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RESEARCH ARTICLE

Bacteriological Profile of Endotracheal Aspirates and their Antibiotic Susceptibility Pattern

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Abstract

Early onset Ventilator associated pneumonia (VAP) is usually less severe, associated with a better prognosis, and is more likely caused by antibiotic- sensitive bacteria. Late-onset VAP, is usually caused by multidrug resistant (MDR) pathogens and is associated with increased morbidity and mortality. Therefore, the local microbial flora causing VAP needs to be studied and appropriate therapy based on the early endotracheal (ET) aspirate culture report can help managing this group of patients. The present study was carried out in the department of Microbiology, JSS Hospital, Mysore from January 2017 to December 2017. Bacterial pathogens and antibiotic resistance pattern of isolates from ET aspirates of patients on mechanical ventilation in Intensive care units were source of data. A total number of 1432 samples were received in the lab for ET aspirate culture. Among these 1432 samples, 1055 showed growth ranging from 10 ^{2}to 10^{6} colony forming units/ml (CFU/ml), 124 had no growth at all and 253 had no significant growth (<10^{2} CFU/ml) Out of 1055 isolates,1023 were Gram negative bacteria and 32 were Gram positive cocci . Acinetobacter, Pseudomonas and Klebsiella were the major pathogens in our study. Majority of them being MDR, maximum sensitivity was observed for Tigecycline and colistin in these isolates. Knowledge of causative microbial flora of VAP along with information on the susceptibility patterns will help in selection of the appropriate antibiotic for therapeutic use for better outcome.

Keywords: VAP, ET aspirates, Early onset, MDR, susceptibility pattern.

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INTRODUCTION

Ventilator associated pneumonia (VAP) is a globally known notorious nosocomial infection that occurs more than 48 hours after a patient begins mechanical ventilation (MV)making recovery more difficult for patients who are already critically ill.[1] Early onset VAP is usually less severe, associated with a better prognosis, and is more likely to be caused by antibiotic- sensitive bacteria. Late-onset VAP, is usually caused by multidrug resistant (MDR) pathogens and is associated with increased morbidity and mortality[2,3]. VAP results from microaspiration, which is primary route of bacterial entry into respiratory tract. The ET tube acts as an obstacle to host defences by inhibiting the action of cilia, swallowing, and spontaneous coughing by the patients. Risk factors that are associated with increased incidence of VAP are body position, state of consciousness, enteral nutrition, prior exposure to antibiotics, use of a nasotracheal tube and pre-existing conditions such as acute respiratory distress syndrome[4].

Typically, diagnosis includes clinical, radiologic and microbiologic evidence of infection. However, the exact criteria for diagnosis varies widely among institutions and thus results in a wide range of reported VAP mortality rates, which is stated to be anywhere from 0-76% [5,6,7]. Many studies from India have investigated the causative organisms of VAP. *Pseudomonas* species, *Acinetobacter* species, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* are the common pathogens with varying prevalence and quite often being MDR [6,8,9]. Therefore, the local microbial flora causing VAP needs to be studied and an appropriate therapy based on the early ET aspirate report can reduce the mortality and cost in managing this group of patients

Aim

In view of increasing incidence of VAP and MDR nature of pathogens in our hospital, this study was taken up to detect the bacteriological profile and sensitivity pattern of isolates from Endotracheal aspirates

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, JSS Hospital, Mysore, Karnataka, for a period of 1 year from January 2017 to December 2017. This was a prospective study. ET aspirates sent from various intensive care units sent to Microbiology laboratory formed the material of our study

Bacterial identification and isolation

The ET samples received in the Microbiology lab were subjected to microscopy and culture. Gram stain of Lower respiratory tract secretions is an immediate procedure that can guide management and it has a reasonable correlation with culture results Semiquantitative culture was done on blood agar and MacConkey agar and incubated overnight at 37°C for 24 to 48 hrs for bacterial growth and isolation. The isolated organisms were processed with Vitek 2 systems for identification as well as Antibiotic susceptibility testing. Colony count of 10^6 CFUml

Table 1. Distribution of microbial isolates in colony forming units per ml

	10^2	10^3	10^4	10^5	10^6 or more
Acinetobacter	26	57	23	192	130
Klebsiella	19	32	20	136	77
Pseudomonas	10	27	9	117	62
E.coli	2	2	2	12	11
Proteus	0	0	0	0	3
Citrobacter	0	3	1	2	0
Enterobacter	0	0	2	3	1
Serratia	2	1	2	10	5
Stenotrophomonas	1	2	4	4	3
Burkholderia	0	0	0	3	3
Morganella	0	1	0	0	1
S.aureus	0	0	8	13	4
Coagulase negative *Staphylococcus*	2	5	0	0	0

or more was considered significant for VAP . ET aspirates yielding growth between 10^{2} to 10^{6} CFU/ml, whose microscopy revealed moderate to plenty of inflammatory cells were also processed.

RESULTS

A total number of 1432 samples were received in the lab for ET aspirate culture. Among these 1432 samples, 1055 showed growth ranging from 10^{2} to 10^{6} CFU/ml or more, 124 had no growth at all and 253 had no significant growth (<10^{2} CFU/ml) Among 1055 bacterial growth isolates, 377 isolates showed growth 10^{6} CFUml or more which is considered significant for VAP and 731 isolates ranging 10^{2} to 10^{6} CFU/ml.

Out of 1055 isolates, 1023 were Gram negative bacteria and 32 were Gram positive cocci. Out of 1023 GNB, 428 were *Acinetobacter* species,284 were Klebsiella species,225 were *Pseudomonas* species, 29 were *E.coli*, 3 *Proteus* spp, 6 *Citrobacter* spp, 6 *Enterobacter* spp, 20 Serratia, 14 *Stenotrophomonas*, 6 *Burkholderia* spp and 2 *Morganella* spp. *Acinetobacter baumanii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the most common isolates in our study. Amongst the 32 gram positive isolates

Table 2. Antibiotic Resistance pattern of Acinetobacter

Antibiotics	Sensitive	Intermediate	Resistant
Piperacillin/Tazobactum	33(7%)	3(0.7%)	392(92%)
Ceftazidine	18(4%)	20(4.6%)	390(92%)
Cefoperazone/ Sulbactum	100(23%)	66(15%)	262(62%)
Cefepime	31(7.2%)	14(3.2%)	383(89.4%)
Aztreonam	10(2.0%)	5(1.16%)	413(95%)
Doripenem	37(8.64%)		391(92%)
Imipenem	34(7.94%)	8(1.86%)	386(90.1%)
Meropenem	39(9.11%)	8(1.86%)	381(89.01%)
Amikacin	17(3.97%)	1(0.23%)	410(95.79%)
Gentamicin	78(18.22%)	32(7.4%)	318(74%)
Ciprofloxacin	30(7.9%)	12(2.80%)	386(90%)
Levofloxacin	32(7.4%)	52(12.14%)	344(80.3%)
Minocycline	242(56.54%)	46(10.74%)	140(32.7%)
Tigecycline	346(80.84%)	75(17.52%)	7(1.63%)
Colistin	412(96.26%)		16(3.7%)
Cotrimoxazole	69(16.12%)		359(84%)

Table 3. showing Antibiotic resistance pattern of Pseudomonas

Antibiotics	Sensitive	Intermediate	Resistant
Ticarcillin/Clavulinic acid	72(32%)	59(26.22%)	94(42.22%)
Piperacillin/Tazobactum	110(48.88%)	25(11.11%)	90(40%)
Ceftazidine	125(55.55%)	20(8.88%)	80(35.55%)
Cefoperazone/ Sulbactum	127(56.44%)	49(21.77%)	49(21.77%)
Cefepime	119(52.8%)	20(8.88%)	86(38.22%)
Aztreonam	81(36%)	59(26.22%)	85(37.77%)
Doripenem	132(58.66%)	3(1.33%)	90(40%)
Imipenem	136(60.4%)	0(0%)	89(39.5%)
Meropenem	126(56%)	13(5.7%)	86(38.22%)
Amikacin	150(66.6%)	71(31.5%)	4(1.7%)
Gentamicin	119(52.88%)	3(1.33%)	32(14.22%)
Ciprofloxacin	118(52.44%)	13(5.77%)	94(41.77%)
Levofloxacin	121(53.77%)	9(4%)	95(42.22%)
Colistin	206(91.55%)	2(0.8%)	17(7.55%)

25 were *Staphylococcus aureus*, 3 *Streptococcus pneumoniae*, 2 Beta hemolytic Streptococci and 2 coagulase negative Staphylococcus.

Majority of Enterobacteriaceae apart from *Klebsiella* isolates were sensitive to Imipenem, Ertapenem, Meropenem, Gentamicin, Amikacin, Tigecycline, Colistin, Cotrimoxazole, Ampicillin. Among the 25 Staphylococcus aureus, 4 were Methicillin resistant Staphylococcus aureus sensitive Vancomycin and Linezolid.

Table 4. Antibiotic resistance pattern of *Klebsiella*

Antibiotics	Sensitive	Intermediate	Resistant
Amoxyclav	41(14.43%)	44(15.49%)	199(70.07%)
Piperacillin/Tazobactum	48(16.9%)	10(3.5%)	226(79.5%)
Cefuroxime	43(15%)	6(2.11%)	235(82.7%)
Cefuroxime axetil	48(16.9%)	5(1.76%)	231(81.3%)
Cefoperazone/ Sulbactum	77(21%)	3(1%)	204(71.83%)
Cefipime	49(17%)	13(4.57%)	222(78.3%)
Ertapenem	98(34.5%)	13(4.57%)	173(60.91%)
Nalidixic acid	74(26.05%)	16(5.63%)	194(68%)
Imipenem	119(41.90%)	63(22.18%)	102(35.91%)
Meropenem	84(29.57%)	10(3.52%)	190(66.90%)
Amikacin	169(59.5%)	40(14.08%)	75(26.40%)
Gentamicin	120(42.2%)	6(2.11%)	158(55.63%)
Ciprofloxacin	83(29.22%)	24(8.45%)	177(62.32%)
Tigecycline	180(63.3%)	64(22.53%)	40(14%)
Colistin	270(95%)	1(0.35%)	13(4.57%)
Cotrimaxozole	102(35.9%)	2(0.70%)	180(63.38%)
Ceftriaxone	40(14.08%)	2(0.70%)	242(85.21%)

Table 5. Range of Values of MIC of Tigecycline and Colistin for most frequent MDR isolates.

Oraganisms	Tigecycline			Colistin		
	Sensitive ≤0.5 - 2	Intermediate >2 – 6	Resistant >8 – 16	Sensitive ≤0.5 - 2	Intermediate >2 – 6	Resistant >8 – 16
Acinetobacter	346	75	7	412	0	16
Klebsiella	180	64	40	270	1	13
Pseudomonas		206	2	17		

DISCUSSION

Prompt identification of organisms from ET aspirates in ventilated patients and accurate selection of antimicrobial agents, based on MIC represent important clinical goals in prevention of VAP. Sound knowledge of susceptibility pattern of the locally prevalent pathogens is necessary for choosing the appropriate antibiotics.

In our study, overall, Gram-negative bacilli predominated over Gram-positive cocci. *Acinetobacter* spp, *Klebsiella* spp followed by *Pseudomonas* were the most common isolates identified. Other significant Gram negative isolates were *E.coli, Serratia, Stenotrophomonas, Enterobacter, Citrobacter, Proteus* and *Burkholderia*. Among Gram positive organisms *Staphylococcus aureus* was most common. The antibiogram pattern of isolates showed that Maximum resistance of *Acinetobacter spp* and *Pseudomonas* were seen in Aztreonam, Cefipime, Doripenem, Imipenem, Ciprofloxacin, Piperacillin/ Tazobactum. Maximum sensitivity was observed with Colistin, Tigecycline and Minocycline. Our findings were similar to the other studies[6,7,8,9]

In last two decades, Gram negative bacteria have become important nosocomial

pathogens throughout the world and is a leading problem in treatment owing to its multidrug resistance. Colistin, which is an old antimicrobial agent, is very active against these. Susceptibility to Colistin was reported as 97.9–100% in various studies. There was least resistance to Colistin in our study. It appeared to be a good option apart from Tigecycline, in the treatment of VAP developed with MDR pathogens.

CONCLUSION

We conclude that VAP in mechanically ventilated patients is on rise and has been continually associated with indiscriminate and irrational use of antibiotics which contribute to emergence of drug resistant strains. Knowledge of their causative microbial flora in a local setting along with information on the susceptibility patterns will help in selection of the appropriate antibiotic for therapeutic use and a better outcome. It is clear that Tigecycline and Colistin become the last resort antibiotics in most cases. Within no time in future, we can expect resistance to these antibiotics also making us reach the dead end. Hence, resistance rates should be pursued closely. Newer alternative drugs revealing promising results to solve the problem is the need of the hour.

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