

Study of Bacteriological Profile in Ventilator Associated Pneumonia

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The present study is to know the frequency, causative bacteria and their sensitivity pattern in S.S. Hospital, Davangere. A prospective study was conducted between April to September 2010 in the department of microbiology, SSIMS and RC, Davangere. Endotracheal aspirate samples were collected from a total of 369 patients under mechanical ventilation for >48hrs who were suspected of having ventilator associated pneumonia (VAP) and were processed and identified by standard laboratory techniques. Quantitative culture threshold of $>10^5$ cfu/ml was considered to diagnose ventilator associated pneumonia in our study. Out of the 369 patients studied, quantitative culture was positive in 166 (44.99%), 143 cases (38.75%) showed no growth and in 60 cases (16.26%) growth was $<10^5$ both of which were included under No Ventilator associated pneumonia (NO-VAP) group. Out of 166 culture isolates, *Pseudomonas* species 56 (33.73%) was the most common, followed by *Klebsiella pneumoniae* 28 (16.87%), *Acinetobacter* species 20 (12.05%), *Escherichia Coli* 16 (9.64%), Meticillin resistant *Staphylococcus aureus* 9 (5.42%) and *Citrobacter freundii* 7 (4.21%). Organisms showed sensitivity to imipenem, ceftazidime, cefoperazone-sulbactam and amikacin. Most common age group affected was 21-30 years (28.46%) and in males with male to female ratio being 1.95:1. Most common indication for admission was poisoning (42%) followed by preterm with low birth weight (18%).

Key words: Ventilator associated pneumonia, Quantitative culture, Preterm.

Patients in the intensive care units are at the risk of dying not only from their critical illness but also from secondary processes such as ventilator associated pneumonia. The mortality attributable to VAP has been reported to range from

0 to 50%.¹ VAP is the second most common hospital acquired infection among neonatal and paediatric patients and even in adult intensive care unit patients on ventilators.² Detection of causative organism is crucial for diagnosis of VAP. This is done by microbiological investigation where in samples obtained by bronchoscopic and non bronchoscopic methods from lower respiratory tract are cultured quantitatively and semiquantitatively.³

Information regarding common pathogens and their antibiogram is essential for

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proper choice of antibiotics. The present study was undertaken to know the frequency, bacterial flora of Ventilator associated pneumonia and their antibiogram.

Methodology

The present study was conducted between April to September 2010 in the department of microbiology, SSIMS and RC, Davangere. A total number of 369 cases on ventilator for more than 48 hours who were suspected of having VAP from neonate, paediatric and adult ICU of S.S. Hospital, Davangere were studied. Patients with pre-existing pulmonary infection at the time of admission, and evidence of sepsis at admission were excluded from the study group.

Endotracheal aspirate sample was collected under strict aseptic precautions by using 5 or 6F infant feeding tube for neonates, 8F suction catheter for paediatric patients and 12F suction catheter for adults, through the endotracheal tube. Sample collected was subjected to gram staining and quantitative culture on MacConkey agar and chocolate agar using sterile standard 4mm bacteriological loop. Inoculated plates were incubated at 37°C overnight and were checked for growth. All those samples which yielded

quantitative culture threshold of $>10^5$ cfu/ml on culture plates were considered under VAP group.⁴⁻⁷ Those samples which showed no growth and which yielded quantitative culture threshold of $<10^5$ cfu/ml were assumed to be due to colonization or contamination and were categorized under NO-VAP group.⁸ Culture isolates were identified by colony morphology, gram stain and biochemical tests.

The antibiotic susceptibility testing of the isolates was done on Mueller-hinton agar using standard Kirby-bauer disc diffusion method.⁹ All the strains of *Staphylococcus aureus* were tested for methicillin resistance by disc diffusion technique using 1µg oxacillin disc on Mueller hinton agar incorporated with 4% sodium chloride and incubating at 35°C for 24 hours.⁹⁻¹¹

RESULTS

In the present study, most common age group affected was 21-30 years (28.46%) followed by 51-60 years (15.45%). Males (66.12%) were more frequently affected than females (33.18%) with male to female ratio being 1.95:1.

Out of the 369 patients studied, 166 cases (44.99%) were positive for quantitative

Table 1. The indication for admission requiring mechanical ventilator support

Cases	Number	Percentage
poisoning	155	42%
Preterm with low birth weight	42	11.38%
Meconium aspiration	26	7.04%
Post operative cases	29	7.86%
Dengue hemorrhagic fever	18	4.87%
Congenital anomaly	16	4.34%
Snake bite	11	2.98%
others	72	19.51%
Total	369	100%

Table 2. Association of gram stain of endotracheal aspirate showing presence of pus cells with VAP and NO-VAP cases

Gram stain	VAP(166)	NO-VAP(203)	Total
Plenty of pus cells	166(100%)	0(0%)	166(44.99%)
Few pus cells	0(0%)	60(61.25%)	60(16.26%)
No pus cells	0(0%)	143(38.75%)	143(38.75%)

P<0.001, highly significant.

culture, 143 (38.75%) showed no growth and 60 (16.26%) were $<10^5$ cfu/ml.

Out of the 166 culture isolates, *Pseudomonas* species 56 (33.73%) was the most common followed by *K. pneumoniae* 28 (16.87%), *Acinetobacter* species 20 (12.05%), *E. coli* 16 (9.64%) and Methicillin resistant *Staphylococcus aureus* (MRSA) 9 (5.42%).

In the present study, *Pseudomonas* spp were sensitive to imipenam (94%), cefoperazone-sulbactam (72%), ceftazidime (68.6%) and amikacin (38%).

Klebsiella spp showed sensitivity to

Imipenam (100%), ceftazidime (78%), cefoperazone-sulbactam (69.4%), ceftriaxone (38.2%) and amikacin (27%).

Acinetobacter spp showed sensitivity to imipenam (62.7%), cefoperazone-sulbactam (24%), ceftazidime (14%) and amikacin (18.3%). *E. coli* showed sensitivity to imipenam (100%), cefoperazone-sulbactam (62%), amikacin (46%).

MRSA isolates showed sensitivity to vancomycin (88.89%), linezolid (100%) and erythromycin (44.44%). *Citrobacter* spp showed sensitivity to cefoperazone-sulbactam (42.9%), imipenam (85.7%).

Table 3. Shows spectrum of organisms associated with Ventilator associated pneumonia

Organism	Number	Percentage
<i>Pseudomonas</i> spp	56	33.73%
<i>K. pneumoniae</i>	28	16.87%
<i>Acinetobacter</i> spp	20	12.05%
<i>E. coli</i>	16	9.64%
Coagulase negative <i>Staphylococci</i> (CONS)	14	8.43%
MRSA	9	5.42%
<i>C. freundii</i>	7	4.21%
Multiple isolates a) <i>Klebsiella</i> spp + <i>Pseudomonas</i> spp (7)	15	9.04%
b) <i>Acinetobacter</i> spp + <i>Klebsiella</i> spp (5)		
c) <i>Pseudomonas</i> spp + <i>Acinetobacter</i> spp (2)		
d) <i>E. coli</i> + <i>Pseudomonas</i> spp (1)		
<i>Proteus mirabilis</i>	1	0.60%
Total	166	100%

DISCUSSION

The present study is a prospective study over a period of 6 months which included 369 clinically suspected VAP patients.

It is very important to know bacteriological profile and also resistance pattern in respective hospital ICU.⁶ Organisms vary among different hospitals.¹² Injudicious use of even prophylactic antibiotics is not routinely recommended in case of VAP because exposure to antibiotics is a significant risk factor for colonization and infection with nosocomial multidrug resistant pathogens.¹² The rational use of antibiotics may reduce colonization and

subsequent VAP with multidrug resistant pathogens. As pathogens vary among hospitals, it is very important to know the incidence of VAP and the associated microbial flora in each setting so as to guide more effective and rational utilization of antimicrobial agents.¹³

The frequency of VAP in the present study is 44.99%, which correlates with the studies of Dey *et al.*,⁴ (45.4%) and mukhopadhyay *C et al.*,¹⁴ (42%), where as Rajashekar *T et al.*,⁷ reported frequency of 73.33%. The difference could be due to different methods used for sample collection, sample size and underlying disease state requiring ventilation.

The most common age group affected was

21-30 years(28.46%) which correlates with the study of Dey A *et al.*,⁴ and in males(66.12%).

In the present study, the predominant bacterial isolate was *Pseudomonas* spp 56(33.73%) followed by *K.pneumoniae* 28(16.87%), *Acinetobacter* spp 20(12.05%), *E.coli* 16(9.64%) , CONS 14(8.43%) and MRSA 9(5.42%) which is comparable with the study of Raghavendra KH *et al.*,¹⁵ , where as Dey A *et al.*,⁴ and Rajshekar T *et al.*,⁷ have reported *Acinetobacter* as the predominant species in their studies.

Increased use of advanced diagnostic and interventional procedures in hospital ICU is responsible for the emergence of multidrug resistant pathogens such as *Pseudomonas* spp and *Acinetobacter* spp in the ICU's. They pose a great problem for the clinician because of their resistance to major group of antibiotics. The isolation of bacteria from clinical specimens may not necessarily indicate infection, but rather may be result of colonization or contamination. This is reflected in our study where bacteria were isolated from endotracheal aspirates of NO-VAP cases 60(16.26%).^{4,6,7,14} Thus bacteria considered as high risk pathogens like *pseudomonas* spp, *Acinetobacter* spp , *Klebsiella* spp, *E.coli* and MRSA isolates figure prominently in the cases of VAP in the present study.

In the present study ,multiple isolate growth pattern(9.04%) is also appreciated similar to other studies.^{4,13,16,17}

In the present study, *Pseudomonas* spp were sensitive to imepenam(94%), cefoperazone-sulbactam(72%), ceftazidime(68.6%) and amikacin(38%).

Klebsiella spp showed sensitivity to Imepenam(100%), ceftazidime(78%), cefoperazone- sulbactam(69.4%), ceftriaxone(38.2%) and amikacin(27%).

Acinetobacter spp showed sensitivity to imepenam(62.7%), cefoperazone-sulbactam(24%), ceftazidime(14%) and amikacin(18.3%).

E.coli showed sensitivity to imepenam(100%), cefoperazone-sulbactam(62%), amikacin(46%).

MRSA isolates showed sensitivity to vancomycin(88.89%), linezolid(100%) and erythromycin(44.44%).

Citrobacter spp showed sensitivity to

cefoperazone-sulbactam(42.9%), imepenam(85.7%).

Rajshekar T *et al.*,⁷ has reported *Pseudomonas* spp sensitive to imepenam(25%), *Klebsiella* spp sensitive to imepenam(100%) and *Acinetobacter* spp sensitive to cefoperazone-sulbactam(100%) and imepenam(80%).

Dey A *et al.*,⁴ has reported *Pseudomonas* spp sensitive to imepenam(50%), *Klebsiella* spp sensitive to cefoperazone-sulbactam(100%) and imepenam(100%), *Acinetobacter* spp sensitive to cefoperazone-sulbactam(78.2%) and imepenam(60.8%), *E.coli* sensitive to imepenam(100%) and amikacin(100%).

This difference in antibiotic sensitivity pattern in different studies could be due to the difference in the strains and the antibiotic policies in the hospitals. Some of the isolates were multidrug resistant due to the production of beta-lactamase, extended spectrum beta-lactamase and metallo beta lactamase.

CONCLUSION

A microbiological study was undertaken to determine the VAP frequency, causative bacteria and antibiotic susceptibility pattern of the isolates in S.S.Hospital, Davangere. Endotracheal aspirate samples from a total of 369 clinically suspected VAP cases were collected and studied.

Out of the 369 clinically suspected VAP cases, 166(44.99%) were positive for quantitative culture.

The presence of plenty of pus cells in gram stain and its role in occurrence of VAP was statistically highly significant in our study.

Out of 166 cases VAP cases, 151 cases showed single isolate growth pattern and 15 cases showed multiple isolate pattern of which *Klebsiella* spp+*Pseudomonas* spp(7) was the most common followed by *Acinetobacter* spp+*Klebsiella* spp(5), *Pseudomonas* spp+ *Acinetobacter* spp(2) and *E.coli*+ *pseudomonas* spp(1).

Gram negative bacteria were the most common organism causing VAP in our study , of which, *Pseudomonas* spp, *Klebsiella* spp, *Acinetobacter* spp and *E.coli* were the most frequent. Among the gram positive cocci, CONS was the predominate isolate followed by MRSA.

The antibiotic sensitivity pattern of gram negative bacteria isolated in our study showed that imipenam, cefoperazone-sulbactam, ceftazidime and amikacin were the most effective drugs. In case of MRSA and CONS, they were sensitive to vancomycin, linezolid and erythromycin.

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