Bacteriological Profile of Endotracheal Aspirates from Patients with Lower Respiratory Tract Infections and Their Antibiotic Resistance Pattern

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ABSTRACT

BACKGROUND

Lower respiratory tract infections (LRTI) in mechanically ventilated patients are associated with high mortality and morbidity. Therefore, identification of the causative agents and their antibiotic susceptibility pattern play a key role in selecting the suitable antibiotics thus improving the condition of the patient. We wanted to study the bacteriological profile of endotracheal aspirate from patients with LRTI and their antibiotic resistance pattern.

METHODS

A total of 100 samples of endotracheal aspirates from patients with LRTI was cultured as per standard microbiological technique. Organisms were identified and antibiotic resistance pattern was studied. The gram-negative isolates were subjected for detection of extended spectrum beta lactamase (ESBL) and metallo beta lactamase (MBL). Methicillin resistance was checked for *S. aureus* as per Central Laboratory Standard Institute (CLSI) guidelines.

RESULTS

In this study acinetobacter species (27.3 %) and klebsiella species (27.3 %) were the predominant organisms that were multidrug resistant. Extended spectrum β lactamase production was reported in 78.12 % of klebsiella species, 66.67 % of *E. coli*, 50 % of acinetobacter spp and 37.5 % of citrobacter species; MBL production was detected in 57.14 % of *P. aeruginosa*. A total of 26 % *S. aureus* isolates were methicillin resistant.

CONCLUSIONS

Multi drug resistance was significantly high within the strains isolated from endotracheal (ET) secretions of LRTI patients. Majority of the MDR strains were ESBLs or MBLs producing gram negative bacilli (GNBs) or methicillin resistant *S. aureus*.

KEY WORDS

Lower Respiratory Tract Infection, Endotracheal Aspirate, Extended Spectrum β Lactamase, Metallo β Lactamase, Methicillin Resistant *Staphylococcus aureus*

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BACKGROUND

Ventilator associated pneumonia (VAP) is a sub-type of hospital acquired pneumonia (HAP) occurring in patients who are receiving mechanical ventilation (MV) for at least 48 hours or more. Although it is a lifesaving procedure for the hospitalised patients its severe drawback is acquiring persistent LRTI leads to high mortality and morbidity rate in patients.

Lower respiratory tract infection is outlined because of the inflammation of the respiratory tract ranging from trachea to the alveoli with sequent multiplication of an infectious agent. Patients who are on mechanical ventilation for a minimum of 48 hours or additional are at a risk of acquiring persistent LRTI resulting in serious health touching issues in patients worldwide. For the effective treatment of LRTI in patients, precise information of microbial investigation, higher and better diagnostic practices and antimicrobial susceptibility pattern are extremely important.

LRTI mainly in developing countries the common cause of infection among patients is pneumonia. LRTI is caused when patients are colonised with bacteria of either exogenous or endogenous origin. Colonisation in lower respiratory tract by gram negative bacteria have been frequently observed in patients after tracheostomy and patients with persistent trachea bronchial colonisation. The type of LRTI depends on various factors viz. epidemiology, pathogenesis and host response. The major respiratory pathogens are gram negative bacilli like *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa*, acinetobacter species, other non-fermentative gram-negative bacilli (NFGNB) and gram-positive organisms like *Streptococcus pneumonia, Staphylococcus aureus* etc.

The endotracheal aspirate from patient with LRTI is collected for microbiological and cultural diagnosis of LRTI. As it is easily performed at bedside and is totally simple, cheap and minimally invasive, it also has a proven acceptable precision and requires minimal speculation for the training of health professionals.[1] Multi drug resistant gram-negative bacteria are the foremost reason for concern in LRTI. Although it is a lifesaving procedure for the hospitalised patients it has a severe drawback of acquiring persistent LRTI leading to high mortality and morbidity rate in patients. The patient who had been intubated, their endotracheal tube aspirate was collected and was processed by standard methods. Many organism's including multiple drug resistant organism are responsible for causing ventilator associated pneumonia.

The misuse of antibiotics is considered as a direct cause of antibiotic resistance worldwide.[2] Multi drug resistance in GNBs may be related to enzymes like extended spectrum beta lactamase (ESBL) or metallo beta lactamase (MBL). Amongst staphylococci, methicillin resistance can be the cause for multi-drug resistance. The antimicrobial therapy for LRTIs is frequently empirical and presumptive. To study the current pattern of the organism causing LRTI, specific microbiological investigations are necessary for proper identification of causative organisms and their antibiotic susceptibility profile. Therefore, the current study was planned to study the bacteriological profile of endotracheal aspirates of hospitalised patients with LRTI and to check the antibiotic resistance pattern with special relevance to ESBL and MBL production in gram negative bacteria and methicillin resistance in *Staphylococcus aureus*.

METHODS

This is a hospital based, cross-sectional study carried out for a period of 2 years from October 2017 to March 2019 in the Department of Microbiology of MGM Medical College and Hospital after the approval from institutional ethics committee. The endotracheal aspirate (ETA) from patients with lower respiratory tract infections was collected by a trained respiratory therapist by non-bronchoscopic technique and processed immediately.

The ETA sample was immediately inoculated on blood agar, MacConkey's agar and the plates were incubated at 37^{o} C for 24 hours. The clinical isolates were identified, and antibiotic sensitivity was performed on Muller-Hinton agar plates by Kirby Bauer's disc diffusion method using suitable antibiotics as per CLSI guidelines.[3]

Test for ESBL Detection[4]

1. Screening Test

ESBL test was performed by using antibiotics like cefotaxime and ceftazidime on Muller-Hinton agar plates. Zone size less than 20 mm for cefotaxime and ceftazidime was screened positive for ESBL. The strains found to be ESBL screen positive were subjected to phenotypic confirmatory tests.

2. Phenotypic Confirmatory Test

The discs of ceftazidime (CAZ-30 µg) and ceftazidime with clavulanic acid (CAC-30 / 10 µg) and cefotaxime (CTX-30 µg) and cefotaxime-clavulanic acid (CTX-30µg) were placed on MHA plate and incubated at 37^{o} C overnight. Increase in zone size by ≥ 5 mm with ceftazidime / clavulanic acid and cefotaxime-clavulanic acid in comparison to ceftazidime or cefotaxime alone, then the strain is confirmed to be an ESBL producer.

Test for MBL Detection[4,5]

MBL production was detected in imipenem resistant isolates by phenotypic tests. The imipenem (IMP) EDTA combined disc test was used. Test organisms were inoculated on to plates of Muller Hinton agar as recommended by CLSI.[4] In the combined disc test, increase in inhibition zone with the imipenem and EDTA disc is ≥ 7 mm than the imipenem disc alone, it is considered as MBL positive.[5]

Test for MRSA Detection[4]

Methicillin resistance was detected by using a cefoxitin disc (30 µg). Plates were incubated at 35^{o} C for 24 hrs. Results were interpreted according to CLSI, a zone of growth inhibition

around cefoxitin disk ≥ 22 mm rules out MRSA; a zone size < 22 mm indicates MRSA.

Statistical Analysis

All the results were analysed statistically. Level of significance was set at 0.05. All P values > 0.05 were considered significant.

RESULTS

Out of 1,440 ET secretions of clinically suspected lower respiratory tract infection patients, 100 cases were enrolled for this project. All the 100 samples yielded mono or polymicrobial growth. A total of 117-gram negative bacteria and 38-gram positive bacteria were isolated during this study. It was observed that acinetobacter spp and klebsiella spp were the foremost predominant isolates (27.3 % each) followed by *Pseudomonas aeruginosa* (17.6 %). Amongst 38 gram positive isolates, the foremost predominant was *Staphylococcus aureus* (50 %).

		No. of Isolates	Percentage
Gram negative bacteria	Acinetobacter spp	32	27.3 %
	Klebsiella spp	32	27.3 %
	P. aeruginosa	21	17.6 %
	Citrobacter spp	11	9.4 %
	E. coli	9	7.6 %
	Enterobacter spp.	9	7.6 %
	GNNF	3	2.5 %
Gram positive bacteria	*Staphylococcus aureus*	19	50 %
	Coagulase negative *Staphylococcus aureus* (CONS)	9	23.6 %
	Streptococcus spp	9	23.6 %

Table 1. Spectrum of Gram-Negative Bacteria and Gram-Positive Bacteria Isolated in ET Secretions

Organism	Antibiotics Showing Maximum Resistance to	Antibiotics Showing Minimum Resistance to
Acinetobacter spp	Ceftazidime (96.8 %) Cefoperazone (93.7 %) Cefazolin (90.6 %)	Amikacin (34.3 %) Ofloxacin (37.5 %) Levofloxacin (3.1 %) Polymyxin B (3.1 %) Tigecycline (9.3 %)
Klebsiella spp	Tetracycline, (90.6 %) Cefuroxime (87.5 %) Ceftazidime (84.3 %) Ticarcillin / Clavulanic acid (65.6 %) Cefixime / Clavulanic acid (56.2 %) Aztreonam (50 %)	Amikacin (15.6 %) Ofloxacin (43.7 %) Cefotaxime (53.1 %) Levofloxacin (3.1 %) Imipenem (6.2 %)
P. aeruginosa	Gentamicin (95.2 %) Tetracycline (90.4 %) Cefoperazone (85.7 %)	Amikacin (4.7 %) Tobramycin (14.2 %) Ofloxacin (23.8 %) Imipenem (14.2 %) Meropenem (14.2 %)
S. aureus	Penicillin (73.6 %) Roxithromycin (68.4 %)	Cefuroxime (26.3 %) Cefazolin (36.8 %) Netilmicin (21 %)

Table 2. Antibiotics (Routine and Higher) Showing Maximum and Minimum Resistance to Organisms

Isolates	No. of Isolates	ESBL Producers No (%)	MBL Producers No (%)
Acinetobacter spp	32	16 (50 %)	0 (0 %)
Klebsiella spp	32	25 (78.12 %)	0 (0 %)
Pseudomonas aeruginosa	21	0 (0 %)	12 (57.14 %)

Table 3. Percentage of ESBL and MBL Producers in GNBs Isolated from ET Secretions

Total *S. aureus*	No. of MRSA (%)	No. of MSSA (%)
19	5 (26 %)	14 (74 %)

Table 4. Percentage of MRSA Isolated from ET Secretions

Multi drug resistance (MDR) is the antimicrobial resistance shown by the organism to over two classes of antibiotics. Prevalence of MDR in various GNBs and gram-positive cocci (GPC) was studied during which multidrug resistance was reported in acinetobacter spp (84.37 %), klebsiella spp (71.87 %) *P. aeruginosa* (33.33 %) and *S. aureus* 5 (26.31 %). (Figure 1)

Prevalence of ESBL and MBL production in MDR and non MDR isolates was studied that showed MDR isolates were the chief ESBL or MBL producers.

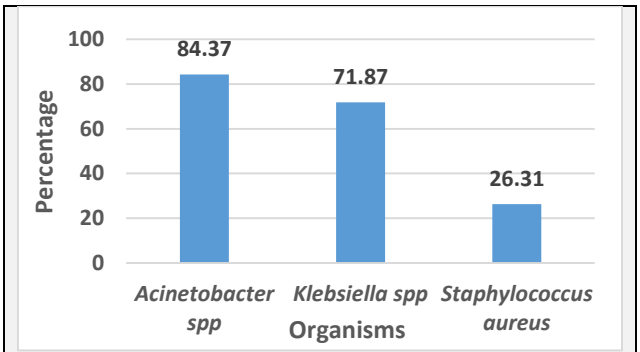

Figure 1. Percentage of MDR in GNBs and GPC

Organisms	ESBL or MBL Production in MDR (%)	ESBL and MBL Production in Non MDR (%)
Acinetobacter spp	16 (50 %)	0
Klebsiella spp	23 (100 %)	2 (22.2 %)
P. aeruginosa	7 (100 %)	5 (35.71 %)

Table 5. Prevalence of ESBL or MBL Production in MDR and Non MDR Isolates

DISCUSSION

Lower respiratory tract infection remains the main reason of concern from several decades resulting in high mortality and morbidity rate worldwide. Lower respiratory tract is colonised chiefly by endogenous and exogenous bacteria.

During this present study, seven totally different gram-negative bacteria were isolated. They were acinetobacter spp 32 (27.3 %) klebsiella spp 32 (27.3 %) followed by *P. aeruginosa* 21 (17.6 %), citrobacter spp 11 (9.4 %), *Escherichia coli* (7.6 %), enterobacter spp 9 (7.6 %) and gram negative non fermenters 3 (2.5 %).

In the study by Deepti Chandra et al[6] the common organisms were klebsiella species, 11 (32.35 %) followed by acinetobacter spp, 7 (20.58 %), *Pseudomonas aeruginosa,* 5 (14.70 %).

In this present study 38 Gram positive bacteria were isolated, out of which *S. aureus* 19 / 38 (50 %) was the foremost predominant organism. Present study correlates with study by Purba Mukherjee and Prativa Biswas[7] that showed *S. aureus* (50 %) as the most predominant pathogen.

In this present study antibiotic resistance pattern of gram negative and gram-positive organism was studied. In our study, acinetobacter spp showed maximum resistance to ceftazidime (96.8 %), cefoperazone (93.7 %) and cefazoline (90.6 %) whereas within the study by Santosh Khanal et al[8] very high resistance to ofloxacin (80 %) and amikacin (78.2 %) was reported.

In the present study, klebsiella spp, showed resistance to tetracycline (90.6 %), cefuroxime (87.5 %) and ceftazidime (84.3 %). Santosh Khanal et al[8] in his study reported a really high resistance to amikacin (54.8 %), cefotaxime (78.6 %), ofloxacin (64.3 %) and imipenem (9.5 %). In our study solely 15 % of klebsiella was resistant to amikacin.

Present study showed *P. aeruginosa* was resistant to gentamicin (95.2 %) and amikacin (80.9 %) which correlates with the study by Azar Dokht K et al within which resistance was reported to gentamicin (69.7 %), amikacin (68.4 %) and imipenem (60.4 %). Similar findings have been additionally reported from Iran in 2013.[9]

Amongst gram positive bacteria, *S. aureus* showed resistance to penicillin (73.6 %) and roxithromycin (68.4 %). Study by Regha IR and Sulekha B also reported resistance to penicillin (84.6 %).[10]

In the current study ESBL and MBL production was studied. ESBL production was predominantly seen in klebsiella spp (78 %) and acinetobacter spp (50 %) and MBL production was observed in *Pseudomonas aeruginosa* (57.14 %). A study by Santosh Khanal et al[8] also showed similar findings. ESBL and MBL producing bacteria are increasing public unhealthiness worldwide and mortality rates are increased because of inadequate empirical therapy.

MRSA is a major infectious agent inflicting LRTI and the identification of MRSA carriage is a vital measure to prevent and control these infections in LRTI. The risk factor for the development of LRTI is the settlement of lower airways. In the present study 5 / 19 (26 %) methicillin resistant *S. aureus* were isolated that correlates with the study by Shrestha RK et al[11] which shows 6 isolates were MRSA.

Multi drug resistance pattern was studied in gram negative and gram-positive bacteria. Out of total 117 bacteria 74 bacteria were multidrug resistant of which predominant bacteria were acinetobacter spp 27 / 32 (84.37 %) and klebsiella spp 23 / 32 (71.87 %). Similar findings were also shown by Santosh Khanal et al[8] where in acinetobacter spp (85.4 %) and klebsiella spp (73.8 %) were the predominant MDR GNBs.

Present study shows multi drug resistance in gram positive bacteria. Out of 19 isolates of *S. aureus*, 5 (26.3 %) isolates of *S. aureus* were multi drug resistant. This is less than the percentage reported by Santosh Khanal et al[8] in which 6 / 12 (50 %) *S. aureus* were MDR.

The potential factors enhancing the emergence of resistant bacteria in hospitalised patients might be due to prolonged stay in hospitals, mechanical devices, previous use of broad-spectrum antibiotics. The emergence of MDR will be prevented by adopting antibiotic policy worldwide. The emergence of MDR pathogens can be prevented by adopting antibiotic institutional policy and dose de-escalation regimes.[12]

In this present study, prevalence of ESBL and MBL production in MDR and non MDR isolates was studied which showed multi drug resistance was related to either ESBL or MBL production in GNBs.

The importance of ESBL or MBL producing strains lies in the fact that they are difficult to treat because they carry plasmids which confer resistance to many other antibiotics.[13]

The pan drug resistant strains are on the verge of arising. The incidence of ventilator associated pneumonia can be prevented by adopting careful intubation techniques, oral intubation, avoid gastric over-distention, maintaining adequate endo tracheal cuff pressure and efficient tracheal toileting may facilitate in minimising the VAP cases.[14]

CONCLUSIONS

LRTI remains an important cause of high mortality and morbidity rate worldwide; therefore, identification of the causative agents and their antibiotic susceptibility pattern will play a key role in selecting the suitable antibiotics for clinicians and will improve conditions.

The present study revealed gram negative bacteria as the major pathogens inflicting LRTI; therefore, by performing aseptic measures within the intensive care unit (ICU), the frequency of LRTI can be controlled.

Besides the high degree of antibiotic resistance in hospitals, the major concern is ESBL and MBL in gram negative bacteria. Routine antibiotic susceptibility testing could fail to notice ESBL and MBL producers; therefore, straight forward ways like confirmatory test method may play a very important role in screening the ESBL and MBL producing isolates.

Methicillin resistance in gram positive bacteria also plays a major role in resistance of *S. aureus*. Periodic and statistical analysis of LRTI pathogens and their antibiogram should be carried out in every institution for effective infection management.

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