitative culture of endotracheal aspiration samples was performed for patients who received mechanical ventilatory support for more than 72 hours. The quantitative culture samples were obtained using a J05029 nonreusable sputum collector (China Medical University). Endotracheal tubes were inserted 24-30 cm into the patient's airway. No suction was used while inserting the tubes, and aspirate specimens were obtained without saline flushing. The samples obtained were sent to the microbiology laboratory for analysis.[4]

After obtaining endotracheal aspiration samples with the mucus collector, sterile isotonic sodium chloride solution was added to make a final volume of 20 mL. Serial dilutions of the endotracheal aspiration samples were prepared in isotonic sodium chloride solution (10^{-1}, 10^{-2}, and 10^{-3}). Then 0.1 mL samples of each dilution was inoculated onto 3 culture media (chocolate, MacConkey, and blood agar). All cultures were incubated at 37°C under aerobic conditions for 48 hours. All isolated microorganisms were identified by biochemical and differential tests. The culture results were semiquantified as "light growth" (10^2 cfu/mL), "moderate growth" (10^3 cfu/mL), and "heavy growth" (10^5 cfu/mL). The 10^5cfu/mL measurement was used a threshold to distinguish colonization from true infection.[4] Antimicrobial susceptibility testing of all isolates was performed by the disk diffusion method, according to National Committee for Clinical Laboratory Standards recommendations.[5]

A total of 335 isolates were obtained from 249 samples, which means that more than 1 species of microorganism was isolated from 46 specimens (18.4%). Each patient contributed 1 sample to the study. Of the 249 patients, 135 (54.2%) were female and 114 (45.8%) were male. The mean age of the patients hospitalized in the intensive care unit was 60.3 years (range, 8-99 years).

Of the 249 specimens, 38 (15.3%) yielded light growth on culture, 65 (26.1%) yielded moderate growth, and 146 (58.6%) yielded heavy growth. There was evidence of VAP in 95 patients (65.1%) in the group whose cultures yielded heavy growth.

Gram-negative bacilli accounted for more than 75% of isolates. Predominant microorganisms were *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter* species. *Staphylococcus aureus* was the most predominant gram-positive bacterium isolated (Table).

More than 45% of *S. aureus* isolates were resistant to ox-

TABLE. Relative Prevalences of the Predominant Microorganisms Isolated From Endotracheal Tube Aspiration Samples From Study Patients

Microorganism	No. (%) of isolates (n = 335)
Klebsiella pneumoniae	67 (20.0)
Pseudomonas aeruginosa	62 (18.5)
Acinetobacter species	60 (17.9)
Staphylococcus aureus	51 (15.2)
Serratia marescens	32 (9.5)
Escherichia coli	22 (6.6)
Enterobacter species	10 (3.0)
Candida albicans	5 (1.5)
Others	26 (7.8)

acillin, but all *S. aureus* isolates were susceptible to vancomycin. The prevalences of resistance to other antimicrobials were as follows: penicillin, 97% of isolates; erythromycin, 9.5%; gentamicin, 9%; tetracycline, 40%; clindamycin, 5%; trimethoprim-sulfamethoxazole, 17.5%; chloramphenicol, 11%; and ciprofloxacin, 1%. Among *K. pneumoniae* isolates, 24% were resistant to ciprofloxacin, but all isolates were resistant to ampicillin. All isolates of *Acinetobacter* species were resistant to ceftazidime, cefixime, and cefazolin. However, all isolates were susceptible to imipenem. The prevalences of resistance to other agents were as follows: ciprofloxacin, 65% of isolates; gentamicin, 76.5%; amikacin, 82.5%; trimethoprim-sulfamethoxazole, 85.7%; and ofloxacin, 87.5%. Thirty percent of *P. aeruginosa* isolates were resistant to imipenem, and 60% were resistant to ciprofloxacin and gentamicin. All isolates of *K. pneumoniae* were resistant to ampicillin, amikacin, ceftriaxone, and ceftazidime; 29% were resistant to imipenem, 73% were resistant to ciprofloxacin, and 83% were resistant to gentamicin.

In our study, gram-negative bacilli, including *K . pneumoniae, Acinetobacter* species, and *P. aeruginosa,* were the colonizing organisms most frequently detected in tracheal tube aspirates, and accounted for 20%, 18.5%, and 17.9% of all isolates, respectively. *S. aureus,* which accounted for 15.5% of all isolates, was the predominant gram-positive organism.

Only a few studies from the Middle East have examined methods of detecting VAP. In a study by Kanafani et al.[6] from a medical center in Beirut, Lebanon, the incidence of VAP was 47%. Gram-negative bacilli accounted for more than 83% of all isolates. In our study, gram-negative bacilli accounted for more than 72% of colonizing isolates recovered from tracheal tube aspirates. In another study by Albert et al.[7] from Germany, gram-negative bacilli accounted for 85% of isolates. The investigators used a cutoff value of 10^5 cfu/mL for differentiate between tracheobronchial colonization and infection. In contrast to these studies, a US study by Babcock et al.[8] found that *S. aureus* (28.4% of isolates) was the predominant organism, followed by *P. aeruginosa.* It is believed that the differences in the etiologic agents of VAP found in different studies are the result of differences in the population of intensive care unit patients, duration of hospital stay, and prior antimicrobial therapy.[2,9] We conclude that gram-negative bacilli are the predominant colonizing microorganisms in tracheal tubes of hospitalized patients in our region. In addition, many of the isolated strains were resistant to commonly used antibiotics.

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Infection Control for Emerging Infectious Diseases in Developing Countries and Resource-Limited Settings

TO THE EDITOR—The articles by Srinivasan et al.[1] and Fung et al.[2] published in the journal provide preparedness and response plans for severe acute respiratory syndrome (SARS) and other emerging infectious diseases in healthcare facilities. We outline 4 practical issues relevant to the adoption and modification of these well-outlined recommendations for groups and institutions in developing countries and resource-limited settings.

Healthcare administration support. The protection of healthcare workers (HCWs) in developing countries is largely neglected in the establishment of national healthcare priorities. However, these countries should not delay the implementation of effective infection control strategies while we await more evidence-based data from such settings. Given the global experience with the SARS outbreaks that occurred both in designated "SARS" hospitals and in "non-SARS" hos-