Research Article

CULTURE PROFILE AND ANTIBIOGRAM OF INFECTIVE ORGANISMS FROM ENDOTRACHEAL SECRETIONS IN MECHANICALLY VENTILATED PATIENTS OF A TERTIARY CARE CENTRE

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Abstract- Introduction: Respiratory infections in critically ill patients are associated with high morbidity and mortality. Patients who are mechanically ventilated are at high risk of acquiring respiratory tract infections due to complex interplay between the endotracheal tube, host immunity and virulence of invading bacteria. To initiate empiric antimicrobial therapy knowledge of local antimicrobial resistance patterns are essential. **Material And Methods:** A cross sectional study of 48 adult patients who were mechanically ventilated for various reasons in ICU of our hospital from June 2015 to May 2017 was undertaken to study profile and sensitive characteristics of infective organisms from endotracheal aspirate obtained after 48 hours of intubation. **Results:** Gram negative bacteria (83.02%) were isolated from most of the patients and Gram positive organisms were 16.98%. The most common being *Acinetobacter spp.* (33.96%), followed by *Klebsiella pneumoniaee* (32.07%) and *Pseudomonas aeruginosa* (15.09%). Gram positive cocci i.e. *Staphylococcus aureus* isolated in (16.98%) patients. Most of these gram negative organisms were susceptible to colistin and tigecycline antibiotics. Most of these gram positive organisms were susceptible to vancomycin and linezolid. **Conclusion:** Gram negative organisms susceptible to colistin, tigecycline and meropenem antibiotics form the predominant isolates in our critical care setup. A local antibiogram for each hospital, based on bacteriological patterns and susceptibilities is essential to initiate empiric therapy, to prevent poor outcomes and help in framing the appropriate institutional antibiotic policy.

Key words- Ventilator associated pneumonia (VAP), nosocomial infections, Endotracheal secretions.

Introduction

Endotracheal intubation is an important procedure for life threatening conditions. The uses of invasive therapeutic procedures have saved many lives but it can also cause life threatening consequences due to severe persistent resistant infections [1]. The invasive therapeutic and diagnostic methods have increased the incidences of nosocomial infections particularly in ICU's [2-5]. Intensive care patients on mechanical ventilation/ orotracheal intubation are frequently colonized with this microbial source of exogenous origin or endogenously from the patients themselves [6-8]. These colonized bacteria cause Ventilator associated pneumonia (VAP) [9,10]. Despite advances in patient care, these changing floras complicate therapy by acquiring drug resistance and altering their sensitivity pattern [11]. An updated knowledge of local epidemiological and susceptibility profile is recommended for guiding the clinicians regarding empirical choice of antibiotics and has become mandatory along with adequate clinical diagnosis and bacterial confirmation [12]. The quantitative endotracheal aspirate culture is a useful non-invasive tool for the diagnosis of VAP pathogens [13]. The present study was undertaken to determine the outcome of VAP and to identify bacteriological profile of infective organisms and their susceptibility pattern associated with duration of mechanical ventilation and length or hospitalization.

Materials and Methods

Type of study: A cross sectional study of adult patients aged above 18 years who were mechanically ventilated for more than 48 hours for various reasons in ICU of our hospital.

Study Period: June 2015 to May 2017.

Ethics statement: The approval to conduct this study was obtained from the Institute Ethics Committee.

Sample collection: Endotracheal secretions were obtained after 48 hours of intubation by sterile suctioning and the secretion was subjected for Gram stain and culture. Non-repeat positive culture samples were subjected to drug susceptibility testing.
Endotracheal aspirate >1ml was collected under aseptic precautions after 48 hours of intubation, using a suction catheter with a mucus extractor and sent to the laboratory immediately for microbiological processing,

Microbiological methods: which involved semi quantitative and quantitative cultures and direct Gram staining of the specimens. The findings were tabulated as type of causative micro-organism and antibiotic sensitivity. For culture, all samples were inoculated on Blood agar and Mac Conkey agar plates using standard sterilized 4mm nichrome wireloop which holds 0.01ml of ETA. EA cultures were quantified using calibrated loops. Plates are incubated overnight at 37°C. Colonies were then counted and bacterial concentrations (CFU/ml) were calculated. Interpretation: each colony corresponded=20,000 CFU/ml. Microorganisms with counts > 10^5 CFU/ml were submitted for identification and antimicrobial susceptibility testing. If no growth was detected on any plate, the incubation was extended for 24 hrs.
Isolates were identified on the basis of colony morphology and biochemical

reactions as per conventional isolation and identification procedure as per the Clinical Laboratory Standards Institute (CLSI) guidelines [14]. Bact/Alert 3D system and VITEK 2 were used where required. Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion method using commercially available discs (HiMedia Laboratories) on Mueller Hinton agar and also β-lactamases production was detected by phenotypic confirmatory disc diffusion test. ATCC strains of *E. coli* 25922 and *K. pneumoniae* ATCC 700603 was used as quality control strains for the detection of β-lactamases production.

Detection of Extended Spectrum β-lactamases (ESBL) Production: All isolates were subjected to screening for ESBL production using 30 µg ceftazidime disc (CAZ) by disc diffusion method with CLSI guidelines [14]. Isolates having zone of inhibition >22 mm and < 22 mm were considered as susceptible and non-susceptible to ceftazidime (CAZ) i.e. ESBL non-producer and producer respectively. Phenotypic confirmatory test for ESBL producers were done by double disc diffusion test (DDDT), for all the ESBL producing isolates as per CLSI guidelines [14].

Double disk diffusion method (DDDT): In this test a disc of ceftazidime (30µg), cefotaxime (30µg) alone and a disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10µg) were used for each isolate. Both the discs were placed on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 37°C. A ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive.

Detection of AmpC β lactamase production: All isolates were subjected for screening for AmpC β-lactamase production using 30 µg cefoxitin disc (CX) by disc diffusion method as of CLSI guidelines [14]. Isolates having zone of inhibition >18 mm and <18 mm mere considered as susceptible and non-susceptible to cefoxitin (CX) i.e., β-lactamase non-producer and producer respectively. All the isolates were subjected to confirmatory tests for AmpC β-lactamase production.

Confirmatory test for AmpC β–lactamases (cefoxitin-cloxacillin double disc synergy test)
The test is based on inhibitory effect of cloxacillin on AmpC enzyme. Disc containing Cefoxitin (30µg) and Cefoxitin cloxacillin (30/200 µg) was used. A difference in the inhibition zone of cefoxitin-cloxacillin minus the cefoxitin alone ≥4mm considered positive for AmpC production.

AmpC E-test: The isolate to be tested was inoculated on MHA by using standard methods. AmpC E-test strip (biomeriéux SA) was placed over the culture. The E-strip contains graded concentrations of Cefoxitin (CX) and Cefoxitin + Cloxacillin (CXX) on opposite sides.A ratio of the minimum inhibitory concentrations (MICs) for CX and CXX ≥8 or a phantom zone formed was considered as a positive result i.e., the isolate is an AmpC β-lactamase producer.

Detection of MBL Production: Screening for carbapenem resistant GNB from the routine clinical samples was done by using 10µg imipenem discs (HiMedia). Isolates having zone of inhibition >16 mm and < 16mm were considered as susceptible and non-susceptible to Imipenem (I) i.e. MBL non-producer and producer respectively. All the isolates were subjected to confirmatory test for MBL production

Imipenem-EDTA Disc method (Combined Disc test): To confirm the MBL production phenotypically in imipenem resistant GNB isolates. MBL activity is inhibited by chelating agents. EDTA is a chelating agent. When in one MHA plate both imipenem and imipenem+EDTA discs are placed, the EDTA present will chelate the metal ion present in the MBL. Hence an increase in the zone of inhibition will be present with the IMP+EDTA disc as compared to only imipenem. ≥7 mm increase in size is taken as positive for MBL production.

Epsilometer test (E-test): E-test was done on all imipenem resistant to calculate

the minimum inhibitory concentration (MIC) for imipenem and to screen for MBL in them [15]. E-test MBL strip is a plastic carrier (5×60mm) calibrated with a reading scale in µg/ml, IP stands for imipenem (4-256µg/ml) and IPI stands for imipenem plus a constant level of EDTA (1-64 µg/ml).The presence of MBL is reflected by a reduction of the IP MIC by 3 log dilutions in the presence of EDTA or the appearance of a phantom zone or deformation of the IP ellipse.

VAP was diagnosed by using modified clinico–pulmonary infection score by Singh, *et al.* [16]. They observed that the empiric antibiotic treatment could be stopped on day 3, if the scoring on m-CPIS is <6 and can be continued for the entire course if m-CPIS is >6.

Statistical Analysis
All the collected data was subjected to SPSS (v2.0) statistical analysis. Data was represented as frequencies & mean with standard deviation.

Observations and Results
A total of 48 patients (29 males, 19 females, mean age 53.02 years, SD = 16.8) were enrolled. Of 48 patients, 11 patients were admitted with Cerebro-vascular accident (CVA). These 11 patients were either hypertensive or diabetic. Total 10 patients were diabetic and 12 patients were hypertensive. Seven patients were admitted with renal failure.
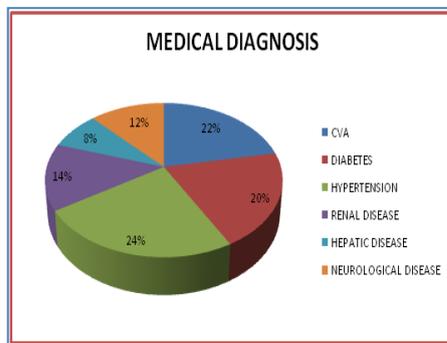

Fig-1 Diagnosis at the time of admission

Diabetes mellitus with hypertension being major risk factor involving 6 (12.5%) cases, next were Stroke with hypertension (10.41%), Septicaemia with Acute kidney injury (6.25%), Diabetes with CVA (6.25%). Septic shock, Alcoholic liver disease, chronic kidney disease (CKD), intracranial haemorrhage, Guillain-Barre syndrome, operated case of craniotomy (4.16%) each. The main surgical cause of ICU shifting was post-surgical. The mean duration of ventilation days was 7.8 days (SD = 2.8) in patients. Organisms isolated from endotracheal aspirate: Among 53 isolates, 9 (17%) were Gram positive organism and 44 (83%) were Gram negative organisms. Poly-microbial in 5 (10.4%) patients and mono-bacterial in 43 (89.6%) patients. Out of the 5 poly-microbial cases, one was due to *Acinetobacter spp.* and *Klebsiella pneumonia (K. pneumoniae)*, another one by *P. aeruginosa* and *Acinetobacter species*. Third one by *K. pneumoniae* and *Pseudomonas aeruginosa*, and fourth one by MRSA and *K. pneumoniae*. Fifth one by MRSA and *Citrobacter koseri*. Among the 48 patients of VAP, *Acinetobacter spp.* (n=18) was the most common organism followed by *K. pneumoniae* (n=17), *P. aeruginosa* (n=8) and MRSA (n=8).
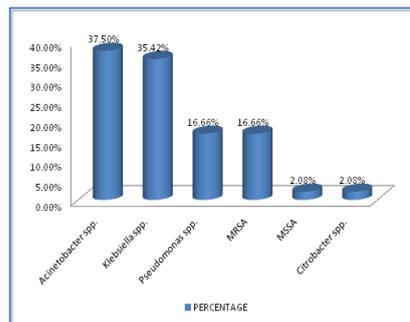

Fig-2 Distribution of micro-organism from endotracheal aspirate in ICUs

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Antibiotic resistance pattern in Gram negative organisms:

Table-1 *Sensitivity and resistance pattern of Gram negative organisms*

Antibiotics	Acinetobacter spp. (18)		K. pneumoniae (17)	
	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)
Amikacin	17 (94.44%)	1 (5.55%)	14 (82.35%)	3 (17.64%)
Gentamicin	16 (88.88%)	2 (11.11%)	13 (76.47%)	4 (23.52%)
Ampicillin	18 (100%)	0 (0%)	17 (100%)	0 (0%)
Norfloxacin	17 (94.44%)	1 (5.55%)	16 (94.11%)	1 (5.88%)
Chloramphenicol	12 (66.66%)	6 (33.33%)	8 (47.05%)	9 (52.94%)
Imipenem	11 (61.11%)	7 (38.88%)	6 (35.29%)	11 (64.7%)
Ceftazidime	17 (94.44%)	1 (5.55%)	17 (100%)	0 (0%)
Ceftazidime-Clavulanic acid	16 (88.88%)	2 (11.11%)	15 (88.23%)	2 (11.76%)
Ceftazidime-Tazobactam	7 (38.88%)	11 (61.11%)	12 (70.58%)	5 (29.41%)
Cefoxitin	17 (94.44%)	1 (5.55%)	16 (94.11%)	1 (5.88%)
Cefotaxime	17 (94.44%)	1 (5.55%)	17 (100%)	0 (0%)
Tigecycline	0 (0%)	18 (100%)	0 (0%)	17 (100%)
Colistin	0 (0%)	18 (100%)	0 (0%)	17 (100%)
Ofloxacin	6 (33.33%)	12 (66.66%)	5 (29.41%)	12 (70.58%)
Piperacillin -Tazobactam	6 (33.33%)	12 (66.66%)	5 (29.41%)	12 (70.58%)

Table-2 *Sensitivity and resistance pattern of Pseudomonas aeruginosa*

Pathogens (no of isolates)	Antibiotics	Resistant (%)	Sensitive (%)
P. aeruginosa (n=8)	Ciprofloxacin	1 (12.5%)	7 (87.5%)
	Gentamicin	2 (25%)	6 (75%)
	Piperacillin	5 (62.5%)	3 (37.5%)
	Carbenicillin	5 (62.5%)	3 (37.5%)
	Imipenem	0 (0%)	8 (100%)
	Ceftazidime	4 (50%)	4 (50%)
	Ceftazidime-Clavulanic acid	2 (25%)	6 (75%)
	Ceftazidime-Tazobactam	1 (12.5%)	7 (87.5%)
	Cefoxitin	7 (87.5%)	1 (12.5%)
	Cefotaxime	1 (12.55%)	7 (87.5%)

Staphylococcus aureus was most resistant to Erythromycin (87.5% resistant), followed by Cotrimoxazole (62.5% resistant) and all isolates were sensitive to Vancomycin and Linezolid (100% sensitive). Multi Drug Resistant (MDR) Organisms: Most of the isolates were Multi Drug Resistant, meaning they were resistant to three or more group of antibiotics. Among 53 organisms isolated from this study, 40 (75.47%) were multi drug resistant organisms, that included ESBL producers, AmpC producers, MBL producers and MRSA. ESBL producing organisms: All the Gram negative organisms (n=44) were subjected to screening tests using Ceftazidime for ESBL production. 37 isolates were resistant to Ceftazidime and 19 (51.35%) of these were ESBL producers which were confirmed by Double Disk Diffusion Method (DDDT).

Table-3 *Distribution of ESBL producing organisms*

Organism	ESBL producer	ESBL non-producer
Acinetobacter spp. (n=18)	10	8
K. pneumoniae (n=17)	5	12
P. aeruginosa (n=8)	3	5
Citrobacter koseri (n=1)	1	0

AmpC producing organism: Screening of AmpC production was done by Cefoxitin resistance, and those were resistant to Cefoxitin, were confirmed for AmpC production by Cefoxitin-cloxacillin Double Disk Synergy Test (CC-DDS). 38 isolates out of the 44 Gram negative isolates, were resistant to Cefoxitin. Three organisms (1 each of *Citrobacter koseri*, *Acinetobacter* spp., and *K. pneumoniae*) were showing AmpC production. MBL producers: All Gram negative organisms (n=44) were subjected to screening tests using Imipenem. 16 isolate out of 44 Gram negative organisms were resistant to Imipenem. Out of which 2 were MBL producers by Imipenem-EDTA disc method (combined disc test) and Imipenem E-strip test. Both the MBL producers were *K. pneumoniae.*

Discussion

In our study endotracheal secretions were sent for bacteriological culture and sensitivity to identify the organisms which would help in initiating and or modifying antibiotic therapy appropriately and help in preventing the occurrence of ventilator associated pneumonia (VAP) or Hospital acquired pneumonia (HAP) and helps bring about favourable outcome. VAP is increasingly found to be associated with multi-drug resistant organisms that explain the high rate of colonization due to these pathogens. The high incidence density of VAP in the study when compared with the studies of developed countries, could be possibly due to the following reasons- It is to be taken into account that most developing countries lack the legal framework or standards governing the implementation of infection control programs. Hand hygiene is not properly followed in most health care facilities. Majority of hospitals in developing countries receive limited financial or administrative support, resulting in a scarcity of necessary funds to deal with infection control. Etiological agents also vary based on type of ICU and patient studied. Therefore, knowing the susceptibility pattern of local microbial isolates will guide the clinicians to choose the appropriate empirical therapy. This may be followed by de-escalation strategy focused on narrow spectrum antibiotics after the culture and sensitivity report. It reduces the colonization and also leading to better outcome of patients with less morbidity and mortality. To reduce the incidence, more efforts also required to increase the knowledge in medical and paramedical staff regarding its prevention like nursing care and judicious use of broad spectrum antibiotics with good infection control practices. In our study gram negative bacteria was the most common isolate with *Acinetobacter spp.* being the most common organism followed by *K. pneumoniae*, *P. aeruginosa* which were sensitive to Colistin, Tigecycline and Meropenem. Culture positivity was more common in elderly male patients with hypertension and diabetes.

Among 53 isolates, 9 (17%) were Gram positive organism and 44 (83%) were Gram negative organisms. Ali Shamshad, *et al.*[17] reported that major pathogenic bacteria isolated were Gram negative organisms (74%); *E. coli*, *Pseudomonas spp.*, *Klebsiella spp.* and *Acinetobacter spp.* were the commonest among them. Mohan, *et al.* [18] in 2013 isolated *Acinetobacter spp.* in 26 cases and *K. pneumoniae* in 9 cases from 48 patients with VAP [15]. A study done by Rajesh Chawla, *et al.,* in 2008 reported that most common etiology of VAP in India was *Acinetobacter* spp. (38%), followed by *K. pneumoniae* (23%) [19]. These findings were well correlated with our study also, where we found that Gram-negative organisms were the most common associated pathogens (83%). *Acinetobacter* spp. (37.50%) and *K. pneumoniae* (35.42%) were the most common organisms isolated from our patients with mechanical ventilation, followed by *P. aeruginosa* (16.66%) and MRSA (16.66%) [Fig-2].

These organisms are particularly common in the Indian hospital settings, where the humid and warm conditions of tropical climate favour infection [20]. These organisms are ubiquitous, persist for months on inanimate surfaces and more importantly, are inherently resistant to the commonly used antibiotics. Thus, they are able to colonize the mucosa of patients and the surfaces of various devices. The production of bio-films by these bacteria also gives them survival advantage by protecting them from antibiotics used in the hospitals [21].

Particularly alarming is the antibiotic susceptibility profiles of these microorganisms, especially of multidrug resistant organisms. In our study, colistin (100% sensitive) and tigecycline (100% sensitive), were the most effective antibiotic against *K. pneumoniae* and *Acinetobacter spp.* [Table-1]. Joseph, *et al.* found colistin was highly active against *Acinetobacter spp* [22]. For β-Lactam/β-Lactam inhibitor combination; tazobactam was more effective than clavulanic acid for both *K. pneumoniae* and for *Acinetobacter spp.* In this study [Table-1]. In the present study, most of the isolates (75.47%) were multidrug resistant (MDR) and most of the Gram negative organisms (51.35%) were ESBL producers [Table-3]. In a study of Saldana Dominic, *et al.*, [23], 52.7% isolates were MDR pathogens. Joseph, *et al.* found 78.7% MDR pathogens, in their study [22]. Our previous

on prevalence and antimicrobial susceptibility showed high incidence of *K. pneumonia* infections as well as Staphylococcal infections [24-28]. Dey, *et al.*, also observed a high prevalence of ESBL producers in their study [29]. *Pseudomonas* and *Acinetobacter spp.* showed multi-drug resistance (MDR), even to carbapenems [21], whereas certain studies reported a lower incidence of meropenem resistance. The high incidence of MRSA in our study correlated well with the study done by Gupta, *et al.*[16] The incidence of MDR isolates was found to be high (75.47%), which indicated the need for appropriate empirical antibiotic treatment effective against MDR organisms.

Out of 44 Gram negative isolates, 38 isolates were resistant to Cefoxitin and among them 3 organisms were showing AmpC production. All the Imipenem resistant isolates were tested for Metallo beta Lactamases (MBL) production. Two were detected to be MBL producers.

The etiologic agents vary according to the population of patients in an ICU, duration of hospital stay, pre-existing illness and airway commensals and prior antimicrobial therapy. To initiate an empiric antimicrobial therapy, knowledge of microbial flora of the locality and their sensitivity and resistance patterns are essential. Such information needs to be analyzed periodically and institution based antibiotic policies formed from time to time and made available to all consultants treating infectious diseases to facilitate better outcomes.

Limitations: The major limitations of our study, in the form of small sample size and single centre data; these findings should be confirmed by further prospective studies with large sample size from other centres.

Conclusion

Gram negative organisms susceptible mostly to Colistin, Tigecycline and Carbapenem group of antibiotics form the predominant isolates in our critical care setup. Initial appropriate empiric antibiotic therapy on admission to ICU helps in decreasing the mortality and duration of ICU stay. An updated local antibiogram for each hospital and ICU based on local bacteriological patterns and susceptibilities is essential to guide optimally dosed initial empiric therapy. With an empiric antibiotic regimen, de-escalation is the key to reduce emergence of resistance. Culture of ET aspirate is easy, cost-effective procedure which helps in identifying the organism. Delays in initiation of antibiotic treatment may lead to poor outcomes. There is a risk of emergence of MDR pathogens with inadequate, inappropriate antibiotic treatment.

Application of research: The microbiological profile & sensitivity pattern of the local community helps in framing the appropriate institutional antibiotic policy for better outcomes.

Research Category: Culture Profile and Antibiogram

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