Microbial Profile and their Susceptibility Pattern in Ventilator Associated Pneumonia in a Tertiary Care Hospital

B. Shanthi, R. Selvi*, V. Sheeba and P. Ponnammal

Stanley Medical College, Chennai – 600001, Tamil Nadu, India

*Corresponding author

Ventilator Associated Pneumonia (VAP) is the most common intensive care unit (ICU) acquired nosocomial infection and it is considered as the second most common hospital acquired infection associated with higher mortality and morbidity. The aim of this study was to determine the microbial profile of pathogens causing VAP and their antibiotic susceptibility patterns over a period of five years in the intensive care unit (ICU) of a tertiary care hospital. Cross-sectional, descriptive study was done on patients who were on mechanical ventilation for more than 48 hours and clinically suspected of having pneumonia for the five consecutive years 2012 to 2016. During the study period significant growth of pathogens were found in 216 / 581 patients. 90.27% were monomicrobial 9.72% were polymicrobial. Pseudomonas aeruginosa was the most commonly isolated gram-negative bacteria 69 / 206 (33.49 %) followed by Klebsiella species 59 (28.64%). An increase in resistance was shown by Pseudomonas aeruginosa and Acinetobacter spp. 58.49 % of Klebsiella spp and 52.00 % of Escherichia coli were ESBL producers. Staphylococcus aureus was the most commonly isolated gram positive bacteria (24), 18 (75%) were Methicillin resistant (MRSA). Good management strategies for VAP like adequate infection control practices include hand washing by hospital personnel, basic cleaning of all surface levels, increased barrier precautions, early accurate diagnosis and more specific antimicrobial use may significantly improve patients’ outcome.

Keywords
Ventilator Associated Pneumonia (VAP), Intensive care unit, Antibiotic resistance

Introduction

Ventilator Associated Pneumonia (VAP) is the most common intensive care unit (ICU) acquired nosocomial infection that develops when a patient is on mechanical ventilation for more than 48 hours and it is considered as the second most common hospital acquired infection associated with higher mortality and morbidity (Kalanuria et al., 2014; American Thoracic Society and Infectious Diseases Society of America, 2005).

The estimated prevalence of nosocomial pneumonia in intensive care units ranges from 10-65% with mortality rates of 13 to 55% (Kollef and Schuster, 1994; Pawar et al., 2003).

The etiologic agents widely differ according to the population of patients in an intensive care unit, duration of hospital stay, and prior antimicrobial therapy (Chastre and Fagon, 2002). Multidrug resistant pathogens such as Pseudomonas spp, Acinetobacter spp and
**Staphylococcus aureus** were the common organism causing Ventilator Associated Pneumonia.

Antimicrobial resistance is an increasing threat in hospitalized patients, and inappropriate empirical antimicrobial therapy is known to adversely affect outcomes in ventilator-associated pneumonia (Hsueh et al., 2005; Rhomberg et al., 2004). Therefore, it is necessary to evaluate antimicrobial usage, incidence, etiology and antimicrobial resistance trends for prominent nosocomial pathogens causing ventilator associated pneumonia in an intensive care unit (ICU).

Hence, the aim of this study was to determine the microbial profile of pathogens causing VAP and their antibiotic susceptibility patterns over a period of five years in the intensive care unit (ICU) of a tertiary care hospital.

**Materials and Methods**

Cross-sectional, descriptive study was done on patients who were on mechanical ventilation for more than 48 hours and clinically suspected of having pneumonia for the five consecutive years 2012 to 2016. The total number of patients included in this study were 581.

For diagnosis of VAP, a colony count of \( \geq 10^5 \) colony forming units (cfu) / ml was considered significant (Ioanas et al., 2001). Any growth below this was assumed as colonization or contamination. Quantitative culture of the endotracheal aspirates was performed and organism isolated was identified based on standard microbiological techniques.

Antimicrobial susceptibility testing was performed on Mueller Hinton agar using Kirby-Bauer disk diffusion method (CLSI, 2012) and Zone diameter was measured and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Ampicillin, Ciprofloxacin, Cefotaxime, Gentamicin, Amikacin, and Imipenem were tested for Enterobacteriaceae. Amikacin, Gentamicin, Ceftazidime, Ciprofloxacin and Imipenem were tested for *Pseudomonas* spp. and *Acinetobacter* spp. Penicillin, Erythromycin, Cefotaxime, Ciprofloxacin, Amikacin and Vancomycin (MIC) were tested for *S. aureus*.

Isolates showing zone diameter of \( \leq 22 \) mm for Cefotaxime and \( \leq 17 \) mm for Ceftazidime were considered as screening test for ESBL producers according to CLSI guidelines and were confirmed by double disk synergy test. Combination disk method using both Cefotaxime and Ceftazidime alone and in combination with clavulanic acid was performed for detection of Extended Spectrum Beta Lactamase (ESBL) among the members of Enterobacteriaceae. Five mm or more increase in zone of inhibition for either Cefotaxime-clavulanic acid or Ceftazidime-clavulanic acid disk compared to the Cefotaxime or Ceftazidime disk respectively was taken as confirmatory evidence of ESBL production.

Cefoxitin (30 μg) disc was used as a surrogate marker for determining methicillin resistance among the staphylococci. ATCC strains of Escherichia coli ATCC 25922, *Staphylococcus aureus* (MSSA) ATCC 25923, MRSA ATCC 33591 and *Pseudomonas aeruginosa* ATCC 27853 strains were used as quality control.

**Results and Discussion**

A total number of 581 patients were included in our study. During the study period significant growth of pathogens were found in
216 / 581 patients. 44/112 (39.28 %) in 2012, 43/114 (37.71%) in 2013, 42/106 (39.62 %) in 2014, 44/127 (34.64 %) in 2015 and 43/122 (35.24 %) in 2016.

Two hundred and thirty seven isolates were identified from 216 VAP patients. 90.27% were monomicrobial 9.72% were polymicrobial. Among 237 bacteria, 206 (86.91%) were gram-negative bacteria. In all the five years *Pseudomonas aeruginosa* was the most commonly isolated gram-negative bacteria 69 /206 (33.49 %) isolates followed by *Klebsiella* species 59 (28.64%), *Acinetobacter* species 26 (12.62 %), *Escherichia coli* 21 (10.19%) *Proteus* species 16 (7.76%), and *Citrobacter* species 15(7.28%).

An increase in resistance was shown by *Pseudomonas aeruginosa* for Gentamycin, Ciprofloxacin and Ceftazidime ranging from 40 % in 2012 to 72.72 % in 2016. Amikacin ranges from 26.66 % in 2012 to 38.46 % in 2015, in 2016 it was 36.36 %. For Imipenem 13.13 % in 2012 to 27.27 %.in 2016.

For *Acinetobacter* spp resistance remains high for most of the antimicrobials ranging from 50 % to 100 % except for Imipenem (16.66 % to 40%).

2012 to 2016 almost all Enterobacteriaceae isolates were resistant to Ampicillin and Gentamycin (50 % - 100 %).

*Klebsiella* spp was totally resistant to Ampicillin (100 %) and showed increased resistance to Gentamycin, Ciprofloxacin and Cefotaxime (50.00 % - 77.73 %).

*Escherichia coli* and *Proteus* spp showed high resistant to Gentamycin, Cefotaxime and Ampicillin (50 % to 100%). All the *Citrobacter* spp were almost resistant to Amikacin, Gentamycin, Ciprofloxacin, Cefotaxime and Ampicillin (50 to 100%).

58.49 % of *Klebsiella* spp and 52.00 % of Escherichia coli were ESBL producers.

*Staphylococcus aureus* was the most commonly isolated gram positive bacteria (24), 18 (75%) were Methicillin resistant (MRSA). Forty percent of the CONS (Coagulase-negative *Staphylococcus aureus*) were resistant to Cefotaxime, Ciprofloxacin and Erythromycin and all the isolates were resistant to Penicilllin. However, all the gram positive cocci were sensitive to Vancomycin.

Fungal isolates were 16, *Candida* species12 and *Aspergillus* species 4.

Ventilator associated pneumonia (VAP) is a major problem and it is one of the most frequently encountered hospital acquired infection in the ICU.

The microbial profile of pathogens causing VAP may differ between hospitals and ICUs. Therefore, surveillance of bacterial susceptibility should be conducted and local epidemiological data should be provided for every ICU.

This information can help in guiding the initial empiric antibiotic therapy, which would be useful in decreasing mortality and preventing development of MDR bacteria (Iregui *et al.*, 2002; Leroy *et al.*, 2003; Clec’h *et al.*, 2004).

In our study initial 3 years the VAP rate was high (39.28 %, 37.71% & 39.62%) compared to the last 2 years (34.64 % & 35.24 %).

Gram negative bacilli were the most common agents responsible for VAP and it is accounted for 86.91 % of the causative agents. Similar results were shown by (Fugon *et al.*, 1989) who reported an incidence of 75 % of gram negative bacilli and (Smsek *et al.*, 2001) who reported an incidence of 72 % of gram negative bacilli.
Distribution of pathogen (2012-2016)

Distribution of VAP pathogen (2012-2016)

| Organism                        | 2012 | 2013 | 2014 | 2015 | 2016 |
|---------------------------------|------|------|------|------|------|
| **GRAM NEGATIVE BACILLI**       |      |      |      |      |      |
| Non-fermentors                  |      |      |      |      |      |
| *Pseudomonas aeruginosa*        | 15   | 14   | 16   | 13   | 11   |
| *Acinetobacter spp*             | 6    | 5    | 4    | 6    | 5    |
| Fermentors - Enterobacteriaceae |      |      |      |      |      |
| *Klebsiella spp*                | 14   | 12   | 13   | 9    | 11   |
| *E. coli*                       | 2    | 5    | 4    | 6    | 4    |
| *Proteus spp*                   | 3    | 4    | 2    | 3    | 4    |
| *Citrobacter spp*               | 4    | 1    | 2    | 3    | 5    |
| **GRAM POSITIVE BACTERIA**      |      |      |      |      |      |
| Coagulase positive-*Staphylococcus aureus* | 4   | 6    | 6    | 5    | 3    |
| Coagulase-negative *Staphylococcus* | 0  | 2    | 1    | 2    | 2    |
| **Total**                       | 48   | 49   | 48   | 47   | 45   |

Antimicrobial Resistance of Non Fermenters

| Antimicrobial agents | Year | AMIKACIN 30 µg | GENTAMYCIN 10 µg | CIPROFLOXACIN 5 µg | CEFTAZIDIME 30 µg | IMIPENEM 10 µg |
|---------------------|------|----------------|------------------|--------------------|-------------------|----------------|
| *Pseudomonas aeruginosa* | 2012 | 26.66          | 53.53            | 40.00              | 53.53             | 13.13          |
|                     | 2013 | 35.71          | 64.20            | 42.85              | 57.14             | 21.42          |
|                     | 2014 | 37.50          | 62.50            | 43.75              | 56.65             | 25.00          |
|                     | 2015 | 38.46          | 69.23            | 38.46              | 61.53             | 23.07          |
|                     | 2016 | 36.36          | 72.72            | 54.54              | 63.63             | 27.27          |
| *Acinetobacter spp*  | 2012 | 66.66          | 83.30            | 50.00              | 50.00             | 16.66          |
|                     | 2013 | 60.00          | 80.00            | 80.00              | 60.00             | 20.00          |
|                     | 2014 | 75.00          | 100              | 75.00              | 75.00             | 40.00          |
|                     | 2015 | 66.60          | 83.33            | 83.33              | 66.66             | 33.00          |
|                     | 2016 | 80.00          | 100              | 80.00              | 80.00             | 40.00          |
Antimicrobial resistance of enterobacteriaceae

| Antimicrobial agents | Year | Amikacin 30 μg | Gentamicin 10 μg | Ciprofloxacin 5 μg | Cefotaxime 30 μg | Ampicillin 10 μg | Imipenem 10 μg |
|----------------------|------|----------------|-----------------|-------------------|-----------------|-----------------|----------------|
| **Klebsiella spp**   | 2012 | 35.71          | 64.2            | 57.14             | 50.00           | 100             | 7.1            |
|                     | 2013 | 41.66          | 66.66           | 58.00             | 58.33           | 100             | 8.3            |
|                     | 2014 | 38.46          | 69.23           | 61.53             | 53.84           | 100             | 15.38          |
|                     | 2015 | 44.44          | 66.66           | 77.73             | 66.66           | 100             | 22.22          |
|                     | 2016 | 45.44          | 72.72           | 72.72             | 63.63           | 100             | 27.27          |
| **Escherichia coli** | 2012 | 50.00          | 50.00           | 50.00             | 50.00           | 100             | 0              |
|                     | 2013 | 40.00          | 60.00           | 40.00             | 60.00           | 80.00           | 20.00          |
|                     | 2014 | 50.00          | 50.00           | 50.00             | 50.00           | 75.00           | 0              |
|                     | 2015 | 33.33          | 66.66           | 50.00             | 50.00           | 66.66           | 16.66          |
|                     | 2016 | 50.00          | 75.00           | 50.00             | 50.00           | 100             | 25.00          |
| **Proteus spp**      | 2012 | 33.33          | 66.66           | 33.33             | 66.66           | 66.66           | 0              |
|                     | 2013 | 50.00          | 50.00           | 50.00             | 75.00           | 50.00           | 25.00          |
|                     | 2014 | 50.00          | 100.00          | 50.00             | 50.00           | 50.00           | 0              |
|                     | 2015 | 66.66          | 66.66           | 66.66             | 66.66           | 66.66           | 33.33          |
|                     | 2016 | 75.00          | 100.00          | 75.00             | 50.00           | 50.00           | 25.00          |
| **Citrobacter spp**  | 2012 | 50.00          | 75.00           | 50.00             | 50.00           | 50.00           | 25.00          |
|                     | 2013 | 100            | 100.00          | 100               | 100             | 100             | 0              |
|                     | 2014 | 50.00          | 50.00           | 50.00             | 50.00           | 50.00           | 50.00          |
|                     | 2015 | 66.66          | 66.66           | 66.66             | 66.66           | 66.66           | 33.33          |
|                     | 2016 | 60.00          | 80.00           | 60.00             | 60.00           | 80.00           | 40.00          |
Antimicrobial resistance of gram positive cocci

| Antimicrobial agents | Year | Penicillin 10u | Erythromycin 15 μg | Cefotaxime 30 μg | Ciprofloxacin 5 μg | Amikacin 30 μg | Vancomycin MIC |
|---------------------|------|---------------|-------------------|-----------------|-------------------|---------------|--------------|
| Staphylococcus aureus | 2012 | 75.00         | 25.00             | 25.00           | 25.00             | 25.00         | 0            |
|                     | 2013 | 100           | 33.33             | 33.33           | 33.33             | 33.33         | 0            |
|                     | 2014 | 100           | 50.00             | 50.00           | 33.33             | 33.33         | 0            |
|                     | 2015 | 100           | 40.00             | 40.00           | 60.00             | 60.00         | 0            |
|                     | 2016 | 100           | 66.66             | 66.66           | 66.66             | 66.66         | 0            |
| Coagulase negative Staphylococcus | 2012 | -              | -                 | -               | -                 | -             | -            |
|                     | 2013 | 100           | 50.00             | 100             | 50.00             | 50.00         | 0            |
|                     | 2014 | 100           | 100               | 0               | 100.00            | 0             | 0            |
|                     | 2015 | 100           | 50.00             | 100             | 100.00            | 50.00         | 0            |
|                     | 2016 | 100           | 100               | 100             | 50.00             | 50.00         | 0            |

It was found that 9.72% of bacterial cultures were polymicrobial, while variable results have been reported in other studies ranging from 13% to 80%.

Among the gram negative organism, in all the five years, *Pseudomonas aeruginosa* was the predominant isolates, accounting for 69 of 206 (33.49%) isolates. The next most commonly isolated bacteria were *Klebsiella* species (28.64%) followed by *Acinetobacter* species (12.62), *Escherichia coli* (10.19) *Proteus* species (7.76), and *Citrobacter* species (7.28). The study conducted by (Rajesh Chawla, 2008) showed 31% of *Pseudomonas aeruginosa* and 20% of *Klebsiella* sp among gram negative bacilli.

Most of the organism remained more or less same over the five years, a small decrease in the incidence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was seen.

Currently, antimicrobial resistance rates are increasing among *Pseudomonas aeruginosa* and *Acinetobacter* species.

An increase in resistance was shown by *Pseudomonas aeruginosa* (ranging from 42.85 percent to 72.72 percent) for Gentamycin, ciprofloxacin and Ceftazidime. For Amikacin 26.66 percent to 38.46 percent and for Imipenem 13.13 percent to 25.00 percent. For *Acinetobacter* spp resistance remains very high for most of the antimicrobials ranging from 50 percent to 100 percent except for Imipenem (16.66% to 40%).

Among the Enterobacteriacea 58.49% of *Klebsiella* spp and 52.00% of *Escherichia coli* were found to be ESBL producers. The emergence of extended spectrum betalactamase (ESBLs) necessitated the increase use of carbapenems, but this increased use of drugs may be contributing to the emergence of multidrug resistant Gram negative bacilli. All the ESBL producing isolates were sensitive to Imipenem in this study.

VAP due to Gram positive bacteria (13.08%) were relatively less. MRSA is another global problem, this study showed among all *Staphylococcus aureus* isolates, 18 (75.00%) isolates were methicillin resistant *S. aureus* (MRSA). It correlates with the study of Naouel Mandani 78.3% were resistant to...
Methicillin. More than forty percent of the CONS (Coagulase negative *Staphylococcus aureus*) were resistant to cefotaxime, ciprofloxacin and erythromycin and all the isolates were resistant to Penicillin.

However all the gram positive cocci were sensitive to vancomycin. Hence Vancomycin should be part of regimen because *Staphylococcus aureus* is the most frequent gram positive isolates with high methicillin resistance rates.

Despite the advancements in antimicrobial regimes, VAP continues to be an important cause of morbidity and mortality. Hence, knowing the local microbial flora causing VAP, their antibiotic resistant pattern and effective infection control practices are essential to improve clinical outcomes.

From this study we can conclude that VAP is an important nosocomial infection *Pseudomonas aeruginosa* was the commonest organism isolated.

Increasing drug resistance rates among gram-negative pathogens that frequently cause ventilator-associated pneumonia have resulted in increased hospital mortality, longer hospital stays, and higher inpatient health care costs.

Hence Good management strategies for VAP like adequate infection control practices include hand washing by hospital personnel, basic cleaning of all surface levels, increased barrier precautions, early accurate diagnosis and more specific antimicrobial use may significantly improve patients’ outcome.

A multidisciplinary approach, coordinated participation of microbiologist, clinician, nursing personnel and hospital infection control team is necessary for management of this nosocomial infection.

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