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Occurrence of Carbapenem resistant enterobacteriaceae in Ventilator Associated Pneumonia cases admitted to tertiary care center of Wayanad- analysis of in vitro efficacy of Modified Hodge test.

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Abstract:
Background*: The emergence and spread of carbapenem resistant enterobacteriaceae in association with conditions like ventilator associated pneumonia which becomes a significant and a major public health concern in the hospital settings. The main objectives of this study is to analyse the microbiological profile of ventilator associated pneumonia in patients attending the tertiary care hospital and study the occurrence of CRE, also to determine the efficacy of modified Hodge test for detection of carbapenemase producing gram negative rods.*
Materials and method:*The study has been conducted for a period of three months with a sample size of 50. The Endotracheal aspirate (ETA) samples were collected with proper aseptic precautions and sent immediately to microbiology laboratory for processing and identified based on standard microbiological techniques. Modified Hodge Test is then performed on positive sample to study its efficacy.*
Report*:A total of 123 patients were prospectively reviewed for the 3 months study period and among them only 53 patients were infected. Metallo-betalactamases was produced by 48.1% and ESBL by 51.9% of non-fermenters. 1.8% of the pathogens in our study were MDR. These MDR pathogens included Gram-negative bacteria producing ESBL and MBL. 21 isolates (38.1%) showed resistance to carbapenem group of drugs (meropenem and imipenem). Among them maximum resistance was shown by Acenitobactor baumannii (47.6%), followed by Klebsiella pnuemoniae (33.3%) and Pseudomonas aeruginosa (19.1%). Among the 21 isolates which showed carbapenem resistance only 16 of them were found to be positive by modified Hodge test (76.1%).*
Conclusion*: The emergence of carbapenemase-producing multidrug resistant (MDR) gram-negative bacteria is major public health problem particularly in the hospital settings. Prevention of VAP may be carried out by early isolation and decreasing the length of stay along with proper knowledge of the MDR organisms and during the shorter duration of this study we did not come across any VAP cases which can be considered as a successful hospital infection control impact. A detailed study on VAP for longer duration is required to determine for a proper understanding Also knowledge of the susceptibility pattern of the local pathogens should guide the choice of antibiotics, in addition to the likelihood of organisms, as there is an increasing prevalence of MDR, MBL and ESBL pathogens.Testing all isolates showing intermediate or sensitive zone diameter on disc diffusion for production of carbapenemases by Modified Hodge test will avoid treatment failures and development of resistance due to unnecessary use of this class of antibiotic for a better future.*
Key words:*ventilator associated pneumonia, enterobacteriaceae, ModifiedHodge test*

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I. Introduction:

Health care-associated infections represent a major health issue to mankind in terms of personal distress, economical loss, morbidity, and mortality.[1,2] Among all HAI, pneumonia is assumed to be one of the leading causes of death.[3] The occurrence of pneumonia is more in the intensive care units (ICUs) chiefly because of utilization of invasive procedures such as mechanical ventilation.[4,5] Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hrs after endotracheal intubation; initiation of mechanical ventilation (MV) including pneumonia developing even after extubation.[6] Ventilator-associated pneumonia indirectly influences the length of stay, cost of treatment, and mortality. Nearly 10%–20% patients on MV for longer than 48 hrs develop VAP.[7,8] VAP is less severe and is likely with a better prognosis and diagnosis during first 4 days, caused by antibiotic sensitive bacteria. Late onset VAP, which develops after 4

days after initiation of MV, is caused by multidrug resistant (MDR) pathogens and associated with increased mortality and morbidity.[9] The common pathogens causing VAP include aerobic Gram-negative rods such as Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, and Escherichia coli. [8,10,11] Treatment of VAP is usually supportive, along with administration of proper antibiotics. The selection of proper antimicrobial agents, active against the VAP pathogens is an important determinant for reducing morbidity and mortality. Appropriate antimicrobial therapy, when initiated early, has shown to reduce mortality among critically ill patients with VAP. Drug resistance is due to production of extended spectrum β-lactamases (ESBL), AmpC β-lactamase, or metallo-β-lactamase (MBL).[8,11] .There is an urgent need of local surveillance data at routine interval as the frequency of specific MDR pathogens causing VAP may vary by hospital, patients' population, type of ICU patients, exposure to antibiotics, and changes over time.

II. Materials and Methods

Sample size: 50
Study location: Tertiary hospital in Meppadi, Wayanad Kerala
Study duration: 10th July 2019 to 10th October 2019
Inclusion criteria: Patients who satisfied the CPIS > 6 and quantitative culture of endotracheal aspirate with growth thresholds greater than equal to 10^6 cfu/mL
Exclusion criteria: Patients on mechanical ventilation for less than 48 hrs; patients in ICU and not receiving ventilator support and have developed pneumonia; patients diagnosed to have lower respiratory tract infections such as pulmonary tuberculosis, chronic obstructive pulmonary disease, acute respiratory distress syndrome, bronchial asthma on admission will be excluded.
After getting approval from the Research and Ethical Committee a cross-sectional study was conducted in the tertiary care hospital, for a period of 3 months. The informed consent was obtained from each patient's next of kin. The patients who were either admitted directly or transferred from other wards such as surgery, medicine, neurology, cardiology, obstetrics and gynecology and pulmonology will be considered. Procedure for Data Collection:
All patients were monitored at frequent intervals for the development of VAP using CPIS scoring. The medical history and data was recorded from their medical records and bedside charts.

Criteria for Diagnosis of VAP
The diagnosis of VAP were based on clinical and microbiological criteria

Microbiological Techniques
Endotracheal aspirate (ETA) samples were collected with proper aseptic precautions and sent immediately to microbiology laboratory for processing and identified based on standard microbiological techniques. Following that Gram stained findings were considered for interpretation of culture report; polymorphonuclear neutrophils > 10 per high power field and > 1 bacteria per oil immersion field and presence of intracellular bacteria. Ziehl–Neelsen stained preparations were be observed to detect possible co-existence of pulmonary tuberculosis. Antibiotic susceptibility testing of these bacterial isolates were carried out by employing Kirby- Bauer disk diffusion method on Muller Hinton agar (MHA) plate according to CLSI guidelines 2013.

MODIFIED HODGE TEST[12]
A 0.5 McFarland dilution of the Escherichia coli ATCC 25922 in 5 ml of broth or saline was prepared. A 1:10 dilution was streaked as lawn on to a Mueller Hinton agar plate. A 10 µg meropenem or ertapenem susceptibility disk will be placed in the center of the test area. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35 ± 2° C in ambient air for 16-24 hours. Quality control of the carbapenem disks were performed according to CLSI guidelines. After 24 hrs, MHT Positive test show a clover leaf-like indentation of the Escherichia coli 25922 growing along the test organism growth streak within the disk diffusion zone.

STATISTICAL ANALYSIS
A test and Fisher's exact test of independence was used to compare the following variables of interest: sex, age, educational level, height, weight, pulse, blood pressure, size of family, annual family income, smoking, drinking, and the history of diseases. All the reported values, two-sided, and value < 0.05 was regarded as statistically significant for all included studies. Logistic regression was used to select significant predictor variables and to estimate odds ratios (ORs) of these variables and, if possible, to predict outcomes.

III. Result

During the 3-month study period from 10th July 2019 to October 10th 2019, a total of 123 patients were prospectively reviewed. Among them only 53 patients were infected. Only the patients who satisfied the criteria as described were part of the study among them, 39 were males and 14 were females in our study. Age-wise distributions of clinically suspected cases were studied and it was found that susceptible patients belonged to the age group 70-80 years, followed by the patients in 60-70 years age group and the patients in more than 72 years age group. In this study total 55 organisms were isolated from culture of endotracheal aspirates.

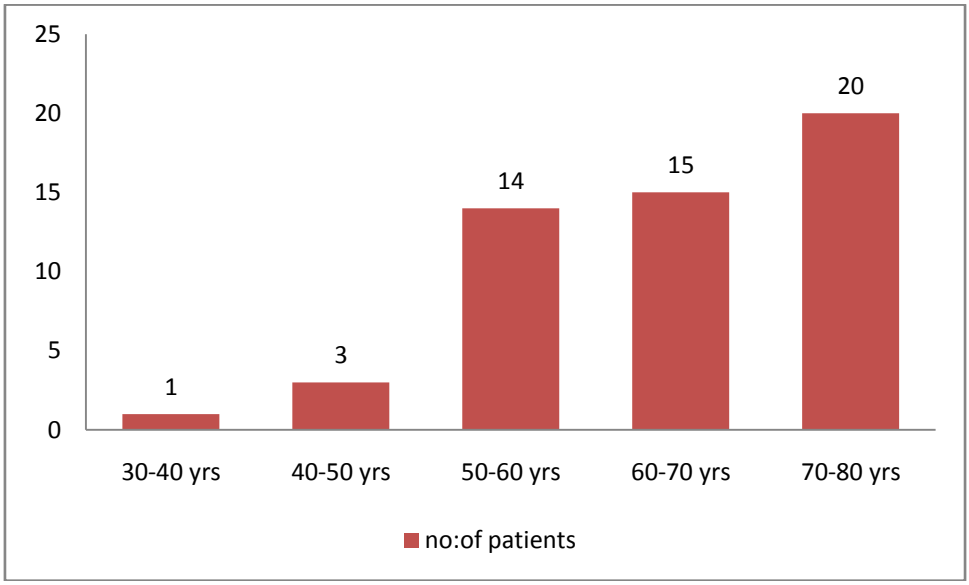
Microbial Pattern in isolated Cases: The isolation was monomicrobial in 50 cases and polymicrobial in 3 cases . Among the isolated organisms, all were Gram negative bacilli, among them most common isolate was *Klebsiella pneumoniae* (36.3%) isolates followed by isolates of *Acinetobacter* (27%), and isolates of Pseudomonas aeruginosa (22%). Total 10 organisms (18.1%) were isolated from ICUs which includes *Klebsiella pneumoniae(4), Acinetobacterbaumannii(3), Pseudomonas(2) and E.coli(1*). .

Detection of ESBLand metallo-betalactamase:
Metallo-betalactamases was produced by 48.1% and ESBL by 51.9% of non-fermenters. 1.8% of the pathogens in our study were MDR. These MDR pathogens included Gram-negative bacteria producing ESBL and MBL.

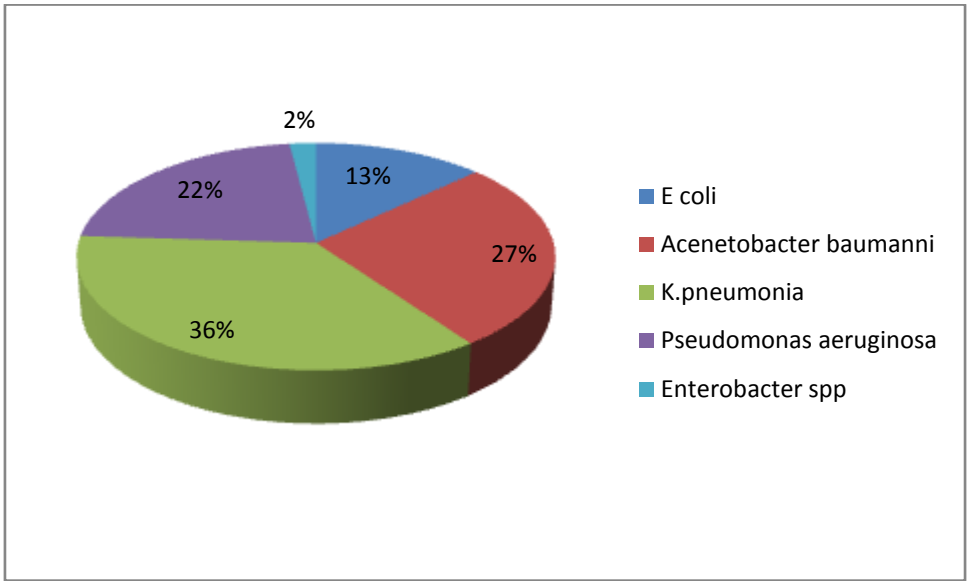
Antibiotic Resistance Pattern: The antibiotic resistance pattern of the various etiological agents is summarized in Table. Ceftazidime and Ampicillin are resistant to most of the isolates. 21 isolates (38.1%) showed resistance to carbapenem group of drugs (meropenem and imipenem). Among them maximum resistance was shown by *Acinetobacter baumannii* (47.6%), followed by *Klebsiella pneumoniae* (33.3%) and *Pseudomonas aeruginosa* (19.1%). Susceptibility pattern of GNB to carbapenems are shown in the Table :5

Age wise distribution of clinically susceptible patients(table:1)

Age wise distribution of classes(yrs)	Frequency
30-40	1
40-50	3
50-60	14
60-70	15
70-80	20



Distribution of isolated organisms



Organisms isolated from ICUs (among total 10) (table:2)

Organism	No:of isolates	Percentage (%)
Klebsiella pneumoniae	4	40
Acinetobacter baumannii	3	30
Pseudomonas aeruginosa	2	20
Escherichia coli	1	10
Total	10	

ESBL and MBL mediated resistance in organism. (table:3)

Organism	No. of isolates	ESBL	MBL	MDR
E. coli	7	6	1	-
Acinetobacterbaumannii	15	5	10	-
Klebsiella pneumoniae	20	12	7	1
Pseudomonas aeruginosa	12	9	3	-
Enterobacterspp	1	1	0	-
Total	55	33	21	1

Antibiotic resistance patterns of isolates (Add the antibiotic list).(table:4)

Organism	No: of isolate	Amp	Amc	Gen	Caz	Cxm	Cip	Ctx	Cot	At	cf	Cfz	Pit	Ak	Ipm	Mer	Net	Cb	Tob	Cpm
Escherichia coli	7	6	4	1	6	6	1	5	4	6	3	0								
Acinetoba-cterbaumannii	15	15	11	7	14	15	5	14	6	15	2	8	9	7	9	10	3	0	0	0
Klebsiellapneumoniae	20	20	14	12	19	20	5	18	14	18	3	8	11	7	7	7	8			
P. aeruginosa	12	0	0	3	11	0	0	0	0	0	1	4	2	3	1	3	0	12	5	1
Enterobac-ter spp	1	1	1		1	1		1												

Susceptibility pattern of Gram-negative bacilli to carbapenems(table:5)

Organism	Total no:of isolates	No:of Isolate susceptible to carbapenem	Percetange(%)
Escherichia coli	7	7	100
Acinetobacterbaumannii	15	5	33.3
Susceptibility in Enterobacteriaceae			
Klebsiella pneumoniae	20	13	65
Psuedomonas aeruginosa	12	8	66.6
Enterobacterspp	1	1	100
Total	55	34	61.8

Resistance pattern Gram negative bacilli to carbapenem (table:6)

Organism	Total no:of isolates	No:of isolates resistant to carbapenem	Percentage of isolate resistant to carbapenem(%)
Escherichia coli	7	0	0
Acinetobacter baumannii	15	10	66.6
Resistance pattern in Enterobacteriaceae			
Klebsiella pneumoniae	20	7	35
Psuedomonas aeruginosa	12	4	33.3
Enterobacterspp	1	0	0
total	55	21	38.1

Bacteria showing positive results in Modified Hodge test(table:7)

Organism	No. of isolates	No:ofCarbapenemResistance	No:ofCarbapenemsensitive	CarbapenemResistance(%)	Carbapenemsensitive(%)
A.baumanni	15	9	6	60%	40%
K. pneumoniae	20	4	16	20%	80%
P.aeruginosa	12	3	9	25%	75%
Total	47	16	31	34%	66%

Among 21 isolates which showed carbapenem resistance only 16 of them were found to be positive by modified Hodge test (76.1%).

ver leaf-like intendation

Negative result of Modified Hodge test

IV. Discussion:

Several studies and surveys have been done on occurrence of carbapenem resistance in Enterobacteriaceae in different parts of the world such as US, China, Austria, Japan, India etc. The reports also show a lot of epidemiological variations. CRE appear to have been uncommon in United States before 1992 and in the Indian scenario, CRE was not reported till recent times. Using the data from the National Nosocomial infection Surveillance (NNIS) system from 1986 to 1990, Gaynes et al (2005) found that 2.3% of 185 Enterobacter isolates tested to be non- susceptible to carbapenem. However over the last few decades, CRE have been reported more commonly. Studies conducted in different parts of India shows varying prevalence for CRE.

Datta et al. (2012) reported a CRE prevalence rate of 7.87% from a study conducted in a tertiary care hospital in North India while Gupta et al (2006) reported carbapenem resistance varying from 17 to 22% among Enterobacteriaceae strains. Wattal et al (2010) also reported a high CRE prevalence rate ranging from 13 to 51% in a tertiary care hospital In Delhi. Thus the significant CRE prevalence recorded in different parts of India emphasize the need for controlling the further dissemination of CRE. Of the CRE identified in the hospital, in this study most of the isolates were obtained from the wards and a significant number from OPD patient samples and then from ICU. This suggest that several CRE isolated may be community acquired.

This study documents a Carbapenem resistance of 38.1% among the total 53 isolates in sputum samples within a period of three months. It shows considerable highest prevalence of resistance among Acenitobacter baumanni (47.6%) followed by Klebsiella pneumoniae, which is not in concordance with the study done by Federico Perez et al (2010) were it revealed a resistance to carabapenem in 90% of the isolated K. pneumoniae and 85% in Acenitobacter baumanni . Similarly the report by Jesse T. Jacob et al (2013) shows carbapenem resistance was more prevalent in K. pneumoniae.

Carbapenems have been considered the treatment of last resort for treatment of AST resort for the management of multi drug resistant infections caused by Enterobacteriaceae. Carbapenamase, which hydrolyzes carbapenems and renders them inactive, have been increasingly reported in Asia, Europe United states and been detected in several other countries. In 2001, LEE et al published a modification of the Hodge test. This was used for detection of carbapenemase production in Pseudomonas species and Acenetobacter species. Among the strains which showed resistance to carbapenem 76.1% were positive with MHT, while some carbapenemase producers were missed by Modified Hodge test. Sensitivity of MHT was 77.4% among genotypically known carbapenemase producers from a study in India (Fomda et al 2014). The findings of our study has the positive results for MHT (76.1 %) similar to these studies and the result correlates with the study of Castan heira et al.(2009) who conclude that MHT may not be a useful screening test for detection of carbapenemase as many MBL producing isolates could not be detected by this test.

Although the MHT is simple and cheap, high false positivity results have been observed, especially in carbapenem-resistant Enterobacteriaceae that produces ESBLs (Carvalhaes et al 2010). However such false positive results were not found in our study as it was compared with the conventional technique. A recent study reported high sensitivities and specificities for the MHT, Cury et al (2012) .The other authors also found a high frequency of inconclusive results. In contrast, Doyle et al (2012) described only 58% sensitivity and 93% specificity. Despite the problems of modified Hodge test and recent increase in rate of fasle-positive and false-negative results with some isolates, the CLSI proposed this test for the confirmation of putative carbapenemase producers (CSLI-2010). However MHT should not be used as a final confirmatory test detection of Carbapenemase production, though it can be a useful screening test for detection of carabapenemase production and can be applied in epidemiological studies. Further studies with larger sample size will definitely throw light into the usefulness of MHT and also in the identification of VAP cases which will definitely prevent the increase in morbidity and mortality.

V. Conclusion:

Carbapenem resistance is comparatively common among ICU patients basically caused by MDR pathogens. During this study VAP cases were not identified. An early isolation followed by prevention of prolonged antibiotic therapy may lead to reduce mortality; associated with VAP. A detailed study on VAP for longer duration is required for a proper understanding of the susceptibility pattern of the local pathogens and this inturn should guide the choice of antibiotics, in addition to the likelihood of organisms, as there is an increasing prevalence of MDR, MBL and ESBL pathogens in the present day world..

Modified Hodge test is an easy and simple test to be performed to detect carbapenemases producing bacteria .There is a very high percentage of carbapenemases producing Gram negative rods in most of the health care facilities. It should be made compulsory that all isolates showing intermediate or sensitive zone diameter on disc diffusion be tested for production of carbapenemases by Modified Hodge test to avoid treatment failures and development of resistance due to unnecessary use of this class of antibiotic for a better future. MHT cannot be considered as a specific test for detection of CRE, but can be made useful in a resource limited setting where molecular diagnostic facility is unavailable and development of resistance due to unnecessary use of this class of antibiotic can be avoided.

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