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Surveillance of ventilator-associated-pneumonia (VAP) in Pediatric Intensive Care Unit (PICU) at a tertiary care teaching hospital from Central India

Microbiology

KEYWORDS: Multidrug resistance,tracheal secretions, mechanical ventilation

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ABSTRACT

Introduction: Pneumonia associated with mechanical ventilation is one of the most important causes of nosocomial infections in paediatric intensive care units . Despite advances in patient care, changing tracheal floras complicate therapy by acquiring drug resistance.

Methods - The tracheal secretions of patients on mechanical ventilation from Paediatric-Intensive-Care- received in Microbiology laboratory were processed by standard bacteriological techniques. The correlation between the organisms isolated from tracheal secretions & organisms causing VAP were studied.

Results : Out of 104 tracheal secretions, 82 (78.84%) showed the bacterial growth.The with most common organism isolated from tracheal secretion of VAP patients was *pseudomonas aeruginosa.* A VAP rate of 8.65 % or 17.34 per 1000 ventilator days was found. Approximately12-50% Gram negative bacilli were Multi drug resistant.

Conclusion- We hope present observation would be helpful in providing useful guidelines for initiation of therapy in patients suspected of developing VAP.

Introduction:

Pneumonia associated with mechanical ventilation means ventilator associated pneumonia (VAP) is one of the most important causes of nosocomial infections in in paediatric intensive care units (PICU), as has been demonstrated by studies evaluating nosocomial infections in pediatrics[1-4]. The role of upper airway secretions contaminated by pathogens which drains to the subglottis area is known to cause VAP in intubated patients on mechanical ventilation. This fact has led to clinical and epidemiological investigations which have, in turn, contributed significantly to the understanding and management of patients suffering from VAP [5-8]. It is estimated that the incidence of VAP in adults is greater than 10% [9].The incidence in children, estimated by the CDC.s National Nosocomial Infections Surveillance study (NNIS), is 20% [7]. The tracheobronchial tree and oropharynx of patients on mechanical ventilation are frequently contaminated by microorganisms[9].The relation between this colonization and pulmonary infection, however, is not yet clear. Johanson et al [10]. showed that 23% of patients colonized by bacteria later developed pulmonary infection.

Despite advances in patient care, these changing floras complicate therapy by acquiring drug resistance and altering their sensitivity pattern. Therefore updated knowledge of local epidemiological and susceptibility profile is recommended for guiding the clinicians regarding empirical choice of antibiotics . So the present study was conducted to analyze the spectrum of aerobic bacteria isolated from endotracheal aspirates of patients on mechanical ventilation , to evaluate the antibiotic sensitivity pattern of these isolates and to correlate the organisms isolated from tracheal secretions & organisms causing VAP.

Material & Methods:

The present study was a prospective study. The study population consisted of all pediatric patients on mechanical ventilation admitted in Pediatric Intensive Care Unit(PICU) of Government Medical College & Hospital ,Nagpur whose tracheal secretions were received in Microbiology laboratory, Government Medical College, Nagpur between August 2015 and March 2016.The approval of Institutional Ethics committee, Government Medical College, Nagpur was obtained. The tracheal secretions and endotracheal tube (ET) secretions were immediately inoculated and streaked onto nutrient agar, 5% sheep blood agar and MacConkey agar (Hi-Media, India)[11]. Plates were incubated aerobically at 37°C for 24 hours. Isolated organisms were processed and identified according to standard bacteriological techniques[12].Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion technique [13]. The drugs used were as per the Clinical and Laboratory Standards Institute 2013 guidelines. Each Gram negative isolate was further subjected for Extended spectrum lactamase production by Disc Potential Test . Each strain of S*taphylococcus aureus* was subjected to determination of Methicillin resistance i.e determination of Methicillin resistant S*taphylococcus aureus* (MRSA) was done by Cefoxitin disc diffusion method[14].These cases on mechanical ventilation were further followed for the development of symptoms of VAP which were further confirmed microbiologically by blood culture , endo-tracheal(ET) tube secretion culture and tracheal secretion culture.The correlation between these organisms isolated from tracheal secretions & organisms causing VAP was studied.

A patient was said to be suffering from VAP if he/she was on mechanical ventilation and developed a new fever and cough with development of purulent sputum, in combination with radiological evidence of a new or progressive pulmonary infiltrate, a suggestive Gram's stain and growth of bacteria in cultures of tracheal aspirate or blood after 48 hours of hospitalization[15].

For data collection and descriptive analysis, two important parameters were considered. They were:[15]

1).Denominator data (population at risk) - This included all patients exposed to ventilator during surveillance period. A total number of ventilator days were thus calculated.

2).Numerator data - This included total number of patients on mechanical ventilation who developed bacteriologically confirmed lower respiratory tract infection means confirmed VAP cases as per the standard definitions.

VAP rate was expressed as the total number of VAP cases per 1000 ventilator days, as calculated by dividing the number of persons developing VAP by the total number of ventilator days and multiplied by 1000.

Results:

A total of 104 tracheal secretions processed 82 (78.84%) specimens

showed bacterial growth. Aerobic Gram negative bacilli (GNB) remained the predominant pathogens isolated with an isolation rate of 91.46% as compared to isolation rate of 8.53% of gram-positive organisms. Among aerobic GNB, *Pseudomonas aeruginosa* 31(37.80%) was the most common organism isolated. Among Gram positive organisms *Staphylococcus aureus* was the only isolate isolated (Table 1)

Table 1: Different organisms isolated from culture of tracheal secretions

S.no	Organism isolated(n=83)	Number(%)
1	*Pseudomonas aeruginosa*	31(37.80)
2	*Klebsiella pneumonia*	19(23.17)
3	*Acinetobacter baumannii*	13(15.85)
4	*Citrobacter freundii*	06(7.31)
5	*E.coli*	06(7.31)
6	*Staphylococcus aureus*	07(8.53)
	Total	82(100)

NOTE : - % - Percentage

Resistance pattern of the Gram negative bacilli isolated from tracheal secretions against β – lactam antibiotic was as shown in table 2

Table 2 - Resistance pattern of the Gram negative bacilli isolated from tracheal secretions against β – lactam antibiotics

	Antibiotics								
Gram negative Bacilli	A(%)	Ac(%)	PIT (%)	Pc(%)	Cn(%)	Cfz(%)	Cpm(%)	Ip(%)	M P(%)
Psuedomonas aeruginosa (n=31)	31(100)	31(100)	04(12.90)	19(61.29)	31(100)	25(80.64)	19(61.29)	04(12.90)	07(22 58)
Klebsiella pneumoniae(n=19)	19(100)	19(100)	03(15.78)	04(21.05)	19(100)	19(100)	06(31.57)	03(15.78)	02(10 52)
Acinetobacter baumannii (n=13)	13 (100)	13 (100)	02(15.38)	03 (23.07)	13 (100)	13 (100)	04 (30.76)	01 (7.69)	02 (15.3
Citrobacter freundii (n=06)	05 (83.33)	05 (83.33)	01 (16.66)	01 (16.66)	05 (83.33)	05 (83.33)	02 (83.33)	01(16.66))	01(16
E.coli (n=06)	04 (66.66)	04 (66.66)	01 (16.66)	01 (16.66)	04 (66.66)	04 (66.66)	02 (33.33)	02 (33.33)	01(1666)

A-Ampicillin, Ac-Amoxycillin-clavulanic acid , PIT – Piperacillin-tazobactum , PC-Piperacillin, Cn-cefoxitin, Cfz-ceftazidime, Cpm-Cefepime , Ip- Imepenem, MRP-Meropenem

Resistance pattern of the Gram negative bacilli isolated from tracheal secretions against fluoroquinolones ,aminoglycoside and polypeptide is as in table 3

Table 3: Resistance pattern of the Gram negative bacilli against fluoroquinolones, aminoglycosides and polypepetides

	Antibiotics		
Gram negative bacilli	Of (%)	Ak (%)	Pb (%)
Psuedomonas aeruginosa (n=31)	15 (48.38)	18 (58.06)	04(12.90)
Klebsiella pneumonia (n=19)	06 (31.75)	10 (52.63)	03(15.78)
Acinetobacter baumannii (n=13)	05 (38.46)	07(53.84)	02 (15.38)
Citrobacter freundii (n=06)	02 (40)	02 (40)	02 (40)

Of-Ofloxacin , Ak-Amikacin , Pb-Polymyxin B

Approximately 12- 50 % of the Gram negative bacilli isolated were Multi drug resistant (MDR).

The β lactamase production among Gram negative bacilli is as shown in table 4

Table 4: β-lactamase production among Gram negative bacilli

S.no	Organisms	Number of ESβL producer organisms(%)
1	*Pseudomonas aeruginosa (n= 31)*	-
2	*Klebsiella pneumonia (n=19)*	14(73.68)
3	*Acinetobacter baumannii (n=13)*	-
4	*Citrobacter freundii (n=06)*	04(66.66)
5	*E.coli (n=06)*	04(66.66)

NOTE :- % - Percentage

Among Gram positive organism, the only isolate was *staphylococcus aureus* with resistance pattern as shown in table 5

Table 5: Antibiotic resistance pattern of *staphylococcus aureus* isolated from tracheal secretions

	Antibiotics								
Organism	E	Cn	Cz	Co	T	G	Cf	Va	Lz
Staphylococcus aureus (n=07)	04 (57.14)	04 (57.14)	04 (57.14)	05 (71.42)	05 (71.42)	04 (57.14)	04 (57.14)	01 (14.28)	01 (14.28)

E-Erythromycin, Cn-Cefoxitin, Cz-Cefazolin, Co-trimoxazole, T-Tetracycline, G-Gentamycin, Cf-Ciprofloxacin, Va-Vancomycin, Lz -Linezolid

80% strains of *Staphylococcus aureus* were MDR with maximum sensitivity towards Vancomycin & Linezolid. Four staphylococcal strains (57.14 %) were MRSA.

Out of these 104 cases , 09 (8.65%) cases were clinically suspected as VAP. The blood culture, endotracheal tube secretion culture & tracheal secretion culture of these nine suspected VAP cases yielded the same organisms with same antibiotic resistance pattern. When correlated, the same organism with same antibiotic resistance pattern was also isolated from tracheal secretion culture of these patients previously. These nine cases(8.65%) were labelled as VAP giving a VAP infection rate 17.34 per 1000 ventilator days (table / figure 6)

Table 6: Ventilator associated pneumonia detected during the period of Aug 2015 to Mar 2016 (n = 104)

Total number of cases studied	Number of VAP cases detected (%)	Total number of ventilator days	Infection rate (per 1000 ventilator days
104	09(8.65%)	519	17.34

NOTE : - % - Percentage

The organisms isolated from VAP cases was as in table 7

Table 7: Different oraganisms isolated from patients developing VAP cases

S.no	Organism (n=9)	Number (%)
1	*Psuedomonas aeruginosa*	03(33.33)
2	*Klebsiella pneumonia*	02(22.22)
3	*Acinetobacter baumannii*	02(22.22)
4	*Citrobacter fruendii*	01(11.11)
5	*Staphylococcus aureus*	01(11.11)

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	Total	09 (100)

NOTE : - % - Percentage

The resistance pattern of the Gram negative bacilli against β – lactam antibiotics isolated from VAP cases is as shown in table 8

Table 8 : Resistance pattern of the Gram negative bacilli against β – lactam antibiotics isolated from VAP cases

Gram negative bacilli (n=08)	Antibiotic							
	A(%)	Ac(%)	PIT (%)	Pc(%)	Cn(%)	Cfz(%)	Cpm (%)	Ip(%
P.aeruginosa (n=3)	03	03	01	02	03	03	03	01
	(100)	(100)	(33.33)	(66.66)	(100)	(100)	(100)	(33.33)
K. pneumonia (n=02)	02	02	01	01	02	02	02	01
	(100)	(100)	(50)	(50)	(100)	(100)	(100)	(50)
Acinetobacter baumannii(n=02)	02	02	01	01	02	02	02	01
	(100)	(100)	(50)	(50)	(100)	(100)	(100)	(50)
Citrobacter fruendii(n=01)	01	01	01	01	01	01	01	01
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)

A-Ampicillin, Ac-Amoxycillin-clavulanic acid , PIT – Piperacillin-tazobactum, PC-Piperacillin, Cn-cefoxitin, Cfz-ceftazidime, Cpm-Cefepime , Ip- Imepenem, MRP-Meropenem

The Resistance pattern of the Gram negative bacilli against fluoroquinolones ,aminoglycoside and polypeptide is as shown in table 9

Table 9: Resistance pattern of the Gram negative bacilli against fluoroquinolones ,aminoglycoside and polypeptide

Gram Negative bacilli (n=08)	Antibiotic		
	Of (%)	Ak (%)	Pb (%)
P.aeruginosa (n=3)	03 (100)	02 (66.66)	00 (00)
K. pneumonia (n=02)	01 (50)	02 (100)	00(00)
Acinetobacter baumannii (n=02)	02 (100)	02 (100)	00(00)
Citrobacter fruendii(n=01)	01 (100)	01 (100)	00(00)

Of-Ofloxacin , Ak-Amikacin , Pb-Polymyxin B

Approximately 60% of GNB isolates were Multi drug resistant (MDR). All the isolates showed maximum sensitivity towards polymyxin B & meropenem.

The antibiotic resistance pattern of *Staphylococcus aureus* isolated from VAP cases is as shown in table 10

Table 10 : Antibiotic resistance pattern of *Staphylococcus aureus* isolated from VAP cases

Organism	Antibiotics								
	E	Cn	Cz	Co	T	G	Cf	Va	Lz
Staphylococcus aures (n=01)	01	01	01	01	01	01	01	00	00
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(00)	(00)

E-Erythromycin, Cn-Cefoxitin, Cz-Cefazolin, Co-trimoxazole, T-Tetracycline, G-Gentamycin, Cf-Ciprofloxacin, Va-Vancomycin, Lz-Linezolid

Discussion : Health care associated infections (HCAI) continue to be a major cause of patient morbidity and attributable. The mechanically ventilated and tracheostomised patients are colonized with bacteria of either endogenous or exogenous origin which might end up in ventilator associated tracheobronchitis or VAP [16].

The Preeminent result of the study was high growth rate with GNB (91.46%) as compared to isolation rate of 8.53% of gram-positive organisms. Bypassing of the upper respiratory tract and imperfect functioning of mucociliary escalator (due to insertion of tube in trachea) impair the immune system. Besides, leakage of secretion around the tube and opening of the binding site for gram negative bacteria may have caused high rate of colonization[17,18]. The present study isolated *Pseudomonas aeruginosa* (33.33%) *followed by Klebsiella pneumoniae* (22.22%) . Koirala et al 2010 [19] also reported *Pseudomonas aeruginosa* as the most common organism whereas Shanmuga et al 2014 [20] reported Klebsiella spp.(35.9%) as the most common isolate from tracheal secretion of mechanically ventilated patients.

The high frequency of Pseudomonas spp. can be justified as the mechanical injury to the tracheal surface (as could occur from endotracheal intubation and suctioning) may expose binding site for the Pseudomonas spp. [21], the binding is further enhanced by Carbohydrate produced by novel tracheobronchial cell when repairing the injury [22].The bio-film inside the endotracheal tube itself may be a surface where bacteria bind avidly [23].The dominant *Pseudomonas* colonization coincided with earlier studies[24] on the other hand ,high frequency of enteric GNB can be referred to entry of these bacteria from gastrointestinal tract to tracheobroncheal tree and increased abdominal volume(as could occur from nasogastric feeding) which refluxes colon bacteria to respiratory tract[25].

The study revealed that all the isolates of *Pseudomonas aeruginosa* were resistant to ampicillin, amoxicillin-clavulanic acid,cefoxitin and more than 60 % resistance was observed towords ceftazidime and 58 % to amikacin. All the isolates of *Acinetobacter baumanii* were resistant to ampicillin, amoxicillin-clavulanic acid,cefoxitin, ceftazidime . The drugs which were effective against these two non-fermenters were piperacillin-tazobactam , imepenem, meropenem & polymyxin B. Koirala et al 2010[19] & Shanmunga et al 2014[20] also reported such a high level of resistance towards cephalosporins and aminoglycosides & also reported the same drugs effective against these organisms as in our study.

Again all the enteric GNB were showing a very high % of resistance to cephalosporins & aminoglycosides. Also the drug against which these organisms showed maximum sensitivity were piperacillin ,piperacillin-tazobactam,imepenem & polymyxin B.These findings were in accordance with Koirala et al 2010[19] & Shanmunga et al 2014[20]

It was observed that more than 12 - 50 % of GNB isolated from tracheal secretions were MDR. Santosh Khanal[26]& Werarek P[27] reported 80 % MDR GNB from tracheal secretion culture. Only drug effective against GNB in our study were piperacillin-tazobactam , polymyxin B & imepenem.This finding is in accordance with other studies [19,10,22,25,26].

In our study approximately 60 – 80 % Staphylococci isolates were MDR whereas other studies [26,27] reported isolation of 80 % MDR GPC from tracheal secretion culture.

All the staphylococci showed maximum sensitivity towards Vancomycin & Linezolid. Out of these 6 staphylococcal strains, 4 (66.66%) were MRSA which is again in accordance with studies by other workers[18,19]. 100 % isolates of *E.coli*, *Citrobacter freundii* & 14(73.68%) strains of *Klebsiella pneumoniae* were identified as ESBL producers. Charles et al 2013[28] reported 100 % isolates of *E.coli* & 40% of *Klebsiella pneumoniae* whereas Joseph et al 2010 [29]50 % of *E.coli* & 67 % of *Klebsiella pneumoniae* as ESβL producers.

We studied 104 patients who were on ventilator, for 519 ventilator days from PICU. Out of them, nine were suspected for VAP and later these nine patients were confirmed microbiologically ensuring an infection rate of 8.65 % or 17.34 per 1000 ventilator days; which was remarkably high when compared to US infection rate of 5.1 per 1000 ventilator days, but comparable to that of a Turkish study, reporting a rate of 20.8% or 24.1 infections per 1000 ventilator days [30,31].Rates of VAP have ranged from 10.5 to 34.8% in different

studies [31,32,33,34,35]

The most common organism isolated from VAP cases was *Pseudomonas aeruginosa*(33.33%) followed by *Klebsiella pneumoniae* (22.22%) and *Acinetobacter baumannii* (22.22%).Study from Nepal[19] reported *pseudomonas spp.* whereas one study from India [20] reported *Klebsiella pneumonia* as the most common organism causing VAP at their Institution. As *Pseudomonas spp.* is a bacterium that is capable of surviving in nature with minimum nutritional requirements, being able to survive for long periods in humidifiers and solutions, it is emerging as the most common organism causing VAP in ICUs.

In the present study,GNB showed maximum resistance towards β-lactum,Cephalosporins & Aminoglycoside group of drugs.100% GNB showed sensitivity towards poymyxin B , again *Staphylococcus aureus* also showed sensitivity to vancomycin & Linezolid only. A study from Greece (Guembe *et. al.,* 2008) [36] , study from India (Goel *et. al.,* 2009) [37]& a study from Turkey (Erden *et. al.,* 2008) [38]also reported the same sensitivity pattern.

A high burden of the multi-drug resistant bacteria were reported, this may be ascribed either to selective decontamination of digestive tract with different antibiotics or empirical use of broad spectrum antibiotics and non adherence to hospital antimicrobial position.

Conclusion –

The present study explains two important microbiological complications in patients on mechanical ventilation. The first is high growth rate. Hence the isolation, identification and antibiotic susceptibility testing of each bacterium from culture of tracheal aspirate will help prompt confirmation of possible aetiological agent ultimately will enhance the treatment guideline.The second is presence of high numbers of MDR. Once MDR is established in hospital, these can persist and acts as a source of Nosocomial infection.Further the resistant bacteria that survive the effect of the antibiotic are able to multiply,spread to others & cause further infections in the family, community and/or health care setting. In turn ,these infections are more resistant To another round of the same antibiotic. Therefore, careful microbial surveillance and in vitro testing of antibiotics before the start of antimicrobial therapy and restricted antimicrobial policy may bolster the prevention and treatment of MDR isolates.

Unfortunately as we are not having the molecular set up we could not process it further.

We hope present observation would be helpful in providing useful guidelines for initiation of therapy in patients suspected of developing VAP .Further there is a need to initiate an Active Surveillance for VAP by Hospital Infection Control Committee at every health care centre

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