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Bacteriological Profile and Antimicrobial Susceptibility Pattern of Endotracheal Tube Secretions of Patients in ICU of A Teritiary Care Hospital

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Abstract

Background

Ventilator-associated respiratory infections in ICU are responsible for high morbidities and mortalities worldwide due to emergence of resistant bacteria. The current study was intended to determine the antibiotic - resistance pattern of bacteria isolated from Endotracheal secretions of such patients.

Materials and Methods

This was a descriptive cross-sectional study carried out in a tertiary care hospital in Assam. The data of profile and antibiotic pattern of bacterial isolates recovered from ET aspirates of ventilated patients during the period of December 2020 to November 2021. The data were analysed using the Statistical Package for Social Sciences (SPSS) software version .Descriptive statistics were applied to find the frequencies and percentages.

Results

In total, 196 bacterial isolates were recovered from ET secretions of 389 ventilated patients.The rate of isolation was predominant among Males(56.63%). Gram Negative Bacilli (GNB) were predominantly (79.59%) isolated. Klebsiella pneumoniae (39.79%) was the most common isolate and most of them were multi-drug resistant strains. Acinetobacter baumannii (19.38%) and Pseudomonas aeruginosa(18.36%) were the other common GNB , where as Staphylococcus aureus was the most frequently isolated Gram Positive bacteria. Carbapenems showed good in-vivo activity against GNB's and Oxazolidinones among GPC's reflecting good choice for empirical therapy.

Conclusion

Gram Negative Bacteria were the predominant isolates in ET secretions of ventilated patients in ICU setup. Klebsiella being the most common one. There

was an alarmingly high rate of antimicrobial resistance among GNB. An Antibiogram specific for healthcare setting is a must that shall be based on bacteriological profile and its sensitivity pattern.

Keywords

Klebsiella, critical care, drug resistance, mechanical ventilation, respiratory infections

Introduction

Hospital associated infections and antibiotic resistance are the important global public health concerns(1,2). Respiratory tract infections in critically ill patients are associated with high morbidity andmortality. Patients who are intubated are at a risk of acquiring respiratory infections due tothe old age, underlying morbid diseases like DM and depressed immunity due to treatmentwith chemo therapeutic drugs .The intubation for mechanical ventilation is one of the life-saving practices employed for patients admitted to ICU's(2). In case of intubated patients, colonization in therespiratory tract is more common (3). Mechanical ventilation is responsible for 6-to-10-foldincrease in the risk of respiratory tract infection. Apart from this, the emergence of MDRpathogens is an additional problem. Various factors like new mutations, selection of resistantstrains and use of high-level antibiotics influence the rapid spread of MDR microbes in theICU. Thus, it is recommended to the clinicians to have updated knowledge of localepidemiological and susceptibility profile for the appropriate use of antibiotics. Therefore, theaims and objectives of the present study were to analyse the microbiological profile of endotracheal secretions in ICU patients on mechanical ventilators.

Material and Methods

It was a descriptive cross-sectional study conducted in the Teritiary care Hospital over a period of 12 months. All the patients who were admitted in ICU and were on mechanical ventilators.

Inclusion Criteria

We collected all the data from the enrolled patients after a proper informed consent, in the form of: Name, Age, Sex, ID, detailed clinical history, Date of admission in ICU, Date of endotracheal tube intubation, Date of sample collection and Details of antibiotics therapy prior to collection of samples.

Rejection Criteria

Wrongly labelled samples, containers which were externally soiled, leaking containers and if the samples collected had >10 squamous epithelial cells per low powerfields as well as bacteria were rejected.

Altogether, we collected 389 samples under strict aseptic precautions.

Collection and Processing of Sample

All the samples were collected using suction catheter introduced into the ET tube and were sent immediately to Microbiology department for Culture and Sensitivity. This sample received was inoculated in thio-glycolate broth and incubated at 37º C for 18- 24 hrs. After incubation, the broth was examined primarily for evidence of any growth of the bacteria by direct Gram-stained smear. Smears were examined for detection of squamous epithelial cells and Poly-morphonuclear neutrophils (PMN) and for any presence of bacteria under low power fields (LPF) and under oil immersion field (100x) microscopy.

Then same broth was used for Culture. A calibrated loop was used to inoculate the sample on the on the sheep BA, Chocolate agar and Mac-Conkey agar (four quadrant streak technique) and incubated at 37° C for 24-48 hrs.

The plates were read on the next day for the growth by observing all the four quadrants. They

suggested approximate number of colony forming units (CFU/ml) of bacteria/ml. The cultures were graded as 1+,2+,3+ and 4+ depending upon the growth. Routine biochemical tests were put up to speciate the organism like TSI test, Citrate utilization test, Indole test, Urease test, Oxidase test, MR test, Catalase test, Coagulase test , Mannitol utilization test etc as per manual methods.

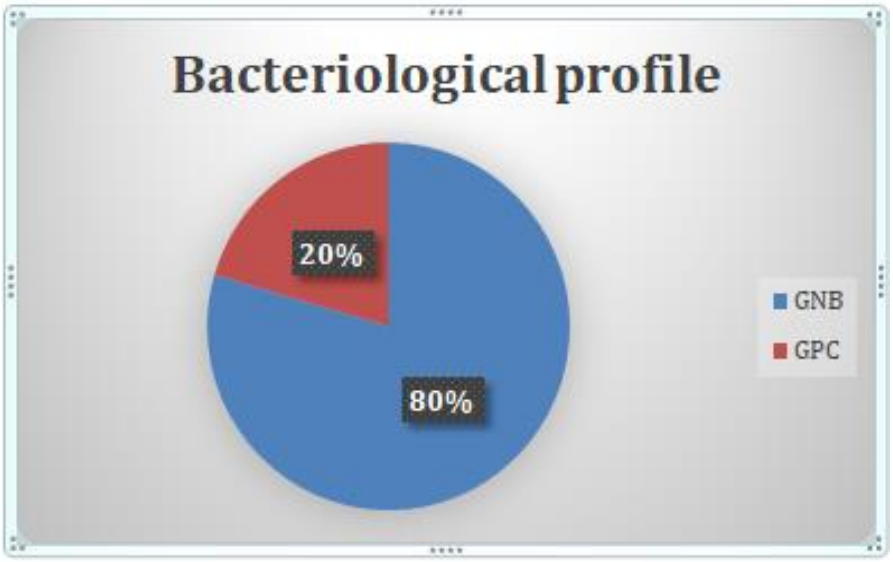
Antibiotic susceptibility testing

The obtained isolate was diluted in sterile NS and matched with Mac Farland's std 0.5, MH agar plates were used for antibiotic susceptibility testing using Modified Kirbe-baur method after doing a lawn culture of the isolate as per CLSI std guidelines. Antibiotic discs used for GNB were- Amikacin, Gentamycin, Netilmicin, Tobramycin, Cefixime, Ceftriaxone, Ciprofloxacin, Ofloxacin, Cotrimoxazole, Piperacillin-tazobactam, Cefaperazone-sulbactum, Ceftazidime, Imipenem, Meropenem, Ertapenem, Aztreonam, Cefotaxime, Polymyxin-B, Colistin and for GPC –Oxacillin, Amoxycillin-clavulonic acid, Piperacillin- tazobactum, Cefoperazone-sulbactum, Cefuroxime, Cefixime, Ceftriaxone, Ceftazidime, Erythromycin, Azithromycin, Amikacin, Gentamycin, Cotrimoxazole, Teicoplanin, Vancomycin, Linezolid discs were used.

Statistical Analysis

Above data were analyzed by statistical software SPSS version 17. A value of $p<0.05$ was accepted as significant.

Comparison between Gram Negative Bacilli and Gram - Positive COCCI



Bacteriological profile of the Endotracheal tube secretions in our study

BACTERIAL ISOLATES	NUMBER OF ISOLATES
1) Klebsiella pneumoniae (ESBL producer)	45 (22.95%)
2) Klebsiella pneumonia (ESBL and AMP C)	33(16.83%)
3) Acinetobacter baumannii	14(7.14%)
4) Acinetbacter baumannii (MBL producer)	24(12.24%)
5) Pseudomonas aeruginosa	16(8.16%)
6) Pseudomonas aeruginosa (MBL inhibitor)	20(10.20%)
7) E. coli	01(0.5%)
8) E .coli (ESBL producer)	03(1.53%)
9) Staphylococcus aureus (MSSA)	12(6.12%)
10) MRSA	28(14.28%)

Antibiotic Sensitivity pattern obtained in our study (GNB)

Antibiotic	Genta	Tobra	Amik	Netil	Cefexime	Ceftriax	Cipro	Oflox	Co-tri
K.pneu ESBL	15 (33.33%)	14 (31.11%)	8 (17.77%)	6 (13.33%)	0 (0%)	1 (2.22%)	4 (8.88%)	4 (8.88%)	2 (4.44%)
K.pneu ESBL +AMP C	9(27.27%)	6(18.18%)	12(36.36%)	12(36.36%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Acineto	3(21.42%)	4(28.57%)	3(21.42%)	2(14.28%)	0(0%)	2(14.28%)	2(14.28%)	1(7.14%)	2(14.28%)
Acineto MBL producer	2(8.33%)	1(4.16%)	1(4.16%)	0(0%)	0(0%)	0(0%)	1(4.16%)	0(0%)	0(0%)
Pseudo	8(50%)	7(43.75%)	8(50%)	6(37.5%)	0(0%)	0(0%)	3(18.75%)	1(6.25%)	0(0%)
Pseudo MBL inhibitor	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(%)	0(0%)
E.coli	1(100%)	1(100%)	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	1(100%)
E.coli ESBL	1(33.33%)	2(66.66%)	2(66.66%)	1(33.33%)	0(0%)	1(33.33%)	2(66.66%)	1(33.33%)	1(33.33%0

Antibiotic Sensitivity pattern obtained in our study (GNB)

Antibiotic	Pip-tazo	Cef-sulb	Ceftazi	Imip	Mero	Ertap	Aztre	Cefotax	Poly-B	Colist
K.pneu ESBL	23(51.11 %)	10(22.22%)	0(0%)	32(71.11 %)	33(73.33 %)	31(68.88%)	0(0%)	0(0%)	27(60%)	28(62.22%)
K.pneu ESBL +AMP C	6(18.18%)	2(6.06%)	0(0%)	32(96.96 %)	31(93.93 %)	27(81.81%)	0(0%)	0(0%)	31(93.93%)	32(96.96%)
Acineto	3(21.42%)	1(7.14%)	1(7.14%)	6(42.85%)	5(35.71%)	2(14.28%)	1(7.14%)	0(0%)	13(92.85%)	13(92.85%)
Acineto MBL producer	3(12.5%)	1(2.94%)	0(0%)	9(37.5%)	8(33.33%)	0(0%)	1(2.94%)	0(0%)	23(95.83%)	23(95.83%)
Pseudo	7(43.75%)	0(0%)	0(0%)	10(62.5%)	11(68.75 %)	1(6.25%)	6(37.5%)	0(0%)	16(100%)	15(93.75%)
Pseudo MBL inhibitor	4(20%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(5%)	0(0%)	20(100%)	20(100%)
E.coli	1(100%)	1(100%)	0(0%0	1(100%)	1(100%)	1(100%)	0(0%)	1(100%)	1(100%)	1(100%)
E.coli ESBL	2(66.66%)	1(33.33%)	1(33.33 %0	2(66.66%)	2(66.66%)	2(66.66%)	0(0%)	1(33.33%)	2(66.66%)	2(66.66%)

Antibiotic profile of GPC in our study

Isolate	OX	AMC	Pip-T	Cef-Sul	CEF	CEFTR	CEFE	CEFTA	AZI	ERY	GEN	AMI	VAN	LIN	TEIC	COTRI
Staph.aureus	12 (30%)	4 (10%)	11 (27.5%)	1 (2.5%)	9 (22.5%)	0 (0%)	0 (0%)	0 (0%)	10 (25%)	7 (17.5%)	15 (37.5%)	11 (27.5%)	38 (95%)	39 (97.5%)	21 (52.5%)	14 (35%)

Results

In this study, Males(111) were significantly affected than the females(85) . Males were notably infected with Klebsiella group (ESBL producer, ESBL and AMPC producer and non-ESBL and non-AMPC producer), Acinetobacter baumannii, Citrobacter, Enterobacter, ESBL producing E. coli, pseudomonas aeruginosa as compared to females.

In our current study, gram negative bacteria were remarkably involved as compared to gram positive bacteria (GNB=156 vs GPC=40). Table -3.

ESBL producing Klebsiella pneumoniae was highly sensitive to Meropenem(73.33%) , Imipenem(71.11%), Ertapenem (68.88%) and Colistin (62.22%). ESBL and AMP C producing Klebsiella were sensitive to Imipenem(96.96%), Colistin (96.96%) and Polymyxin -B(93.93%) and Meropenem(93.93%). Acinetobacter baumannii (both MBL and non-MBL producer) were significantly sensitive to Polymyxin-B and Colistin respectively(MBL- Polymyxin -B(95.83%), Colistin(95.83%) and non-MBL-Polymyxin-B(92.85%) and Colistin(92.85%).

On the other hand Pseudomonas aeruginosa(non-MBL inhibitor) were highly sensitive to Polymyxin-B(100%), Colistin(93.75%) in addition to Meropenem(68.75%) and Imipenem(62.5%) sensitive, where as Pseudomonas(MBL inhibitor) were highly sensitive to Polymyxin-B(100%), Colistin(100%) only. Again E .coli(ESBL producer) were highly sensitive to Imipenem, Meropenem, Ertapenem, Polymyxin -B, Colistin, Tobramycin, Amikacin, Ciprofloxacin and

Piperacillin-tazobactum 66.66%each. Also we isolated both MRSA and MSSA which showed high sensitivity to Linezolid(97.5%), Vancomycin(95%) followed by Teicoplanin(52.5%) and moderate sensitivity to Gentamycin(37.5%) and Amikacin(27.5%).

Discussion

In our study, the incidence of positivity was 53.47%(208 out of 389 cultures). In the various studies done by Saha et al(3), Sharma and Sharma(4) and Jamil et al (5) the incidence of positivity were found to be 59.26%, 31.92% and 93.8% respectively.Incidence of positivity in our study was as follows-males was 111(56.63%) as compared to females 85(43.36%) ,which was similar to the studies done by Ghosh et al(6), Saha et al(3).

In our study,incidence of prevalent bacteria were Klebsiella pneumoniae (39.79%) followed by Acinetobacter baumannii (19.38%), Pseudomonas aeruginosa (18.36%). In a similar study by Deepti Gupta et al(7) enteric Gram Negative aerobic rods were found to be the most frequent isolates with Klebsiella pneumoniae being the most common species followed by non-fermentative GNB such as Acinetobacter baumannii and Pseudomonas aeruginosa . Malik et al (8) and Chandra et al(9) have also reported K. pneumoniae as the most common isolate in the lower respiratory tract specimens of the ventilated patients. In

another study done at Bathinda(7) , also showed K. pneumoniae to be the commonest isolate seen in ET secretions. These findings suggest that GNB were more frequently associated with nosocomial infections, especially in criticalli ill patients and are difficult to treat because of their high drug resistance nature.

The maximum use of antibiotics in ICU patients drives the emergence of MDR strains. Therefore, the primary goal in all ICU's is to reduce the antimicrobial resistance and there by improve the patient"s outcome of illness in terms of reducing health care expenses, length of ICU stay and mortality. In our study,MDR among the Gram Negative Bacteria isolates of ET secretions was alarmingly high(Table-2). The most common MDR Gram Negative bacteria in our study were Klebsiella. Pneumoniae (39.79%) , Acinetobacter baumannii (19.38%), Pseudomonas aeruginosa (18.36%) , Staphylococcus aureus (20.40%) and E.coli (2.04%). Among MDRO's , ESBL production was observed maximally in K.pneumoniae (>60%) and E.coli (50-60%), while Carbapenem resistance was predominantly noticed among Acinetobacter baumannii (>60%) in Enterobacteriaceae family. These findings were consistent with the report of Malik et al.(8).

Klebsiella spp is emerging as a major MDR pathogen in nosocomial infections especially in critically ill patients. In the present study ,more than 80% Klebsiella spp showed resistance to Ciprofloxacin, Cotrimaxozole, Cephalosporines. Among Aminoglycisides, ESBL Klebsiella spp were more resistant to Tobramycin(31.11%) and Amikacin (17.77% sensitive only.) when compared to Gentamycin(33.33%-S). However, all Klebsiella spp (Both ESBL and AMP C as well as non-ESBL and non-AMP C producers) were highly sensitive to Imipenem (84%), Meropenem(83.6%) followed by Polymyxin -B (76.96%) and Colistin (79.59%).

In our study , Acinetobacter baumannii showed uniformly high-level resistance to most of the tested antibiotics (60-100%) except for Polymyxin -B (94.3%), Colistin(94.3%) followed by Imipenem(40.17%), Meropenem(34.52%). Similar high-level resistance of Acinetobacter baumannii to these drugs and high sensitivity to Colistin was reported by Jamil et al (12) in Bangladesh. This alarming low susceptibility of A. baumannii to most of these drugs, and the rapid emergence of MDR clones was probably due to overuse in critically ill patients and persistence in ICU settings. In contrast, P.aeruginosa showed relatively good susceptibility to Polymyxin-B (100%), Colistin(96.87%) similar to the report of Malik et al.However, decreased susceptibility of P.aeruginosa to Carbapenems(<40%) and aminoglycosides(20%) was noted. These findings indicate that the GNB have become increasingly resistant to multiple drugs which may be due to the facts of cross-infections and other factors such as overuse of antibiotics. Therefore,it emphasizes the regular characterization of bacteria and their resistance pattern for standardizing the most appropriate empirical therapy to control the drug resistant strains.

In the current study, 70% of Staphylococcus aureus were MRSA. All S.aureus spp showed high susceptibility to Linezolid(97.5%), Vancomycin (95%) and moderate sensitivity to Teicoplanin(52.5%), Gentamycin(37.5%) and Piperacillin-tazobactum(27.5%). These results were in-line with the reports of Chandra et al and Gupta et al.

Conclusion

In conclusion, we found the predominance of GNB in ET secretions with Klebsiella.pneumoniae

being the most common isolate. One of the grave concerns associated with nosocomial infections in ICU settings are the upsurge in MDR respiratory pathogens which are difficult to treat. Therefore, combined approach of strict antibiotic policy, judicious use of antibiotics and refined infection containment procedures are indispensible to overcome antibiotic resistance and to maintain a low level of resistant organisms. In addition, there is also a need for prioritization of molecular diagnostic techniques, being used routinely, for the most accurate and rapid identification of pathogens and improved management of severe infections caused by drug-resistant pathogens.

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