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RESEARCH ARTICLE

BACTERIOLOGICAL STUDY OF VENTILATOR-ASSOCIATED PNEUMONIA IN A TERTIARY CARE HOSPITAL.

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Abstract

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection diagnosed in the intensive care units (ICUs). Microorganisms responsible for ventilator associated pneumonia vary from place to place. VAP requires a rapid diagnosis and initiation of the antibiotic treatment.The present study was carried out to detect bacteria commonly associated with VAP and determine their susceptibility patterns including beta-lactamases production. Out of a total 1438 patients intubated for more than 48 hours, 302 patients (21.0%) were clinically diagnosed as VAP cases. The VAP rate was found to be 2.06 cases per 1000 ventilator days. *A. baumannii* (38.11%), *P. aeruginosa* (26.74%), *K. pneumoniae* (14.85%), *S.aureus* (11.38%), *E. coli* (3.96%), *C. freundii* (1.98%), *A. lwoffi* (1.98%) and *P. mirabilis* (0.99%) were isolated. Amongst the gram negative isolates, 15.64% were ESBL, 11.73% were AmpC and 13.96% were MBL producers. Early diagnosis of VAP along with their sensitivity pattern will help as an epidemiological marker for initial prophylaxis and treatment planning for mechanically ventilated patients.

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Introduction:-

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection diagnosed in the intensive care units (ICUs).[1] It is defined as pneumonia occurring more than 48 hours after endotracheal intubation and initiation of mechanical ventilation (MV).[2] VAP is the most frequent infection occurring in 9 to 24% of the intubated patients admitted in ICU.[3,4] It has been associated with an attributable mortality of approximately 30% depending on the pathogen isolated,[5,6] which may exceed 50%.[7,8]

Microorganisms responsible for ventilator associated pneumonia vary from place to place. Common pathogens include aerobic gram-negative bacilli (GNB), such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and Acinetobacter species;[9] gram positive cocci such as *Staphylococcus aureus.*[5]

There have been reports of increased occurrence of multi-drug resistant pathogens including carbapenem resistance bacteria in health care settings in recent times.

To date, however, there are very few studies in India evaluating VAP. Thus, this study was carried out to detect the bacteria commonly associated with VAP and determine their susceptibility patterns.

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Material and methods:-

The present study was carried out at a tertiary care hospital and approved by the Ethical committee of Indira Gandhi Government Medical College, Nagpur.

During the two and a half year study period, a total of 1438 patients admitted to the ICUs were intubated for more than 48 hours. Out of them, 302 patients were clinically diagnosed to have VAP. Thus, patients on MV for more than 48 hours having temperature of >38 ^{o}C or <36 ^{o}C, leukopenia or leukocytosis or having purulent tracheal secretions were included in the study.

Endotracheal tube aspirates were obtained with sterile precaution using a 22 inch No.14F suction catheter and collected in a mucous collector.[10] The samples were processed and the isolates were identified by standard microbiological procedures.[11] Antimicrobial susceptibility testing was done by Kirby-Bauer disk diffusion method[10] as per CLSI guidelines.[12]

All the gram negative isolates (*Enterobacteriaceae* group, Acinetobacter spp. and *P. aeruginosa*) were tested for extended spectrum β-lactamases (ESBL) and AmpC β-lactamases production[10,13] by initial screening and phenotypic confirmatory test (PCT) as per CLSI guidelines.[10] ESBL production was detected by combined disk test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed.[10] Amp C production was detected by AmpC disk test.[14] Carbapenemases production in *Enterobacteriaceae*[10] and non-fermentative gram negative[15] isolates were tested by performing both initial screening test and phenotypic confirmatory test (Modified Hodge test). MBL production was detected by minimum inhibitory concentration (MIC) testing by agar dilution method using meropenem; and MIC reduction testing using meropenem and meropenem-EDTA as per CLSI guidelines.[10,16,17] MBL testing was also done using double disk synergy test (Imipenem, Meropenem and Ceftazidime).[18,19]

Methicillin resistant *S. aureus* (MRSA) production was detected by Cefoxitin disk diffusion testing[10,20,21] and Minimum inhibitory concentration testing of oxacillin[10,13,22] by agar dilution method using oxacillin-salt screen agar containing 6µg/ ml oxacillin and 4% NaCl.[25]

Statistical analysis

Data analysis was done by Chi square test with appropriate (yate's) correction to see the significance of difference using SPSS software; $p \leq 0.05$ was considered significant.

Table I:- Bacteria isolated from VAP with antibiotic resistance pattern (%) (n=302)

Organisms (n)	AMP	AMC	CAZ	CTX	CX	CPM	PIT	MEM	GEN	AMK	TET	CIP	COT	AT	*CL
K. pneumoniae (30)	100	100	96.66	86.66	76.66	83.33	20	16.66	62.5	33.33	76.66	66.66	93.33	70	-
E. coli (8)	100	100	87.5	87.5	62.5	75	50	0	50	37.5	50	62.5	100	62.5	-
C. freundii(4)	100	100	75	75	0	25	25	0	50	25	100	25	50	75	-
P. mirabilis (2)	100	100	50	50	0	0	0	0	0	0	-	50	0	50	-
A. baumannii (77)	-	-	100	100	-	100	62.33	32.46	87.01	75.32	80.51	93.50	93.50	-	-
A. lwoffi (4)	-	-	100	100	-	75	50	0	100	50	50	75	100	-	-
P. aeruginosa (54)	-	-	85.18	-		81.48	37.03	12.96	46.29	51.85	-	81.48	-	81.48	0

Gram-positive	PEN	CX	E	CD	GEN	AMK	TET	CHL	CIP	RIF	LZ	VAN	OXA	-	-
S. aureus (23)	86.95	39.13	60.86	39.13	69.56	26.08	65.21	4.34	78.26	0	0	0	39.13	-	-

Result:-

Of the 302 patients, VAP rate was found to be 2.06 cases per 1000 ventilator days. *A. baumannii* (38.11%) was the commonest organism isolated followed by *P. aeruginosa* (26.74%) and *K. pneumoniae* (14.85%). *S. aureus* (11.39%) was the most common gram-positive bacteria associated with VAP. Table I shows organisms isolated from VAP cases along with their antibiotic resistance pattern.

VAP – Ventilator-associated pneumonia AMP – Ampicillin, AMC - Amoxyclav, CAZ - Ceftazidime, CTX - Cefotaxime, CX - Cefoxitin, CPM-Cefipime, PIT – Piperacillin-tazobactam, MEM - Meroenem, GEN – Gentamicin, AMK – Amikacin, TET – Tetracycline, CIP – Ciprofloxacin, COT – Cotrimoxazole, AT - Aztreonam, CL – Colistin, PEN – Penicillin, ERY – Erythromycin, CHL – Chloramphenicol, RIF – Rifampicin, LZ – Linezolid, VAN – Vancomycin, OXA – Oxacillin.

*Colistin resistance was detected by MIC using agar dilution method

Non-fermenters (66.83%) were the most predominant pathogens causing VAP, members of *Enterobacteriaceae* and gram-positive bacteria (*S. aureus*) causing VAP were 42.99% and 11.38% respectively. VAP episodes due to gram-positive bacteria (11.38%) were relatively less common as compared to gram-negative bacilli (88.61%).

Detection of ESBL, AmpC β-lactamase and Metallobetalactamase

ESBL production was detected in 25% of *Enterobacteriaceae* isolates and 12.59% of the Non-fermenters**.** AmpC β-lactamases were produced by 34.09% and 4.44% of the members of *Enterobacteriaceae* and non-fermenters respectively. MBL production was seen in 11.36% of the *Enterobacteriaceae* isolates while 14.81% of the non-fermenters were MBL producers (Table II).

Table II:- ESBL, AmpC β - lactamase and MBL production among the gram-negative bacteria

Bacteria (n)	ESBL	AmpC β-lactamase	MBL
Klebsiella pneumoniae (30)	9	12	5
Escherichia coli (8)	2	2	0
Citrobacter freundii (4)	0	0	0
Proteus mirabilis (2)	0	0	0
Acinetobacter baumannii (77)	15	4	17
Acinetobacter lwoffi (4)	0	0	0
Pseudomonas aeruginosa (54)	2	2	3

ESBL – Extended spectrum β-lactamase, MBL – Metallo-β-lactamase

Detection of MRSA

MRSA were found to be 39.13% by oxacillin MIC testing by agar dilution method. The results of both the methods i.e. cefoxitin susceptibility testing by disk diffusion method and oxacillin MIC testing by agar dilution method, are similar. Thus, among 23 *S. aureus* isolates studied, nine (39.13%) were found to be MRSA and 14 (60.86%) were MSSA.

Discussion:-

VAP is an important nosocomial infection among ICU patients receiving MV along with multi-drug resistant pathogens causing VAP are a major concern in any kind of ICU set up. The rate of VAP obtained in our study is 2.06 cases per 1000 ventilator days similar to a study conducted by Bowton DL *et al*.[23] However, a study by Panwar et al[24] reported the VAP rate as 26 per 1000 ventilator days.

A. baumannii (38.11%) was the commonest isolate followed by *P. aeruginosa* (26.74%) and *K. pneumoniae* (14.85%). Other organisms were *S. aureus* (11.39%), *E. coli* (3.96%), *Citrobacter freundii* (1.98%), *A. lwoffi* (1.98%) and *Proteus mirabilis* (0.99%). Our findings were similar to the study done by Dey A and Bairy I.[9]

Most of the *Enterobacteriaceae* isolates were sensitive to meropenem, piperacillin-tazobactum and amikacin; most non-fermentors being sensitive to piperacillin-tazobactum and colistin; and *S. aureus* being sensitive to vancomycin. Thus, the use of these antibiotics can be advocated in our area. This finding also emphasises the need for stringent preventive measures for VAP, as the treatment of an established VAP becomes very expensive.[25]

We observed that the most effective antibiotic for gram negative isolates was meropenem, while for *S.aureus* isolates was vancomycin. Piperacillin-tazobactam was highly active against *Acinetobacter* spp., while colistin has good activity against *Pseudomonas* spp. Our findings were similar to the other studies.[26,27,28]

MBL was produced by most of the non-fermenters, especially *A. baumannii* consistent with other studies.[29] Some *K. pneumoniae* isolates also showed MBL production similar to a study by Galani *et al*.[30] Similarly ESBL and AmpC β-lactamases were produced by a large proportion of the *Enterobacteriaceae*.[25] We found 39.13% MRSA amongst *S. aureus* isolates. Mathews *et al*[21] reported 34.4% MRSA while Alqurashi *et al*[26] reported 40% MRSA in their studies. These isolates also shown cefoxitin resistance by Kirby-bauer disk diffusion method. A study by Arora et al[31] shown that by cefoxitin disk, oxacillin screen agar and MIC testing, methicillin resistance was detected in 115 (46%) strains. CLSI[12] also states the same and advocates the use of cefoxitin disk diffusion testing, MIC to oxacillin by agar dilution and oxacillin screen agar for deciding oxacillin resistance.

The presence of these MDR pathogens highlights the need for treatment of the VAP cases with second-line antibiotics effective against them.

When the results of our study were compared with the findings of other studies, it was clear that the incidence of VAP and its resistance pattern have been changing with due course of time. Geographical variation and difference in patient population studied could be the possible factor for variability. The emergence of multi-drug-resistant pathogens causing VAP has made its treatment very difficult and, in some cases, impossible. The observed high number of GNB showing ESBL, AmpC, MBL production; *S. aureus* with methicillin resistance in VAP cases reflects its emerging resistance pattern. Thus, the antimicrobial policy needs to be updated from time to time.

Early diagnosis of VAP along with their sensitivity pattern will help as an epidemiological marker for initial prophylaxis and treatment planning for mechanically ventilated patients. In recent years, there has been a rapid increase in the incidence of VAP among the patients admitted to the ICUs for various clinical conditions and so is the increase in the multi-drug resistant pathogens due to their prolonged stay in the ICU leading to increased mortality and morbidity.

Hence, it is the hour of need to prevent ventilator associated pneumonia caused by hospital-acquired multi-drug resistant strains by formulating appropriate hospital antibiotic policy and proper infection control practices.

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