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ANALYSIS OF THE MICROBIOLOGICAL PROFILE AND IT'S RESISTANCE PATTERN IN SUBJECTS WITH VENTILATOR ASSOCIATED PNEUMONIA

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ABSTRACT

Background: Major cause of mortality in low-income countries is respiratory infections which are also the third most common cause of death globally. VAP (Ventilator-associated pneumonia) is a vital form of hospital-acquired pneumonia which signifies pneumonia seen in subjects that are mechanically ventilated for a minimum of 48 hours following tracheostomy or tracheal intubation

Aims: The present study was conducted to assess the microbiological profile in subjects with Ventilator-associated pneumonia and to assess the bacterial profile in these subjects. The study also assessed the antibiotic susceptibility pattern of bacteria linked with ventilator-associated pneumonia.

Material and methods: In 124 subjects with a confirmed diagnosis of ventilator-associated pneumonia either clinically or radiographically, endotracheal aspirates were collected and were assessed for antimicrobial susceptibility, culture, or microscopy testing.

Results: High resistance was seen for CTX, CIP, AXV, and AMP with respective incidence of 90% (n=27), 83.3% (n=26), 93.3% (n=28), and 96.6% (n=29) subjects with E.coli, 78% (n=39), 52% (n=26), 84% (n=42), and 96% (n=48) subjects for Klebsiella, 64.28% (n=27), 64.28% (n=27), 80.95% (n=34), and 90.47% (n=38) subjects for Enterobacter, and 64.28% (n=9), 71.42% (n=10), 71.42% (n=10), and 85.71% (n=12) Enterobacteriaceae family. In Pseudomonas spp. An increase in resistance was seen for IPM, AK, PTZ, and CFS with incidence of 13% (n=7), 19% (n=11), 16% (n=9), and 19% (n=11) subjects for, whereas, for NGFNB, the incidence was high for most of the antimicrobials except for PB and IPM with respective incidence of 3.50% (n=2) and 13% (n=7) respectively. For Methicillin resistance Staph aureus, highest resistance was seen for PEN and AMP in 92.85% (n=13) study subjects followed by CIP and AXV in 64.28% (n=9) study subjects. In CONS, highest resistance was

seen for PEN in 91.6% (n=11) subjects followed by 83.3% (n=10) subjects, and for AXV and COT with 58.3% (n=7) study subjects each. For streptococcus spp., highest resistance was seen for COT in 66.6% (n=6) subjects followed by PEN in 33.3% (n=3) subjects, and for AMP in 22.2% (n=2) subjects. Foe enterococcus spp., highest resistance was seen for CIP and AMP for 66.6% (n=4) subjects each followed by ERY in 50% (n=3) subjects, and PEN in 33.3% (n=2) subjects

Conclusion: The present study concludes that more common isolates were gram-negative compared to the gram-positive. Also, significant resistance was seen for cephalosporins, trimethoprim-sulfamethoxazole, and tetracyclines. Ventilator-associated pneumonia being a major cause of morbidity and death globally, VAP cases should be detected and early treated promptly.
Keywords: Microbial resistance, monomicrobial, MRSA, resistance pattern, Ventilator Associated Pneumonia

INTRODUCTION

The second most common cause of infection in hospitalized subjects is VAP (Ventilation Associated Pneumonia) whereas the most common cause is UTI (urinary Tract Infection).Ventilation Associated pneumonia is most commonly seen in the subjects admitted to ICU (intensive care units) of the hospital. ICU-associated pneumonia is usually seen in admitted subjects within the first 48 hours, whereas, Ventilation associated pneumonia is commonly seen after 48 hours of admission to the hospital and mechanical ventilation.Ventilation Associated pneumonia is included in the broader terminology of 'nosocomial pneumonia'.[1]

It is vital to detect the causative organism for Ventilation Associated Pneumonia to get appropriate treatment. For microbiologic investigations to diagnose Ventilation Associated Pneumonia, the samples collected are either non-bronchoscopic or bronchoscopic taken from the lower respiratory tract which is cultured either semiquantitatively or quantitatively. In subjects receiving mechanical ventilation, VAP complicates nearly 8% to 30% of the cases.[2]

Ventilation Associated Pneumonia can lead to complications with high mortality in nearly 10% to 20% of subjects needing mechanical ventilation. The rate of Ventilation Associated Pneumonia is assessed as episodes seen/per 1000 days on a ventilator and it greatly differs in different geographical regions from low cases in the USA with the episode of 4.4/1000 cases to high cases of 13-51/100 days on a ventilator in other geographic regions. In developed countries, the incidence of Ventilation Associated Pneumonia is quite low, whereas, a very high incidence is still seen in less-developed or developing countries. In a study conducted in the Indian setup in two separate ICU settings, the VAP incidence was seen to be 15.8 and 30.7/1000 ICU days, whereas, another study reported it to be 53.2/1000 days.[3]

The etiologic factors leading to Ventilation Associated Pneumonia include common pathogens seen in the hospital are gram-positive pathogens including staphylococci and candida like fungal agents, and non-fermenters, Acinetobacter, and Pseudomonas spp and other members of the Enterobacteriaceae family. The multidrug-resistant strains' emergence is associated with Ventilation Associated Pneumonia is usually linked to the non-judicial use

of broad-spectrum antibiotics in the early phase of ICU settings in countries that are economically developing.[4]

The present study was conducted to assess the microbiological profile in subjects with Ventilator-associated pneumonia and to assess the bacterial profile in these subjects. The study also assessed the antibiotic susceptibility pattern of bacteria linked with ventilator-associated pneumonia.

MATERIALS AND METHODS

The present retrospective study was conducted to assess the microbiological profile in subjects with Ventilator-associated pneumonia and to assess the bacterial profile in these subjects. The study also assessed the antibiotic susceptibility pattern of bacteria linked with ventilator-associated pneumonia. The study population was comprised of the ICU subjects admitted to the Institute.

The inclusion criteria for the study were subjects on mechanical ventilation for more than 48 hours, radiographic infiltration evidence of pneumonia, and clinically diagnosed ventilation-associated pneumonia. The study included a total of 124 subjects from both genders with microbiologic and clinical evidence of ventilation-associated pneumonia based on a Clinical Pulmonary Infection Score (CPIS)[5] with a value of more than 6.

For sample collection, endotracheal aspirates were collected by suction catheter by gentle suctioning from the endotracheal tube which was then sent for the quantitative culture analysis. Significance was considered from a colony count of $\geq 10^5$ CFU (colony forming units). Growth seen below this count was taken as contamination or colonization. Following CLSA (Clinical and Laboratory standard Institute) guidelines, antimicrobial susceptibility tests were conducted and interpreted and the isolate identification was done with standard tests.

For antibiotic susceptibility in non-fermenters and Enterobacteriaceae family, antibiotics used were tigecycline (TGC), polymyxin-B (PB), netilmicin (NET),piperacillin-tazobactam (PTZ), imipenem (IPM), gentamicin (GEN), cotrimoxazole (COT), ciprofloxacin (CIP), chloramphenicol (CHL),cefoperazone-sulbactam (CFS), ceftazidime (CTZ),cefepime (CPM),cefotaxime (CTX),aztreonam (AZT), amoxicillin-clavulanate (AXV), ampicillin (AMP), and amikacin (AK). For Pseudomonas isolates, the exclusions were TGC, COT, CHL< CXT (cefoxitin), AXV, and AMP. In Gram-positive pathogens, antibiotics included were linezolid (LIZ), vancomycin (VAN), erythromycin (ERY),GEN, CIP, COT, CXT,and penicillin (PEN), AXV, and AMP. The surrogate marker for assessing MRSA (methicillin resistance streptococcus aureus) was 30 µg CXT. Also, for antimicrobial susceptibility tests, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922 control strains were used.

In selected cases, where guidelines of CLSI were not applicable for disc diffusion technique, the strategies adopted were: VAN susceptibility for *Enterococcus* spp and *Staphylococcus* spp, were assessed using British Society for Antimicrobial Chemotherapy

guidelines,[6] Galani recommendation[7] was used for PB, and CLSI interpretative guideline for Cefoperazone for CFS.

The collected data for clinical and laboratory parameters were taken from the previous medical data record of the institute including the ventilated days number, clinical findings, radiological findings, and CPIS scores were taken from ICU and medical records. The collected data were subjected to the statistical evaluation using SPSS software version 21 (Chicago, IL, USA) and one-way ANOVA and t-test for results formulation. The data were expressed in percentage and number, and mean and standard deviation. The level of significance was kept at $p<0.05$.

RESULTS

The present retrospective study was conducted to assess the microbiological profile in subjects with Ventilator-associated pneumonia and to assess the bacterial profile in these subjects. The study also assessed the antibiotic susceptibility pattern of bacteria linked with ventilator-associated pneumonia. The study included a total of 124 subjects from both genders with microbiologic and clinical evidence of ventilation-associated pneumonia based on a Clinical Pulmonary Infection Score (CPIS)[5] with a value of more than 6. On assessing the distribution of the pathogens in the present study it was seen that 256 gram-negative pathogens were isolated from the study subjects, most commonly isolated pathogen was NFGNB seen in 24.60% (n=63) subjects followed by Pseudomonas spp in 22.26% (n=57) subjects, Klebsiella spp in 19.53% (n=50) subjects, Enterobacter spp in 16.40% (n=42) subjects, E. coli in 11.71% (n=30) subjects, and Enterobacteriaceae in 5.46% (n=14) study subjects respectively.For gram-positive pathogens, lesser (n=53) pathogens were isolated most commonly seen isolate was Staphylococcus aureus seen in 26.41% (n=14) subjects followed by CONS and candida spp in 22.64% (n=12) subjects, Streptococcus spp in 16.98% (n=9) subjects, and Enterococcus spp in 11.32% (n=6) study subjects (Table 1).

For the resistant strains in the study subjects with ventilator associated pneumonia, it was seen that a high resistance was seen for CTX, CIP, AXV, and AMP with respective incidence of 90% (n=27), 83.3% (n=26), 93.3% (n=28), and 96.6% (n=29) subjects with E.coli, 78% (n=39), 52% (n=26), 84% (n=42), and 96% (n=48) subjects for Klebsiella, 64.28% (n=27), 64.28% (n=27), 80.95% (n=34), and 90.47% (n=38) subjects for Enterobacter, and 64.28% (n=9), 71.42% (n=10), 71.42% (n=10), and 85.71% (n=12) Enterobacteriaceae family. Apart from Enterobacter spp. Increased resistance was seen for IPM, CFS, and PTZ which are commonly used drugs in the ICU. For PB, no strain of Klebsiella, Enterobacter, or Enterobacteriaceae was has shown any resistance as shown in Table 2.

Concerning the resistance form for the NGFNB (to non-fermentative gram-negative bacilli) and pseudomonas spp, in the study subjects which were the two most commonly seen hospital-associated microbes, it was seen that in Pseudomonas spp. An increase in resistance was seen for IPM, AK, PTZ, and CFS with the incidence of 13% (n=7), 19% (n=11), 16% (n=9), and 19% (n=11) subjects, whereas, for NGFNB, the incidence was high for most of the antimicrobials except for PB and IPM with the respective incidence of 3.50% (n=2) and 13% (n=7) respectively as shown in Table 3.

On assessing the antimicrobial resistance in Gram-positive pathogens in the study subjects, it was seen that Methicillin resistance to Staph aureus, highest resistance was seen for PEN and AMP in 92.85% (n=13) study subjects followed by CIP and AXV in 64.28% (n=9) study subjects. In CONS, highest resistance was seen for PEN in 91.6% (n=11) subjects followed by 83.3% (n=10) subjects, and for AXV and COT with 58.3% (n=7) study subjects each. For streptococcus spp., highest resistance was seen for COT in 66.6% (n=6) subjects followed by PEN in 33.3% (n=3) subjects, and for AMP in 22.2% (n=2) subjects. For enterococcus spp., the highest resistance was seen for CIP and AMP for 66.6% (n=4) subjects each followed by ERY in 50% (n=3) subjects, and PEN in 33.3% (n=2) subjects as depicted in Table 4.

DISCUSSION

The present retrospective study was conducted to assess the microbiological profile in subjects with Ventilator-associated pneumonia and to assess the bacterial profile in these subjects. The study also assessed the antibiotic susceptibility pattern of bacteria linked with ventilator-associated pneumonia. The study included a total of 124 subjects from both genders with microbiologic and clinical evidence of ventilation-associated pneumonia based on a Clinical Pulmonary Infection Score (CPIS)[5] with a value of more than 6. On assessing the distribution of the pathogens in the present study it was seen that 256 gram-negative pathogens were isolated from the study subjects, most commonly isolated pathogen was NFGNB, seen in 24.60% (n=63) subjects followed by Pseudomonas spp in 22.26% (n=57) subjects, Klebsiella spp in 19.53% (n=50) subjects, Enterobacter spp in 16.40% (n=42) subjects, E. coli in 11.71% (n=30) subjects, and Enterobacteriaceae in 5.46% (n=14) study subjects respectively. For gram-positive pathogens, lesser (n=53) pathogens were isolated most commonly seen isolate was Staphylococcus aureus seen in 26.41% (n=14) subjects followed by CONS and candida spp in 22.64% (n=12) subjects, Streptococcus spp in 16.98% (n=9) subjects, and Enterococcus spp in 11.32% (n=6) study subjects. These results were consistent with the findings of Goel V et al[7] in 2012 and Krishnamurthy V et al[8] in 2013 where authors reported similar distribution of pathogens in subjects with ventilator-associated pneumonia.

Concerning the resistant strains in the study subjects with ventilator associated pneumonia, it was seen that a high resistance was seen for CTX, CIP, AXV, and AMP with respective incidence of 90% (n=27), 83.3% (n=26), 93.3% (n=28), and 96.6% (n=29) subjects with E.coli, 78% (n=39), 52% (n=26), 84% (n=42), and 96% (n=48) subjects for Klebsiella, 64.28% (n=27), 64.28% (n=27), 80.95% (n=34), and 90.47% (n=38) subjects for Enterobacter, and 64.28% (n=9), 71.42% (n=10), 71.42% (n=10), and 85.71% (n=12) Enterobacteriaceae family. Apart from Enterobacter spp. Increased resistance was seen for IPM, CFS, and PTZ which are commonly used drugs in the ICU. For PB, no strain of Klebsiella, Enterobacter, or Enterobacteriaceae was has shown any resistance. These findings were in agreement with the results of Noyal MJ et al[9] in 2009 and Mukhopadhyay C et al[10] in 2010 where authors reported similar antimicrobial resistance in gram-negative microbes in their studies.

For the resistance form for the NGFNB (to non-fermentative gram-negative bacilli) and pseudomonas spp, in the study subjects which were the two most commonly seen hospital-

associated microbes, it was seen that in Pseudomonas spp. An increase in resistance was seen for IPM, AK, PTZ, and CFS with incidence of 13% (n=7), 19% (n=11), 16% (n=9), and 19% (n=11) subjects for, whereas, for NGFNB, the incidence was high for most of the antimicrobials except for PB and IPM with respective incidence of 3.50% (n=2) and 13% (n=7) respectively. On assessing the antimicrobial resistance in Gram-positive pathogens in the study subjects, it was seen that Methicillin resistance to Staph aureus, highest resistance was seen for PEN and AMP in 92.85% (n=13) study subjects followed by CIP and AXV in 64.28% (n=9) study subjects. In CONS, highest resistance was seen for PEN in 91.6% (n=11) subjects followed by 83.3% (n=10) subjects, and for AXV and COT with 58.3% (n=7) study subjects each. For streptococcus spp., highest resistance was seen for COT in 66.6% (n=6) subjects followed by PEN in 33.3% (n=3) subjects, and for AMP in 22.2% (n=2) subjects. For enterococcus spp., the highest resistance was seen for CIP and AMP for 66.6% (n=4) subjects each followed by ERY in 50% (n=3) subjects, and PEN in 33.3% (n=2) subjects. These results were similar to the studies of Lee MS et al[11] in 2013 and Safdar N et al[12] in 2005 where authors have reported comparable antimicrobial resistance in gram-negative and gram-positive microbes as of the present study.

CONCLUSION

Within its limitations, the present studies conclude that more common isolates were gram-negative compared to the gram-positive. Also, significant resistance was seen for cephalosporins, trimethoprim-sulfamethoxazole, and tetracyclines. Ventilator-associated pneumonia being a major cause of morbidity and death globally, VAP cases should be detected and early treated promptly. The present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with larger sample size and longer monitoring period will help reach a definitive conclusion.

REFERENCES

1. Kalanuria AA, Zai W, Mirski M. Ventilator associated pneumonia in the ICU. Crit Care. 2014;18:208.
2. Dudeck MA, Weiner LM, Allen-Bridson K, Malpiedi PJ, Peterson KD, Pollock DA, et al. National Healthcare Safety Network (NHSN) report, data summary for 2012, Device-associated module. Am J Infect Control. 2013;41:1148–66.
3. Khilnani GC, Jain N. Ventilator-associated pneumonia: changing microbiology and implications. Indian J Crit Care Med. 2013;17:331–2.
4. Charles MP, Easow JM, Joseph NM, Ravishankar M, Kumar S, Umadevi S. Incidence and risk factors of ventilator-associated pneumonia in a tertiary care hospital. Australas Med J. 2013;6:178–82.
5. Fartoukh M, Maitre B, Honoré S, Cerf C, Zahar JR, Brun-Buisson C. Diagnosing pneumonia during mechanical ventilation: the clinical pulmonary infection score revisited. Am J Respir Crit Care Med. 2003;168:173–9.
6. Clinical and Laboratory Standards Institute 2012. *Performance standards for antimicrobial susceptibility testing. 22nd international supplement*. M100-S22. Wayne, PA, USA: CLSI; 2012.

7. Goel V, Hogade SA, Karadesai S.Ventilator-associated pneumonia in a medical intensive care unit: Microbial etiology, susceptibility patterns of isolated microorganisms and outcome Indian J Anaesth. 2012;56:558-62
8. Krishnamurthy V, Vijaykumar G.S., and Nagaraj E.R. Phenotypic and Genotypic Methods for Detection of Extended Spectrum β Lactamase Producing Escherichia coli and Klebsiella pneumoniae Isolated from Ventilator-Associated Pneumonia J Clin Diagn Res. 2013; 7:1975–8.
9. Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of non fermentative Gram-negative bacteria. Indian J Med Res. 2009;129:707-12
10. Mukhopadhyay C, Krishna S, Shenoy A, Prakashini K. Clinical, radiological and microbiological corroboration to assess the role of endotracheal aspirate in diagnosing ventilator-associated pneumonia in an intensive care unit of a tertiary care hospital, India. Int J Infect Control 2010;61:991-19.
11. Lee MS, Walker V, Chen LF, Sexton DJ, Anderson DJ et al. DICON The Epidemiology of Ventilator-Associated Pneumonia in a Network of Community Hospitals. A Prospective Multicenter Study. Infect Control Hosp Epidemiol.2013;34:657-62.
12. Safdar N, Dezfuliane, Collard H, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review.Crit Care Med. 2005;33:2184-93.

TABLES

S. No	Microorganisms	Percentage (%)	Number (n)
Gram-negative	NFGNB	24.60	63
	Pseudomonas spp	22.26	57
	Enterobacteriaceae	5.46	14
	Enterobacter spp	16.40	42
	Klebsiella spp	19.53	50
	E.coli	11.71	30
	Total	100	256
Gram-positive	Candida spp	22.64	12
	Enterococcus spp	11.32	6
	Streptococcus spp	16.98	9
	CONS	22.64	12
	Staphylococcus aureus	26.41	14
	Total	100	53

Table 1:Distribution of gram-negative and gram-positive pathogens in the ventilator-associated pneumonia subjects

AMA (anti-microbial agent)	E. coli		Klebsiella Sp.		Enterobacter sp.		Enterobacteriaceae	
	n=30	%	n=50	%	n=42	%	n=14	%
PB	1	3.33	0	0	0	0	0	0
CIP	25	83.3	26	52	23	54.76	10	71.42

COT	22	73.3	37	74	27	64.28	11	78.57
CHL	3	10	9	18	13	30.95	5	35.71
AZT	7	23.3	15	30	12	28.57	2	14.28
NET	5	16.6	11	23	13	30.95	4	28.57
GEN	14	46.6	22	39	23	54.76	10	71.42
AK	9	30	11	22	18	42.85	9	64.28
IPM	2	6.66	9	18	7	16.6	2	14.28
PTZ	6	20	14	28	13	30.95	1	7.14
CFS	5	16.6	16	32	13	30.95	2	14.28
CPM	7	23.3	15	30	16	38.09	6	42.85
CTZ	7	23.3	15	30	17	40.47	5	35.71
CTX	27	90	39	78	27	64.28	9	64.28
AXV	28	93.3	42	84	34	80.95	10	71.42
AMP	29	96.6	48	96	38	90.47	12	85.71

Table 2: Antibiotic resistance in the study subjects to the Enterobacteriaceae family

AMA (anti-microbial agent)	**NGFNB**		**Pseudomonas spp.**	
	n=63	**%**	**n=57**	**%**
PB	1	1.58	2	3.50
CIP	48	76.19	14	24.56
COT	50	79.36		-
CHL	21	33.33		-
AZT	33	52.38	7	12.28
NET	29	46.03	9	15.78
GEN	45	71.42	14	24
AK	43	68.25	11	19
IPM	21	33.33	7	13
PTZ	35	55.55	9	16
CFS	25	39.68	11	19
CPM	44	69.84	8	14
CTZ	36	57.14	18	32
CTX	56	88.88	18	31
AXV	53	84.12		-
AMP	59	93.65		-

Table 3: Antibiotic resistance in the study subjects to non-fermentative gram-negative bacilli and Pseudomonas sp.

AMA (anti-microbial agent)	**Staph aureus**		**CONS**		**Streptococcus spp.**		**Enterococcus spp.**	
	n=14	**%**	**n=12**	**%**	**n=9**	**%**	**n=6**	**%**
LIZ		-		-		-		-
VAN		-		-		-	1	16.6
CXT	7	50	5	41.6		-		-
PEN	13	92.85	11	91.6	3	33.3	2	33.3
ERY	7	50	5	41.6	1	11.1	3	50
CIP	9	64.28	6	50	1	11.1	4	66.6

COT	7	50	7	58.3	6	66.6	1	16.6
GEN	7	50	4	33.3	-	-		-
AXV	9	64.28	7	58.3	1	11.1	1	16.6
AMP	13	92.85	10	83.3	2	22.2	4	66.6

Table 4: Antimicrobial resistance seen in Gram-positive pathogens in the study subjects